LBL--24379
DE88 014865

Applications of High Resolution ^3H NMR Spectroscopy.

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Tritium is an excellent nucleus for NMR observation, but its use in the chemical and biological sciences is still limited.[1] A list of some of the properties which make tritium a desirable NMR nucleus are given in the Table, and the only real disadvantage in the eyes of traditional NMR users is its radioactivity. This one barrier to routine use is readily overcome by use of some simple precautions, such as the double encapsulation of samples to prevent contamination upon accidental breakage of NMR tubes. In general, "onepulse" tritium measurements can quickly and cleanly give the chemical shift and relative abundance of tritons in a labelled sample, with tritium levels as low as several hundred μCi in an NMR sample. Except for two notable exceptions[2,3], tritium NMR has been confined to simple "onepulse" experiments, but much more information is available, and the numerous advances in NMR hardware and the controlling software have made possible NMR techniques for extracting that information. In short, the large number of multipulse techniques currently in use have the potential for giving a great deal of conformational and coupling information, by way of the interaction of 3-H and 1-H atoms. Only a few of these methods have so far been applied to tritium, but their use will have many advantages.

Advantages of ^3H as an NMR Nucleus

1.	Spin 1/2	narrow lines
2.	High magnetic moment and γ	highest resonant frequency, good dispersion
3.	High Sensitivity	21% higher than protons, 125 times deuterons
4.	Low natural abundance ($< 10^{-16}$)	no background
5.	Can use proton assignments	vast library of proton information is available

In catalysis the use of the simplest NMR experiment means that hydrogen isotope exchange is readily monitored, with the relative incorporation at each position of a substrate yielding specificity rules for the catalyst as well as some mechanistic detail. A graphic example is given in Figure 1, where toluene has been labelled by metal-catalysed exchange with T_2 over bulk Raney Nickel, Platinum and Palladium catalysts, at room temperature. Labelling is predominantly in the methyl positions, but the platinum result also shows a large degree of ring saturation to give methyl-cyclohexane. The pattern of labelling in the methyl group and the aromatic ring of each substrate can also be analysed, and can give guidance as to the predominant mechanisms over each metal.

Hydrogenation and halogenation replacement reactions are the cornerstone of high level tritium labelling procedures. However, little is known about concomitant side-reactions, and these are extremely important when specific labelling is required. Observation of tritium NMR peaks from supposedly "unlabelled" positions obviates these extra mechanisms, and allows the choice of appropriate precursors and reaction conditions for the desired tritiation. A simple example is given in Figure 2, with the hydrogenation of β-methylstyrene to give n-propylbenzene (the reaction is shown in Figure 2A). The expected product would give a proton-decoupled tritium NMR spectrum as shown in Figure 2B, with a doublet at the chemical shift of the α and β-CH_2 positions. The experimental spectrum is given in Figure 2C, and shows significant incorporation in the methyl position, as well as additional signals in the α and β-CH_2 regions of the spectrum. Application of various multiple pulse techniques to the analysis of this reaction product yields a great deal of information about the isotopic content of the sample, and allows some speculation as to the mechanism of the methyl incorporation.[4]

Important new uses of tritium NMR spectroscopy include its use as an aid in spectral elucidation of proton NMR spectra[5] and for monitoring of the conversion of intermediates in biological systems.[6] The conversion of glucose (labeled at the C-1 position) to lactate by erythrocytes has been monitored by tritium NMR with the following results:

The ^3H NMR spectrum of the starting material consists of peaks for the α anomer at 5.19

ppm and the β anomer at 4.61 ppm, with an intensity ratio of 40:60. In the presence of erythrocytes the α anomer was consumed at a faster rate than was the β, and the kinetics of the growth and decay of tritiated components can be modeled. The anaerobic conversion of glucose to lactate was clearly demonstrated by the appearance and growth of two new signals, one at 1.32 ppm from the tritium label on the lactate methyl group and the other at 4.9 ppm from tritons that are exchanged into water as HTO. In experiments using rat erythrocytes, a signal from a transient metabolite was observed at 4.0 ppm, and, based on its chemical shift and previous biochemical studies, this intermediate has been tentatively identified as 2,3-diphosphoglycerate.

