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One-dimensional (1D) variants of two-dimensional (2D) nmr techniques can frequently provide the required information in a much shorter period of time than the 2D experiment from which they were derived. Application of the 1D homonuclear Hartmann-Hahn (1D-HOHAHA) experiment to establish proton-proton connectivity networks in highly overlapped four-spin systems in the proton nmr spectra of polynuclear aromatics is described. Selective subspectra are contrasted to the data obtained in a COSY experiment on the same molecule, benzo[*a*]benzothieno[2,3-*c*]quinoline. Results from the 1D-HOHAHA technique are especially useful when component resonances from several spin systems are heavily overlapped. Relayed 1D-HOHAHA provides the means of exploiting small, long range coupling pathways of polynuclear aromatics.

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Introduction.

Two-dimensional nmr techniques have facilitated the solution of structural problems and have allowed the unequivocal assignment of nmr spectra that would have been impossible a scant few years ago. In the area of heterocyclic chemistry, the most difficult nmr spectral assignment problems are, quite frequently, those involving polynuclear aromatic heterocycles. We have made extensive use of two-dimensional nmr techniques in the assignment of such molecules [1-4]. There are instances, however, where selective establishment of one or two proton connectivity pathways would be sufficient, in conjunction with heteronuclear chemical shift correlation spectra, to allow a total

spectral assignment. Work by Kessler [5] and Freeman [6] have addressed the reduction of 2D experiments to their selective one-dimensional (1D) counterparts. One useful member of this growing family of techniques is the one-dimensional variant [7,8] of the two-dimensional TOCSY [9] or HOHAHA [10] experiment. The 1D analog, possibly known more commonly as 1D-HOHAHA, is shown schematically in Figure 1 in its most recently reported form [11], which provides the option of a relay step performed in the fashion of Eich, Bodenhausen and Ernst [12] following the isotropic mixing portion of the experiment.

Results and Discussion.

Application of the 1D-HOHAHA pulse sequence to a typical polynuclear heteroaromatic problem is demonstrated using benzo[*a*]benzothieno[2,3-*c*]quinoline (**1**) [13]. As might be expected on the basis of the chemical structure, the two bay region protons, H1 and H13, are well resolved doublets resonating downfield at 8.82 and 9.01 ppm, with no assignment to be inferred from these shift data. The balance of the proton resonances of the two four-spin systems are heavily overlapped with only the apparent "triplets" resonating at 7.42 and 7.67 ppm resolved. The two remaining triplets and the terminal spins, H4 and H10, are all overlapped as shown by the highly digitized 300 MHz COSY spectrum presented as Figure 2. Unfortunately, the acquisition time for such a spectrum is considerable and may, in some cases, still fail to provide the required connectivity information. A far more time efficient alternative is afforded by the 1D-HOHAHA experiment.

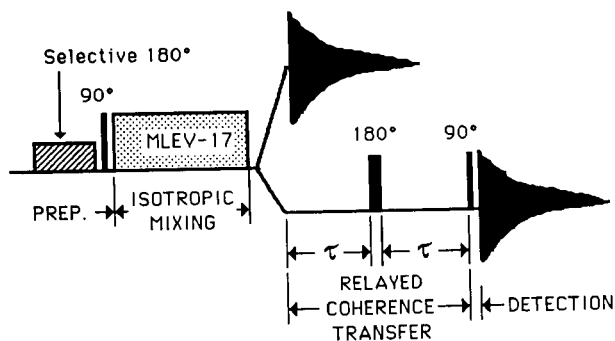


Figure 1. Schematic representation of the one-dimensional homonuclear Hartmann-Hahn (1D-HOHAHA) pulse sequence. In the upper trace, acquisition is initiated immediately following the MLEV-17 interval. The MLEV-17 sequence was flanked by 1.2 msec trim pulses. In the lower trace, a relay interval analogous to that initially described by Eich, Bodenhausen and Ernst [12] is employed with acquisition following. Phases are cycled according to Table 1.

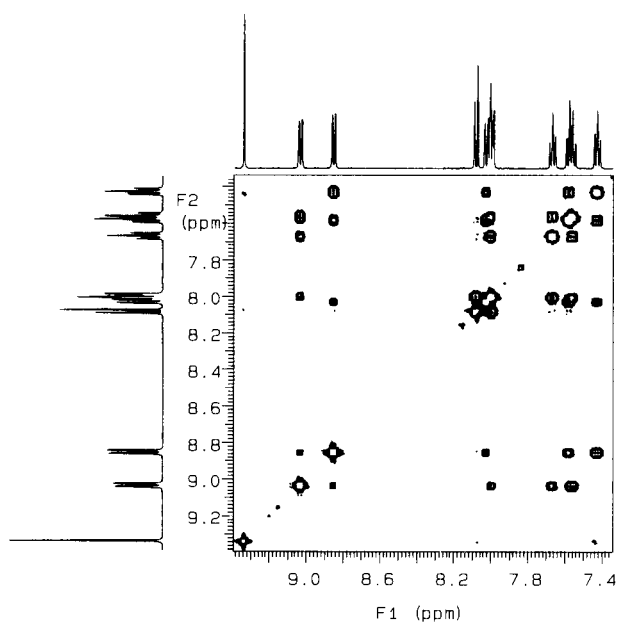
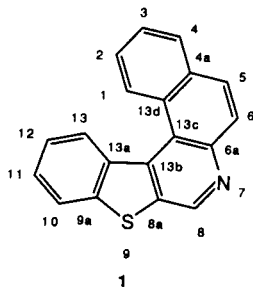


