J. Am. Chem. Soc. **1983**, *105*, 7523-7527

$^{13}C\{^{2}H\}$ Polarization Transfer Studies: New NMR Methods for Isotopic Labeling Studies

Peter L. Rinaldi* and Nicholas J. Baldwin

Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106.

Received May 9, 1983

Abstract: A new methodology for NMR labeling studies is described which makes advantageous use of both the ease and inexpense of ^{2}H incorporation and the large ^{13}C chemical shift range for later identification of the ^{2}H label's position in the molecule. Selective excitation of ^{13}C NMR resonances of deuterated carbon atoms is obtained by transfer of the ^{2}H spin polarization to ^{13}C via special NMR pulse sequences. Experimental details are provided for using $^{13}C\{^{2}H\}$ insensitive nuclei enhanced by polarization transfer (INEPT) and distortionless enhancement by polarization transfer (DEPT) NMR experiments to observe CD, CD_2, and CD_3 resonances.

Isotopic labeling studies are commonly used to elucidate mechanistic and structural features in chemistry and biochemistry, either as a tracer or as a structural probe for a selected environment in a large molecule. One method by which this has been accomplished is by selectively labeling sites of interest with enriched NMR active nuclei such as ^{13}C[1] or ^{2}H.[2] Although ^{2}H is generally easier than ^{13}C to incorporate into organic molecules and ^{2}H labeled reagents are much less expensive, ^{2}H spectra of isotropic systems have the disadvantage of much smaller NMR chemical shift dispersion.

In a previous paper,[3] a new strategy was demonstrated for performing isotopic labeling studies of selected sites, which takes advantage of both the ^{13}C chemical shift dispersion and the expediency of ^{2}H incorporation. In those cases where ^{2}H labeling of a site is easier, polarization transfer from ^{2}H to ^{13}C via an INEPT (insensitive nuclei enhanced by polarization transfer) pulse sequence[4,5] provides the needed selectivity to observe only the resonances of the ^{13}C nuclei with directly attached ^{2}H.

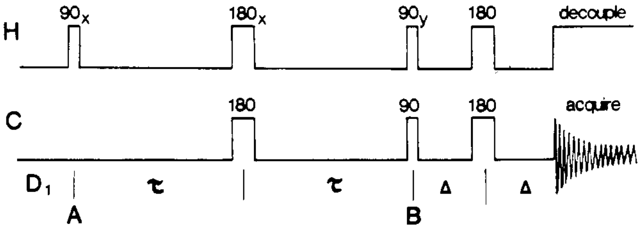
In this paper, experimental considerations necessary for implementing the $^{13}C\{^{2}H\}$ INEPT experiment for observation of CD, CD_2, and CD_3 are described, the parallels between $^{13}C\{^{1}H\}$- and $^{13}C\{^{2}H\}$-INEPT are discussed, and the utility of a new polarization transfer experiment, distortionless enhancement by polarization transfer (DEPT),[6] for observation of ^{13}C bound to ^{2}H is demonstrated.

Results and Discussion

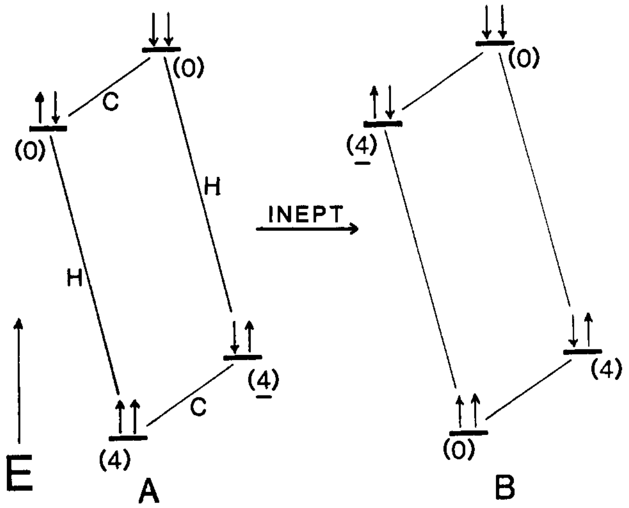
The utility of the $^{13}C\{^{2}H\}$ INEPT experiment is illustrated by the spectra of 1-phenylethanol (PE). The 200-MHz ^{1}H spectrum of PE and the 30.7-MHz ^{2}H spectrum of PE-d_5 (aryl position deuterated) are shown in Figure 1a,b, respectively. The chemically distinct ortho, meta, and para protons or deuterium nuclei have very similar chemical shifts in their respective spectra, even at relatively high field (4.7 T). This makes it impossible to identify the position of a single label from a standard one-pulse NMR experiment.

