TRITIUM LABELLING OF ANTIDEPRESSANTS WITH REGARD TO THEIR CHEMICAL STRUCTURE

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SUMMARY

Different methods of tritium incorporation have been applied to 14 available tricyclic antidepressant drugs. The specific activities of the labelled compounds are compared. The results are discussed by comparison with hydrogen-deuterium exchange and with regard to the chemical structure of these derivatives.

INTRODUCTION

The use of antidepressant drugs having a tricyclic structure has been recognized by pharmacologists and psychiatrists alike and it is generally accepted that this class of compounds has a specific effect in the treatment of psychiatric depressive illness[1].

Tricyclic antidepressants differ structurally from the phenothiazines antipsychotic agents only by the replacement of the sulfur atom in the phenothiazine nucleus by an ethylenic linkage, so that they are formed from two benzene rings fused to a central seven membered ring. This replacement led to a skewed three dimensional structure instead of a flat two dimensional structure, typical of the phenothiazine derivatives[2]. In addition, this modification changed the clinical activity from antipsychotic to antidepressant.

The most potent compounds were found in the dihydrobenzoazepine (I),

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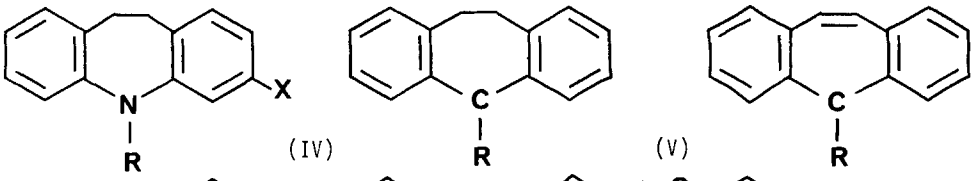
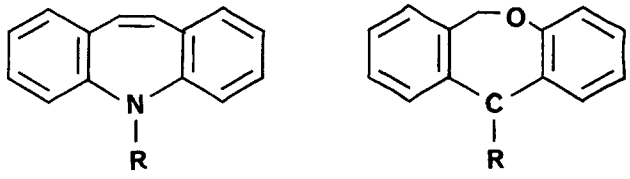
(I) (II) (III)



(IV) (V)



Iminobibenzyl (Ia)	R: -H	X: -H
Desipramine (Ib)	R: $-(CH_2)_3NHCH_3$	X: -H
Imipramine (Ic)	R: $-(CH_2)_3N(CH_3)_2$	X: -H
Trimipramine (Id)	R: $-CH_2CH(CH_3)CH_2N(CH_3)_2$	X: -H
Clomipramine (Ie)	R: $-(CH_2)_3N(CH_3)_2$	X: -Cl
Desmethylclomipramine (If)	R: $-(CH_2)_3NHCH_3$	X: -Cl
Didesmethylclomipramine (Ig)	R: $-(CH_2)_3NH_2$	X: -Cl
10,11-Dihydrobenzocycloheptane (IIa)	R: =CHH	
Amitriptyline (IIb)	R: $=CH(CH_2)_2N(CH_3)_2$	
Nortriptyline (IIc)	R: $=CH(CH_2)_2NHCH_3$	
Hepzidine (IId)	R: =CH(O-C$_6$H$_4$-N-CH$_3$)	
Cyproheptadine (IIIa)	R: =C(C$_6$H$_4$-N-CH$_3$)	
Cyclobenzaprine (IIIb)	R: $=CH(CH_2)_2N(CH_3)_2$	
Dibenzoheptadienol (IIIc)	R: =CHOH	
Opipramol (IV)	R: $-(CH_2)_3$-N(C$_4$H$_8$)-N-CH_2CH_2OH	
Doxepin (V)	R: $=CH(CH_2)_2N(CH_3)_2$	

dibenzocycloheptadiene (II) and dibenzocycloheptatriene (III) series. Derivatives of the type (II) and (III) consisted in the replacement of the nitrogen atom by the isosterically similar carbon in the central ring.

It appears that the labelling at high specific activities of neuroleptic drugs has become very important because the fast development of radioimmunoassay procedures which are applied in the determination of drugs concentration in human fluids[3]. In spite of the extended use of tritiated tricyclic antidepressants, it is surprising that very little has been reported[4] on labelling methods of these derivatives, excepting our own work. Despite the close similarity in their pharmacological and therapeutical potency, the modifications in their chemical structure were sufficient to void the attractive possibility of a single process of tritiation which could be applicable to all these derivatives. We wish to emphasize that in the present work we expressly wanted to avoid multi-step syntheses involving the handling of small quantities of very "hot" material. In this paper, we report the diversity of our labelling attempts, the difficulties encountered, the incompatibilities between chemical structure and methods of tritiation and the specific solutions suggested in order to obtain most of the tritiated antidepressants at high specific activity. In all cases, we started from available drugs.

