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^{3}H NMR Studies of Hydrogen Isotope Exchange Reactions

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Chapter 1

^{3}H NMR STUDIES OF HYDROGEN ISOTOPE EXCHANGE REACTIONS

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CONTENTS

I.   INTRODUCTION

A.   General

A large number of publications now exist where ^{3}H NMR spectroscopy has been used as an analytical tool, but only a small proportion of these papers reflects an interest in the catalytic exchange method by which the subject compound was tritiated. Conversely, the field of isotope exchange is a large one, but only a small proportion of the results reported include ^{3}H NMR

analyses. Perhaps this is a stage in the development of the ^{3}H NMR technique, from its initial discovery,[1] through its methodological development,[2] to its application in areas of greatest relevance.[3] The point is clear, that very few techniques offer the power of ^{3}H NMR spectroscopy for characterising exchange mechanisms - by clearly showing the labelling pattern in the substrate of interest. It is also clear that the majority of reports of ^{3}H NMR studies concern compounds labelled by synthetic techniques such as hydrogenation or tritio-dehalogenation rather than exchange methods, and the former techniques have already been well reviewed.[3]

Previous reviews of the exchange literature with respect to ^{3}H NMR analysis have been brief overviews.[4-6] This review will bring together the published NMR results and emphasize the new information that the availability of the technique has afforded. In the case of several labelling techniques there are only a small number of results, and these have been tabulated. Other techniques are much better represented in the literature, and a selection of data will be presented in these instances. Many results confirm theories proposed from mass spectrometry data, but there are subtleties that this analytical technique could not reveal.

The positional information available from ^{3}H NMR study is theoretically available from either ^{1}H or ^{2}H NMR analyses. An early study of platinum catalysed exchange used fully deuterated substrates and proton NMR analysis of H_2O exchange products to give positional information of very high integrity.[7] This technique has not been widely used despite its obvious value, presumably because of the additional step of beginning with fully deuterated substrate. ^{2}H NMR spectroscopy has been used to analyse the products of homogeneous metal catalysed exchange reactions,[8] and the technique is very useful for small molecules. A comparison of deuterium and tritium NMR spectra of similarly labelled glucose products are shown in Figure 1, and the superior resolution and dispersion of tritium is obvious.

B.   [3]H NMR Spectroscopy

After the initial high resolution ^{3}H NMR spectrum was described,[1] there was a long delay until the technique was reinvestigated[9] and many of the methodological questions resolved.[10,11] It should be noted that the development of tritium NMR spectroscopy coincided with advances in instrumentation that also allowed many other low abundance nuclei such as ^{13}C to be routinely observed. Once the methodological matters were clarified, and the power of the technique was obvious, the major point to be settled was the accuracy of orientations derived from ^{1}H-decoupled ^{3}H NMR studies. It is clear that nuclear Overhauser enhancements from ^{1}H will affect the reporting of ^{3}H intensities,[12] but in most cases differential enhancements are not observed.[13] In this, and more recent work,[14] it was shown that care should be exercised with analysis of very highly tritiated substrates or where tritium atoms do not have protons nearby.

A series of reviews have covered practical and theoretical aspects of ^{3}H NMR spectroscopy,[2,15-19] culminating in an excellent compilation of both techniques and results.[3] Table 1 gives a listing of a number of useful properties of tritium, especially in comparison to the other hydrogen isotopes. Containment of tritiated samples was originally a point of major concern,[1]

Figure 1. NMR spectra of D-glucose in water. (A). Proton spectrum of C-1 tritiated D-glucose; the large peak is due to HDO. (B). Proton decoupled deuterium NMR spectrum of C-1 labelled glucose; DMSO is an integration marker. (C). Proton coupled tritium spectrum of C-1 labelled glucose; labelled by the same catalytic technique as (B).
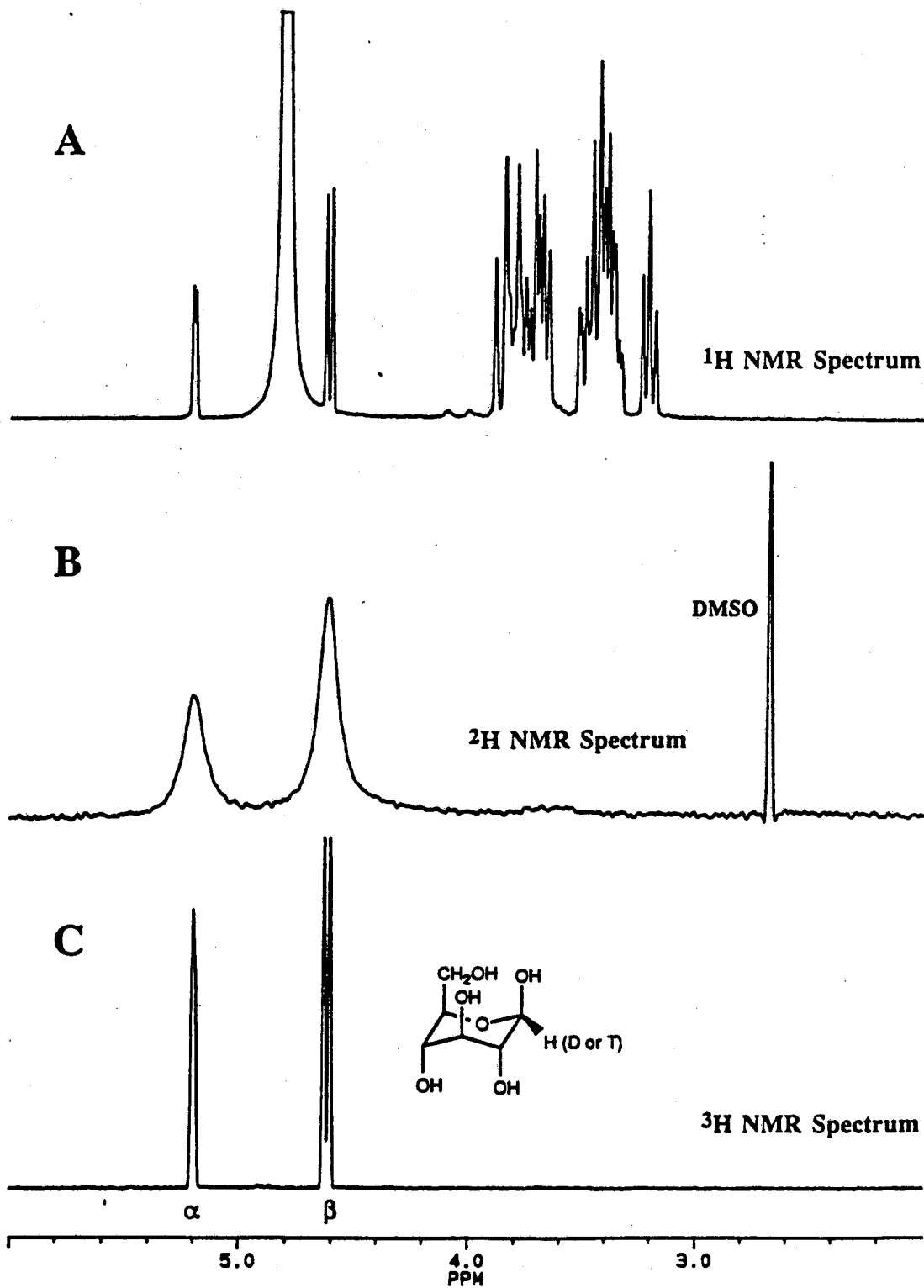
but several simple and effective systems are currently being used.[3,20] Suffice it to say here that the handling and health physics of analysing tritiated samples is an important but trivially solved consideration, and that ^{3}H NMR spectroscopy can be readily executed in most laboratories having a pulsed Fourier transform NMR spectrometer.

Table 1

NMR Properties of ^{3}H

Nucleus	Natural Abundance	Spin	γ	Resonant[a] Frequency	Relative[b] Sensitivity
^{1}H	99.984	1/2	26.7519	300.13	1.0
^{2}H	0.0156	1	4.1064	46.07	9.65 x 10^{-3}
^{3}H	<10^{-16}	1/2	28.5336	320.13	1.21
^{13}C	1.11	1/2	6.7263	75.46	1.59x10^{-2}

δ range 0-20ppm $J_{HT}=J_{HH} \times \gamma_T/\gamma_H$ J range 0-20Hz

$J_{TT}=J_{HH} \times (\gamma_T)^2 \times (1/\gamma_H)^2$

Isotope effects 1°~ 9Hz (at 7T), 2°~ 4Hz (at 7T)

T_1 : 0-10sec T_2 : 0-10sec

Radiation properties

β(100%)   0.0186MeV   range: 4.5-6mm in air

Maximum specific activity: 28.76 Ci/mmole (1063 GBq/mmole)

a - 7.1 Tesla field. b - Sensitivity given for equal numbers of nuclei in the same field.

II.   ACID CATALYSIS

A.   Mineral Acids

Acids have been used to promote labelling of organic compounds since the 1930's.[21] The application of acids to alkane exchange was pursued by a number of groups including Ingold,[21-23] Burwell,[24-28] Beeck,[29-31] and Kursanov.[32] Since exchange only occurred when the subject hydrocarbon contained a tertiary carbon, and label was not incorporated into the tertiary carbon position, the proposed mechanism[31] was thought to involve three steps - initiation, exchange and propagation. The hydrogen on the tertiary carbon atom was removed to form a tertiary carbonium ion in the initiation step, and exchange of isotope into the carbonium ion proceeded by formation of an olefin and subsequent reprotonation by the acid catalyst. An ion was thought to terminate its exchange cycle by hydride (H$^-$) transfer from the tertiary carbon of a neutral molecule, thereby also propagating the exchange.

Since alkanes not containing tertiary carbon atoms were not readily labelled, most acid-catalysed exchange studies have been concentrated on aromatic substrates. Labelling is generally

thought to occur by electrophilic substitution, and there is a huge literature[33-36] based on nitration, chlorination and other such synthetic organic procedures which is immediately applicable.

Most fundamental research on acid-catalysed systems was complete before the advent of routine ^{3}H NMR spectroscopy. There are a number of published tritiation results[3,37] which rely on acids such as CF_3CO_2T, which were first explored in the 1960's.[38] Unfortunately, complex acids such as $T(F_3BOPO_3H_2)$[39] which have been reported as exceptionally powerful, have not been used in reactions where products were analysed by ^{3}H NMR spectroscopy.

General trends in electrophilic aromatic substitution reactions are summarized below, and will be illustrated in tritium NMR studies in this and following sections:

(1) groups such as $-CH_3$, $-NH_2$, $-OCH_3$, $-NHCOCH_3$ *etc* activate the aromatic nucleus and direct exchange to the ortho and para positions.

(2) halogens and -CH=CH-X are much less activating than the groups in (1), but are o/p directing.

(3) $-N(CH_3)_3$, -CHO, $-NO_2$, -CN and $-CF_3$ groups are deactivating and meta directing.

(4) no known groups are activating and meta directing.

A number of results from the literature are given in Table 2. The vinblastine sulfate study[37] was one of the first clear illustrations of the power of the ^{3}H NMR technique for resolution of catalytic labelling patterns. It also required care with reaction and workup of the compound (2 hrs, room temperature), which is light sensitive. The structure of the compound is given in Figure 2(a), and the tritium and proton NMR spectra in Figures 2(b) and (c) respectively. It is easily seen that degradative assignment of the orientation of exchange would have been a daunting task. A very similar anti-cancer drug, vincristine, was similarly labelled and assigned.[2]

Specific labelling of a series of aliphatic acids was achieved[40] by the use of $HTSO_4$ and relatively forcing conditions. All the incorporated tritium was shown to be in the positions α to the carbonyl group. A similar position in glutamic acid (the α, or 2 position), which also bears the amino substituent, was specifically labelled by acid exchange.[3] In the same way, the 3 position of 8-methoxypsoralen (adjacent to the carbonyl) held 80% of the incorporated tritium.[3]

The labelling of dopamine[41] serves to illustrate several rules of electrophilic substitution reactions. The varying extent of labelling in the aromatic positions is a result of the substituent effects on the tritiation as outlined in the four rules above. Comparison of (1) and (2) clearly shows that the 5-position, which is meta to two substituents, is the least labelled of the three aromatic positions.
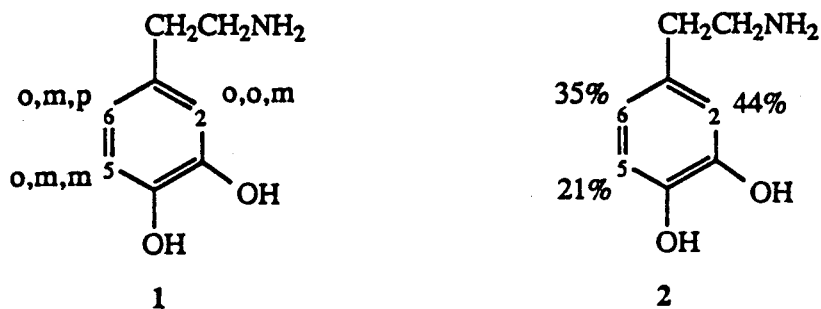
Figure 2. (A). Structure of Vinblastine Sulfate. (B). Proton decoupled tritium NMR spectrum. (C). Proton NMR spectrum. (Reproduced by permission, The Royal Society of Chemistry).
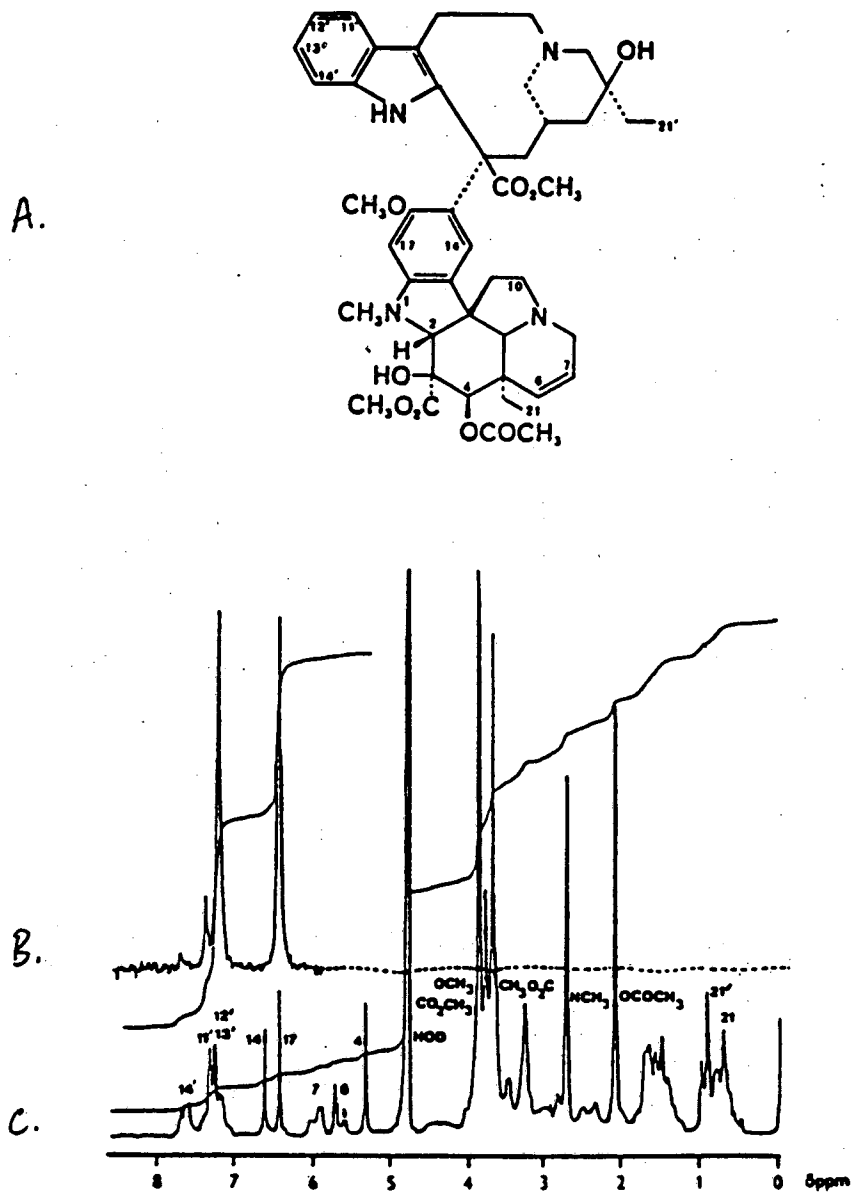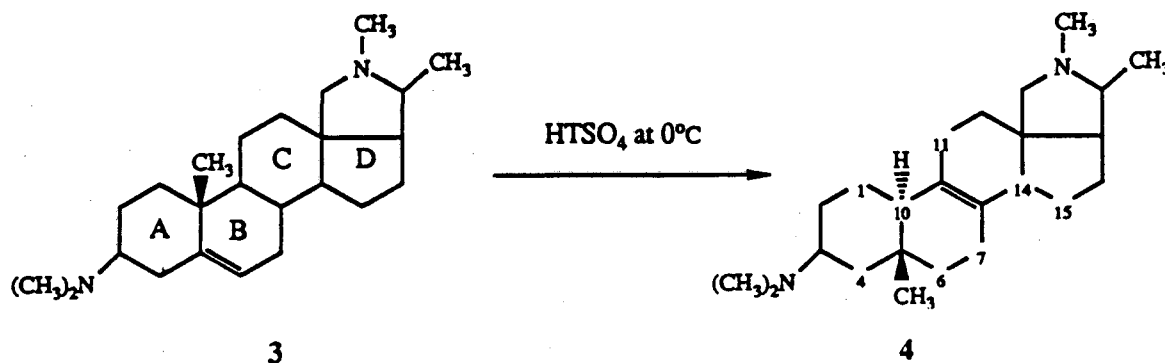
Table 2

