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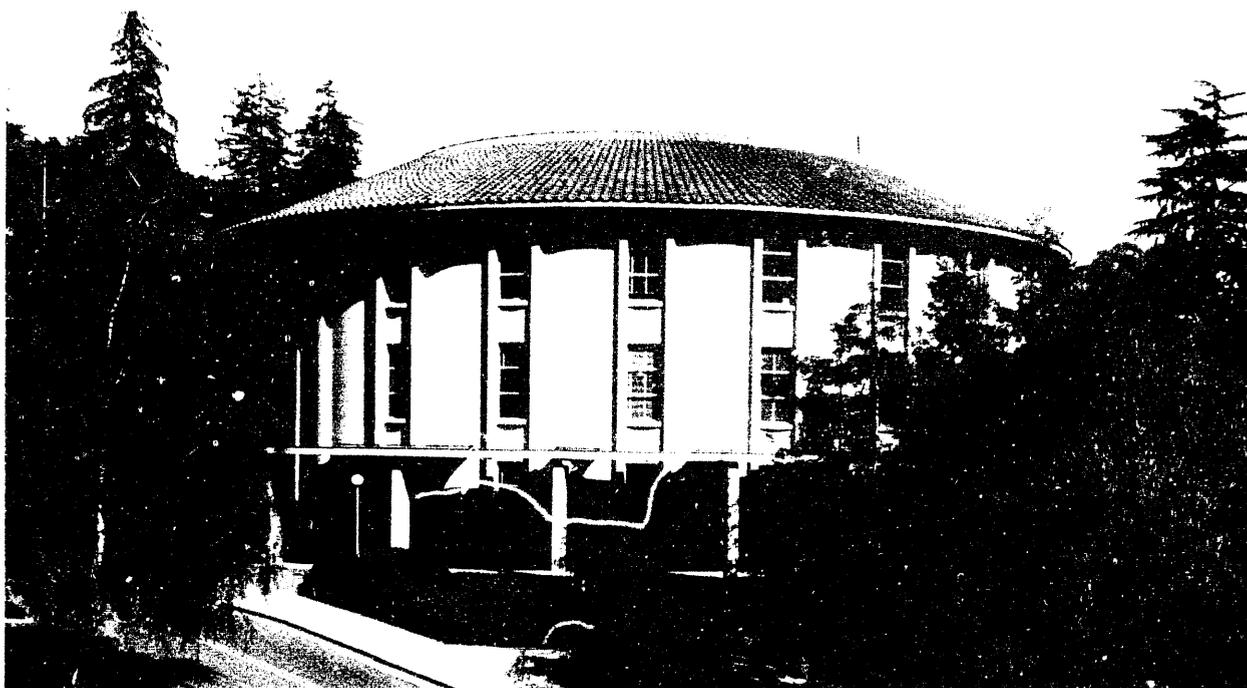
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### Tritiation Methods and Tritium NMR Spectroscopy

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## Tritiation Methods and Tritium NMR Spectroscopy

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### Abstract

The diversity of pharmaceuticals required for research and clinical studies is driving the constant development of new or refined tritiation techniques. The position of the National Tritium Labelling Facility (NTLF) as a site where numerous groups collaborate or visit to label their own substrates means that the techniques used there are often pushed to the limits, and the pressure to improve procedures is great. We are also interested in proton transfer methods which have not been used to any great extent in tritiation chemistry, but which are reasonably commonplace in general organic chemistry. In all of these studies NMR spectroscopy, and tritium NMR spectroscopy in particular, is a valuable tool for the understanding of mechanisms and the quality control of products.

### 1. INTRODUCTION

There are a number of widely accepted methods for tritiation of organic compounds [1], generally falling into the categories of exchange [2] or synthetic techniques [3]. Exchange methods usually rely on a catalyst to promote labelling, and are often limited by the achievable specific activity (S.A.) of the products. Nevertheless, advances in mechanistic knowledge and technical improvements in these methods continue and their value is clear [4].

