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COMMUNICATIONS

Structure Determination and Spectral Assignment by Pulsed Polarization Transfer via Long-Range ^{1}H–^{13}C Couplings

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Two-dimensional heteronuclear chemical-shift correlation (*1–4*) has proven to be a very effective way for identifying which proton is bonded to which ^{13}C nucleus. Unfortunately, the experiment is much less effective in determining long-range connectivity (*5*), i.e., correlating shifts from protons and ^{13}C nuclei that are separated by two or more bonds. A possible way for determining two-bond connectivity is the two-dimensional RELAY experiment (*6–9*). Although this experiment is considerably less sensitive than conventional heteronuclear chemical-shift correlation, it can provide very useful information.

In practice, there is a great interest in determining three-bond ^{1}H–^{13}C connectivity, as this would allow "bridging" of nonprotonated carbons, and nuclei other than carbon, for example, oxygen and nitrogen. In most practical cases, none of the techniques mentioned above are very effective at doing this. It will be demonstrated here, that a modified version of the refocused INEPT experiment (*10–12*) is a very sensitive and simple way for determining two- and three-bond connectivity. It has been demonstrated elsewhere that this modified INEPT experiment is also very useful for enhancing sensitivity of nonprotonated ^{15}N nuclei (*13*).

For a description of how the regular INEPT experiment functions, the reader is referred to the original literature (*10–12*). Here the effect of replacing all nonselective proton pulses in the INEPT experiment by "soft" pulses ($\gamma H_1 \approx 25$ Hz) will be discussed. The pulse scheme is sketched in Fig. 1. As will be shown below, the effect of this sequence can easily be understood. The first soft 90°_x proton pulse rotates the magnetization of one preselected proton into a position parallel to the y axis of the rotating frame (Fig. 2a). Consider the case where there is a ^{13}C nucleus in the molecule that has a long-range coupling, $^{\mathrm{lr}}J_{\mathrm{CH}}$, with the proton considered. The proton magnetization will then evolve during the first half of the delay Δ_1, under the influence of chemical shift, static magnetic field inhomogeneity, homonuclear J coupling, and coupling with the ^{13}C nucleus (Fig. 2b). For clarity, the $^{\mathrm{lr}}J_{\mathrm{CH}}$ interaction is assumed to be larger than the homonuclear proton–proton coupling in the diagram. The selective 180°_y proton pulse then rotates the proton magnetization vectors 180° about the y axis (Fig. 2c) and all the effects of ^{1}H–^{1}H and ^{1}H–^{13}C J coupling, static field inhomogeneity, and chemical shift would refocus at the end of the interval, Δ_1. However, at the midpoint of the interval, Δ_1, a ^{13}C 180° pulse flips the spin state of

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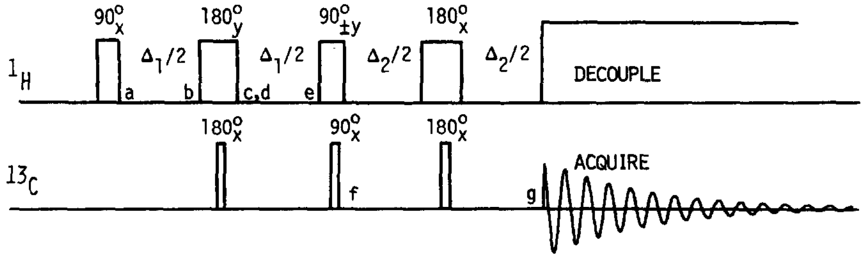

FIG. 1. Pulse scheme of the selective INEPT experiment. All proton pulses are soft pulses ($\gamma H_1 \approx 25$ Hz), applied to a preselected proton resonance. The phase of the second 90° ^{1}H pulse is alternated along the $\pm y$ axis in successive experiments, and data are accordingly added and subtracted. For optimum polarization transfer from one proton to one ^{13}C nucleus with a scalar coupling J, the optimum condition for transfer is $\Delta_1 + 2\tau_{90} = \Delta_2 + \tau_{90} = 1/(2J)$, where τ_{90} is the duration of the soft 90° proton pulse ($\approx$10 msec).

the coupled ^{13}C nucleus, and therefore, the effect of the heteronuclear J coupling will not refocus at time Δ_1, but the magnetization components will be aligned along the $\pm x$ axis if the delay Δ_1 is chosen equal to $1/(2^{1r}J_{CH})$.

Analogous to the conventional INEPT experiment, a 90°_y (^{1}H), 90°_x (^{13}C) pulse pair transfers all proton magnetization to the ^{13}C nucleus (*10*). As described in Ref. (*10*), the ^{13}C doublet components, corresponding to the ^{1}H in the α and β spin states, will be aligned oppositely along the $\pm y$ axis (Fig. 2f). A soft proton pulse, in combination with a (nonselective) ^{13}C pulse, applied at the midpoint of the interval, Δ_2, will align the two vectors along the x axis of the rotating frame (Fig. 2g), if $\Delta_2 = 1/(2^{1r}J_{CH})$ (*14*). At this point, broadband proton decoupling and data acquisition are started.