Labelling by Acid-catalysed Exchange

Compound	Method	Orientation, %	Ref.
Vinblastine Sulfate	CF_3CO_2H/HTO/2 hrs	$11'$-8; $12',13'$-46; $14'$-2; 17-44	37
Vincristine Sulfate	CF_3CO_2H/HTO/2 hrs	$11'$-11.4; $12',13'$-76.1; $14'$-5.7; 17-6.8	2
Palmitic Acid	$HTSO_4$/64 hrs 100°C	α-CH_2-100	40
Myristic	$HTSO_4$/64 hrs 100°C	α-CH_2-100	40
Lauric	$HTSO_4$/64 hrs 100°C	α-CH_2-100	40
Stearic	$HTSO_4$/64 hrs 100°C	α-CH_2-100	40
Glutamic Acid	Acid/HTO	2-100	3
8-Methoxypsoralen	Acid/HTO	3-79.8; 5-8.9; 7-11.3	3
Dopamine HCl	HCl/HTO	2-44; 5-21; 6-35	41
Kainic Acid	Acid/HTO	(i) CH_3-75; $=CH_2$-25 (ii) CH_3-32; -CH_2-CO_2H-45; 5-CH_2-18; $=CH_2$-5	41
Strychnine Sulfate	CF_3CO_2H/HTO	2-24; 4-23; 11α-27; 11β-26	41
Benzo[a]pyrene	CF_3CO_2H/HTO	1-9.6; 2-6.2; 3-9.6; 4-12.4; 5-6.7; 6-8.4; 7-7.3; 8-6.7; 9-12.4; 10-4.2; 11-4.2; 12-12.4	3
7-Methylbenz[c]acridine	Acid/HTO	CH_3-71.4; ring-6.3,8.7,9.5,3.2,0.8	3
12-Methylbenz[a]acridine[a]	Acid/HTO	CH_3-24.3; ring-60.1,10.1,2.3,3.2	3
Colchicine	Acid/HTO	4-100	3
Imipramine	$CF_3CF_2CO_2H$/HTO	4,6-45; other aromatics-55	42
Isoconessine	$HTSO_4$/0°C	C1,C4,C6,C7,C10,C11, C14,C15,C19-all labelled	43

Aromatic positions in compounds such as benzacridines, imipramine[42] and colchicine have also been exchanged, with extremely specific labelling in the case of the latter substrate (Table 2).[3] The $HTSO_4$-catalysed rearrangement and tritiation of isoconessine (4, from conessine, 3) has been studied[43] and yielded highly labelled product, with nine resolved lines in the tritium NMR spectrum. Comparison with ^{2}H and ^{13}C NMR, and mass spectra of similarly deuterated substrates led to the conclusion that the product was tritiated on nine carbons (17 possible hydrogens), and that there were a considerable number of multiply tritiated molecules in the product mix. The carbons which were thought to bear tritium atoms include the 19-methyl group (near the 4 and 6 carbons) and the numbered positions on the structure below (4):

3   HTSO₄ at 0°C   4

Acid catalysis is rapid and effective, but it should be kept in mind that tritium incorporated at low pH may also be lost under similar conditions. That is, positions labelled by acid (or base) catalysis may not be stable under biological conditions, where local pH excursions may be large.

B. Lewis Acids

The possibility of using extremely reactive superacids and Lewis acids as isotope exchange catalysts has been recognized for many years. As with the mineral acid work, much of this research took place before the advent of routine ^{3}H NMR analyses, so positional information is scant.

Tritiation of alkanes[44] by HTO/EtAlCl$_2$ exchange was shown to occur preferentially at the CH positions, with considerably less labelling in CH$_2$ and CH$_3$ groups (Table 3). The aromatic labelling induced by EtAlCl$_2$ is rapid,[45] and is reported to be random, but until recently there was no published tritium NMR data to support this contention. Certainly, near-random tritium distributions were observed for the tritiation of 1,4-dimethylnaphthalene in the presence of both BBr$_3$ and EtAlCl$_2$[47] (see Table 3). Later work[48] with BBr$_3$ as the catalyst suggested that an o/p orientation in aromatic centres was obtained, supporting an electrophilic mechanism for this catalyst.

The most systematic study of Lewis acid catalysed exchange by ^{3}H NMR spectroscopy is that of Garnett, Long and Lukey.[49,50] A series of simple organic compounds were tritiated and the positions of labelling assessed (Table 3). The studies with BBr$_3$ as catalyst clearly show that the orientation of exchange in aromatic centres is o/p in every case where data was obtained.[49] Alkyl substituents were not labelled, and compounds such as α,α,α-trifluorotoluene, which are deactivated towards electrophilic attack, were not labelled. Important features of the BBr$_3$ system in contrast to EtAlCl$_2$ labelling, are:

(1)   that the aromatic labelling patterns are distinctly different (o/p vs random), and

(2)   that labelling may still be effected with BBr$_3$ even when it has previously been hydrolysed with HTO.

This latter point is very different from the EtAlCl$_2$ system, where the HTO appears to effect labelling by destruction of a catalyst-substrate complex, and the order of addition of reactants is critical.[45]

A wide variety of polycyclic aromatic hydrocarbons labelled with tritium are required for studies of the mechanisms of carcinogenesis. A range of compounds have been successfully labelled by application of the EtAlCl$_2$ technique with high specific activity HTO.[51,52] A number of these compounds have recently been analyzed by ^{3}H NMR spectroscopy,[53] and a typical spectrum is shown in Figure 3, acquired on 2mCi of purified substrate. The orientation in this and other (similar) substrates is almost random, and supports the widely accepted theory of the lack of specificity of aromatic labelling by EtAlCl$_2$.

Despite the wide use[54] of Lewis acids as tritiation catalysts, and their application to a number of classes of compounds,[52,55] very little orientational information exists in the literature.

Figure 3. NMR spectra of Fluoranthene tritiated by Lewis acid catalysed exchange.
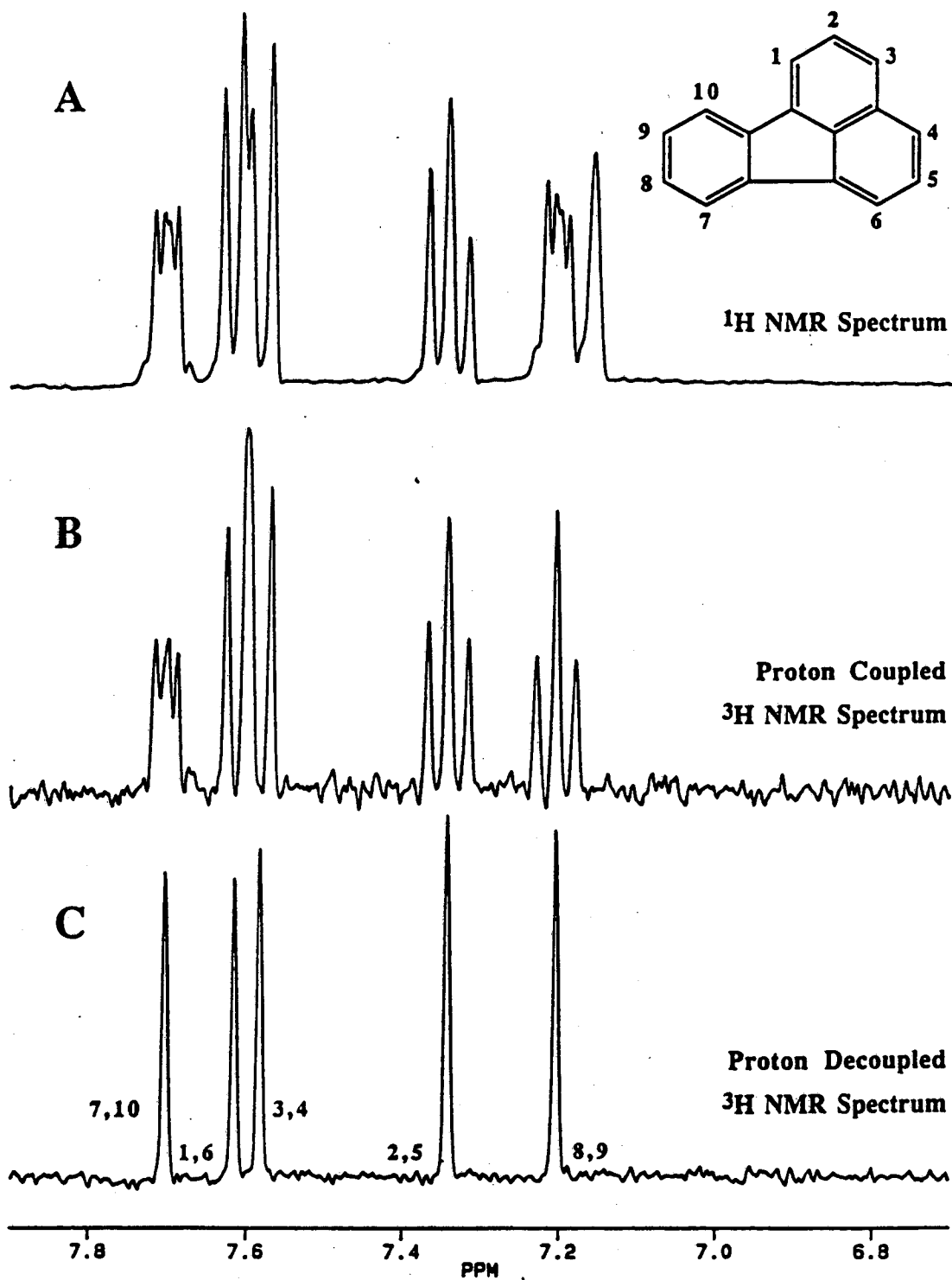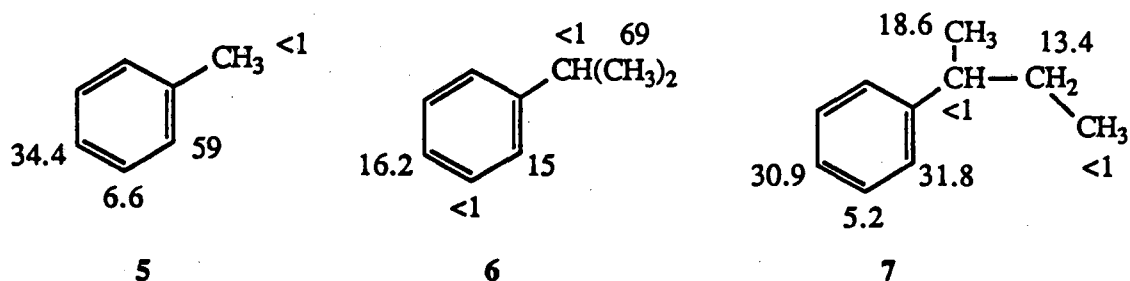
Table 3

Labelling by Lewis Acid Catalysts

Compound	Catalyst	Orientation, %	Ref.
2,3 Dimethylbutane	EtAlCl$_2$	CH-17; CH_3-83	44
3-Methylpentane	EtAlCl$_2$	CH-21; CH_2-29; CH_3-50	44
Methylcyclohexane	EtAlCl$_2$	CH-28; CH_2-32; CH_3-40	44
Bromobenzene	EtAlCl$_2$	o-38; m-36; p-25	48
Toluene	BBr$_3$	o-60; m<10; p-40; CH_3<3	48
iso-Propylbenzene	BBr$_3$	o-36; m-10; p-19; CH-16; CH_3-18	48
Bromobenzene	BBr$_3$	o-52; m<3; p-47	48
Naphthalene	BBr$_3$	α-76; β-24	48
Chlorobenzene	BBr$_3$	o-43; $m+p$-57	46
1,4 Dimethylnaphthalene	BBr$_3$	2,3-39; 5,8-33; 6,7-28; CH_3<1	47
1,3,5 Trimethylbenzene	BBr$_3$	2,4,6-100; CH_3<1	47
Chlorobenzene	EtAlCl$_2$	o-41; m-39; p-20	47
1,4 Dimethylnaphthalene	EtAlCl$_2$	2,3-32; 5,8-33; 6,7-35; CH_3<1	47
1,3,5 Trimethylbenzene	EtAlCl$_2$	2,4,6-100; CH_3<1	47
1-Chloronaphthalene	BBr$_3$	2-15; 3-4; 4-10; 5-25; 6-4; 7-15; 8-26	47
Toluene	EtAlCl$_2$	o-36.6; m-40; p-24.4; CH_3<0.1	50
Fluorobenzene	EtAlCl$_2$	o-42.4; m-35.2; p-22.3	50
Bromobenzene	EtAlCl$_2$	o-41.2; m-37.2; p-21.6	50
Naphthalene	EtAlCl$_2$	α-55.2; β-44.8	50
Toluene	BBr$_3$	o-63; m-10.2; p-26.9; CH_3<0.1	49
n-Propylbenzene	BBr$_3$	o-61; m-8.4; p-30.5; $alkyl$<0.1	49
iso-Propylbenzene	BBr$_3$	o-62; m-5.4; p-32.7; $alkyl$<0.1	49
iso-Butylbenzene	BBr$_3$	o-42.8; m<0.1; p-57.3; $alkyl$<0.1	49
t-Butylbenzene	BBr$_3$	o-60.4; m-8.6; p-31.0; $alkyl$<0.1	49
Cyclohexylbenzene	BBr$_3$	o-60.4; m-6.8; p-32.8; $alkyl$<0.1	49
Diphenylmethane	BBr$_3$	o-66.6; m<0.1 p-33.3; $alkyl$<0.1	49
Fluorobenzene	BBr$_3$	o-31.6; m<0.1; p-68.4	49
Chlorobenzene	BBr$_3$	o-54.6; m<0.1; p-45.5	49
Bromobenzene	BBr$_3$	o-52; m<0.1; p-48.0	49

C.   Zeolite Catalysis

Zeolites are crystalline aluminosilicates, and have long been known to have catalytic properties which allow them to act like very strong mineral acids. A detailed study of the hydrogen isotope exchange capabilities of these catalysts was undertaken with ^{3}H NMR spectroscopy as the major analytical tool.[56]

Preliminary results[57,58] for exchange of aromatic substrates with HTO over the large-pore zeolite, HNaY, showed very clearly that the orientation of labelling was that due to electrophilic aromatic substitution. As shown for toluene (5) aromatic exchange was confined almost exclusively to the ortho and para positions, and alkyl exchange was not observed. This pattern was generally true of straight-chain alkylbenzenes, but branched-alkyl aromatics gave alkyl exchange confined to the β-carbon atoms of molecules branched at the α-carbon (see 6, 7), in addition to the ortho/para aromatic labelling. Such a substitution pattern is expected where exchange involves hydride transfer between the reactant molecule and an α-carbonium ion, as proposed for alkane

exchange with strong mineral acids.[30,31] Incorporation of tritium into the carbonium ion may take place by deprotonation to an olefinic intermediate and reprotonation.[30,59]

$$CH_3 \quad <1$$
34.4 / 59
6.6

5

$$<1 \quad 69$$
$$CH(CH_3)_2$$
16.2 / 15
<1

6

$$18.6 \quad CH_3 \quad 13.4$$
$$CH-CH_2$$
$$<1 \qquad CH_3$$
30.9 / 31.8 / <1
5.2

7

It was also observed that exchange between aromatic substrates was facile over HNaY and other zeolites, such as H-mordenite and HZSM-5.[58,60] Tritiated benzene and specifically tritiated toluene were used to characterize the mechanism of this aromatic-aromatic labelling.[61] As previously reported from deuteration studies,[62] exchange took place by transfer of isotope from one aromatic to the zeolite, followed by incorporation into the second aromatic centre. The extent of exchange observed in any given substrate depended on its adsorption and exchange in competition with the isotope source. Alkanes were not efficiently labelled with either an organic or water as the isotope source,[57,60] in contrast to metal-catalysed labelling systems.

