Implementation of Selective Heteronuclear 2D J-Resolved Method on Standard NMR Spectrometers and Selective Proton Pulse Calibration

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Some currently used methods for determining the long-range carbon-proton coupling constants are discussed. We chose the selective heteronuclear 2D J-resolved method because of its good sensitivity and resolution in F_1 dimension. We implemented the method on a standard NMR spectrometer with transmitter working at fixed power and no facilities for shaped pulse generation. Selective proton pulse was achieved by using suitable attenuators for the decoupler output power attenuation. The selective proton pulse was calibrated by the indirect method for ^1H resonance observation, which allows precise determination of the pulse length. The proposed methodology can be applied to any NMR spectrometer provided that the decoupler output power can be switched by computer at least between two levels in few μsec. The implementation was tested on a sample of 5-Me-8-OH-quinoline, for which long-range carbon-proton coupling constants have not yet been determined.

INTRODUCTION

Precise determination of carbon-proton coupling constants is of considerable importance in conformational and structural analysis. However, the measurement of long-range carbon-proton coupling constants, their extraction from the spectrum and their assignment are burdened with considerable difficulties.

In principle, carbon-proton couplings may be obtained from proton satellite spectra. These can be quite complex and only the simplest molecules are amenable to satellite spectral analysis.

They may also be determined from the natural abundance ^{13}C NMR spectra. However, the low sensitivity of the ^{13}C nucleus and multiplets in carbon-proton coupled spectra, which may be overlapped in an indiscernible manner, make their determination difficult or even impossible. Besides, in both of the above mentioned cases, different sites in a molecule may involve several long-range couplings of comparable magnitude[1] and the assignment of the observed splitting to the appropriate carbon and proton nuclei may not be straightforward and sometimes even not possible.

These difficulties can be overcome by using appropriate one-dimensional (1D) or two-dimensional (2D) NMR methods for precise determination of all long-range couplings of carbon nuclei with a particular proton in the molecule. We shall briefly discuss several of them that are currently used. These are the selective heteronuclear (HET) 2D J-resolved,[2] semiselective 1D INEPT,[3] selective 2D INEPT,[4] soft ^1H, ^{13}C-COSY[5] and modified heteronuclear shift correlation methods proposed by Bauer *et al.*.[6]

The overall sensitivity of the semiselective 1D INEPT, soft ^1H, ^{13}C-COSY and Bauer's method is low. In the first two cases, this is due to the presence of many passive couplings and in the last case because loss of transverse magnetization may happen during the relatively long delays in the pulse sequence. The advantage of Bauer's experiment is that selective proton pulses are not required, and we get all coupling constants at once.

Selective 2D INEPT and selective HET 2D J-resolved methods offer better sensitivity. The sensitivity of the former method is even four times greater than the sensitivity of the latter, because in the first case magnetization is transferred from protons to carbon nuclei while in the second case carbon magnetization is observed during the detection and evolution period. Besides, in the selective 2D INEPT spectra no singlets, that could interfere appear as in the selective HET 2D J-resolved spectra. A disadvantage of the selective 2D INEPT experiment is the application of the extra delay before acquisition to bring antiphase carbon magnetization into in-phase magnetization. This is necessary because proton decoupling is used during acquisition. When various couplings of carbon nuclei with a particular proton in the molecule are present, it is difficult to optimize this delay so as to give good results for all values of coupling constants. In contrast, the selective HET 2D J-resolved experiment gives equally good results for all values of coupling constants between carbons and particular proton.

For the determination of the long-range couplings of protonated carbons, the modified selective HET 2D-J resolved method[7] can be used. This method is based on the replacement of the initial 90 ° carbon pulse of the standard selective HET 2D J-resolved method[2] by a nonselective polarization transfer optimized for one bond couplings which increases the sensitivity of the experiment. However, this method is not useful for the determination of long-range couplings of the non protonated carbons.

