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A new NMR method for measuring proton–proton spin coupling constants: *J*-resolved HMQC

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Abstract—A new NMR pulse sequence that incorporates *J*-scaling into HMQC enables easy analysis of strongly coupled proton spin systems. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Protons involved in a strongly coupled spin system display complex splitting patterns,[1] and detailed analysis of their coupling constants frequently requires computer assisted simulation. In order to overcome this problem, we developed a new HMQC technique named *J*-resolved HMQC, which enables easy analysis of strongly coupled proton spin systems by exploiting a proton directly attached to ^{13}C in the spin system.

In a strongly coupled spin system (H_A–^{12}C–^{12}C–H_B) with the chemical shift difference between H_A and H_B being very small ($J_{HAHB} \geqq \Delta\delta\ H_AH_B$), the coupling constant between H_A and H_B cannot be directly determined from their splitting patterns due to higher order couplings. On the other hand, in the same proton spin system where H_A is attached to ^{13}C (H_A–^{13}C–^{12}C–H_B), the ^{13}C satellite signals of H_A (H_{A1} and H_{A2}) appear at both sides of the H_A signal (attached to ^{12}C) with the chemical shift difference between H_{A1} and H_B being increased by $J/2$ (usually in the range of 60–80 Hz).[2] Thus, H_{A1} and H_B constitute a weakly coupled spin system ($J_{HAHB} < \Delta\delta\ H_{A1}H_B$) enabling direct measurement of the H_A–H_B J coupling constant from the splitting pattern of H_{A1}.

Such splitting patterns due to $J_{HA–HB}$ can in principle be observed with HMQC cross peaks which contain information on H–H J splittings;[3–5] it is difficult, however, to measure the magnitudes of the H–H J couplings directly from the cross peaks due to poor digital resolution in the F_2 or F_1 dimension. By incorporating the J- scaling method[6–9] into the HMQC pulse sequence, the formal magnitude of H–H J coupling constants in the J-resolved HMQC spectra can be increased by a factor of 'n' that is usually set to 20–30. Measurement of the H–H J coupling constants then becomes straightforward. This new technique is particularly useful for determination of the geometrical configurations of conjugated double bonds, whose protons usually form a strongly coupled spin system. Another approach using ^{13}C satellite signals and a different pulse sequence has recently been reported for easy analysis of strongly coupled spin systems.[10]

Pulse sequence

Two pulse sequences, J_{HH}-resolved-HMQC and J_{CH} resolved-HMQC, are shown in Fig. 1. The magnetization in the HMQC pulse sequence evolves with carbon chemical shift and proton–proton spin couplings. The Δt_1 is usually set to several milliseconds in this experiment, and thus the $t_1$max value cannot be so long as to enable the observation of the H–H J coupling constants in the F_1 dimension. For example, $t_1$max would need to be set to a value longer than 500 ms for observation of the small spin coupling (2 Hz).[7] By incorporating the J-scaling method into the HMQC pulse sequence, this problem can be easily overcome. The J-scaling pulse ($-nt_1/2$–180(H,C)-$nt_1/2$–) is introduced after the spin evolution period of HMQC (t_1) (Fig. 1, lower; J_{CH}-resolved HMQC). In this pulse sequence, the magnetizations of chemical shifts and H–H coupling constants and C–H coupling constants evolve with t_1, $(n+1)t_1$ and nt_1, respectively. The scaling factor (n) must be set to allow for nt_1max being larger than $1/J$, as in the case of

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the conventional 2D-J-resolved method).[11–13] As a result, the spin coupling constants are amplified by a factor of n for J_{CH} and n+1 for J_{HH}. In the J_{HH}-resolved HMQC pulse sequence (Fig. 1, upper), the 180(zC) component is omitted from the J-scaling pulse. As an application of this new technique, the J-resolved-HMQC spectra of fumaric acid with a high order spin system are shown in Fig. 2 (right, F_1-decoupled; left, non-decoupled). In the J_{CH}-resolved-HMQC spectrum, 45 degree tilted cross peaks are observed at the chemi-

