J. Pharm. Pharmacol. 1979, 31: 508–511 Received December 12, 1978

Catalytic tritiation of drugs and analysis of the tritium distribution by ^3H n.m.r. spectroscopy

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Propranolol, phenobartitone, diphenylhydantoin, amphetamine, imipramine and propoxyphene have been tritiated using tritiated water and platinum catalyst (from the dioxide and sodium borohydride), and the pattern of labelling has been ascertained by ^3H n.m.r. spectroscopy. The results show that this exchange procedure can lead to satisfactory incorporation of tritium at 'stable' aromatic positions. For imipramine where incorporation of tritium was low, an alternative acid-catalysed procedure was satisfactory.

Drugs labelled with either radioactive or stable isotopes find wide use in studies of absorption, distribution and metabolism. Before labelling, the choice of isotope and the site(s) to be labelled are important considerations. Carbon isotopes (^{13}C and ^{14}C) are relatively expensive and their incorporation requires special syntheses which can be most demanding in terms of skill and time. In contrast, hydrogen labelling (^2H or ^3H) can readily be achieved by exchange or addition procedures, and total costs are low. A potential disadvantage has hitherto been the lack of easy direct means of establishing the integrity of the label under biological or other reaction conditions. However, tritium is an ideal nucleus for magnetic resonance examination. Consequently the development over the last decade of tritium n.m.r. spectroscopy (Chambers et al 1978) as a safe and rapid technique has materially altered the situation. With its power to delineate precisely and directly the pattern of tritium labelling even at relatively low levels of incorporated radioactivity, the n.m.r. method makes the use of generally tritiated compounds most attractive.

Considerable knowledge of the effectiveness of different catalysts for hydrogen exchange has been accumulated (Calf & Garnett 1975). As part of our catalytic and ^3H n.m.r. studies (Elvidge et al 1979) we have found platinum, freshly prepared by reduction of the oxide, to be a most effective catalyst for introducing tritium from tritiated water into aryl ring-positions. It seemed logical to extend these investigations to drugs based upon aromatic structures, as shown (1–6). With imipramine (5) where the degree of tritium incorporation was considered unsatisfactory, advantage was taken of a recently reported acid-catalysed procedure using heptafluorobutyric acid (Hanzlik et al 1978).

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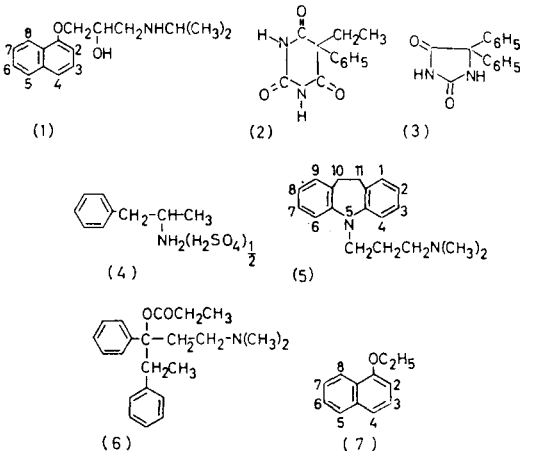

The drugs chosen have widely different uses. Propranolol (1) is a β-adrenoceptor blocking agent and phenobarbitone (2) has anticonvulsant, hypnotic and sedative properties; diphenylhydantoin (3) is widely used in the treatment of epilepsy, amphetamine (4) is a stimulant for the central nervous system, imipramine (5) possesses antidepressant activity and propoxyphene (6) is a widely used analgesic.

METHODS

Tritiation of drugs: The reaction mixtures were sealed in small tubes and heated, as specified in Table 1. The general purification procedure entailed treating the resulting contents with acetone–water (or ethanol–water), filtration from the catalyst, and lyophilization of the filtrate. For amphetamine, imipramine and propoxyphene the product was recrystallized; in the other cases purification was effected by thin layer chromatography e.g. in ethylacetate–ethanol (60:40) on silica gel.

For the acid-catalysed exchange of imipramine,

Table 1. Experimental conditions for tritiation of the drugs using 10 μl of tritiated water at 40 Ci ml^{-1}.

Compound wt (mg)	Catalyst wt (mg)	Solvent	Temp (°C)	Time days	Sp. act. (mCi mmol^{-1})
Propranolol hydrochloride 20	35	HAc(glac.)	85	3	150
Phenobarbitone 20	35	Dioxan	85	2·5	80
Diphenyl hydantoin 20	35	HAc(glac.)	85	2	180
Amphetamine Sulphate 20	35	HAc(glac.)	85	3	125
Imipramine hydrochloride 20	35	HAc(glac.)	85	3	70
Propoxypene hydrochloride 20	35	HAc(glac.)	60	2·5	120

the drug hydrochloride (25 mg) was dissolved in heptafluorobutyric acid (0·5 ml) and tritiated water (10 μl, 50 Ci ml^{-1}) in a small tube which was then sealed and heated for 6 days at 105 °C. The product was extracted into ether; after being dried (Na_2SO_4) the ether was evaporated under reduced pressure leaving the tritiated imipramine hydrochloride (specific activity 210 mCi mmol^{-1}).