High level tritiation reactions (with products in the range 1-100 Ci/mmol) rely on traditional chemical reactions such as hydrogenation, dehalogenation, methylation or hydride reduction. Although frequently used and widely studied in general chemistry applications, these approaches have limitations for the practicing radiochemist:

- a) Hydrogenation reactions are usually restricted to multiple C-C bond precursors, and many other functional groups (*e.g.* -NO<sub>2</sub>, halogens, -N<sub>3</sub>, other multiple bonds) are reducible under common reaction conditions.
- b) Dehalogenation is most effective for Aryl-I or Aryl-Br precursors, but suffers from the same drawbacks as hydrogenation.
- c) Methylation obviously requires the non-methyl precursor, and by definition is restricted to final products with a methyl in a position accessible by current methylation protocols.
- d) Hydride chemistry is well developed, but only tritiated NaBH<sub>4</sub> is commercially available, generally at 50-75% of the theoretical maximum S.A.

At the NTLF the limitations of available techniques are regularly encountered, and the needs or ideas of visitors lead to the pursuit of new and improved methods. The ability to conduct multinuclear NMR analyses of reagents and products is an essential facet of our approach, and in a tritiation reaction <sup>3</sup>H NMR analysis [5] is considered mandatory. Three ongoing projects offering improved substrate specificity are discussed.

## 2. HIGHLY TRITIATED WATER

There are immediate uses for T<sub>2</sub>O, CH<sub>3</sub>COOT or CF<sub>3</sub>COOT in tritiation reactions:

- Exchange with highly tritiated solvents under acid, base or metal-catalyzed conditions [6,7]
- Quenching of Grignard salts [1] or lithiated derivatives [8,9].

These reactions have advantages over the traditional methods, yielding regiospecifically labelled, high S.A. products without concomitant loss of functionality in the substrate.

The manufacture of T<sub>2</sub>O is not a project to be taken lightly, since tritiated water is readily taken up by man, and a small dose (30 μL, *ca.* 80 Ci) could be fatal. After some preliminary experiments with PdO we chose the combustion of PtO<sub>2</sub> [10] as the most convenient method for our purposes, since it is simple, rapid, and the total yield of water may be controlled through the reaction stoichiometry.

The following example will illustrate the generation of high level tritiated water, and give information regarding the species formed. Platinum oxide (Adams catalyst, PtO<sub>2</sub>.xH<sub>2</sub>O, 5 mg, 22 μmole) was added to bulb A of the reaction flask (Figure 1) and the apparatus was rigorously evacuated (with the PtO<sub>2</sub> heated by a heat gun). With Tap 1 closed, tritium gas was added to the vessel to 300 mm Hg pressure. On subsequently opening Tap 1 there was a bright flash followed by a rapid pressure decrease. When the pressure stabilized tap 3 was closed, bulb B was placed in liquid nitrogen and all areas of the vessel were heated with a heat gun. When transfer was complete, excess T<sub>2</sub> was removed and 1 mL of dry dioxane-d<sub>8</sub> injected into bulb B. After vigorous stirring an aliquot of the solution was removed for liquid scintillation counting (LSC) and NMR analysis.

<sup>3</sup>H (320 MHz) and <sup>1</sup>H (300 MHz) NMR analyses are shown in Figure 2. It is clear from the <sup>3</sup>H coupled <sup>1</sup>H spectrum in Figure 2A that both H<sub>2</sub>O and HTO species were present in the product mixture. <sup>3</sup>H decoupling collapsed the doublet ( $J_{HT}=7.3\pm 0.6$  Hz) due to <sup>1</sup>H-<sup>3</sup>H coupling in HTO to leave two singlets (Figure 2B) with intensity ratios H<sub>2</sub>O:HTO = 14.5:34.6. The change in <sup>1</sup>H chemical shift on introduction of tritium into the water molecule was  $\Delta\delta=14.6\pm 0.6$  Hz. Similarly the <sup>3</sup>H spectrum showed evidence of both HTO and T<sub>2</sub>O species (with T<sub>2</sub>O to higher field, Figure 2C). Proton decoupling collapsed the TH coupling ( $J_{TH}=7.1\pm 0.4$  Hz), and gave the intensity ratio of the two species (Figure 2D) as HTO:T<sub>2</sub>O = 34.6:100. The primary isotope effect on the <sup>3</sup>H  $\delta$  was  $\Delta\delta=17.9\pm 0.4$  Hz, compared with the primary isotope effect in a hydrocarbon of 8.8-9.8 Hz (at 320 MHz) [11,12].

