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Determination of Heteronuclear Long-Range $^{1}H-^{13}C$ and $^{1}H-^{15}N$ Coupling Constants of Harman by Modified *J*-HMBC 2D NMR Techniques

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Abstract—Selective and double-selective *J*-HMBC 2D methods were developed and employed to determine the coupling constants of harman (**1**). The $^{1}H-^{13}C$ and $^{1}H-^{15}N$ long-range coupling constants could be accurately determined without interference from the $^{1}H-^{1}H$ *J*-modulation effect. We also applied selective *J*-HMBC 2D to 2-fluoropyridine (**2**) to confirm effectiveness.

Introduction

The measurement of spin–spin coupling constants, together with NOE (nuclear Overhauser effect) by NMR spectroscopy, is very helpful for the structure elucidation and conformational analysis of organic compounds.[1–10] The heteronuclear multiple bond correlation (HMBC) technique combined with a pulse field gradient (PFG)[11] provides highly sensitive detection of the long-range coupling correlation for low-natural-abundance nuclei such as ^{15}N (0.37%),[12–15] as well as ^{13}C (1.1%). Recently, a new method, *J*-HMBC 2D using a modified PFG HMBC pulse sequence, has been developed for the measurement of heteronuclear long-range coupling constants.[16] This method has the advantage of detecting long-range coupling constants of nuclei to which no proton is attached, and is applicable to compounds having very complex ^{1}H NMR spectra, thanks to the distinct 2D separation. It has been applied to measure accurately the $^{1}H-^{15}N$ coupling constants of mono-substituted pyridines.[17] In this paper we wish to report the determination of heteronuclear long-range $^{1}H-^{13}C$ and $^{1}H-^{15}N$ coupling constants of harman (**1**), the basic framework of β-carboline alkaloids, by using the *J*-HMBC 2D method. We have also developed improved methods, selective and double-selective pulse excitation *J*-HMBC 2D, to obtain more accurate values of the coupling constants.

Keywords: harman; 2-fluoropyridine; long-range coupling constant; $^{1}H-^{13}C$ HMBC; $^{1}H-^{15}N$ HMBC; *J*-HMBC 2D.

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Results and Discussion

J-HMBC 2D

The principle of the *J*-HMBC 2D method is as follows. The HMBC signal intensity is given by a sine function of the pulse interval time (Δ) and coupling constant (J_{HX}) (Eq. (1)).

$$\text{Intensity} = |A \sin(\pi J_{HX} \Delta)| \quad (1)$$

Therefore, the *J* value (coupling constant) can be derived from a least-squares approximation by fitting a sine curve to the signal amplitude of the HMBC correlation peak depending on the characteristic sin (πJ_{HX}) with increasing evolution time (Δ). The pulse sequence for this method is shown in Fig. 1a. However, a poor correlation coefficient, *R*-value, was obtained in the case of 2-substituted pyridine[17] owing to the $^{1}H-^{1}H$ *J*-modulation effect. We therefore developed new methods, selective and double-selective pulse excitation *J*-HMBC 2D, in order to avoid this interference. The pulse sequences of these methods are shown in Fig. 1b and c.

Long-range coupling constants of harman (1)

NMR spectroscopic studies of harman (3-methyl-4-carboline) (**1**), the basic framework of β-carboline alkaloids, have been reported,[18,19] including measurement of the $^{1}H-^{13}C$ long-range coupling constants based on ^{1}H coupled[20] and selective ^{1}H decoupled[21] ^{13}C NMR experiments. However, evaluation of the coupling constants was restricted by the difficulty of distinguishing long-range correlations from