Proton detected heterocorrelation methods for the determination of long-range carbon-proton coupling constants also exist and they have better sensitivity because of the proton, instead of carbon, detection.[8] Poor resolution in F_1 dimension is a disadvantage of the basic proton detected heterocorrelation method.[8] This can be overcome by using a narrower spectral range in F_1 dimension when the folding of remote resonances does not disturb the part of the spectrum to be observed or by the application of shaped pulses for excitation of only a part of the whole carbon spectrum.[9] This is possible in the recent versions of NMR spectrometers designed to produce shaped pulses. In the case when the spectrometers have no facilities to produce shaped pulses and have a transmitter working at fixed power, rectangular decoupler pulses of long duration can be employed for selective proton excitation. Although this approach is not ideal, since unwanted proton resonances may be excited by the side lobes of the rectangular pulses,[10] it can give satisfactory results if the spectrum is not too crowded. Besides, it can easily be implemented on a standard NMR spectrometer, as shown below.

Selective proton pulses can, in principle, be achieved by setting the decoupler output power (DOP) to the value that will generate the required $\gamma B_1/2\pi$ pulse amplitude. When the lowest $\gamma B_1/2\pi$ achieved cannot fulfill the request for the frequency range

of the order of 25 Hz or less around the exact resonance condition for a chosen proton, a soft-pulse train[11] or suitable attenuators for the attenuation of decoupler output can be used. The second approach was adopted in our laboratory. Suitable attenuators were inserted between the decoupler output and probe decoupler input of a Varian VXR 300 spectrometer. The attenuation factor of the attenuator used is limited by successful decoupling of protons from carbons during acquisition.

In this scheme, it is necessary to redetermine the power of the decoupler needed for Waltz or efficient decoupling of protons from carbons and to determine the length of the selective 180 ° pulses. Pulse calibration may be performed using one of the known methods[11,12] or by the indirect method for ^{1}H resonance observation. In the indirect method, the decoupler serves as a transmitter. For our purpose, the decoupler pulses should be sent directly into the probe decoupler input. The signal is then observed in the observed coil. As a consequence of such a connection, the sensitivity drops, which is not crucial in the case of ^{1}H resonance, but it prevents all additional attenuation. By the indirect method for ^{1}H resonance observation, the same physical connection of the decoupler to the probehead as in the case of the desired heteronuclear application was achieved. Using this method, the decoupler pulse length can be determined directly from the observation of proton spectra, while by the known methods[11,12] ^{13}C spectra are observed and ^{13}C satellites, which may be unresolved in crowded proton spectra, must be selectively irradiated. We have compared the indirect method for ^{1}H resonance observation with the Selective Population Inversion (SPI) method.[11] According to our experience, the intensities of proton resonances observed using our method are more sensitive to the length of the selective proton pulse than the intensities of ^{13}C resonances observed by the SPI method. Thus, our method allowed more precise determination of the selective 180 ° proton pulse length.

EXPERIMENTAL

Being interested in a method for determining the ^{13}C–^{1}H long-range couplings that is independent of coupling values, with good sensitivity and good resolution in F_1 dimension, we performed a selective HET 2D J-resolved experiment on a Varian VXR 300 MHz spectrometer. As the test sample, 0.3M solution of 5-Me-8-OH-quinoline (Figure 1) in CDCl3 was used. The ^{13}C–^{1}H long-range coupling constants of this sample have not yet been determined. The pulse sequence for proton »flip« experiment[13] with composite 90 °(x) 180 °(y) 90 °(x) selective proton pulse[14] was used:

^{1}H   180 °   –dec
^{13}C   90 ° $t_1/2$ 180 ° $t_1/2$ –acq.
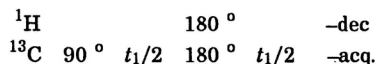
Two parameters for the determination of DOP used for the selective proton pulse during evolution and for Waltz decoupling during acquisition were inserted in the pulse sequence. The experimental details were: 64 experiments of 100 scans each, delay of 5 s between scans, acquisition time for one scan 0.14 s, spectral width in F_1 20 Hz and in F_2 3500 Hz, zero-filling in F_1 to 1K points, line broadening of 1Hz in F_2 and Lorentzian to Gaussian window in F_1, 19 dB at-
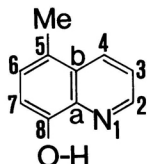

Figure 1. 5-Me-8-OH-quinoline.

tenuator, $\gamma B_1/2\pi$ value of 25 Hz and 20 ms long selective 180 ° proton pulse. The measuring time was 9 hours. Although a relatively long preparation period is necessary for the establishment of a nuclear Overhauser enhancement,[2] good results were obtained also with a delay of 2 s between scans. This shortened the measuring time to 4 hours. The selective proton pulse length may be slightly different for protons at different sites of the molecule, so it should be checked separately for each proton. The selective 180 ° proton pulse for all protons of 5-Me-OH-quinoline was 20 ms long. The precise calibration of the selective 180 ° proton pulse, which can be obtained by the indirect method for ^{1}H resonance observation is crucial for successful determination of long-range couplings using the HET 2D J-resolved method.

