

Determination of Heteronuclear Coupling Constants via Semiselective Two-Dimensional J Spectroscopy

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The earliest types of two-dimensional NMR experiments that received widespread attention were the proton-flip and the gated-decoupler experiments (1-8). Conceptually those early experiments are rather simple to understand, but the use of those experiments in practical cases often is complicated by data storage requirements and sensitivity considerations. Only in the case where the spectroscopist is interested in determining the multiplicity of a certain carbon-13 site is the use of the two-dimensional experiment straightforward; in such cases only a very low resolution of the carbon-13 multiplet structure along the F_1 axis is necessary, and hence the data matrix can be kept small. The sensitivity will be fairly good in this application because the carbon-13 intensity is spread over a maximum number of only four multiplet components. However, this application of two-dimensional J spectroscopy has been out-dated by even more sensitive and experimentally more convenient "spectral editing" experiments (9-11).

The use of the original 2D J -spectroscopy experiments for the exact determination of coupling constants requires a very fine digitization along the F_1 frequency axis; this leads to a very large size of the required data matrix, unless the acquisition time along the t_2 axis is made very short, which in turn decreases the sensitivity of the experiment dramatically. The intensity of a certain carbon-13 nucleus in this high- F_1 -resolution application will generally be spread over a large number of multiplet components, which decreases the sensitivity even further. All problems mentioned above can be overcome by using a modified version of the proton-flip experiment in which only one proton is "flipped" by the proton pulse (which in this case is selective) in the center of the evolution period (12). This allows the accurate determination of all heteronuclear long-range couplings with this particular proton.

In this communication we describe two experiments which rely on the same principle as the selective proton-flip experiment and which allow the accurate determination of either all direct ^1H - ^{13}C coupling constants by removal of the long-range splittings or the determination of all long-range couplings while removing all the direct ^1H - ^{13}C splittings.

The experimental pulse scheme is set out in Fig. 1. As in the original proton-flip experiment (3, 4), broadband low power ^1H irradiation during the preparation period provides a NOE effect, enhancing the sensitivity, and high power proton noise decoupling is employed during the detection period. A carbon-13 180° pulse is applied

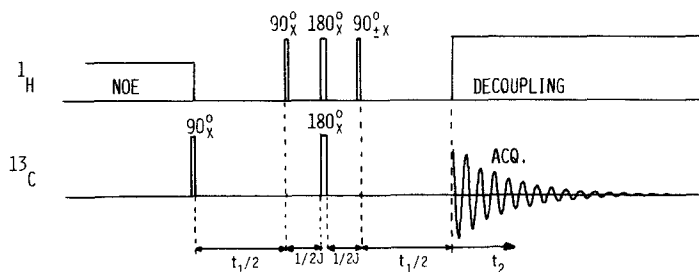


FIG. 1. Pulse scheme for the semiselective proton-flip experiments. In the case where the final proton 90° pulse is applied along the positive x axis, only direct couplings will be observed. In the case where the final 90° pulse is applied along the negative x axis, only long-range couplings will be observed.

in the center of the evolution period. However, the proton 180° pulse in the center of the evolution period of the original proton-flip experiment is now replaced by the $90_x^\circ - \frac{1}{2}J - 180_x^\circ - \frac{1}{2}J - 90_{\pm x}^\circ$ sequence, where J is the magnitude of the direct ^1H - ^{13}C coupling constant in hertz. For an isolated ^{13}C - $^1\text{H}_n$ set it is readily seen that in the case where the final pulse is applied along the positive x axis, the spin state of the proton(s) will be inverted, i.e., experience a flip. However, magnetization vectors from protons not directly coupled to the ^{13}C will undergo only small dephasing during the short intervals, $\frac{1}{2}J$, at a rate determined by the homonuclear proton-proton couplings and the long-range proton-carbon couplings. The final 90_x° pulse brings those magnetization vectors back along the positive z axis, and therefore the sequence has the effect of not changing the spin state of those protons.

The picture sketched above is a simplified one, but it can be shown with simple density matrix calculations that it is valid, given the assumption that the homonuclear proton-proton and the heteronuclear long-range proton-carbon couplings are very much smaller than the direct proton-carbon coupling constant. Only protons with a directly coupled carbon-13 nucleus will be flipped, and the detected magnetization will be modulated in amplitude by the frequency of the direct coupling only. The multiplets along the F_1 axis of the two-dimensional spectrum will all be simple singlets, doublets, triplets, or quartets, depending on the number of protons directly coupled to the carbon-13 considered. This makes it possible to allow multiple folding along the F_1 axis without introducing overlap, and therefore reducing the size of the data matrix considerably.

In a similar way it can be seen that in the case where the final 90° proton pulse is applied along the negative x axis, only protons which are not directly coupled to a carbon-13 nucleus will have their spin state flipped, and hence the detected magnetization will be modulated by the heteronuclear long-range couplings only. In this case a sampling frequency along the t_1 axis equal to about 40 Hz, giving a spectral width of ± 20 Hz in the F_1 dimension, will usually be sufficient, and no fold-over will occur. The number of resonances in the F_1 dimension is again reduced compared with the original proton-flip experiment, and hence sensitivity is improved.

