

Distortionless Enhancement of NMR Signals by Polarization Transfer

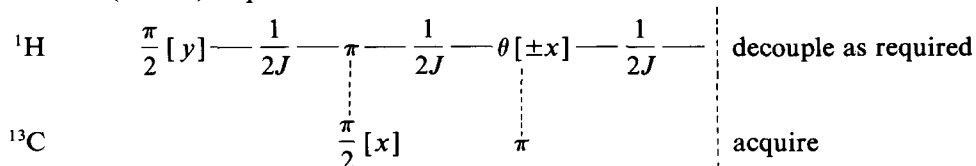
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Carbon-13 proton-polarization transfer signals generated by the extended INEPT pulse sequence have the following deleterious characteristics (1, 2). (a) There is a gross distortion of relative intensities of the coupled spectra compared with that observed using standard FT NMR. For example, a CH₂ 1:2:1 triplet becomes an enhanced 1:0:1 multiplet with phases dependent on the free-precession time before acquisition. (b) Signal enhancement in coupled and decoupled spectra varies depending on the free-precession times between pulses and the ¹³C-¹H coupling constant. The latter vary between different CH_n groups and thus generation of methine (CH), methylene (CH₂), or methyl (CH₃) subspectra using INEPT (3) will be inexact (4).

In this paper we briefly describe the distortionless enhancement by polarization transfer (DEPT) sequence



and note the following characteristics of the DEPT sequence. (a) CH carbon signals exhibit maximum enhancement of γ_H/γ_C at $\theta = \pi/2$, CH₂ carbons exhibit a maximum enhancement at $\theta = \pi/4$, and CH₃ carbons a maximum enhancement of $1.15\gamma_H/\gamma_C$ at $\theta = 0.196\pi$, respectively. (b) For coupled spectra, peak intensities are observed with their characteristic normal ratio and the lines have the same phases as in the normal FT spectrum. For example, a CH₃ (1:3:3:1) quartet becomes an enhanced 1:3:3:1 quartet, as shown in Fig. 1. The enhancement can be varied uniformly, i.e., the same for each line within a multiplet, by adjusting θ . (c) The variation with θ for CH, CH₂, and CH₃ have the following mathematical dependencies: $\sin \theta$ for CH; $\sin 2\theta$ for CH₂; and $\sin \theta + \sin 3\theta$ for CH₃. As a consequence, CH₂ and CH₃ signals are zero when $\theta = \pi/2$ and CH₂ signals are inverted by changing θ from $\pi/4$ to $3\pi/4$; CH and CH₃ signals are unaffected. These dependencies can be used to generate individual CH, CH₂, and CH₃ subspectra by appropriate combinations of spectra recorded at $\theta = \pi/4, \pi/2, \text{ and } 3\pi/4$, as in Fig. 2. This subspectral editing is more exact than for INEPT because of the dependence

