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Short Research Article

Improved analysis of tritiated samples via a ^{3}H-NMR cryo-probe†

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Abstract: Tritium (^{3}H) NMR spectra of a number of compounds prepared via metal-catalyzed H/T reduction/exchange of unsaturated hydrocarbons are presented. These show the considerable benefits that emerge from using a tritium cryo-probe. Copyright © 2007 John Wiley & Sons, Ltd.

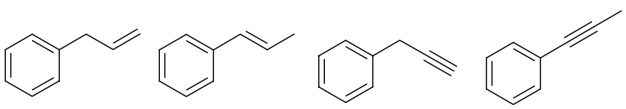
Keywords: tritium; NMR; cryo-probe; hydrogenation; H/T exchange; deuterium tritide

Introduction

Three years ago, at the previous IIS meeting in Boston, we reported[1] on the benefits that could be obtained by using a high-field magnetic resonance spectrometer (Bruker DRX-500) in conjunction with a tritium cryo-probe. Satisfactory ^{3}H-NMR spectra from samples containing only 11 μCi of radioactivity could be obtained and the improvement in signal to noise ratio (S/N) by using the cryo-probe was 4.3 ± 0.7, equating to an approximate 20-fold saving in time. In that study *ortho*-methoxy acetophenone, specifically labelled in the acetyl group by base-catalyzed H/T exchange was used as the test substrate. Here we report on an extension to these studies using compounds or mixtures that have tritium distributed amongst a range of different molecular sites.

Recently, we have been engaged in a project dealing with the heterogeneous metal-catalyzed hydrogenation of a number of unsaturated compounds.[2] Earlier studies[3] had shown that a rapid terminal methylene H/T exchange process can accompany the main hydrogenation process. This opens up the possibility that by using a deficit of isotopic hydrogen one can obtain a product specifically tritiated in the terminal group, provided appropriate separation procedures are available. For such an investigation maximum ^{3}H-NMR sensitivity is essential and this is where the tritium cryo-probe proved ideal.

Four compounds: allylbenzene **1**; *trans*-β-methylstyrene **2**; 3-phenylprop-1-yne **3**; and 1-phenylprop-1-yne **4** as well as two catalysts (5% Pd/C and 1% Pd/Al_2O_3) were used in the present study. For various reasons we required the products to be labelled with both deuterium and tritium.



1 2 3 4

Experimental

The experimental procedure used for the hydrogenation reactions is given below.

Sodium hydroxide (ca. 100 mg, 1 pellet) was dissolved in D_2O (20 ml) and solid $NaBD_4$ (155 mg) added. A portion (10 ml) of this solution was then used to dissolve solid $NaBT_4$. Aliquots (0.5 ml) of the resulting solution were then used for the preparation of low specific activity DT gas for the hydrogenation reactions using a modification of a previously described apparatus[4] in which the D_2O solution of boro[D&T]hydrides was subjected to acid hydrolysis by contacting a chloroform/toluene mixture containing CH_3CO_2D. The DT gas (9 ml) resulting from each hydrolysis was then introduced via a hydrogen-tight septum into a previously evacuated pressure tube (Discover, 10 ml

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capacity) charged with the hydrogenation substrate (20–80 mg), the catalyst (10 mg for 5% Pd/C or 50 mg for 1% Pd/Al_2O_3) and tetrahydrofuran (1 ml). Each hydrogenation reaction was then stirred for 24 h at room temperature. After filtration and careful evaporation ^{1}H-decoupled ^{3}H-NMR spectra were recorded in chloroform using a Bruker DRX-500 spectrometer with a standard 5 mm selective excitation proton/tritium probe for tritium observation at 533.5 MHz or the corresponding tritium cryo-probe.

Results and discussion

Table 1 summarizes the signal to noise (S/N) data from the analysis of ^{3}H-NMR samples from hydrogenation reactions with the four substrates. The mean enhancement in the S/N ratio of 4.3 is the same as that observed previously although the somewhat higher uncertainty ($\pm$ 1.3) might reflect the more complex spectra here (up to seven signals) as compared to the singlet observed for the tritiated ketone.

The practical utility of the cryo-probe is demonstrated in Figure 1, where the presence of over-reduction in the reaction products from the hydrogenation of 3-phenyl-1-propyne was not observable using traditional tritium NMR analysis but is clearly identified in the corresponding tritium cryo-probe spectrum.

