TRITIUM NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. PART 13 [ref. (1)]. TRITIUM LABELLED NEUROCHEMICALS

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SUMMARY

A series of 50 neurochemicals, important for the investigation of neurotransmission processes, has been labelled with tritium, using in all 8 methods. Tritium nuclear magnetic resonance spectroscopy has been employed to determine the positions and extent of labelling in these neurochemicals which can be important for their use in tracer studies. The specificity of the labelling processes is discussed.

INTRODUCTION

Knowledge of the mechanisms of neurotransmission is essential for improving the alleviation of pain and for the understanding and possible control of disorders of the nervous system such as

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Huntington's chorea, Parkinsonism, depressive illness, and schizophrenia. The transmission of nerve signals occurs in two main ways (2). Along nerve cell axons it is accomplished electrochemically, rapid progressive depolarisation of the cell membrane resulting in passage of an electrical impulse. Across the synapses between neurons, however, transmission is established by the release of a chemical neurotransmitter, and subsequent uptake of this by the succeeding neuron. A similar communication system exists between neurons and muscle or other tissue under nervous control.

There are a number of neurotransmitters (3), each characteristic of a particular type of neuron (4) and, as indicated, they are compounds biosynthesised and stored within the ends of axons, to be released on receipt of the electrical impulse. In common with hormones and drugs, neurotransmitters exert their effects by interacting with specific receptor sites on the target tissue. Demonstration of this is being achieved through the use of receptor-specific compounds that are radio-labelled at high specific activity, generally with the tritium isotope. Receptor site and binding studies involve approaches using three main types of ligand. One is to use labelled neurotransmitters. A second is to use labelled agonists which are compounds that mimic the physiological response of the natural neurotransmitter more or less closely. Through their use different kinds of receptor for a single neurotransmitter have been identified. A third approach is to use labelled antagonists : these are compounds that interact specifically with receptors yet elicit no response. One of several aspects of the use of specific antagonists is in the identification of inhibitory neurotransmitters (5).

The present Paper lists some eight methods for the labelling of

a wide variety of neurochemicals with tritium. The specificity of these methods and thence of the labelling in the products has been examined by ^3H n.m.r. spectroscopy, this being a highly convenient and non-destructive method for determining both the positions and extent of tritium labelling (6). Precise knowledge of the distribution of tritium in the tracer compounds employed can frequently be of vital importance for the correct interpretation of biochemical studies (7). This has proved true for experiments using tritiated steroids (8), sugars (9), carcinogenic hydrocarbons (10) and aminoacids (11), and is proving equally true for investigations with tritiated neurochemicals (12).

EXPERIMENTAL

The reaction conditions for the specific tritiation of a range of 50 neurochemicals (or ligands) of current interest are listed in Table 1. In every case, labile tritium, as e.g. in amino or hydroxy groups, was removed by treatment with water. The compounds are grouped according to the main classification of receptor types, and for each group the natural neurotransmitter (where known) is indicated in parentheses.

Each compound was examined by ^3H n.m.r. spectroscopy in order to define the pattern of labelling (Table 2). The various solutions (mostly 20 - 25 mCi and of high specific radioactivity) were lyophilised. Each sample was redissolved in a deuteriated solvent (80 µl) to provide for field-frequency locking, and a trace of TMS or DSS (as appropriate) was added as internal ^1H standard. The solutions were separately loaded by syringe into 3 mm diam. combination tubes (Wilmad, SK 1374A) which were then sealed and mounted in Teflon spacers inside standard 5 mm n.m.r.

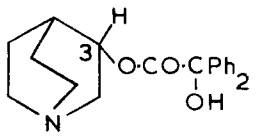
TABLE 1

Preparation of tritiated neurochemicals (ligands)

No.	Ligand	Receptor type (neurotransmitter)	Labelling method
1.	L-Quinuclidinyl benzilate	Muscarinic (acetylcholine)	Cat. tritiodebromination of the 4-bromobenzilate
2.	DL-Quinuclidinyl benzilate		Na borotritide reduction of 3-quinuclidone and trans-esterification with methyl benzilate
3.	Atropine		Wilzbach, T_2
4.	Dopamine HCl	Dopamine (dopamine)	(a) Cat. tritiation of protected amino-styrene (b) Exchange, THO/HCl
5.	ADTN.HCl		Exchange, T_2/PdO-$BaSO_4$
6.	Spiperone		Cat. tritiodebromination of the N-(4-bromophenyl) compound
7.	L-Noradrenaline	α-Adrenergic (noradrenaline, adrenaline)	From [^{3}H]dopamine using dopamine β-hydroxylase
8.	DL-Noradrenaline HCl		Na borotritide reduction of noradrenalone
9.	L-Adrenaline HCl		Enzymatic methylation of L-[^{3}H]noradrenaline
10.	DL-Adrenaline HCl		Na borotritide reduction of adrenalone
11.	WB 4101		Cat. tritiodebromination of the bromophenoxy compound
12.	9,10-Dihydroergocryptine		Cat. tritiation of ergocryptine
13.	Clonidine		Cat. tritiodebromination of the 4-bromophenyl compound
14.	Prazosin HCl		Cat. tritiodebromination of the bromo compound
15.	L-Dihydroalprenolol	β-Adrenergic	(a) Cat. tritiation of L-alprenolol (b) Cat. tritiation of 4,6-dibromoalprenolol
16.	DL-Propranolol		Cat. tritiodebromination of the 4-bromo compound