RESULTS AND DISCUSSION

Benzylic exchange

In a previous work[5], we found that a rapid hydrogen-tritium isotopic exchange occurred on the ethylenic sites of bibenzyl when catalyzed by pre-reduced PdO. We also found that this catalytic labelling was controlled essentially by two steps: activation of the catalyst by reductive tritiation followed by isotopic exchange on the metal surface, where the first step was rate-determining in the exchange process. A relationship between the volume of solvent and the efficiency of labelling was also measured. We applied systematically this process of *in situ* catalyzed general labelling[6] to a wide variety of antidepressant compounds (table 1).

Table 1: Hydrogen-tritium exchange catalyzed by pre-reduced PdO.

Compound	Specific activity* Ci/mmol
Imipramine (Ic)	12.8 - 20.4
Desipramine (Ib)	14.7 - 18.4
10,11-Dihydrobenzocycloheptane (IIa)	35.0
Trimipramine (Id)	10.2
Clomipramine (Ie)	12.0
Desmethylclomipramine (If)	6.2
Didesmethylclomipramine (Ig)	6.7
Doxepin (V)	0.03
Hepzidine (IId)	3.4
Amitriptyline (IIb)	1.1
Nortriptyline (IIc)	1.4

*The specific activities are given for purified products.
Experimental conditions: 0.1 mmol substrate in 0.3 mL solvent (methanol or dioxane), 0.1 mmol PdO; temp.: 25°C.; reaction time: time needed to reduce PdO to Pd by gaseous tritium (5 min. to 40 min.).

From the results given in table 1, it appeared that molecules with a dihydrobenzoazepine skeleton underwent relatively high isotopic exchange. However, when the molecular structure was of the dibenzocycloheptadiene type, which includes a double bond between the carbon in position 5 and the aliphatic moiety, low specific activities were reached. Trying to explain this behaviour, we also performed the hydrogen-deuterium exchange in bibenzyl (VI), 10,11-dihydrodibenzocycloheptane (IIa), iminobibenzyl (Ia), imipramine (Ic) and nortriptyline (IIc).

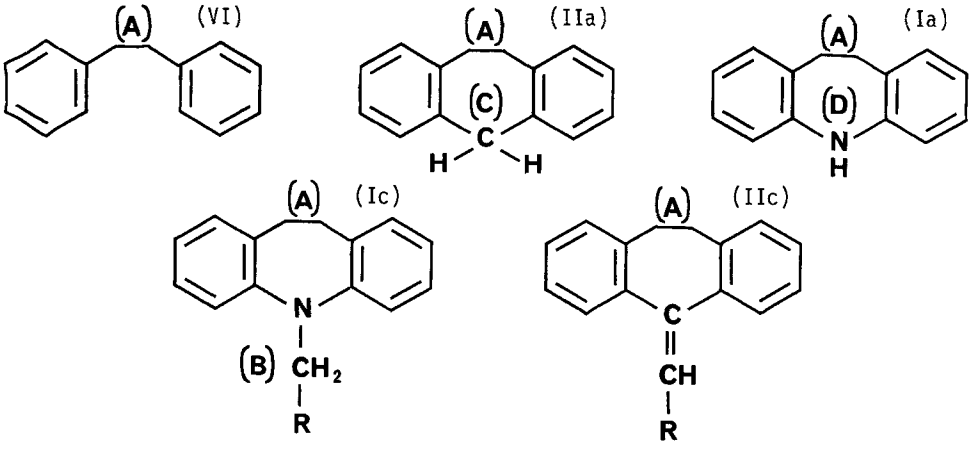


Table 2: Hydrogen-deuterium exchange catalyzed by pre-reduced PdO.

Compound	Number of hydrogen atoms exchanged*			
	[A](3 ppm)	[B](3.8 ppm)	[C](4 ppm)	[D](6 ppm)
Bibenzyl (VI)	2.4	---	---	---
10,11-Dihydrodibenzo-cycloheptane (IIa)	2.4	---	0.6	---
Iminobibenzyl (Ia)	1.1	---	---	0.15
Imipramine (Ic)	0.9	0.2	---	---
Nortriptyline (IIc)	0.65	---	---	---

*determined by integration of the NMR spectra.

