SYNTHESIS OF DEUTERIUM-LABELED IMIPRAMINE USING ACID-CATALYZED EXCHANGE REACTION

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SUMMARY

Synthesis of three forms of selectively deuterated imipramine with high isotopic purity using acid catalyzed hydrogen-deuterium exchange reaction is described. Deuterated imipramine labeled at the positions of 2,4,6 and 8 [IP-d_4(I)] was prepared directly by heating imipramine in 10% DCl-D_2O at 80° for 8hr. Imipramine labeled at all of the eight aromatic positions (IP-d_8) was synthesized from iminodibenzyl-1,2,3,4,6,7,8,9-d_8 which was prepared by treating iminodibenzyl (IDB) in 37% DCl-D_2O at 160° for 24hr. And imipramine labeled at the positions of 1,3,7 and 9 [IP-d_4(II)] was obtained by "back-exchange" of IP-d_8 under the protio condition according to the exchange procedure of IP-d_4(I).

Key words : Imipramine-1,3,7,9-d_4, Imipramine-1,2,3,4,6,7,8,9-d_8, Iminodibenzyl-1,2,3,4,6,7,8,9-d_8, Acid-catalyzed exchange reaction, NMR Spectrum

INTRODUCTION

The application of stable isotopically labeled drugs in clinical pharmacokinetic studies has rapidly increased in recent years[1-3]. One of the major advantages of using stable isotopically labeled drugs is that these studies can be conducted in patients during and without interruption of therapy by substituting the normal dose with a pulse dose of the labeled drug.

Imipramine is a member of tricyclic antidepressant and is usually administrated to depressive patients daily. The measurement of plasma or serum levels of this drug as a means of monitoring and titrating clinical response is often desirable for effective and safe drug therapy. It is well known that elimination rates or total body clearance of imipramine vary markedly among patients[4,5]. In

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order to study the pharmacokinetics of the drug in patients during long term treatment, the most reliable method for studying changes in drug disposition is to employ stable isotope methodology. Success depends upon the availability of suitable isotopically labeled compounds for the pharmacokinetic studies using GLC-mass spectrometry. This paper describes the preparation of three forms of deuterium labeled imipramine using hydrogen-deuterium exchange reaction.

EXPERIMENTAL

Deuterium chloride (DCl, 99 atom D%) and deuterium oxide (D_2O, 99.75 atom D%) were obtained from E. Merck. ^{1}H-NMR spectra were determined on a Varian EM-390 90 MHz NMR Spectrometer using carbon tetrachloride as a solvent with tetramethylsilane as the internal standard. Mass spectra were recorded on a Hitachi Double Focusing Mass Spectrometer M-80 (ionization energy : 70 eV, direct inlet).

Determination of conditions for the exchange reaction

A solution of imipramine hydrochloride (100 mg) or iminodibenzyl (100 mg) in DCl-D_2O (3 ml) was heated in a nitrogen-filled sealed glass tube under the various conditions shown in Tables I and II. The reaction mixture was adjusted to pH 10 with 3N NaOH at 0°, and extracted three times with n-hexane (30 ml). The combined extract was washed well with water, dried over anhydrous sodium sulfate, and then evaporated to dryness under reduced pressure. The extent of deuteration of imipramine and iminodibenzyl were followed by ^{1}H-NMR Spectroscopy.

Imipramine-2,4,6,8-d_4

A solution of imipramine hydrochloride (500 mg, 1.58 mmol) in 10% DCl-D_2O (15 ml) was heated at 80° for 8hr in a nitrogen-filled sealed glass tube. The mixture was adjusted to pH 10 with 3N NaOH at 0° and extracted three times with n-hexane (60 ml). The combined extract was washed well with water and dried over anhydrous sodium sulfate. The solution was evaporated to dryness under reduced pressure to give imipramine-2,4,6,8-d_4 as a yellowish oil (448 mg, 99.7%). To a solution of the material in acetone (30 ml) was added acetone (5 ml) saturated with hydrogen chloride gas at 0° and the solution was evaporated to dryness under reduced pressure at room temperature. The residue was recrystallized from

acetone to give imipramine-2,4,6,8-d_4 hydrochloride.