17.	β-Alanine	Gaba-ergic (γ-aminobutyric acid)	(a) Na borotritide reduction of ethyl cyanoacetate (b) Cat. tritiation of ethyl cyanoacetate
18.	4-Aminobutyric acid		Cat. tritiation of protected Δ^2-ene intermediate
19.	L-2,4-Diaminobutyric acid HCl		Cat. tritiation of 3-cyanoalanine
20.	cis-3-Aminocyclohexane carboxylic acid		Cat. tritiation of protected Δ^4-ene intermediate
21.	Muscimol		Thermal decarboxylation of ibotenic acid in THO
22.	DL-Nipecotic acid		Cat. tritiation of protected guvacine
23.	Isoguvacine HBr		Na borotritide reduction of the protected pyridine-4-carboxylic acid
24.	Strychnine sulphate	Glycine (glycine)	Exchange, THO-CF_3CO_2H
25.	L-Glutamic acid	Glutamate/ aspartate (glutamic acid and aspartic acid)	Reductive amination of 2-oxoglutaric acid
26.	DL-Glutamic acid		Exchange of azlactone deriv. with THO
27.	Kainic acid		Exchange, THO
28.	DL-Aspartic acid		Cat. tritiation of protected Δ^2-ene
29.	D-Aspartic acid		Enzymic resolution of previous product
30.	L-Aspartic acid		(a) Enzymic resolution of the DL[2,3-^{3}H]aspartic acid (b) Cat. exchange, THO
31.	5-Hydroxytryptamine creatinine sulphate	Serotonin (5-hydroxy-tryptamine)	Cat. exchange, THO
32.	Lysergic acid diethylamide		Cat. tritiodebromination of the 2-bromo compound
33.	Imipramine		Cat. tritiodechlorination of the 3-chloro compound
34.	Mianserin		Cat. tritiodebromination of the 8-bromo compound

35.	Morphine	Opiate (enkephalin)	Cat. tritiodeiodination of the 1-iodo compound
36.	Dihydromorphine		Cat. tritiation of [1-^{3}H]morphine
37.	Diacetylmorphine		Acetylation of [1-^{3}H]morphine
38.	Etorphine		Cat. tritiation of Δ^{15}-ene precursor
39.	Diprenorphine		Na borotritide reduction of Δ^{15}-ene precursor
40.	Adenosine	Purinergic (purines)	(a) Na borotritide reduction of adenosine-5'-carboxylate, then bromination and tritio-debromination (b) Exchange of [5'-^{3}H]adenosine with T_2/PdO-$BaSO_4$
41.	Theophylline		Exchange, T_2/PdO-$BaSO_4$
42.	Caffeine		Exchange, T_2/PdO-$BaSO_4$
43.	Histamine di HCl	H_1 and H_2 (histamine)	Cat. tritiodeiodination of 2,5-di-iodohistamine
44.	Pyrilamine ascorbate	H_1	Cat. tritiodebromination of the 5-bromo compound
45.	Cimetidine phosphate	H_2	Reaction of [methyl-^{3}H]methylamine with suitable intermediate
46.	Diazepam	'Anxiety'	Reaction of desmethyldiazepam with [^{3}H]methyl iodide
47.	Flunitrazepam		Reaction of desmethylflunitrazepam with [^{3}H]methyl iodide
48.	Piperidine HCl	Miscellaneous	Cat. tritiation of Δ^3-ene
49.	Taurine		Na borotritide reduction of ethyl glycinate, reaction with HBr and then Na_2SO_3
50.	DL-Octopamine HCl		Cat. tritiodebromination of 3,5-dibromo-octopamine



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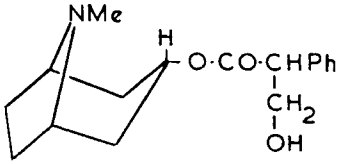

