Use of ^{13}C N.M.R. Spectral Editing Techniques as a Possible Procedure to follow the Fate of Deuterium Atoms in Mechanistic and Biosynthetic Studies

David M. Doddrell,*† James Staunton, and Ernest D. Laue

University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW, U.K.

Multipulse techniques have been developed for spectral editing of ^{13}C n.m.r. spectra of deuteriated compounds by combining proton polarization transfer methods and deuterium spin-echo techniques; the technique permits the generation of ^{13}C n.m.r. subspectra of only those carbons coupled to one, two, or three deuterium nuclei.

Deuterium has been widely used as a tracer in biosynthesis in recent years and its importance is reflected in the many approaches which have been developed to detect its presence in the metabolites under investigation.[1a] The isotope can be detected directly by ^{2}H n.m.r. spectroscopy or indirectly by ^{13}C n.m.r. spectroscopy in those cases where the hydrogen isotope is placed either alpha or beta to a carbon reporter nucleus.[1b] This indirect approach capitalises on the greater resolution which is inherent in ^{13}C n.m.r. spectroscopy and is therefore capable of discriminating more effectively between various labelled sites in molecules multiply-labelled with deuterium and also between molecules bearing one, two, or three deuteriums at a particular site. However, in complicated cases with many labelled sites even this technique will prove inadequate because of overlap between the resonances of interest. We now report a novel approach which allows selective generation of carbon resonances according to the number of deuterium and proton spins with which they are coupled. This technique has therefore the potential for much enhanced resolution and sensitivity in the spectra of complicated metabolites such as macrolides and ionophore antibiotics.

The complexity of the problem is illustrated in Figure 1(f)

† *Present address:* School of Science, Griffith University, Nathan 4111, Australia.

