Enhancement of the NMR Spectra of Insensitive Nuclei using PENDANT with Long-range Coupling Constants

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The recently reported NMR pulse sequence PENDANT, that enables the simultaneous detection of all insensitive nuclei (S), of a given type, in a compound and the enhancement of the signals arising from those nuclei coupled to sensitive nuclei (I), is developed further. The sequence has been modified to enable the detection of spin–spin coupled multiplets without phase distortions. Its normal form, for use with short range coupling constants, has been adapted to facilitate the S–N enhancement of insensitive nuclei through the use of long range coupling constants. The developments are illustrated by reference to $\{^1H\}$–^{13}C and $\{^1H\}$–^{29}Si spectra.

The recently reported NMR pulse sequence PENDANT[1] (polarization enhancement nurtured during attached nucleus testing) offers the synthetic chemist a method of obtaining, *e.g.*, ^{13}C NMR spectra that exhibit all of the beneficial features of the now classical sequences such as APT,[2] INEPT[3] and DEPT,[4] and some benefits of more advanced sequences such as SEMUT.[5] However, not only does it yield spectra showing alternating phases of isolated (quaternary), CH, CH_2 and CH_3 carbon resonances but it also enables the simultaneous detection of all of these resonances, unlike INEPT and DEPT. Moreover, for the last three resonance types it provides enhancements comparable to those obtained using INEPT and DEPT.

A superficial comparison of PENDANT and the refocussed version of INEPT[6] (a comprehensive discussion of this and the other classical sequences referred to above has been presented by Ernst *et al.*)[7] could result in the mistaken belief that the former differs from the latter sequence simply by the addition of a single S (*e.g.* ^{13}C), pulse. In fact the extra initial pulse (*a*) completely changes the spin dynamics in PENDANT relative to refocussed INEPT, (*b*) results in a totally different polarization transfer step that ultimately allows the detection of isolated insensitive nuclei at the same time as those attached to sensitive nuclei and (*c*) through the use of the final $5/8J$ rather than $3/8J$ evolution delays results in some detected magnetization components moving towards the detection axis rather than away from it and makes the choice of the compromise average value of $\langle J \rangle$ less critical.

In its basic form PENDANT yields well phased {I}decoupled S spectra. However, the corresponding coupled spectra reveal phase distortion of individual multiplet components. The present paper explains how to remove these phase distortions, how to use the sequence to enhance the resonances of NMR insensitive nuclei through the use of long range coupling constants and provides some comparisons of PENDANT with INEPT and DEPT.

Symmetrization of PENDANT. Although PENDANT is applicable to any insensitive nucleus (coupled to sensitive nuclei), reference will be made to ^{13}C coupled to ^1H for the initial discussion. The basic PENDANT sequence is, ignoring the bold features in brackets,

^1H (I) $\pi/2(+x)$ $1/4J$ $\pi(+x)$ $1/4J$ $\pi/2(-y)$ [**A**] $5/8J$ $\pi(+x)$ $5/8J$ (decouple)

^{13}C (S) $\pi/2(+x)$ $1/4J$ $\pi(+x)$ $1/4J$ $\pi/2(-y)$ [**B**] $5/8J$ $\pi(+x)$ $5/8J$ [**C**] acquire $(+y)$

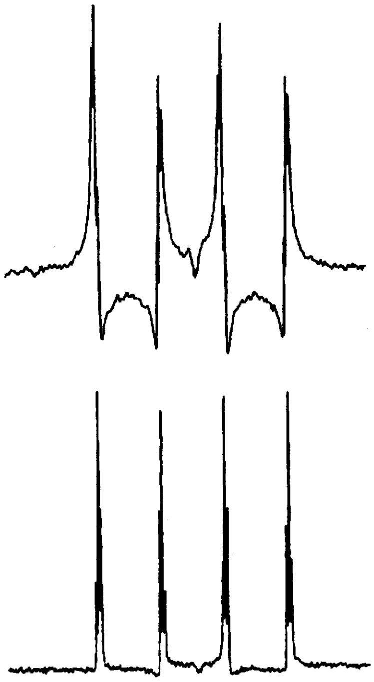

Fig. 1 Directly coupled ^{13}C PENDANT spectra of the CH_3 group in ethylbenzene (*a*) original and (*b*) revised versions

where $+y$ is the detection phase in the normal rotating frame that has $+z$ as the direction of the polarizing magnetic field, B_0.