Since the HTO peak appears in both the <sup>1</sup>H and <sup>3</sup>H spectra the abundance of H<sub>2</sub>O and T<sub>2</sub>O species may be related, and an estimate made of the S.A. of the tritiated water [13] *e.g.* combining the <sup>1</sup>H and <sup>3</sup>H intensity information:

$$\begin{array}{l} \text{Intensity} \quad \quad \quad \text{H}_2\text{O} : \text{HTO} : \text{T}_2\text{O} = 14.5 : 34.6 : 100 \\ \% \text{ of each} \quad \quad \quad \text{H}_2\text{O} : \text{HTO} : \text{T}_2\text{O} = 7.9 : 37.7 : 54.4 \\ \text{S.A.} \quad = (\% \text{ T}_2\text{O} \times 57.52) + (\% \text{ HTO} \times 28.76) + (\% \text{ H}_2\text{O} \times 0.00) \\ \quad \quad = (31.3) + (10.8) + (0.0) = 42.1 \text{ Ci/mmole} \end{array}$$

Since the theoretical maximum S.A. = 57.52 Ci/mmole, the NMR results suggest *ca.* 73% of the expected tritium content was recovered. Liquid scintillation counting measurements gave a similar gross yield of tritium (*ca.* 1.75 Ci out of a maximum calculated 2.53 Ci; 44 μmole T<sub>2</sub>O; *ca.* 1 μL). Tritiated water made in such a manner may be readily used or converted to CH<sub>3</sub>COOT or CF<sub>3</sub>COOT by reaction with the appropriate anhydride [14].

## 3. TRITIATED DIIMIDE

Tritiated diimide (HN=NT) is known to be the active agent in a range of selective hydrogenation reactions, the earliest of which was the combination of hydrazine with oleic esters to yield stearic esters [15]. Diimide chemistry has been extensively studied [16], and the preferred source of the reagent is by the proton-catalyzed decomposition of potassium

azodicarboxylate (PADA) [17]. Hydrogenation of carbon-carbon multiple bonds proceeds stereospecifically (>95%) *via cis*-addition [18,19], and  $N_2D_2$  has been used for the production of a range of selectively deuterated compounds [20]. In addition to stereoselectivity, diimide has high substrate specificity: hydrogenation of symmetrical double bonds (acetylenes,  $C=C$ ,  $N=N$ ) is achieved while unsymmetrical multiple bonds ( $-CN$ ,  $-NO_2$ ,  $N=C$ ,  $S=O$ ,  $C=O$ ) and other functional groups ( $Ar-I$ ,  $Ar-Br$ ,  $Ar-Cl$ ,  $R-Br$ ,  $R-SH$ ,  $R-S-S-R$ , *etc.*) are not attacked [21]. Despite these features (especially the ability to hydrogenate sulphur-containing compounds), tritium has rarely been used in diimide reactions [22,23], and when it has the S.A. of products was modest (0.5-1 mCi/mmole [23], or  $1.7 \times 10^{-3}\%$  of maximum theoretical S.A., 57.52 Ci/mmole).