Another illustration of the use of tritum NMR spectroscopy is shown by studies on the effect that the binding of maltose has on the solution structure of maltose binding protein (MBP).[7] ^{1}H NMR experiments are limited by the huge number of lines in the NMR spectrum (even at 500 MHz) and as a consequence attempts to characterize the binding site have been restricted. Maltose was specifically labeled in the C-1 position of the reducing glucose moiety by catalytic exchange and ^{3}H NMR analysis indicated an extremely clean product.

In preliminary binding studies, approximately equimolar concentrations of tritiated maltose and the binding protein have been studied by both ^{3}H and ^{1}H NMR spectroscopy. Addition of a molar excess of maltose leads to ^{3}H signals for both the free and bound maltose, and the simplicity of the tritium spectrum clearly illustrates the utility of tritiated substrates for studying substrate binding. The relative binding affinities of the anomeric forms of the substrate and the kinetics of the binding can be assessed without the background of the numerous protein signals. In addition, further characterization of the protein binding site is possible by application of heteronuclear NOE techniques.

These experiments demonstrate the ease of use and broad relevance of high resolution tritium NMR spectroscopy. In particular, the technique is essential for labelling studies to assure purity of products and to give mechanistic information. The glucose results demonstrate the power of tritium NMR spectroscopy as a non-invasive probe of an *in vivo* system, giving fresh insight into the well-studied glycolytic pathway. The study of maltose binding protein shows the potential

of tritium NMR spectroscopy for defining the nature of binding sites, and the kinetics of intermolecular interactions.

Acknowledgements

Many thanks to the other members of the NTLF staff, especially Hiromi Morimoto and Mel Klein, for their help in many aspects of this work. This research was supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant P41 RR01237-06, and by the Department of Energy under Contract DE-AC03-76SF00098.

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Figure 1

^1H Decoupled 320 MHz Tritium NMR Spectra of Toluene, Labelled by Exposure to T_2 over Reduced Metal (100 mg) for 5 Hours at Room Temperature.