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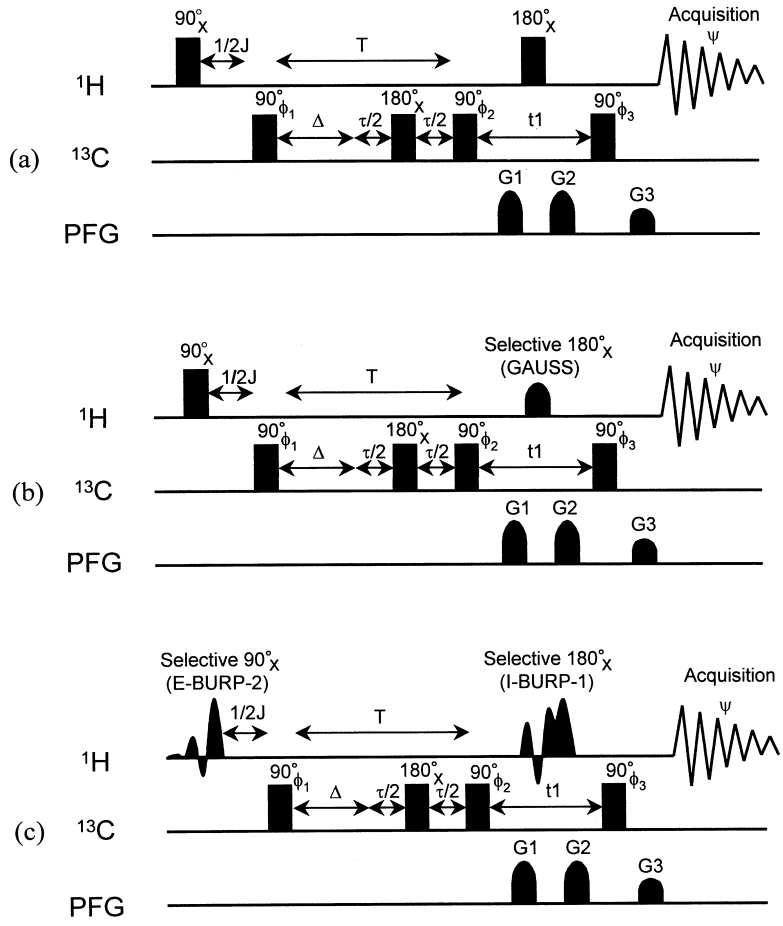
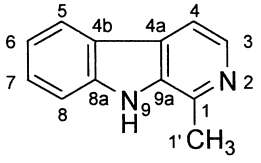


Figure 1. Pulse sequence for the gradient-selected *J*-HMBC 2D experiments: (a) conventional pulse sequence; (b) selective *J*-HMBC 2D pulse sequence; (c) double-selective *J*-HMBC 2D pulse sequence. Pulse interval time τ is varied to set different Δ values at constant time *T*. The ^{13}C 180° pulse is a $90°_X$–$180°_Y$–$90°_X$ composite pulse. The gradient ratios are G1:G2:G3=2:2:1 or 5:3:4. The following phase cycle was used: $\phi_1=x; -x$; $\phi_2=x, -x$; $\phi_3=x,x,-x$; $\psi=x, -x,-x,x$.



(1)

Scheme 1.

Table 1. ^{13}C and ^{15}N NMR assignments (^{1}H and ^{13}C chemical shifts are referenced to TMS as an internal standard; ^{15}N chemical shifts are referenced to neat CH_3NO_2 (0 ppm) as an external standard) of harman (**1**)

Position	δ_H	δ_C	δ_N
1		142.1	−73.6
2			
3	8.26(d, *J*=5.5 Hz)	137.5	
4	7.94(d, *J*=5.5 Hz)	112.6	
4a		126.9	
4b		121.1	
5	8.21(d, *J*=7.7 Hz)	121.7	
6	7.25(t, *J*=7.7 Hz)	119.2	
7	7.56(t, *J*=7.7 Hz)	127.8	
8	7.65(d, *J*=7.7 Hz)	111.9	
8a		140.4	
9			−264.6
9a		134.5	
1′	2.83 (s)	20.4	

specific ^{1}H using ^{1}H decoupled ^{13}C NMR. In this study we subjected this compound to *J*-HMBC 2D (Scheme 1).