The method is demonstrated here on pyridine, a compound for which the heteronuclear coupling constants have been extensively investigated in the past (7, 13, 14). Experiments were performed on a Nicolet-360 spectrometer controlled by a Nicolet

293A pulse programmer, using a 12 mm sample tube and a 50% v/v mixture of pyridine in acetone- d_6 . The proton 90° pulse length was carefully calibrated using the method described in Ref. (15) and determined to be equal to 53 μ sec. The delay between the proton 180° pulse and the 90° pulses was set equal to 3 msec. Only one acquisition, during which 1024 complex data points were acquired, was performed for each length of the evolution parameter t_1 , and 64 different t_1 values were used. The length of the t_1 increment was 38 msec in the experiment for the determination of the direct couplings, giving a spectral width of ± 13.16 Hz. In the experiment for the measurement of the long-range couplings the length of the increment was equal to 33.33 msec, giving a spectral width of ± 15 Hz. The delay time between experiments was equal to 6 sec giving a total measuring time of about 7 min for each of the two 2D experiments. After the first Fourier transformation the data matrix contains useful information only at the carbon-13 chemical shift frequencies; the second Fourier transformation is therefore restricted to only those sections through the data matrix for which the F_1 traces carry the desired coupling information. A complete two-dimensional spectrum is not computed.

The experiment for the determination of the direct couplings in pyridine gave the doublets shown in Fig. 2a-c, and gave J values in excellent agreement with those given by Hansen and Jakobsen (13) and Gunther *et al.* (14). Figure 2d-f shows the long-range multiplets for the different ^{13}C sites obtained with the semiselective long-range experiment. Again the measured values are in good agreement with the literature values and are given in Table 1. However, the multiplet of carbon C2 shows a pattern which does not correspond to the pattern in the conventional proton-

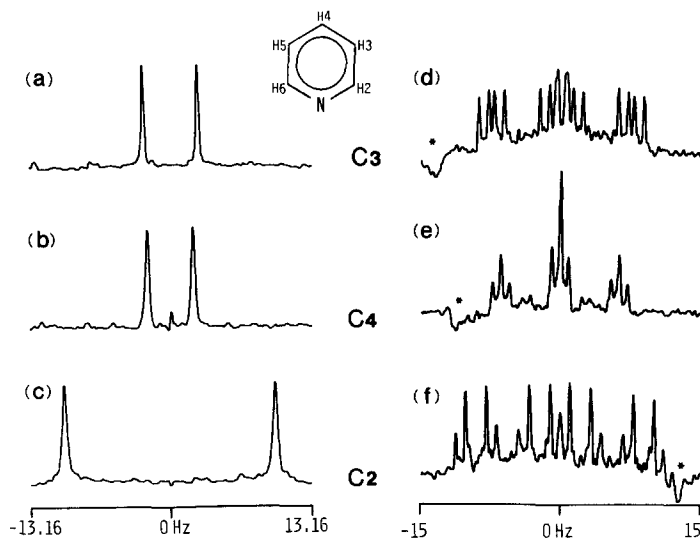


FIG. 2. Cross sections parallel to the F_1 axis through the two-dimensional spectra of pyridine. Spectra a-c show the multiplet patterns for the carbons C3, C4, and C2 with the long-range splittings suppressed. The doublet components have been folded three times. Spectra d-f show the long-range splittings for the carbons C3, C4, and C2 with the direct couplings suppressed. Small artifacts due to pulse imperfections are indicated with an asterisk.

TABLE I
ABSOLUTE VALUES OF ^{13}C - ^1H COUPLING
CONSTANTS IN PYRIDINE AS MEASURED
FROM THE SPECTRA IN FIG. 2 AND
FROM THE LITERATURE

	Measured ^a	Literature ^b
J_{C2H2}	177.56	177.63
J_{C2H3}	^c	3.12
J_{C2H4}	^c	6.85
J_{C2H5}	^c	0.92
J_{C2H6}	^c	11.16
J_{C3H2}	8.42	8.47
J_{C3H3}	163.01	163.04
J_{C3H4}	1.04	0.84
J_{C3H5}	6.51	6.56
J_{C3H6}	1.65	1.65
J_{C4H2}	6.31	6.34
J_{C4H3}	0.88	0.70
J_{C4H4}	162.19	162.41

^a The sample concentration was 50% v/v in acetone- d_6 .

^b Ref. (13). The sample concentration was 70% v/v in a mixture of acetone- d_6 and TMS (2:1).

^c Due to second-order effects, these values cannot be measured directly from Fig. 2.

coupled carbon spectrum. This is due to strong coupling effects between the protons H3 and H5, which are rendered inequivalent by the presence of a carbon-13 nucleus at site C2. Because the proton-proton coupling between protons H2 and H6 is only 0.15 Hz, the strong coupling effect between protons H2 and H6 is much less severe, and values for the coupling constants with carbon C3 can be extracted straightforwardly from the multiplet structure shown in Fig. 2d. To determine the values for the coupling constants to carbon C2 from the multiplet structure in Fig. 2f, a suitable simulation program is required, which is presently not yet available. Small artifacts, marked with an asterisk, are visible in Fig. 2d-f and are attributable to imperfections of the carbon-13 pulses. Those can, in principle, easily be cycled out by performing four experiments for each value of t_1 with different rf phases (16), but this is not easily accomplished with our pulse programmer.

In a future publication we will analyze the effect of strong coupling between the protons, and discuss the theory and limitations of the experiment in more detail.

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