Iminodibenzyl-1,2,3,4,6,7,8,9-d_8

A solution of iminodibenzyl(2.00 g, 10.2 mmol) in 37% DCl-D_2O (60 ml) was heated at 160° for 24hr in a nitrogen-filled sealed glass tube. The reaction mixture was adjusted to pH 10 with 10N NaOH at 0° and extracted three times with n-hexane (100 ml). The combined extract was washed well with water, dried over anhydrous sodium sulfate, and then evaporated to dryness under reduced pressure. The exchange reaction was repeated according to the procedure as described above. Iminodibenzyl-1,2,3,4,6,7,8,9-d_8 was obtained as yellowish crystals (1.60 g, 76.9%).

Imipramine-1,2,3,4,6,7,8,9-d_8

Imipramine-1,2,3,4,6,7,8,9-d_8 was synthesized according to the procedure of W. Schindler et al.[6] Sodium (0.4 g) was stirred in liquid ammonia (30 ml) in the presence of $Fe(NO_3)_3 \cdot 9H_2O$ (3 mg) for 1hr at about -40°. To the sodium amide thus obtained was added dropwise a solution of iminodibenzyl-1,2,3,4,6,7,8,9-d_8 (1.60g, 7.87 mmol) in dry benzen (30 ml) at room temperature and the mixture was heated at 78° for 1hr. To the mixture was added dropwise 3-dimethylaminopropyl chloride (1.20 g, 10.0 mmol) in dry benzene (20 ml) at room temperature, and the mixture was refluxed for 8hr. After decomposing excess sodium amide cautiously with water, the product was extracted three times with 2N HCl (30 ml). The combined extract was adjusted to pH 10 with 5N NaOH and then extracted with diethylether. The solution was washed well with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was chromatographed using silica gel with a mixture of chloroform and methanol (3:1) as eluant to give imipramine-1,2,3,4,6,7,8,9-d_8 as a yellowish oil (1.03 g, 45.4%). The HCl salt of this material was prepared according to the procedure of imipramine-2,4,6,8-d_4 hydrochloride described above.

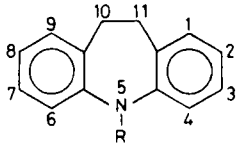
Imipramine-1,3,7,9-d_4

A solution of imipramine-1,2,3,4,6,7,8,9-d_8 (445mg, 1.54 mmol) in 10% HCl-H_2O (15 ml) was heated at 80° for 8hr in a nitrogen-filled sealed glass tube. The mixture was then worked up in the same manner as the prcedure of imipramine-

2,4,6,8-d_4 to give Imipramine-1,3,7,9-d_4 as a yellowish oil (428 mg, 97.5%). The HCl salt of this material was obtained in about 60% yield.

RESULTS AND DISCUSSION

There have been several reports concerning the synthesis of deuterium labeled imipramine. The structure of imipramine is as follows:



Imipramine: R=$^{1'}CH_2^{2'}CH_2^{3'}CH_2N(CH_3)_2$

Iminodibenzyl: R=H

J-P. Dubois et al.[7] and J. R. Geigy[8] synthesized imipramines labeled with two deuterium atoms at the benzyl positions of the dibenzazepine ring. Deuterium atoms of these positions are chemically stable. Problems due to the metabolic loss of deuterium atoms negligible because of hydroxylation at the labeling positions of this drug is not prevalent in humans[9]. However, neither of these labeled compounds seems to be suitable for the accurate and selective mass spectrometric analysis because of the interference from $(M+2)^+$ ion originated from the natural abundance of isotopes in the non-labeled imipramine, where M^+ is the molecular ion. In the mass spectrometric analysis by chemical-ionization (CI) selected ion monitoring, even three deuterium atoms per molecule (imipramine-d_3) may be insufficient. In the CI mass spectra of the region of the monitoring ion at m/z 281 $[(M+1)^+]$, an ion at m/z 279 $[(M-1)^+]$ is produced from the loss of a hydrogen atom from the molecular ion. The corresponding ion due to the loss of one deuterium atom from imipramine-d_3, $(M+3-2)^+$ would coincide with the $(M+1)^+$ ion of undeuterated imipramine and would constitute an interference from imipramine-d_3. It was necessary to prepare labeled imipramine with high deuterium content, introducing at least four deuterium atoms per molecule. Imipramine labeled with two, three or six deuterium atoms in the methyl group of dimethylaminopropyl side chain were also synthesized[10,11]. The materials, however, exhibited significant physicochemical or biological isotope effect on thin layer

chromatographic (TLC) purification[12] and on the demethylation process in the metabolism of imipramine to desipramine[13].