Although zeolites can activate tritium gas for exchange into organic substrates at modest temperatures,[63] the process is made much more efficient by the presence of a transition metal such as platinum or palladium.[58,61] The methyl position of toluene was efficiently labelled over PdY, and the orientations generally could be seen to be influenced both by guest metal and the zeolite. In addition to vastly increasing the uptake of tritium by a given substrate over a time period, the presence of metal caused positions and molecules not normally exchanged over zeolites to be labelled (compare 8 and 9 with 5). Of particular interest was the efficient and relatively general labelling of alkanes (10).

$$16.3$$
$$CH_3$$
42 / 29.2
12

8

$$78.3$$
$$CH_3$$
15.7 / 6
<1

9

$$31.2$$
$$H_3C-{}^{2}\diagdown \diagup {}_{3} \diagup CH_3$$
33.6 \quad 35.2

10
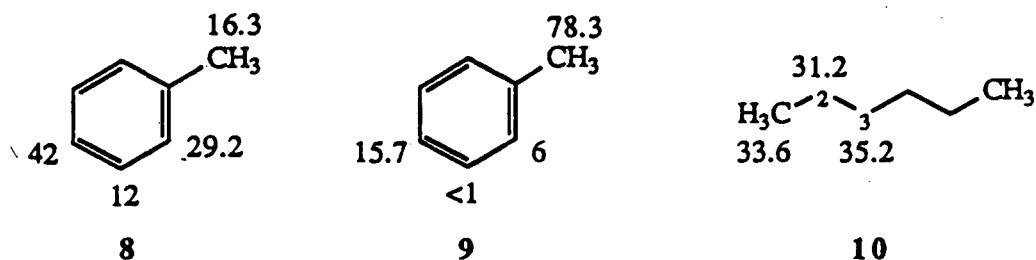
The appearance of "metal character" in labelling distributions was apparent with HTO as isotope source as well as with T_2, as indicated by extensive methyl exchange over Pd-Mordenite zeolite (11). However, as is well known, metal catalysis is poisoned by the presence of air, and

methyl exchange (a metal catalysis feature) is quenched when air is included in the reaction tube (12).[66]
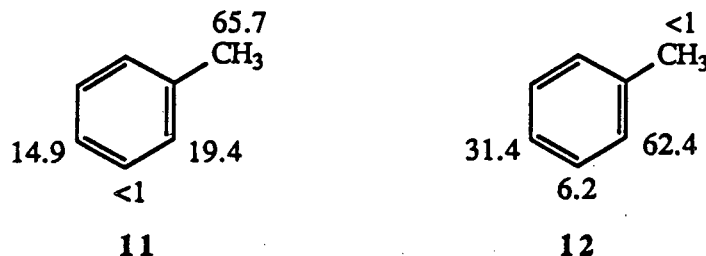
In summary of zeolite catalysis, a range of simple organic substrates may be readily labelled[56] using a variety of isotope sources. Alkanes and the straight-chain alkyl portions of alkylbenzenes require the presence of a metal with the zeolite to promote exchange. Pyridine also required metal presence, but other heterocyclic compounds such as thiophene and furan were readily exchanged. Exchange results were found to rely upon the following factors:

(1) the form of zeolite: HNaY, H-Mordenite, HZSM-5 with Pt, Pd or Ni substitution
(2) the isotope source - HTO, C_6D_6, C_6H_5T, T_2
(3) the presence or absence of air in the reaction vessel.

Hence, the activation of tritium gas and its availability to the zeolite lattice is expedited by the presence of metal. In the case of HTO or organic isotope sources the metal and zeolite catalysts are in direct competition, but the metal contribution can be controlled by the admission or exclusion of air.

D.   Catalysis by Aluminophosphates

Direct exchange of tritium gas with organic substrates over aluminophosphates has been reported recently.[64,65] In comparison to the zeolite systems, where exchange with tritium gas was slow with the unmodified zeolite,[60,66] the AlPO-5 catalyst gave 5-20% incorporation of tritium over a few days at temperatures of 100-180°C.[64] The more remarkable facet of the exchange was the orientation of exchange in labelled substrates, as given in Table 4. Even though the acid properties of AlPO-5 have not been stressed in comparison to zeolites, the orientations show clearly that electrophilic aromatic substitution is likely to be the mechanism of labelling. However, the para position of toluene shows much more exchange than the ortho, and this is the first report of such a unique orientation.[64] This is also observed for labelling of bromo- and chlorobenzene, but not fluorobenzene.

The same orientation is observed when HTO is the isotope source,[65] but some of the specificity is lost as the temperature is raised in order to increase total incorporation. This was also a feature of zeolite labelling, and is expected from the kinetics of electrophilic aromatic substitution.

The use of the AlPO catalyst in a modified Wilzbach experiment with T_2[65] gave approx. 20% incorporation of tritium in toluene with excellent purity of the product (Table 4). The orientation of labelling shows almost random exchange in the aromatic ring, but a significant amount of tritium in the methyl positions (6%).

Table 4

Tritium Distribution in Compounds labelled over AlPO-5 Catalyst

| Compound		Time (hr)	Temp. (°C)	Activity (mCi/mL)	Orientation, %	Ref.
Toluene	T_2	24	100	48	o-7.2; m<2; p-93; CH_3<1	64
Toluene	T_2	168	100	153	o-9.6; m-6.2; p-84; CH_3<1	64
Toluene	T_2	72	180	175	o-36; m-8.2; p-55; CH_3<1	64
m-Xylene	T_2	72	100	83	C2-25; C4,6-38; C5<1; CH_3<1	64
Naphthalene	T_2	72	100	31	α-100; β<1	64
Furan	T_2	72	100	36	α-100; β<1	64
2,3 Dimethylbutane	T_2	72	100	17	Methine-100; Methyl<1	64
Toluene	HTO	136	125	23	o<1; m<1; p-100; CH_3<1	65
Toluene	HTO	136	140	84	o-14.2; m<1; p-86; CH_3<1	65
Toluene	HTO	136	180	784	o-38; m-5; p-58; CH_3<1	65
Chlorobenzene	T_2	72	150	46	o<1; m<1; p-100	65
Bromobenzene	T_2	72	150	75	o<1; m<1; p-100	65
Fluorobenzene	T_2	72	150	35	o-58; m<1; p-42	65
Toluene[a]	T_2	120	RT	1410	o-36; m-36; p-21; CH_3-6	65

a - modified Wilzbach experiment with 2Ci of T_2 and 0.03mL of substrate

Once again, these results show that "solid acids" such as the crystalline aluminosilicates (zeolites) or aluminophosphates (AlPO's) may be used to give high levels of acid labelling in simple organic compounds. The methods have not been extended to larger molecules, and it remains to be seen whether efficient exchange will be observed in molecules excluded from the structural pores.
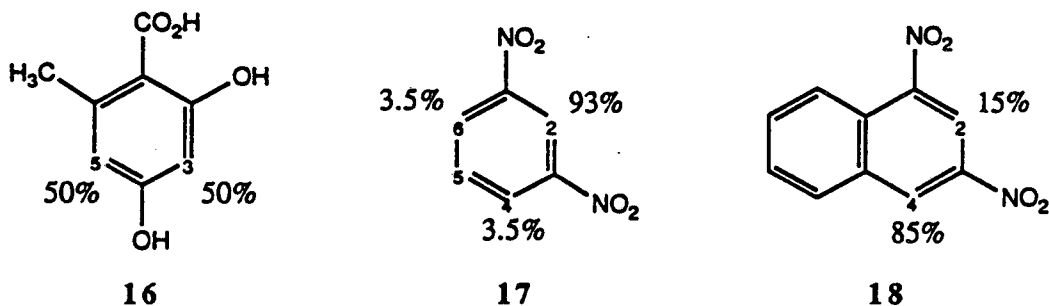
III.   BASE CATALYSIS

Most organic compounds can be regarded as carbon acids, even if very weak, and treatment with sufficiently strong base can lead to hydrogen isotope exchange.[67] Labelled compounds produced in this way may be used as tracers provided that the activity of the compound and the basicity of its solution medium are known. A series of studies by Jones and co-workers, recently reviewed,[68] has explored the many factors affecting the detritiation of heterocyclic compounds in solution.

Table 5 contains the results of a large number of substrates labelled by base catalysis, and subsequently analysed by ^{3}H NMR spectroscopy. As is well known, the method provides a very specific procedure for introducing tritium adjacent to a carbonyl group, as illustrated in the labelling of acetophenone,[11] stearic acid,[40] diethyl malonate,[69] and a large series of substituted (2-acetyl) thiophenes.[70,71] In addition, a number of steroids (13, 14)[72] and 15,16-dihydrocyclopenta[a]-phenanthren-17-ones (15)[73] have been specifically labelled (Table 5) by use of the same characteristic, and (in some cases) subsequent chemical modification.

13   **14**   **15**
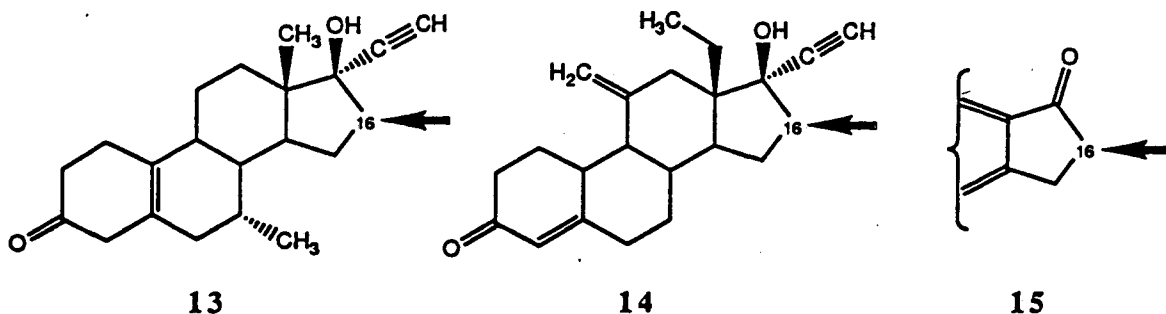
Similarly, compounds containing terminal triple bonds are specifically labelled on the terminal (methine) carbon,[10,74] and a series of nitriles have been tritiated on the 2 carbon.[10,11] Application of this technique leads to specific benzyl exchange in substrates such as phenylacetylene, phenylacetonitrile or benzoselenazole (Table 5). Aromatic tritiation has only been reported in a limited number of cases,[75-77] and the orientations are given below. The even distribution in orsellinic acid (16) probably just reflects equilibration of isotope, while the marked orientations in the other two substrates (17, 18) were used to propose canonical forms of intermediates,[77] and to provide evidence for carbenoid type delocalization in nitroaromatic systems. In addition, the results illustrate the particular usefulness of the method for labelling nitroaromatics, which are difficult to label by heterogeneous metal and other catalytic techniques.

16   **17**   **18**

Base catalysed labelling has the advantages of providing rapid and specific exchange under conditions of mild temperature and pressure. The maximum specific activities attainable are controlled by the isotope source, and this can be an important disadvantage (as is also true of acid systems). However, since the results for base catalysed exchange can easily provide very specific labelling, and the parameters governing detritiation in a number of classes of compounds are well understood, it is surprising that the technique has not been more widely used and characterized by ^{3}H NMR spectroscopy. In particular, since the NMR technique allows the monitoring of multiple positions within one molecule, it is a little surprising that ^{3}H NMR studies of reactions in progress have not been conducted.

Table 5

Base Catalysis of Hydrogen Isotope Exchange

Compound	Method	Orientation, %	Ref.
Acetophenone	NaOH/20°C/18 hrs	CH_3-100	11
Stearic Acid	NaOH/160°C/16 hrs	α-CH_2-100	40
Diethyl Malonate	Na_2CO_3/RT/7 days	CH_2-100	75
Diethyl Malonate	Na_2CO_3/RT/2 days	CH_2-100	69
Sodium Acetate	NaOH/90°C/168 hrs	CH_3	11
Pentan-3-one	Na_2CO_3/60°C/3 days	2,4-100	74
α-Chloroacetophenone	Na_2CO_3/ /24 hrs	α-$ClCH_2$-100	74
α-Bromoacetophenone	Na_2CO_3/20°C/18 hrs	α-$BrCH_2$-100	74
Acetone	NaOH/20°C/18 hrs	CH_3-100	11
Sodium Malonate	NaOH/20°C/18 hrs	2-100	10
Isobutyric acid	NaOH/150°C/2 days	2-100	74
Sorbic Acid[a]	Pyridine/steam/2 hrs	α-36,γ-64	78
7αMethyl Norethinodrel	CH_3ONa/80°C/2 hrs	16 α/β-100	72
3-Oxo-desogestrol	CH_3ONa/80°C/2 hrs	16 α/β-100	72
15,16-Dihydrocyclopenta[a]-phenanthren-17-ones	NaOH/RT/2-3 days	16-100	73
2-Acetyl thiophene derivatives	NaOH/RT/48 hrs	Acetyl-100	70
3-Carboxy 2-acetyl thiophenes	NaOH/RT/48 hrs	Acetyl-100	71
Adenosine 3' monophosphate[b]	HTO/85°C/18 hrs	8-100	3
1-Methyl Inosine[b]	HTO/85°C/18 hrs	8-100	3
Phenylacetylene	NaOH/RT/36 hrs	Methine-100	74
Undec-10-yn-1-oic acid	NaOH/45°C/48 hrs	11-100	74
2-Methylbut-3-yn-2-ol	NaOH/45°C/48 hrs	4-100	74
Prop-2-yn-1-ol	NaOH/20°C/18 hrs	3-100	10
Prop-2-en-1-ol	NaOH/20°C/18 hrs	3-100	10
Phenylacetonitrile	Na_2CO_3/ /24 hrs	α-CHTCN-100	74
Acetonitrile	NaOH/20°C/18 hrs	CH_3 -100	11
Propionitrile	NaOH/20°C/18 hrs	2-100	10
Malononitrile	NaOH/20°C/18 hrs	2-100	10
Dimethylsulfoxide	NaOH/90°C/18 hrs	CH_3-100	11
Benzyl methyl sulfoxide	NaOH/RT/36 hrs	α-CH_2-100	74
Nitromethane	NaOH/20°C/18 hrs	CH_3-100	11
Chloroform	0.2N NaOH/20°C/1 hr	1-100	10
1,3 Dinitrobenzene	CH_3ONa/45°C/4 hrs	2-93, 4-7	76
1,3 Dinitronaphthalene	NaOH/RT/300 hrs	2-15, 4-85	77
Orsellinic Acid	NaOH/RT/4 days	3,5-50 each	75
2-Picoline	NaOH/20°C/18 hrs	1'-100	10
2 Methyl resorcinol	NaOH/20°C/18 hrs	4-100	10
Pyridine-1-oxide	NaOH/75°C/30 hrs	2-100	10
Quinoline-1-oxide	NaOH/75°C/20 hrs	2-100	10
Isoquinoline-1-oxide	NaOH/75°C/20 hrs	1,3-100	10
Benzoxazole	0.2N NaOH/20°C/1 hr	2-100	10
Benzothiazole	0.2N NaOH/20°C/1 hr	2-100	10
Benzoselenazole	0.2N NaOH/20°C/1 hr	2-100	10

a - Reaction of crotonaldehyde and malonic acid to give labelled product.
b - Simple heating with HTO is sufficient to label many purines.[68]

