

Deceptively Simple NMR Spectra of Contiguously ^{13}C -enriched Compounds*

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Contiguously ^{13}C -enriched compounds are widely used in biosynthetic studies, since they effectively label a bonded pair of carbon atoms, rather than an individual atom. Such compounds are referred to as »bond labeled« compounds. With such substrates, the course of biosynthesis can be followed using ^{13}C -NMR spectroscopy. When the enriched carbon atoms are chemically but not magnetically equivalent, the ^{13}C -NMR spectra are unusual, and such spectra have been labeled »deceptively simple«. Furthermore, the use of standard NMR experiments such as DEPT (Distortionless Enhancement by Polarization Transfer) is complicated by the presence of homonuclear coupling between ^{13}C nuclei. At natural abundance, the ^{13}C nucleus is magnetically dilute and shows no homonuclear coupling. This paper analyzes the ^{13}C -NMR spectra of a series of molecules derived from fully ^{13}C -enriched acetylene, and explores some of the complications that arise in applying pulse NMR methods.

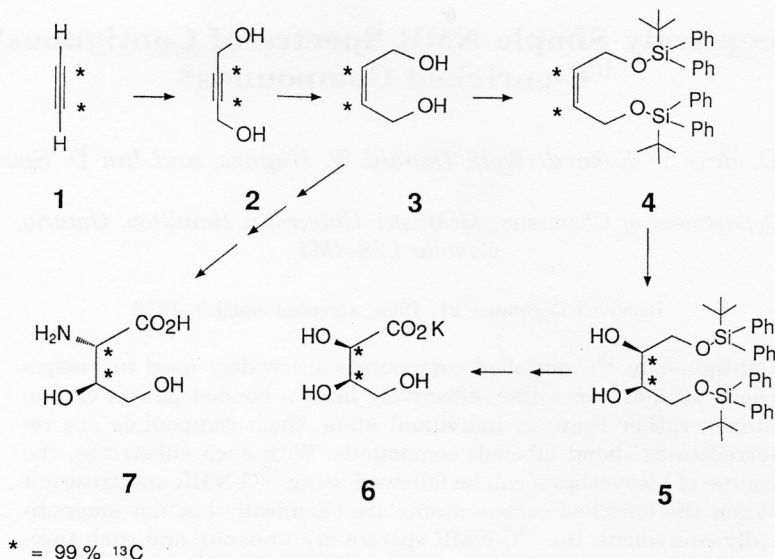
INTRODUCTION

Biosynthetic studies have benefitted greatly from ^{13}C -NMR spectroscopy. While enrichment at a single site allows the fate of an individual carbon atom to be studied, full enrichment of contiguous carbon atoms permits the fate of a chemical bond to be studied, since full homonuclear ^{13}C - ^{13}C spin coupling will persist only if the bond remains intact. Studies with such

* Dedicated to Professor Vladimir Prelog in honour of his 90th birthday.

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»bond-labeled« substrates make it possible to follow the transfer of multi-carbon units during a biosynthetic process. The availability of ^{13}C -enriched starting materials and the power of modern NMR instruments have made ^{13}C -NMR spectroscopy one of the major techniques used to trace a biosynthetic pathway.



Scheme 1.

Bond-labeled precursors have played an important role in studying the biosynthesis of vitamin B₆ (pyridoxine) in *Escherichia coli*.¹² As part of this work the synthesis of [2,3- $^{13}\text{C}_2$]-4-hydroxy-L-threonine (**7**), by an eight-step reaction sequence from [1,2- $^{13}\text{C}_2$] acetylene (**1**), was recently reported,³ and the preparation of potassium [2,3- $^{13}\text{C}_2$]-D-erythroate (**6**), via [2,3- $^{13}\text{C}_2$]but-2-ene-1,4-diol (**3**) and some of its derivatives,⁴ was also carried out (Scheme 1). For compounds in which the enriched carbon atoms are chemically inequivalent *e.g.*, (**6**) and (**7**), the ^{13}C signals of the natural abundance carbon nuclei near the enriched sites display the expected appearance of doublets. However, compounds with identical groups on either side of the ^{13}C - ^{13}C labeled bond, such as [2,3- $^{13}\text{C}_2$]but-2-yne-1,4-diol (**2**) and [2,3- $^{13}\text{C}_2$]-*meso*-1,4-bis(*tert*-butyldiphenylsilyloxy)-butane-2,3-diol (**5**), as well as (**3**), (**4**) and other compounds,³ were found to have peculiar ^{13}C -NMR spectra. The unusual appearance of the signals due to carbon atoms 1 or 4 in the ^{13}C -NMR spectra of these compounds is evident from Figures 1 and 2. The spectra of these compounds and the associated spin systems are the subject of this paper.