When an average value of the spin-coupling constant $\langle ^1J_{CH} \rangle$ of around 135–145 Hz is used, the quaternary and CH_2 magnetizations yielding the coupled spectra roughly parallel the $+y$ axis. However, the components of the CH and CH_3 resonance magnetizations lie at significant angles (ideally $\pi/4$) to the $-y$ axis. It is because of the latter fact that the multiplet components show phase distortion, although, because components symmetrically disposed about the chemical shift centre have essentially mirror image phase distortions [see Fig. 1(*a*)], the decoupled spectra are well phased (all spectra presented herein were obtained using a Bruker AC 300 multinuclear spectrometer). Naturally, in order to provide optimum analytical value, PENDANT should be capable of use in the coupled mode to yield no phase distortion of the multiplet components. This can be achieved readily in two ways.

First, the basic PENDANT multiplets can be symmetrized.

Various methods of reducing phase distortions in coupled spectra have been proposed and are discussed by Ernst *et al.*[7] Of these, that of particular relevance here is the use of ^{1}H π pulses on alternate scans to reduce phase distortions in DEPT. PENDANT benefits from an extension of the principles implicit in this approach. In the absence of phase cycling (possible through an adaptation of standard phase cycling for refocussed INEPT), the approach is to arrange for each multiplet component to be relabelled in frequency terms and components with the original frequencies detected in the alternative quadrant (*e.g.*, $+y$, $-x$ instead of $+y$, $+x$). These manipulations are readily achieved by making $[\mathbf{A}] = [\mathbf{B}] = [\mathbf{C}] = \pi(+y)$, and result in the detection of distorted coupled spectra that are the mirror image of the basic PENDANT sequence. If FIDs (free-induction decays) from the latter and from the modified sequence are superimposed repeatedly the transformed spectra are symmetrical and without phase distortion. This is illustrated in Fig. 1(*b*) which was obtained, like that in Fig. 1(*a*), for the CH_3 carbon in ethylbenzene with the rotating frames of both ^{1}H and ^{13}C exactly at the appropriate resonance frequencies and with the precise value of ${}^1J_{CH}$ being used: an intersequence relaxation delay of 80 s was used to ensure that ^{1}H relaxation to equilibrium was complete.

If ideal experimental conditions such as those just mentioned could be enforced on all resonances in a typical spectrum the multiplet appearance of the coupled resonances would appear as: CH, doublet with J spacing; CH_2, main doublet with 2 J spacing and with a small central absorption; CH_3, quartet with equal intensity J spaced components. In practice, the appearance of the CH_3 multiplet depends on the chemical shift to rf (radio frequency) carrier frequency difference for both ^{1}H and ^{13}C, as well as the actual coupling constants. Nevertheless, when using normal compromise experimental conditions the multiplet remains symmetrical, with relative intensities around 2:1:1:2: the appearance of the CH and CH_2 ^{13}C resonances change little from the ideal.

A second, and probably more beneficial, method of symmetrizing the detected PENDANT multiplets is to add a ^{1}H π/2 'purging' pulse immediately before acquisition as in the INEPT$^+$ sequence.[8] This has the effect of restoring the true binomial intensities to the detected multiplet components.