Table 1 shows the assignments of ^{1}H, ^{13}C and ^{15}N NMR signals of this compound. The values of ^{1}H–^{15}N long-range coupling constants measurements (natural abundance) obtained by conventional *J*-HMBC 2D are shown in Table 2.

Table 2. ^{1}H–^{15}N long-range coupling constants (Hz) (coupling constants are absolute values; accuracy is estimated to be ±0.5 Hz; the values in parenthesis are correlation coefficient) of harmane (**1**) by *J*-HMBC 2D

Position	3-H	4-H	8-H	1′-H
N-2	11.4 (0.9890	1.5 (0.981)		3.0 (0.998)
N-9			<0.5	3.1 (0.936)

As can be seen from this Table, good *R*-values were obtained owing to fortuitously low interference by ^{1}H–^{1}H *J*-modulation. However, in the case of ^{1}H–^{13}C long-range coupling, poor results ($R<0.9$) were observed at 6-, 7- and 8-H (Table 3). This is ascribed to the ^{1}H–^{1}H *J*-modulation effect because the signals of these *o*-coupled protons are concentrated within 0.4 ppm in the spectrum (Fig. 2).

Selective and double-selective *J*-HMBC 2D

In order to avoid the influence of ^{1}H–^{1}H *J*-modulation, an

Table 3. $^{1}H-^{13}C$ long-range coupling constants (Hz) (coupling constants are absolute values; accuracy is estimated to be ±0.5 Hz; the values in parenthesis are correlation coefficient) of harmane (**1**) obtained by *J*-HMBC 2D

Position	3-H	4-H	5-H	6-H	7-H	8-H	1′-H
C-1	11.6 (0.987)						6.5 (0.985)
C-3		2.5 (0.941)					
C-4	8.5 (0.952)						
C-4a	7.3 (0.951)	1.5 (0.957)	2.8 (0.920)				
C-4b		2.4 (0.988)	1.3 (0.988)	8.5 (0.858)		5.4 (0.956)	
C-5					8.3 (0.431)		
C-6			1.7 (0.928)		0.6 (0.974)	6.8 (0.884)	
C-7			7.2 (0.939)	2.3 (0.910)		8.1 (0.628)	
C-8				8.1 (0.778)	0.2 (0.738)		
C-8a			7.6 (0.989)		9.8 (0.978)		
C-9a	1.1 (0.997)	6.8 (0.952)					3.0 (0.992)
C-1′	0.3 (0.989)	0.2 (0.991)					

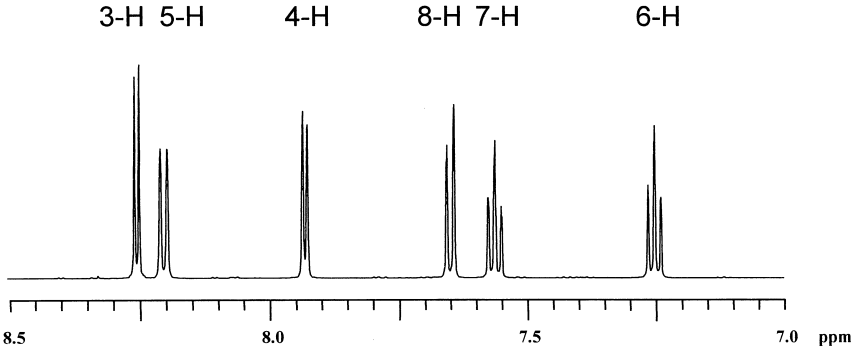


Figure 2. ^{1}H NMR spectrum of harman (**1**) in the aromatic region.