The selectivity of the $^{13}C\{^{2}H\}$ INEPT experiment is demonstrated in Figure 2, where the normal ^{13}C spectrum of a 5% solution of PE in $CDCl_3$ (Figure 2a) is shown, along with the $^{13}C\{^{2}H\}$ INEPT spectrum of a similar solution of PE-d_5 in $CHCl_3$ (Figure 2b). The $^{13}C\{^{2}H\}$ INEPT spectrum is completely selective for ^{13}C which is bound to ^{2}H. No detectable signal from protonated solvent is observed despite its greater than 20fold molar excess. Similarly, the nondeuterated methyl and methine carbon resonances are not detected. Preliminary $^{13}C\{^{2}H\}$ INEPT studies indicated that suppression of nondeuterated carbon resonances by at least four orders of magnitude is achievable.[3]

Scheme I



Scheme II. C-H Coupled Spin System



The second important factor of the spectrum in Figure 2b is that unlike the ^{1}H and ^{2}H spectra, signals from each of the chemically distinct deuterated positions are clearly resolved. It is therefore possible to identify the positions of ^{2}H labels using the $^{13}C\{^{2}H\}$ polarization transfer. To evaluate the practical aspects involved in exploiting this NMR technique, the polarization transfer mechanism must be examined in some detail. Mechanistic details of $^{13}C\{^{1}H\}$ INEPT have been described before,[4,7] so only those features relevant to the $^{13}C\{^{2}H\}$ INEPT technique will be discussed here.

$^{13}C\{^{1}H\}$ INEPT. The INEPT pulse sequence, as diagrammed in Scheme I, was originally developed to enhance the sensitivity

---

(1) (a) Stothers, J. B. In "Topics in Carbon-13 NMR Spectroscopy"; Levy, G. C., Ed.; Wiley-Interscience: New York, 1974; Vol. 1, pp 229-286. (b) McInnes, A. G.; Walter, J. A.; Wright, J. L. C.; Vining, L. C. *Ibid.* 1976; Vol 2, pp 123-178.

(2) Mantsch, H. M.; Saito, H.; Smith, I. C. P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1977**, *11*, 211.

(3) Rinaldi, P. L.; Baldwin, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 5791.

(4) Morris, G. A.; Freeman, R. J. *J. Am. Chem. Soc.* **1979**, *101*, 760.

(5) Morris, G. A. *J. Am. Chem. Soc.* **1980**, *102*, 428.

(6) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

(7) Pegg, D. T.; Doddrell, D. M.; Bendall, M. R. *J. Chem. Phys.* **1982**, *77*, 2745.

0002-7863/83/1505-7523$01.50/0

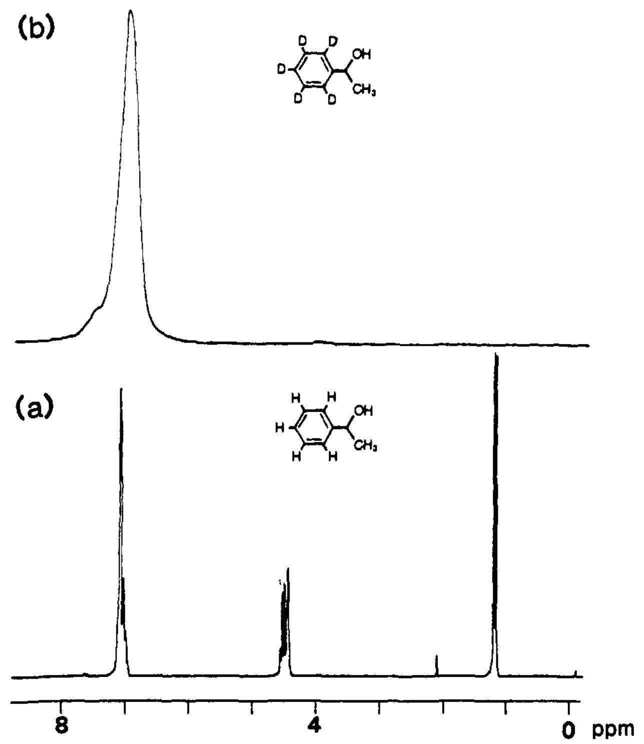


Figure 1. 200-MHz ^{1}H NMR spectrum (a) of 1-phenylethanol (PE) in $CDCl_3$ 30.7-MHz ^{2}H NMR spectrum (b) of PE-d_5 in $CHCl_3$. Note that the chemically distinct aryl resonances are not resolved in either spectrum.

