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## Communications

# Pulsed Field Gradient Long-range COSY Experiment: Combined Use of Gradient and Fixed Delay for the Detection of Long-range Couplings

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Since the gradients probe-heads have been commercially available in 1991, many gradient-based experiments are spanning a range of applications which are useful in resolving many chemical problems.[1-3] Among the many advantages of using gradient in NMR experiments, the most significant aspect is the coherence pathway selection using gradient pulses instead of phase cycling.[4] Therefore, the data acquisition time can be significantly decreased for the properly concentrated samples. The gradient experiments are well established for the normal vicinal coupling constants.[5,6] However, the long-range coupling experiments (> $^3J$) are rarely applied in the field of gradient-based experiments. The earlier experiment of the weaker long-range $^4J$ and $^5J$ proton connectivities[7] has only been observed in gradient COSY experiment by the changes of gradient strength and gradient time.[8]

Generally, the transfer and detection process of long-range couplings are the most efficient at time $t_1 = t_2 = T_2$, leading to an unacceptably large data matrix. In order to avoid large data matrix, fixed delays ($\Delta$) are used at the end of the evolution and beginning of the detection periods, changing the optimum setting for the center of the pseudo echo to the points $t_1 = T_2 - \Delta$ and $t_2 = T_2 - \Delta$.[9] On the other hand, we have to perform several different experiments with different $\Delta$ values to observe all the long-range couplings because each nucleus has different $T_2$ value. Therefore, the detection of long-range coupling needs sometimes a significant amount of instrument time.

Here, we report the novel gradient method to detect the long-range coupling by combining the gradient and fixed delay. In order to replace the phase cycling, the pulsed field gradients were implemented into right after the end of the fixed delay of the pulse sequence used for small coupling constants,[9] respectively, as shown in the Figure 1.

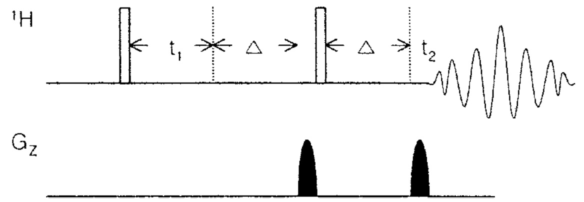
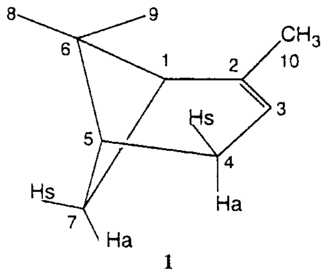


**Figure** 1. Pulse sequence for gradient long-range COSY (gradient ratio; 1 : 1).

Experiment was performed on a 0.01 M solution of $\alpha$-pinene (**1**) which has several possible long-range coupling pattern.[10] A 5 mm Willmad NMR tube was used, and 0.01 M solution in benzene-$d_6$ was used at an ambient probe temperature of *ca.* 21-22 °C. Data acquisitions were done at 500.23 on a Bruker AVANCE-500 spectrometer with a 5 mm BB z-gradient probe. The $^1H$-$^1H$ gradient COSY-45 (1 scan) and long-range COSY (4 scans, fixed delay $\Delta$ = 500 ms) spectra were obtained using the sequences, cosygp and cosylr, respectively, supplied by Bruker. For the gradient long-range COSY (1 scan, fixed delay $\Delta$ = 500 ms), the sine shaped