Alternation of the phase of the second 90° ^{1}H pulse along the $\pm y$ axis, in combination with addition and subtraction of acquired data ensures suppression of signals that

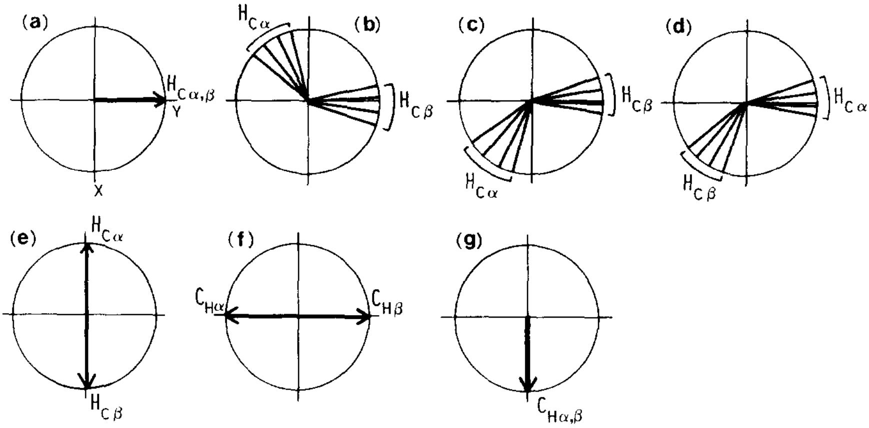

FIG. 2. Schematic diagram of the behavior of the proton magnetization vectors, $H_{C\alpha}$ and $H_{C\beta}$ and the ^{13}C magnetization vectors $C_{H\alpha}$ and $C_{H\beta}$ during the selective INEPT experiment, sketched in Fig. 1. The indices "Cα," "Hα," etc., denote the state of the coupled spin. Diagrams (a)–(g) correspond to the times (a)–(g) in Fig. 1. The effects of proton chemical shift, ^{1}H–^{13}C and ^{1}H–^{1}H coupling are taken into account (see text).

do not originate from the INEPT transfer (*12*). Note that, in analogy with the conventional INEPT experiment, the ^{13}C magnetization has been enhanced by a factor of four, even for nonprotonated carbons. In practice, the length of the delays Δ_1 and Δ_2 is not optimized for all different (and unknown) values of $^{lr}J_{CH}$, and relaxation will also occur during the relatively long intervals, Δ_1 and Δ_2. Therefore, a smaller enhancement, of a factor of about two or three, is usually observed. However, the main point is that the spectrum will only show resonances from ^{13}C nuclei that have a long-range coupling of the order of $1/(2\Delta_{1,2})$ with the preselected proton. Three-bond C–H couplings depend on the dihedral angle and are usually of the order of 3–8 Hz, and can therefore conveniently be used in this selective INEPT experiment.

For selective INEPT transfer from two equivalent methylene protons, or from a methyl group, the length of the delay, Δ_2, has to be set to $1/(4^{lr}J_{CH})$ and $1/(5^{lr}J_{CH})$, respectively (*12*). The length of the delay, Δ_1, remains $1/(2^{lr}J_{CH})$. If the rf field strength of the soft proton pulses, expressed in hertz, is much smaller than the direct J_{CH} coupling constant, the ^{13}C satellites of this proton will not be affected by the soft proton pulses and the resonance of the carbon, directly coupled to the selected proton, will not appear in the selective INEPT spectrum. If the soft proton pulses do affect one of the ^{13}C satellites of another C–H pair, sometimes a small residual signal for this carbon is observed.

Experiments were performed on a sample of 50% v/v 1,2-dihydronaphthalene in C_6D_6, in a 5 mm sample tube at 12°C, using a NT-270 spectrometer. Proton pulse widths were calibrated (*15*, *16*) using a sample of dichloroacetic acid ($^{lr}J_{CH} \approx 2.4$ Hz).

For all ^{13}C spectra, two scans were recorded (total time 11 sec), with opposite phase of the second $90^\circ_{\pm y}$ pulse. For selective transfer from the methylene protons, the delays Δ_1 and Δ_2 were set to 50 and 30 msec, respectively, and for transfer from the olefinic protons both delays were set to 50 msec.

Figure 3a shows the proton spectrum and assignment of 1,2-dihydronaphthalene. In this spectrum, the resonances from protons H5, H6, H7, and H8 are not very well resolved and are strongly coupled. Even if those resonances were well resolved, it would be very hard to distinguish between the resonances from H5 and H8 (and from H6 and H7), because couplings to protons H1 and H4 are very small (< 0.3 Hz), and those small couplings could be either four- or five-bond couplings. Figure 3b shows the regular INEPT spectrum for the olefinic and aromatic region. The assignment of the various resonances in this spectrum, using selective INEPT, will be discussed below. The two methylene resonances (C1 and C2) are not shown because their assignment is trivial, although, of course, the selective INEPT experiment could also be used to confirm their assignment. Figure 3c shows the selective INEPT spectrum, pulsing protons H1, and contains two resonances from protonated carbons (which must be C8 and C3), and the two quaternary carbon resonances from C9 and C10. Transfer from protons H2 (Fig. 3d) must show C9 and C4 (three bonds) and possibly C3 (two bonds). Comparison with Fig. 3c then immediately identifies C10, C9, C4, C3, and C8. The low-intensity signal for C10 in Fig. 3d is apparently due to a small four-bond coupling. Transfer from H3 (Fig. 3e) confirms the assignment of C10, and transfer from H4 (Fig. 3f) additionally shows resonance C5. The two-bond couplings between H3 and C4 (0.5 Hz) and between H4 and C3 (0.1 Hz) are too small to give

