MAGNETIC RESONANCE IN CHEMISTRY, VOL. 36, 211–216 (1998)

Utilizing NMR Spectroscopy for Assessing Drug Enantiomeric Composition

Martin Thunhorst and Ulrike Holzgrabe*

Pharmaceutical Institute, University of Bonn, Kreuzbergweg 26, D-53115 Bonn, Germany

Received 17 June 1997; revised 11 September 1997; accepted 26 September 1997

ABSTRACT: Heptakis(2,3-di-*O*-acetylated)-β-cyclodextrin was tested for its utilizability to check the enantiomeric purity of the chiral protonated phenethylamines, such as selegiline, amphetamine and norephedrine, by means of NMR spectroscopy. For all compounds, the method turned out to be a powerful and rapid tool, which should be considered more frequently in drug analysis.

KEYWORDS: NMR; ^{1}H NMR; phenethylamines, heptakis(2,3-diacetyl)-β-cyclodextrin; enantiomeric purity

INTRODUCTION

National authorities and the European Pharmacopoeia Commission have recently aimed to introduce NMR spectroscopic methods for the determination of the isomeric composition and enantiomeric purity of drugs, because these methods were found to be superior to the method of specific optical rotation used in all pharmacopoeias.[1] Since enantiomers of a chiral drug can exhibit different pharmacological and toxicological profiles,[2] the proof of enantiomeric purity of chiral drugs is pivotal. Several NMR methods have been described as appropriate for this purpose,[3,4] e.g. methods utilizing lanthanide shift reagents (LSR),[5,6] chiral solvating agents such as (−)-(*R*)-2,2,2-trifluoro-1-(9-anthryl-ethanol)[7–9] and derivatization procedures.[10] The NMR methods were found to have some advantages over HPLC for chiral analysis, i.e. the time of method development is shorter, the methods are more robust and the time of analysis is shorter because the NMR instruments are always ready for measurement, without any preceding conditioning or calibration procedure being necessary.[1]

The use of cyclodextrins (CDs) as chiral resolution agents in NMR spectroscopy has been well established in the last decade.[11–15] Regarding the application of CDs and corresponding derivatives as chiral shift reagents, the formation of diastereomeric complexes between the CD and a chiral substrate in D_2O leads to the possibility of observing the corresponding signals of each enantiomer in its complex with the CD and, consequently, it allows the quantification of the enantiomeric excess (*ee*) in a sample.

Advantages of CDs in chiral NMR analysis are the water solubility of the samples, no signal broadening effects, in contrast to LSR,[6] and the narrow chemical shift range of CDs.[16] In previous studies we have established heptakis(2,3-*O*-diacetyl)-β-CD (Diac-CD) as a powerful tool to discriminate between the enantiomers of chiral phenethylamines in clinical use by means of NMR spectroscopy and capillary electrophoresis.[17–19] Since good signal splitting of the phenethylamines studied was observed in each case, the purpose of this study was to establish whether it is possible to apply the conditions of measurement to other structurally related chiral drugs. Selegiline (formerly deprenyl), an antiparkinsonian drug, was chosen as a test compound. Since only the *R*-isomer of selegiline inhibits the monoamine oxidase irreversibly,[20] this isomer has to be used in high enantiomeric purity. The transparency statement of the selegiline monograph[21] describes (*R*)-methamphetamine, (*R*)-amphetamine, (*R*)-desmethylselegiline, norephedrine and (*S*)-selegiline as impurities. In this context, the European Pharmacopoeia Commission is seeking a robust and cheap test which allows one to find a content of (*S*)-selegiline of $<2\%$ in a batch. In addition, methamphetamine and amphetamine are metabolites of selegiline.[20] From this selection of compounds, selegiline, amphetamine and norephedrine were chosen to check whether it is possible to determine the *ee* by NMR spectroscopy upon Diac-CD complexation (see Fig. 1).