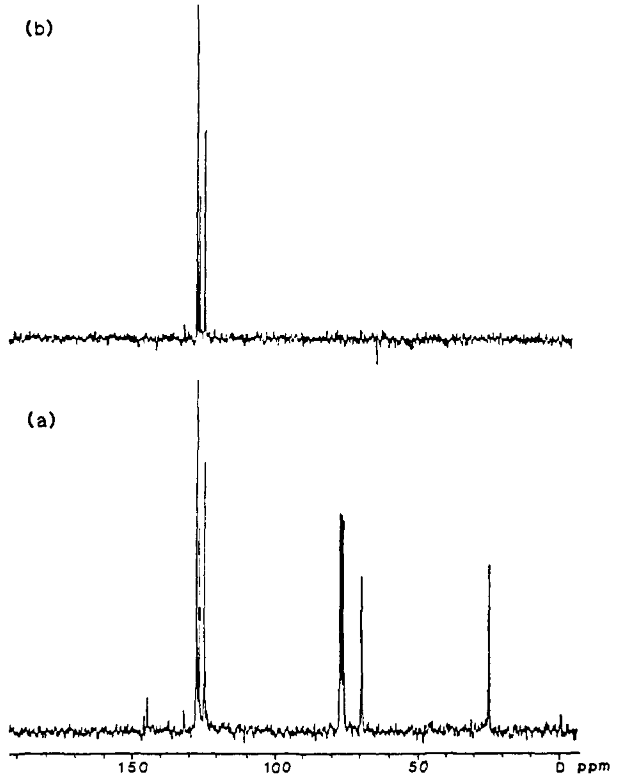
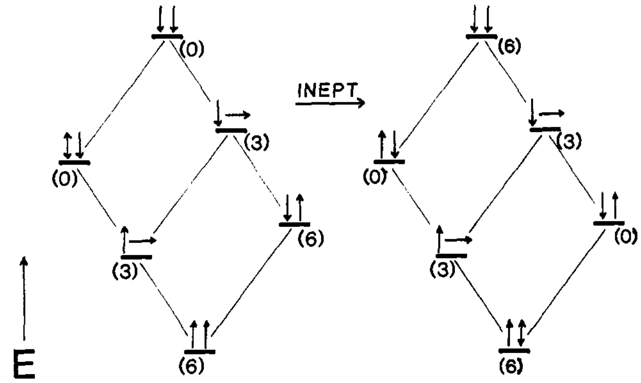


Figure 2. 50-MHz ^{13}C NMR spectra of 1-phenylethanol (PE) in chloroform, obtained using a spectral window of 5000 Hz, an acquisition time of 0.4 s, and a line broadening of 3.5 Hz: (a) normal proton decoupled spectrum of 5% PE in $CDCl_3$ obtained with a total experiment time of 4 min, 40 transients, a repetition time of 6 s, and a 90° pulse; (b) $^{13}C\{^2H\}$ INEPT spectrum of PE-d_5 (deuterated in the aromatic position) in $CHCl_3$ obtained with a total experimental time of 4 min, 240 transients, and a 1 s repetition time.

Scheme III. C-D Coupled Spin System



for NMR observation of ^{13}C, ^{15}N, and other difficult to detect nuclei (S spins) having attached protons or other nuclei (I spins) with higher gyromagnetic ratios (γ). To achieve this end, the I spins are allowed to express their precession due to J coupling with S spins for a period of time (2τ) exactly long enough so that those spins attached to α S spins are $^1/_2$ cycle ahead of those attached to β S spins. When brought along the z axis at point B in the pulse sequence, one ^{1}H population is inverted as diagrammed in Scheme II.[8] The result is that now the population differences described by the carbon transitions are comparable to the proton population differences in the initial state A. In this way, a factor of four ($\gamma_{^1H}/\gamma_{^{13}C}$) enhancement in sensitivity is achieved relative to the normally acquired ^{13}C signal without nuclear Overhauser enhancement.

$^{13}C\{^2H\}$ INEPT. Similar arguments apply for $^{13}\{^2H\}$ INEPT. The effects of the INEPT experiment on the spin populations in a CD system are shown in Scheme III. The relative excess populations of the spin states are indicated by the numbers in parentheses. As before, contributions due to excess carbon spins need not be considered here.[8]

Since the ^{2}H nuclear spin $I = 1$, the population differences which are transferred to ^{13}C are described by those between the highest and lowest energy spin states ($m = -1$ and $m = +1$, respectively). There is no phase coherence of ^{2}H spins with $m = 0$ (spins perpendicular to H_0), and so there is no net polarization to transfer from this spin state. The relative population difference between the $m = -1$ and $m = +1$ states in the CD system is greater than that between the $m = +^1/_2$ and $m = -^1/_2$ states if ^{2}H would have had $I = {^1/_2}$. However, the same total number of spins is distributed between three states, $m = +1$, 0, and -1, in the CD system compared to two states in a spin $^1/_2$ system. The relative populations of the $m = -^1/_2$ and $+^1/_2$ states for an $I = {^1/_2}$ nucleus are:

$$(1 - \gamma\hbar H_0/2kT)*n_0/2$$

and

$$(1 + \gamma\hbar H_0/2kT)*n_0/2$$

respectively, where n_0 is the total number of nuclei in the system, $\hbar$ k is the Boltzmann constant, and T is the temperature. In an $I = 1$ system, the relative populations of the $m = -1$, 0, and $+1$ states are:

$$(1 - \gamma\hbar H_0/kT)*n_0/3,\ n_0/3,\ \text{and}\ (1 + \gamma\hbar H_0/kT)*n_0/3$$

The population differences between the energy levels from which polarization transfer is obtained in the $I = 1$ system is 2/3*

---

(8) The complete energy level diagram contains a subpopulation of carbons equally distributed between α and β states, as well as an excess of carbon spins in the α states. As discussed in previous papers,[9-11] the subpopulation of excess carbons is unmodulated by coupled protons during polarization transfer pulse sequences, and its contribution to the observed signal is removed by phase cycling in a multiacquisition experiment. Therefore, it is appropriate to consider only the modulated carbon spins (those in equal numbers in the higher and lower carbon energy states).