RESULTS

We have achieved also lower $\gamma B_1 2/\pi$ values and better selectivity by using lower DOP. The selectivity of 40 ms long selective proton pulse was tested by the SPI method. The result is given in Figure 2, where it is shown that the selective proton pulse does not invert the populations of ^{13}C satellite of the selected proton when its offset from the selected ^{13}C satellite is larger than 10 Hz. So, the selectivity of the selective
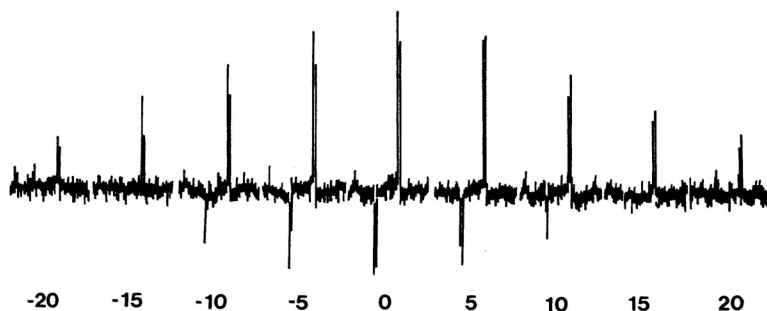

Figure 2. Selectivity test by the SPI method. Under the spectra, the values of the selective proton pulse offset from the selected ^{13}C satellite are given.

TABLE I

*[1]H and ^{13}C chemical shifts in ppm from TMS
of 5-Me-8-OH quinoline in CDCl₃*

Ring position	δ_H/ppm	$\delta^{13}C$/ppm
2	8.775	147.08
3	7.461	121.14
4	8.248	132.75
b		124.11
5		127.48
6	7.260	127.46
7	7.067	109.13
8		150.36
a		138.49
Me	2.553	17.68

pulse is approximately 20 Hz. This is in agreement with the $\gamma B_1/2\pi$ value 12.5 Hz, determined from the length of the selective 180 ° proton pulse, which was 40 ms. We used a higher $\gamma B_1/2\pi$ value, equal to 25 Hz, for selective proton inversion of 5-Me-8-OH-quinoline, because better selectivity was not required, since the proton resonances are separated by at least 50 Hz. The duration of the selective proton pulse is shorter at higher $\gamma B_1/2\pi$ values, which shortens the pulse sequence duration and, therefore, prevents additional loss of transverse magnetization due to ^{13}C relaxation.

The results are given in Table I, Table II and Figure 3. The assignment of ^1H and ^{13}C resonances (Table I) is based on the assignments in DMSO-d_6[15], confirmed by ^{13}C–^1H shift correlation experiment.[16] In the first column of Table II the values are given of carbon-proton splittings belonging to a particular carbon in the 1D ^{13}C spectrum

TABLE II

*Long-range carbon-proton coupling constants of
5-Me-8-OH-quinoline in CDCl₃, obtained from
carbon-proton 1D spectrum and by the selective
HET 2D-J resolved experiment*

$n_{J(C,H)}$	1D ^{13}C	Selective HET 2D-J
$^4J(8,4)$		1.6
$^3J(2,4)$	7.6	7.8
$^2J(b,4)$		3.8
$^3J(a,4)$		5.7
$^4J(6,4)$		0.6
$^3J(8,6)$	8.9	9.5
$^3J(b,6)$		8.0
$^4J(a,6)$		1.5
$^4J(4,6)$		1.3
$^5J(3,6)$		1.7
$^2J(7,6)$		1.6
$^2J(2,3)$	3.6	3.9
$^5J(6,3)$		0.8
$^2J(4,3)$		0.8
$^4J(a,3)$		7.8
$^3J(b,3)$		7.9
$^2J(8,7)$	3.3	3.9
$^5J(2,7)$		1.1
$^4J(b,7)$		7.7
$^3J(a,7)$		5.9
$^2J(6,7)$		1.8
$^2J(3,2)$	9.0	9.0
$^3J(a,2)$		11.0
$^4J(b,2)$		1.0
$^5J(7,2)$		0.8
$^3J(4,2)$	5.7	5.8
$^3J(6,Me)$	5.4	5.4
$^3J(b,Me)$		6.2
$^4J(7,Me)$		0.8
$^4J(a,Me)$		1.0
$^5J(8,Me)$		1.0