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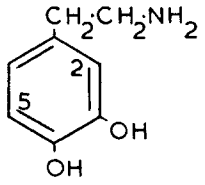

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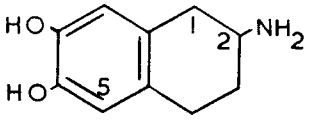

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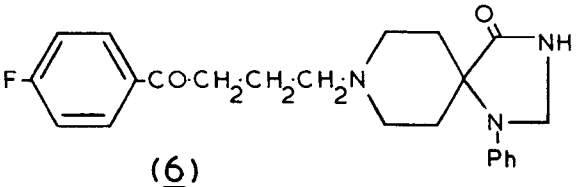

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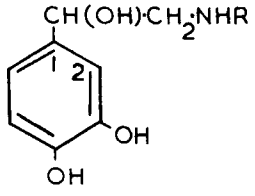
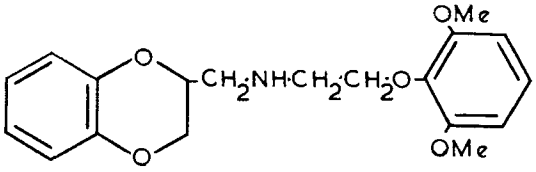


(7) R = H

(9) R = Me



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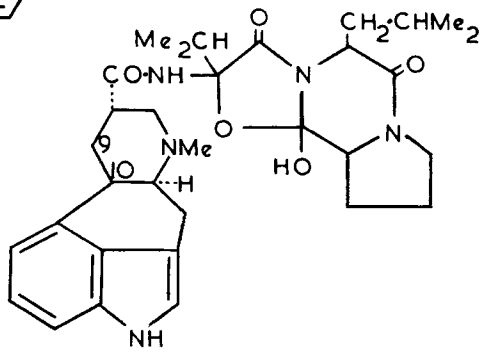

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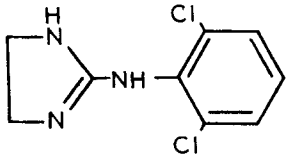

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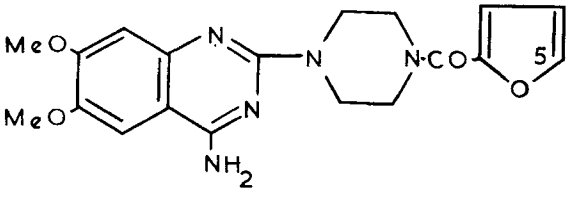

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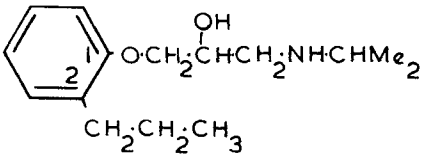

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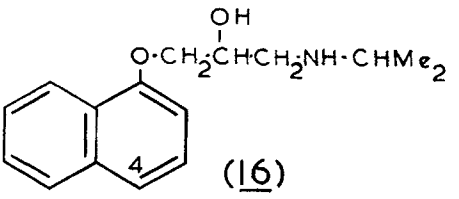

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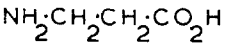

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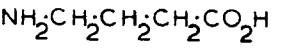

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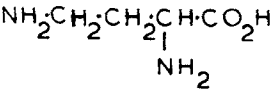

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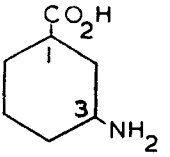

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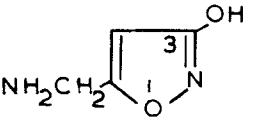

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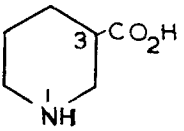

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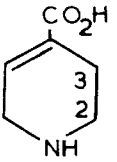

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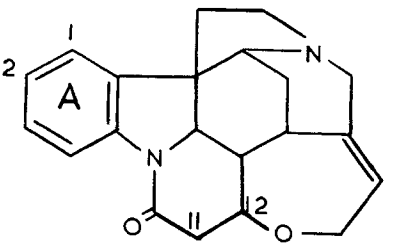

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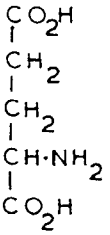

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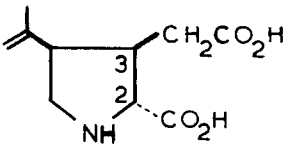

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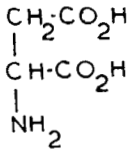

