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Isotope effects in liquid chromatography of imipramine and desmethylimipramine

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ABSTRACT

The effects of tritium substitution in imipramine (IMI) and N-desmethylimipramine (DMI) on their liquid chromatography are described. Increased elution times for tritiated analogues of IMI and DMI were observed using isocratic reversed-phase HPLC techniques. It was shown that the retention of labelled molecules depends on number and position of the tritium atoms. An explanation of the observed phenomena is offered.

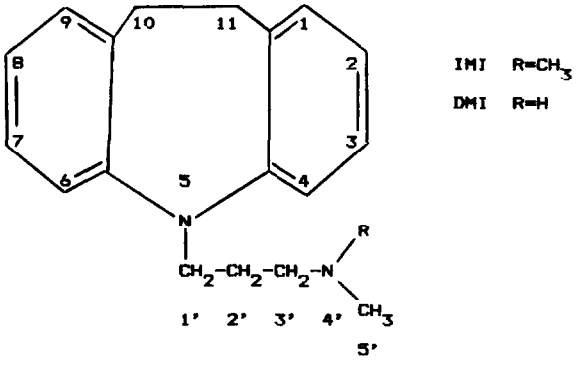
INTRODUCTION

Isotope effects in the chromatography of labelled compounds have repeatedly been observed and a labelled compound can demonstrate more or less mobility in comparison with the corresponding unlabelled analogue. Most commonly the substitution of protium atoms by heavier isotopes resulted in a decrease in chromatographic retention [1–4].

However, for some preparations, in particular for tricyclic and tetracyclic antidepressants, different trends have been observed [5,6]. Deuterated mianserin and Org GC 94 (1,3,4,14b-tetrahydro - 2,7 - dimethyl - 2*H* - dibenzo[*b*,*f*]pyrazino [1,2-*d*][1,4]oxazepine maleate) on a normal-phase column were eluted after the corresponding protium analogues. The behaviour of deuterated imipramine (IMI) on thin layers of silica, alumina and cellulose has been investigated in detail [7]. A decrease in IMI mobility was observed when protium atoms in N-methyl groups were replaced with deuterium.

Tritium-labelled analogues of such compounds have been used as radioligands in serotonin receptor investigations and in the screening of potential antidepressants [8,9].

This paper is devoted to a discussion of the observed isotope effects on the chromatographic behaviour of tritiated IMI and desmethylimipramine (DMI) and the corresponding unlabelled compounds on normal- and reversed-phase columns.



EXPERIMENTAL

The chromatographic separations were performed on columns with a reversed stationary phase (LiChrosorb RP-18; Merck, Darmstadt,

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Germany) (5 μm, 250 × 4 mm I.D.) and a normal stationary phase (TSK NH_2-60; Toyo Soda, Japan) (5 μm, 250 × 4.6 mm I.D.). An LKB (Bromma, Sweden) liquid chromatograph was used with UV detection at 254 nm. The radioactivity of the fractions obtained after chromatographic separation was measured by liquid scintillation counting with a Beckman LS 9800 instrument.

The HPLC conditions were as follows: temperature, 22°C; eluents, ethanol–water (95:5, v/v) containing 0.1–0.9% of triethylamine (TEA) for the reversed-phase column and heptane–chloroform–2-propanol (83:10:7, v/v/v) for the normal-phase column; and flow-rate, 0.2–0.5 ml/min. All chemicals were obtained from Sojuzkhimreaktiv (Moscow, Russian Federation). The eluents were degassed with helium before use.

TLC separations were performed on commercially precoated plates (Silufol UV254, 15 × 15 cm; Kavalier, Sklárny, Czechoslovakia). The solvent was ethyl acetate–2-propanol–ammonia solution ($d = 0.904$ g/ml) (40:30:3, v/v/v).

Tritiated IMI and DMI were obtained in our laboratory by solid-state catalytic exchange with gaseous tritium. Chemical homogeneity and identity with the unlabelled substances were tested by UV spectrophotometry and thin-layer radiochromatography. The locations of the labels were determined by ^3H NMR spectroscopy (Bruker AC250 instrument, 266.8 MHz).