IV. GAS EXPOSURE TECHNIQUES

A. Wilzbach

The energy of the β-decay of tritium can sometimes be sufficient to activate an organic compound towards exchange with a tritium gas atmosphere.[79] Generally the percentage of the total tritium that is incorporated into the substrate is very small, and usually a large quantity and variety of other radioactive products are formed in addition to the desired product. As a consequence, a great number of variations on the technique have been tried,[80] and some of the more successful ones will be discussed in the next few sections.
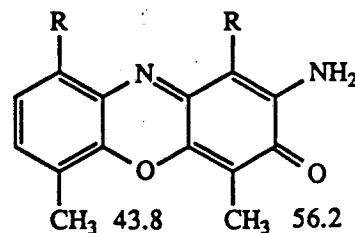
In rare cases specific and efficient Wilzbach labelling is observed as illustrated in the following examples:
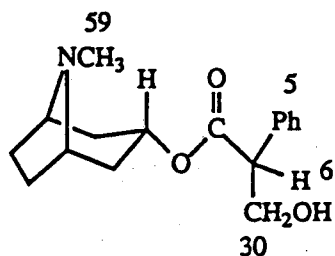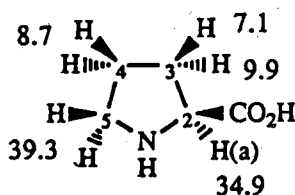
**19**   **20**   **21**

In the case of atropine (**19**) significant quantities of CTH_2, CT_2H, and CT_3 species were observed,[17] as well as the magnetic non-equivalence of the tritons of the $-CH_2-OH$ group (adjacent to the chiral $-CH-Ph$ group). The positions close to a nitrogen were once again most labelled in proline (**20**) with 75% of the incorporated tritium being on a carbon attached to nitrogen.[3] The Wilzbach exchange of Actinomycin D^2 (where R represents a cyclic peptide) gave surprisingly specific labelling (**21**), and once again multiple tritiums were incorporated on each carbon atom, as revealed by the ^3H NMR spectra.

B. Microwave Induced Exchange

Microwave discharge activation (MDA) was first published as a variation of gas exposure or Wilzbach labelling,[81] and has attracted a lot of attention as a potentially useful general labelling process.[80,82] Once again, very little ^3H NMR data has been published on the orientation of exchange induced by microwave techniques.[83,84] However, a number of studies on the best conditions for labelling have explored the critical parameters influencing yield and specific activities obtained.[83,85] The best reported results are those arising from the modification engineered by Peng and co-workers, whereby a support is used in the exchange reaction, and the microwave activated species are no longer acting directly on the exposed substrate.

In preliminary studies of peptide labelling using this system[83] it was reported that samples of Gly-Gly-Leu with specific activities of 1-5 Ci/mmole were obtained, and ^3H NMR evidence suggested most of the ^3H was in the CH_3 groups of the Leucine residue.

Studies of peptide labelling by Ehrenkaufer[82,86,87] have given several clues as to the mechanism of the exchange. It was noted[84] that the form of the substrate greatly affected the efficiency of labelling[82]: changing from the zwitterionic form of L-Valine to the neutral sodium salt gave a 45-fold increase in specific activity of product.[87] Similarly, the neutral form of L-Proline experiments revealed that the orientation is also influenced by the overall charge on the molecule (zwitterion 22, Na salt 23)[84] where * denotes that the 3β resonance was insufficiently resolved from the 4 tritons in the NMR spectra for quantitation.

22

23
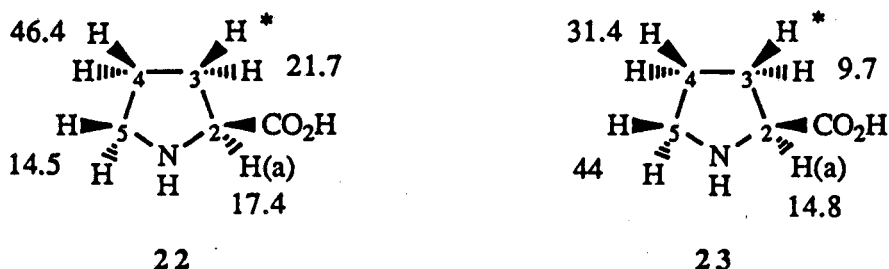
Studies of the labelling of steroids[88,89] have shown that the backbone of the molecule appears to provide protection against degradation during tritiation by microwave activated tritium. In addition, the presence and nature of supports was found to influence the orientation of labelling, as determined by ^{3}H NMR study.[89] Use of 5% Ru on silica-alumina pellets gave predominantly 2β labelled progesterone, whilst labelling without metal on the support yielded mainly the 2α tritiated product.

More recent work with the MDA system has shown that remarkably pure products with specific labelling may be obtained. Figure 4 shows the ^{3}H and ^{1}H NMR spectra of n-propylbenzene labelled by exposure to T_2 while supported on the same silica-alumina supported Ni catalyst as previously studied.[83] The pattern of labelling strongly favours ortho/para incorporation, which suggests attack by a T^+ species, as proposed by Peng.[83] This is one of a series of simple organic substrates in which ^{3}H NMR analyses show similarly marked orientations.[90]

The method has also been valuable for labelling of a series of polycyclic aromatic hydrocarbons,[90] with similar high specific activities and excellent purity. An example is given in Figure 5 with the NMR analyses of phenanthrene, showing relatively even incorporation of tritium.

Although the parameters governing yield, purity and level of tritium incorporation by the MDA technique are still being pursued, the preceding results suggest that it may have great value as a simple, high level labelling process. In addition to the simple organic substrates, carcinogens[90] and steroids[88,89] labelled in this manner, benzodiazepines[91] have also been successfully tritiated.

$\left(T_3^+ \right)$

B

ortho

para

meta

Proton Decoupled ^{3}H NMR Spectrum

A

CH_2 — CH_3

CH_2

^{1}H NMR Spectrum

Aromatic

CH$_3$

α-CH$_2$ β-CH$_2$

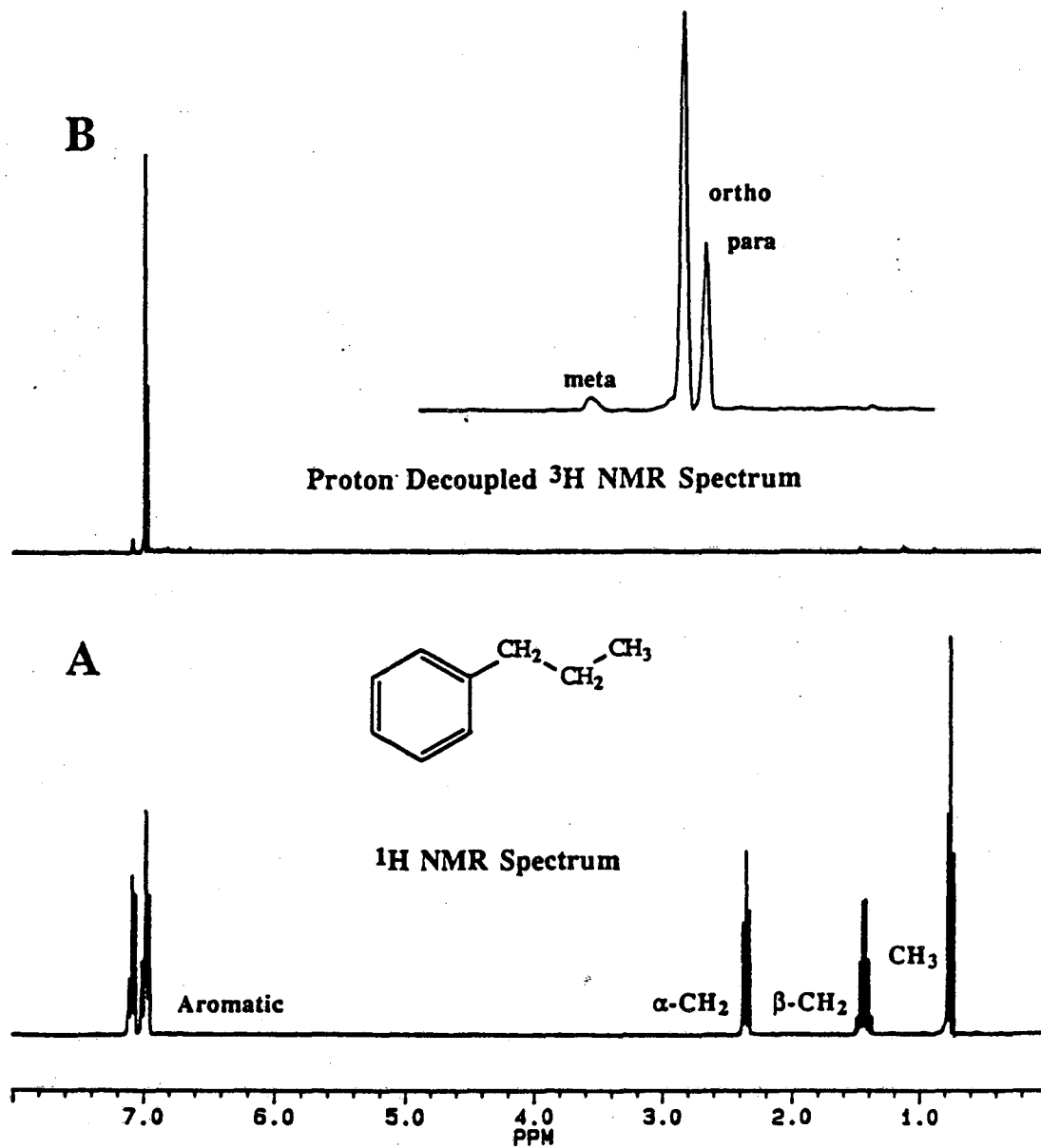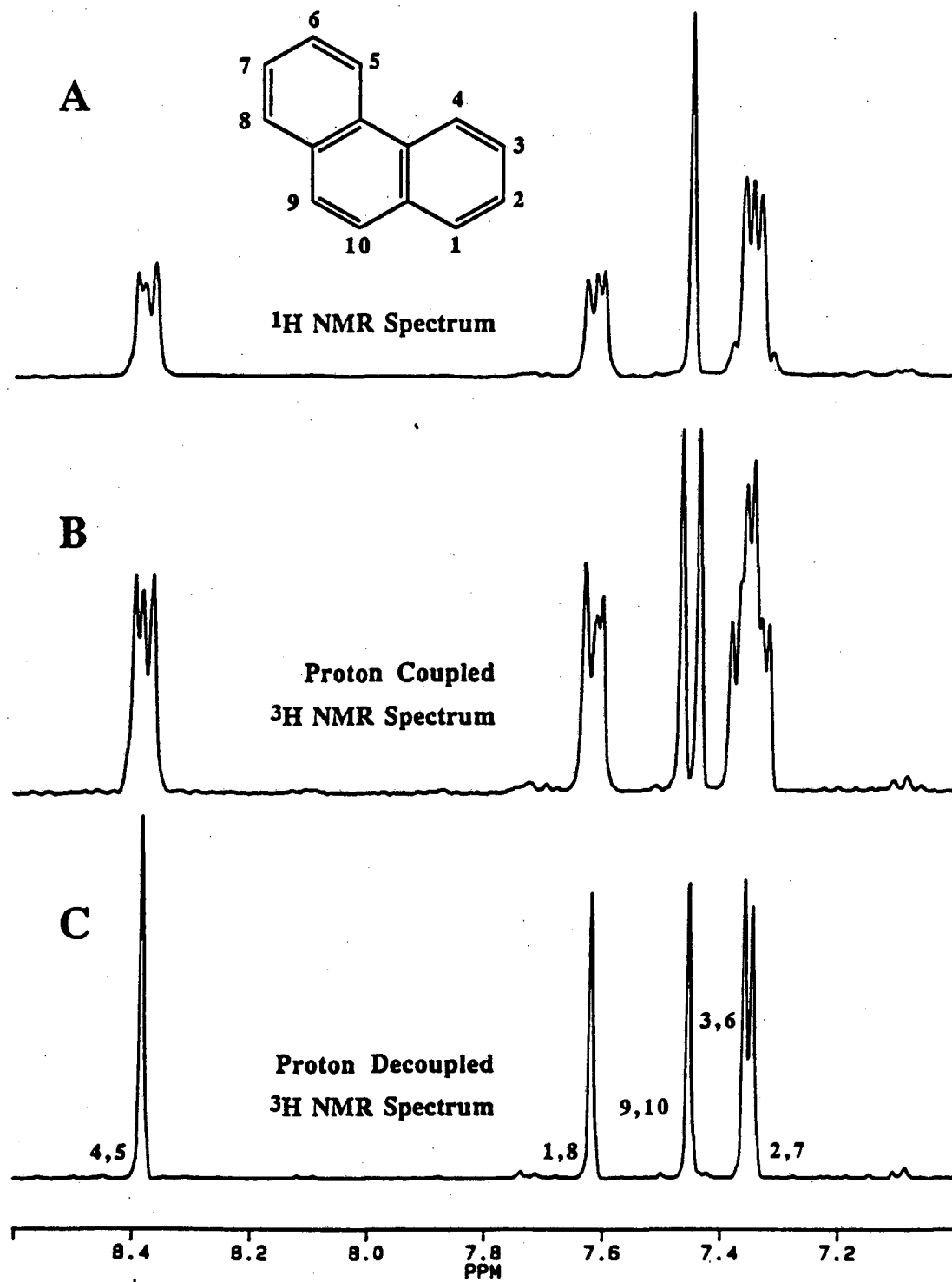7.0   6.0   5.0   4.0   3.0   2.0   1.0
              PPM

Figure 5. NMR spectra of Phenanthrene tritiated by the MDA labelling technique.

C.   Thermal Atom Labelling

Another variation on the gas exposure technique involves allowing tritium atoms produced by atomization of tritium gas on a hot tungsten wire to impinge on a substrate. These thermal tritons have been reported[92,93] to label molecules at high specific activity and with retention of biological activity; however, details on the purification and radiochemical identification of the products were sparse.

The technique has recently been reinvestigated[94,95] with the substantial benefit of ^{3}H NMR analytical capability. Initially benzene and m-xylene were used as model aromatic substrates for labelling studies under a variety of conditions, a tungsten wire was used for excitation, and volatile products were analysed by gas chromatography in addition to ^{3}H NMR spectroscopy. Reaction of benzene (frozen, -196°C)[94] showed predominant saturation with 93% of the labelled products being cyclohexane (Figure 6(b)) - labelled benzene was present in trace amounts only. The standard mass markers for possible products are shown in Figure 6(a). In contrast, when the benzene was held at -60°C, (and the surface of the substrate was considered mobile) the relative amount of saturation decreased, but cyclohexane was still the major product with appreciable amounts of hexane and polymers (Figure 6(c)). Labelled benzene accounted for 20% of the incorporated tritium; 1,4 cyclohexadiene was formed, but 1,3 cyclohexadiene was absent. NMR analysis of the reaction products (Figure 7) clearly supports the gas chromatographic data.