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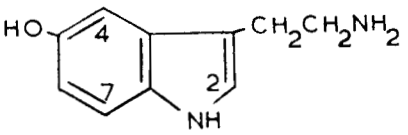

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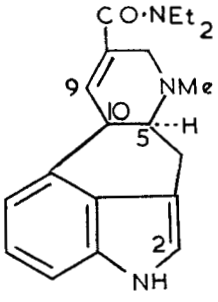

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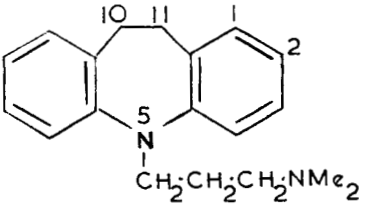

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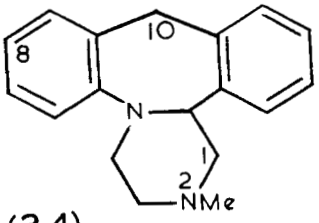

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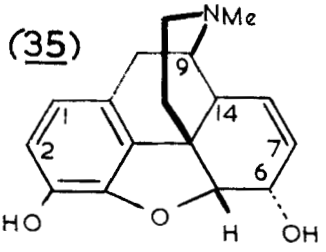

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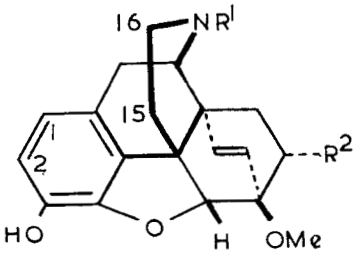
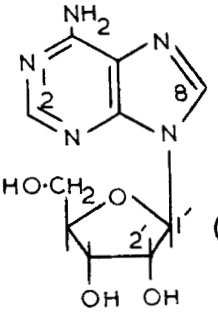


(38) R^1=Me, R^2= 

(39) R^1=  , R^2=CMe_2OH



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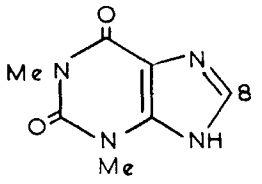

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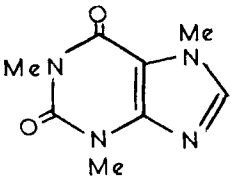

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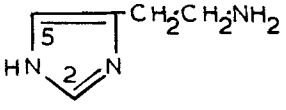

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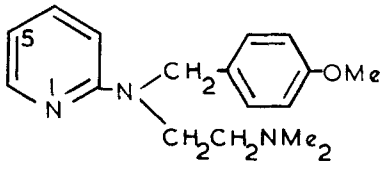

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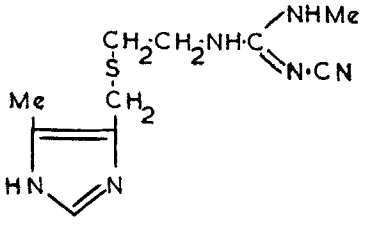

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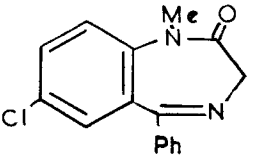

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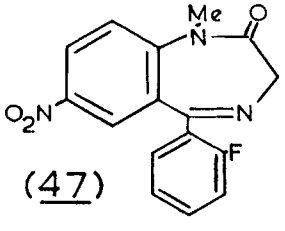

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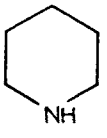

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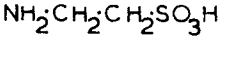

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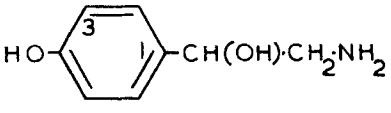

tubes : the latter were capped (13). The ^3H n.m.r. spectra were obtained (mostly with ^1H noise decoupling) at 25°C with a Bruker WH 90 pulse spectrometer operating at 96 MHz (90 MHz for ^1H) and with quadrature detection. The flip angle was usually 30° , the repetition interval 1.6 s, and, depending on the radioactivity present, between 10^2 and 3.5 x 10^4 transients were acquired. The data were stored in 4 K channels and Fourier transformed to provide spectral display widths of up to 13 p.p.m. Referencing was to a ghost reference generated from the ^1H resonance frequency of the internal standard (measured at 90 MHz) by multiplying by the Larmor ratio 1.06663974 (14).