This methodology is still under development, and will be published in full elsewhere. An example is given here to illustrate the validity of the literature claims for substrate specificity, and to show the potential of the technique in combination with the production of highly tritiated water (discussed above). Methyl *p*-nitrocinnamate (10 mg, 50  $\mu$ mole) was placed in a reaction flask with PADA (50 mg, 250  $\mu$ mole) and evacuated. Tritiated acetic acid (22  $\mu$ L, 380  $\mu$ mole, 10 mCi) in 0.5 mL dioxane was added, and the reaction mixture stirred overnight. The solvents were subsequently removed under vacuum, the residue dissolved in  $CH_3OH$  and redried. The resultant solids were dissolved in  $C_6D_6$  and filtered through glass wool before NMR and LSC analyses. The  $^1H$  spectrum of the starting material is shown in Figure 3A, and the product mixture in Figure 3B. Significant conversion to the saturated product (*ca.* 70-80%) was achieved without loss of the nitro or ester functional groups, and the  $^3H$  spectrum in Figure 3C clearly shows that all the incorporated tritium was in the two  $CH_2$  positions.

#### 4. TRITIATED TRIBUTYL TIN HYDRIDE

Organotin hydrides such as tri-*n*-butyltin hydride (TBTH) are capable and versatile reagents [24,25] which are easily prepared and stable. In the area of tritium labelling TBTH has four obvious applications:

- dehalogenation of aryl halides to arenes
- transition metal catalyzed conversion of acyl chlorides to the corresponding aldehydes
- free radical cyclization reactions, and
- rearrangement reactions such as the production of deoxy sugars from pyranosyl halides.

There is a single report on the use of tritiated TBTH [26] produced by two methods; hydrolysis of  $(Bu^n)_3Sn-Li$  with highly tritiated water, and by  $NaB^3H_4$  reduction of  $(Bu^n)_3Sn-Cl$ . The borohydride reduction was considered the more facile procedure, and gave eventual products of mild S.A. (71.4 mCi/mmole) in good yield.

We chose to generate  $(Bu^n)_3Sn-^3H$  from the reaction of  $Li-T$  [27] with  $(Bu^n)_3Sn-Cl$ , and to characterize the product by NMR spectroscopy. After preliminary experiments with  $^2H$ , and study by  $^1H$ ,  $^2H$  and  $^{119}Sn$  NMR methods, fully tritiated  $(Bu^n)_3Sn-^3H$  was synthesized and fully characterized. The  $^1H$  spectra of a commercial sample of TBTH and  $(Bu^n)_3Sn-^3H$  were compared, and the  $Sn-H$  resonance at  $\delta = 4.7$  ppm was absent in the tritiated sample. The  $^3H$  spectrum of  $(Bu^n)_3Sn-^3H$  showed a large singlet at  $\delta = 4.7$  ppm due to spin zero  $Sn-T$  species (83%), and small doublets for  $^{117}Sn-T$  (7.67%,  $J = 1610$  Hz) and  $^{119}Sn-T$  (8.68%,  $J = 1685$  Hz) species respectively. The  $^{119}Sn$  (111.9 MHz) spectrum showed a doublet ( $^{119}Sn-^3H$ ,  $J = 1685$  Hz) of multiplets (arising from the long range  $^{119}Sn-^1H$  couplings to the  $Bu^n$  groups).

#### 5. CONCLUSION

We have used a simple process for the production of highly tritiated water and characterized the product species by  $^1H$  and  $^3H$  NMR spectroscopy. The water is readily manipulated and used in subsequent reactions either as  $T_2O$ ,  $CH_3COOT$  or  $CF_3COOT$ . Development of tritiated diimide has progressed to the point where *cis*-hydrogenated products at 1-20 Ci/mmole S.A.

are possible. Tri-n-butyl tin tritide has been produced at >95% tritium content and well characterized by multinuclear NMR techniques. It remains to apply these reagents to exemplary tritiation reactions, and full details will be published in specialty Journals.

## ACKNOWLEDGEMENTS

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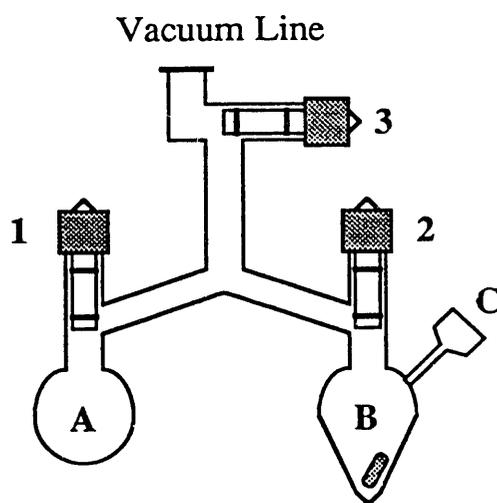


Figure 1. Apparatus used for production of highly tritiated water.