Detection of 'Isolated' Insensitive Nuclei Using PENDANT with SHORT-range Coupling Constant Values.—It is particularly worthy of note that the routine use of PENDANT using short range couplings has revealed many cases where the signal intensities obtained for quaternary ^{13}C are significantly greater than those obtained by direct detection. A probable explanation for this may lie in the fact that few quaternary carbons are truly isolated from the rest of the spin system so that long range couplings may afford partial polarization transfer, even though PENDANT is used with a typical short range coupling constant value. What must be recognized is the fact that other ^{1}H nuclei may couple with that which is long range coupled to the ^{13}C. For example, consider the situation in which a ^{1}H doublet arises from a long range J_{CH} coupling of 20 Hz and another proton couples directly with the first proton with $J_{HH} = 10$ Hz. In this case the outer and inner multiplet resonances of the proton that is long-range coupled to the ^{13}C will be 15 and 5 Hz, respectively, from the ^{1}H shift frequency. Consequently, if PENDANT is used with a short range coupling of 135 Hz, the magnetization components can evolve through angles of 20° and 6.6° and partial polarization transfer could be possible. To check this in practice it is necessary to compare PENDANT intensities with those in a 'direct' detect experiment and ensure that in both cases the evolution times are the same and the long range coupled ^{13}C multiplets lie at the same angles to the detection axis. In order to approximate these requirements the 'direct' detect spectra can be obtained from PENDANT by omitting the first 90° $(+x)$ ^{1}H and the two simultaneous 90° $(-y)$ ^{1}H and ^{13}C polarization transfer pulses. An appropriate comparison is shown in Fig. 2 for the quaternary carbon of ethylbenzene. The significantly greater intensity derived from PENDANT over the sequence with the polarization transfer removed is clearly evident.

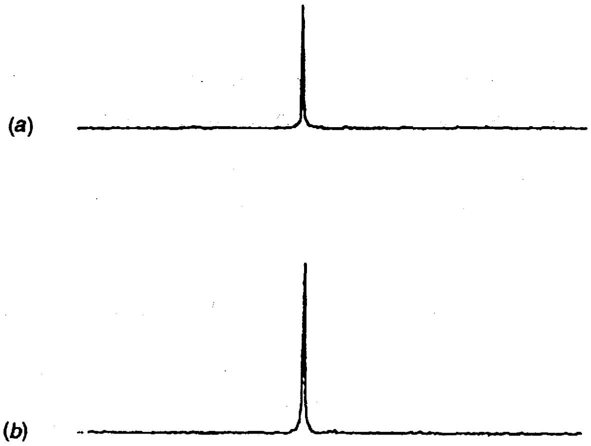

Fig. 2 Comparison of the ^{13}C quaternary carbon resonance of ethylbenzene obtained using (*a*) PENDANT with the polarization transfer step removed, and (*b*) PENDANT, both with $\langle J \rangle = 135$ Hz

Evidently, when a single PENDANT experiment is used to obtain resonances from 'isolated' insensitive nuclei and those directly coupled to sensitive nuclei this offers immense savings in time over INEPT or DEPT, which both have to be combined with other separate experiments to enable the detection of the 'isolated' nuclei.

Use of PENDANT with Long Range Couplings.—Although pulse sequences such as PENDANT, INEPT and DEPT, that involve polarization transfer from sensitive to insensitive nuclei, are normally used with short-range coupling constants, they can in principle be used with long-range coupling constants. However, it is known that this method of use is not efficient. Necessarily, in such a case, if the coupling constant is of the order of 20 Hz and an evolution angle of say 90° is required for each component of a simple J split doublet, this would require an evolution time of *ca.* 0.025 s. This, being nearly an order of magnitude greater than that appropriate to short-range couplings of *ca.* 145 Hz, reduces the efficiency of the classical pulse sequences. An additional fact, that appears not to be widely appreciated, is that when an insensitive nucleus is long-range coupled to more than three equivalent sensitive nuclei and the sensitive and insensitive nuclei are set at exact resonance and the precise long range coupling constant is used with INEPT and PENDANT, multiplet components can cancel. On decoupling the sensitive from the insensitive nuclei little if any signal from the latter can be observed. This can be demonstrated readily for the ^{29}Si resonance of Me_4Si using $J = 6.7$ Hz. However, if, as in routine use, the nuclei are moved away from exact resonance some signal can be observed. The signal intensities obtained using INEPT (or DEPT) with long range coupling constants can be increased dramatically by increasing the value of J above the true value.

On the other hand, PENDANT is sufficiently adaptable to enable the problems mentioned to be overcome more simply. In order to illustrate this, attention is drawn to the final $5/8J$ $\pi(+x)$ $5/8J$ section of the PENDANT sequence.