Under optimal conditions, the ratio of products could be modified to the point where the fraction of tritiated benzene was 60% of the product radioactivity.[95] Substituting platinum wire for tungsten further increased tritium exchange in benzene to 70%, with only small amounts of tritiated cyclohexane formed. Despite these promising improvements, specific radioactivity levels obtained under all conditions were several orders of magnitude below those previously reported.[92,93]

Table 6

Thermal Atom Labelling of Benzene and m-Xylene in the Presence of Various Metal Wires

Metal	% Incorporation into Benzene	mCi	% Incorporation into m-Xylene	mCi
Palladium	-	-	55	2.3
Nickel	-	-	55	1.0
Rhodium	51	1.3	45	0.1
Iridium	56	0.6	45	2.2
Tungsten	58	5.7	46	25.0
Titanium	-	-	32	5.0
Platinum	72	0.4	<5	0.5

Figure 6. Radio-gas chromatography traces of products of thermal atom labelling of benzene: (A). Mass markers for possible products are (1) hexane, (2) contaminant, (3) cyclohexane, (4) cyclohexene, (5) 1,3 cyclohexadiene, (6) 1,4 cyclohexadiene, (7) benzene, and (8) bicyclohexyl. (B). Radioactivity trace, labelling at 77K. (C). Radioactivity trace, labelling at 213K.
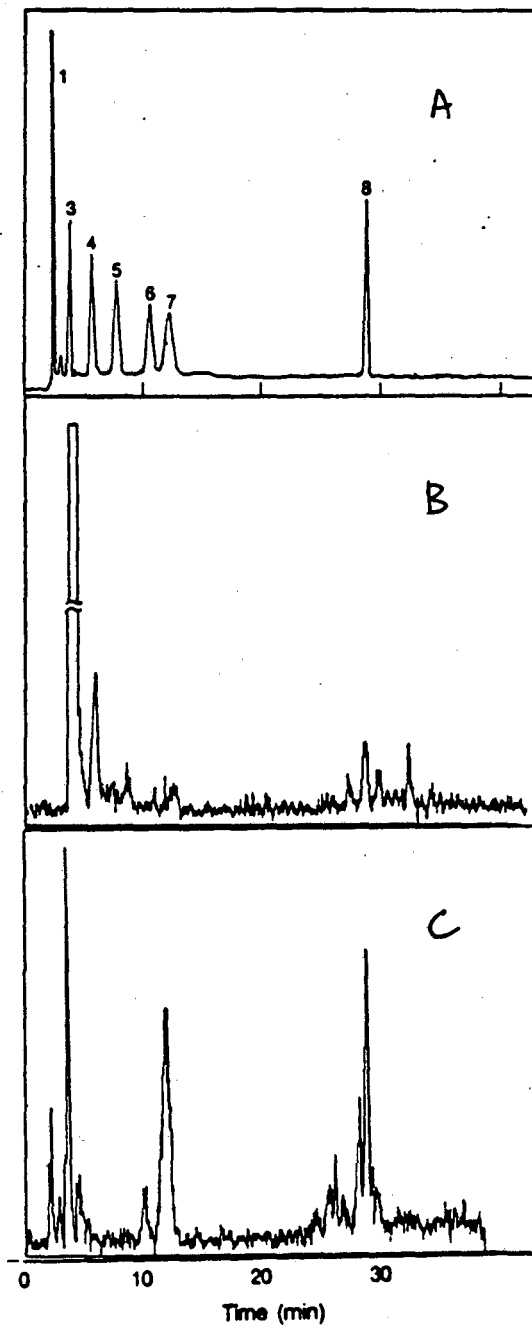
Figure 7. NMR spectra of the labelled products for which the gas chromatography analyses were shown in Figure 6.
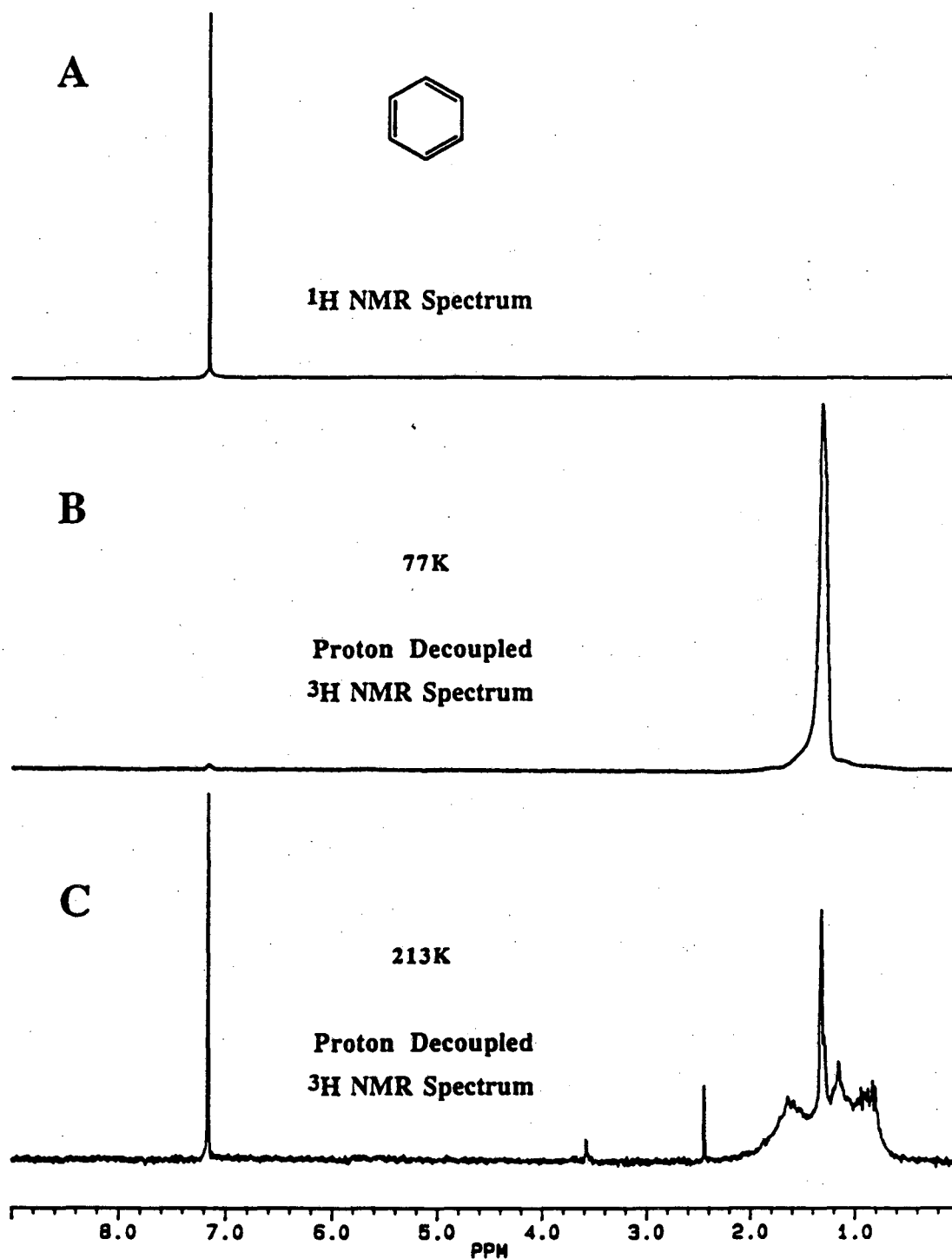
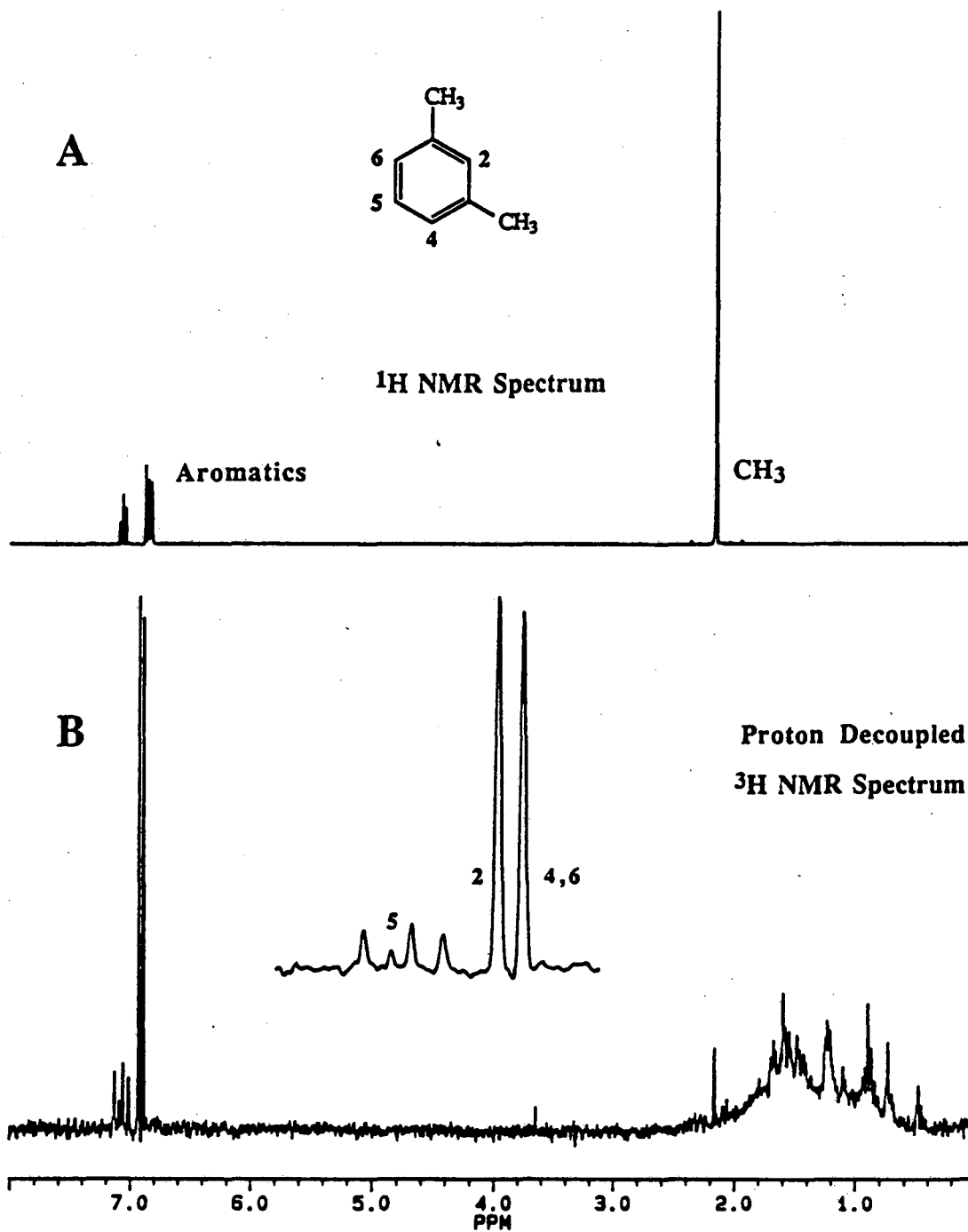**Figure 8.** NMR spectra of m-Xylene tritiated by exposure to thermal atoms.

Figure 9. NMR spectra of Phenylalanine-Leucine labelled by exposure to thermal atoms.

A

HDO

^{1}H NMR Spectrum

B

Proton Decoupled
^{3}H NMR Spectrum
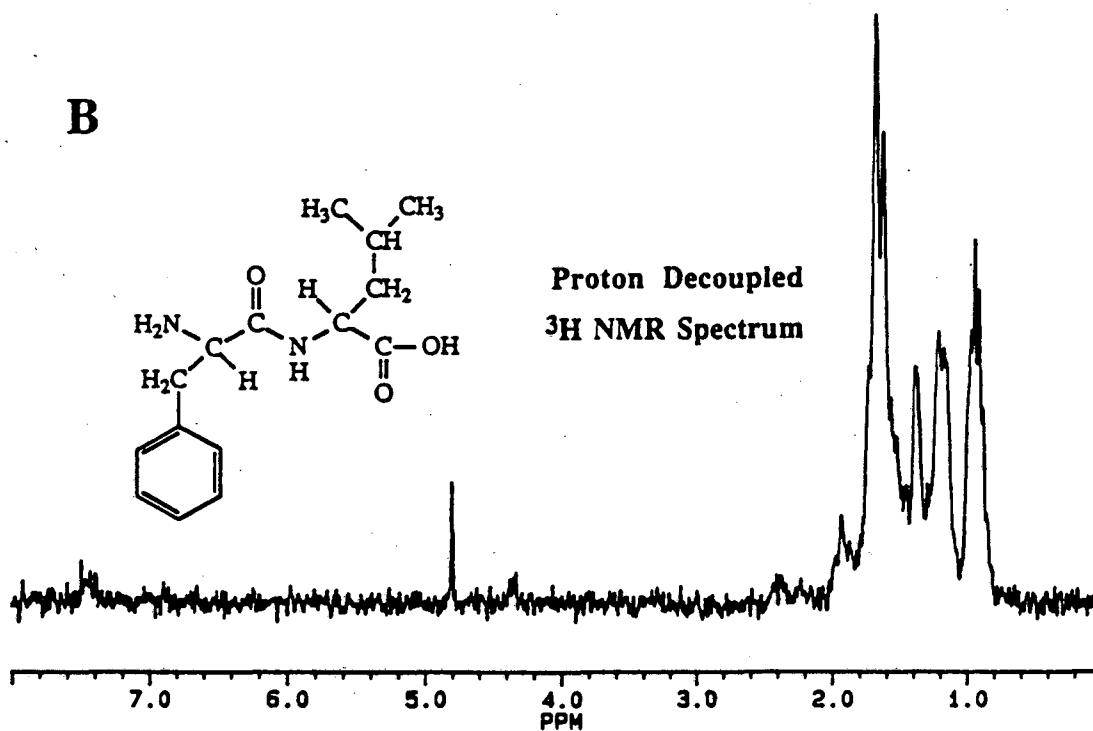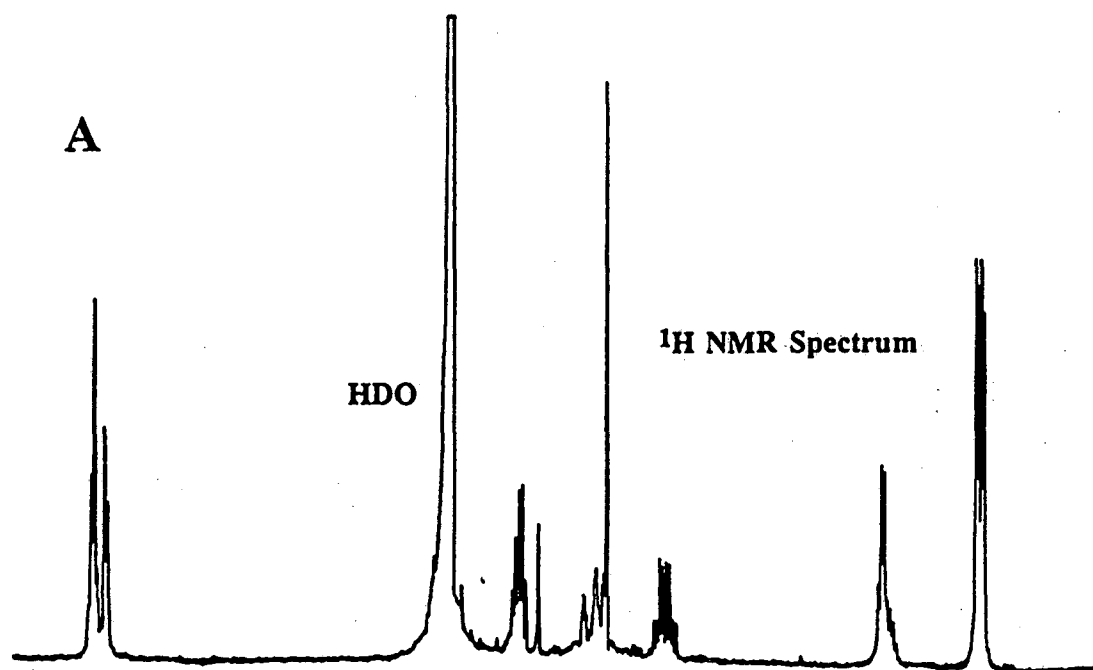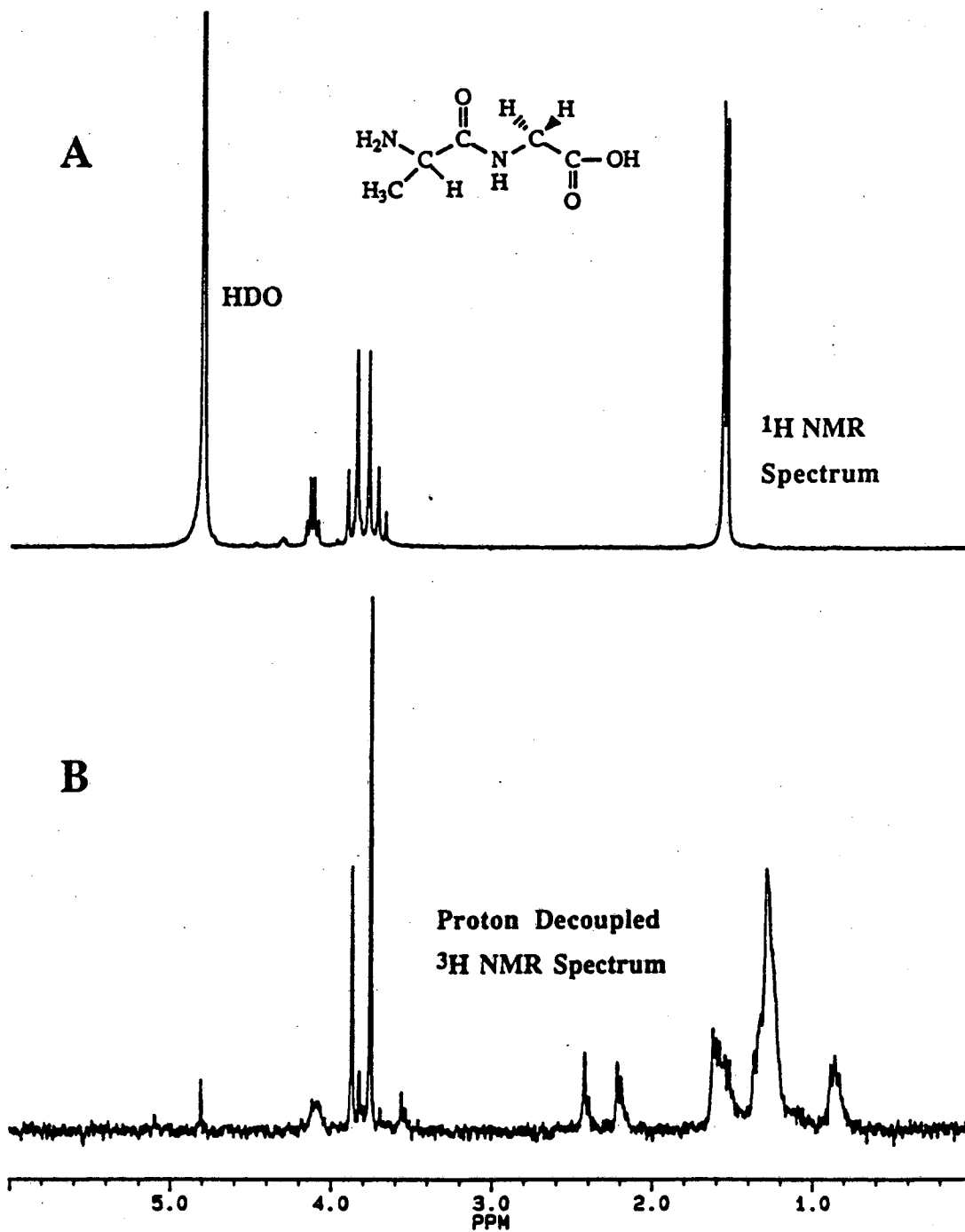
7.0 6.0 5.0 4.0 3.0 2.0 1.0
PPM