(9) Maudsley, A. A.; Muller, L.; Ernst, R. R. J. Magn. Reson. 1977, 28, 463.

(10) Bodenhausen, G.; Freeman, R. J. Magn. Reson. 1977, 28, 471.

(11) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M. J. Magn. Reson. 1981, 45, 8.

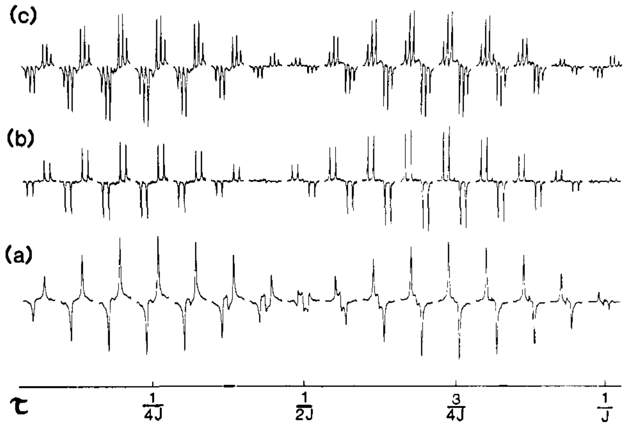


Figure 3. Coupled $^{13}C\{^2H\}$ INEPT NMR spectra of benzene-d_6 (a), methylene-d_2 chloride (b), and acetone-d_6 (c) as a function of polarization transfer delay (τ). Data acquisition was started at point B in Scheme I. Maximum polarization transfer is achieved with $\tau = 1/4J$ for all three (CD, CD_2, and CD_3) systems.

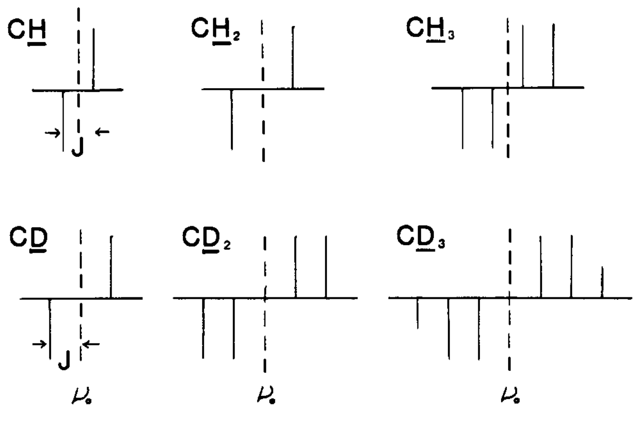
$(\eta_0\gamma\hbar H_0/kT)$ to $1/2*(n_0\gamma\hbar H_0/kT)$ in the $I = {}^1/_2$ system. Thus the intensities of the signal in the $^{13}C\{^2H\}$ INEPT experiment will be $4/3(\gamma_{^2H}/\gamma_{^{13}C}) = 4/5$ relative to the normal ^{13}C spectrum without NOE.

There is no loss in sensitivity with the $^{13}C\{^2H\}$ INEPT experiment compared to the normal proton decoupled ^{13}C experiment with NOE despite the apparent unfavorable enhancement ratio (4/5 relative to 3 for the normal spectrum with NOE). The quadrupolar ^2H nucleus typically has relaxation rates that are an order of magnitude faster than the corresponding protonated ^{13}C nucleus. Since it is the ^2H population difference that must be reestablished before repeating the INEPT sequence, faster repetition rates can be used, allowing ten times the number of accumulations to be obtained relative to the normal $^{13}C\{^1H\}$ spectrum. This results in a further threefold improvement in the signal-to-noise ratio. Consequently, the sensitivity of the $^{13}C\{^2H\}$ INEPT experiment is comparable to that of the normal single pulse experiment with NOE of the corresponding ^{13}C–^1H spin system. This is demonstrated by the spectra in Figure 2a,b, which both required 4 min of accumulation time.

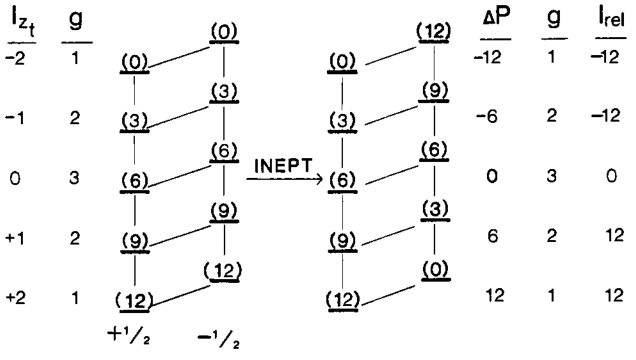
Only the optimum times for τ and Δ delays need to be determined. Since these will in general depend on both the magnitude of the J coupling and the multiplicity of the coupled ^{13}C and ^2H signals, these delays will be treated separately for CD, CD_2, and CD_3 signals.

