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Phase sensitive 3D J-resolved HMBC experiment for spectral assignment and measurement of long-range heteronuclear coupling constants

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Abstract

A phase sensitive 3D J-resolved HMBC experiment has been developed to obtain well-separated multiple-bond and one-bond heteronuclear correlation spectra. This experiment greatly facilitates spectral assignment and accurate measurement of the long-range ^{1}H–^{13}C J coupling constants by a least-squares method.

Heteronuclear long-range coupling constants provide valuable structural information on dihedral angles via the Karplus equation.[1] Difficulties in measuring these quantities, which are caused by the poor sensitivity and low natural abundance of ^{13}C and by complex multiplet structures, hinder their use in practice. The 2D HMBC experiment[2] has long been employed for spectral assignment and the measurement of long-range heteronuclear couplings. Efforts have been made to improve the sensitivity of this experiment. These include the use of pulsed field gradients,[3] constant time methods,[4,5] and variation of the initial delay.[6] Here, we describe a phase-sensitive 3D J-resolved HMBC experiment which correlates ^{1}H and ^{13}C nuclei separated by either one-bond or multiple-bonds, and allows accurate measurement of the heteronuclear long-range J-coupling constants. In this experiment, the delay for long-range heteronuclear magnetization transfer is replaced by a J-evolution period with chemical shifts refocused so that magnetization transfer between ^{1}H and ^{13}C, with different long-range $^{3}J_{CH}$, is optimized.[6] This results in an overall enhancement in sensitivity as well as an improvement in the spectral dispersion of the HMBC experiment. To minimize the sensitivity loss caused by gradient diffusion in the pulse sequence proposed by Davis et al.,[3] we designed a different scheme with application of the pulsed field gradients. Application of this 3D J-resolved HMBC experiment should facilitate spectral assignment and the measurement of long-range ^{1}H–^{13}C couplings, particularly in systems with poor sensitivity and severe overlaps.

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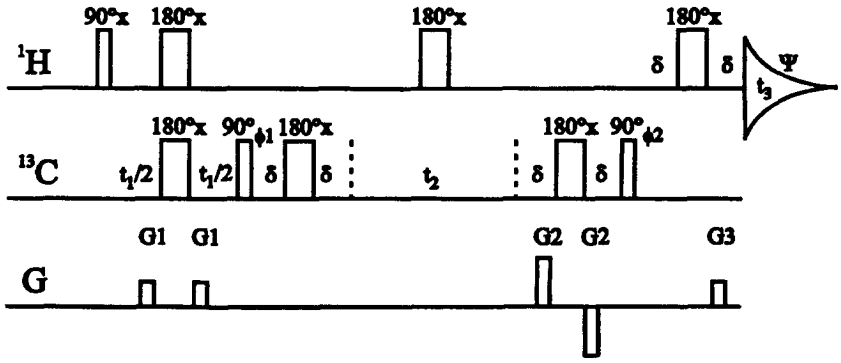

Figure 1. The phase cycling used are φ1=0, 2; φ2=0, 0, 2, 2; Rec Ψ=0, 2, 2, 0. The duration of z gradients G1, G2 and G3 were 1, 2 and 2 ms, respectively; and their strengths were 4.75, 23.75 and 11.94 G/cm, respectively. Quadrature detection in t_2 dimension was achieved by recording P- and N-type spectra separately

Fig. 1 illustrates the pulse sequence of the 3D J-resolved HMBC experiment. The preparation period of the 2D HMBC experiment[2] is now replaced by an evolution period for one-bond and multiple-bond J couplings. There are two advantages of this arrangement over the method proposed by Furihata et al.[6] Firstly, chemical shifts during the J evolution period are refocused so the spectral width of this dimension can be reduced to $^1J_{CH}$ Hz only. This has the benefit of avoiding possible peak cancellation, due to spectral folding, as well as increasing the digital resolution.[7] The spectral width in the J dimension can be further reduced to optimize the sensitivity and digital resolution as long as the folded $^1J_{CH}$ peaks do not overlap the $^3J_{CH}$ cross peaks. For example, a 100 Hz spectral width will result in no overlap between the $^1J_{CH}$ and $^3J_{CH}$ cross peaks since the $^1J_{CH}$ values are around 130 Hz and $^3J_{CH}$ values are less than 20 Hz. Secondly, 2D HMQC and HMBC spectra, which are optimal in sensitivity for a wide range of $^1J_{CH}$ and $^3J_{CH}$ values, can be obtained by summing the sections of the 3D spectrum along the J (F_1) dimension corresponding to the well separated one-bond and long-range J couplings. The 2D HMBC spectrum obtained in this way is free of interference from the one-bond correlation peaks. The separation of the one- and multiple-bond correlation peaks is more effective than that in the traditional experimental method, which uses a one-bond $^1J_{CH}$ filter that cannot purge the strong one-bond ^1H–^{13}C correlation peaks completely due to the variation in $^1J_{CH}$ coupling constants.[8]

Pulsed field gradients are applied in the second evolution period for the selection of the coherence pathway and they also serve to suppress t_1-noise generated by solvent signals and untransferred magnetizations. In the original HMQC experiment,[3] gradients are placed in two carbon 180° refocusing periods on both sides of the ^{13}C evolution period, and another pulsed field gradient is applied before detection to select the coherence pathway. Here the gradient diffusion effect will lead to line width broadening and a loss of sensitivity. This effect will be particularly severe for NMR study of smaller molecules.[9] An alternative method is shown in Fig. 1, in which the pulsed field gradients are applied in such a way that minimizes the gradient diffusion effect and also suppresses the t_1-noise.