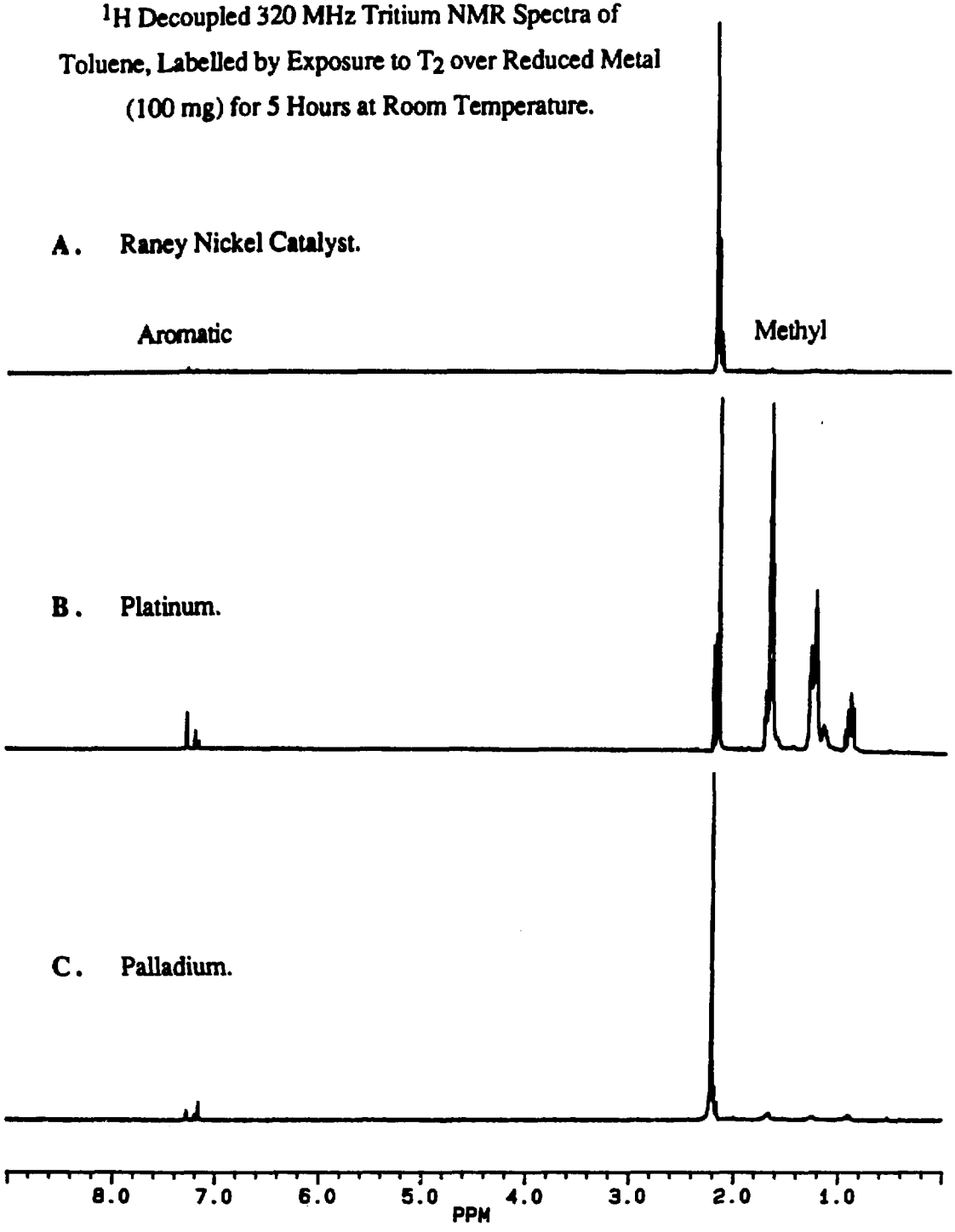
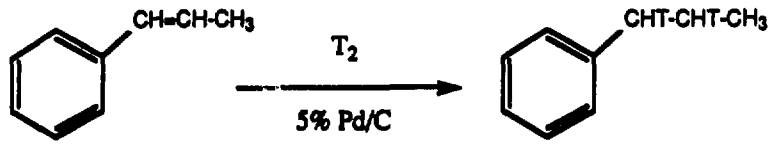


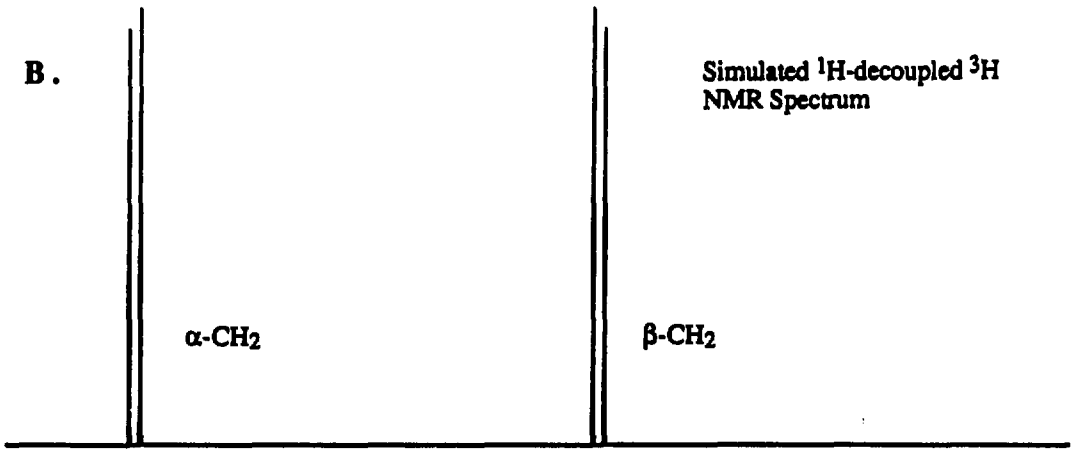
Figure 2.

Hydrogenation of β-Methylstyrene to Yield n-Propylbenzene

A.



B.



C.