EXPERIMENTAL

All spectra were recorded using a Bruker AMX 500 spectrometer operating at 500.137 MHz with a sample temperature of 26 °C. The chemical shifts were referenced to the HDO signal at 4.650 ppm; 128 scans over a frequency width of 4132.2 MHz were collected into 32K data points, giving a digital resolution of 0.13 Hz per point. Sufficient quantities of the phenethylamines with and without Diac-CD were dissolved in D_2O to

* *Correspondence to*: U. Holzgrabe, Pharmaceutical Institute, University of Bonn, Kreuzbergweg 26, D-53115 Bonn, Germany.
E-mail: holzgrabe@uni-bonn.de

CCC 0749-1581/98/030211–060 $17.50

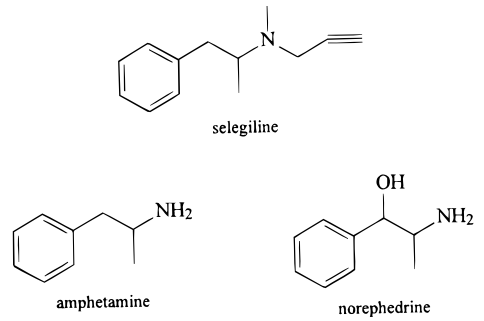

Figure 1. Structures of the racemic phenethylamines studied.

give 12 mmol concentrations of each racemate in a 5 mm tube. In order to obtain a Job plot, NMR spectra of mixtures of selegiline and Diac-CD in various ratios were recorded according to Branch *et al.*[17]

Diac-CD was synthesized according to the literature,[17] selegiline was a gift from ASTA Medica (Frankfurt, Germany), norephedrine was purchased from Aldrich (Steinheim, Germany) and amphetamine and dexamphetamine were generous gifts from Dr M. Neugebauer (Pharmaceutical Institute, University of Bonn, Bonn, Germany).

RESULTS AND DISCUSSION

In previous studies, acetylated CDs have been shown to be appropriate for discriminating between the enantiomers of several chiral phenethylamines.[17,18] The previously optimized conditions for NMR measurements were used in this study. The ^{1}H NMR spectral assignments of all derivatives and the changes in the chemical

Table 1. Chemical shifts of the phenethylamines and $\Delta\delta$ values induced upon complexation with Diac-CD

Compound	Arom. H	Benzyl H		C*H*-CH_3	CH-C*H*$_3$	N-C*H*$_3$	N-C*H*$_2$
(*R*,*S*)-Selegiline	7.296	2.802	3.094	3.870	1.174	2.847	4.000
(*R*,*S*)-Selegiline + Diac-CD	7.328	—[a]	3.067	3.987	1.209/1.244	2.935	4.095
$\Delta\delta$ (ppm)	0.032		−0.027	0.117	0.035/0.070	0.088	0.095
Amphetamine	7.278	2.847		3.534	1.203	—	—
Amphetamine + Diac-CD	7.304	2.914/2.950		3.608/3.654	1.235/1.245	—	—
$\Delta\delta$ (ppm)	0.026	0.067/0.103		0.074/0.102	0.032/0.042		
Norephedrine	7.359	4.865		3.593	1.095	—	—
Norephedrine + Diac-CD	7.361	4.874/4.895		3.604	1.097	—	—
$\Delta\delta$ (ppm)	0.002	0.009/0.030		0.011	0.002		

[a] Hidden.