Figure 10. NMR spectra of Alanyl-Glycine tritiated by exposure to thermal atoms.

Comparison of the labelling of benzene and m-xylene over a variety of wires (Table 6) showed very different reactivities for the two substrates. ^3H NMR analysis of the product formed with the tungsten wire and m-xylene as substrate is given in Figure 8, along with the ^1H NMR spectrum of the sample. Appreciable tritium is associated with m-xylene (some in the methyl position), and it appears there are also small amounts of other xylenes which are labelled in aromatic positions (6 peaks; 3-m-xylene; 1-p-xylene; 2-o-xylene). However, the majority of tritiated materials again appear to be saturation products (1-2ppm).

Since the main objective of radiation-induced or gas exposure techniques such as thermal atom labelling is to tritiate materials not readily accessible by other techniques, two dipeptides were studied.[94,95] The dipeptide solids were adsorbed onto filter papers, and exposed to the thermal tritons produced on a hot tungsten wire. Phenylalanyl leucine (Figure 9) showed a large amount of ring saturation (0.8-2ppm), but little exchange. Similarly, the NMR spectra of labelled alanyl-glycine in D_2O (Figure 10) suggested that only a little of the tritium was associated with the parent compound.

In summary, the major effect of thermal atom irradiation on solid aromatic centres is saturation. Even under conditions where the surface of the substrate is mobile and appreciable exchange is observed, the resultant mixture contains highly labelled but saturated products. Thus, when dealing with very large substrates, rigorous criteria of radiochemical purity and structural analysis are essential.

V. METAL CATALYSIS

A.   Heterogeneous Metal Catalysed Hydrogen Isotope Exchange with HTO or Other Solvents

More substrates have been labelled by exchange over metal catalysts than by any other exchange technique. Consequently, the technique is also well represented in the ^3H NMR literature. The general technique is well reviewed,[96,97] and the purpose here is to show the detail available from the use of ^3H NMR spectroscopy.

The technique was first reported in the 1930's, and applied to a broad range of substrates following the seminal work of Garnett.[98] The mechanism of exchange with water as the isotope source has been thoroughly investigated,[96] and, although other metals have been studied, platinum appears to be the most active. The great majority of reported work is with reduced platinum oxide (Adams catalyst, or Platinum black). As with the other exchange techniques, this work was completed before the availability of the ^3H NMR technique. Recent investigations have served to confirm the majority of the earlier mechanistic proposals,[99] and to clarify a number of other points.[102,103] The mechanism of exchange in aromatic centres is thought to involve reversible dissociation of a π-complex to form a σ-bond, and desorption with incorporation of isotope (Figure 11). Sterically hindered positions will not form the complexes as readily as unhindered, and are therefore less labelled. This mechanism is supported by all the published ^3H NMR data, and the differences in steric hindrance are easily observed even amongst a series of halobenzenes,[99] as shown in Table 7. Protons adjacent to still larger substituents such as the ortho protons of t-butylbenzene, or in doubly hindered positions (as in m-xylene) show almost no exchange.

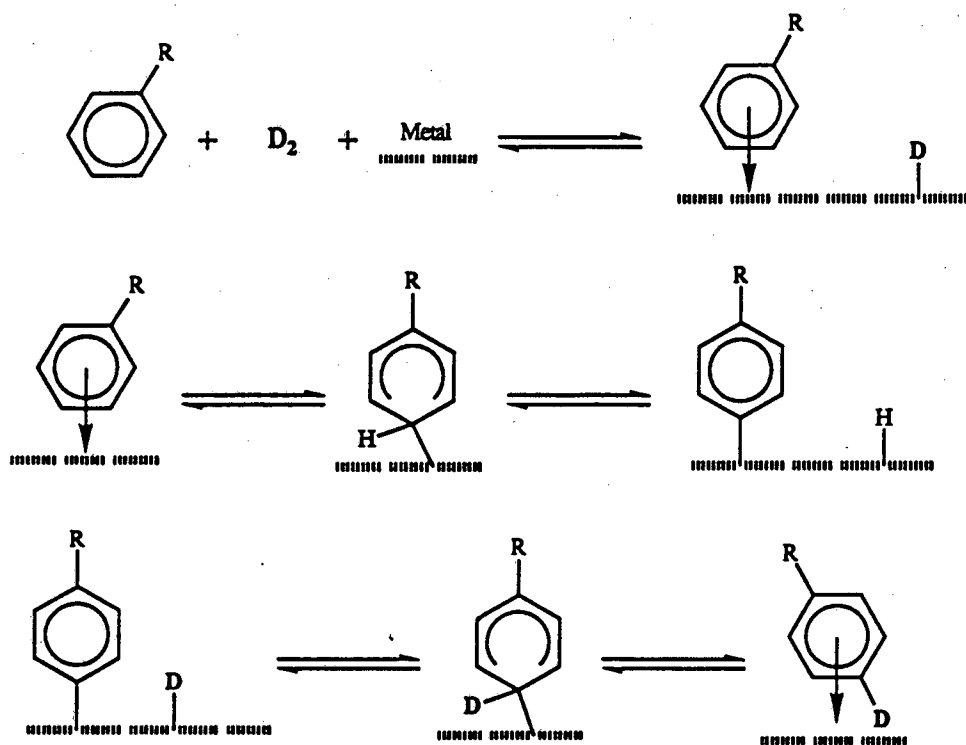**Figure 11.** Dissociative π-complex mechanism of exchange of aromatic substrates.

Figure 12. π-Allylic mechanism of exchange of alkylaromatic substrates.
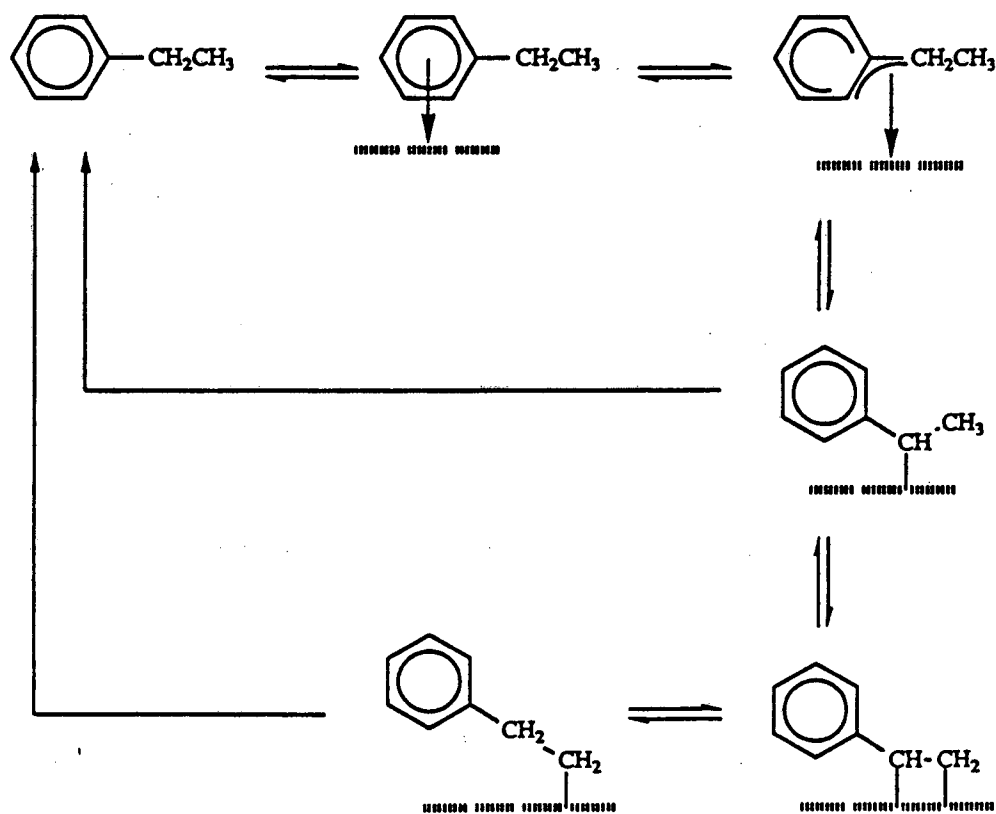
Table 7

Heterogeneous Metal Catalysed Exchange with HTO

Compound	Catalyst	Orientation, %	Ref.
Benzene	Pt	-	101
Toluene	Pt	o-19.6; m-30; p-18; CH_3-33	99
m-Xylene	Pt	2-6; 4,6-16; 5-11; CH_3-66	47
Ethylbenzene	Raney Ni	CH_3-25; CH_2-75	74
n-Propylbenzene	X	α-CH_2-100	3
Fluorobenzene	Pt	o-16.6; m-58; p-25	99
Chlorobenzene	Pt	o-8; m-60; p-32	99
Naphthalene	X	α-49; β-51	3
1,4 Dimethylnaphthalene	Pt	2,3-14; 5,8-4; 6,7-39; CH_3-43	47
Phenanthrene	X	1,8-18; 2,7-21; 3,6-23; 4,5-19; 9,10-19	3
Triphenylene	X	1,4,5,8,9,12-59; 2,3,6,7,10,11-41	3
Pyrene	X	1,3,6,8-36; 2,7-29; 4,5,9,10-35	3
Anthracene	X	1,4,5,8-44; 2,3,6,7-38; 9,10-18	3
Benzo[e]pyrene	X	1,8-19; 2,7-14.5; 3,6-20; 4,5-14.5 9,12-18; 10,11-14	3
7,12 Dimethylbenz[a]-anthracene	PtO$_2$	7-CH_3-6; 12-CH$_3$-1.5; 9,10-27.1 5,3,2-43; 4-17.6; 8-4.9; 6,11,1<1	100
11-Methyl-15,16-dihydro-cyclopenta[a]phen-anthren-17-one	Pt	16-CH_2-19; 11-CH_3-23.1; 15-CH_2-14.8 2,3-17.4; 12-1.9; 6-8.7; 7-3.2 4-11.9; 1<1	104
5-Hydroxytryptamine Creatinine sulfate	X	2-19; 4-21; 6-22; 7-22; CH_2N-16	41
2'-Deoxyadenosine	X	2-15; 8-85	3
Propanolol hydrochloride	Pt	2-31; 3-10; 4-13; 6,7-46	42
iso-Quinoline	Pt	1-41.2; 3-45; 4-2.6; 5-3.2; 6-2.8 7<0.1; 8-5.3	102
Lutidine	Pt	CH_3-76; 3,5-7; 4-17	103
Phenanthridine	Pt	1,10-2; 2-14; 3,8-34; 4-11; 6-10; 7-13 9-16	103
2-Picoline	Pt	CH_3-47; 3-13; 4-13; 5-15; 6-12	103
Pyridine	Pt	2,6-43.6; 3,5-22.6; 4-33.8	102
Pyridoxine hydrochloride	X	CH_3-40; 4-CH_2OH-4.9; 6-55.1	3
Nicotine D-bitartrate	X	2-11.6; 4-8.3; 5-12; 6-11; 2'-12.4 3'a-5.1; 3'b-3.2; 4'a-6.5; 4'b-1.9 5'a-18.8; 5'b-9.2	3
Tryptamine hydrochloride	X	2-56.5; ring-18.5 and 24.9	3
L-Phenylalanine	Pt	β-CH_2-26; α-CH-2; o-27; m-29; p-16	106
L-Proline	X	2-29; 3α-6; 4α-5; 4β-5; 5α-28; 5β-26	107
D,L-Threonine	X	2-71.6; 3-28.4	3
L-Tryptophan	Pt	sidechain-8; 2-7; ring-85	105
L-Aspartic Acid	X	2-39.3; 3-25.2; 3-35.5	41
Shale oils	Raney Ni	General	108

X - Catalyst not given in publication, but most probably reduced PtO$_2$.

Alkanes exchange slowly relative to aromatic centres,[96] and the mechanism is thought to involve direct σ-bond formation. The alkyl groups of alkyl aromatics are also labelled by metal-catalysed exchange, and this exchange is both rapid and has a distinctive pattern. Initial adsorption is thought to occur by π-complexation, before formation of a π-allyl complex and an alkyl metal-

carbon bond, as shown in Figure 12. Further exchange in the side-chain, beyond the α-position, could occur as shown in the Figure. Two results (24, 25) illustrate this type of labelling:
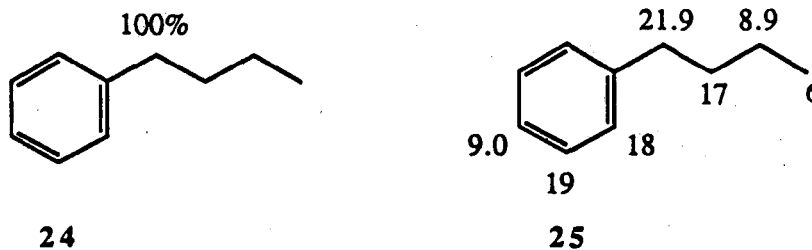

There is also considerable variation amongst the group VIII metals for aromatic vs alkyl labelling, as shown above for Raney nickel (24) and platinum (25) exchange of n-butylbenzene.

