New Method for NMR Signal Enhancement by Polarization Transfer, and Attached Nucleus Testing

John Homer* and Michael C. Perry

Department of Chemical Engineering and Applied Chemistry, Aston University, Aston Triangle, Birmingham, UK B4 7ET

A new NMR pulse sequence that permits signal enhancement of insensitive nuclei by polarization transfer from sensitive nuclei is presented and is illustrated by its application to ^{13}C NMR spectroscopy where resonances from ^{13}C in all chemical environments (including quaternary carbon nuclei) can be detected simultaneously.

Even with the availability of many elegant 2D NMR pulse sequences[1] 1D sequences are still most often used in routine investigations of insensitive nuclei. For ^{13}C studies the attached proton test (APT),[2] or its counterparts,[3] and distortionless enhancement by polarization transfer (DEPT)[4] techniques are widely used. Both have disadvantages. While APT enables the detection of ^{13}C in all chemical environments, it suffers from signal enhancement being limited by the nuclear Overhauser effect (NOE).[5] Although DEPT results in greater signal enhancement by polarization transfer,[1] it does not permit the detection of quaternary carbon nuclei at the same time as other carbon nuclei. Many workers choose, therefore, to undertake time-comsuming studies relying on both techniques. A sequence is described here that embodies the advantages of both techniques by enabling polarization enhancement that is nurtured during attached nucleus testing (PENDANT).

The APT sequence relies on the evolution of spin–spin coupled single quantum coherence transition magnetization vectors to achieve alternating signs of signals from quaternary, methyne, methylene and methyl group ^{13}C nuclei. Irradiation of ^{1}H nuclei can result in a significant NOE enhancement of ^{13}C resonances from the last three functional groups by a factor up to 1.998. Quaternary ^{13}C resonances normally show less enhancement. The spin–lattice relaxation times (T_1) of the observed nuclei govern the inter-pulse sequence delay (ideally $5T_1$). As T_1 for quaternary ^{13}C can be several tens of seconds, a much lower than ideal inter-sequence delay is often used so that all, particularly the quaternary ^{13}C, resonances are reduced in intensity.

Polarization transfer from ^{1}H to ^{13}C can be used both to increase ^{13}C signal intensities and transfer the dependence of the inter-sequence delay onto the shorter ^{1}H T_1s. If conventional polarization transfer to all relevant carbon nuclei is performed simultaneously, the problem of refocusing (through normal evolution delays) all spin-coupled components of individual nuclei in the same phase sense, while detecting quaternary ^{13}C resonances, might appear intractable. Undoubtedly, this problem impinged on the use of multiple quantum coherence transitions[1] in the DEPT sequence. Although this does not permit the simultaneous detection of quaternary ^{13}C, it does have the advantage of permitting polarization transfer to varying degrees so that editing of the observed spectra may be achieved with similar effect to APT.

Evidently, there is a need for a pulse sequence that results in polarization enhanced and edited spectra that contain resonances from all detectable nuclei. This is possible through the evolution of single quantum coherence transitions and the unusual polarization transfer principles implicit in PENDANT, the pulse sequence for which is as follows:

^{1}H	$\pi/2(+x)$		$\pi(+x)$		$\pi/2(-y)$		$\pi(+x)$		decouple
^{13}C	$\pi/2(+x)$	$1/4J$	$\pi(+x)$	$1/4J$	$\pi/2(-y)$	$5/8J$	$\pi(+x)$	$5/8J$	acquire
	A	B	C	D	E	F	G	H	

Although the details of this will be explained elsewhere, some features will now be explained in simplified outline, with reference to the normal NMR rotating frame of reference (z-axis, direction of polarizing magnetic field; y-axis, detection) and assuming that the frequency of the rotating frame is at the exact chemical shift frequency for each resonance.

Step A nutates all vectors to the y-axis, along which that for the quaternary ^{13}C ideally remains or is inverted by 180°. Steps B, C and D allow evolution of the coupled vectors, with chemical-shift refocusing, to dispositions suitable for polarization transfer. This is effected during step E. Contrary to apparent popular assumption, it is not necessary for the insensitive nucleus to have z-magnetization prior to polarization transfer from the sensitive nucleus, and the steps preceding E are specifically directed to result in nuclei such as ^{13}C having no initial z-magnetization before polarization transfer. During step E the ^{1}H pulse (set with *ca.* twice the length of the ^{13}C $\pi/2$ pulse for the example given later) places the proton magnetization components opposed along the z-axis and the ^{13}C pulse may, for simplicity, be considered to return the magnetization components of ^{13}C nuclei strongly coupled to protons, opposed back to the $\pm x$-axes. Steps F–H allow vector evolution to suitable orientations for chemical-shift refocusing and detection. Ignoring the small changes to the evolution angles that the preceding steps introduce, the quaternary and methylene ^{13}C vectors would become aligned along the $+y$-axis prior to decoupling and detection, with the methyne and methyl vectors at 45° to the $-y$-axis. Before