RESULTS AND DISCUSSION

Table 2 summarises the ^3H n.m.r. results and reports the patterns of labelling in the various neurochemicals, achieved by the labelling methods used (Table 1). Many of the ^1H decoupled ^3H n.m.r. spectra were simple and so capable of interpretation by inspection, knowing the corresponding ^1H chemical shifts. The latter were available either from standard spectra (15) (as e.g. for compounds 7,9,17,18, etc), from the information in Tables (16) (as e.g. for compounds 1, 6, 11, 15, 20, etc), or from our own measurements of the ^1H n.m.r. spectrum of unlabelled material (as e.g. for compounds 5, 22, 23, 27). In other cases assignments were made from the coupling information provided by proton-coupled ^3H n.m.r. spectra (e.g. for compounds 3, 4, 24, 31) or from specific proton decoupling (as for compound 24). Quantitative information was obtained directly from signal intensities, there being little or no differential nuclear Overhauser effect under the operating conditions (17).

TABLE 2

^{3}H N.m.r. results for tritiated neurochemicals listed in Table 1

Ligand	Solvent	Chemical shift (δ)	Assignment	Relative intensity (%)
(1)	D_2O	7.30	phenyl-4	100
(2)	d_6DMSO	4.86	quinuclidinyl-3	100
(3)	$CDCl_3$	2.18,2.20	N-Me (CHT_2,CH_2T)	59
		3.73	benzylic CH	6
		3.77,4.13	O-CH_2	30
		7.29,7.35	phenyl	5
(4a)	D_2O	2.82	Ar-CH_2	48
		3.18	N-CH_2	52
(4b)	D_2O	6.79	6	35
		6.87	2	44
		6.93	5	21
(5)	D_2O	1.79	3 ax	6
		2.15	3 eq	4
		2.71	1 ax	26
		2.73	4	44
		3.01	1 eq	17
		6.71	5,8	3
(6)	d_6DMSO	6.77	phenyl-4	100
(7)	D_2O	3.2	N-CH_2	63
		4.8	O-CH	37
(8)	D_2O	4.82	O-CH	100
(9)	D_2O/CD_3CO_2D	3.27	N-CH_2	61
		4.90	O-CH	39
(10)	D_2O	4.89	O-CH	100
(11)	d_6DMSO	6.68	trialkoxybenzene ring-H	100

Ligand	Solvent	Chemical shift (δ)	Assignment	Relative intensity (%)
(12)	d_6DMSO	2.47	9	13
		2.61		37
		2.75	10	43
		2.93	8	4
		3.35	CH.CO of dipeptide	3
(13)	D_2O	7.45	phenyl-4	100
(14)	d_6DMSO	7.93	furanyl-5	100
(15a,b)	d_6DMSO	(a) 0.86 m (b) 0.83 m	propyl-3	(a) 64 (b) 50
		1.53 m 1.50 m	propyl-2	29 22
		2.54 m 2.52 m	propyl-1	7 5
		6.93	ring 4-,6-	23
(16)	d_6DMSO	7.54	4	100
(17a)	D_2O	3.14	$\underline{N}$-CH_2	100
(17b)	D_2O	2.51	CH_2	49
		3.14	$\underline{N}$-CH_2	51
(18)	D_2O	1.84	3-CH_2	48
		2.24	2-CH_2	52
(19)	D_2O	2.30	3-CH_2	15
		3.25	4-CH_2	85
(20)	D_2O	1.33	some or all of	25
		1.37	4 ax, 5 ax, 6 ax	18
		1.88		17
		1.96	4 eq, 5 eq, 6 eq	15
		1.99		25
(21)	d_6DMSO	3.56	$\underline{N}$-CH_2	100
(22)	D_2O	1.73	4 ax	6
		1.99	4 eq	58
		2.56	3 ax	36
(23)	D_2O	3.29	2-CH_2	24
		3.37		44
		3.86	6-CH_2	32

Ligand	Solvent	Chemical shift (δ)	Assignment	Relative intensity (%)
(24)	$CDCl_3$	2.65	11 β	27
		3.15	11 α	26
		7.20	2	24
		8.12	4	23
(25)	D_2O	2.00	3-CH_2	66
		2.08	3-CH_2	26
		3.73	2-CH	8
(26)	D_2O	3.73	2-CH	100
		(i) (ii)		(i) (ii)
(27)	D_2O	1.7 1.84	Me	75 32
		3.36	CH_2-CO_2-	45
		ca. 3.86	5-CH_2	18
		4.35	CH-CO_2-	trace
		4.75 4.90	=CH_2	25 5
		5.0 5.10	=CH_2	
(28)	D_2O	2.63	3-CH_2	36
		2.76	3-CH_2	30
		3.84	2-CH	34
(29)	D_2O	2.68	3-CH_2	45
		2.81	3-CH_2	23
		3.88	2-CH	32
(30)	D_2O	(a) 2.66 (b) 2.89	3-CH_2	(a) 45 (b) 36
		2.77 2.93	3-CH_2	33 25
		3.85 3.97	2-CH	22 39
(31)	d_6DMSO	3.07	side chain	16
		6.89	6	22
		7.12	4	21
		7.31	2	19
		7.43	7	22
(32)	d_6DMSO	7.22	2	100
(33)	d_6DMSO	3.06	10,11	20
		7.17	3,9	80