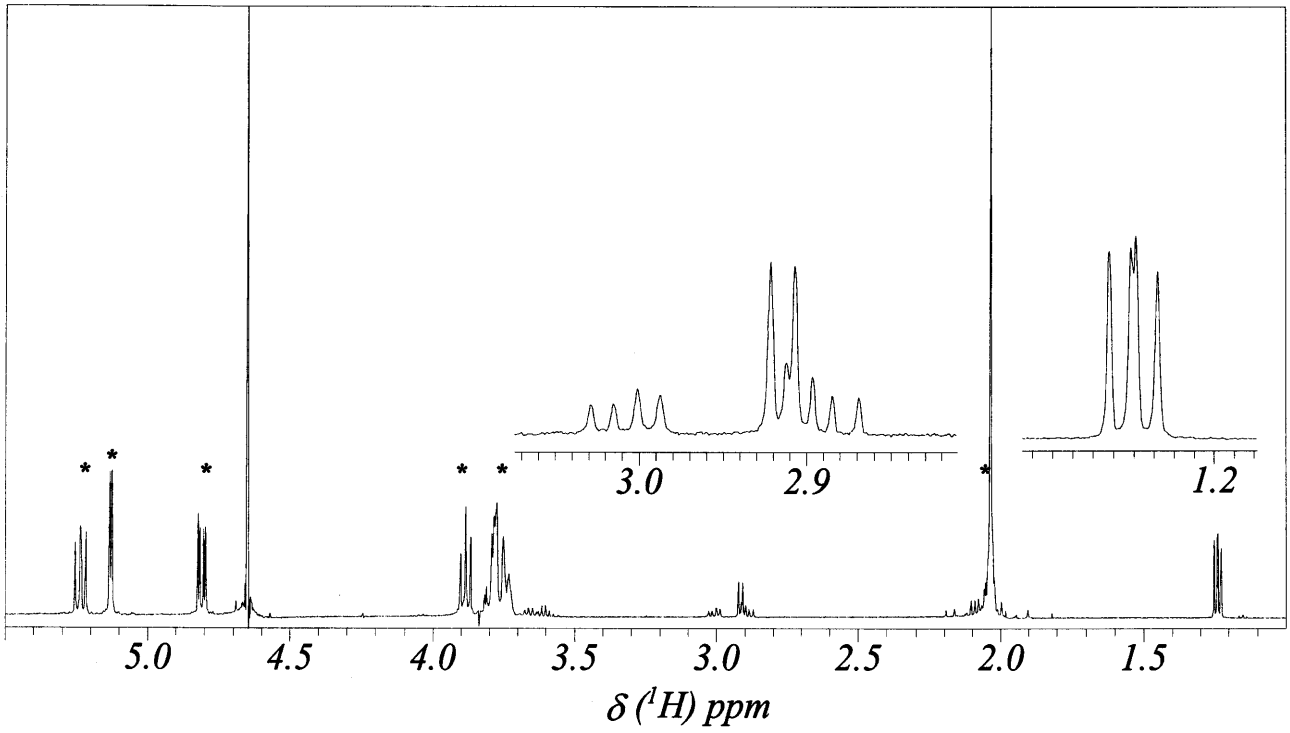

Figure 2. ^{1}H NMR spectrum of amphetamine in the presence of Diac-CD. Asterisks indicate signals of Diac-CD.

