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J-Resolved HMBC, a new NMR technique for measuring heteronuclear long-range coupling constants

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Abstract

A new NMR pulse sequence that incorporates J-scaling into HMBC enables efficient measurement of heteronuclear long-range coupling.

Keywords: NMR; HMBC; J-resolved HMBC; heteronuclear long-range coupling.

Heteronuclear long-range coupling constants have recently been successfully utilized in stereochemical investigations of acyclic systems such as maitotoxin[1] and dysiherbaine.[2] These approaches relied on the determination of coupling constants between protons and carbons separated by two or three chemical bonds ($^2J_{C\text{–}H}$ or $^3J_{C\text{–}H}$). The methods utilized for this purpose are HETLOC,[3] selective J-resolved 2D,[4] or quantitative HMBC.[5] We now report a useful new NMR technique named J-resolved HMBC.

HMBC[6] is one of the most powerful NMR techniques for detecting heteronuclear long-range correlations. The cross peaks in HMBC spectra, in principle, contain information on heteronuclear long-range coupling constants. The magnitudes of long-range C–H coupling constants, however, cannot be practically determined from the multiplets of the cross peaks due to insufficient digital resolution. Increasing the number of data points to avoid this problem is not the method of choice because of the combined effects of ^1H–^1H and ^1H–^{13}C J-modulations which prevent improvement of the formal resolution.

In order to overcome this problem, we have modified conventional HMBC to give two new NMR techniques, J-resolved HMBC-1 and J-resolved HMBC-2, by incorporating J-scaling pulse sequence.[7] The basic principle of these techniques is to amplify formal magnitudes of the coupling constants by a factor of n (n=20 to 30) by J-scaling, thereby allowing measurement of such enlarged long-range coupling constants easily by HMBC.

The two pulse sequences, J-resolved HMBC-1 and J-resolved HMBC-2, are shown in Fig. 1. The magnetization during the HMBC pulse sequence is evolved with the carbon chemical shift and pro-

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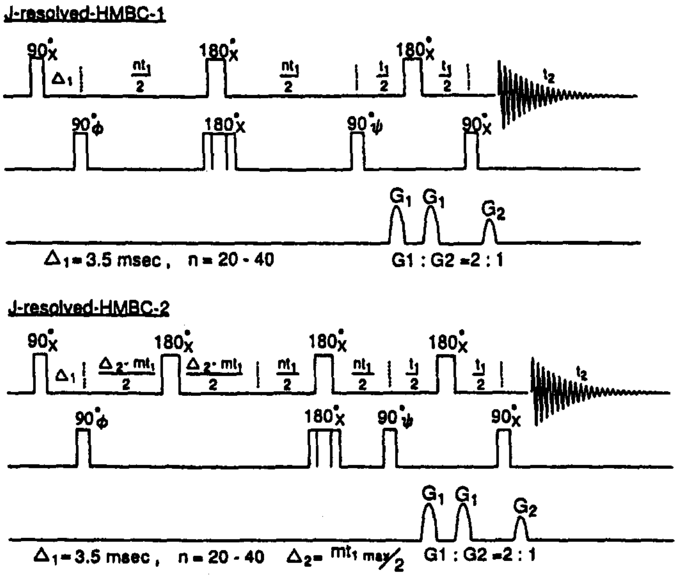


Figure 1.

ton–proton spin couplings, but not with proton–carbon spin couplings. Since Δt_1 and $t_1$max in HMBC experiments are usually set to several μsec and 20–30 msec, respectively, proton–proton spin couplings cannot be detected in the f_1-dimension due to insufficient digital resolution. To measure such spin coupling constants, $t_1$max needs to be set to several hundred msec to increase the digital resolution. However, the evolution time t_1 is not practically evolved to give such a large $t_1$max value due to the decrease of S/N.

In J-resolved HMBC-1, t_1 is evolved over 300–500 msec to enable the measurement of proton–carbon spin coupling constants in the f_1 dimension, and the spin evolution time (Δ_2) of the HMBC pulse sequence is replaced by a J-scaling pulse ($-nt_1/2$–180(H,C)–$nt_1/2$-) (Fig. 1). As a result, the magnetization is evolved with t_1, $(n+1)t_1$ and nt_1 for chemical shift, proton–proton spin couplings and proton–carbon couplings, respectively, and thus the spin couplings $J_{H\text{-}H}$ and $J_{C\text{-}H}$ can be detected in the f_1 dimension as the values of $(n+1)\ J_{H\text{-}H}$ and $nJ_{C\text{-}H}$. In 2D-J-resolved spectroscopy, which is utilized for measuring coupling constants, $t_1$max must be set to a value larger than $1/J$. If $t_1$max were smaller than $1/J$, a condition required by the sampling theorem is not satisfied and the spin coupling constants cannot be determined. Thus, in the J-resolved HMBC method, the scaling factor (n) must be set so as to give the nt_1max value larger than $1/J$ as in the evolution time of 2D-J-resolved spectroscopy.