One of the great advantages of metal-catalysed exchange over other techniques is its applicability to a broad range of substrates. Notably these include compounds containing hetero-atoms, which are often poorly labelled by other methods. A selection of results for nitrogen-containing substrates, polycyclic aromatic hydrocarbons, amino acids and drugs are included in Table 7. Several other features of metal-catalysed exchange are borne out in the orientations derived from ^{3}H NMR study of the labelled products.

Comparison of the two results for 3-picoline (26, 27) are very informative.[102] It is clear that primary adsorption involves the nitrogen atom, and most exchange is adjacent to this atom. Steric hindrance by the methyl group obviously affects exchange adjacent to that group. Even at higher temperatures (130 vs 60°C, 27 vs 26) these effects are still apparent, although somewhat masked by the loss of specificity due to more vigorous exchange conditions. In general, orientational effects may be obscured after long reaction times or at high temperatures, but the sensitivity of the ^{3}H NMR technique allows analysis very early in the exchange cycle (i.e. ≤ 20% incorporation of tritium), when initial conditions exist and only low levels of isotope are incorporated.
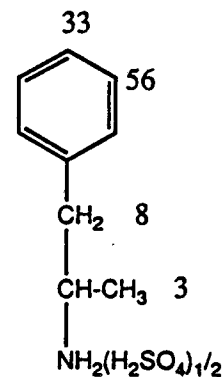

The analysis of 7-methylbenz(a)anthracene (28) is a good example of the variation in steric constraints over all the positions of one molecule. Amino acids and many drugs are readily labelled, and favour the least hindered, aromatic positions over aliphatic hydrogens for exchange (29, 30).[42,105]

28

CH_3
19%

29

F
β CH_2 11
α CH 9
H_2N C=O
OH

30

33
56
CH_2 8
$CH-CH_3$ 3
$NH_2(H_2SO_4)_{1/2}$
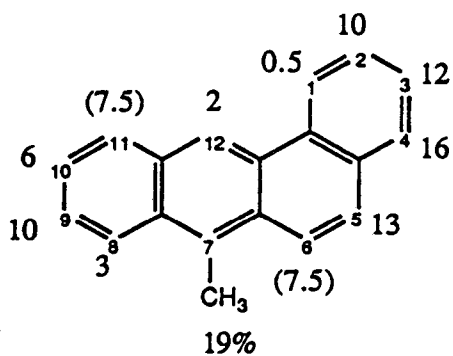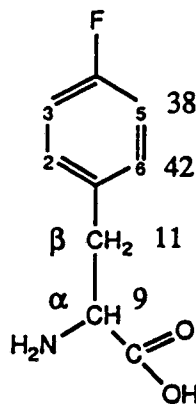
Limitations of the heterogeneous metal HTO labelling techniques include the inability to label nitro or iodo-containing substrates, racemisation of many optically active compounds and disproportionation of some reactants.[96]
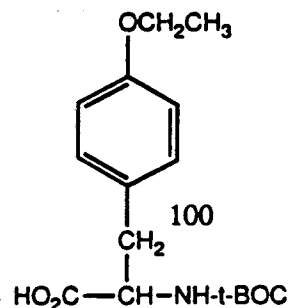
B.   Heterogeneous Metal-Catalysed Exchange with T_2

This process was first published as a catalysed Wilzbach experiment.[109,110] The technique has several advantages, notably the possibility of introducing large amounts of tritium from a carrier free isotope source (T_2). However, this is also a disadvantage, since many substrates are catalytically hydrogenated under the reaction conditions. The fact that the use of a tritiated solvent as isotope source precludes these unwanted reactions has far outweighed the fact that extremely high specific activities are difficult to attain with the HTO method.

Evans et al [111] pioneered a variation of the tritium gas technique which yields high incorporation, specific labelling and very little degradation or hydrogenation. Briefly, the simple technique involves stirring a buffered solution (pH 7) of the substrate in the presence of an atmosphere of tritium gas and a supported metal catalyst for several hours. The method has been used to good effect in labelling a wide variety of substrates including purines, purine nucleosides and nucleotides, aromatic amines and amino acids, carbohydrates and steroids. A selection of results are given in Table 8, and the remarkable feature of the results is the specificity of the exchange, as illustrated by the following orientation data for glucose (31) and a blocked amino acid (32).[112]

31

CH_2 OH
OH
T 100
OH OH

32

OCH_2CH_3
CH_2 100
$HO_2C—CH—NH-t-BOC$
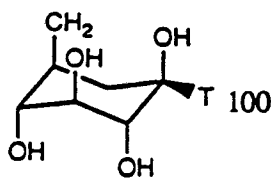
Table 8

Heterogeneous Metal Catalysed Exchange with T_2

Compound	Catalyst	Orientation, %	Ref.
Methotrexate	Pd/CaCO$_3$	7-100	116
Adenine β-D-arabinoside	PdO/BaSO$_4$	2-48; 8-52	3
Adenosine hydrochloride	PdO/BaSO$_4$	5'-38,22; 2-4; 8-36	41
Adenosine	PdO/BaSO$_4$	2-22; 8-78	3
Adenosine cyclic 5' monophosphate	PdO/BaSO$_4$	8-100	3
Adenosine 5' triphosphate	PdO/BaSO$_4$	5'-62.2; 2-10.6; 8-27.2	3
Caffeine	PdO/BaSO$_4$	8-100	41
β,γ-Methylene ATP	-	2-73.8; 8-26.2	3
Theophylline	PdO/BaSO$_4$	8-100	41
Estradiol 17-cyclopentyl ether	Pd/C	6β-33; 7α-1; 9α-66	72
Estriol	Pd/C	6α-61; 9-39	117
Estriol	Pd/C	2-26.4; 4-19; 6α-31; 9-24	117
Estrone β-D-glucuronide	Pd/C	6α-59.2; 9-40.8	3
Estrone sulfate	Pd/C	6α-50; 9-50	117
2-Hydroxyestradiol	-	6α,β-58; 9-42	3
2-Hydroxyestrone	-	6-52.6; 9-42.7; 16-4.7	3
2-Amino-6,7-dihydroxyl 1,2,3,4 tetrahydronaphthalene (ADTN)	PdO/BaSO$_4$	1ax-26; 1eq-17; 3ax-6 3eq-4; 4-44; 5,8-3	41
2,4 Diamino pteridine-β-carboxylic acid	Pd/CaCO$_3$	7-100	116
Folic acid	Pd/CaCO$_3$	7-59; 9-CH$_2$-41	116
Pyridoxine hydrochloride	-	CH$_3$-63.4; 4-CH$_2$OH-22.4 5-CH$_2$OH-2; 6-12.2	3
Tyramine hydrochloride	PdO/BaSO$_4$	side-chain 2-100	3
5-mers			118
D-Fucose	PdO/BaSO$_4$	1α-34; 1β-66	3
L-Fucose	PdO/BaSO$_4$	1α-34.4; 1β-65.6	3
2-Deoxy-D-glucose	PdO/BaSO$_4$	1α-50; 1β-50	3
D-Galactose	PdO/BaSO$_4$	1α-34.3; 1β-65.7	3
D-Glucosamine	PdO/BaSO$_4$	1α-63; 1β-37	3
D-Glucose	PdO/BaSO$_4$	1α-37.5; 1β-62.5	119
D-Mannose	PdO/BaSO$_4$	1α-61; 1β-39	3
n-Hexylpropionate	Rh black	CH$_3$'s most labelled	5
n-Pentylpropionate	Rh black	CH$_3$'s most labelled	120
t-Boc-O-Ethyl-D-Tyrosine	Pd/BaSO$_4$	Benzyl-100	112
Toluene	Pt black	o<1; m-6.4; p-3; CH$_3$-90	66
n-Hexylbenzene	Pt black	o<1; m-20; p-10.1; α-CH$_2$-48 β-CH$_2$-15; γ-CH$_2$-3.2 δ,ε-CH$_2$'s-2.8; CH$_3$<1	66
iso-Propylbenzene	Pt black	o<1; m-40; p-19; CH-21 CH$_3$-20.4	66
n-Hexane	Pt black	CH$_3$-84; 2-CH$_2$-31.8; 3-CH$_2$-29.2	66
n-propane	Raney Ni	CH$_3$/CH$_2$ = 2.4	113
Dibenz[a,j]acridine	Pd/CaCO$_3$	H14	121
Dibenz[a,h]acridine	Pd/CaCO$_3$	H14	121
Dibenz[c,h]acridine	Pd/CaCO$_3$	H7	121
Thyroliberin (TRF)	Pd/Al$_2$O$_3$	C-2,C-5(1/6)	122
Triethylsilane	Raney Ni	SiH-37; CH$_2$-38; CH$_3$-25	114
Tetramethylsilane	Raney Ni	CH$_3$-100	114
Hexamethyldisiloxane	Raney Ni	CH$_3$-100	115
Chlorodimethylsilane	Raney Ni	SiH-57; CH$_3$-43	114

Figure 13. Proton decoupled tritium NMR spectra of Toluene labelled by exposure to tritium gas over reduced metal for five hours at room temperature.
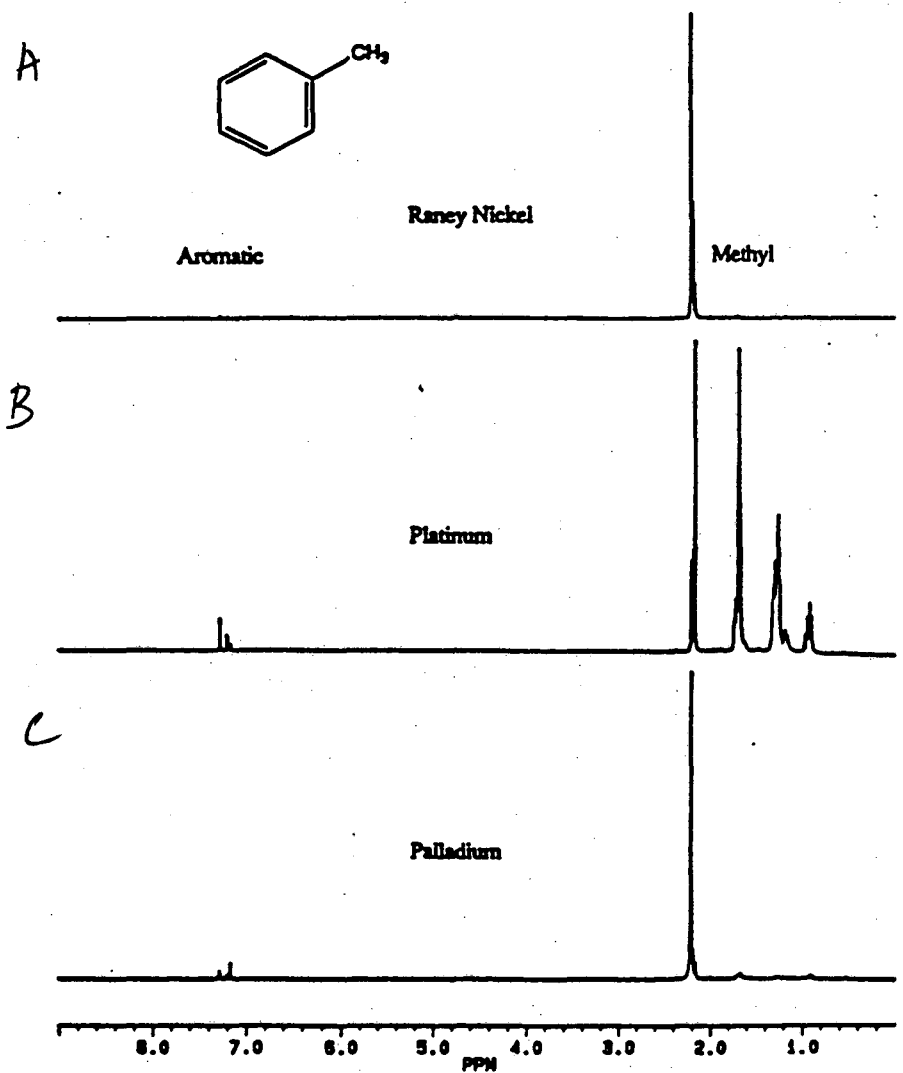
XBL 883-1021

Figure 14. Expanded views of the aliphatic (2.02-2.32ppm) and aromatic (7.06-7.36ppm) regions of the spectra in Figure 13.
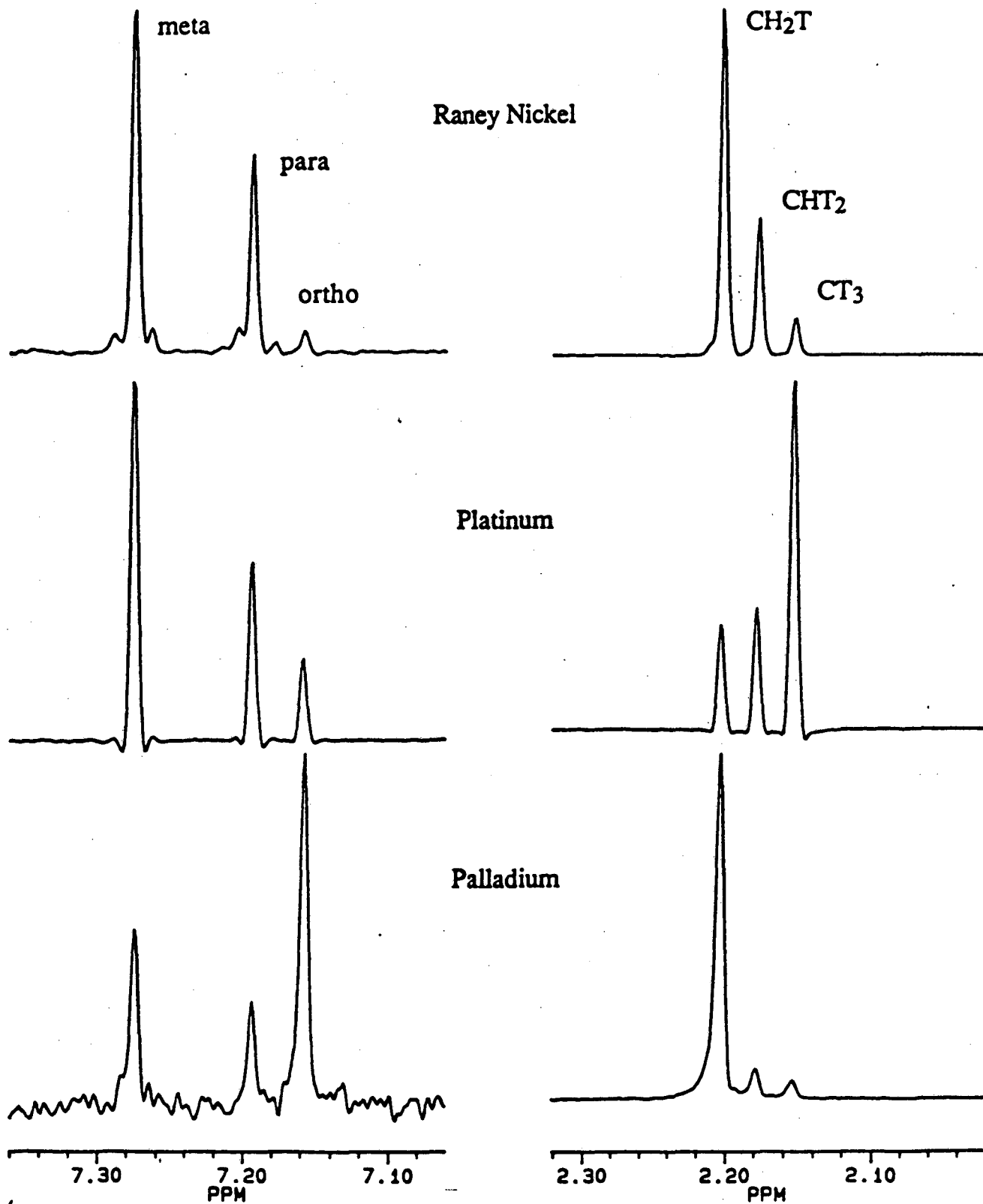
XBL 8711-4713

In studies of unsupported metal catalysts Raney nickel has been demonstrated as an effective catalyst for the labelling of alkanes,[113] aromatic compounds[66] and silanes.[114,115] The variation of labelling patterns between metals can be very marked with tritium gas as isotope source, as previously noted with HTO. However, in general aromatic centres are not the predominant site of labelling as noted in Pt/HTO exchange, and most metals tend to the high α-CH incorporation previously observed with Raney nickel as the catalyst and HTO as isotope source.