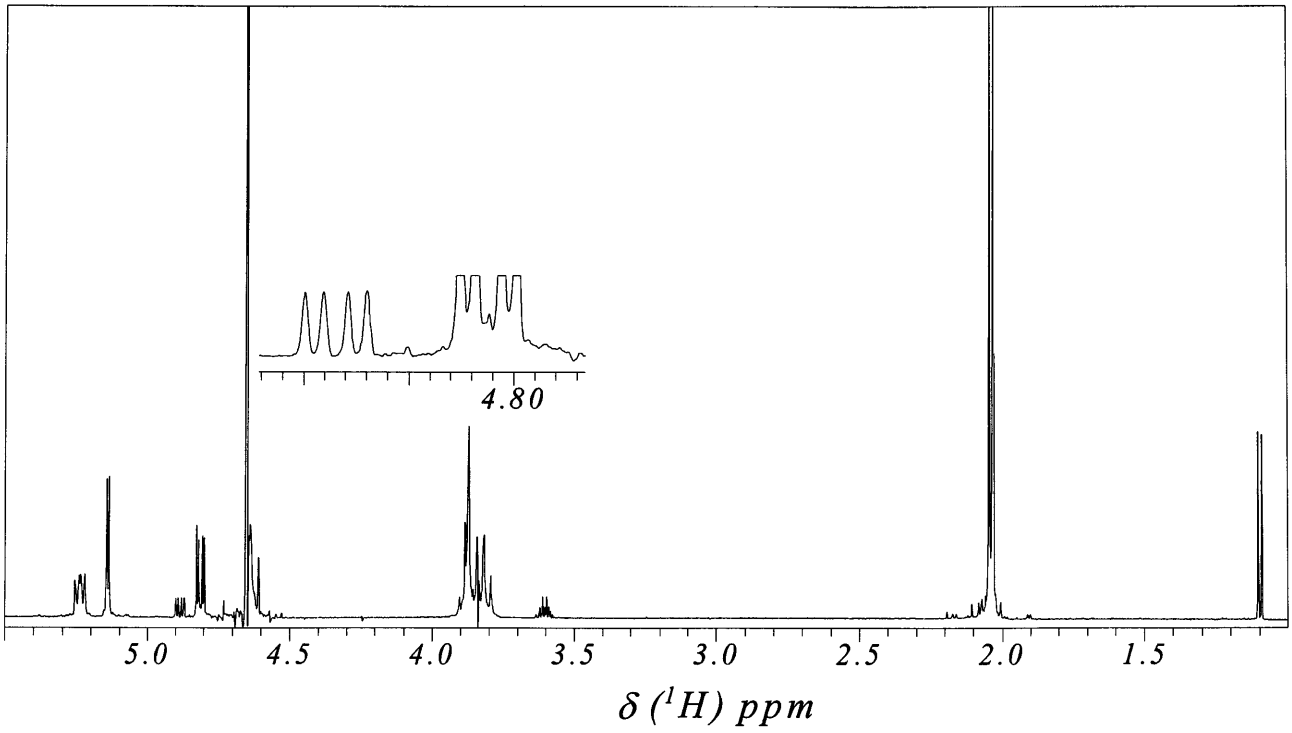

Figure 3. ^{1}H NMR spectrum of norephedrine in the presence of Diac-CD.

shifts ($\Delta\delta$ values) induced by Diac-CD are given in Table 1. In general, the signals of the CD and the analytes were clearly separated. A significant shift of almost all signals can be seen for all compounds, indicating complexation between the CD and the phenethylamines.

Almost all signals of the racemic amphetamine are split in the presence of Diac-CD. Two sextets for the *N*-methine hydrogen (at *ca.* 3.6 ppm) can be found (Fig. 2); one is very close to a CD signal, which makes an integration of the signals very difficult. In addition, the benzylic hydrogens (at *ca.* 2.9 ppm) show different signal