of 5-Me-8-OH-quinoline, obtained without proton decoupling. Values of the couplings missing in the first column were not observed in the 1D ^{13}C spectrum. The particular carbon-proton splitting can be reliably assigned only on the ground of the selective proton »flip« as it can be gathered from the second column.

From 1D ^{13}C coupled spectrum the coupling constant of methyl carbon with H6 only could be obtained. In selective HET 2D J-resolved experiment, we have used a narrower spectral width in F2 dimension to get better resolution. This required the elimination of the methyl carbon signal from the experiment. Even so, the resolution was insufficient to resolve the chemical shift difference of 0.02 ppm between C5 and C6.
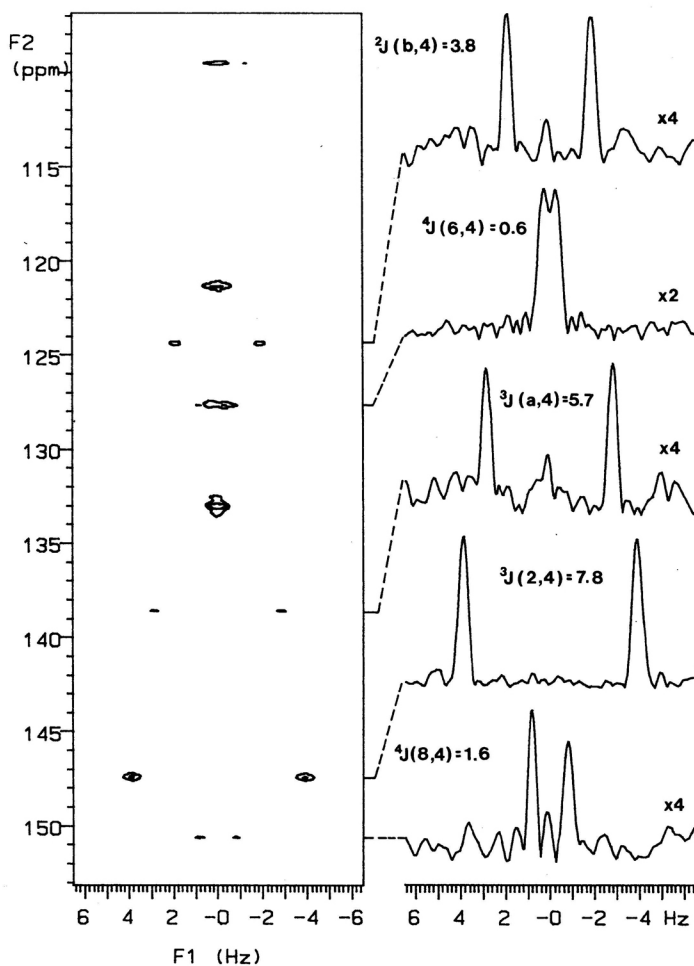