Since the peaks in the J (F_1) and detection (F_3) dimensions are antiphase in nature, delayed acquisition can be used in these dimensions[10] to further optimize the sensitivity of correlations with small $^3J_{CH}$ couplings. In addition, the apparent sensitivity and resolution of long-range correlations can be further enhanced if backward extension in the t_1(J) dimension is carried out by the linear prediction method to partially remove the long-range J couplings.[10]

To demonstrate the method described above, we have measured a 3D J-resolved HMBC spectrum of a natural product, 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl heidagenin-28-*O*-β-D-glucopyranoside (G4), which is isolated from the roots of *Pulsatilla patens* var. *multifuda*. The struc-

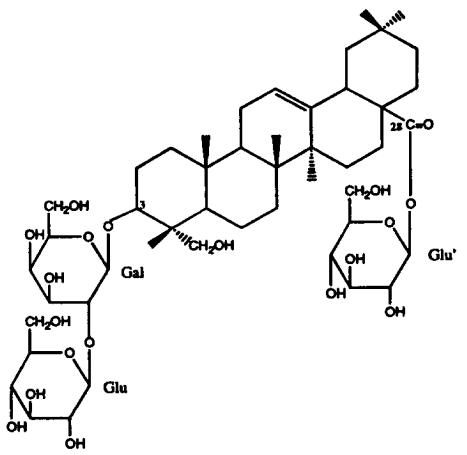

Figure 2.

ture of G4 is shown in Fig. 2. The 3D experimental conditions were as follows: $F_1(J)\times F_2(^{13}C)\times F_3(^{1}H)$=125×22637.5×4500 Hz, data matrix is 12 (real)×200 (complex)×1024 (complex), the value of t_1 was varied from 40 ms to 136 ms with increments of 8 ms in order to optimize the magnetization transfer based on $^{3}J_{CH}$, eight scans were measured per transient, and the total measuring time was 14.5 h. The experiment was conducted on a Varian Unity INOVA 500 MHz spectrometer running under VNMR software. The data were processed with the nmrPipe software package.[11] A cosine bell window function was applied in the t_3 dimension, followed by zero-filling to 2K complex data points, Fourier transformation and phasing. In the $t_1(J)$ dimension, linear prediction was used to extend the data by four points in the front and by 12 points at the end. An additional zero point was added in the front by right-shifting the data. This was followed by applying a squared sine bell window function and zero-filling to 128 data points (real). Processing of the t_2 (^{13}C) dimension was carried out with a cosine bell window function and zero-filling to 512 data points.

One-bond and multiple-bond ^{1}H–^{13}C correlation spectra of G4 were obtained by projection from the 3D spectrum. ^{1}H–^{13}C planes, with J ranges from 83 to 203 Hz, were used to project the one-bond ^{1}H–^{13}C correlation spectrum shown in Fig. 3A. The 2D multiple-bond long-range ^{1}H–^{13}C correlation spectrum shown in Fig. 3B was obtained from planes with J ranging from 0 to 20 Hz. It should be noted that the long-range ^{1}H–^{13}C correlation spectrum did not contain any one-bond correlation peaks. This clearly demonstrates the advantage of the 3D J-resolved HMBC experiment over the conventional 2D HMBC method in terms of reduction in spectral overlap, hence greatly facilitating the spectral analysis of complicated natural products.

The 3D J-resolved HMBC experiment can also be very useful for accurate measurements of the long-range $^{3}J_{CH}$ coupling constants by fitting the one-bond correlation peaks to the corresponding long-range correlation peaks. This approach, in which the reference spectrum is recorded simultaneously, is more convenient and accurate than the conventional method in which a reference spectrum is recorded by a different experiment, e.g. a TOCSY experiment.[12] The long-range $^{3}J_{CH}$ couplings measured in our experiment requires that the relaxation properties of the long-range and the one-bond resonances are the same. This condition is approximately satisfied in NMR studies of organic molecules. It should be noted that since the resolution of the detection dimension (F_3) is much higher than that of the J (F_1) dimension, the long-range $^{3}J_{CH}$ coupling constants can be more accurately measured in the detection dimension.

