= 단신 =

NOE 실험의 대용으로 응용될 수 있는 PFG Long-range COSY NMR 실험

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Application of Pulsed Field Gradient Long-range COSY-NMR for the Assignment of Geminal Protons on Rigid System: supplemental method of NOE Experiment

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Abstract : Antiphase character of cross peaks in long-range COSY is modulated by changing the fixed delay time and used to assign diastereotopic methylene protons on rigid systems which could produce unpredictable NOE phenomena because of complicate coupling spins.

Key words : NOE, Diastereotopic methylene, PFG long-range COSY.

1. Introduction

The application of coupling constants is very important to get the structural and conformational information of molecules.[1] Therefore, the small coupling constant sometimes can be a very important clue to confirm the structural conformation.[2] However, their application to structure elucidation has appeared only rarely in the literature. This lack of utility can probably be traced back to the inherent difficulty in their measurement. Generally, the proton couplings over four, five and more bonds show only small splittings of a few tenths hertz or less[2] and these small splittings such as 0.1 Hz or even smaller are detectable without

difficulties by modern NMR spectrometer. Although many new experiments have been introduced to facilitate the extraction of this useful scalar coupling information, most of their methods are not used frequently because of difficulty to use.

Since the gradients probe-heads of NMR have been commercially available in 1991, many gradient-based experiments are still spanning range of applications which are useful in resolving many chemical questions.[3-5] Among the many advantages of using gradient in NMR experiments, the most significant advantage is the coherence pathway selection using gradient pulses instead of phase cycling.[6] Therefore, the data acquisition time can be significantly decreased for properly concentrated samples. On the basis of this advantage, we previously reported a new pulse sequence of the Pulsed Field Gradient (PFG) long-range COSY experiment for the

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detection of long-range coupling.[7] In the COSY pulse sequence, the cross peaks transferred from A to X is proportional to $\sin(\pi J_{AX} t_1)\exp(-t_1/T_2)$. Therefore, the cross peaks obtained from unresolved small coupling will be very small and the transferred vector components giving cross peaks start out in antiphase which produces a net magnetization proportional to $\sin(\pi J_{AX} t_2)\exp(-t_2/T_2)$.[8] Thus, the fixed delays ($\triangle$) inserted at the end of evolution and beginning of the acquisition times in long-range COSY can prevent large data matrix and give optimum condition for the pseudo echo to detect very small couplings. Although we do not know the real coupling constants in this experiment, we can expect the net magnetization of antiphase giving cross peaks will be modulated by changing the fixed delay time.

Here we report that the PFG long-range COSY technique by changing the fixed delay time is a supplemental or potentially general method of unambiguously assigning diastereotopic methylene protons on rigid systems which could produce unpredictable NOE9 phenomena because of complicate coupling spins.

2. Experimental

The experiment was performed on a 0.01 M solution of α-pinene (1) which has very complicated coupling pattern and also has several possible long-range coupling pattern.[10-11] A 5 mm Wilmad NMR tube was used, and 0.01 M solution in benzene-d_6 was used at an ambient probe temperature of *ca.* 21-22 ℃.

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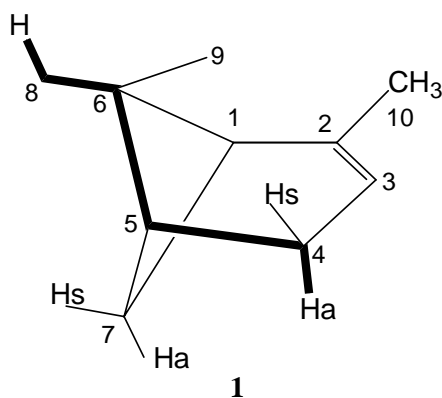
Data acquisitions were done at 500.23 MHz on a Bruker AVANCE-500 spectrometer with a 5 mm BB z-gradient probe. The spectra with fixed delay $\triangle$=300, 400, 500 ms (1 scan) were obtained using ^{1}H-^{1}H PFG long-range COSY sequence. For the PFG long-range COSY, the sine shaped gradient pulses 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 1K× 512 data points was zero-filled to give 1K×1K points and then processed by sinusoidal multiplication in each dimension followed by symmetrization of the final matrix. The 1H 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 64K data points over a 3500 Hz spectral width to give *ca.* 0.05 Hz digital resolution using a 30°pulse.