Optimum τ Delays for CD, CD_2, and CD_3 Systems. It was previously shown that the optimum delay τ for polarization transfer from ^1H to quadrupolar nuclei such as ^{11}B is dependent on the spin of the observed nucleus.[12] This is not the case when polarization transfer is from the quadrupolar nucleus I (^2H in this case) to the $I = {}^1/_2$ nucleus (^{13}C). If observed, both ^1H and ^2H would appear as doublets with resonances at $\nu_0 \pm J/2$. These two magnetization components precess in opposite directions in the xy plane of the rotating frame, with frequencies $\pm J/2$ in a manner identical with that of the protons as described previously.[4,7] Therefore, the conditions necessary to obtain antiparallel alignment of these components are identical with those of $^{13}C\{^1H\}$. For $\tau = n/4J$ maximum polarization transfer will be observed if n = 1, 3, 5, ..., and nulled signals will be obtained if n = 2, 4, 6, ... Attenuated polarization transfer will be achieved for all other values of τ.

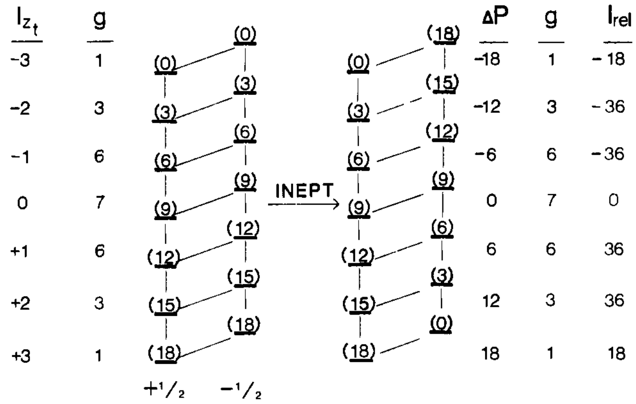
Scheme IV



Scheme V. CD_2



Scheme VI. CD_3



The numbers in parentheses in Scheme III indicate the relative populations of the modulated magnetization component in a CD spin system. From the population differences of the carbon transitions, relative signal intensities of −1:0:+1 are expected for the ^{13}C triplet of a CD group; this is illustrated in Scheme IV. The $^{13}C\{^2H\}$ INEPT spectra of benzene-d_6 as a function of τ are shown in Figure 3a; the expected multiplicity and intensity modulation are observed.

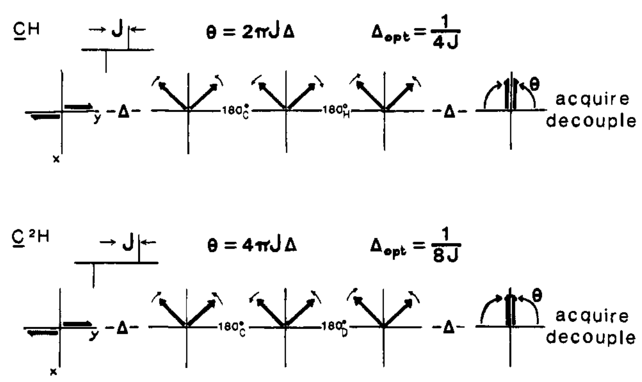
Identical arguments apply for determining the optimum delay τ for observing CD_2 and CD_3 groups. For $\tau = n/4J$, maximum sensitivity enhancement will be achieved with n = 1, 3, 5, ..., nulled signals will be obtained with n = 2, 4, 6, ..., and attenuated sensitivity enhancement will be achieved for other values of τ. Relaxation also plays a role in determining the amount of enhancement, so that for larger values of n as the ^2H magnetization vectors dephase in the xy plane due to T_2 relaxation, the total sensitivity enhancement becomes further attenuated. Therefore ideally, $\tau = 1/4J$ should be used.

The relative intensities of the multiplet components for CD_2 and CD_3 groups will be described by both the relative modulated Boltzmann populations of the energy levels and the number of degenerate states which occupy each energy level. The effect of the INEPT sequence on the populations of a CD_2 spin system is shown in Scheme V, with the INEPT modulated Boltzmann populations shown in parentheses. The first and second columns in Scheme V describe the total ^2H magnetization component ($Iz_t = \sum m_z$) and the degeneracy of the state with that Iz_t, respectively. After the INEPT sequence the energy levels describe five ^{13}C

---

[12] Pegg, D. T.; Doddrell, D. M.; Brooks, W. M.; Bendall, M. R. *J. Magn. Reson.* **1981**, *44*, 32.

Scheme VII



transitions with relative population differences (ΔP) of $-12{:}-6{:}0{:}+6{:}+12$. However, when these population differences are weighted by the degeneracy (g) of each transition (next to last column in Scheme V), relative signal intensities (I_{rel}) of $-1{:}-1{:}0{:}+1{:}+1$ in the last column are predicted for the CD_2 multiplet in the $^{13}C\{^2H\}$ INEPT experiment.