To obtain the long-range $^{3}J_{CH}$ couplings, two 1D cross-sections in F_3 dimension, which contain the $^{3}J_{CH}$ and $^{1}J_{CH}$ correlation multiplets for a particular proton, were extracted from the 3D spectrum. Both

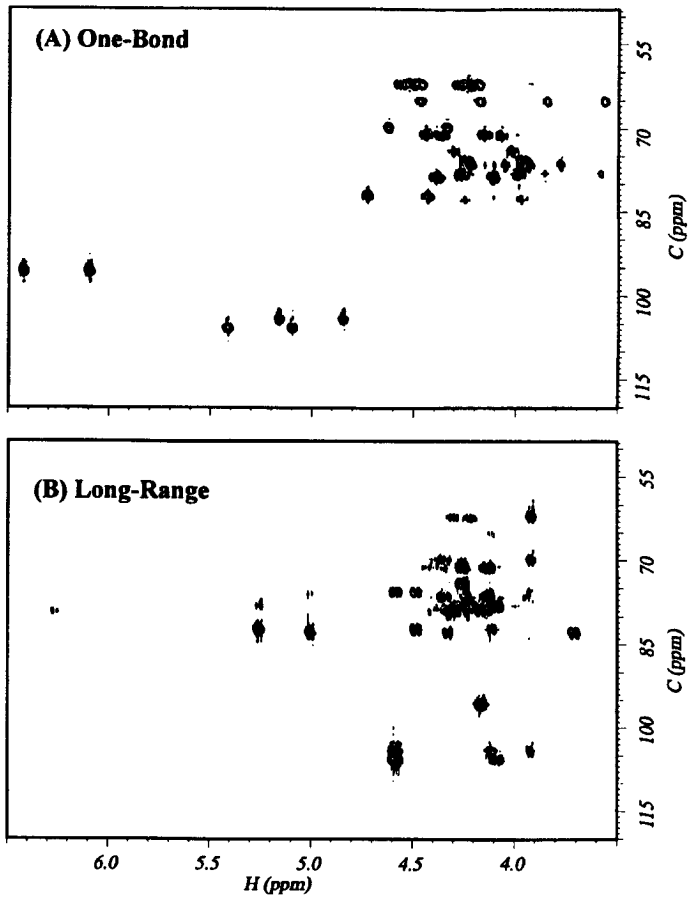

Figure 3.

cross-sections were subjected to FFT interpolation to 16 K points, followed by baseline correction. The center of the selected multiplet in the $^{1}J_{CH}$ cross-section was estimated and then shifted by 1/2 a trial $^{3}J_{CH}$ value. The original multiplet was duplicated, inverted and shifted by 1/2 the trial $^{3}J_{CH}$ value in the opposite direction. The combined multiplet was fitted to the experimental $^{3}J_{CH}$ multiplet cross-section by linear least-squares fitting. Since the center of the experimental multiplet cannot be determined precisely, the center position and the trial $^{3}J_{CH}$ value are both used as variable parameters in the least-squares fitting procedure. The center position and the trial $^{3}J_{CH}$ value which gave the minimal Chi-square goodness of fit value were considered to be the center, and the $^{3}J_{CH}$ coupling constant of the corresponding $^{3}J_{CH}$ multiplet. Fig. 4 shows the $^{3}J_{CH}$ coupling constants of the three glycosidic bonds ($^{3}J_{\text{Glu H1'} \rightarrow \text{Gal C2'}}$, $^{3}J_{\text{Gal H1'} \rightarrow \text{C3}}$ and $^{3}J_{\text{Glu' H1'} \rightarrow \text{Gal C28}}$) in G4 obtained by using the method described above. Two shifted one-bond multiplets (upper trace) were added to each other and the resulting multiplet was matched with the long-range multiplet (lower trace).

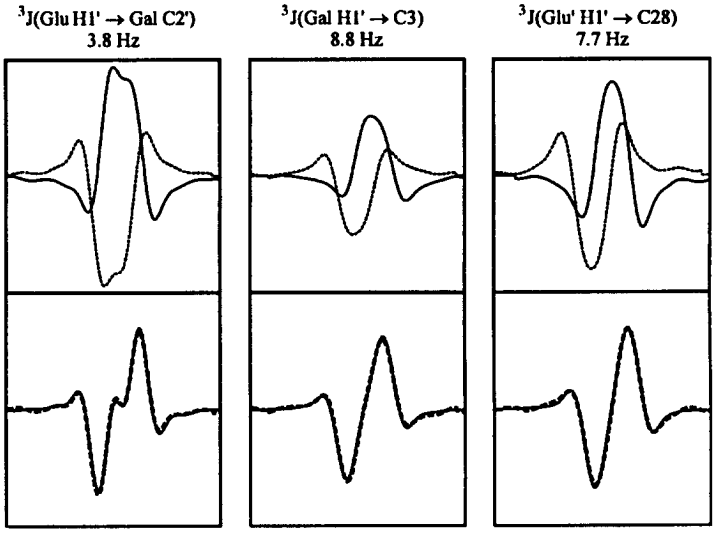

Figure 4.

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