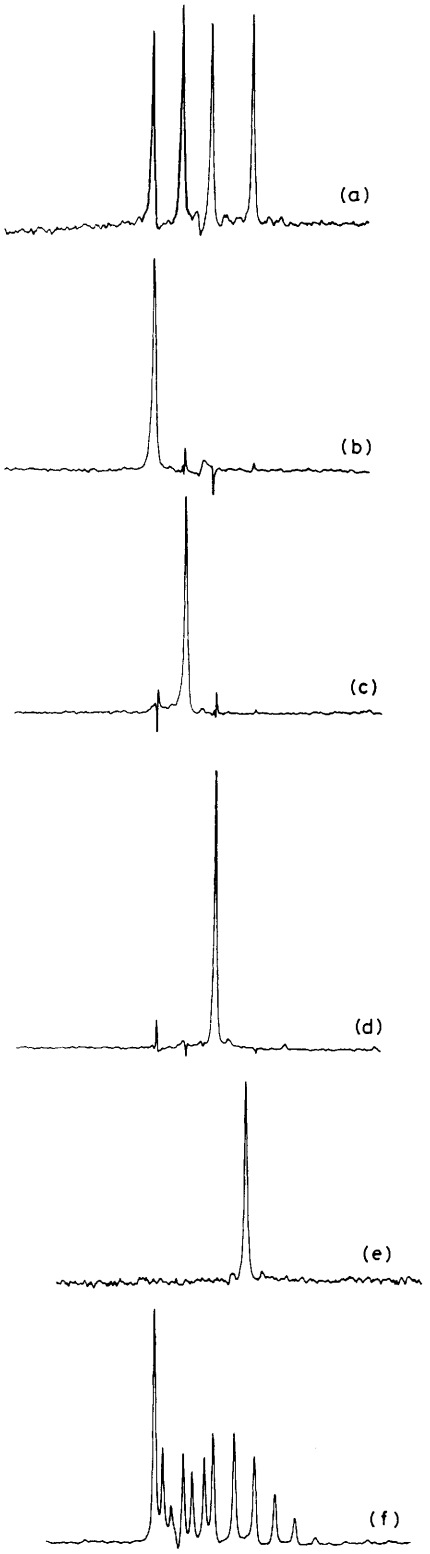

Figure 1. (a) Methyl region of the proton-decoupled, deuterium-decoupled ^{13}C n.m.r. spectrum of a mixture of the compounds $PhCH_nD_{3-n}$ after 32 pulses with a recycle time of 45 s. (b) CH_3 Subspectrum recorded using DEPT. (c) CH_2D Subspectrum recorded using DEPT. (d) CHD_2 Subspectrum recorded using DEPT. (e) CD_3 Subspectrum recorded using the first part[4] of the composite pulse sequence given in Figure 2(b). (f) Proton-decoupled, deuterium-coupled spectrum after averaging 128 pulses. In all cases, the spectral width was 500 Hz. Internal hexafluorobenzene (20%) was used as a ^{19}F lock. The composition of the mixture was such that the most intense resonance from each species had about the same intensity.

which shows the methyl region of the proton-decoupled ^{13}C n.m.r. spectrum of a mixture of compounds $PhCH_nD_{3-n}$ ($n = 0, 1, 2, 3$) which was used as one test sample. This spectrum can be simplified somewhat by deuterium decoupling [Figure 1(a)] but even in this simple test sample four lines (singlets) are still observed for the methyl. Such spectra could be further simplified by generating subspectra containing one line per subspectrum by spectral editing based on the number of remaining attached protons [Figure 1(b)—(e)]. Such a procedure is attractive as it is not sensitive to rapid deuterium relaxation which could in principle hinder attempts to achieve the same result by application of D–^{13}C polarization-transfer techniques.[2]

To achieve the results presented in Figure 1 we used the DEPT pulse sequence[3] to generate the proton-decoupled, deuterium-decoupled CHD_2, CH_2D, and CH_3 subspectra and our recently published[4] pulse sequence which is selective for non-protonated carbon signals to generate the deuterium-decoupled CD_3 subspectra. (These two pulse sequences are shown in Figure 2 as the first part of the composite pulse sequences.) However, these techniques do not distinguish CHD_2 and CHD resonances from other CH resonances, CH_2D resonances from non-deuteriated CH_2 resonances, or CD_3, CD_2, and CD resonances from other non-protonated carbon resonances. It is precisely this result which is required in mechanistic studies involving deuterium labelling and we report that it can be achieved by adding the pulse sequence segment, equation (1),[5] to the above mentioned pulse sequences to yield the

$$\left.\begin{array}{cccc} -\frac{1}{4J_{CD}} & -\pi[C] & -\frac{1}{4J_{CD}} & - \end{array}\right| \begin{array}{l}\text{acquire } ^{13}C \\ \text{receiver add/} \\ \text{subtract}\end{array} \qquad (1)$$
$$\pi[D,1,0]$$

composite pulse sequences shown in Figure 2. The pulse sequence segment given in equation (1) inverts the phase of the ^{13}C resonances associated with the odd values of the total deuterium spin quantum number; if spectra are recorded without deuterium decoupling, a CD group appears as a 1 : 1 'doublet', a CD_2 as a 1 : 1 'doublet', and a CD_3 as a 1 : 6 : 6 : 1 'quartet'. Ideally lines associated with the even values of the total deuterium quantum numbers are eliminated; small residual peaks may remain depending on the quality of the deuterium π pulse (see Figure 3). Our overall approach can be summarised as follows: a degree of spectral editing is achieved based on the number of protons scalar coupled to the carbon and a further spectral simplication is then achieved using the pulse segment given in equation (1) to eliminate signals from carbons not coupled to deuterium.

Both composite pulse sequences have been tested experimentally and the results of applying the composite pulse sequence given in Figure 2(a) are shown in Figure 3. Note the satisfactory elimination of all the desired resonances of the test samples which consisted of [α-$^2H_1$]toluene or [α-$^2H_2$]-toluene in the presence of 2,2,4-trimethylpentane. (Such a mixture might approximate the product of mechanistic studies of hydrogenation using deuterium gas.) As a bonus, in this case, the composite pulse sequence recycle time is determined by the much shorter proton relaxation times. On our instrument we could not both pulse and decouple deuterium. Even so, the degree of spectral editing is certainly sufficient to determine the presence of deuterium at a level of 5% or greater. The availability of deuterium decoupling would probably halve this figure.