RESULTS AND DISCUSSION

There have been many reports on the HPLC of tricyclic antidepressants (for a review, see ref. 10). In reversed-phase chromatography, buffer eluents are usually used. We tried to find eluents without any salts for easier subsequent isolation of the labelled compounds from the column effluent. Suitable results were obtained with ethanol–water (95:5, v/v) containing 0.1–0.5% of triethylamine (TEA) for reversed-phase and heptane–chloroform–2-propanol (83:10:7, v/v/v) for normal-phase HPLC. In both instances considerable fractionation of the chromatographic zones of tritiated IMI and DMI, depending on their molar activity, was observed.

In the solid-state catalytic exchange reaction with gaseous tritium, a set of products with different extents of protium–tritium substitution were formed. On HPLC, such samples give a set of unresolved peaks corresponding to products with different numbers of tritium atoms in the molecule. Fig. 1 shows typical curves. Separate fractions of the effluent were analysed by ^3H NMR, UV spectrophotometry and thin-layer radiochromatography to determine their chemical homogeneity and identity with unlabelled IMI and DMI. All the analyses confirmed that the separate peak fractions of the labelled compounds differ only in molar activity, *i.e.*, in the number of tritium atoms. The molar activity data are given in Table I. The volume of the fractions was random.

In most reports in the literature, hydrogen isotope effects lead to a decrease in the retention volumes of labelled compounds in comparison with the unlabelled analogues [3,4]. However, in this work, for both reversed and normal phases the labelled compounds had longer retention times than the unlabelled analogues. The observed order of elution allows some conclusions to be drawn about the retention mechanism.

It has been reported that the main role in the retention of basic compounds on reversed phases is played by residual polar silanol groups [11]. Our data confirm this: the order of elution of the labelled and unlabelled compounds on the normal- and reversed-phase columns is the same, indicating an identical retention mechanism on both columns. Small amounts of strongly polar modifiers such as triethylamine play a specific role. Usually such additives are used for the suppression of undesirable interactions of the components to be separated with the residual silanol groups of reversed-phase sorbents. We observe a strong dependence extent of fractionation of the labelled compounds on the amount of triethylamine used in the mobile phase. In Table II the capacity factors are given both for unlabelled IMI (standard) and for the beginning and the end of the unresolved peak sequence of the different isotopic forms of labelled IMI. It is obvious that such a suppression of the interaction of the chromatographed compound with the silanol groups of the reversed-phase sorbent