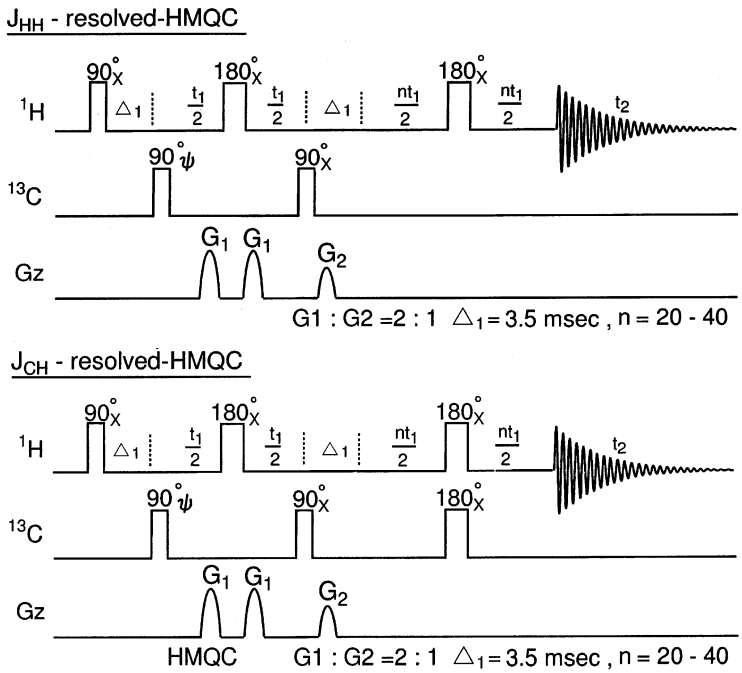

Figure 1.

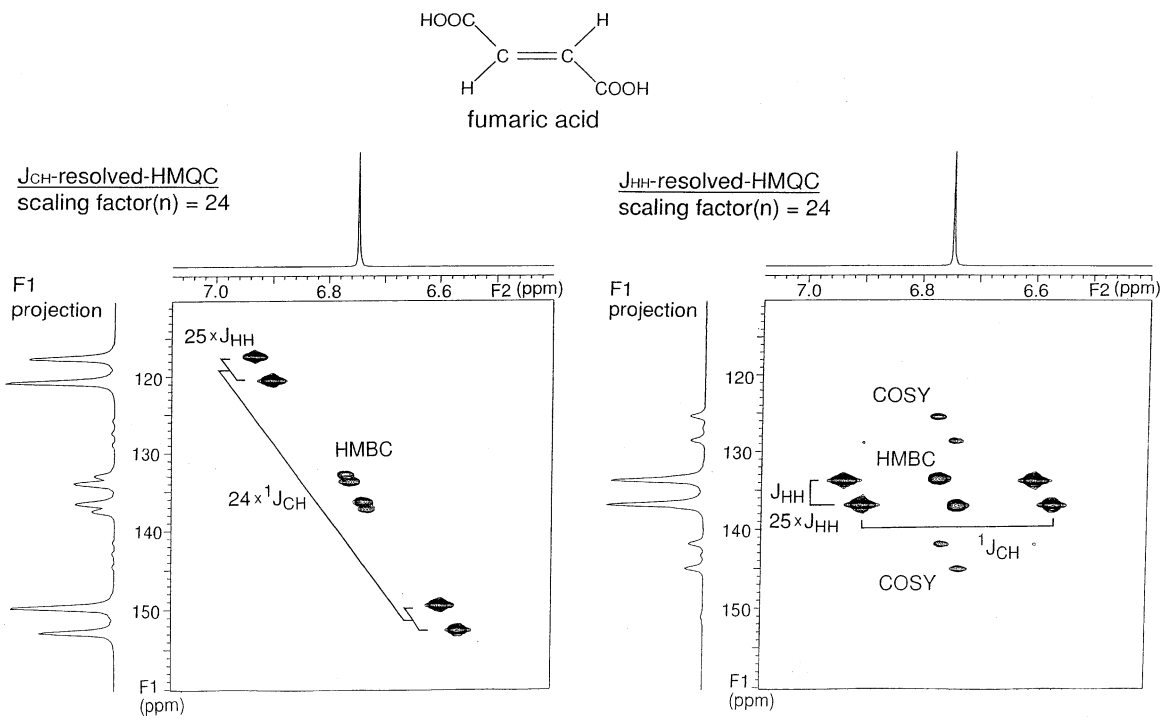

Figure 2.

