The HMQC-NOESY Experiment Without Decoupling During Acquisition for Detection of nOe Between Pseudo-Equivalent Protons

Lyle W. Castle, Milton D. Johnston, Jr., Charalampos L. Camoutsis and Raymond N. Castle
Department of Chemistry, University of South Florida
Tampa, Florida 33620-5250
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The pseudo-symmetric structure of benzo[*f*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline causes overlap of the resonances corresponding to H1 and H15 and also those of H11 and H12. We report here the observation of nOe between these pseudo-equivalent protons using the HMQC-NOESY experiment acquired without decoupling during acquisition.

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Results and Discussion

One of the inherent challenges of making chemical shift assignments on pseudo-symmetric molecules is that pseudo-symmetrically similar proton resonances usually appear overlapped in the ^{1}H spectrum. Under these circumstances it is virtually impossible, using 1D-homonuclear techniques, to obtain nOe (nuclear Overhauser enhancement) between two such resonances for either structural characterization or

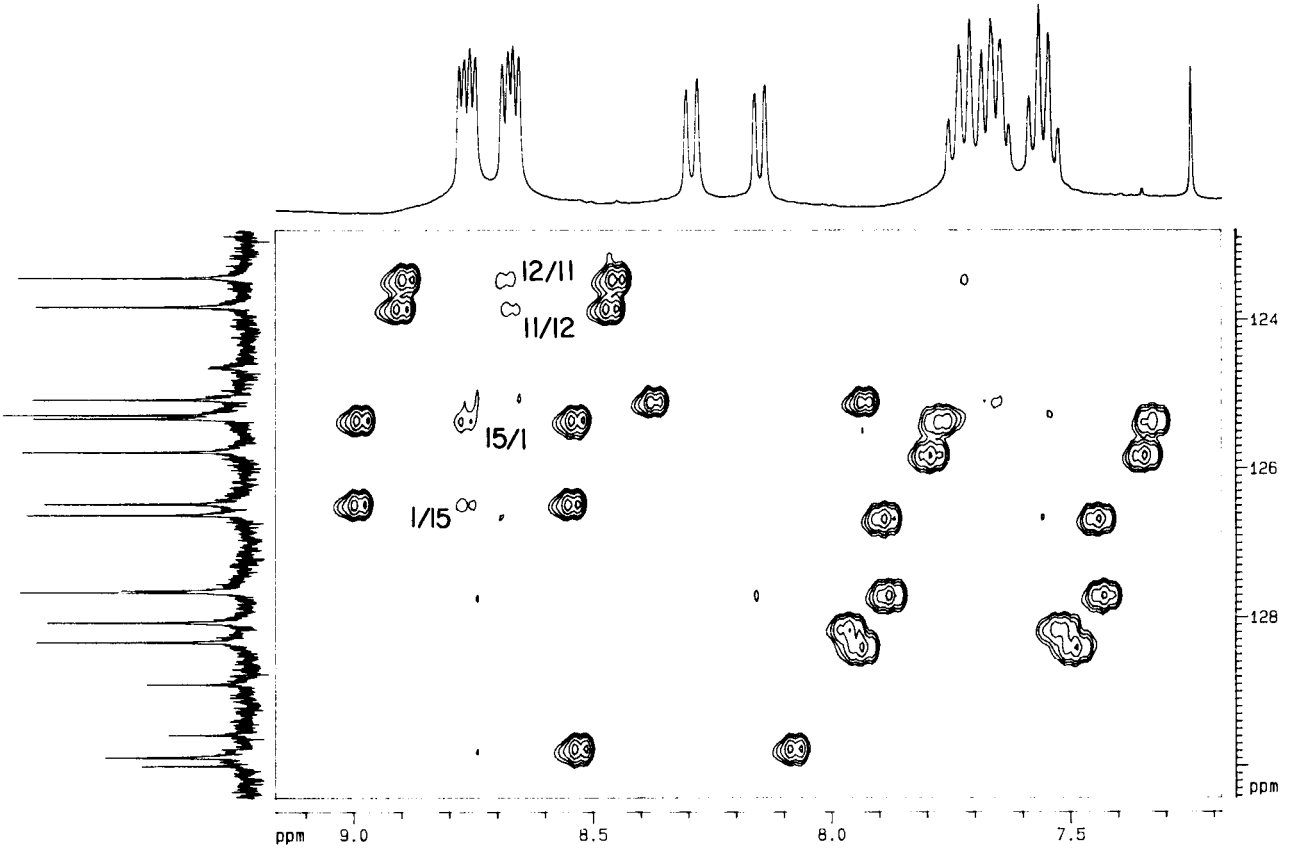
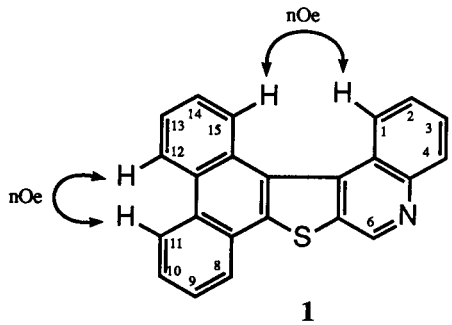