A similar description of the CD_3 system (Scheme VI) leads to the prediction of relative population differences of $-18{:}-12{:}-6{:}0{:}+6{:}+12{:}+18$ between the energy levels which describe ^{13}C transitions. After weighting by the degeneracy of each level (1:3:6:7:6:3:1), relative intensities of $-1{:}-2{:}-2{:}0{:}+2{:}+2{:}+1$ are predicted for the seven-line pattern in the coupled $^{13}C\{^2H\}$ INEPT spectrum of a CD_3 group.

Experimental $^{13}C\{^2H\}$ INEPT spectra of neat methylene-d_2 chloride and acetone-d_6 are shown in Figure 3b,c, respectively. These exhibit the predicted relative intensities. Additionally, the expected identical intensity modulations are observed for the CD, CD_2, and CD_3 multiplets. Maxima are observed when $\tau = n/4J$ where $n = 1, 3, 5, \ldots$, and attenuated signals are observed for other values of τ, with nulls observed for $n = 2, 4, 6, \ldots$.

Optimum Refocussing Delay Δ. Decoupled ^{13}C spectra are usually more desirable than the coupled spectra shown in Figure 3, since higher signal-to-noise levels and simpler spectra are obtained. However, if ^2H decoupling is applied immediately after the polarization transfer sequence (point B in Scheme I), positive and negative components of the multiplets would cancel to eliminate the signal. To prevent this problem, a refocussing sequence is inserted before acquisition of the ^{13}C spectrum with decoupling is attempted.[5,12] In the $^{13}C\{^1H\}$ INEPT experiment the ^1H decoupler must be left off during the entire sequence except during the acquisition time. In the $^{13}C\{^2H\}$ INEPT experiment it is the ^2H decoupler which must be left off except during the acquisition time. When it is desirable to perform simultaneous ^1H decoupling, no adverse consequences are predicted if ^1H irradiation is performed during the entire sequence.

The refocussing period serves to align the opposing ^{13}C magnetization components so that they do not cancel when decoupling is applied. The vector diagrams of the magnetization in the xy plane for CH and CD systems are shown in Scheme VII. The optimum signal intensity for the CH system is obtained when the two ^{13}C magnetization components are allowed to precess through a 90° angle relative to one another during each of the Δ periods. The same argument applies for the CD system. However, since carbon is coupled to ^2H with $I = 1$, the INEPT modulated ^{13}C magnetization components at point B in the sequence consist of two vectors precessing at $\pm J$ as opposed to two components precessing at $\pm J/2$ in the corresponding CH system. The ^{13}C components precess twice as fast in the CD system, requiring a delay $\Delta = 1/8J$ to precess through an angle of 45°, compared to the $\Delta = 1/4J$ required in the CH system. Once maximum polarization transfer is achieved, the fraction of this maximum signal retained after the refocussing period can be expressed by the vector sum of the magnetization components at the start of data acquisition. For a CD system, this is:

$$I_{CD} \propto \sin(2\pi J_{CD}\Delta) \tag{1}$$

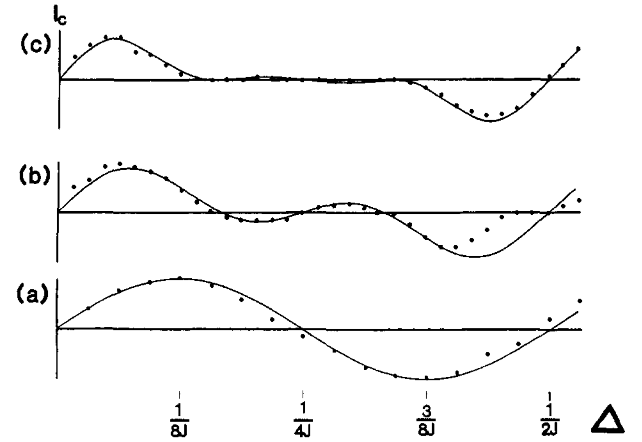
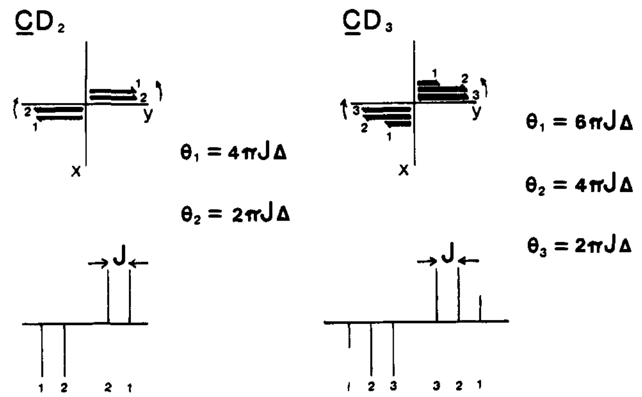


Figure 4. Decoupled $^{13}C\{^2H\}$ INEPT signal intensities for (a) CD, benzene-d_6, (b) CD_2, methylene-d_2 chloride, and (c) CD_3, acetone-d_6 as a function of the refocussing delay Δ, showing both calculated (—) and experimental (•) values. Best predicted polarization transfer is achieved with $\Delta = 1/8J$, $1/16J$, and $1/16J$ for the CD, CD_2, and CD_3 resonances, respectively. Best compromise value for observation of all three types of carbons simultaneously occurs at $\Delta = 3/40J$.