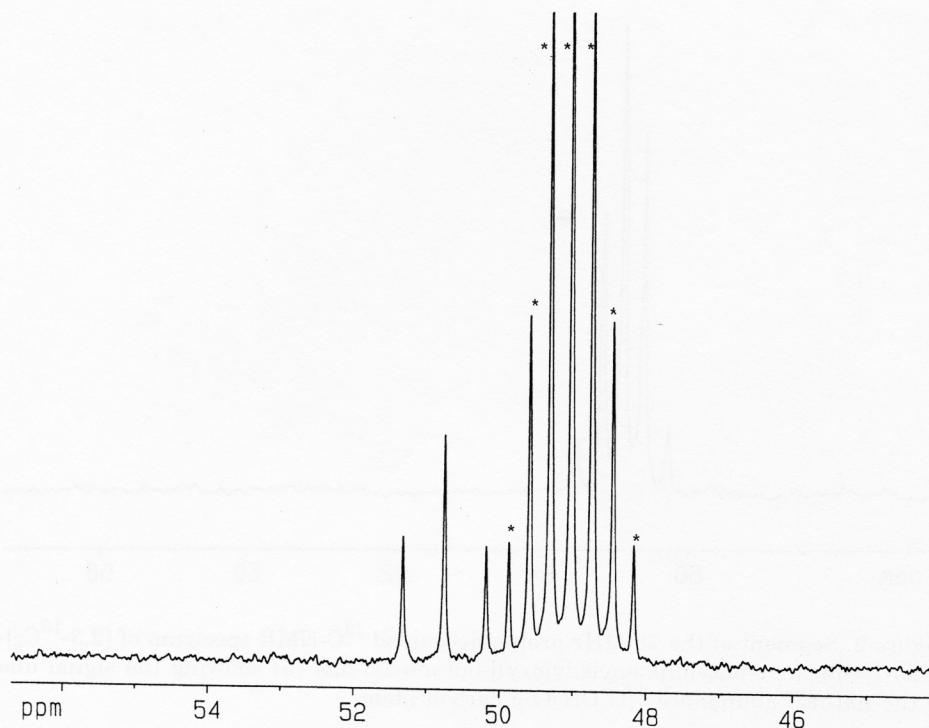


Figure 1. Segment of the 125 MHz proton-decoupled ^{13}C -NMR spectrum of [2,3- $^{13}\text{C}_2$]but-2-yne-1,4-diol (**2**) showing the pseudo-triplet signal at 50.8 ppm due to the natural abundance ^{13}C CH_2OH carbon atom. The septet centred at 49.1 ppm (indicated by asterisks) is due to the methanol- d_4 solvent.

STRONG COUPLING IN THE ^{13}C -NMR SPECTRA OF ^{13}C -ENRICHED COMPOUNDS

The basic NMR parameters of the compounds whose spectra are shown in Figures 1 and 2 are very similar to those in the corresponding natural abundance compounds. The main reason why the spectra of the enriched compounds are dramatically different is that there are two coupled ^{13}C atoms in the same molecule, with similar chemical shifts. As a result, the spin system becomes strongly coupled.⁵ Strong coupling occurs when differences in the Larmor frequencies (*i.e.*, chemical shifts in frequency terms) of two nuclei become comparable in magnitude to scalar coupling constants. In normal NMR terminology the two enriched carbon atoms and their natural abundance neighbour form an ABX spin system.

The ABX spin system can show a bewildering array of unusual behaviour.^{5,6} This includes unexpectedly complex spectra (\gg virtual coupling \ll) and