The exquisite detail provided by careful ^3H NMR analysis of reaction products is illustrated by the data in Figure 13. Toluene was labelled at room temperature over unsupported Pt, Pd and Ni, and the majority of the tritium was incorporated in the methyl groups in all three cases. Over platinum catalyst a large amount of hydrogenation also took place, yielding highly tritiated methyl cyclohexane (Figure 13B, 0.5-1.8ppm). The expanded methyl and aromatic regions of the spectra reveal additional information about the labelling processes under these reaction conditions (Figure 14). In particular, the multiply labelled methyl species would suggest that exchange is rapid over all catalysts relative to desorption. This is especially true in the case of platinum, where CT_2 and CT_3 labelled methyl groups are very abundant, and hydrogenation is also a major feature of the reaction. Analysis of the ring labelling patterns shows that slow exchange due to steric hindrance of the ortho position is obvious with platinum and nickel, but the toluene labelled over palladium has a large amount of ortho labelling. This effect was not observed when exchange was conducted at higher temperature and lower tritium pressure,[56,66] as given in Table 8.

These subtle differences, revealed only by ^3H NMR analyses, may suggest that there are several slightly different mechanisms of metal-catalysed exchange, and the predominant process (and therefore orientation) depends on exact conditions such as the particular metal, pH, isotope source and reaction temperature.

Recent work[123] has shown that nitrobenzene may be labelled in heterogeneous metal/T_2 systems, in contrast to the well-known lack of reactivity of this substrate in the comparable HTO experiment. Since the only obvious difference between the two cases is the presence of adsorbed OH on the metal surface in HTO exchange, it is not clear why the results differ. This phenomenon has been observed previously,[124] when the presence of water was found to influence the orientation of hexane exchange with D_2 over clean platinum surfaces.

In summary, metal/T_2 exchange procedures offer the opportunity for high specific activity products, and remarkable specificity in labelling, but have not been fully pursued.

C.   Homogeneous Metal Catalysed Exchange with HTO

The inability to label substrates such as nitrobenzene, naphthalene and acetophenone by heterogeneous exchange led to development of the homogeneous metal systems.[125,126] These catalytic systems were first proposed with tetrachloroplatinate as the metal salt, with acetic acid present to ensure a single phase for the aqueous isotope source, catalyst and organic substrate. Facile exchange was observed at 80°C, and the orientations of labelling were very similar to those from heterogeneous metal techniques.

Table 9

Homogeneous Tetrachloroplatinate Catalysed Exchange with HTO

Compound	Catalyst	Orientation, %	Ref.
1,3 Dinitronaphthalene	K_2PtCl_4	$C5$-10; $C6$-45; $C7$-45	77
Toluene	Na_2PtCl_4	$o<0.1$; m-22.8; p-23.0; CH_3-34.2	50
Ethylbenzene	Na_2PtCl_4	$o<0.1$; m-61.2; p-38.8; $alkyl<0.1$	50
n-Propylbenzene	Na_2PtCl_4	$o<0.1$; m-55.4; p-27.3; α-4.4; β-3.0; CH_3-10.2	50
n-Butylbenzene	Na_2PtCl_4	$o<0.1$; m-57.0; p-29.9; $CH_2<0.1$; CH_3-12.9	50
n-Hexylbenzene	Na_2PtCl_4	$o<0.1$; m-61.2; p-30.2; α-$CH_2<0.1$; $C2$-$6<0.1$; CH_3-8.7	50
n-Heptylbenzene	Na_2PtCl_4	$o<0.1$; m-57.0; p-27.1; α-CH_2-5.4; CH_2-10.0; CH_3-8.7	50
iso-Propylbenzene	Na_2PtCl_4	$o<0.1$; m-59.0; p-29.9; $CH<0.1$; CH_3-10.8	50
iso-Butylbenzene	Na_2PtCl_4	$o<0.1$; m-59.2; p-32.3; $CH_2<0.1$; CH-0.1 CH_3-8.4	50
sec-Butylbenzene	Na_2PtCl_4	$o<0.1$; m-55.2; p-30.7; $CH<0.1$; $CH_2<0.1$ α-CH_3-4.2; γ-CH_3-9.6	50
t-Butylbenzene	Na_2PtCl_4	$o<0.1$; m-62.2; p-37.9; $CH_3<0.1$	50

A selection of results using tetrachloroplatinate catalyst are given in Table 9. There are several features of the orientations which should be stressed, and are shown in the Figures below:[50]
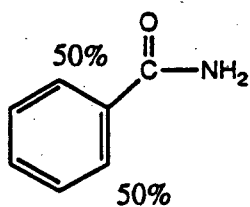

33   **34**   **35**
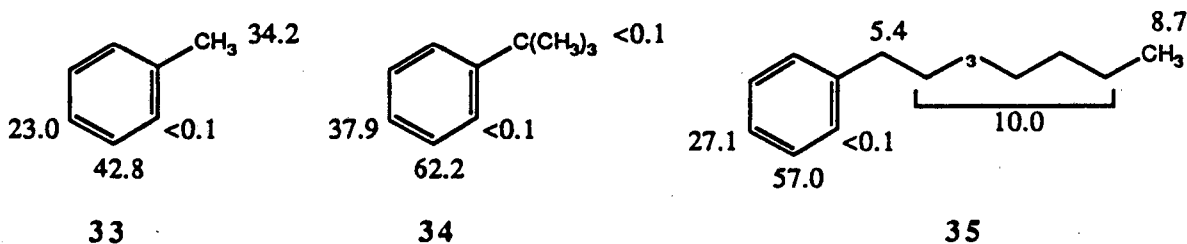
Firstly (33), ortho deactivation appears more pronounced than in the case of heterogeneous catalysis; it would seem more difficult for the substrate to complex with the multiply substituted metal atom in homogeneous catalysis, than with a metal surface as in the heterogeneous catalysis case. Secondly, bulky alkyl groups cause some deactivation of meta positions (as well as ortho, of course) in comparison to para labelling (34). The labelling of long-chain alkylbenzenes shows a curious pattern of alkyl labelling, where the terminal (CH_3) positions appear to be as well tritiated as the α-CH_2 hydrogens (35). To the everlasting credit of the authors[127] this was first detected by difference ^1H NMR spectroscopy. It is a small effect, but is clearly shown by ^3H NMR spectroscopic studies, and close analysis of the alkylbenzene results in Table 9 suggests that it may be observed for all chain-lengths greater than 3-carbons, or with a reasonable amount of chain branching. The phenomenon was explained[128] in terms of a "terminal abstraction π-complex" (TAPC) mechanism which requires initial complexation through the aromatic centre and curling around of the chain for exchange in remote positions. Since alkanes also exchange under the reaction conditions,[129] it's entirely possible that the result is caused by direct competition of
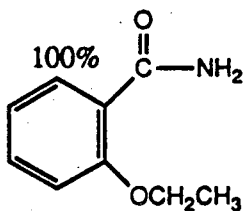
35

aromatic and alkyl complexation. One of the major advantages of homogeneous catalysis over heterogeneous is illustrated by the labelling of 1,3 dinitronaphthalene.[77]

Other metal salts have been shown to catalyse exchange under homogeneous conditions, and iridium[130] and rhodium[131] were used to label both alkylbenzenes and alkanes. Generally, salts other than platinum require more stringent conditions, especially for alkane labelling. These conditions can often cause metal precipitation, at which point the exchange observed may be heterogeneously catalysed, or be due to the acid present. A selection of results for Ir^{3+} as catalyst are given in Table 10.[50] Several features are immediately clear: comparison of the two toluene results reveals that orientational differences may be obscured after long exchange times. Survey of the results shows that alkyl exchange was never observed, in contrast to the results with tetrachloroplatinate as catalyst. As an extension of this, one might expect that alkane exchange would be slow, and this is the case.[50] As reported for heterogeneous platinum catalysis,[99] the labelling of halobenzenes shows the effect of steric hindrance of the halogen eg. the larger the halogen, the less ortho labelling observed.
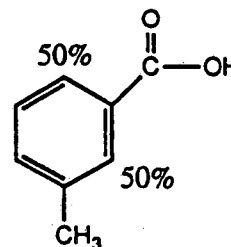
Table 11 lists a number of homogeneous labelling results with $RhCl_3$[47,132] and $(Ph_3P)_3RuCl_2$[133] as catalysts. The orientation of exchange in aromatic rings with $RhCl_3$ as catalyst is markedly different from other homogeneous metal systems,[47,132] and a mechanism has been proposed to explain this difference.[134,135] In any case, advantage has been taken of the phenomenon to yield very specifically labelled benzamides and benzoic acids,[132] as shown below (36-38):

A series of primary and secondary alcohols have been specifically tritiated by the application of $(Ph_3P)_3RuCl_2$ as catalyst.[133] The specificity of the labelling is excellent for primary alcohols, but some specificity loss occurs for secondary alcohol substrates.

Homogeneous metal catalysed exchange systems offer several advantages over the related heterogeneous technique. Nitro and other substrates which poison heterogeneous systems are readily tritiated. Most substrates are soluble in the solvent systems used for homogeneous catalysts, and so there is no fear of extra solvents poisoning exchange or competing with the substrate. Disadvantages include the difficulty of retrieving labelled products, and the mid-to-low specific activities attainable, as a result of the proton rich solvent systems used in homogeneous exchange.

Table 10

Homogeneous Iridium Catalysed Exchange with HTO

Compound	Catalyst	Orientation, %	Ref.
Toluene[a]	Na_3IrCl_6	o-18.8; m-43.0; p-38.3; $alkyl$<0.1	50
Toluene[b]	Na_3IrCl_6	o-23.8; m-47.4; p-28.9; $alkyl$<0.1	50
iso-Propylbenzene	Na_3IrCl_6	o-24.0; m-35.0; p-41.0; $alkyl$<0.1	50
iso-Butylbenzene	Na_3IrCl_6	o-18.6; m-48.8; p-32.6; $alkyl$<0.1	50
s-Butylbenzene	Na_3IrCl_6	o-10.0; m-54.4; p-35.7; $alkyl$<0.1	50
t-Butylbenzene	Na_3IrCl_6	o-18.0; m-52.6; p-29.3; $alkyl$<0.1	50
Cyclohexylbenzene	Na_3IrCl_6	o-7.8; m-56.4; p-35.7; $alkyl$<0.1	50
o-Xylene	Na_3IrCl_6	$3,6$-41.2; $4,5$-58.8; CH_3<0.1	50
m-Xylene	Na_3IrCl_6	2-21.4; $4,6$-25.2; 5-28.2; CH_3<0.1	50
1,2,4 Trimethylbenzene	Na_3IrCl_6	3-84.6; 5-8.9; 6-6.5; CH_3<0.1	50
Fluorobenzene	Na_3IrCl_6	o-20.4%; m-39.0; p-40.7	50
Chlorobenzene	Na_3IrCl_6	o-13.8%; m-41.4; p-44.9	50
Bromobenzene	Na_3IrCl_6	o-6.6%; m-63.0; p-30.4	50
Naphthalene	Na_3IrCl_6	α-20.8%; β-79.2	50
Biphenyl	Na_3IrCl_6	o-16.4; m-56.4; p-24.8	50

a. 8 hours - 16.1% Approach to Eqm.
b. 264 hours - 54.2% Approach to Eqm.

Table 12

Homogeneous Rhodium or Ruthenium Catalysed Exchange with HTO

Compound	Catalyst	Orientation, %	Ref.
iso-Propylbenzene	$RhCl_3$	$2,6$-47; 4-42; CH_3-10	47
1,3,5 Trimethylbenzene	$RhCl_3$	$2,4,6$-100; CH_3<1	47
Benzamide	$RhCl_3$	$2,6$-97	132
Benzoic acid	$RhCl_3$	$2,6$-99	132
2-Ethoxybenzamide	$RhCl_3$	6-96	132
2-Hydroxybenzamide	$RhCl_3$	6-66; $3,5$-34	132
2-Hydroxybenzoic acid	$RhCl_3$	6-9; $3,5$-91	132
4-Methoxybenzoic acid	$RhCl_3$	$2,6$-99	132
2-Methoxybenzoic acid	$RhCl_3$	6-98	132
3-Methylbenzoic acid	$RhCl_3$	$2,6$-98	132
4-Methylbenzoic acid	$RhCl_3$	$2,6$-99	132
4-Oxo-4H-chromene- 2-carboxylic acid	$RhCl_3$	3-97	132
Ethanol	$(Ph_3P)_3RuCl_2$	α-100	133
1-Heptanol	$(Ph_3P)_3RuCl_2$	α-86; β-14	133
3-Phenyl-1-propanol	$(Ph_3P)_3RuCl_2$	α-96; β-4	133
1-Octadecanol	$(Ph_3P)_3RuCl_2$	α-88; β-4; $other$-8	133
Benzyl alcohol	$(Ph_3P)_3RuCl_2$	α-100	133
2-Pentanol	$(Ph_3P)_3RuCl_2$	α-CH-14; 1-CH_3-48; 3-CH_2-38	133
2-Decanol	$(Ph_3P)_3RuCl_2$	α-CH-18; 1-CH_3-49; 3-CH_2-34	133
2-Hexadecanol	$(Ph_3P)_3RuCl_2$	α-CH-20; 1-CH_3-47; 3-CH_2-33	133

D.   Homogeneous Metal Catalysed Exchange with T_2

There are no reported examples of this type of exchange, although the first report of homogeneous alkane exchange[129] with D_2O also discussed attempts to exchange alkanes with D_2. The over-riding reason for the lack of activity in this area is probably due to reports that metal complexes will be reduced to the heterogeneous metal by the presence of hydrogen (tritium) gas.[97] This may be the case for some systems, but the success of Wilkinson's catalyst for homogeneous hydrogenation reactions, and the recent reports of ^3H[136] and ^2H NMR studies[137] of hydrogen complexed by metals in homogeneous organometallic systems suggests that T_2 exchange should be possible. If realized, this eventuality would quickly remove the specific activity limitations imposed by the use of HTO as isotope source. It is also clear that development of a homogeneous tritium gas exchange technique will have to take account of such factors as the exchange of solvents, *etc.*

VI.   SUMMARY

Hydrogen isotope exchange techniques are many and varied, and can be exceptionally powerful. Extremely specific labelling may be obtained, as in the case of base systems or $Pd/BaSO_4$ catalysis. High incorporation may also be observed (up to 10's of Ci/mmole), but not always in the same case as specific labelling. The availability of extremely high specific activity HTO (max. 2650 Ci/g) would eliminate all the specific activity limitations of the catalytic systems discussed here. However, this reagent should be treated with caution since the LD_{50} of HTO is reported to be approximately 1Ci/kg in man,[138] and 70Ci of T_2O is only 27μL (70kg=157lb man).

Exchange techniques offer the ability to label a substrate without any prior or subsequent chemical synthesis, and with complicated biological molecules this is often essential. In a similar way, ^3H NMR spectroscopy[3] offers the opportunity to non-destructively assay the results of labelling experiments on ever-smaller quantities of radiochemical. Although the sensitivity of the NMR technique will never rival that of liquid scintillation counting, modern high field spectrometers allow the observation of μCi quantities of tritium.

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