For a multiplet component that is separated from the chemical shift position by $nJ/2$, where n is an integer, the final total evolution time of $5/4J$, neglecting the final refocussing

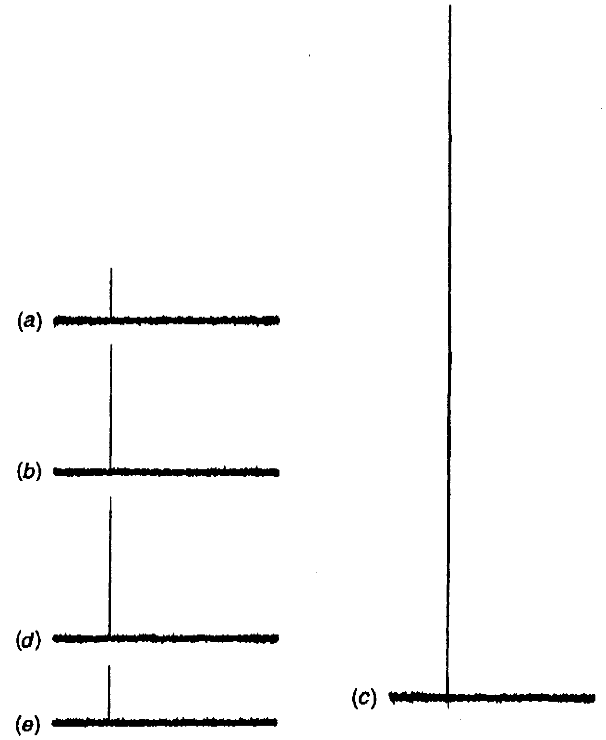

Fig. 3 ^{29}Si spectra of tetramethylsilane obtained using PENDANT with final evolution delays of (*a*) 3/8J, (*b*) 5/8J and (*c*) 5/80J, and using INEPT with final evolution delays of (*d*) 5/8J and (*e*) 3/8J

pulses, in the original PENDANT sequence allows the magnetization vector to evolve through angles of n225°. Thus, *e.g.*, a high frequency multiplet component with $n = 1$ would be detected 45° to the $+y$ axis and a high frequency component with $n = 5$ would be detected at 45° to the $-y$ axis. This results in the two disadvantages alluded to above. First, the coupled spectrum will show components having opposed phases, even after the removal of distortion. Secondly, the decoupled signal will lose much of its intensity through the cancellation of signals, even under off-resonance conditions. These problems can be overcome quite simply by reducing the 5/8J evolution delays to 5/80J. The evolution angles of multiplet components now become n22.5°. Consequently, if the sequence is modified in this way n can be as high as eight before opposing phases of multiplets are seen or indeed there is any loss of signal on decoupling: in other words multiplets containing components separated from the shift centre by less than 4J can readily be detected without disadvantage and will have the same phase as the still detectable signal from a resonance that has no coupling to any other nuclei. In other words the final 5/8J delays in PENDANT should be changed to 1/16J when using long range coupling constants. The $\{^1H\}$–^{29}Si PENDANT spectra (eight scans) in Fig. 3 reveal the advantage of changing the final delays from 5/8J to 1/16J: similar observations are found when using INEPT, for which the advantage mentioned earlier of using 5/8J rather than 3/8J is also clearly demonstrated. Similar benefits apply to the detection of quaternary ^{13}C and Fig. 4 illustrates this for the quaternary carbon of ethylbenzene. Fig. 4(*a*) shows the normal PENDANT resonance while Fig. 4(*b*) presents that obtained using the 1/16J delays to enable detection of the result of polarization transfer to the ^{13}C nucleus: both experiments were conducted on resonance and with the same non-optimized average value of J of 8 Hz.

Evidently, the use of PENDANT with long-range coupling constants facilitates the targetted search for appropriate nuclei that otherwise yield weak resonances in routine investigations.