3. Results and discussion

The unique structure of bicyclic or fused organic compounds such as compound 1 is generally rigid with complicated proton coupling pattern because of the unusual long-range couplings.[11] Furthermore, the strong coupling between the spins and the spreading of NOE enhancements led by extended spin systems gives misleading NOE results. Several cases are already reported that the chemical shift of axial and equatorial ring protons in these systems could not be predicted on the basis of general rule obtained from cyclohexane system.[11,13] Compound 1 also has those unique complexities mentioned above including ambiguous NOE results which show enhancement of 4-Hs and one part of 4-Ha by the irradiation of 9-methyl protons. Those NOE ambiguities originated from molecular structure itself could not be avoided by NMR experiments.

Three different partial 2-D PFG long-range COSY spectra of compound 1 were obtained with $\triangle$ = 300, 400, and 500 ms and are shown with 1-D projection in the *Fig*. 1. Although some of the diagonal peaks from diastereotopic methylene protons (4-Ha and 4-Hs)
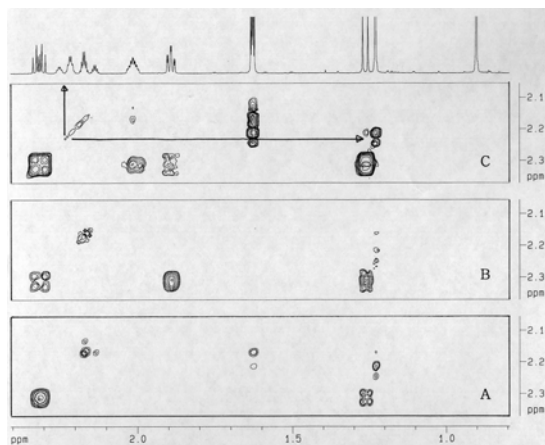
Fig. 1. Partial PFG long range COSY spectra with different fixed delay (1 scan). A. 300 ms fixed delay. B. 400 ms fixed delay. C. 500 ms fixed delay.

did not appear with $\triangle = 300$ and 400 ms, the cross peaks between the 8-methyl protons (1.26 ppm) and the down-field multiplet of doublet (2.23 ppm) in the multiplet of double-doublet are clearly shown. Furthermore, the intensities of cross peaks produced from the antiphase are modualteded with the fixed delay times as expected. Thus, spectrum obtained with $\triangle = 400$ ms had smaller cross peaks than that of 300 ms but spectrum of 500 ms had bigger cross peaks than that of 300 ms. In this experiment, the spectrum of 600 ms fixed delay (not shown) did not give cross peak and that is not unusual for the experiments which produces the cross peaks from the net magnitization of antiphase.[14] With these results we can conclude that the proton resonated at the down-field multiplet of doublet is placed in the zig-zag arrangement formed from the one of 8-methyl protons as designated by solid line in the structure. Thus, we can unambiguously ascertain that the 4-Ha (pseudo-axial) had down-field chemical shift unlike the general rule obtained from cyclohexane.

In conclusion, we could obtain several PFG long-range COSY spectra with different fixed dealy very quickly (4 spectra within 40 min.) without any information of unresolved long-range coupling constant

and assigned easily the ambiguous methylene protons on α-pinene (1) with the aid of simple molecular model such as Dreiding Stereomodels.[15] On this kind of system, several fixed delay experiments will give at least one result because the cross peak of long-range COSY has the antiphase modulation. Instead of using NOE experiment, this method, therefore, could be a supplemental or potentially general method for unambiguously assigning diastereotopic methylene protons on rigid systems.

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