Ligand	Solvent	Chemical shift (δ)	Assignment	Relative intensity (%)
(34)	d_6DMSO	6.88	8	100
(35)	d_6DMSO	6.59	1	100
(36)	d_6DMSO	1.35	7, 8	64
		6.67	1	36
(37)	d_6DMSO	6.77	1	100
(38)	d_6DMSO	1.85	15	43
		2.75	16	57
(39)	$CDCl_3$	2.37	16	100
(40a)	D_2O	3.80, 3.87	5'-CH_2	69
		8.33	8	31
(40b)	D_2O	3.82	5'-CH_2	38
		3.89		22
		8.26	2	4
		8.35	8	36
(41)	D_2O	7.85	8	100
(42)	D_2O	7.89	8	100
(43)	d_6DMSO	7.53	5	71
		9.00	2	29
(44)	d_6DMSO	6.65	pyridinyl-5	100
(45)	d_6DMSO	2.68	N-Me	100
(46)	d_6DMSO	3.26	N-Me	100
(47)	d_6DMSO	3.36	N-Me	100
(48)	D_2O	1.57, 1.61	3-,4-,5-CH_2	93
		1.69, 1.72		
		3.10	2,6	7
(49)	D_2O	3.21	1-CH_2	59
		3.38	2-CH_2	41
(50)	D_2O	6.98	phenyl-3,5	100

The assignments for atropine base (3) (Table 2), made initially from chemical shift values calculated from Tables [only the shifts for the tropane ring in the sulphate have been detailed (18)] were confirmed and amplified by examination of the proton coupled ^{3}H n.m.r. spectrum (19a). This showed the expected triplet (now at δ 2.23) with J (gem. H,T) = 12.8 Hz from the $\underline{N}\text{-}CH_2T$ triton, and a doublet (at δ 2.20, with the same splitting) from the $\underline{N}\text{-}CHT_2$. In addition there was a singlet at δ 2.18 (previously obscured in the decoupled spectrum) necessarily from the $\underline{N}\text{-}CT_3$ group. The oxymethyl group resonances appeared as two sets of four lines centred at δ 4.13 and 3.77 (the nuclei responsible are non-equivalent because of the adjacent chiral centre) and the tritiated benzylic CH group also gave 4 lines, centred at δ 3.74, of which two were almost hidden by superposition. Each set of four lines is the X part of an ABX spectrum, the A and B lines being in the ^{1}H spectral region.

For [^{3}H]dopamine (4b) which had been labelled by exchange only in the ring, the three lines (Table 2) were assigned from the proton coupled ^{3}H n.m.r. spectrum. This showed in order of increasing chemical shift a double doublet with J (ortho H,T) = 8.5 and J (meta H,T) = 2.2 Hz, a doublet with J = 2.2 Hz, and a doublet with J = 8.5 Hz. Hence the signals were from tritium in the 6-, 2-, and 5-positions respectively. Confirmation came from the ^{1}H n.m.r. spectrum of unlabelled dopamine (as hydrochloride in D_2O) in which the ring signals at δ 6.76, 6.87 and 6.95 showed multiplicities analogous to those above [with J (H,H) = 8 and 2 Hz].

Compound (5), 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrochloride was examined by ^{1}H n.m.r. spectroscopy in deuteriated

water which effected deuteriation of the hydroxy and amino groups (and so removal of the signals). A quintet at δ 3.6 was necessarily from H-2 (ψ-axial) and a double doublet at δ 3.03 (J = 16, 5 Hz) was then from H-1 (ψ-equatorial). The ^3H n.m.r. assignments for the labelled compound (5) followed, as in Table 2. Thus all ring hydrogen positions except H-2 were tritiated by the exchange process.

Compound (11), 2-(2,6-dimethoxyphenoxyethylaminomethyl)benzo-1,4-dioxin, was expected to brominate only at the free ortho-positions in the dimethoxybenzene ring under the mild conditions employed. The tritiodebromination product showed in its proton decoupled ^3H n.m.r. spectrum a single sharp line at δ 6.68. The chemical shift calculable from Tables of benzene substituent constants is δ 6.67. Hence both reaction stages had been specific.