In J-resolved HMBC, nt_1max must be developed from 333 msec (3 Hz) to 500 msec (2 Hz) according to the magnitude of the spin coupling constant to be observed. Therefore, it should be noted that S/N of J-resolved HMBC is inferior to that of the conventional HMBC where $t_1$max is typically set to 20–30 msec.

The J-resolved HMBC-1 spectrum of monazomycin[8,9] is shown in Fig. 2. The large coupling constant 5.5 Hz between 47-H and C49 obtained from $25\times J=135$ Hz indicated a *trans* relation between them. The small ^{1}H–^{13}C long-range couplings between 47-H and 48-Me, 46-Me and C45 (less than 3 Hz) show *gauche* relationships between 47-H and these carbons.

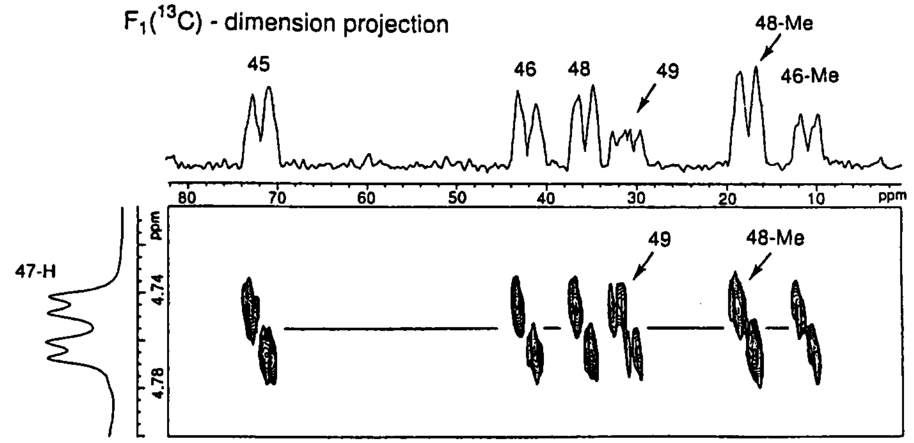
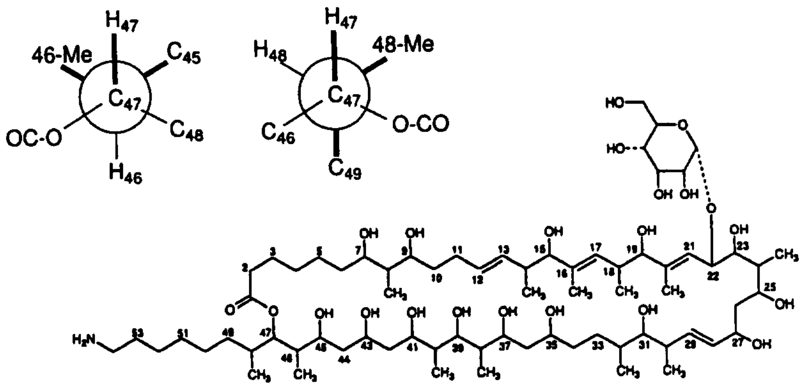


Figure 2.



Monazomycin

In J-resolved HMBC-2, an additional pulse (Δ_2-$mt_1/2$–180(H)–Δ_2-$mt_1/2$) is incorporated in order to decouple proton–proton couplings (Fig. 1). When m is set to n+1, J-modulation by proton–proton coupling is fixed by the effect of the constant time method,[10] and the proton–proton couplings are decoupled. Therefore, only nJ_{C-H} is observed, enabling easy spectral analysis. It should be noted, however, that S/N of the spectra is considerably decreased by the effect of the constant time method.

When $m \leq n$, the value of the formal J_{C-H} is increased by n times, while the value of J_{H-H} is scaled down to n+1−m times. Therefore, a shorter Δ_2 value can be set as compared to the case where m is set to n+1, and it becomes possible to improve S/N by this experimental manipulation. An additional effect of this experimental setting is the scaling down of J_{H-H} to prevent overlapping of proton signals. This effect is important when complicated cross peaks must be analyzed.

Fig. 3 shows cross peaks observed with 3-H in the J-resolved HMBC-2 spectrum of portmicin whose stereochemical structure had been established by X-ray analysis.[11] The proton spectral data of portmicin had indicated a *gauche* relationship between 3-H and 4-H ($J_{3,4}$=1.5 Hz), but no information could be obtained with regard to the stereochemical relation between 3-H and 4-Me and C5. The large coupling constant between 3-H and 4-Me, which was determined to be $^3J_{C-H}$=5.2 Hz based on the observed value (25×J_{C-H}=129.7 Hz), clearly established that 4-Me and 3-H are in an *anti* relationship.[12] In addition, $^3J_{C-H} \leq 3$ Hz observed with C1 and 2-Me to 3-H indicated *gauche* relationships between 3-H and these two carbons.