Figure 1. HMQC-NOESY spectrum of **1** acquired without decoupling during the acquisition period. In the HMQC portion of the sequence those protons directly attached to ^{13}C are labeled and give rise to the direct responses. Because the decoupler is off during acquisition the direct responses appear as doublets with a $^1J_{CH}$ of approximately 166 Hz. The nOe signals, generated during the NOESY portion of the sequence, result from protons directly bonded to ^{12}C. Because the direct responses are coupled to ^{13}C, they do not interfere with the nOe signals which appear negatively phased in the center of direct responses.

assignment purposes. For example, presaturation of one resonance will also saturate the other negating the possibility of observing nOe, using difference-spectroscopy, between protons of this type. A 2D-NOESY experiment generally will not overcome this obstacle because both signals will be overlapped in the second dimension as well.

It was recently suggested [1,2] and then reported [3] that an HMQC-NOESY experiment, acquired without heteronuclear decoupling during the acquisition period, generates detectable nOe between equivalent



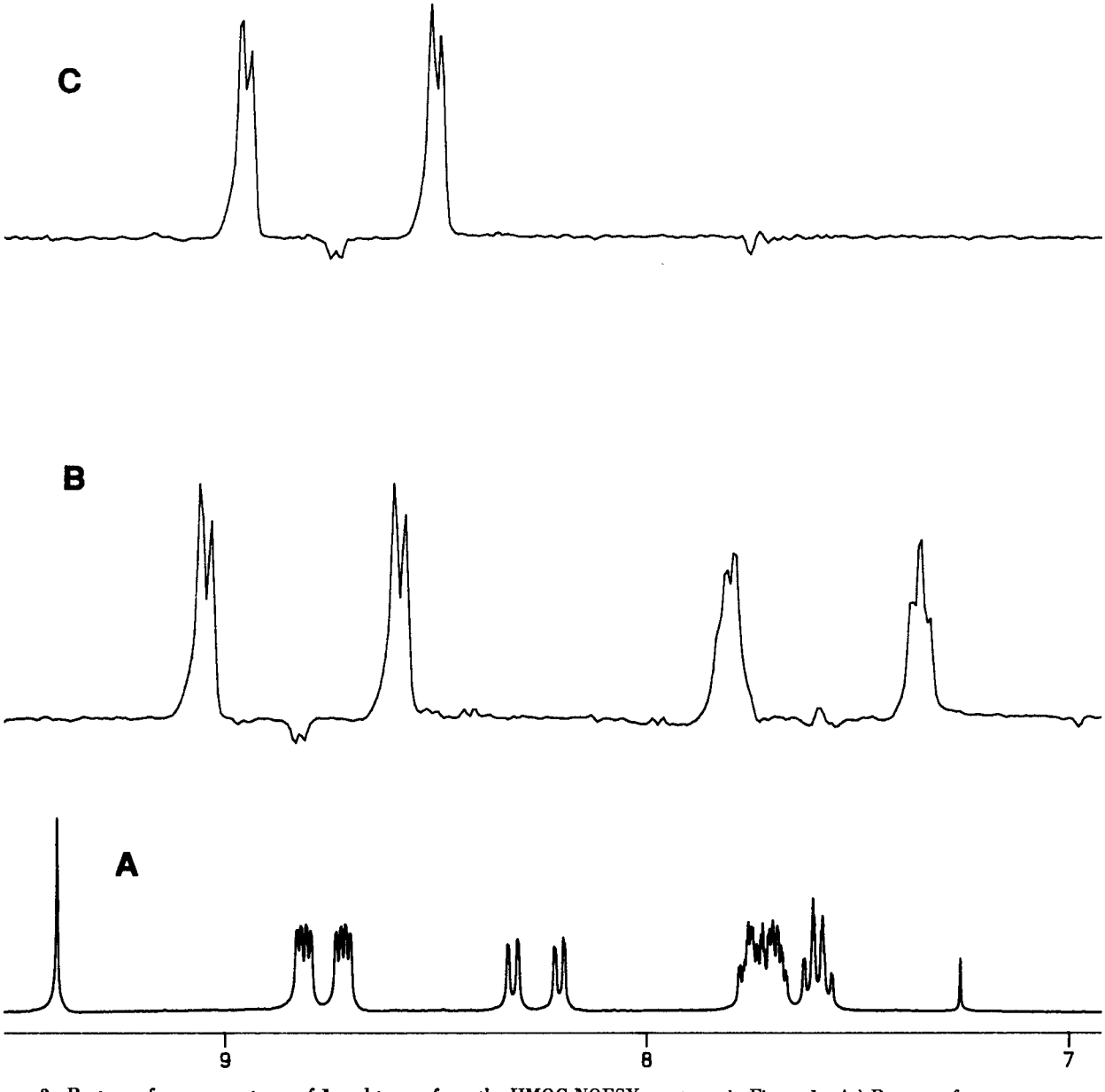


Figure 2. Proton reference spectrum of **1** and traces from the HMQC-NOESY spectrum in Figure 1. A.) Proton reference spectrum. B.) Trace from the HMQC-NOESY spectrum at the chemical shift of C1 at 125.3 ppm. The negatively phased nOe response had an intensity of 5.3 %. C.) Trace from the HMQC-NOESY spectrum at the chemical shift of C11 at 123.4 ppm. The negatively phased nOe response had an intensity of 4.5 %.

protons. During the HMQC portion of the sequence, protons that are directly attached to ^{13}C are labeled giving rise to the direct responses. Because the decoupler is off during acquisition the direct responses appear as doublets with a $^{1}J_{CH}$ of approximately 125-180 Hz. The nOe signals, generated during the NOESY portion of the sequence, result from protons that are directly bonded to ^{12}C. As a result, the nOe responses appear as negative-phased signals centered between the ^{13}C coupled direct responses, thereby allowing the observation of nOe between either equivalent or overlapping proton resonances.