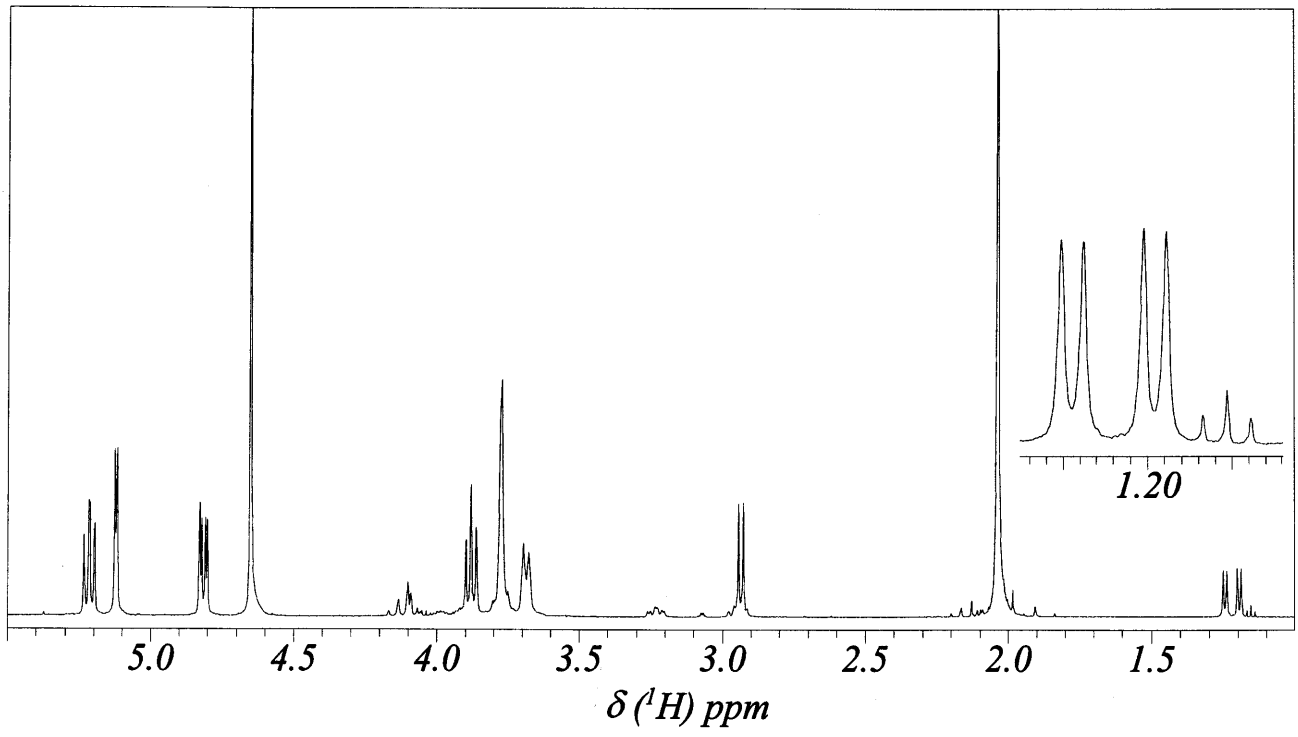

Figure 4. ^{1}H NMR spectrum of selegiline in the presence of Diac-CD (traces of ethyl acetate).

patterns for each isomer, which supports the hypothesis of a different orientation of the enantiomers in the CD cavity.[19] Whereas the uncomplexed racemic amphetamine shows a simple doublet for the benzylic hydrogens and a sextet for the neighbouring methine hydrogen, two doublets of doublets can be detected for one isomer, indicating the inequivalence of the geminal hydrogens in the case of CD complexation. The doublet of high intensity for the other enantiomer gives evidence of greater flexibility in the presence of the CD. The *C*-methyl groups are split, but not well separated.

In the case of racemic norephedrine, the benzylic hydrogen (at *ca.* 4.9 ppm) shows a splitting and complete resolution upon complexation with Diac-CD (Fig. 3). Additionally, the CD signals are clearly separated from the norephedrine signals. No other signal splitting can be detected.

With the exception of the signals of the aromatic hydrogens, all signals of the racemic selegiline are strongly shifted and split in the presence of Diac-CD (Fig. 4), indicating deep penetration of the molecule into the torus of the CD. Whereas the benzylic hydrogens and the hydrogens next to the nitrogen show unresolved signals upon complexation, the doublets of the C-CH_3 group at about 1.2 ppm are well separated. A Job plot utilizing the $\Delta\delta$ values of either the H-5 or Diac-CD or of the chiral CH-CH_3 hydrogen of selegiline (at *ca.* 3.9 ppm) revealed a defined 1:1 composition of the selegiline–CD complex (Fig. 5), which is in accordance with the results obtained for the corresponding phenethylamines.[17] This finding indicates the reproducibility of the NMR method, which is imperative for the analysis of the enantiomeric composition of a drug upon complexation with a CD.

In order to check whether the integration of split signals is suitable for determining the enantiomeric composition of the drugs, defined mixtures of the enantiomers (95:5, 97.5:2.5 and 98.75:1.25, corresponding