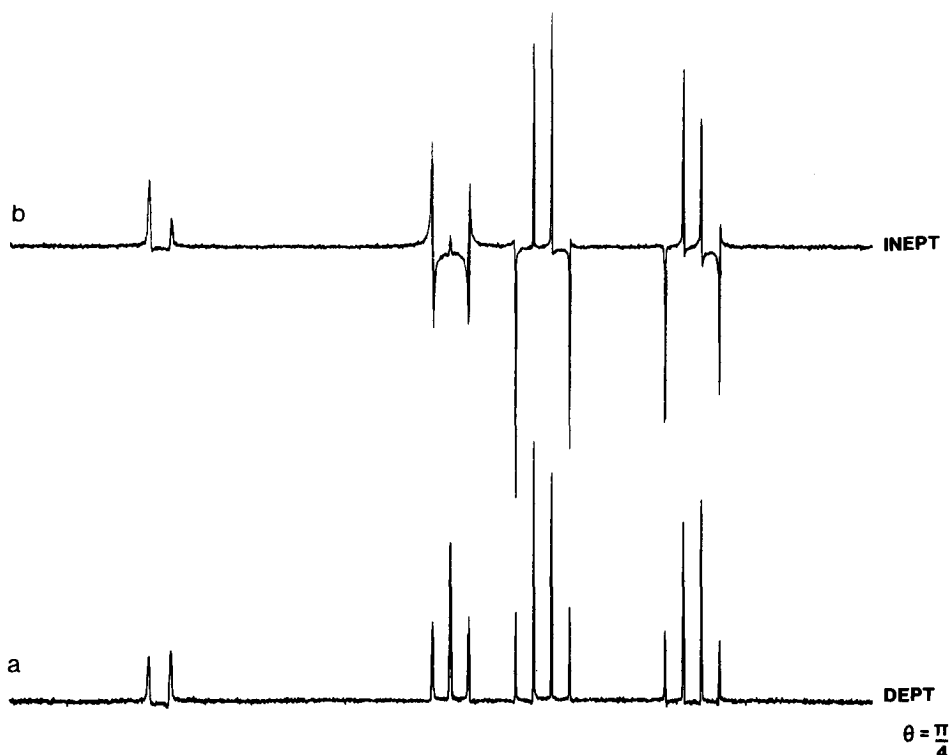


FIG. 1. (a) Proton-coupled DEPT spectrum of 2-chlorobutane with $(2J)^{-1}$ set at 3.9 msec. 40 scans have been averaged with a spectral width of 6024 Hz. (b) Proton-coupled INEPT spectrum of 2-chlorobutane with the final free-precession period set at $(2J)^{-1}$ as above. This time should produce the following phase characteristics for CH, CH₂, and CH₃ groups: CH signals in phase, phase defined here at 0°; CH₂ "doublet" with one line shifted by 90° and the other by 270°; CH₃ quartet with inner lines at 0° and outer at 180°. There are clearly instrumental phase errors which are more serious for INEPT than for DEPT.

on the magnitude of θ for DEPT rather than a dependence on the magnitude of a free-precession period (and thus ${}^1J_{\text{H-}^{13}\text{C}}$) as in INEPT. In practice, because of inhomogeneity in the θ pulse, compensation by combining spectra in ratios slightly different from the theoretical needs to be employed. The fact that we have achieved good editing implies that this compensation can be allowed for in a uniform manner. This is because the θ pulse homogeneity depends on the coil geometry of the spectrometer probe and is constant for all groups in a sample and for different samples. The experimental parameters noted in Fig. 2 are thus constant for different samples. (d) Coupled CH, CH₂, and CH₃ subspectra may be obtained in the same way. This is not possible using the INEPT sequence. (e) The DEPT pulse sequence employs two fewer pulses than the INEPT sequence with waiting time Δ , and because the accuracy of any pulse sequence increases as the number of pulses is reduced, we expect DEPT to be superior. This is evident by the better phase relationships in the coupled DEPT spectrum compared to the coupled INEPT spectrum, Fig. 1.