The distribution of the deuterium atoms showed that the exchange occurred mainly and preferentially at the benzylic positions in bibenzyl and dihydrodibenzo-cycloheptane. This effect is observed when the π-charge-transfer of electrons from the aromatic rings to the transition metal catalyst is comparable to the charge-transfer in benzene.

The presence of a double bond between the central carbon and the aliphatic chain (nortriptyline IIc) resulted in a drastic decrease of the number of hydrogen atoms exchanged. It seems that a high conjugation effect takes place between the aromatic rings and the seven membered ring, causing the delocalization of the double bond. A consequence of this electronic rearrangement is, very probably, a cause of the deactivation of the benzylic groups and the weakening of their allylic character. This can be explained by analogy with the naphthalenic system, where the conjugation of the aromatic system improves the π-electrons charge-transfer to the activated catalyst and the molecule is, therefore, more strongly adsorbed on its surface. The exchange is, thus, slowed because the second reagent (i.e. heavy water or deuterium gas) is preferentially displaced from the catalyst surface[7].

Furthermore, the reductive attack on the double bond did not, in fact, start so long as the PdO catalyst was not completely reduced to Pd. Under our experimental conditions, even after the formation of the activated catalyst, the olefinic bond underwent reduction slowly.

In the derivatives having nitrogen as the central atom, the unpaired electrons undergo overlapping with the π-electrons of the aromatic rings. As appears from molecular models, at least, one aromatic ring of the tricyclic system is co-planar with the nitrogen-containing ring. The resulting conjugative effect consisted of a positive charge on the nitrogen and different resonative forms of the aromatic ring with a negative charge. As a consequence of this mesomerism, the activity of the benzylic positions will be lowered and olny a partial isotopic exchange will occur, as shown from the NMR results (table 2). However, even such a lowered exchange provided tritiated derivatives with a specific activity of 10 - 20 Ci/mmol which may be considered high enough for research purposes (table 1).

Another explanation of the relatively low exchange could be a stronger bonding of the antidepressant to the catalyst, due to the unpaired electrons, and thus, the weakened activation of T_2O on the metal surface.

Chloro derivatives

Detailed results are given in table 3 for the general labelling of these compounds.

Table 3

Hydrogen-tritium exchange of chloro derivatives, catalyzed by pre-reduced PdO.

Compound	% dechlorinated* compound	% other radioactive* impurities	spec. activity** Ci/mmol
Clomipramine (Ie)	60	---	12.1
N-Desmethylclomipramine (If)	51.5	26.5	6.2
N-Desmethylclomipramine***	18	2	1.6
N-Didesmethylclomipramine (Ig)	65	---	6.7

* percentage calculated from the total radioactivity obtained; determined from the relative peak areas after radioscanning of analytical t.l.c. plates.
** the specific activities are given for the purified products.
*** after addition of 0.2 mL of conc. HCl before the exchange reaction.

It is evident that the *in situ* formation of palladium black provided a highly activated catalyst which, in the presence of tritium gas, enhanced dechlorination, together with the simultaneous competitive isotopic exchange. On the other hand, the increasing formation of TCl caused a partial and additive inhibition effect of the catalyst. This latter effect slowed the rate of dehalogenation. In addition, the chlorine atom linked to the aromatic ring caused some delocalization which deactivated the benzylic positions. As a consequence of these combined effects, the chloro derivatives could only be tritiated with low specific activities (table 1, compounds Ie, If and Ig).

An illustrative experiment was performed with desmethylclomipramine (If) where concentrated HCl was added before the catalyst activation with tritium. Two expected effects were observed (table 3): an important reduction of the percentage of dechlorination and a considerable drop in isotopic exchange. However, this latter effect could also be due to a high isotopic dilution by HCl.

Steric hindrance

Another factor influencing the ability of exchange is the conformation of the aliphatic chain, where its ramification (table 1, compound Id) or the presence of an heterocyclic moiety (table 1, compound IId) has a decreasing effect on the labelling efficiency. This could be explained by an active participation of the aliphatic moiety in the catalytic process as a function of its spatial conformation. When no steric hindrance occurs, at least, one aromatic ring is in the same plane as the aliphatic chain, as described by Maxwell et al.[2a]. So, an easier and a closer contact with the catalyst surface can be reached, resulting in a faster isotopic exchange.