For n.m.r. analysis the tritiated drugs (5–15 mCi) were dissolved in a deuteriated solvent, a trace of tetramethylsilane (TMS) was added, and the ^3H and ^1H spectra were recorded (at 25 °C) at 96 and 90 MHz, respectively (the former with ^1H-decoupling), employing a Bruker WH90 Fourier transform spectrometer equipped with quadrature detection and disc storage.

Pulse widths were 1·5–3 μs and the repetition interval 1·6–3·4s as appropriate. A display spectral width of ca 13 p.p.m., usually at 21·31 Hz cm^{-1} for ^3H spectra and 20·00 Hz cm^{-1} for ^1H spectra was used to provide identical p.p.m. scales. Triton shifts, δ_T (p.p.m.) were measured from $\nu_{TMS} \times (\omega_T/\omega_H)$ (Bloxsidge et al 1979). In integrating the n.m.r. signals great care is taken to avoid saturation: the integrals obtained are of course good average values, as a result of the multi-scan FT method. Any additional errors arising from possible differential nuclear Overhauser effects (n.O.es) are generally small and can be ignored (Bloxsidge et al 1977). The average overall accuracy will be ±5%.

RESULTS AND DISCUSSION

The proton decoupled ^3H n.m.r. spectrum of propranolol (1) (Fig. 1a) shows that only the naphthalene ring is labelled (cf. Fig. 1b). The line at δ 7·54 could at once be assigned to the 4-position because in a separate study (Chambers, Evans, Elvidge, Jones, unpublished results) we found this was the chemical shift of the triton in [4-^3H] propranolol which had been specifically labelled by tritiodehalogenation. To assign positions to the other three signals, 1-ethoxynaphthalene (7) was taken as a model compound for propranolol and tritiated under the same conditions as were used for propranolol itself. The pattern of labelling (Fig. 1c) is similar and on the basis of the ^1H spectrum (Fig. 1d) and of the ^1H spectra (in $CDCl_3$) of 1-ethoxy- (Sadtler 1971a) and 1-methoxy-naphthalene (Sadtler 1973b) it can be seen that positions 5 and 8 are not labelled, probably as a result of steric hindrance. It also seems likely that the chemical shifts for positions 6 and 7 are the same. The upfield signal at δ 6·86 can be assigned to position 2. Consequently from the signal intensities, the tritium distribution in propranolol can be calculated (Table 2).

The results for phenobarbitone (2) and diphenylhydantoin (3) (Fig. 2) are similar to one another—each ^3H spectrum (with ^1H decoupling) consists simply of two signals which can be identified with the *meta*- and *para*-positions of the benzene ring; no labelling of the *ortho*-positions occurs. The

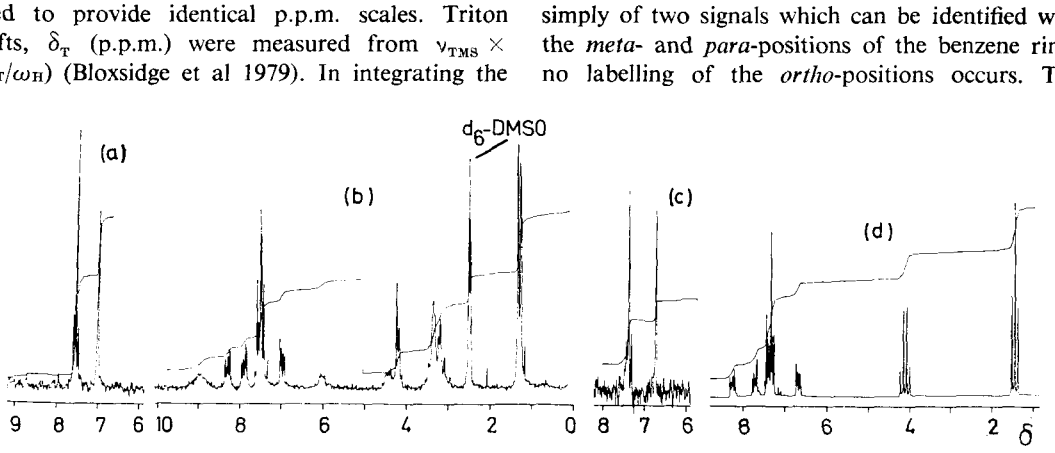

FIG. 1. N.m.r. spectra of [G-^3H] propranolol. (a) ^3H (^1H decoupled) spectrum. (b) ^1H spectrum and n.m.r. spectra of [G-^3H] 1-ethoxynaphthalene. (c) ^3H (^1H decoupled) spectrum. (d) ^1H spectrum.

Table 2. Tritium incorporation and distribution in the labelled drugs.