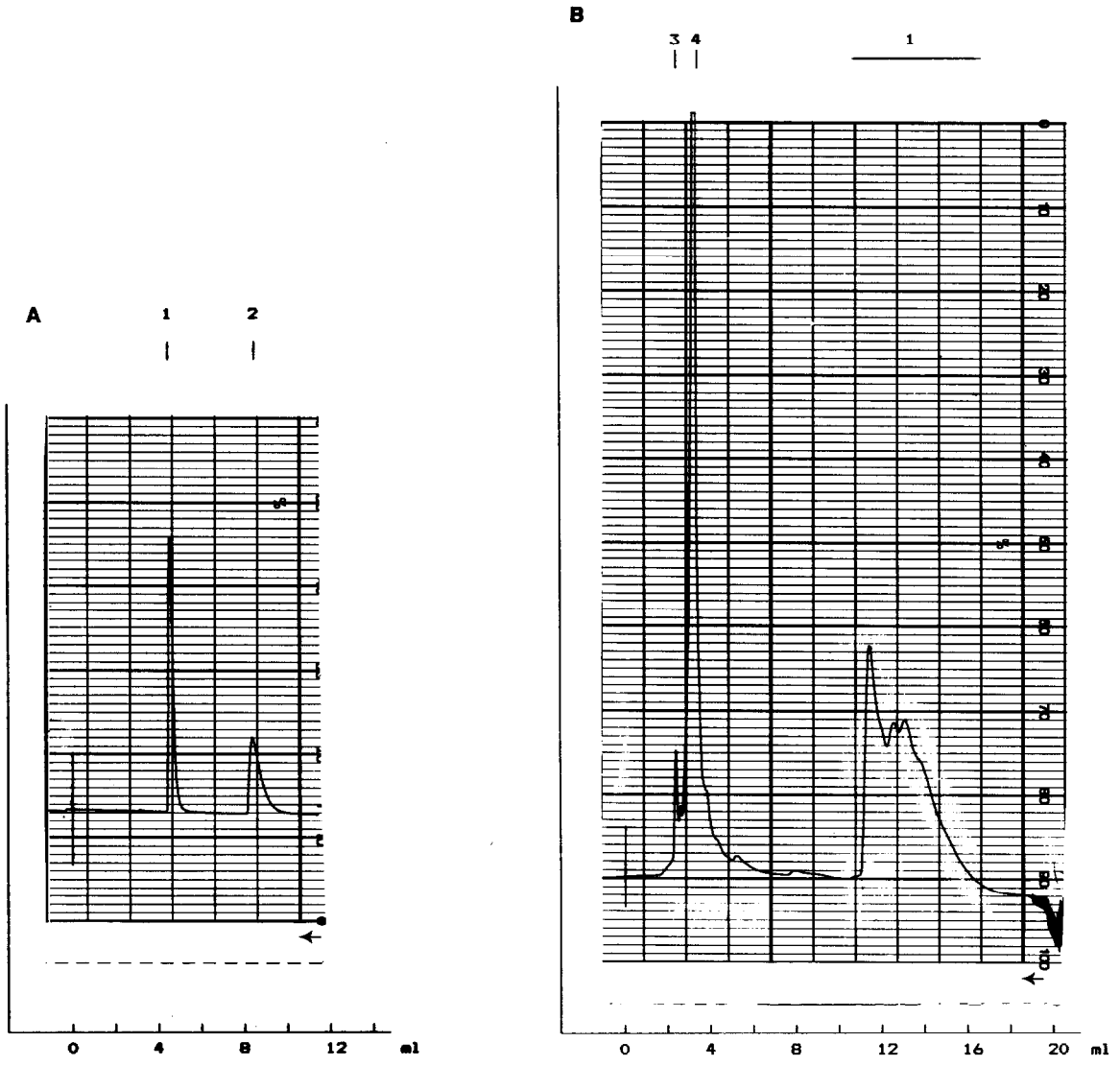

Fig. 1. Typical chromatogram for (A) standard mixture of unlabelled IMI and DMI and (B) sample of tritiated IMI. Chromatographic conditions: column, LiChrosorb RP-18; eluent, ethanol–water (95:5, v/v); flow-rate, 0.3 ml/min; temperature, 22°C. Addition of TEA to the eluent: (A) 0.9% and (B) 0.15%. Peaks: 1 = IMI; 2 = DMI; 3,4 = unidentified compounds from the reaction mixture.

results in a decrease in the fractionation of labelled IMI.

The participation of the polar groups of the sorbent in retention in this work may be due to the formation of hydrogen bonds between the nitrogen atoms of IMI or DMI and the protons of the surface OH groups of the sorbent. Such an interaction is very significant, as evidenced by the stronger retention of DMI than IMI [on the reversed-phase column using ethanol–water (95:5, v/v) containing 0.9% of TEA, the capacity factors are 2.27 and 0.81, respectively (see Fig. 1A)], although usually the substitution of the methyl group by a hydrogen atom leads to a decrease in retention on a reversed-phase column owing to the increase in the polarity of the compound. In IMI and DMI, the substitution of the methyl group by a hydrogen atom and the

TABLE I

MOLAR ACTIVITY OF THE SEPARATE FRACTIONS OF UNRESOLVED PEAKS OF TRITIATED IMI AND DMI

Run No.	Sample	Eluent[a] and sorbent	Fraction No.	Molar activity, Ci/mmol	Activity output[b] (%)
1	[^{3}H]IMI	A, reversed phase	1	75	34
			2[c]	158	25
2	[^{3}H]IMI (second fraction from run 1)	A, reversed phase	1	55	19
			2	162	28
			3[c]	192	10
3	[^{3}H]DMI	B, reversed phase	1	25	7
			2	71	39
			3	102	22
			4	137	20
			5[c]	170	6
4	[^{3}H]DMI	B, reversed phase	1	27	7
			2	43	10
			3	92	34
			4	113	19
			5[c]	126	20
5	[^{3}H]IMI	A, reversed phase	1	–	–
			2	67	4
			3	211	31
			4[c]	208	20
6	[^{3}H]IMI	C, normal phase	1	246	8
			2	322	29
			3[c]	340	22

[a] Eluents: A = ethanol–water (95:5, v/v) + 0.15% TEA; B = ethanol–water (95:5, v/v) + 0.5% TEA; C = heptane–chloroform–2-propanol (83:10:7, v/v/v).
[b] Proportion of the total peak activity in the fraction.
[c] Subsequent fractions were not analysed.