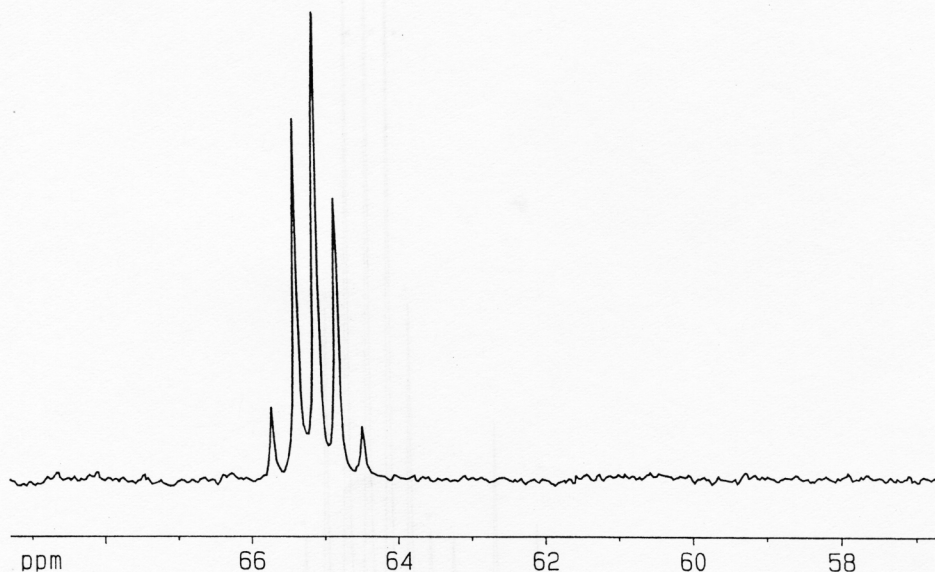


Figure 2. Segment of the 75 MHz proton-decoupled ^{13}C -NMR spectrum of [2,3- $^{13}\text{C}_2$]-*meso*-1,4-[bis(*tert*-butyldiphenylsilyloxy)]-butane-2,3-diol (**5**) showing the signal due to the natural abundance ^{13}C CH_2OSi carbon atom.

spectra that have less structure than one would predict («deceptively simple»). These effects have been widely observed in both organic and inorganic systems at low field,⁷ but tend not to be noticed when spectra are obtained on newer, high-field NMR spectrometers. All these effects can be calculated quite simply, but the intuitive understanding is not as easy.^{8,9} The present cases represent deceptively simple spectra.

The two enriched carbon atoms in compounds **2**, **3**, **4**, and **5** are chemically, but not magnetically, equivalent. Apart from isotope effects, the enriched carbon atoms have the same chemical shift, but have different couplings to a given nucleus outside the pair. In all these cases the given nucleus is the natural abundance ^{13}C at position 1 or 4. Note that the isotopomer with ^{13}C at position 1 has ^{12}C at position 4, and *vice versa*, so that it yields a three-spin system. The degree of strong coupling in an ABX spin system is determined by the relative magnitudes of J_{AB} , [$J_{\text{AX}} - J_{\text{BX}}$] (in this case, $^1J(\text{C-C})$, [$^2J(\text{C-C}) - ^3J(\text{C-C})$]) and the difference in Larmor frequencies, *i.e.* the chemical shift difference of nuclei A and B, in Hertz. The difference in Larmor frequencies is proportional to the magnetic field, so that strong coupling is not a significant problem with high-field magnets. In the present case, however, the chemical shift difference is zero regardless of field strength, so that the spin systems are always strongly coupled.

One rationalization for the spectra in Figures 1 and 2 is that the two enriched nuclei are so strongly coupled (relative to their Larmor frequency difference) that, to the outside world, they become equivalent. The signal of carbon 1 then shows a triplet structure with a splitting (as distinct from a coupling)¹⁰ which is the average of $^1J_{12}$ and $^2J_{13}$. However, the two enriched nuclei are not magnetically equivalent, and as a result additional lines, such as those in Figure 2, appear in the spectrum. If isotope effects are neglected, this is an AA'X spin system. Strictly speaking, there is a small isotope shift, so it is an ABX system. The positions and intensities of the additional lines are strongly dependent on the spin parameters, but are usually of such a low intensity that they are not observed. Provided that all the lines can be observed, a complete analysis of the spin parameters is possible. Otherwise only $(^1J_{12} + ^2J_{13})/2$ can be determined. In upper case of [2,3- $^{13}\text{C}_2$]but-2-yne,1,4-diol (**2**) the one-bond ^{13}C - ^{13}C coupling between carbon atoms 1 and 2 is typically about 70 Hz and the two-bond coupling between carbons 1 and 3 is about 10 Hz. The observed splitting in the pseudo-triplet is 41 Hz.

PULSE NMR EXPERIMENTS WITH CONTIGUOUSLY ENRICHED COMPOUNDS

Almost all the standard pulse experiments involving ^{13}C are based on the assumption that the nuclei are magnetically dilute, that is, that there are no other ^{13}C nuclei nearby in a given molecule. This is indeed the case in natural abundance carbon compounds, and it simplifies the application and analysis of experiments such as DEPT¹¹ (Distortionless Enhancement by Polarization Transfer). In the instance of bond-labeled compounds, *e.g.* **2** and **5**, the enriched carbon atoms are coupled to each other, and the spectra may be quite different from those obtained from the corresponding natural abundance compounds. Homonuclear coupling as well as strong coupling can considerably complicate pulse NMR experiments and can lead to various interesting artifacts.¹² It is useful to illustrate some of these complications in order to show how counter-intuitive they can be.

The DEPT experiment¹¹ is a way of transferring nuclear spin polarization from protons to ^{13}C nuclei, *via* the scalar coupling. This leads to a better signal/noise ratio in the ^{13}C -NMR spectrum, and also permits the sorting of carbon atoms according to their multiplicity. Under normal circumstances, DEPT will suppress signals from carbon atoms which are not directly bonded to protons. For compounds with ^{13}C at natural abundance, the experiment is well-understood and widely used.