PtO_2 catalyst

In a previous work[8], we determined that the *in situ* general labelling, catalyzed by pre-reduced PtO_2 in place of PdO, acted as a milder and slower

Table 4: Isotopic exchange catalyzed by pre-reduced PtO_2.

Compound	% of reduced form*	% of other impurities*	% of final compound*	specific activity** Ci/mmol
Desipramine (Ib)	---	8	92	0.4
Nortriptyline (IIc)	25	25	50	0.7
Opipramol (IV)	100	---	---	---
Cyproheptadine (IIIa)	40	5	55	2.8
Cyclobenzaprine (IIIb)	18	8	74	0.5

*percentage calculated from the total radioactivity obtained; determined from the relative peak areas after radioscanning of analytical t.l.c. plates.
**the specific activities are given for the purified products.

experimental conditions: 0.1 mmol substrate in 0.3 mL methanol; 0.05 mmol PtO_2; temp.: 25°C.; reaction time: 16 hours.

hydrogen-tritium exchange process. Taking in consideration that some specific antidepressant compounds have a very sensitive double bond close to the seven membered ring and, therefore, undergo reductive tritiation before exchange, we applied the PtO_2-general labelling on four selected derivatives. The labelling of these derivatives had previously failed using PdO as catalyst. For comparison, the same tritiation was also performed on desipramine (Ib) and the results are given in table 4.

In spite of its higher selective properties as a catalyst, PtO_2 was found to be of poor efficiency in the isotopic exchange process at the benzylic positions. With nortriptyline (IIc), nearly the same specific activity was reached as with PdO (table 1).

In the case of opipramol (IV) which is structurally stilbene-like, the rapid and complete reduction of the double bond could not be stopped or, even, partially controlled. Molecular models showed that the rigidity caused by this double bond strained the tricyclic structure in such a way that the three rings were nearly in the same plane.

The experimental results obtained by tritiation of cyproheptadine (IIIa) and cyclobenzaprine (IIIb) were of particuliar interest and agreed with our expectations. The electronic rearrangement provided a delocalization effect of the two double bonds. This effect resulted in a slowing down of the tritium reduction on the stilbene-like double bond positions, as observed, and the desired tritiated derivatives could be reached, but at low specific activities(table 4).

Bromination

On the basis of the positive results obtained for the molecular bromination of a series of phenothiazine derivatives, followed by tritio debromination[10], we also attempted a direct halogenation approach on the tricyclic antidepressant compounds. We performed preliminary brominations on bibenzyl (VI), iminobibenzyl (Ia) and imipramine (Ic) and determined by NMR that the bromine mainly substituted one or two active hydrogen atoms in the ethylenic positions of the central ring. Catalytic hydrogenation of these bromo compounds resulted in obtaining the starting materials once again. It has to be mentioned that the bromination does not occur in the aromatic rings, in contrast with the results of bromination on phenothiazine derivatives[11]. It seems that the "butterfly" spatial conformation of the tricyclic

antidepressants[2], together with the benzylic activation, determined the preferential attack of the bromine on these latter positions. These promising results encouraged us to perform also the bromination on the unsaturated derivatives.

In a first series of experiments, we determined the behaviour of some representative compounds, namely dibenzoheptadienol (IIIc), amitriptyline (IIb) and opipramol (IV),under the experimental conditions of tritio dehalogenation. Two parallel experiments were performed with each of these compounds: a) Pd/C catalyzed hydrogenation without and b) in the presence of a base (triethylamine or NaOH). In the first case, complete saturation of the double bond occurred rapidly (20 - 90 min.) where, under alkaline conditions, the reductive reaction was completely inhibited.

On the basis of these results, the tritiation procedure was performed as follows: a) the bromination of the unlabelled molecule resulting in obtaining the bromo derivative, separated as its free base; b) the selective catalytic removal of the halogen atom by tritium gas in alkaline solution. The addition of a base effectively inhibited completely the catalyst surface to any attack on the olefinic bond[12]. This inhibition of the catalyst was selective and did not affect the simultaneous promotion of tritio debromination through alkaline neutralization of the acid formed.

Specific tritiation

By application of this method, the tritiation of bromo amitriptyline and bromo nortriptyline provided the labelled compounds tritiated in positions 10 and 11 with a specific activity of 15.8 Ci/mmol and 14.2 Ci/mmol, respectively (table 5).
The same procedure of bromination - debromination also enhanced the specific activities of the saturated compounds. The results were particularly attractive when the bromo intermediate could be isolated and purified before the tritiation step, as for desipramine and imipramine.