Table 4. Comparison of long-range coupling constants (Hz) (coupling constants are absolute values; accuracy is estimated to be ±0.5 Hz; the values in parenthesis are correlation coefficient) between 6,7,8-H and related carbons of harman (**1**) obtained by *J*-HMBC 2D, selective J-HMBC and double selective *J*-HMBC 2D

Position	Method	C-4b	C-5	C-6	C-7	C-8	C-8
6H	*J*-HMBC	8.5 (0.858)			2.3 (0.910)	8.1 (0.778)	
	Selective *J*-HMBC	8.5 (0.960)			2.3 (0.994)	7.9 (0.989)	
7H	*J*-HMBC		8.3 (0.431)	0.6 (0.974)			9.8 (0.978)
	Selective *J*-HMBC		5.8 (0.591)	1.5 (0.561)			9.7 (0.993)
	Double selective *J*-HMBC		8.2 (0.978)	0.2 (0.976)			9.7 (0.964)
8H	*J*-HMBC	5.4 (0.956)		6.8 (0.884)			
	selective *J*-HMBC	5.7 (0.829)		7.6 (0.829)			
	Double selective *J*-HMBC	1.2 (0.992)		7.3 (0.994)			

improved pulse sequence (Fig. 1b) for selective *J*-HMBC 2D, which can excite a focused region of proton frequency with a GAUSS pulse,[22] was designed. Though the selective pulse excitation caused no significant change at the closely located 7- and 8-H, a remarkable improvement was seen at 6-H (Table 4). The lack of effectiveness at 7- and 8-H may be due to the very small chemical shift difference [7−H(δ_H 7.56) and 8−H(δ_H 7.65)].The E-BURP-2[23] and I-BURP-1[23] pulses were employed for further selective excitation in this proton region. The double-selective *J*-HMBC 2D (Fig. 1c) is compared with the conventional *J*-HMBC 2D in Fig. 3 for the case of harman (**1**). The F2 slice data exhibiting cross sections of the correlated signals between 7-H and 5-C recorded at Δ=70 and 170 ms by the two methods are presented in Fig. 3. No signal change due to the $^{1}H-^{1}H$ *J*-modulation effect was observed in the case of double-selective *J*-HMBC 2D. The advantage of using this pulse was also clear from the HMBC signal intensity change, compared with that of the conventional *J*-HMBC 2D method. The derived function exhibits a reasonably well-developed sine curve, which is almost identical with the calculated one (R=0.978) in the case of double-selective *J*-HMBC 2D, suggesting that there is little interference from the $^{1}H-^{1}H$ *J*-modulation effect in this measurement. The $^{1}H-^{13}C$ coupling constants measured by using this double-selective method are shown in Table 4.

Application to 2-fluoropyridine

As mentioned above we previously showed that low-natural-abundance $^{1}H-^{15}N$ long-range coupling constants can be measured using PFG *J*-HMBC 2D, for example in the case of mono-substituted pyridines.[17] However, inconsistent signal intensities with a poor R-value (less than 0.95) were observed for 2-substituted pyridine, whereas good correlations were obtained for 3- and 4-substituted pyridines. This can be ascribed to the $^{1}H-^{1}H$ *J*-modulation effect caused by the closely located *o*-coupled protons, as

