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Short communication

Determination of the diastereoisomeric purity of D,L- and *meso*-HM-PAO by ^{13}C-NMR spectroscopy

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Abstract

D,L-hexamethylpropyleneamine oxime (HM-PAO) is well known to be the effective isomer when HM-PAO is used as a radiopharmaceutical. Its diastereoisomeric purity is of great importance because *meso*-impurity decreases the concentration of the ^{99m}Tc-complex in the brain. The described investigation shows that ^{13}C-NMR spectroscopy is a suitable analytical method for the determination of the diastereoisomeric purity of HM-PAO. It also can be used for assessment of the relative ratio of both isomers in diastereoisomeric mixtures. It is important to note that the patterns of behaviour of both isomers in ^{13}C-NMR spectra are the same in all solvents tested. The method is simple, fast and explicit.

Keywords: Hexamethylpropyleneamine oxime; Diastereoisomeric purity; ^{13}C-NMR; ^{99m}Tc-ligand

1. Introduction

Technetium complexes of hexamethylpropyleneamine oxime (HM-PAO, **1**) are of interest as commercial radiopharmaceuticals for the diagnosis of ischemia in the heart and the brain as an agent for cerebral perfusion imaging [1–7]. There are data in the literature showing that high quality cerebral blood flow images can be obtained by ^{99m}Tc-HM-PAO, as the complex can cross the intact blood brain barrier [6–9]. While the influence of stereochemistry on the pharmacological action of drugs are well known [10,11], it has been shown recently that the stereochemistry of the HM-PAO affects the distribution properties of the ^{99m}Tc-complexes employed in diagnostic nuclear medicine [12,13].

HM-PAO (**1**) exists in two diastereoisomeric forms, D,L- and *meso*- (Fig. 1), and it has been shown [1,3,7] that the D,L-isomer provides a ^{99m}Tc-complex with superior brain uptake and retention, compared with the complexes generated, either from the *meso*-isomer, or from the isomeric mixture. As the *meso*-impurity in the ligand decreases concentration of the ^{99m}Tc-complex in the brain, the purity of the D,L-ligand is of great importance.

HM-PAO (**1**) has two chiral centers, giving rise to *meso*-, D- and L-forms. Separation of D,L- and *meso*-diastereoisomers formed by a two-step reaction procedure was achieved by repeated fractional recrystallisation. As most of these fractions are in some degree mixed and their purity cannot be judged solely by means of TLC or melting points, the appropriate analytical technique have to be used. ^{1}H-NMR spectroscopy was found to be effective [14], relying on the difference in chemical shifts and multiplicities of the signals for the methyl group in position 6 (Fig. 1). This chemical shift difference, however, is very small and these signals are overlapped which makes their assignment difficult. On the other hand the chemical shift difference between the signals for methylene group C-atoms in D,L- and *meso*-forms of HM-PAO in ^{13}C-NMR spectra is significant which allows their explicit differentiation. On this basis

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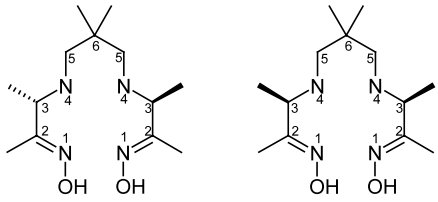

Fig. 1. Diastereoisomers of HM-PAO.

we now report an efficient simple method for establishment of the diastereoisomeric purity of HM-PAO by means of ^{13}C-NMR spectroscopy.

2. Results and discussion

HM-PAO was synthesised by a two-step procedure [3,15–20] from commercially available materials as an equal mixture of diastereoisomers, D,L- and *meso*-forms. Repeated recrystallisation from ethyl acetate permits the separation of the pure isomers. As the *meso*-form has lower solubility in this solvent than the D,L-one two very unequally populated fractions were isolated after the first run. Each fraction was recrystallised many times from the same solvent to give the pure *meso*-isomer (m.p. 147–8 °C) from the first fraction and pure D,L-form (m.p. 130–1 °C) from the second one.

NMR spectra were recorded on a Bruker DRX 250 spectrometer in different solvents (CD_3OD, DMSO-d_6, $CDCl_3$). Chemical shifts (δ) are quoted in ppm using TMS as internal standard.

Assignment of signals in the ^{13}C-NMR spectra of the pure diastereoisomers of HM-PAO was made on the basis of ^{1}H–^{13}C heteronuclear correlation (HSQC) experiments using the known proton assignments. As can be seen in Table 1, apart from two, most of the signals have very close values for the different isomers. The geminal methyl groups in position 6 (Fig. 2) provide two carbon signals for the *meso*-form in DMSO while in the D,L-one a single signal is observed. As these signals overlap in the diastereoisomeric mixture their use for determination of diastereoisomeric purity is inappropriate. On the other hand the carbon signals of the methylene groups (C-5) are quite suitable because of the significant shift difference between the two isomers. As can be seen in Table 1, the methylene group signal in the D,L-isomer is shifted downfield as compared with the *meso*-form and this pattern is valid in all solvents tested. As can be seen in Fig. 2 and in Table 1, the shift differences between these signals are 0.57 and 0.46 ppm in CD_3OD and DMSO-d_6, respectively.