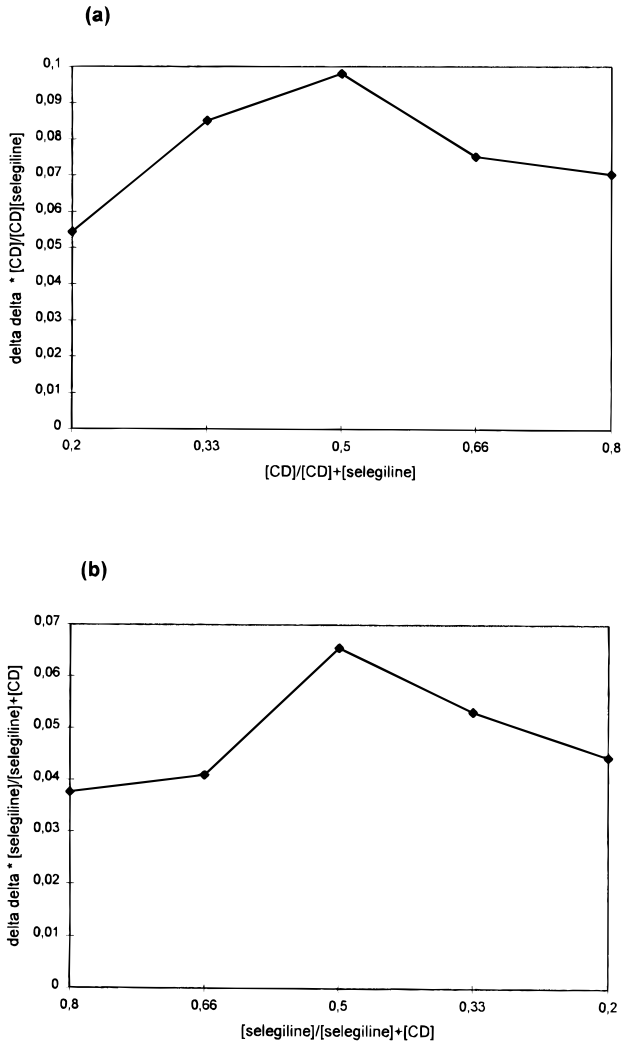

Figure 5. Job plot of selegiline in the presence of Diac-CD. Abscissa: ratio of Diac-CD to selegiline (1 = 1:4; 2 = 1:2; 3 = 1:1; 4 = 2:1; 5 = 4:1). Ordinate: $\Delta\delta$ values of (a) hydrogens of Diac-CD and (b) hydrogens of selegiline.

Table 2. Analysis of synthetic mixtures of (R)- and (S)-phenethylamines by ^{1}H NMR spectroscopy with Diac-CD[a]

Amount added (μl)		Amount found % of each enantiomer	(% of each enantiomer)
Dexamphetamine	*rac.*-Amphetamine	*S*:*R*	*S*:*R*
900	100	95:5	94.7:5.3
1900	100	97.5:2.5	97.2:2.8
1950	50	98.75:1.25	98.5:1.5
(*S*)-Norephedrine	*rac.*-Norephedrine	*S*:*R*	*S*:*R*
900	100	95:5	94.4:5.6
1900	100	97.5:2.5	97.8:2.2
1950	50	98.75:1.25	98.86:1.14
(*R*)-Selegiline	(*S*)-Selegiline	*R*:*S*	*R*:*S*
950	50	95:5	94.2:5.8
1950	50	97.5:2.5	97.5:2.5
1975	25	98.75:1.25	98.8:1.2

[a] All solutions were prepared with 12 mmol stock solutions of the single enantiomer or the racemate.

to 90, 95 and 97.5% *ee*, respectively) of all compounds and Diac-CD were prepared and ^{1}H NMR spectra recorded. In the case of amphetamine and norephedrine, the signals of the benzyl hydrogens, and in the case of selegiline, the signal of the *C*-methyl groups were used for integration and calculation of the enantiomeric excess (Fig. 6). As can be seen from Table 2, the results of the integration of the above-mentioned signals are in fairly good accord with the weighed composition of the enantiomers.

