

Doubling the Sensitivity of INADEQUATE for Tracing Out the Carbon Skeleton of Molecules by NMR

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Probably the most attractive NMR experiment for structure determination of natural products and organic molecules in general is INADEQUATE.^{1,2} This experiment excites double-quantum coherence (2QC) in ¹³C–¹³C pairs and reconverts it to observable single-quantum coherence (1QC) for detection. The two-dimensional (2D) version^{2,3} of the experiment tuned for one-bond J_{CC} coupling constants is especially appealing because the resulting 2D spectrum very clearly maps out all pairs of covalently bound carbon atoms, i.e., the carbon skeleton of the molecule.

There is usually little dispute about the basic attractiveness of INADEQUATE, but there also is little dispute about its severe drawback, namely low sensitivity at the natural abundance level. Apart from the magnetogyric ratio of ¹³C, only one molecule in more than 8000 contributes to the spectrum. It is not uncommon that a structural problem in organic chemistry could be solved elegantly by an INADEQUATE spectrum which, unfortunately, cannot be recorded with the amount of material available or because of low solubility. Therefore, the method would benefit greatly from increased sensitivity.

Our basic idea for sensitivity enhancement is quite simple. A pair of ¹³C nuclei gives rise, in the ω_2 dimension of a 2D INADEQUATE spectrum, to two doublets split by the J_{CC} coupling constant at the respective ¹³C chemical shifts. All four peaks occur at the same frequency in the ω_1 dimension, namely the sum of the two ¹³C chemical shifts. If the intensity in the ω_2 doublets could be concentrated to just one of the two lines, the sensitivity could be enhanced. If the magnitudes of the J_{CC} s are of interest, they are readily obtained by measuring the ω_2 frequency differences between the $\omega_1 = 2\omega_2$ double-quantum diagonal and the two peaks of a given ω_1 frequency. The two numbers differ by J_{CC} .

Before turning to pulse sequences, another important feature of modern multidimensional NMR spectroscopy should be considered, namely pulsed field gradients (PFGs),^{4–6} which have proven very effective for suppression of solvent signals and various artifacts. While originally applications of PFGs had a penalty of a factor of $1/2^{1/2}$ in S/N ratio, a new generation of experiments^{7–11} have emerged in which no sensitivity is lost apart from effects of relaxation and pulse imperfections. Obviously, it is worthwhile to consider this feature in the attempt to improve INADEQUATE.

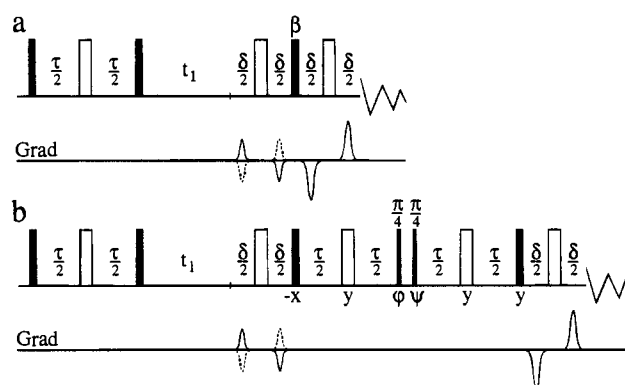


Figure 1. Pulse sequences (a) INADEQUATE with $\beta = 2\pi/3$ (echo) and $\beta = \pi/3$ (antiecho) and (b) INADEQUATE CR with “left line”, $\{\varphi = y, \psi = -x\}$ (echo) and $\{\varphi = -y, \psi = -x\}$ (antiecho) and “right line”, $\{\varphi = -y, \psi = x\}$ (echo) and $\{\varphi = y, \psi = x\}$ (antiecho). Unless otherwise indicated above the pulses, filled and open bars represent $\pi/2$ and π pulses, respectively. The delay τ is ideally equal to an odd multiple of $(2J_{CC})^{-1}$. Established INADEQUATE phase cycles can be used for INADEQUATE CR by cycling the entire mixing sequence (including the π pulses in the gradient delays) with the offsets given below the pulses, as one would do for the β pulse in (a). For example, the first experiment has all pulses of phase x except for the six pulses with another phase indicated. A four-step phase cycle $x, y, -x, -y$ of the first three pulses in concert then requires alternating the receiver phase. Extension to 16 steps is achieved by cycling the entire mixing sequence through $x, y, -x, -y$ while the receiver reference phase runs $x, -y, -x, y$. Sixty-four steps could be achieved by cycling the phase of the π pulse in the first gradient delay through $x, y, -x, -y$ at constant receiver phase.