Scheme VIII



by analogy with the corresponding CH system.[13]

Similar arguments apply for the CD_2 and CD_3 systems; however, these systems have four and six magnetization components which contribute to the observed signal (Scheme VIII). A sinusoidal contribution for each pair of magnetization components at $\pm J$, $\pm 2J$, and $\pm 3J$ can be written. Each component is weighted by its respective contribution to the total magnitude of the multiplet intensity. Analytical expressions for the magnitudes of the xy components for these systems appear in 2 and 3. These

$$I_{CD_2} \propto \tfrac{1}{2}\sin(2\pi J_{CD}\Delta) + \tfrac{1}{2}\sin(4\pi J_{CD}\Delta) \tag{2}$$

$$I_{CD_3} \propto \tfrac{2}{5}\sin(2\pi J_{CD}\Delta) + \tfrac{2}{5}\sin(4\pi J_{CD}\Delta) + \tfrac{1}{5}\sin(6\pi J_{CD}\Delta) \tag{3}$$

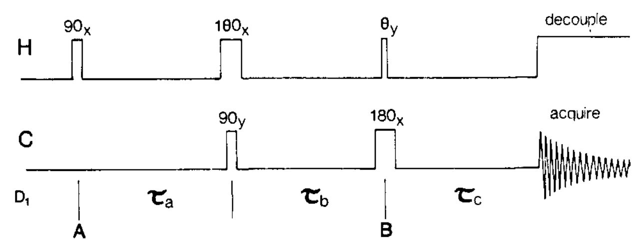
functions are plotted on top of the experimental intensities for CD, CD_2, and CD_3 resonances in Figure 4. It is easy to see that no single value of Δ simultaneously gives optimum signal intensities for all three (CD, CD_2, and CD_3) systems. However, an excellent compromise is achieved with $\Delta = 3/40J$, where CD, CD_2, and CD_3 systems have comparable intensities. This is shorter than the value of $\Delta = 1/3J$ which has been found to be optimum for the corresponding CH, CH_2, and CH_3 systems.[12]

By proper selection of Δ spectral editing can be achieved in the CH_n systems. Because the signal intensities of CH, CH_2, and CH_3 resonances are modulated differently by the refocussing period, selection of $\Delta = 3/8J$ provides a decoupled $^{13}C\{^1H\}$ INEPT spectrum in which CH and CH_3 signals are positive and CH_2 signals are inverted. Selection of $\Delta = 1/4J$ provides a spectrum

---

[13] Burum, D. P.; Ernst, R. R. *J. Magn. Reson.* **1980**, *39*, 162.

Scheme IX



with only CH signals.[13] While it is not possible to observe significant differences between the CD_2 and CD_3 intensity modulations, it is possible to selectively observe only CD signals by choosing a delay $\Delta = 1/6J$.

$^{13}C\{^2H\}$ DEPT. Like the INEPT experiment, the DEPT pulse sequence achieves enhancement of less sensitive observed nuclei by transferring polarization from a more sensitive decoupled nucleus. The mechanism by which this is accomplished is identical with the INEPT mechanism, that is by using J coupling to achieve antiparallel alignment of two ^1H magnetization components in the xy plane of the rotating frame. Only the sequence used to accomplish this antiparallel alignment is different. The mechanism for $^{13}C\{^1H\}$ DEPT has been detailed elsewhere.[6,7] The significant differences pertinent to observation of $^{13}C\{^2H\}$ DEPT spectra will be described here.

The DEPT sequence is illustrated in Scheme IX. At point B, the spins of the excess proton populations are antiparallel and display no chemical shift information (as at point B in the INEPT sequence), but the coupled carbon spins lie in the xy plane antiparallel and displaced from the x axis by the angle precessed due to the ^{13}C chemical shift during the second delay period (τ_b). To refocus this chemical shift precession, the carbon spins are rotated 180° and allowed to precess a time (τ_c) exactly equivalent to τ_b. Additionally, to focus the antiparallel carbon magnetization components, they are allowed to precess under the influence of the proton spins to which they are attached (the antiparallel proton spins are brought along the z axis with the final θ pulse). However, due to different degrees of protonation, carbons will precess at different rates so that either the time τ_c or the magnitude of the coupling with the proton x magnetization must be reduced to refocus carbon resonances with higher multiplicity. Since the DEPT sequence requires $\tau_a = \tau_b = \tau_c$, the proton z magnetization must be varied be using a variable final decoupler pulse width (θ).