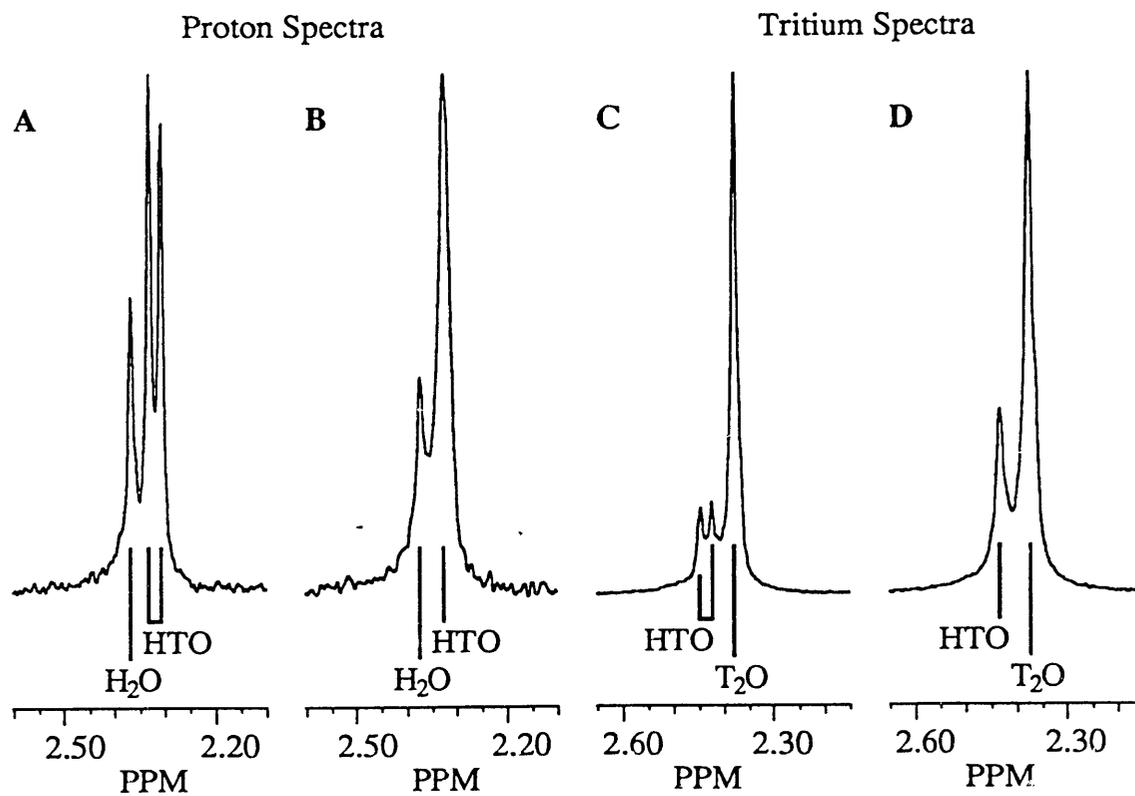
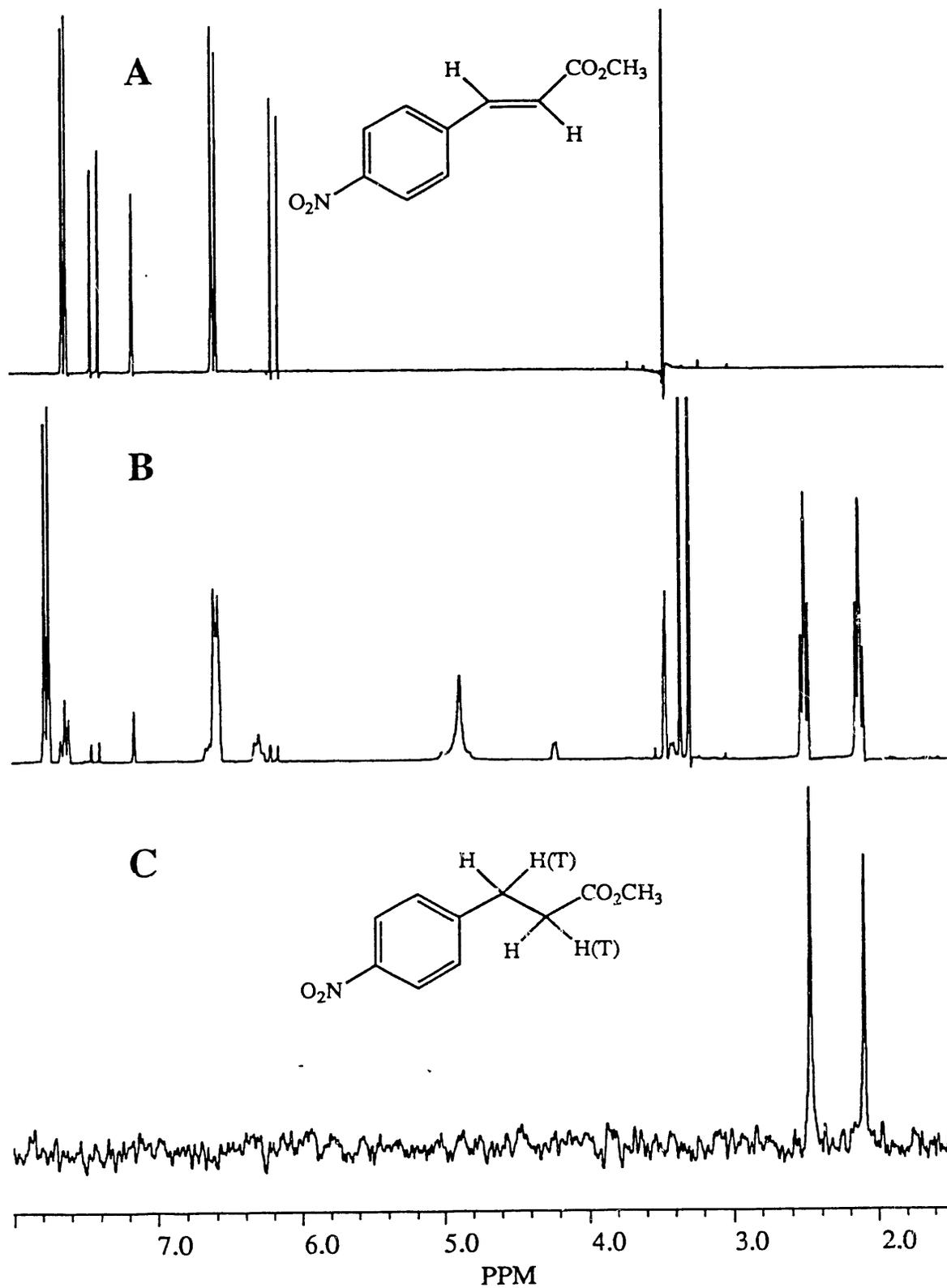


Figure 2. NMR spectra of highly tritiated water dispersed in dioxane-d<sub>8</sub>. A). 300 MHz <sup>1</sup>H spectrum, B). <sup>3</sup>H decoupled <sup>1</sup>H spectrum, C). 320 MHz <sup>3</sup>H NMR spectrum, D). <sup>1</sup>H decoupled <sup>3</sup>H spectrum.



**Figure 3.** A).  $^1\text{H}$  NMR spectrum of methyl p-nitrocinnamate (starting material) in  $\text{C}_6\text{D}_6$ , B).  $^1\text{H}$  spectrum of the products from the diimide reaction, C).  $^1\text{H}$  decoupled  $^3\text{H}$  spectrum (320 MHz) of the reaction products (250  $\mu\text{Ci}$ ).

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