If DEPT is used to enhance the intensity of carbon atoms 1 and 4 of **2** or of **5**, the spectra show a phase modulation in the pseudo triplet. This effect is quite common when there is homonuclear coupling in a molecule.

In a compound such as [2,3- $^{13}\text{C}_2$]but-2-yne-1,4-diol (**2**), DEPT, based on the long-range couplings, might also be used to enhance the signal of carbon atoms 2 and 3, and to reduce the signals of the non-enriched carbons. As with carbon atoms 1 and 4, the methylene protons are coupled in a deceptively simple way to the enriched sites. In the related molecule, propyne 13 , the two-bond ^1H - ^{13}C coupling is in the range of -10.6 Hz, and the three-bond coupling is $+4.8$ Hz. Again, the pseudo triplet shows a splitting which is the average of the two-bond and three-bond couplings, but because of the opposite signs of the couplings, they partially cancel. In compound (**2**), the observed splitting is 1.3 Hz. The DEPT experiment requires a delay to be set to $1/(2J_{\text{CH}})$. In a case such as the present, it is not clear which number should be used for the coupling constant in setting the delay.

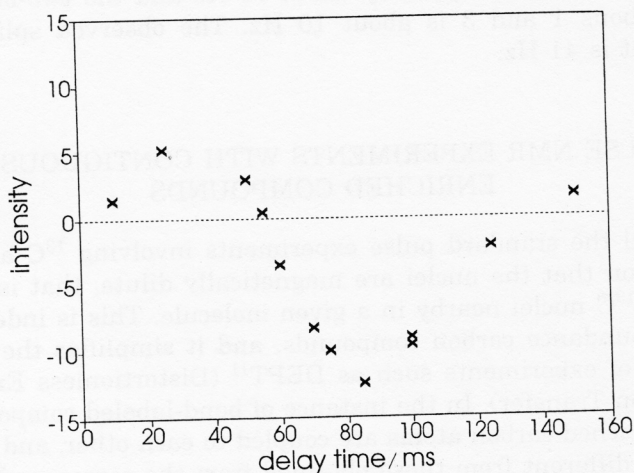


Figure 3. Intensity (arbitrary units) of the proton-decoupled signal due to enriched carbon atoms 2 and 3 of [2,3- $^{13}\text{C}_2$]but-2-yne-1,4-diol (**2**), as a function of the delay in a DEPT experiment.

Figure 3 shows the experimental results. A series of DEPT 135° measurements were performed on compound (**2**), in which the delay was varied from 10 ms to 150 ms. Figure 3 shows the intensity of the signals due to the enriched carbon atoms, as a function of the delay time. The data follow a sinusoidal curve, with a period of approximately 130 ms. This makes the first maximum of the sinusoid 32 ms, leading to an effective J_{CH} of about 15 Hz. It is clear from Figure 3 that an experiment set up by using the observed splitting of 1.3 Hz would be completely wrong.

CONCLUSIONS

Full ^{13}C enrichment of contiguous carbon atoms can lead to unusual ^{13}C -NMR spectra, some unexpectedly complex, others deceptively simple. In a deceptively simple spectrum, what look like simple multiplets are, in fact, only the intense parts of much more complex signal patterns. Failure to recognize these effects can lead to misleading reports of NMR parameters, and to inefficient use of pulse NMR experiments. The strong-coupling effects are relatively easy to calculate, once they have been recognized. The important point is to be aware of their existence.

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SAŽETAK

Varljivo jednostavni NMR spektri spojeva obogaćenih sa ^{13}C na susjednim atomima

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Spojevi obogaćeni sa ^{13}C na susjednim atomima široko se rabe u biosintetskim istraživanjima budući da oni efektivno obilježavaju vezani par ugljikovih atoma, a ne samo jedan atom. Takovi spojevi nazivaju se spojevima »obilježenim na vezi«. S takovim supstratima može se slijediti biosinteza s pomoću ^{13}C -NMR spektroskopije. Kada su obogaćeni ugljikovi atomi kemijski, ali ne i magnetski, ekvivalentni, tada su ^{13}C -NMR spektri neobični i označavaju se kao »varljivo jednostavni«. Nadalje, upotreba standardnih NMR eksperimenata kao što je DEPT (Distortion Enhancement by Polarization Transfer) komplicira se pojavom homonuklearnog sprezanja između jezgara ^{13}C . U prirodnom spoju jezgra ^{13}C jako je razrijeđena, pa ne pokazuje homonuklearno cijepanje. U ovom radu analiziraju se ^{13}C -NMR spektri niza molekula izvedenih od potpuno obogaćenog ^{13}C -acetilena, i istražuju se neke komplikacije koje nastaju pri primjeni pulsni metoda NMR.