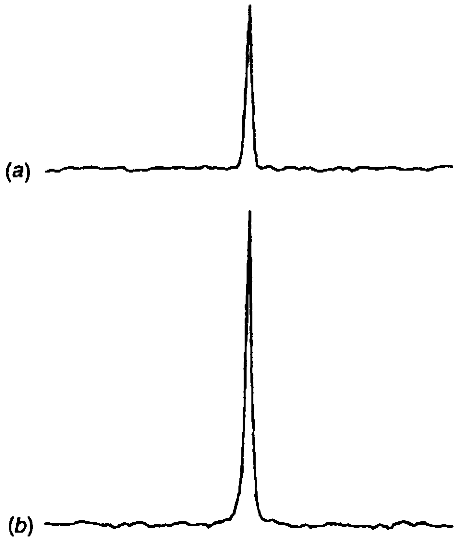

Fig. 4 ^{13}C PENDANT spectra of the *ipso*-carbon in ethylbenzene obtained using (*a*) short-range and (*b*) long-range coupling constants

Procedure for Optimizing PENDANT for use with Long-range Coupling Constants.—Although the optimization of polarization transfer sequences for long-range coupling in the presence of relaxation can be complex,[9] the optimization of PENDANT can be simplified in practice.

The experimental factors that govern the efficiency of PENDANT when used with long range couplings are the calibration of the ^{1}H (I) and ^{13}C (S) 90° and 180° pulse widths, and the average values of J. Although the ^{1}H pulse widths can be readily calibrated it may be less easy to do this for some insensitive nuclei. Fortunately, this does not present any great problem because PENDANT is particularly tolerant to mis-calibrated 90° and 180° pulse widths and will accommodate errors of the order of 15%, unlike DEPT which requires precise calibration. It is, however, important for the efficiency of the polarization transfer step to ensure that the 90° pulse time for ^{1}H is approximately twice that for the insensitive nucleus. Having set appropriate pulse times, a suitable average value of J should be estimated. On detecting a signal the value used in 1/16J should be left constant while that used in 1/4J varied to accommodate off-resonance effects until maximum (polarization transfer) signal intensity is obtained: often the optimum value of $\langle J\rangle$ is found to be different from the true value of J and investigations have shown that values of J in the range 10–16 Hz serve as a convenient starting point for signal optimization in PENDANT studies of ^{29}Si $\cdots$ ^{1}H, ^{15}N $\cdots$ ^{1}H and ^{13}C $\cdots$ ^{1}H. Optimization of the insensitive nucleus 90° pulse width is then straightforward.

In order to illustrate further the various points made above, ^{29}Si spectra (constant phase and amplitude) of a mixture of cyclic carbasilanes shown in Fig. 5 were obtained. Fig. 5(*a*) is the PENDANT spectrum obtained with eight scans using an intersequence delay of 3 s, and $\langle J\rangle = 16$ and 8 Hz in the first and second set of evolution delays respectively. Fig. 5(*b*) is the corresponding NOE suppressed (^{29}Si having a negative NOE when ^{1}H is irradiated) single pulse (90°) or 'direct' detect spectrum. After having established, through inversion recovery experiments, that the longest ^{29}Si T_1 in the sample was 16 s, the single pulse (90°) NOE suppressed spectrum in Fig. 5(*c*) was obtained using an intersequence delay of 80 s. Fig. 5(*d*) shows the result of using DEPT (135°): the variation of $\langle J\rangle$ between 4 and 20 Hz produced little variation in the results. This does not mean, of course, that DEPT cannot be used for studies that depend on long-range coupling constants, but to optimize these for the carbasilane mixture it proved beneficial to increase J to