CONCLUSION

The conditions of NMR measurements that were previously optimized for other phenethylamines were found to be appropriate for the discrimination of the enantiomers of selegiline, amphetamine and norephedrine. In all these cases, signal groups could be found for the determination of the enantiomeric purity. Thus, CD derivatives and especially 2,3-diacetylated CDs are powerful chiral solvating agents. Several gas and high-performance liquid chromatographic and capillary electrophoresis methods have recently been reported for the determination of isomeric composition of amphetamine and norephedrine[22–27] and selegiline.[24,28] Since several parameters had to be optimized when using such methods, e.g. temperature, eluent, derivatization or modifier, method development takes a long time. In addition, chiral columns, highly purified solvents and other chemicals can be costly. Taking these disadvantages of the chromatographic and electrophoretic methods on the one hand and the robustness

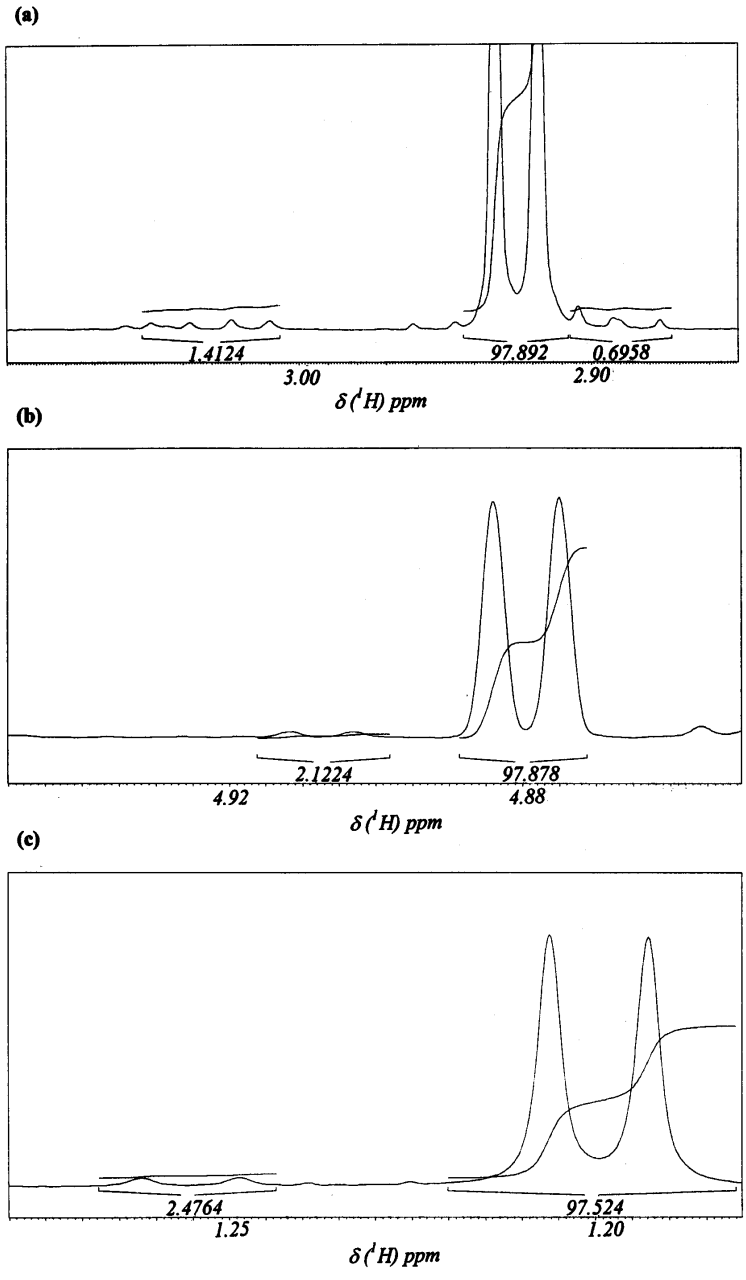

Figure 6. ^{1}H NMR spectra of the phenethylamine–Diac-CD (97.5 : 2.5) showing (a) the signals of benzyl hydrogens of amphetamine, (b) the signals of benzyl hydrogens of norephedrine and (c) the signals of the methyl group of selegiline.

of NMR spectroscopy on the other into account, it can be concluded that NMR spectroscopy should be considered more frequently in drug analysis.