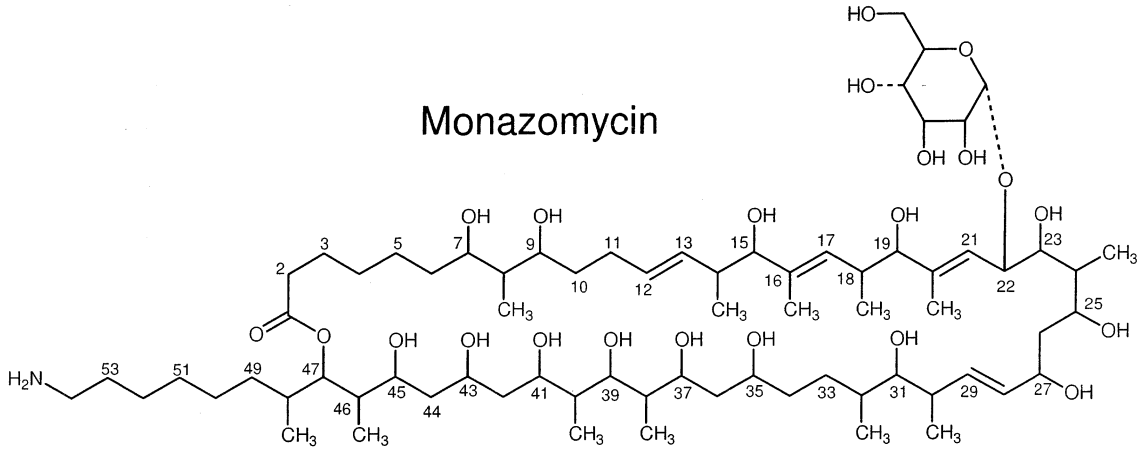

Figure 3.

cal shift of their appended carbon signal. The *J*-splittings of these cross peaks are observed clearly as a result of amplification of J_{CH} and J_{HH} values by *J* scaling with the *n* value being set to 24.

In the F_1 dimension of the J_{HH}-resolved-HMQC (Fig. 2, right) where J_{CH} is decoupled, only $(n+1)J_{HH}$ is observed with additional ghost cross peaks characteristic of *J*-resolved spectra.[12] It is to be noted that the signal to noise ratios remain same for J_{HH}-resolved-HMQC and J_{CH}-resolved-HMQC spectra when the $t_1$max and the measurement time are kept identical. If decoupling of J_{CH} in the F_2 dimension is needed for J_{HH}-resolved-HMQC and J_{CH}-resolved-HMQC, the broad band decoupling pulse can be implemented during the acquisition period.

Application of *J*-resolved-HMQC to the antibiotic monazomycin

The *J*-resolved-HMQC spectra of the olefinic region of monazomycin[14] (Fig. 3) are shown in Fig. 4.[15] Since the two protons H_{28} and H_{29} are strongly coupled to each other, their geometrical configuration could not be determined by analysis of the 1D NMR spectrum. In the J_{CH}-resolved HMQC spectrum[15] (Fig. 4, lower spectrum) however, these two protons are observed as a doublet of doublets ($25\times J=375$ Hz and $25\times J=160$ Hz in the F_1 dimension) with additional very large $^1J_{CH}$ splitting ($24\times J=3651$ Hz). From this result, the coupling constants are determined to be $J_{HH}=15$ Hz and $J_{HH}=6.4$ Hz indicating a *trans* configuration for the C_{28} and C_{29} double bond. The relation of H_{12} and H_{13} was also determined to be *trans* from their coupling constant of $J_{HH}=15$ Hz.

Decoupling of J_{CH} in the F_1 dimension gave the J_{HH}-resolved HMQC spectrum (Fig. 4, upper spectrum). Each cross peak appears at their appended carbon chemical shifts, and the C–H *J*-splittings are observed along the F_2 dimension. It is to be noted that overlapping of the cross peaks and appearance of ghost peaks caused by decoupling make the spectra more complicated. Therefore, use of J_{CH}-resolved-HMQC is generally recommended.

In the HMQC method, the $t_1$max is usually allowed to evolve for about 20 ms. On the other hand, nt_1max must be developed from 333 ms (3 Hz) to 500 ms (2 Hz) in the *J*-resolved-HMQC method, depending on the magnitude of the spin coupling constants to be observed.[13] Consequently it should be kept in mind that S/N of the *J*-resolved-HMQC spectra becomes lower than that of the conventional HMQC method.[3]