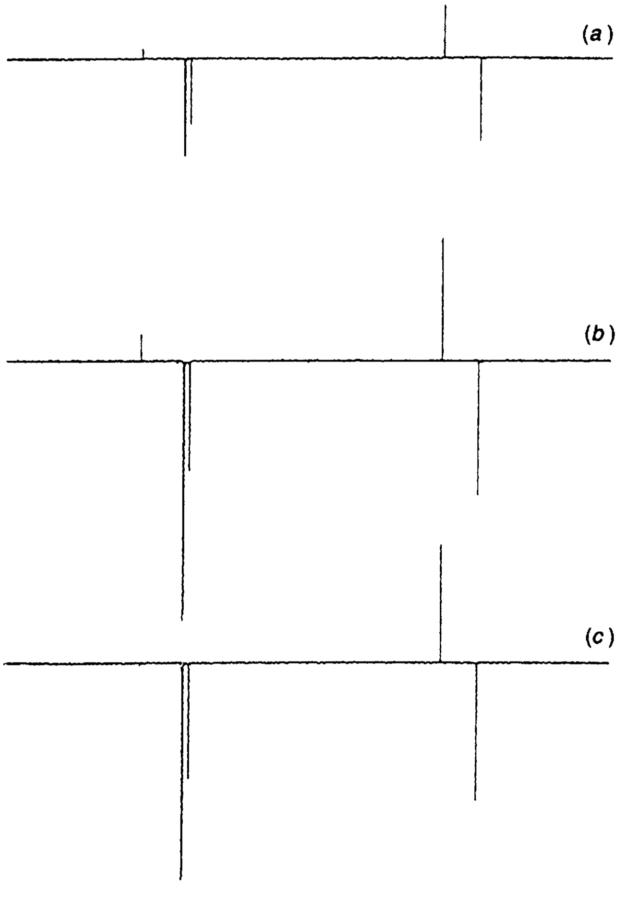

Fig. 1 Comparison of the ^{1}H-decoupled (*a*) APT (with NOE), (*b*) PENDANT (not phase cycled) and (*c*) DEPT (135°) ^{13}C spectra of ethylbenzene under equivalent conditions

decoupling the ^{13}C from the ^{1}H nuclei, the multiplet components within each resonance band are of approximately equal intensity, but in the case of the methylene resonance the central transition is suppressed. Despite this distortion of the normal coupling patterns, the decoupled spectra, that are most commonly studied, are free from distortion.

Detailed theoretical evaluation of PENDANT reveals that it should provide ratios of the resultant total signal intensities relative to the normal intrinsic signals, for the methyne, methylene and methyl ^{13}C resonances, comparable to the corresponding factors that should be achieved by DEPT under ideal conditions.

Fig. 1 shows the decoupled APT (NOE-enhanced), PENDANT and the DEPT (polarization transfer angle 135°) ^{13}C spectra of ethylbenzene obtained using a Bruker AC 300 NMR spectrometer, operating under equivalent conditions (constant amplitude and phase) in each case. PENDANT was not phase cycled, and the absence of spectral distortion demonstrates its relative insensitivity to carrier-to-shift frequency unlike in the case of the multiple quantum coherence based DEPT.[4] In each case an average value of $\langle J \rangle = 135$ Hz was used, but note that to achieve the same versatility as APT in accommodating the normal range of coupling constants when using this value of $\langle J \rangle$, the value used for PENDANT needs to be increased to 144 Hz.

PENDANT does provide all of the features mentioned earlier for a pulse-sequence alternative to both APT and DEPT in normal $\{^{1}H\}$–^{13}C studies. Of additional interest is the fact that the use of appropriate values for $\langle J \rangle$ corresponding to long-range couplings permits the improvement in the *S/N* of remote nuclei: a particularly interesting example is the case of ^{29}Si in carbasilanes when using $\langle J \rangle = 8$ Hz.[6] A significant feature of PENDANT is that it is particularly tolerant to missetting the lengths of the π and $\pi/2$ pulses involved.

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