The above techniques should prove useful in mechanistic studies involving deuterium; the only limitation will be rapid deuterium relaxation expected in some compounds. [We estimate that the above techniques will work satisfactorily

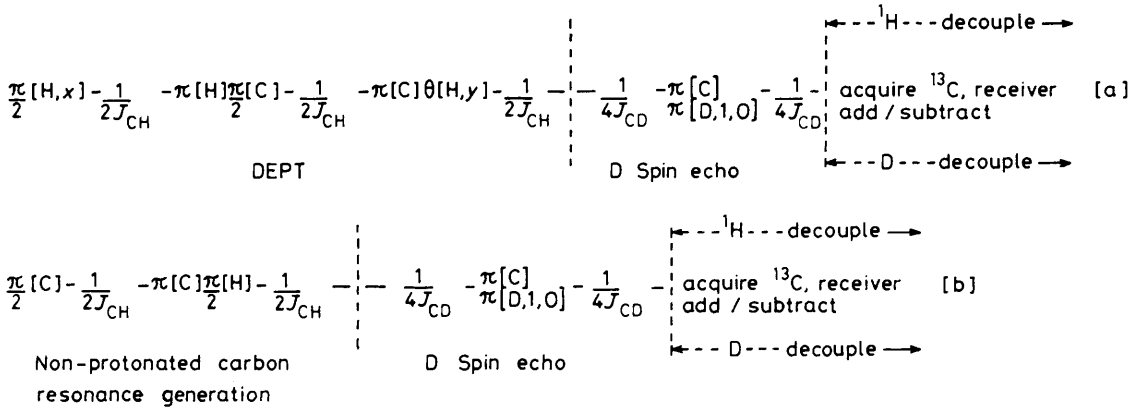

Figure 2. Composite pulse sequences.