^{13}C-NMR spectra of impure samples of both isomers give an illustration of the usefulness of these signals for determination of the diastereoisomeric purity of HM-PAO. As can be seen in Fig. 3, even a small impurity in both isomers can be detected by this technique. In both cases the signal of the minor form is clearly monitored in a spectral region free of other signals which proves the validity of the method.

In conclusion, ^{13}C-NMR spectroscopy represents a fast, simple and explicit technique that can be used as a reliable method for establishment of the diastereoisomeric purity of HM-PAO ligand.

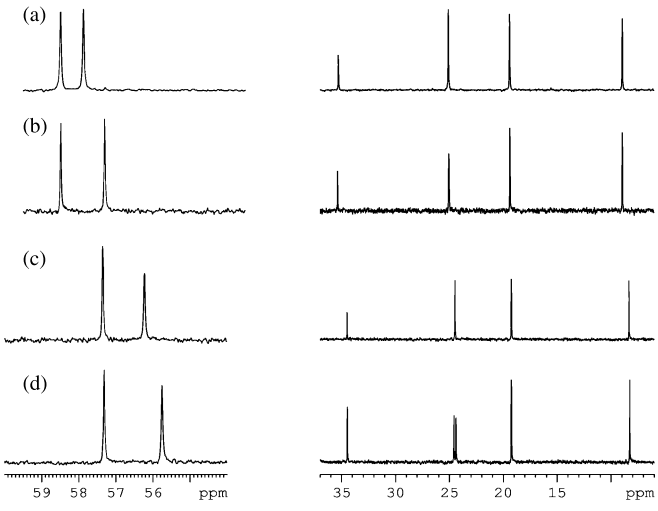

Fig. 2. 250 MHz ^{13}C-NMR spectra of D,L- (a, c) and *meso*-isomers (b, d) of HM-PAO ligand in CD_3OD (a, b) and DMSO-d_6 (c, d).

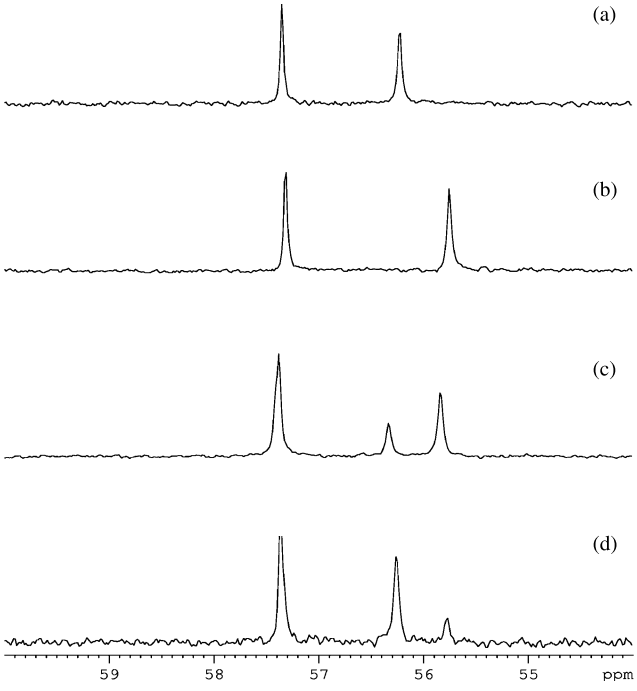

Fig. 3. 250 MHz ^{13}C-NMR spectra in DMSO-d_6 of HM-PAO ligand: (a) pure D,L-isomer; (b) pure *meso*-isomer; (c) impure *meso*-isomer; (d) impure D,L-isomer.

Table 1
Assignment (HSQC), chemical shifts (δ, ppm) and coupling constants (J, Hz) of D,L- and *meso*-diastereoisomers of HM-PAO

Isomer	Solvent	CH_3-6	CH_3-6	C-6	CH_2-5	CH_2-5 AB	CH-3	CH-3	CH_3-3	CH_3-3	CH_3-2	CH_3-2	C-2	OH
D,L	CD_3OD	25.09	0.882 s	35.29	58.37	2.323 2.211 11.6	58.98	3.227 q	19.39	1.164 d, 6.6	8.95	1.769 s	161.35	–
	DMSO-d_6	24.48	0.783 s	34.49	56.22	2.175 2.061 11.4	57.35	3.150 q	19.25	1.068 d, 6.6	8.32	1.643 s	158.49	10.258
	$CDCl_3$[a]	–	0.876 s	–	–	2.333 2.259 11.6	–	3.261 q	–	1.184 d, 6.6	–	1.819 s	–	–
meso	CD_3OD	25.03[b]	0.877 0.869 s	35.36	57.80	2.310 2.220 11.6	58.98	3.226 q	19.36	1.164 d, 6.8	8.95	1.768 s	161.47	–
	DMSO-d_6	24.58 24.38	0.776 s[b]	34.44	55.76	2.164 2.075 11.3	57.31	3.127 q	19.25	1.066 d, 6.8	8.27	1.643 s	158.52	10.250
	$CDCl_3$[a]	–	0.893 0.857 s	–	–	2.337 2.271 11.4	–	3.238 q	–	1.186 d, 6.7	–	1.827 s	–	–

[a] Not enough soluble for a carbon NMR spectrum in analytically reasonable time.
[b] A singlet is observed.

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