Acknowledgements

Thanks are due to Mr Tomberg (Institute of Physiological Chemistry, University of Bonn) for carefully recording the NMR spectra and Dr S. K. Branch (School of Pharmacy and Pharmacology, University of Bath, UK) for valuable discussions and carefully reading of the manuscript.

REFERENCES

1. B. A. Dawson and G. L. Mattok, *Pharmeuropa* **9**, 347 (1997).
2. G. Bejeuhr and U. Holzgrabe, *Dtsch. Apoth. Ztg.* **134**, 2133 (1994), H. J. Roth, *Dtsch. Apoth. Ztg.* **137**, 1030 (1997); S. Batra, M. Seth and A. P. Bhaduri, *Prog. Drug Res.* **41**, 192 (1993).
3. D. Parker, *Chem. Rev.* **91**, 1441 (1991).
4. H. Y. Aboul-Enein, *Anal. Lett.* **21**, 2155 (1988).
5. V. E. U. Costa and M. Axt, *Magn. Reson. Chem.* **34**, 929 (1996).
6. J. W. Jaroszewski and A. Olsson, *J. Pharm. Biomed. Anal.* **12**, 295 (1994).
7. P. M. Lacroix, B. A. Dawson, R. W. Sears and D. B. Black, *Chirality* **6**, 484 (1994).
8. G. M. Hanna and C. A. Lau-Cam, *J. Pharm. Biomed. Anal.* **11**, 665 (1993).
9. G. R. Weisman, *Asymm. Synth.* **1**, 153 (1983).
10. B. A. Dawson and D. B. Black, *J. Pharm. Biomed. Anal.* **13**, 39 (1995).
11. D. Greatbanks and R. Pickford, *Magn. Reson. Chem.* **25**, 208 (1987).
12. A. F. Casy and A. D. Mercer, *Magn. Reson. Chem.* **26**, 765 (1988).
13. K. B. Lipkowitz, S. Raghothama and J. Yama, *J. Am. Chem. Soc.* **114**, 1554 (1992).
14. A. F. Casy, A. D. Cooper, T. M. Jefferies, R. M. Gaskell, D. Greatbanks and R. Pickford, *J. Pharm. Biomed. Anal.* **9**, 787 (1991).
15. J. Klein, H. Hartenstein and D. Sicker, *Magn. Reson. Chem.* **32**, 727 (1994).
16. A. F. Casy, *Trends Anal. Chem.* **12**, 185 (1993).
17. S. K. Branch, U. Holzgrabe, T. M. Jefferies, H. Mallwitz and M. Matchett, *J. Pharm. Biomed. Anal.* **12**, 1507 (1994).
18. S. K. Branch, U. Holzgrabe, T. M. Jefferies, H. Mallwitz and F. J. R. Oxley, *J. Chromatogr.* **758**, 277 (1997).
19. U. Holzgrabe, H. Mallwitz, S. K. Branch, T. M. Jefferies and M. Wiese, *Chirality* **9**, 211 (1997).
20. R. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, pp. 306–307 and references cited therein. Academic Press, San Diego (1992).
21. *Pharmeuropa* **8**, 22 (1996).
22. J. T. Cody, *Forensic Sci. Int.* **80**, 189 (1996).
23. E. Szöko, J. Gyimesi, L. Barcza and K. Magyar, *J. Chromatogr. A* **745**, 181 (1996).
24. B. A. Ingelse, F. M. Everaerts, J. Sevcik, Z. Stransky and S. Fanali, *J. High Resolut. Chromatogr.* **18**, 348 (1995).
25. I. S. Lurie, *J. Chromatogr.* **605**, 269 (1992).
26. Z. Wang, Y. Sun and Z. Sun, *J. Chromatogr. A* **735**, 295 (1996).
27. S. Cladrowa-Runge, R. Hirz, E. Kenndler and A. Rizzi, *J. Chromatogr. A* **710**, 339 (1995).
28. J. Sevcik, Z. Stransky, B. A. Ingelse and K. Lemr, *J. Pharm. Biomed. Anal.* **14**, 1089 (1996).