The 2D INADEQUATE experiment most suitable for incorporation of PFGs is the one proposed by Mareci and Freeman.¹² Their pulse sequence, extended with gradients, is shown in Figure 1a; that shall be the reference experiment. Our new experiment, INADEQUATE CR (composite refocusing), which indeed achieves the objectives stated above, is outlined in Figure 1b. The experiment achieves the highest possible sensitivity set by the singular-value-decomposition bound on spin dynamics.¹³

For both pulse sequences in Figure 1, two separate data sets must be recorded with different gradients, i.e., the solid and dashed gradients following t_1 for echo and antiecho, respectively, in Figure 1. The echo and antiecho parts of the sequence in Figure 1a require $\beta = 2\pi/3$ and $\beta = \pi/3$, respectively. In contrast, the echo and antiecho parts of INADEQUATE CR differ in the phase φ , as indicated in the caption to Figure 1. Echo and antiecho data sets are combined and processed as described elsewhere.^{8–11} The details of the spin physics behind INADEQUATE CR will be described in a separate publication.

An experimental comparison between the two experiments of Figure 1 is shown in Figure 2 with spectra of menthol. Theoretically (i.e., neglecting relaxation, pulse imperfections, and possible strong coupling), the sensitivity of INADEQUATE CR is $8(6)^{1/2}/9 = 2.18$, higher than in the reference experiment. Experimentally, the sensitivity enhancement factor (measured

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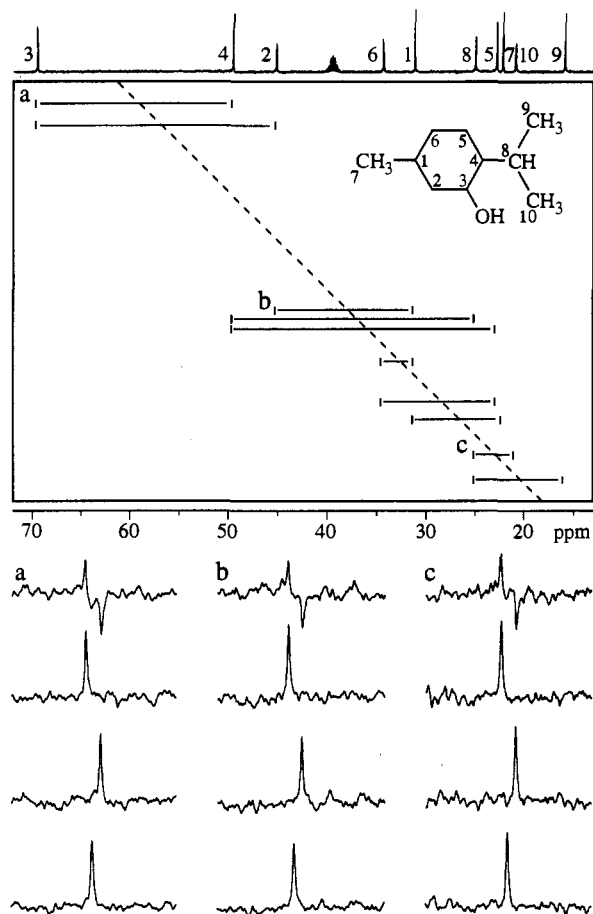


Figure 2. ^{13}C NMR spectra of menthol dissolved in $\text{DMSO}-d_6$ (300 mg in 0.7 mL), recorded on a Bruker AMX-2 400 MHz spectrometer at ambient temperature using a 5 mm probe with the innermost coil tuned for ^1H . On top is a normal 1D ^{13}C spectrum. The 2D contour plot is an INADEQUATE CR spectrum with polarization of the left doublet lines and a, b, and c referring to the traces below that stem from three different 2D spectra. The four rows of ω_2 traces are in the order INADEQUATE, INADEQUATE CR left, INADEQUATE CR right, and INADEQUATE CR merged (shifts, ± 17.1 Hz), the latter scaled down by a factor of 2. Note the phase distortions in the INADEQUATE traces, caused by J_{CC} modulation in the second gradient delay. All three spectra were recorded under identical conditions with exactly the same experimental and processing parameters: data matrix 256×8192 , apodized with a cosine square in t_1 and 6 Hz line broadening in t_2 zero-filled to 1024×16384 prior to 2D Fourier transformation; $\tau = 14.7$ ms; $\delta = 4.3$ ms; number of scans, 64; relaxation delay, 4.5 s.