To understand how this novel polarization-transfer pulse sequence works it is

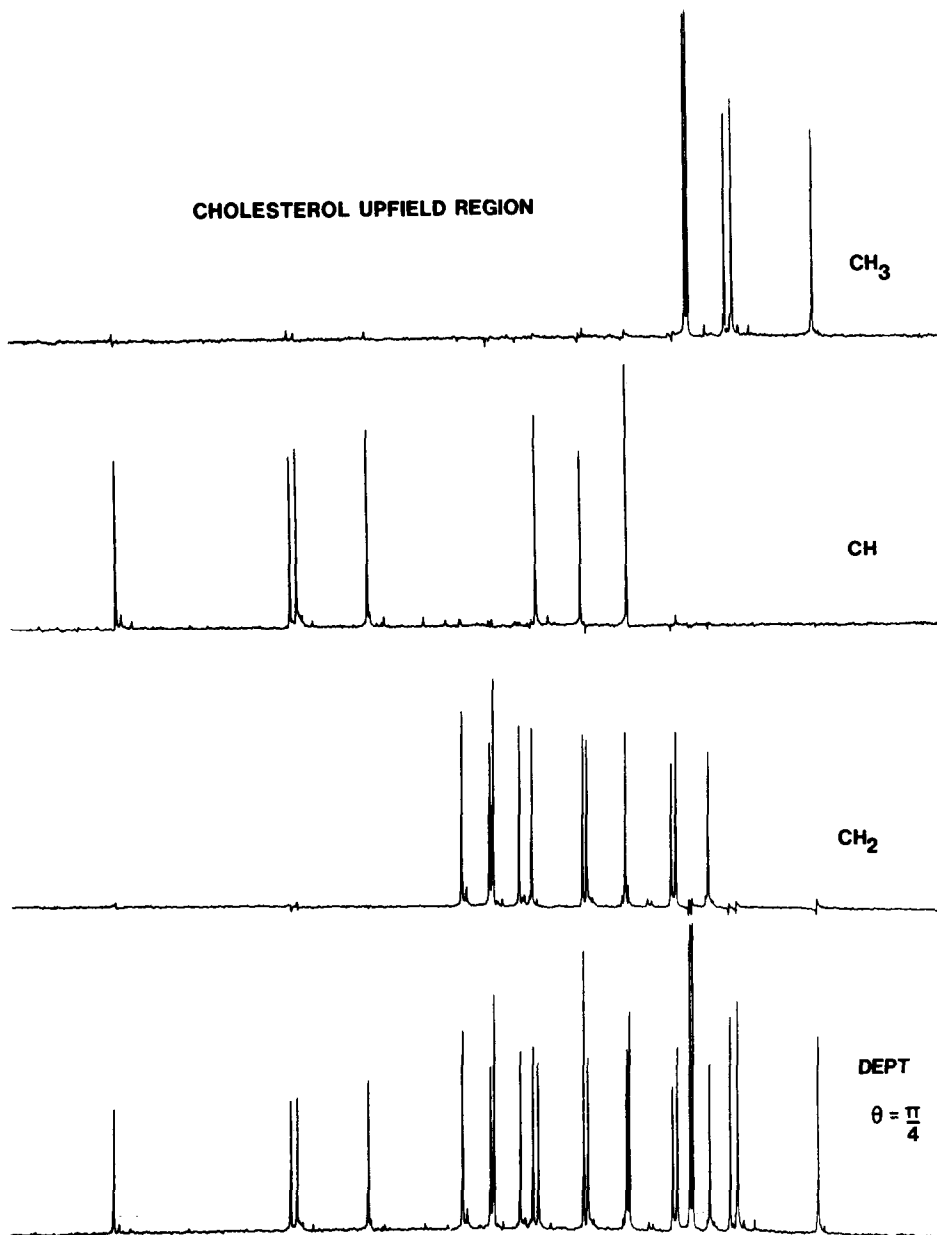


FIG. 2. Three DEPT spectra of a 0.5 M CDCl_3 solution of cholesterol were determined at $\theta_1 = \pi/4$, $\theta_2 = \pi/2$, and $\theta_3 = 3\pi/4$. 100 scans were averaged with a spectral width of 6024 Hz. The recycle time was 5 sec. An average $^1J_{\text{H-}^{13}\text{C}}$ value of 135 Hz was assumed in setting the $(2J)^{-1}$ time. The θ_1 spectrum is shown. The CH, CH₂, and CH₃ subspectra (also shown) were generated by the respective combinations $[\theta_2 - z(\theta_1 + x \cdot \theta_3)]$, $(1/2)(\theta_1 - x \cdot \theta_3)$, and $[(1/2)(\theta_1 + x \cdot \theta_3) - y \cdot \theta_2]$, where the experimental parameters, x , y , and z were determined by obtaining the optimum cancellation of unwanted signals in the subspectra. The spectra were obtained using a standard 10-mm broadband probe on a Bruker CXP-300 spectrometer for which $x = 1.15$, $y = 0.75$, and $z = 0.03$ compared to the theoretical values of 1.00, 0.71, and 0.00, respectively.

appropriate to first consider the refocusing (π) pulses. Because of the beautiful symmetry of the DEPT sequence only two refocusing pulses are required to eliminate the effects of chemical shift off resonance. Each refocusing pulse is applied at the middle of the period in which the respective heteronuclear spins are in the rotating plane. These pulses do not refocus the scalar-coupled interactions because they do not occur during any of the $(2J)^{-1}$ sec periods. Secondly, the phase of the second $\pi[H]/2$ pulse is alternated and the signals from alternate scans are subtracted to eliminate the normal ^{13}C magnetization signals in the same way as for INEPT (3).

To consider the mechanism in detail it is instructive to consider its application to a CH spin system. For simplicity, we assume that all pulses are on resonance and thus the refocusing pulses can be ignored. Furthermore we will ignore the ^{13}C magnetization and thus the alternation of the second $\pi[H]/2$ pulse.