Figure 3. Selective heteronuclear 2D J-resolved spectrum of 5-Me-8-OH-quinoline in CDCl₃. The H4 proton was selectively irradiated. The weak artefacts at F₁-0 of Cb, Ca and C8 traces are due to pulse imperfections.
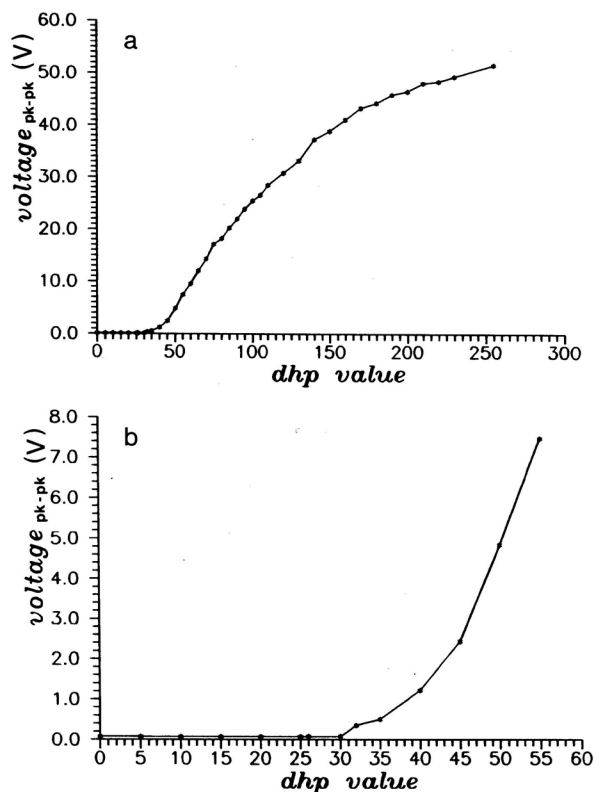
Figure 4. Dependence of the decoupler output peak to peak voltage relative to the decoupler high power (DHP) value, which determines the decoupler output power on Varian VXR 300 MHz spectrometer: a) whole region and b) the lowest part of the region.

An important feature of the HET 2D J-resolved experiment is that resonance lines along F_1-axis appear with natural width, because the static magnetic field inhomogeneity is eliminated by the refocusing pulse applied to the ^{13}C at time $t_1/2$. This facilitates the observation of splittings of an order of magnitude of 1 Hz and, thus, makes it possible to determine carbon-proton couplings in 5-Me-8-OH-quinoline over four and five bonds, even for the nonprotonated sites a and b.

We might also obtain good results by using the proton detected heterocorrelation method[8] for the determination of long-range proton-carbon couplings of 5-Me-8-OH-quinoline because we can use smaller spectral width in F_1 dimension allowing folding of Me-carbon. Using this method, we could obtain all the coupling constants at once. However, for the determination of couplings of samples with clearly resolved proton multiplets and where folding of ^{13}C resonances would seriously disturb the part of the spectrum to be observed, the selective HET 2D J-resolved method is preferable.

The selective HET 2D J-resolved method can be implemented on each NMR spectrometer that permits switching the DOP by computer at least between two levels in a few μsec. Spectrometers with a C class output decoupler amplifier, like the Varian

VXR 300 MHz spectrometer, usually have no regulation of DOP at low decoupler levels (Figure 4). In this case, it is recommended to measure the DOP dependence relative to the decoupler level to find out the region of possible DOP regulation.

CONCLUSION

We have shown that standard NMR spectrometers with transmitter working at fixed power and no facilities for producing shaped pulses can be improved by using suitable attenuators for the attenuation of DOP. In this simple and inexpensive way, selective rectangular proton pulses can be generated by decoupler. The indirect method for ^{1}H resonance observation that we have introduced for selective proton pulse calibration allows precise determination of the selective proton pulse length. The scheme was employed for selective proton excitation in the HET 2D J-resolved method and gave good results, as shown by the determination of the long-range proton-carbon couplings of 5-Me-8-OH-quinoline.

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POVZETEK

Vpeljava selektivne HET 2D-J metode na standardni NMR spektrometer in kalibracija selektivnega protonskega pulza.

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Predstavili smo metode, ki se običajno uporabljajo za določanje ^{13}C–^{1}H sklopitvenih konstant prek več vezi. Izbrali smo selektivno HET 2D-J metodo, ki se odlikuje po dobri občutljivosti in resoluciji v F_1 dimenziji. To metodo smo vpeljali na standarden JMR spektrometer, , ki nima možnosti oblikovanja pulzov in ima »transmitter«, ki dela pri fiksni moči. Selektivni protonski pulz smo dosegli z uporabo atenuatorjev za dušenje izhodne moči »decouplerja« in ga kalibrirali z indirektno metodo za opazovanje ^{1}H resonance, ki omogoča natančno določitev dolžine selektivnega pulza. Predlagano metodologijo lahko vpeljemo na vsak spektrometer, ki ima možnost računalniškega spreminjanja izhodne moči »decouplerja« vsaj med dvema nivojema v času nekaj μsec. Izvedbo smo preizkusili na 5-Me-8-OH-kinolinu, na katerem ^{13}C–^{1}H sklopitvene konstante prek več vezi še niso bile določene.