as the average over all peaks) was 16% short of the theoretical value, while the selectivity as to polarizing only one of the doublet lines was excellent for all carbons. The latter fact is in accordance with a theoretical analysis showing that INADEQUATE CR is extremely robust toward mistuning of the delays, $\tau = (2J_{\text{CC}})^{-1}$, in the CR mixing sequence. For a two-spin system with J_{CC} 20% off, the intensities of the wanted and unwanted doublet lines are respectively 96.35% and 0.38% of the wanted component for perfect tuning of the delays. Even when J_{CC} is off by an unrealistic 40%, the corresponding numbers are 86.02% and 2.95%. (This does not take into account scaling by $\sin(\pi J_{\text{CC}}\tau)$ of the excitation sequence, which also scales the normal INADEQUATE spectrum.) If no gradients are employed, the spin echo parts with the gradients in the pulse sequences of Figure 1 can be left out, and in the sequence of Figure 1a, one then should use a $\beta = \pi/2$ mixing pulse. Compared to this experiment, INADEQUATE CR is theoretically exactly twice as sensitive.

An issue to be addressed in connection with INADEQUATE

CR is whether or not the fact that only one of the two doublet components occurs can lead to ambiguities. We have not been able to identify ambiguities of this type that would not also be a problem in normal INADEQUATE. However, should that occur, they could be resolved by recording both INADEQUATE CR spectra (left or right line of the J_{CC} doublets). On an equal time basis, the sensitivity of the CR approach is for that case up by a factor $8(3)^{1/2}/9$ compared to the conventional method. However, the full CR sensitivity gain of a factor $8(6)^{1/2}/9$ is recovered by merging¹⁴ the left and right CR spectra, i.e., coaddition after shifting by $J_{\text{CC}}/2$ and $-J_{\text{CC}}/2$, respectively, as shown in the bottom traces of Figure 2. When the frequency shifts correspond to $\pm J_{\text{CC}}/2$ exactly, the merged spectrum has the appearance of a homonuclear decoupled spectrum.

It has been suggested¹⁵⁻¹⁸ to enhance the sensitivity of INADEQUATE by transferring the ^{13}C - ^{13}C 2QC to ^1H 1QC for detection. That approach can, however, be problematic for unknown molecules, as connectivities from pairs of quaternary carbons will be missed when $^1J_{\text{CH}}$ is used for heteronuclear coherence transfer and occasionally missed when much less efficient $^nJ_{\text{CH}}$ is used. These sometimes crucial connectivities appear in INADEQUATE CR when proton saturation is used to build up NOE.

Nevertheless, it is of interest to estimate the relative sensitivity of ^1H - and ^{13}C -detected INADEQUATE. That must be on the basis of disregarding the quaternary pairs that are absent in the former. According to Ernst et al.,¹⁹ the relative sensitivity of ^1H and ^{13}C NMR based on one-pulse experiments, singlet lines, and identical relaxation times is $(\gamma_{\text{H}}/\gamma_{\text{C}})^{5/2} = 32$. The two INADEQUATE experiments relevant for comparison are the ^1H -detected one of Weigelt and Otting¹⁸ and the INADEQUATE CR in Figure 1b preceded by an INEPT²⁰⁻²² transfer of ^1H magnetization to ^{13}C . This INEPT transfer reduces the relative sensitivity to roughly $32/4 = 8$. In addition, the Weigelt and Otting experiment excites equal amounts of plus and minus zero-quantum coherence and $\pm 2\text{QC}$, of which only one of the two 2QC components is recovered, i.e., one-fourth of the original magnetization (ignoring relaxation and pulse imperfections). The corresponding number for INEPT INADEQUATE CR is at worst $1/2^{3/2}$ relative to a hypothetical homonuclear decoupled spectrum. (This holds for ^{13}C - ^{13}C pairs where one of the carbons is a quaternary. For pairs where both carbons are protonated the factor is $1/2^{1/2}$. That leads to a revised relative sensitivity of $8(2)^{3/2}/4 = 4(2)^{1/2}$. This number is, however, reduced further by the fact that there is no multiplet structure in INADEQUATE CR spectra, whereas the corresponding ^1H spectra usually exhibit several J_{HH} couplings, hence distributing the intensity on more resonances.

In conclusion, we have presented a novel experiment which about doubles the sensitivity of INADEQUATE. The new experiment should find widespread application for tracing out the carbon skeleton of organic molecules in isotropic liquids and possibly for extracting molecular conformational information from anisotropic liquid (e.g., liquid crystals).²³

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