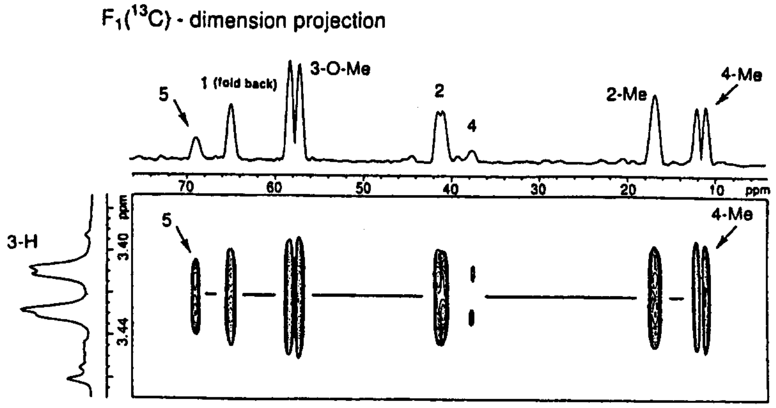
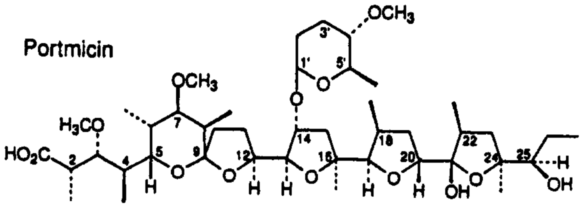
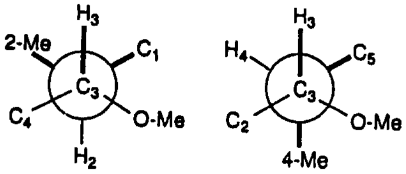
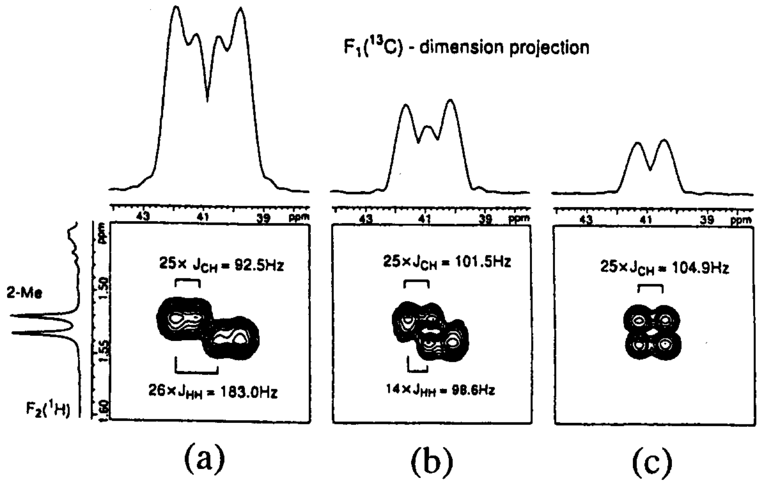


Figure 3.





Effects of proton decouplings in *J*-resolved HMBC-2: The cross peaks between 2-Me-H and C2 of portmicin observed under different conditions[13] are shown in Fig. 4. When *m* is set to 0 (Fig. 4a), $26 \times J_{\text{H-H}}=183.0$ Hz and $25 \times J_{\text{C-H}}=92.5$ Hz are observed, while complete proton decoupling is seen



(a) (b) (c)

Figure 4.

(Fig. 4c) with only $25\times J_{C-H}$=104.9 Hz. On the other hand, an intermediate value m=12 (Fig. 4b) gave $25\times J_{C-H}$=101.5 Hz and $14\times J_{H-H}$=98.6 Hz. From these spectra, $J_{C-H}\fallingdotseq 4$ Hz, $J_{H-H}\fallingdotseq 7$ Hz were calculated. It should be noted that S/N is decreased in the order of Fig. 4a–c as seen by the peak heights of the cross peaks in the projection data of f_1-dimension. Therefore, application of J-resolved HMBC-2 to complicated compounds with short T_2 must be made with this disadvantage in mind.

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9. Experimental conditions: $f_1\times f_2$=23 000×2900 Hz, points=512×1024, scaling factor (n)=25, nt_1max=55 msec, scans=128, digital resolution (f_1)/n=1.8 Hz, total acquisition time=28 h, sample=25 mg/0.4 ml (CD_3OD).
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12. Experimental conditions: $f_1\times f_2$=14 000×2250 Hz, points=230×1024, scaling factor (n)=25, nt_1max=410 ms, scans=64, digital resolution (f_1)/n=2.4 Hz, total acquisition time=6 h, sample=20 mg/0.4 ml (C_6D_6).
13. Experimental conditions: (a) J-resolved HMBC-1, n=25, m=0. (b) J-resolved HMBC-2, n=25, m=12. (c) J-resolved HMBC-2, n=25, m=26. Other experimental parameters were the same as for Ref. 12.