The pseudo-symmetric nature of benzo[*f*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline (1) causes overlap of pseudo-symmetrically opposed protons at each end of the bay regions (*i.e.*, overlap of resonances corresponding to the proton pairs 1/15 and 11/12). Because of the overlap nOe cannot be observed between these proton pairs using conventional homonuclear methods. However, using the HMQC-NOESY experiment, acquired without decoupling during acquisition (Figure 1), we observe nOe cross peaks between the proton pairs 1/15 and 11/12. The nOe signals between H1 and H15 then provide a connectivity link with the phenanthrene moiety of 1 and ultimately allows all chemical shift assignments to be correlated directly back to the singlet corresponding to H6 [4].

The HMQC-NOESY and -ROESY are the only experiments presently available to detect nOe between unresolved protons. The disadvantage of this experiment is that it is very time consuming. The spectrum shown in Figure 1 required an acquisition time of 44 hours and the nOe/rOe signals are still relatively weak (Figure 2).

EXPERIMENTAL

The ^{1}H and HMQC-NOESY spectra were acquired on a Bruker AMX 360 MHz NMR spectrometer operating at an observation frequency of 360.130 MHz for ^{1}H and 90.560 for ^{13}C. All experiments were performed using an inverse-geometry 5-mm broad band probe. Both the ^{1}H and ^{13}C 90° pulses were calibrated, and were 7.2 and 14.5 msec, respectively.

The proton spectrum was recorded with 16384 data points and all the chemical shifts were referenced to TMS. For better sensitivity the carbon spectrum was acquired using a standard-geometry 5mm broad band probe where the 90° ^{13}C pulse was calibrated to 8.3 μsec. The carbon spectrum was recorded with 64K data points and zero-filled to 128K data points. For a ^{13}C reference, the center peak of the 1:1:1 multiplet of deuteriochloroform was assigned a value of 77.0 ppm.

The HMQC-NOESY spectrum was acquired using the Bruker pulse program (*invbnotp*) modified such that the decoupler was off during acquisition [1]. F_2 was acquired with 512 points and F_1 with 128 points and upon processing F_1 was zero-filled to achieve a data matrix of 512 by 256 points. Both dimensions were subjected to cosine squared multiplication prior to Fourier transformation. A mixing time of 250 msec was used for the generation of nOe.

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