Precise Carbon-13 N.M.R. Multiplicity Determination

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Summary A simple excitation sequence allows fast recognition of carbon multiplicity in ^{13}C n.m.r. spectra of large molecules, with high precision and sensitivity.

Routine ^{13}C n.m.r. analyses, namely chemical shift and carbon multiplicity determinations, are usually performed on proton noise-decoupled, and proton off-resonance decoupled spectra. However, the last technique is often difficult to use for complex molecules. Some new methods allow better analysis of ^{13}C n.m.r. spectra,[1,2] but are not normally usable with routine spectrometers, as they use software or hardware alterations (*e.g.* decoupler phase-shift). We present in this communication a method allowing easy identification of quaternary, CH, CH_2, and CH_3 carbons in ^{13}C n.m.r. spectra, with a sensitivity similar to that of proton noise-decoupled techniques. The basic features of this multiplicity separation method have already been used by Campbell *et al.*[3] for ^1H n.m.r. studies on proteins.

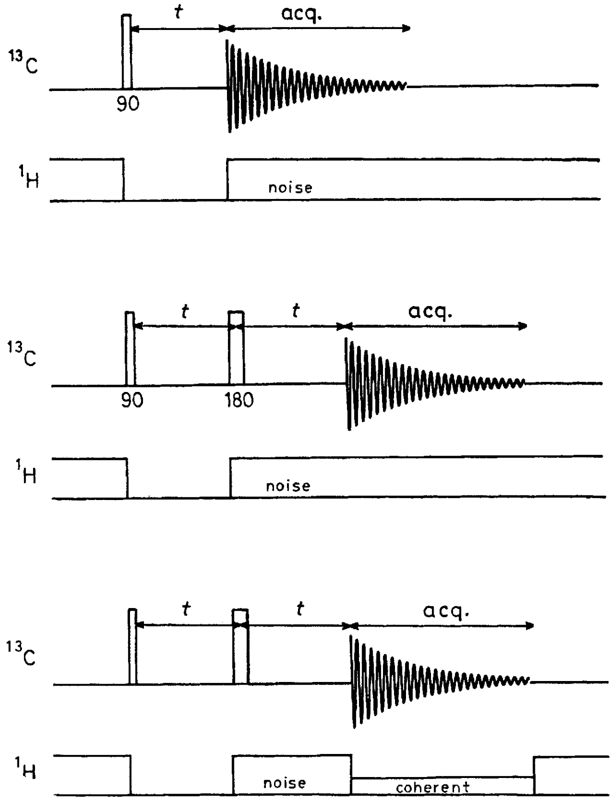


Figure 1. Pulse sequences. (a) Simple sequence of multiplicity separation; (b) modified sequence to avoid phase distortion; (c) sequence for observation of off-resonance multiplets.

The fundamental experiment is depicted in Figure 1a. The sequence is a standard proton-decoupled carbon sequence except that during a short period *t* before the acquisition the proton decoupler is turned off. Analysis of carbon magnetization in the rotating frame (Figure 2) clearly shows that a variety of information can be gained depending upon the choice of the *t* value. If *t* takes the value $1/J(^1H-^{13}C)$ the CH and CH_3 signals are inverted, whilst the CH_2 and quaternary carbon signals are positive.

In general, sp^3 carbons are coupled to protons with a coupling constant *J* of about 125 Hz, and selecting a value of 8 ms for *t* allows complete recognition of the parity of all carbon peaks. However, the delay introduced before acquisition, leading to a truncated free-induction decay (F.I.D.), produces a severe frequency-dependent phase error and line-shape distortion. Sequence 1b (Figure 1) allows elimination of this by utilisation of a spin echo.

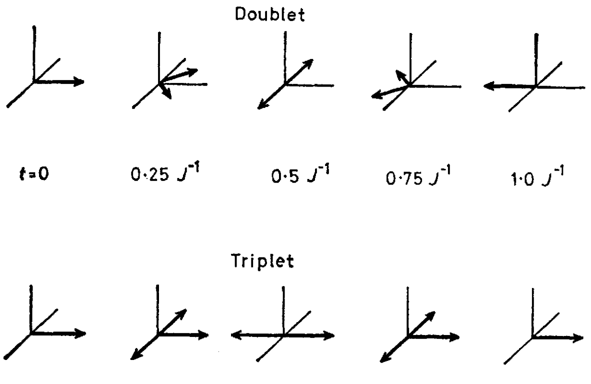


Figure 2. Behaviour of doublet and triplet magnetization with the same coupling constant *J*, after a 90° excitation pulse, in the rotating frame.

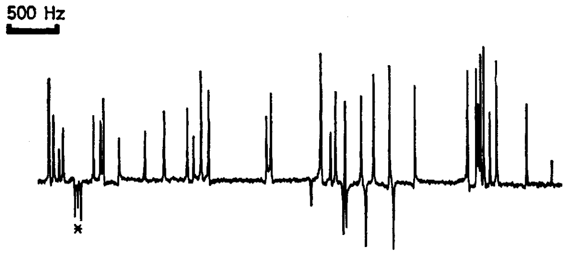
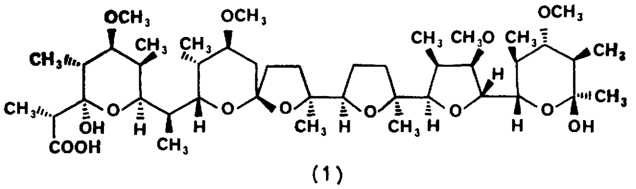


Figure 3. Upfield part of the ^{13}C (62·86 MHz) n.m.r. spectrum of emericide (**1**). Phase corrections have been made so that CH and CH_3 resonances appear as positive, and quaternary and CH_2 carbons as negative signals. *$CDCl_3$ resonance.

A typical example is given in Figure 3 on emericide (**1**),[4] an ionophorous antibiotic. This example shows that the CH and CH_2 resonances, separated by only 10 Hz, are well resolved. It gives a better selectivity and sensitivity than classical off-resonance experiments[5] and has a broader field of application than selective T_1 experiments.[6] The sequence 1b produces positive and negative peaks depending upon parity of the number of protons, while the simple spin echo with continuous decoupling produces only positive peaks. It is therefore evident that one sequence 1b followed by one fully decoupled spin echo gives an F.I.D. where only signals due to quaternary and CH_2 carbons are observed. In the same way, one sequence 1b followed by one inverted decoupled spin echo produces a spectrum of signals of even parity only. In practice, separating even and odd parity peaks is especially useful when using the



(1)

modified sequence 1c (Figure 1) where the acquisition occurs under proton low-power single-frequency decoupling.

In general, the efficiency of low-power heteronuclear decoupling applied to the identification of CH_2 carbons (^1H–^{13}C chemical shift correlation) is very low if CH_2 resonances are coincident with those of CH and CH_3 carbons, and if both protons have different chemical shifts; the carbon signal often appears as either a broad quadruplet, if the low power decoupling is off-resonance, or a doublet if one of the two protons is fully decoupled. Both patterns have insufficient amplitude to be clearly visible in the middle of the CH or CH_3 multiplets. However, use of sequence 1c applied in alternation with decoupled spin-echo leads to the observation of CH_2 multiplets only, and allows proton–carbon chemical shift correlations to be made in some difficult cases.

During the preparation of this manuscript a different method for separating carbon peaks according to their multiplicity was described.[7] It is based on a different extension of the spin-echo sequence; and seems to be more difficult to use on routine spectrometers.

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