Several methods have been developed for the deuteration of aromatic rings of imipramine, including the use of CF_3COOD-or C_3F_7COOD-D_2O, CH_3COCl-D_2O-DCl and CH_3COOD-D_2O in the presence of $RhCl_3$ as catalyst[14-16]. Under these exchanging media, hydrogens at the positions of 2,4,6 and 8 of imipramine [(IP-d_4(I)] were displaced with deuterium atoms. The isotopic purity of d_4-form, however, was not high, including d_2- and d_3-forms in the material. The material may also be involved with an isotope effect on hydroxylation at 2-position such as in the metabolism of imipramine to 2-hydroxyimipramine or 2-hydroxydesipramine, which is a major metabolic pathway in humans[17]. It was suggested that there is a significant selectivity in the deuteration of aromatic rings by exchange reaction and that the hydrogens at the positions of 1,3,7 and 9 should be chemically and metabolically stable.

In our study to synthesize deuterium labeled imipramine for pharmacokinetic studies, special attention was paid to the selective deuterium labeling at the positions of 1,3,7 and 9 [IP-d_4(II)]. These positions were chosen not only because they offered the possibility of introducing four deuterium atoms but also because they seemed not to suffer from serious problems due to loss of labeling atoms and isotope effects. An attempt was first made to synthesize two kinds of imipramines deuterated at all of the eight aromatic positions (IP-d_8) and at the positions of 2,4,6 and 8 [IP-d_4(I)] with high isotopic purity using exchange reaction, because IP-d_4(II) should be obtained by "back-exchange" of IP-d_8 under the protio conditions according to the procedure of IP-d_4(I).

We employed DCl-D_2O as a solvent-reagent system, which has been widely used to deuterate aromatic compounds electrophylically. The extent of deuteration of imipramine was followed by ^{1}H-NMR spectroscopy and the deuterium contents were exactly determined by electron impact (EI) mass spectrometry. The ^{1}H-NMR and mass spectra of deuterated imipramine treated with 10% DCl-D_2O at 80° for 8hr and unlabeled reference are given in Figs. 1 (A and B) and 2 (A and B). In Fig. 1B, proton signals other than aromatic regions of deuterated imipramine are the same

as those of unlabeled reference in Fig. 1A. Both spectra of deuterated imipramine showed that the material contained four deuterium atoms in the aromatic rings (99%). It is well established that electron-donor substituents such as a dimethylamino group in the aromatic ring accelerate electrophilic deuteration principally by activation of the ortho and para positions[18]. It was reasonable to assume that four deuterium atoms entered the ortho and para positions of the N-phenyl-N-alkylanilino moiety in the imipramine molecule [IP-d_4(I)]. These positions were also deuterated completely by heating imipramine in 10% DCl-D_2O at 110° for 8hr (99%). In the reaction of 10% DCl-D_2O at 80° for 1hr,

Table I The Chemical Yields and The Extent of Deuteration at The Aromatic Positions of Imipramine under Various Exchange Reaction Conditions Using DCl-D_2O as A solvent-Reagent System

Reaction Temp.(°C)	DCl(%)	Reaction Time(hr)	Chemical Yield(%)	Deuterium Atoms per Molecule
80		1.0	90	3.6
		4.0	90	4.0
	10		90	4.0
	20		90	4.0
	37		90	4.0
110	10		75	4.0
	20	8.0	60	4.0
	37		50	4.0
140	10		11	(4.7)*
	20		0.5	(5.6)*
	37		not recovered	(6.8)*

( )* : The number of deuterium atoms per iminodibenzyl molecule.

Table II The Chemical Yields and The Extent of Deuteration at The Aromatic Positions of Iminodibenzyl under Various Exchange Reaction Conditions Using DCl-D_2O as A Solvent-Reagent System

Reaction Temp.(°C)	DCl(%)	Reaction Time(hr)	Chemical Yield(%)	Deuterium Atoms per Molecule
140	37	24	90	7.3
160	37	24	87	7.9
180	37	18	70	8.0*

* : Further deuteration was occurred at the positions of 10, 11 of iminodibenzyl.