Depending on the tritium content of the hydrogen used in reducing ergocryptine to the dihydro compound (12), so the product would be expected to be doubly 9,10-labelled or singly labelled in the possible two positions. The line at δ 2.75 is almost certainly from the benzylic 10-position and the lines at δ 2.61 and 2.47 may well be from species with label in the 9-positions, respectively cis and trans to that at the 10-position. The minor labelling is probably at the 8-position (δ 2.93) and in the dipeptide moiety at the position adjacent to carbonyl (δ 3.35), judging from the chemical shifts.

Whilst assignments in cis-3-aminocyclohexanecarboxylic acid (20) are somewhat uncertain because of the lack of detailed ^1H reference information, those for nipecotic acid (22) are unambiguous. The most intense ^3H n.m.r. signal at δ 1.99 in the proton decoupled spectrum of

(22) is a doublet (J = 4 Hz) superimposed on a singlet. The signal at δ 2.56 is a doublet with the same splitting, in agreement with axial-equatorial T,T-coupling in doubly labelled [cis-3,4-$^3H_2$] species which account for 72% of the total label. The rest is distributed mainly as 4 eq-labelled species (22%) and to a small extent as 4 ax-labelled species. The assignments for [^{3}H]isoguvacine (23), 1,2,3,6-tetrahydro-4-pyridinecarboxylic acid, followed from the ^{1}H n.m.r. spectrum of the unlabelled hydrobromide which could readily be interpreted (δ 2.63, 3-H_2 ; 3.43, 2-H_2 ; 3.94, 6-H_2 ; 6.97, 5-H) with the help of the splitting pattern.

The assignments for [^{3}H]strychnine sulphate (24) were derived from comparison of the ^{1}H n.m.r. spectrum of the base, measured at 250 MHz (20), with the ^{1}H spectrum of the sulphate at 90 MHz for which the aromatic ring A protons gave signals at δ 7.26, 7.40, 7.44, and 7.97. Confirmation of the assignments in Table 2 came partly from the proton coupled ^{3}H n.m.r. spectrum and partly from a specific ^{1}H irradiation experiment. The proton coupled ^{3}H n.m.r. spectrum showed a doublet at δ 8.13 with J(ortho H,T) = 8.1 Hz from the 4-triton, a triplet with similar splitting at δ 7.21 as expected from a triton at the 2- or the 3-position, and a double doublet at δ 3.16 [with J(H,T) = 18.5, 8.6 Hz] as expected from an α-orientated 11a-triton coupled geminally to H-11b and vicinally to H-12 (which is α-orientated). The spectrum also showed a double doublet at δ 2.66 [J(H,T) = 18.5, 3.1 Hz] from the β-orientated 11b-triton, again a multiplet signal in agreement with the assignment. Specific ^{1}H irradiation of 4-H at δ 8.1 failed to decouple the triplet at δ 7.21 in the ^{3}H n.m.r. spectrum, indicating that the triton responsible for this last signal was not in the 3-position

adjacent to 4-H. Hence the 2-assignment was made.

The tritium assignments for [^{3}H]kainic acid (27), 2-carboxy-4-isopropenyl-3-pyrrolidineacetic acid, were made from the ^{1}H n.m.r. chemical shifts measured under similar conditions [δ 1.76 s, Me ; 2.41 m, 3-, 4-H ; 3.06 m, CH_2-CO_2- ; 3.37 and 3.64, 5-H_2 ; 4.11 d (J 3 Hz), 2-H ; 4.76 and 5.05, CH_2=].

For the 5-hydroxy[^{3}H]tryptamine salt (31), the four ring-triton signals in the proton decoupled ^{3}H n.m.r. spectrum were assigned with the help of the proton coupled spectrum (19b), the splitting pattern being unambiguous.

Because the H-1 and H-2 chemical shifts in morphine and derivatives are identical or closely similar (21), the single sharp line at δ 6.59 from the labelled morphine (35) was not diagnostic of 1-labelling. Use was therefore made of the differential shielding on adjacent benzene positions provided by an acetoxy group (ca + 0.6 ortho, + 0.3 meta). That the derived 3,6-diacetyl[^{3}H]morphine (37) showed only one sharp peak at δ 6.77 in the proton decoupled ^{3}H n.m.r. spectrum then demonstrated that the label was indeed confined to the 1-position. The same conclusion followed of course for the 7,8-dihydromorphine (36) which had been made from (35).