TABLE II

DEPENDENCE OF THE CAPACITY FACTORS OF IMI ON CONCENTRATION OF TRIETHYLAMINE IN THE ELUENT

Eluent, ethanol–water (95:5, v/v); reversed-phase column.

TEA concentration (%)	Capacity factor	
	Unlabelled compound	Tritiated compound
0.02	8.9	9.3–16.6
0.06	4.9	5.1–8.7
0.10	4.0	4.2–7.2
0.20	3.0	3.0–5.4
0.30	2.5	2.5–4.6

corresponding increase in polarity produced suitable conditions for the formation of stronger hydrogen bonds between the 4′-nitrogen atom and surface OH groups of the sorbent. The important role of this nitrogen atom in the retention of the molecule was noted by Heck *et al.* [7]. They showed that under the conditions of thin-layer chromatography, replacement of the protons on the benzene ring in the IMI molecule by deuterium has no influence on retention, whereas incorporation of deuterium in the N-methyl groups decreases the chromatographic mobility. Our data confirm this conclusion.

The results of the ^{3}H NMR analysis of the distribution of tritium atoms for tritiated DMI in the three fractions with different molar activities

TABLE III

^{3}H NMR DATA ON TRITIUM ATOM DISTRIBUTION IN DMI MOLECULES FOR DIFFERENT FRACTIONS OF PEAK (RUN NO. 7)

Fraction No.	Molar activity (Ci/mmol)	Proportion of activity for different positions of tritium atoms in the molecule (%)			
		Benzene ring	10- and 11-positions	3'-Position	5'-Position
1	82	31	30	8	31
2	108	27	23	14	36
3	101	23	22	17	38

are given in Table III. The fractions were collected during elution of the series of unresolved peaks. It is seen that the retention is largest for molecules in which tritium atoms are concentrated in the 3'- and 5'-positions.

Analysis of the results obtained allows the following conclusion to be drawn about the mechanism of the isotope effect in the present instance. The N-4' atom plays a significant role in the retention of the molecule owing to the formation of hydrogen bonds with surface OH groups of the sorbent. Replacement of protons at C-3' and C-5' by tritium make these carbons more electronegative. In turn, this leads to an increased electronegativity of the N-4' atom and promotes the formation of stronger hydrogen bonds with the surface OH groups of the sorbent. The latter decreases the chromatographic mobility of the labelled molecules.

This behaviour of labelled compounds of the given class and difference in the capacity factors of the labelled and unlabelled compounds may be employed to obtain preparations with desired molar activities by chromatographic procedures. Such preparations can be used as tracer compounds in pharmacokinetic and other studies.

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REFERENCES

1 N. Tanaka and E.R. Thornton, *J. Am. Chem. Soc.*, 98 (1976) 1617.
2 R. Baweja, *J. Liq. Chromatogr.*, 9 (1986) 2609.
3 R. Baweja, *Anal. Chim. Acta*, 192 (1987) 345.
4 W.J.S. Lockley, *J. Chromatogr.*, 483 (1989) 413.
5 J.J. De Ridder and H.J.M. Van Hal, *J.Chromatogr.*, 121 (1976) 96.
6 C.N. Filer, R. Fazio and D.G. Ahern, *J. Org. Chem.*, 46 (1981) 3344.
7 H.A. Heck, R.L. Simon and M. Anbar, *J. Chromatogr.*, 133 (1977) 281.
8 J.J. Clark and J.D. Jauaice (Editors), *Principles of Psychopharmacology*, Academic Press, New York and London, 1978.
9 M.D. Mashkovskii, *Pharmacology of Antidepressants* (in Russian), Meditsina, Moscow, 1983.
10 R. Fazio, E. Spina and F. Pisani, *J. Liq. Chromatogr.*, 10 (1987) 223.
11 B. Pfleiderer and E. Bayer, *J. Chromatogr.*, 468 (1989) 67.