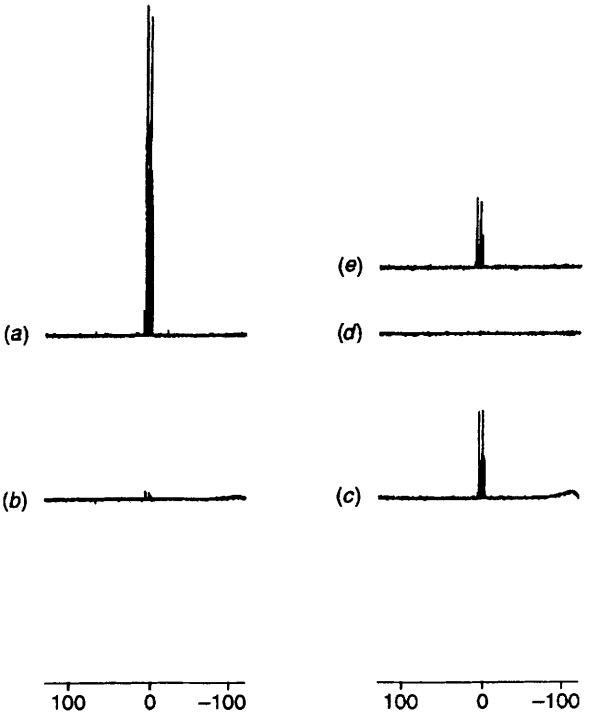

Fig. 5 ^{29}Si NMR spectra of a mixture of six and eight membered cyclic carbasilanes under equivalent experimental conditions: (*a*) PENDANT, (*b*) NOE suppressed, both with 3 s relaxation delay, (*c*) NOE suppressed, with 80 s relaxation delay, (*d*) DEPT (135°) and (*e*) DEPT with $\langle J \rangle$ increased to 40 Hz, both with 3 s relaxation delay. Chemical shifts are relative to Me_4Si.

ca. 40 Hz [see Fig. 5(*e*)]. Similar observations were made when using INEPT.

Figs. 5(*a*), (*b*) and (*d*) illustrate the advantages of PENDANT over both single pulse NOE suppressed and DEPT procedures under equivalent conditions. Figs. 5(*a*) and (*c*) reveal the significant savings in time that are to be gained by the use of the modified version of PENDANT rather than the other methods.

One particularly interesting feature of Fig. 5 is that unlike the NOE suppressed spectra [(*b*) and (*c*)] the PENDANT, (*a*), spectrum does not reveal a ^{29}Si resonance from the glass NMR sample tube used. As is to be expected the more intense signal from the glass in Fig. 5(*c*) compared with that in Fig. 5(*b*) is attributable to the much longer relaxation delay used in the former case. The reason why the PENDANT spectrum beneficially yields little signal from the glass originates from the use of only 90° and 180° pulses in the sequence. If spin–lattice relaxation of the ^{29}Si occurs along the z-axis during the first evolution and refocussing period, the 90° $(-y)$ pulse returns it to the $-x$ axis where it cannot be detected. Of more importance is the fact that developing magnetization during the second evolution and refocussing period is inverted to the $-z$ axis so that its recovery to equilibrium along the $+z$ axis is retarded: successive transients, therefore, progressively minimize the observable signal.

A second interesting feature is the absorption intensities in Figs. 5(*a*) and (*c*). In the latter case a relaxation delay of $\approx 5T_1$ was used so that the intensities can be used quantitatively. It is noticeable that the relative intensities in the PENDANT spectrum [Fig. 5(*a*)] correspond closely with those in Fig. 5(*c*). In the case of the specific sample (air saturated) studied here, the T_1s of the protons to which the ^{29}Si relaxations are transferred will all be similar and the relative intensities of the various absorptions become quantitatively meaningful. No doubt, in other appropriate compounds, PENDANT relative intensities may be used in a directly quantitative fashion, but such use should not be used indiscriminately.

Conclusions

Additions to the basic PENDANT sequence have been devised that enable it to produce NMR spectra of insensitive nuclei (coupled to sensitive nuclei) without phase distortion of the multiplet components of spin–spin coupled resonances. Additionally, the sequence has been adapted successfully to enable the detection of phase undistorted spectra of insensitive nuclei through the use of long-range couplings. The potential of the latter development is revealed through the ability to obtain a ^{29}Si spectrum of a 0.045 mol dm^{-3} [$Si(CH_3)_2$ repeat unit] solution of polydimethylsilane that was obtained with 1000 scans with an intersequence delay of 3 s. A further advantage of this method is that the ^{29}Si signal from glass is suppressed.

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