Since INEPT and DEPT are comparable up to point B, the same $\tau_a = \tau_b = 1/2J$, will suffice regardless of the decoupled nucleus' spin. However, as with Δ in INEPT the ^{13}C multiplicity and, consequently, the ^2H spin will influence the optimum θ pulse. Recent work[14] establishes a direct correspondence between the DEPT θ and the INEPT Δ for CH_n systems (i.e., $\theta = 2\pi J\Delta$). We have found this same relationship. By substituting θ for $2\pi J\Delta$ in eq 1–3, curves relating the final decoupled ^{13}C signal intensity with θ are generated which fit the data for $^{13}C\{^2H\}$ DEPT spectra of methine, methylene, and methyl carbons acquired with varying θ (Figures 5a,b,c, respectively). Although no single value will optimize all three types of carbons, a compromise value of 27° provides equivalent signal intensities.

Conclusion

Both the INEPT and DEPT NMR pulse sequences can be useful for polarization transfer for ^2H to ^{13}C. One of the advantages of the $^{13}C\{^2H\}$ polarization transfer experiments is that they allow the use of ^2H as an isotopic tracer in labeling studies while at the same time permitting the exploitation of the larger ^{13}C chemical shift range for later identification of the label's position. The faster repetition rate permitted by the faster relaxing ^2H and a modest signal enhancement provided by the polarization transfer make these experiments comparable in sensitivity with the normal single pulse ^{13}C NMR experiment with NOE of the corresponding protonated species. In a general system, the optimum delays in the INEPT sequence are $1/4J$ and $3/40J$ in the polarization transfer and refocussing periods of the pulse sequence, respectively. In a general system, the delay in the DEPT sequence should be set to $1/2J$ and the last ^2H pulse should be set to θ = 27°.

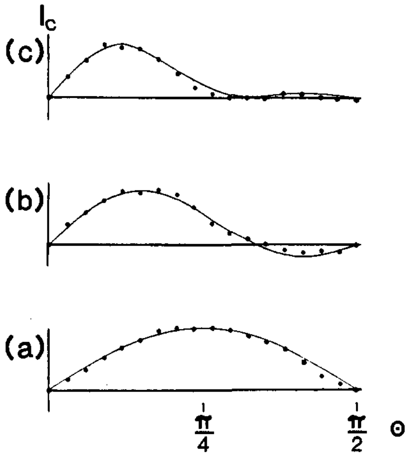


Figure 5. Decoupled $^{13}C\{^2H\}$ DEPT signal intensities for (a) CD, benzene-d_6, (b) CD_2, methylene-d_2 chloride, and (c) CD_3, acetone-d_6 as a function of the final θ pulse, with a constant value of $\tau = 1/2J$, showing both calculated (—) and experimental (•) values. Optimum signal intensity is obtained with θ = 45° for the CD resonance, 27° for the CD_2 resonance, and 22.5° for the CD_3 resonance.

Several factors limit the general application of these experiments. Since the polarization transfer mechanism requires resolvable J coupling between ^2H and ^{13}C, the polarization transfer is ineffective when the quadrupolar T_2 is less than ca. 20 ms. Also, in those situations where T_2 (of ^2H particularly) is on the order of $1/J_{CD}$, INEPT is preferable to DEPT as the former is shorter.[7] In the absence of internal molecular motion such as methyl group rotation, this limits application of the technique to compounds with rotational correlation times of less than 10^{-9} s (M_r < ca. 1000). If only partial deuteration (e.g., CHD instead of CD_2) is achieved, triple resonance must be used or the residual protons will result in additional splitting of the ^{13}C resonances. This is not always disadvantageous, since this allows identification of the extent of ^2H incorporation.

Experimental Section

All spectra were obtained on a Varian XL-200 NMR spectrometer. ^1H spectra were obtained at 200 MHz with 5-mm sample tubes, and ^2H and ^{13}C spectra were obtained at 30.7 and 50 MHz, respectively, with 10-mm sample tubes. The standard INEPT pulse program provided with the instrument's software package was used. A PASCAL pulse program for the DEPT sequence was written at Case Western Reserve University with guidelines described by Pegg et al.[6] Polarization transfer from ^2H required that the ^2H and ^1H transmitter boards which normally occupy the lock and decoupler slots, respectively, be interchanged. The decoupler output was then attached to what is normally the ^2H lock channel on the probe. When ^2H decoupling was required, a two stage band reject filter was used between the observe preamplifier and the probe to remove the 30.7-MHz decoupler signal and pass the 50-MHz observe signal to the detector. An additional 30.7-MHz band-pass filter was inserted in the decoupler line just before the probe to remove any signals other than the desired 30.7-MHz ^2H frequency. Typically, 90° pulse widths of 14 and 300 μs were obtained for ^{13}C and ^2H, respectively. Other experimental parameters are given in the figure captions.

Acknowledgment. This work was supported by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Research Corporation. We also acknowledge the National Science Foundation (Grant No. CHE-80-24633) and the sponsors of the Major Analytical Instruments Facility at Case Western Reserve University for funds used to purchase the XL-200 NMR spectrometer used in these studies.

---

(14) Pegg, D. T.; Doddrell, D. M.; Bendall, M. R. *J. Magn. Reson.* **51**, *264*, 1983.