Drug (n.m.r. solvent)	Chemical shift δ p.p.m.	Position(s) labelled	Relative Incorp. (%)
Propranolol (1)	7·54 (7·56)*	4	13 (8)*
(d_6-DMSO)	7·50 (7·49)	6, 7	46 (42)
	7·43 (7·44)	3	10 (13)
	6·97 (6·86)	2	31 (37)
Phenobarbitone (2)	7·43	Aromatic-*meta*	60
($CDCl_3$)	7·37	,, -*para*	40
Diphenylhydantoin (3)	7·43	Aromatic-*meta*	61
(d_6-DMSO)	7·38	,, -*para*	39
Amphetamine (4)	7·40	Aromatic-*meta*	56
(D_2O)	7·33	,, -*para*	33
	2·86	CH_2	8
	1·24	CH_3	3
Imipramine (5)	7·48	4, 6	45
(CF_3COOD)	7·28	1,2,3,7,8,9	55
Propoxyphene (6)	7·40	Aromatic	100
(D_2O)	7·34		

* Values in brackets refer to 1-ethoxynaphthalene.

results for amphetamine (4) are different from the other drugs investigated in that small, but significant, amounts of tritium are incorporated into the aliphatic part of the molecule (Fig. 3a). It is probable that with a reduced reaction time labelling will be confined to the benzene ring. Steric hindrance evidently ensures that there is no labelling of the *ortho*-positions.

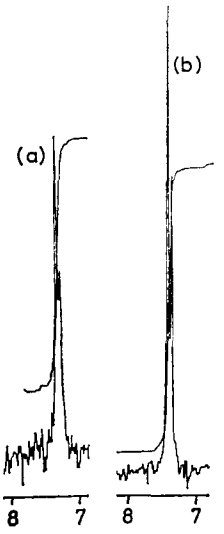

FIG. 2. ^3H N.m.r. spectra (^1H decoupled) of (a) [G-^3H] Phenobarbitone. (b) [G-^3H] diphenylhydantoin.

Tritium incorporation into imipramine (5) was confined to the aromatic rings but was so much less than for the other compounds (Table 1) that alternative methods of labelling were sought. Hanzlik et al (1978) have recently advocated heptafluorobutyric acid as an exchange catalyst, and indeed this gave very satisfactory results with imipramine (Fig. 3c). Comparison with the ^1H spectrum (Fig. 3d) shows that the tritium was confined to the aromatic positions. In view of the identity of the chemical shifts for the *meta* and *para*-positions only the total tritium incorporation at these positions could be obtained directly. Steric hindrance at the *ortho*-positions seems to be less important than in some of the other compounds studied in the present work.

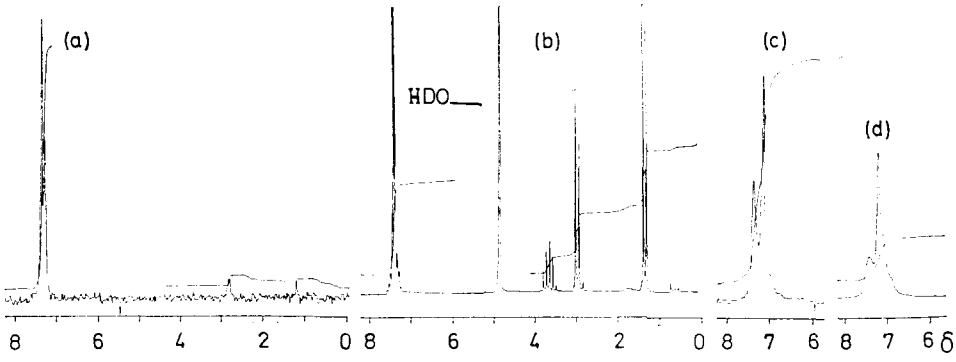

FIG. 3. N.m.r. spectra of [G-^3H] amphetamine. (a) ^3H (^1H decoupled) spectrum. (b) ^1H spectrum and n.m.r. spectra of [G-^3H] imipramine, prepared by acid-catalysed exchange. (c) ^3H (^1H decoupled) spectrum. (d) ^1H spectrum.

The final example, propoxyphene (6), is labelled at both *meta* and *para*-positions of the aromatic ring; as the ^{1}H signals from both rings are superimposed it is not possible to say whether one ring has been labelled at the expense of the other.

The present study underlines the usefulness of one-step catalytic procedures for preparing tritiated compounds, especially when used in conjunction with ^{3}H n.m.r. spectroscopy. Although such methods are usually thought of as a way of preparing generally-labelled compounds, it is clear that, by suitable choice of catalyst and optimization of experimental conditions, a remarkable degree of specificity can be achieved. Similar studies using some of the newly developed catalytic methods e.g. microwave discharge activation (Ehrenkaufer et al 1978) should be equally rewarding as the pattern of labelling could provide information of mechanistic importance. Procedures using tritium gas (Evans et al 1974; Buchman & Pri-Bar 1978; Ehrenkaufer et al 1978) are particularly attractive as high specific activities are readily attainable.

Acknowledgements

We thank the University of Isfahan, Iran, for leave of absence and a scholarship (to M. S.) and Professor R. P. Hanzlik (University of Kansas) for providing a pre-print of his heptafluorobutyric acid work.

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