**1**

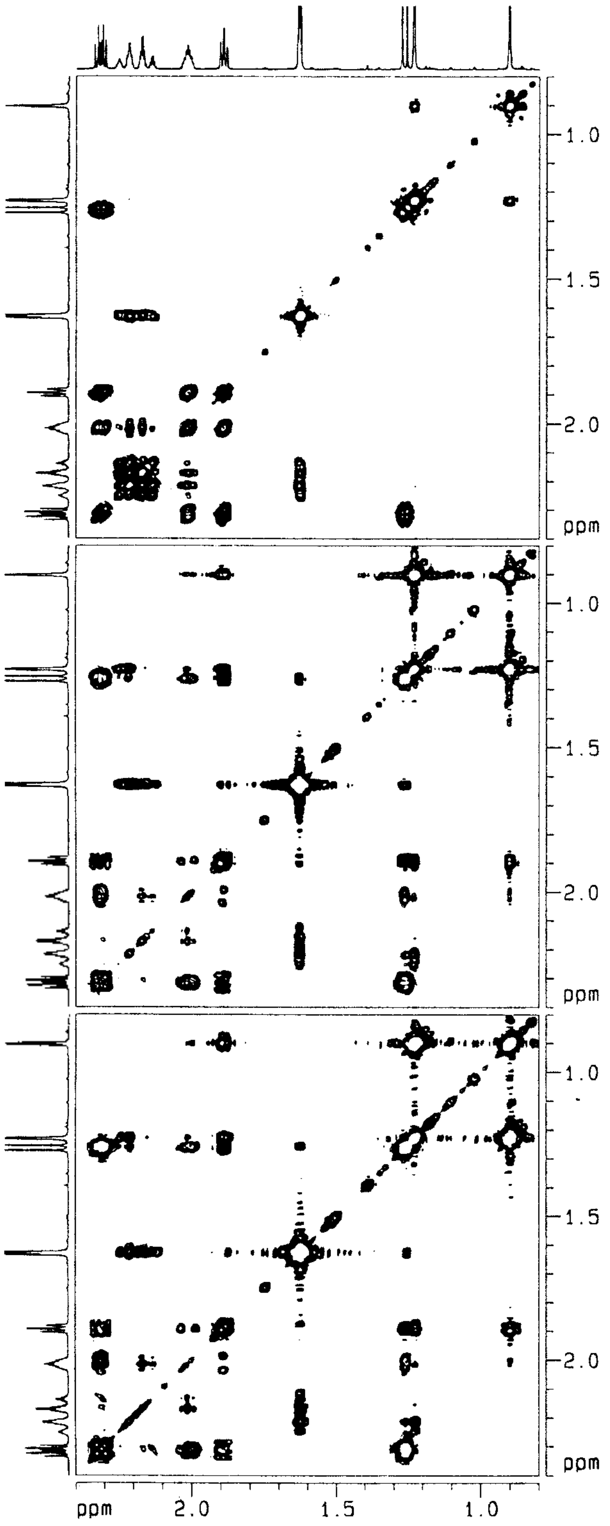


**Figure 2**. Top: Gradient COSY-45 spectrum (1 scan). Middle: Long-range COSY spectrum ($\Delta$=500 ms, 4 scans). Bottom: Gradient long-range COSY spectrum ($\Delta$=500 ms, 1 scan).

gradient pulses were used with 10% strength of 50 Gs/cm gradient unit supplied by Bruker.[11] The gradient pulse and recovery times were 1 ms and 0.4 ms, respectively. An initial matrix of 1 K × 512 data points was zero-filled to give 1 K ×1 K points and then processed by sinusoidal multiplication in each dimension followed by symmetrization of the final matrix. The $^1$H chemical shifts are referenced in ppm relative to TMS. but were measured against the solvent peak at 7.15. The $^1$H spectra were collected as 64 K data points over a 3500 Hz spectral width to give *ca.* 0.05 Hz digital resolution using a 30° pulse.

All three different partial 2-D spectra with 1-D projection are shown in the Figure 2. The long-range COSY spectrum (middle) showed several additional cross peaks which came from the $^4$J and $^5$J coupling and could not be seen in the normal gradient COSY spectrum (top). The gradient long-range COSY spectrum (bottom) obtained with 1 scan has all the long-range correlation peaks in comparison with normal long-range COSY spectrum (middle) as shown in the Figure 2. In fact, the signal to noise ratio of the cross peak between the 4-Ha proton ($\delta$, 2.23) and 8-$CH_3$ protons ($\delta$, 1.23) in normal long-range COSY spectrum (360 : 1) is about 3 times better than that of gradient long-range COSY spectrum (110 : 1). However, the signal sources of phase cycling long-range COSY experiment are both the N- and P-type pathways while the gradient method in this experiment can only obtain one type pathway.[12] With this fact. if four transients rather than one were used for the gradient experiment, the signal to noise ratio would be expected to be double. Thus, this gradient experiment also showed a significant advantage in the signal to noise ratio of about 30% over its phase-cycled counterpart. Furthermore. the gradient experiment is well known to reduce the $t_1$ noise in 2D-spectra.[1] Therefore. even 10 mmole solution sample was enough to measure for the detection of long range ($^5$J, *ca.* 0.4 Hz) with 1 scan in this gradient long-range COSY experiment.

In conclusion. the presented new gradient long-range COSY experiment can be easily utilized to detect the long-range coupling in comparison with the previous method[8] and the conventional phase-cycled counterpart.

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