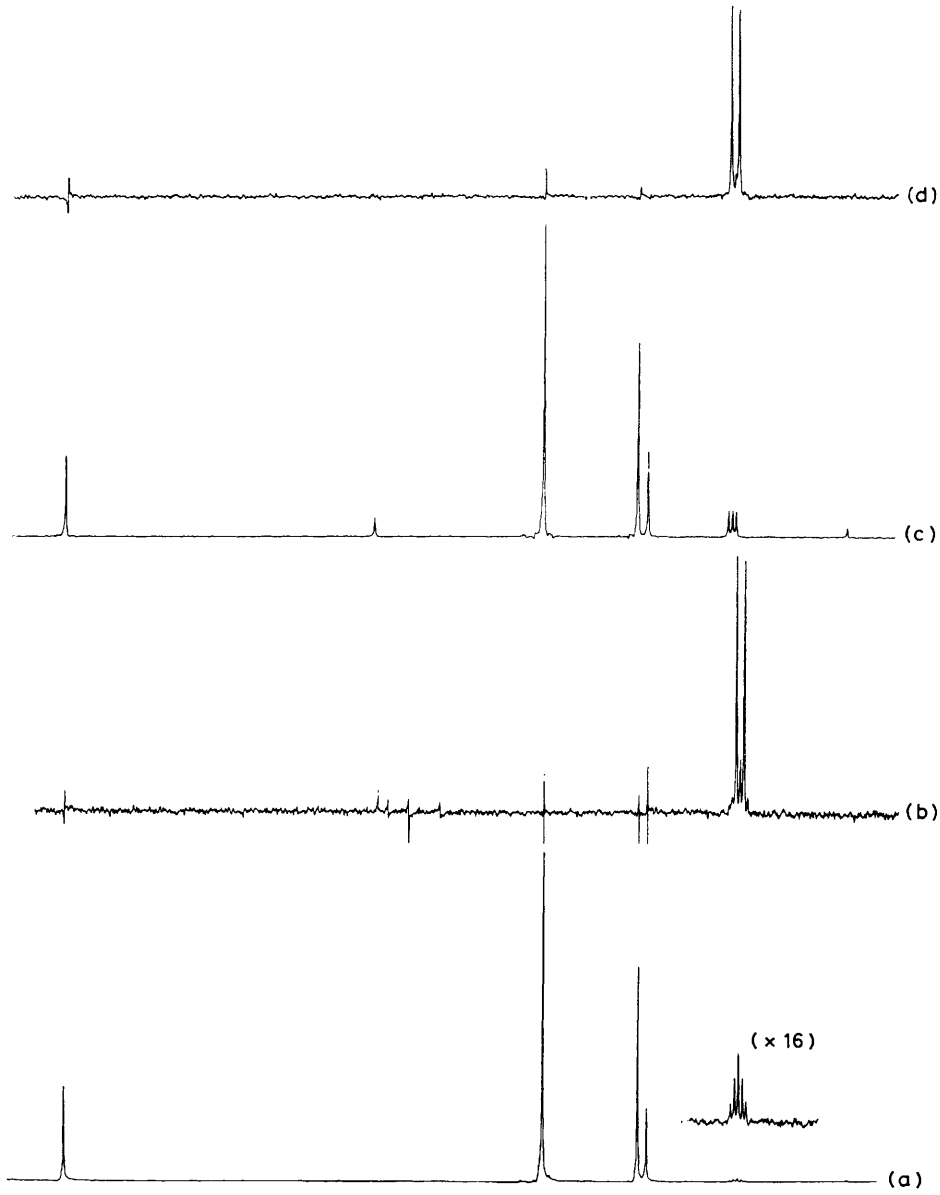

Figure 3. (a) Proton-decoupled ^{13}C n.m.r. spectrum of a mixture containing approximately 7% [α-$^{2}H_2$]toluene, 15% hexafluorobenzene, and the remainder 2,2,4-trimethylpentane. 32 Scans were averaged using the DEPT sequence with $\theta = \pi/4$; $t_{90}(H) = t_{90}(C) = 33.5$ μs. (b) Edited spectrum of the mixture used in (a) using the composite pulse sequence given in Figure 2(a), 1600 scans, $\theta = \pi/2$. (c) Proton-decoupled ^{13}C n.m.r. spectrum of a mixture containing approximately 30% [α-$^{2}H_1$]toluene, 15% hexafluorobenzene, and the remainder 2,2,4-trimethylpentane. 16 Scans were averaged using the DEPT sequence with $\theta = \pi/4$. (d) Edited spectrum of the mixture used in (c) using the composite pulse sequence given in Figure 2(a); the 64 scans averaged were composed of the following: 16 scans [$\theta = \pi/4$, (D pulse = 0), receiver +], 16 scans [$\theta = 3\pi/4$, (D pulse = 0), receiver −], 16 scans [$\theta = \pi/4$, π(D), receiver −], and 16 scans [$\theta = 3\pi/4$, π(D), receiver +]; $t_{90}(D) = 47$ μs.

when the deuterium T_2 is longer than 75 ms, that is for a $T_2 > 3(2J_{CD})^{-1}$ with J_{CD} ca. 20 Hz. Deuterium–carbon polarization transfer techniques, as well as being inherently insensitive and more instrumentally demanding, will require a longer lower limit of T_2.]

This work was performed while one of us (D. M. D.) was on study leave from Griffith University, Brisbane, Australia. D. M. D. thanks the Royal Society for the award of a Nuffield Fellowship. We thank the S.E.R.C. for financial support.

Received, 25th January 1983; Com. 117

References

1 (a) M. J. Garson and J. Staunton, *Chem. Soc. Rev.*, 1979, **8**, 539; (b) C. Abell and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1981, 856.
2 P. L. Rinaldi and N. I. Baldwin, *J. Am. Chem. Soc.*, 1982, **104**, 5791.
3 D. M. Doddrell, D. T. Pegg, and M. R. Bendall, *J. Magn. Reson.*, 1982, **48**, 323; D. T. Pegg, D. M. Doddrell, and M. R. Bendall, *J. Chem. Phys.*, 1982, **72**, 2745.
4 M. R. Bendall, D. T. Pegg, D. M. Doddrell, S. R. Johns, and I. Willing, *J. Chem. Soc., Chem. Commun.*, 1982, 1132.
5 D. M. Doddrell, J. Staunton, and E. D. Laue, *J. Magn. Reson.*, 1983, **52**, 523.