Stereochemical investigation of natural products requires determination of proton spin coupling constants in the proton NMR spectra. When spin coupling constants cannot be determined directly due to proton signals overlapping, the coupling constants can be estimated from the cross peaks of the COSY, HMQC,[4] and HSQC,[5] and other methods which observe the spin couplings in F_2 dimension. The advantage of *J*-resolved-HMQC over these approaches is to detect spin coupling constants in the F_1 dimension giving a data matrix equal in size to that of a conventional HMQC measurement. *J*-resolved-HMQC is measured in the absolute mode, as for the *J*-resolved-2D spectra, and so the time domain data is processed with Lorentz–Gauss multiplication in both t_1 and t_2 dimensions to obtain spectra with good S/N and eliminate dispersive wings in magnitude spectra.[13]

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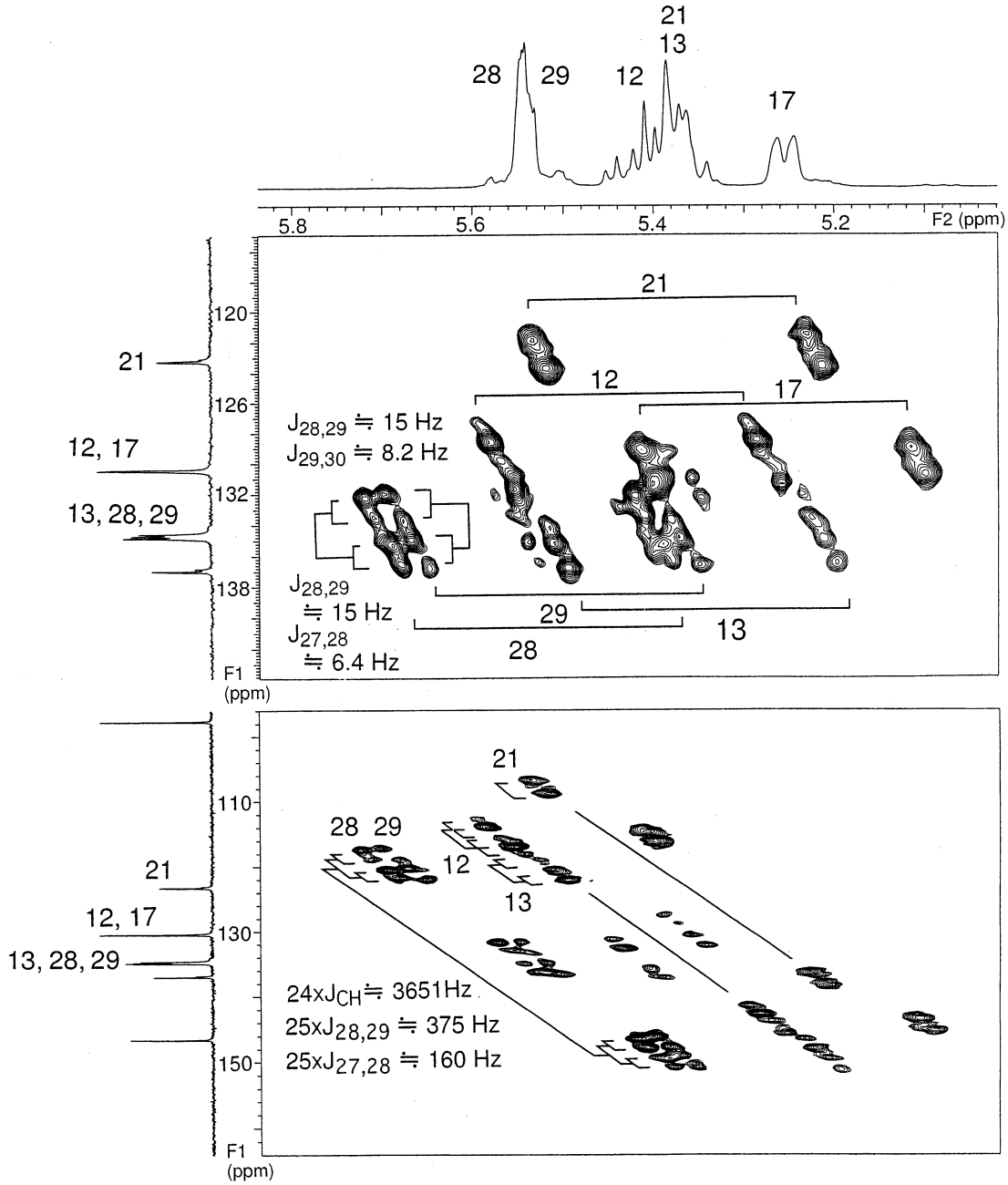

Figure 4.

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