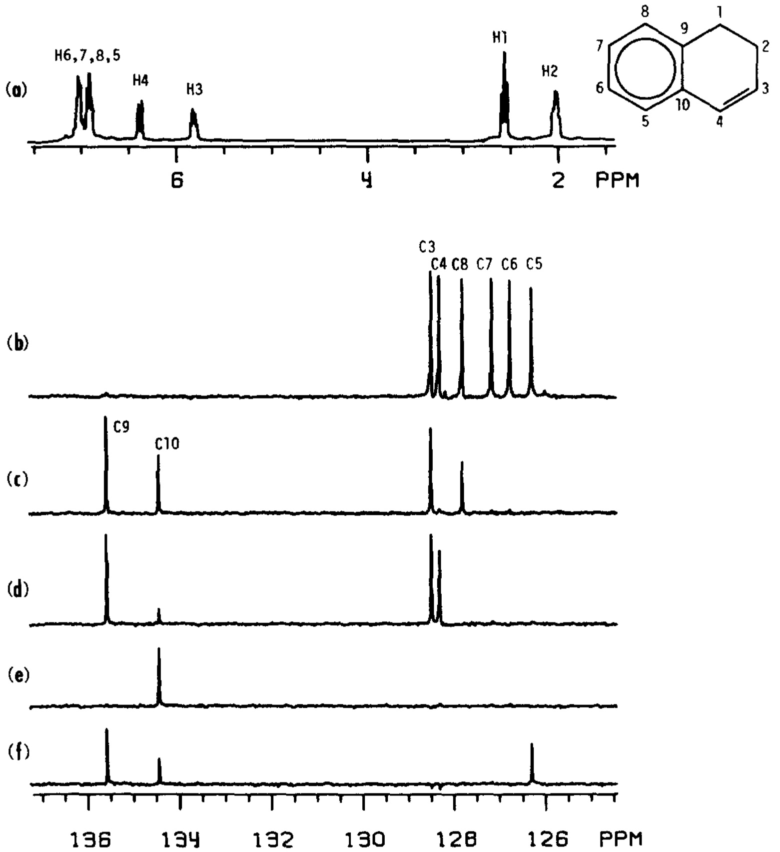

FIG. 3. Spectra of 1,2-dihydronaphthalene. (a) Regular proton spectrum and assignment; (b) regular ^{13}C INEPT spectrum of the low-field region; (c)–(f) selective INEPT spectrum by transfer from protons H1, H2, H3, and H4, respectively. All ^{13}C spectra are the result of two accumulations (11 sec).

rise to a detectable selective INEPT signal. Using the RELAY experiment (*6–9*) allowed assignment of C6 and C7, after H5 and H8 were assigned *via* correlations (*3*) with C5 and C8, respectively.

The illustration of the selective INEPT experiment given above clearly demonstrates the potential power of this approach: assignment information is obtained for both protonated and nonprotonated carbons, and sensitivity is of the same order as that of the regular INEPT spectrum. However, the shortcoming of the experiment is also evident: resonances from protons H5, H6, H7, and H8 are too close to allow a selective INEPT experiment. In practice, one will often need only a limited number of selective INEPT spectra to complete an assignment or a structure determination, and overlapping proton resonances will therefore seldom constitute a serious problem. The only restriction for the experiment is that resonances from protons coupled to the selected proton are more than approximately 30 Hz away from the proton resonance selected. The overlap with a resonance from a proton not coupled to the proton of interest does not affect the polarization transfer, but will merely increase the number of observed ^{13}C resonances.

It has been found experimentally, using large-diameter sample tubes, that the effect of the static magnetic field inhomogeneity that gives rise to spinning sidebands in the proton spectrum, can significantly decrease the sensitivity of the experiment. This occurs because the proton transverse magnetization will not completely refocus if $\Delta_1/2 + 3\tau_{90}/2$ is not equal to an integer multiple of the rotor period, T_R, of the spinner (*17–19*), where τ_{90} is the duration of a soft proton 90° pulse.

It is recommended that Δ_1 and Δ_2 (not counting the length of the soft proton pulses) are not set to values larger than 25 msec, because for large long-range couplings, nulling of the signal could occur if Δ_1 or Δ_2 equals $1/{}^{lr}J_{CH}$ (*12*).

In conclusion, it can be stated that the selective INEPT experiment provides a very sensitive and simple way to determine C–H connectivity information. The selective INEPT experiment is closely related to selective polarization transfer (SPT) experiments (*20–22*), but it appears that the selective INEPT experiment is more convenient to use in the case of complex coupling networks and nonresolved resonances.

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