Comparison of the ^{3}H n.m.r spectrum of the [^{3}H]piperidine salt (48) with the ^{1}H n.m.r. spectrum of piperidine (22) indicated that the label was mainly in the 3(5)- and 4-positions, with some 7% residing in the 2(6)-positions. A little allylic exchange had thus occurred during the hydrogenation of the 3,4-double bond in the precursor.

Specificity of Labelling - Sodium borotritide reductions. Reduction of ketones by this reagent introduced label only into the resultant

methylene group, as shown by the ^{3}H n.m.r. spectra of the products (2), (8) and (10). Equally specific was the reduction of esters, as for product (40a) and thence (40b), and for the preparation of 2-amino[1-^{3}H]ethanol by reduction of glycine ethyl ester (followed by back exchange of labile tritium) : that the label was subsequently 1,2-scrambled in the derived taurine (49) is a result of the further transformations proceeding through a symmetrical aziridine intermediate (7,23). The borotritide reduction of the nitrile group in ethyl cyanoacetate (followed by back exchange of tritium in the resulting amino function) placed label solely in the β-methylene group of the product, β-alanine (17a). Borotritide treatment of a protected pyridine to give [2,6-^{3}H]isoguvacine (23) also effected regiospecific labelling, as did similar treatment of the Δ^{15}-enamine precursor to give diprenorphine (39). These reductions presumably proceeded through imine tautomers so that, after back exchange of labile tritium, label resided only in the methylene group next to the nitrogen function.

Catalytic tritiodehalogenation. This procedure introduced label with complete regiospecificity in each of the preparations of compounds (1), (6), (11), (13), (14), (16), (32), (34) (35), (40a), (43), (44), and (50). However, with 3-chloroimipramine there was some exchange labelling of the benzylic methlene groups which accompanied the reductive replacement of halogen so that the product was [3(9),10(11)-^{3}H]imipramine (33).

Enzymic reactions. The hydroxylation of [^{3}H]dopamine using dopamine β-hydroxylase to give labelled L-noradrenaline (7) and the enzymic methylation of the latter to yield labelled L-adrenaline (9) proceeded

with complete retention of the original label. However, in the enzymic resolutions of DL[2,3-^{3}H]aspartic acid (28) small losses of label occurred and the D- (29) and L-aspartic acid (30a) so prepared differed somewhat in the relative amounts of label retained in the 2- and 3-positions.

Reductive amination. The single example, no. 25, indicated that the method is not entirely specific : much label was introduced into the α-position to the original precursor oxo group, as in product (25).

Decarboxylation. In the presence of tritiated water this process can give specific labelling as for product (21), although particular conditions may also effect exchange elsewhere (24).

Exchange with tritiated water. Activated positions were labelled efficiently, as demonstrated by the preparations of compounds (4b), (24), (26), (30b) and (31). The specificity varied with the substrate and conditions. Thus dopamine (4b) was tritiated only in the ring whereas 5-hydroxytryptamine (31) was tritiated also in the benzylic position of the side chain. Under mild conditions, kainic acid gave a product (27)(i) which was tritiated in the allylic methyl group and also, unexpectedly, in the terminal vinyl methylene group as shown unambiguously by the n.m.r. spectra, discussed above. Conditions evidently more vigorous effected labelling also in the activated β-position of the 3-acetic side-chain and the 2-position, as well as in the 5-position, as shown by the ^{3}H n.m.r. spectrum of the product (27)(ii) (see Table 2).

Exchange with tritium gas in the presence of a catalyst. This gave specific labelling in the 8-position of the purine ring, as for theophylline (41) and caffeine (42). Where the purine 2-position was free as with adenosine, exchange occurred there also to a small extent, as found in the product (40b). For 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (5), (ADTN), the label had been introduced predominantly in the benzylic 1- and 4-positions, with a small amount entering into all the other positions except the 2-position which bears the amino group.

Wilzbach exchange. Occasionally this simple method gives remarkedly selective labelling as in the N-methyl and hydroxymethyl groups of atropine (3).

Catalytic reduction of multiple bonds. Depending on catalyst and conditions there can be concomitant exchange so that label is also introduced into positions allylic to the original multiple bond. Examples of such products were dihydroalprenolol (15), β-alanine (17b), 2,4-diaminobutyric acid (19), 3-aminocyclohexanecarboxylic acid (20) and piperidine (48). Activated positions remote from the multiple linkage may also be labelled, as found for dihydroergocryptine (12). In many cases, however, the label is introduced regiospecifically, as with compounds (4a), (18), (22), (36) and (38), although occasionally the distribution of label between the two positions which orginally constituted the ends of the multiple linkage may be far from equal, as in DL-aspartic acid (28).

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