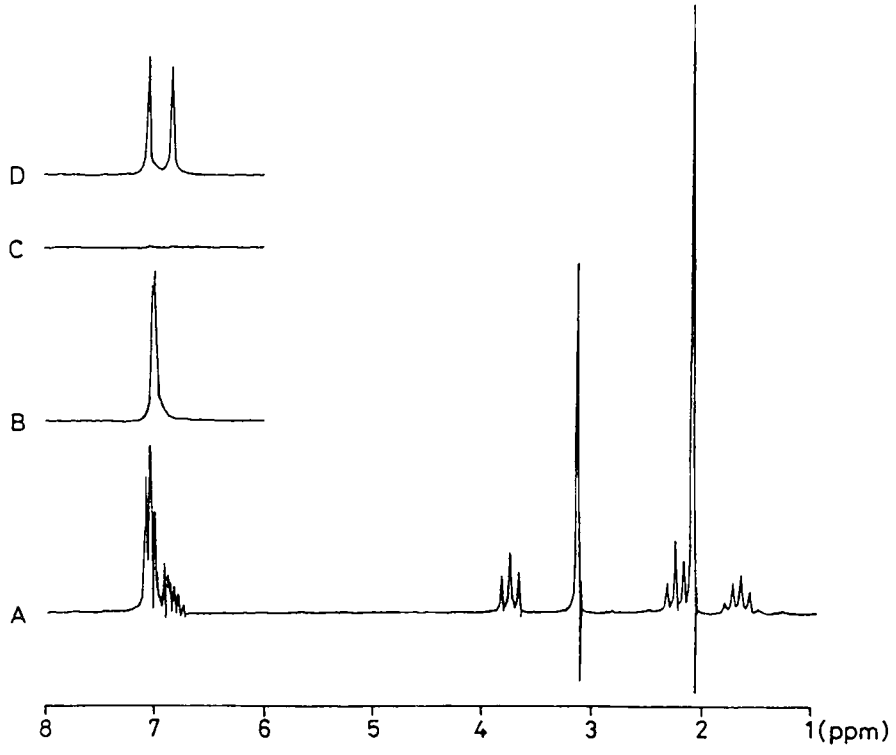


Fig. 1 ^{1}H-NMR Spectra of Imipramine and Deuterated Imipramines Labeled at The Aromatic Positions

A: IP, δ6.70-7.05 (8H, m, ArH), δ3.74 (2H, t, J=7.0 Hz, 1'-CH_2), δ3.14 (4H, s, 10,11-CH_2), δ2.24 (2H, t, J=7.0 Hz, 3'-CH_2), δ2.07 [6H, s, $N(CH_3)_2$], δ1.65 (2H, quin., J=7.0 Hz, 2'-CH_2); B: IP-d_4(I), δ7.00 (2H, s, 3,7-CH), δ6.98 (2H, s, 1,9-CH); C: IP-d_8; D: IP-d_4(II), δ7.07 (2H, s, 2,8-CH), δ6.86 (2H, s, 4,6-CH). Proton signals other than the aromatic regions of deuterated imipramines (B, C and D) were the same as those of IP (A).

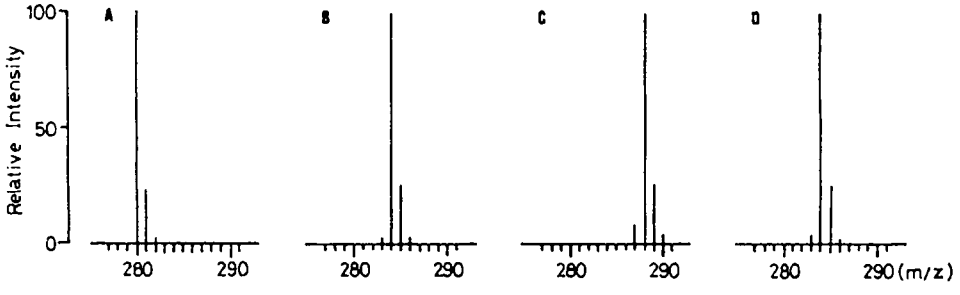


Fig. 2 Mass Srectra in The Molecular Ion Regions of Imipramine and Deuterated Imipramines Labeled at The Aromatic Positions