Bromination of doxepin provided, surprisingly, a compound in which the bromine atom was incorporated in the aliphatic moiety, including its double bond. Iodination of this compound followed by tritiation under the usual conditions provided the desired labelled derivative, mainly tritiated in position 10 but also in the aromatic rings, with a specific activity of 19.6 Ci/mmol.

Table 5: Specific tritiations

Starting compound	Tritiation method	Lab. position(s)	Final compound	Spec. act. Ci/mmol
Amitriptyline (IIb)	debromination	10 -11	Amitriptyline	14.2
Clomipramine (Ie)	debromination	---*	Clomipramine	22.1
Clomipramine (Ie)	deiodination	---*	Clomipramine	7.8
Cyclobenzaprine(IIIb)	partial saturation	10 - 11	Amitriptyline	19.5
Desipramine (Ib)	debromination	10 - 11	Desipramine	84.9
Doxepin (V)	deiodination	10 (mainly)	Doxepin	19.6
Imipramine (Ic)	debromination	10 - 11	Imipramine	45.0
Nortriptyline (IIc)	debromination	10 -11	Nortriptyline	15.8

*the labelled positions were not determined by NMR but are supposed to be located on carbons 10 and 11.

In another experiment, cyclobenzaprine (IIIb), which has two double bonds close to the seven membered ring, was selectively reduced to give tritiated 10,11-amitriptyline with a specific activity of 19.5 Ci/mmol. As discussed in a preceding paragraph, the selective tritiation could be performed because of the relatively higher reactivity of this latter olefinic bond.

In the case of opipramol (IV) which has an olefinic bond between the positions 10 and 11, as expected, tritium addition occurred in place of exchange. Tritium-labelled compounds with a stilbene-like structure could be obtained only by a multi-step synthesis.

CONCLUSION

Most of the saturated tricyclic antidepressants could be easily tritiated by the *in situ* pre-reduced PdO catalyst method. Generally, the specific activities reached are high enough for the use of these compounds in drug metabolism research.

Unsaturated compounds must be labelled via an halogenation intermediate step, when high specific activities are desired. The same method is very useful when applied to saturated derivatives.

Compounds with two double bonds could be obtained only by "hot" syntheses if high specific activities have to be reached. The only way to label compounds with a stilbene-like structure is by multi-step "hot" synthesis.

EXPERIMENTAL

Ultra-violet spectra were recorded on a Perkin-Elmer UV-visible spectrophotometer, Model 402 and NMR spectra on a Varian EM 360 spetrometer. Radiochemical and chemical purity were determined by radiochromatogram scanning on thin-layer chromatography (t.l.c.) plates on a Berthold Dunnschicht Scanner II, Model LB 2722; total and specific activity were measured on a Packard Tri-Carb Liquid Scintillation spectrometer, Model 3375.

Tritiations and deuterations

The tritiation and deuteration experiments were performed on a vacuum manifold, as previously described[9]. The experimental conditions were as described for PdO catalyzed exchange[6], for PtO_2 catalyzed exchange[8] and for tritium dehalogenation[10], respectively.

Purification and analytical control

The purification and the analytical checking of the different compounds was performed on pre-coated silica gel plates, using 2 or 3 of the following solvents systems: 1. methanol-ammonia (100:1.5); 2. benzene-methanol (80:20); 3. chloroform-acetone-triethylamine (50:40:10); 4. hexane-diethylamine (90:10); 5. heptane; 6. carbon tetrachloride; 7. acetone-methanol (88:12); 8. benzene-methanol-ammonia (95:15:5); 9. cyclohexane-acetone-diethylamine (60:30:10); 10. ethyl acetate-acetone-ammonia (20:20:0.5).

Compound	System	Compound	System
Desipramine (Ib)	1, 4, 9	10,11-Dihydrobenzo-cycloheptane(IIa)	5, 6
Imipramine (Ic)	1, 4	Amitriptyline (IIb)	1, 7
Trimipramine (Id)	1, 7	Nortriptyline (IIc)	1, 4, 8
Clomipramine (Ie)	1, 7	Hepzidine (IId)	2, 3
Desmethylclomipra-mine (If)	1, 4, 8	Cyproheptadine (IIIa)	2, 4
Didesmethylclomi-pramine (Ig)	1, 4, 8	Cyclobenzaprine(IIIb)	1, 10
		Opipramol (IV)	1, 4
		Doxepin (V)	2, 3

The purity of the compounds was also controlled by UV spectroscopy.

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