In the first $(2J)^{-1}$ period the Boltzmann-excess proton spins are split into two equally populated groups, depending on whether the coupled ^{13}C spins are up or down, and at the end of this period the protons in one group are along the $+y$ axis, the others along the $-y$ axis of the doubly rotating reference frame. The $\pi[C]/2$ pulse then places each ^{13}C spin in alignment with its coupled proton, that is, relative to the y axis the CH molecules are equally split into states $|+, +\rangle$, and $|-, -\rangle$, where the sign before the comma refers to the ^{13}C state, and after the comma to the associated proton state. During the second $(2J)^{-1}$ period the states evolve to the superpositions $2^{-1/2}|+, +\rangle + i2^{-1/2}|-, -\rangle$ and $2^{-1/2}|-, -\rangle + i2^{-1/2}|+, +\rangle$, respectively. Note the coherence involved here: If a proton is found in state $|+\rangle$ or $|-\rangle$ then the coupled ^{13}C must also be found in the same state, that is, the amplitude of the state $|+, -\rangle$ is zero, implying no single spin flips. The final $(2J)^{-1}$ period will merely change the phase of the signal components arising from ^{13}C spins precessing relative to the rotating frame in a direction determined by whether the attached proton is found parallel or antiparallel to the z axis after the $\theta[H]$ pulse, that is, whether it is found parallel (\uparrow) or antiparallel (\downarrow) to an axis at angle $\phi = 90 - \theta$ to the y axis just before the $\theta[H]$ pulse. When the above superpositions are rewritten in terms of the basis proton states $|\uparrow\rangle$ and $|\downarrow\rangle$ along this axis they become, respectively,

$$2^{-1/2}\left(\cos \frac{1}{2} \phi |+\rangle - i \sin \frac{1}{2} \phi |-\rangle\right)|\uparrow\rangle + 2^{-1/2}\left(\sin \frac{1}{2} \phi |+\rangle + i \cos \frac{1}{2} \phi |-\rangle\right)|\downarrow\rangle \quad [1]$$

and

$$2^{-1/2}\left(i \cos \frac{1}{2} \phi |+\rangle - \sin \frac{1}{2} \phi |-\rangle\right)|\uparrow\rangle + 2^{-1/2}\left(i \sin \frac{1}{2} \phi |+\rangle + \cos \frac{1}{2} \phi |-\rangle\right)|\downarrow\rangle, \quad [2]$$

where $|+\rangle$, $|-\rangle$ are the carbon states still relative to the y axis.

Now in general, the state

$$\cos \frac{1}{2} \alpha |+\rangle + i \sin \frac{1}{2} \alpha |-\rangle$$

represents a carbon nucleus in a state in the x - y plane at angle α to the y axis.

Thus from Eqs. [1] and [2] the carbon spins associated with protons in state $|\uparrow\rangle$ are at angles $-\phi$ and $+\phi$ to the y axis, giving a total upfield signal component proportional to $\cos \phi = \sin \theta$. Similarly the net downfield signal vector is in the opposite direction and is also proportional to $\sin \theta$. The final $(2J)^{-1}$ period brings these equal signals into phase, giving a total of ^{13}C signal proportional to $\sin \theta$, but enhanced by $\gamma_{\text{H}}/\gamma_{\text{C}}$.

Similar, but more complicated theory which will be presented elsewhere shows as above that the CH_2 and CH_3 signals have their normal 1:2:1 and 1:3:3:1 ratios and have magnitudes proportional to $\sin 2\theta$ and to $\sin \theta + \sin 3\theta$.

Note the correspondence of the above expressions with those for the decoupled signal obtained from the INEPT sequence after waiting a period Δ (2). In fact, the replacement of θ by $\pi J\Delta$ gives exact correspondence. Also the overall enhancements of the decoupled INEPT signals are the same as for *each* line of the *coupled* multiplet following the DEPT sequence.

It is clear that the DEPT sequence overcomes some important limitations of the INEPT pulse sequence while retaining the inherent advantages of polarization-transfer NMR spectroscopy. In particular, DEPT will find widespread use in generating enhanced coupled spectra, spectral editing, and signal-to-noise enhancement.

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