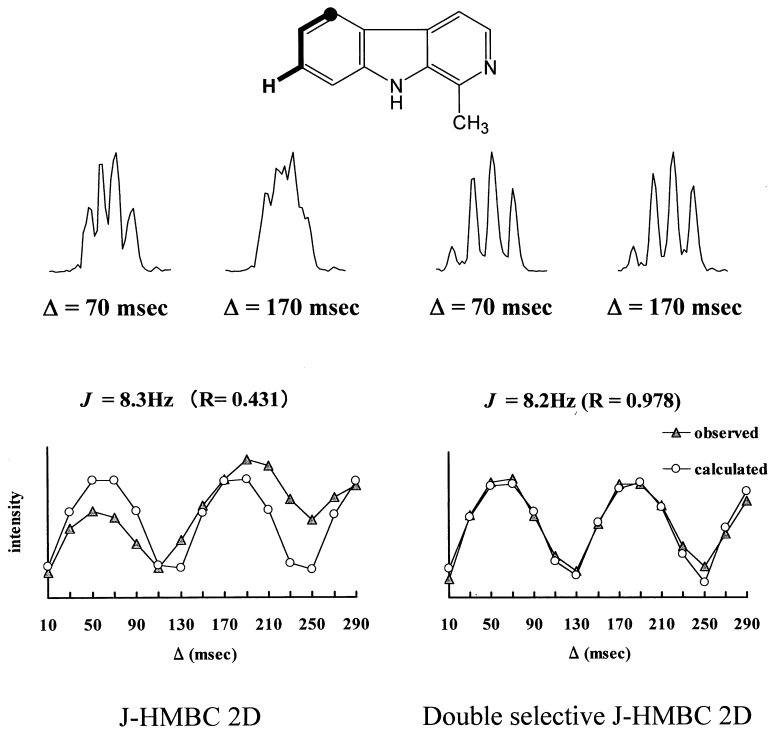


Figure 3. Effect of the selective excitation pulse in *J*-HMBC 2D experiments on harman. Middle: Comparison of F2 slices of the cross peak between 7-H and 5-C at Δ=70 and 170 ms. Bottom: Comparison of relative intensity changes of the observed cross peaks and the calculated values by the method of least squares.

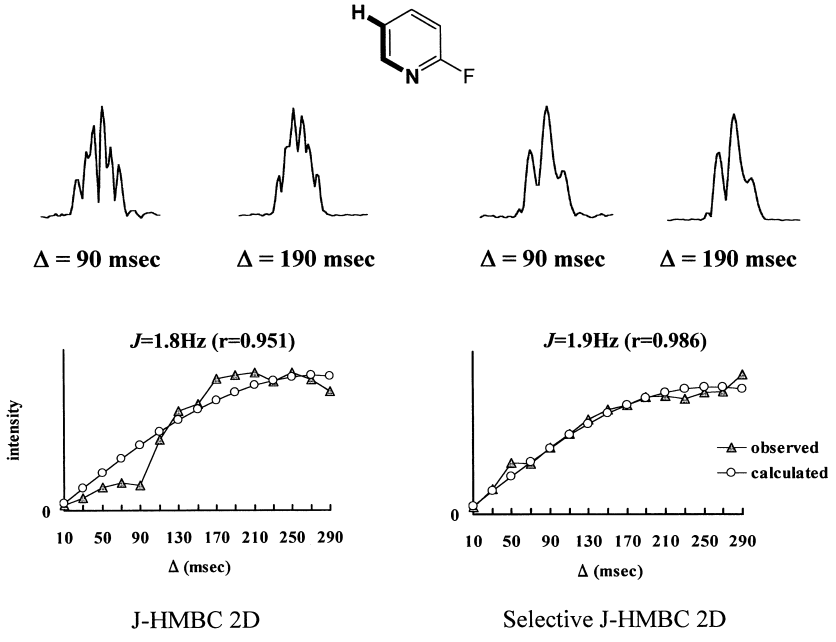


Figure 4. Effect of the selective excitation pulse in *J*-HMBC 2D experiments on 2-fluoropyridine. Middle: Comparison of F2 slices of the cross peak between 5-H and N at Δ=90 and 170 ms. Bottom: Comparison of relative intensity changes of the observed cross peaks and the calculated values by the method of least squares.

observed in the case of harman. Therefore, we applied selective *J*-HMBC 2D to 2-fluoropyridine (Scheme 2). We observed a clear improvement of the *R*-value for the 5-H–N correlation (Fig. 4). This result confirmed that the method using the selective pulse is also useful, reducing the effect of $^{1}H-^{1}H$ *J*-modulation on measurements of $^{1}H-^{15}N$ long-range coupling constants.

Scheme 2.