With some substrates (the example of allylbenzene is given in Figure 2) there is evidence of H/T exchange having accompanied the hydrogenation reaction. Of course, the hydrogenation-product:exchange-product ratio can be varied by changing the substrate:DT ratio, and with the necessary procedures in place, compounds with tritium (or deuterium) in the terminal

Table 1 Improvements in signal to noise ratio from using the tritium cryo-probe

Substrate	Catalyst	S/N enhancement
Allylbenzene, **1**	5% Pd/C and 1% Pd/Al_2O_3	2.7, 5.6, 5.4
trans-β-Methylstyrene, **2**	5% Pd/C	3.7
3-Phenyl-1-propyne, **3**	1% Pd/Al_2O_3	5.8
1-Phenyl-1-propyne, **4**	5% Pd/C	2.8
	Mean $\pm$ SD	4.3 $\pm$ 1.3

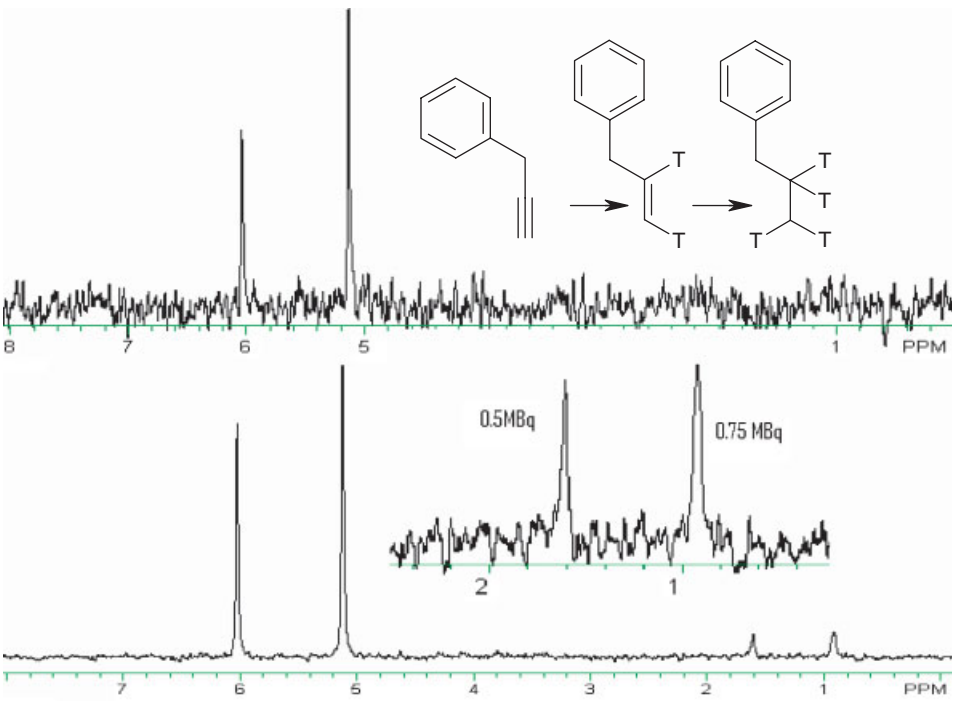

Figure 1 Products from the hydrogenation of 3-phenylprop-1-yne with DT gas analyzed by both ^{3}H-NMR (upper spectrum) and ^{3}H-NMR with cryo-probe (lower spectrum and expansion). Figure available in colour online at www.interscience.wiley.com

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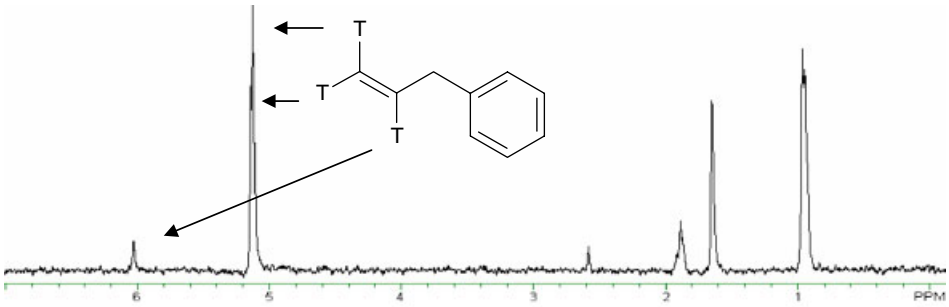

Figure 2 ^{3}H-NMR with cryo-probe of the products of partial hydrogenation of allylbenzene (1) with DT gas over 1% palladium on alumina. Figure available in colour online at www.interscience.wiley.com

alkene function can be prepared, a useful and additional route to such compounds.

Although tritium is the most sensitive of all NMR active nuclei one still requires several μCi of radioactivity per site for a satisfactory spectrum, and acquiring the spectrum still takes many hours. Liquid scintillation counting and accelerator mass spectrometry on the other hand are considerably more sensitive, but provide no information as to the site(s) labelled. The task therefore is to increase the NMR sensitivity so that samples at the sub-μCi levels of radioactivity can be analyzed.

Until recently the problem of noise was addressed either by using a filter and/or reducing the temperature, but revolutionary signal processing technology,[5] brought about by pioneering research in quantum physics, now makes it possible to isolate and identify ultra-weak signals embedded in complex background noise. The first application of this approach has been to gene expression microarrays but work is already underway to extend the limit of quantification of gas chromatography–mass spectrometers.[6]

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