A: IP; B: IP-d_4(I); C: IP-d_8; D: IP-d_4(II)

the deuterium content was approximately 90% and the deuteration was almost completed after the reaction period of 4hr (97%). The chemical yields after the reaction of 8hr were almost quantitative. However, when either the temperature or the concentration of DCl in D_2O was elevated, the yields were significantly decreased. It was confirmed by TLC that imipramine was decomposed to iminodibenzyl (IDB) quantitatively. ^{1}H-NMR spectrum of the recovered IDB showed further deuteration at the positions of 1,3,7 and 9 of IDB. The results indicate that IDB-d_8 should be synthesized at temperatures above 140° using 37% DCl-D_2O. IP-d_8 should be subsequently synthesized by condensation of IDB-d_8 with 3-dimethylaminopropyl chloride, though IP-d_8 could not be obtained directly by the exchange reaction. The effect of the reaction temperature on the exchange reaction of IDB and the respective chemical yields are shown in Table II. ^{1}H-NMR spectra of the sample obtained, when heated at 160° and 180°, showed the complete exchange with deuterium atoms of IDB, while at 140°, about 14% of hydrogens at the positions of 1,3,7 and 9 remained unaffected. In the mass spectra of IDB treated at 160°, the ratios of the intensities [$(M+9)^+$ or $(M+10)^+$ / $(M+8)^+$] were practically the same as those estimated from the natural abundance of unlabeled IDB [$(M+1)^+$ or $(M+2)^+$ / M^+]. However, the ratios of deuterated IDB treated at 180° were larger than those of deuterated IDB treated at 160°, apparently indicating further deuteration at the positions of 10 and 11 of IDB. Based upon the above experiments, IP-d_8 and IP-d_4(II) were synthesized. IDB was heated in 37% DCl-D_2O at 160° for 24hr in a nitrogen-filled sealed glass tube. IDB-d_8 was extracted with n-hexane under the basic condition, washed well with water and then dried. In order to obtain IDB-d_8 with a higher deuterium content, the exchange reaction was repeated. ^{1}H-NMR and mass spectra of this compound showed that IDB was completely deuterated at all of the eight aromatic positions without serious scrambling of deuterium atoms at the positions of 10 and 11. A solution of IDB-d_8 thus obtained and 3-dimethylaminopropyl chloride was refluxed for 8hr in the presence of sodium amide to give IP-d_8 without loss of deuterium atoms. Subsequent back-exchange between IP-d_8 and 10% HCl-H_2O at 80° for 8hr gave IP-d_4(II). In Figs. 1 and 2 (C and D) are shown partial ^{1}H-NMR and mass spectra of

IP-d_8 and IP-d_4(II). The mass spectrum of IP-d_4(II) was the same as that of IP-d_4(I). The corresponding ^{1}H-NMR spectra, however, showed that IP-d_4(II) was selectively deuterated at the positions of 1,3,7 and 9. In Table III are shown the isotopic purity of three forms of deuterium labeled imipramines. The chemical purity was confirmed by TLC and GLC-mass spectrometry. The percentages of d_2-d_7 species in IP-d_4(I) and (II), and of d_6-d_{11} species in IP-d_8 were calculated from the relative intensities of the corresponding peaks in the range of m/z 270-300 in the mass spectra.

Table III Isotopic Purity of The Three Forms of Deuterated Imipramine Labeled at The Aromatic Positions

	IP-d_4(I) (%)	IP-d_4(II) (%)		IP-d_8 (%)
d_2	0.0	0.0	d_6	0.0
d_3	3.2	3.8	d_7	7.5
d_4	94.2	94.7	d_8	88.8
d_5	2.5	1.3	d_9	2.8
d_6	0.1	0.1	d_{10}	0.8
d_7	0.0	0.0	d_{11}	0.0

The present procedures provide simple and unexpensive methods for the syntheses of selectively deuterium labeled imipramines with high isotopic purity and should be applicable for deuteration of other tricyclic antidepressants such as desipramine. These deuterated imipramines should be useful for the pharmacokinetic studies of imipramine in humans by a stable isotope tracer technique.

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