Conclusion

The *J*-HMBC 2D method can afford precise values of $^{1}H-^{13}C$ and $^{1}H-^{15}N$ long-range coupling constants. The use of selective or double-selective *J*-HMBC 2D proved to be effective to reduce interference with the measurements owing to the $^{1}H-^{1}H$ *J*-modulation effect. The optimum method can be chosen depending on the chemical shifts and the complexity of the signals in the ^{1}H NMR spectrum. It is also possible to focus the observation region onto specific ^{1}H and ^{13}C, which are strongly correlated to the excited proton, in contrast to the conventional *J*-HMBC 2D, which covers the whole region of the observable frequency. There is a slight reduction of sensitivity in the selective or double-selective methods compared to the conventional *J*-HMBC 2D. Considering its applicability to various sorts of compounds,[24] selective *J*-HMBC 2D may be the most practical method for samples available in limited amounts.

Experimental

Harman (**1**) (mp 137–138°C) was purchased from Nacalai Tesque Co., Japan. DMSO-d_6 (99.9 atom %D) was purchased from Isotec Inc., USA. 2-Fluoropyridine (**2**) was purchased from Tokyo Kasei Kogyo Co. Ltd., Japan.

All NMR data were recorded on a JEOL JNM-LA600 spectrometer equipped with a 5 mm NALORAC HX inverse probe at 303 K. Chemical shifts are given in δ values in ppm, and coupling constants in Hz. Chemical shifts of ^{1}H and ^{13}C nuclei were referenced to tetramethylsilane as an internal standard (0 ppm), and ^{15}N nuclei were referenced to neat nitromethane as an external standard (0 ppm).

$^{1}H-^{13}C$ and $^{1}H-^{15}N$ experiments on harman were performed using a 0.55 mol ml^{-1} solution (harman 50 mg /DMSO-d_6 0.5 ml). Fifteen conventional *J*-HMBC 2D spectra were acquired with 8 scans per increment for a 4096 (F2)×256 (F1) data matrix for ^{13}C and 64 scans per increment for a 2048 (F2)×64 (F1) data matrix for ^{15}N with a 300 ms constant time (τ max) and varying the pulse interval time (Δ) stepwise from 10 to 290 ms. Pulse repeat time (pulse delay time+acquisition time) was set at 2 s.

Fifteen $^{1}H-^{13}C$ selective and double selective *J*-HMBC 2D spectra of harman were acquired with 32 scans per increment for a 2048 (F2)×64 (F1) data matrix with a 300 ms constant time (δ max) and varying the pulse interval time (Δ) stepwise from 10 to 290 ms. Pulse repeat time (pulse delay time+acquisition time) was set at 2 s.

$^{1}H-^{15}N$ experiments on 2-fluoropyridine were performed using a 0.5 mol ml^{-1} solution. Fifteen selective *J*-HMBC 2D spectra of 2-fluoropyridine were acquired with 64 scans per increment for a 2048 (F2)×64 (F1) data matrix with the same pulse conditions described for the measurement of harman.

All spectra were processed with a shifted Blackman Harris window[25] for the F2 and F1 dimensions after zero filling to the F1 dimension. The signal amplitudes were determined from peak heights recorded using the peak picking program of the JEOL JNM-LA600 data processing software. The obtained data were fitted to a sine curve by the least-squares method.

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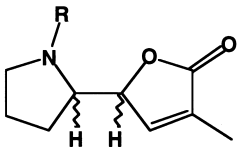
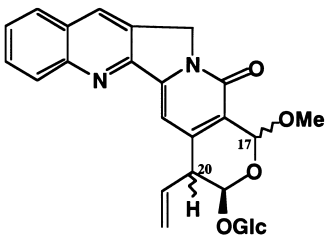
24. For example, *erythro* and *threo* isomers of 5-pyrrolidinyl-γ-lactone and camptothecin derivatives from *Ophiorrhiza pumila* were analyzed (unpublished data).



5-pyrrolidinyl-γ-lactone



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