Figure 2. COSY spectrum of benzo[1]benzothieno[2,3-c]quinoline (**1**) recorded at 500 MHz and symmetrized. Despite the relatively high digital resolution (512 x 512 points) the connectivity pathways derived from the H1 and H13 resonances (9.01 and 8.72 ppm, respectively) are still undecipherable.



The 1D-HOHAHA spectra shown in Figure 3 were recorded on an approximately 2 mg sample at 500 MHz and represent a time investment of about fifteen minutes for all traces, including the reference spectrum shown in the center of the figure. Going outward from the center, the spectra shown had isotropic mixing times of 15, 30 and 60 msec, respectively. Those above the reference spectrum were recorded by applying the selective 180° pulse to the doublet resonating at 9.01 ppm, while those shown below the reference spectrum arose from a selective 180° pulse application to the doublet resonating at 8.82 ppm. Both sets of spectra were recorded with the detection interval immediately after the isotropic mixing period (see upper pulse sequence pathway, Figure 1). Utilization of the relayed coherence transfer portion of the pulse sequence will be considered later.

In both sets of experimental 1D-HOHAHA spectra, the intensity of the resonance to which the selective 180° pulse was applied decreased as a function of increasing

mixing time duration. Referring to the series of traces derived by initial irradiation at 8.82 ppm, the triplet resonating furthest upfield at 7.42 ppm gains intensity in the first in the 15 msec experiment, with weak intensity responses at 7.57 ppm and 8.00 ppm. By increasing the duration of the isotropic mixing period to 30 msec, the "triplet" resonating at 7.57 ppm took on significant intensity followed by the doublet resonating at 8.01 ppm, which took on full intensity in the 60 msec trace. Virtually identical behavior was noted in the other set of experiments, beginning from the doublet resonating at 9.01 ppm. Thus, in a relatively short time, we attained connectivity information (Figure 3) with digital resolution comparable to that derived from a COSY spectrum (Figure 2) with an accumulation time of several hours.

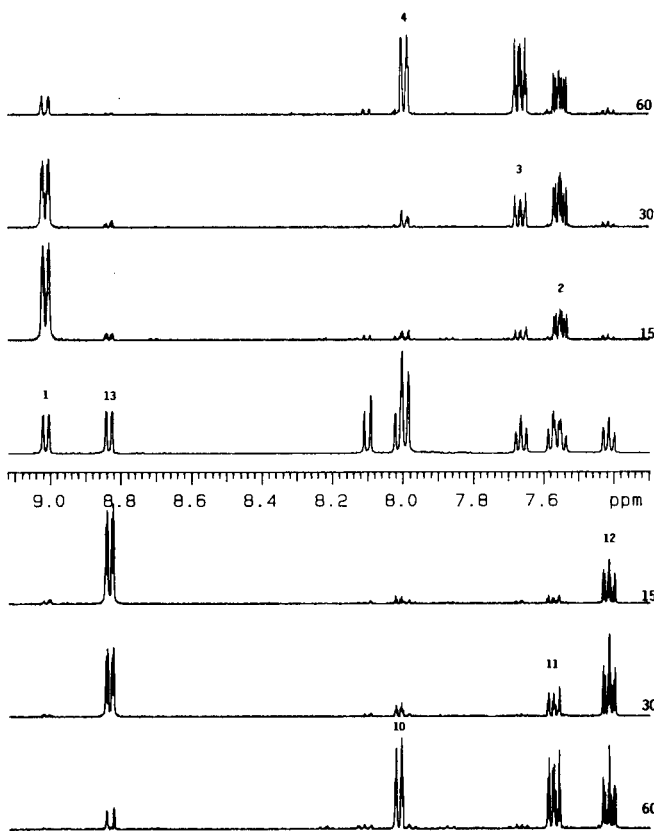
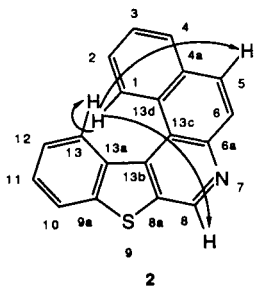


Figure 3. Selective 1D-HOHAHA and reference spectra of benzo[1]benzothieno[2,3-c]quinoline (**1**). The normal, high resolution reference spectrum is shown in the center. Traces shown above the reference spectrum were recorded by selective irradiation of the H1 proton resonating at 9.01 ppm and had isotropic mixing times of 15, 30 and 60 msec respectively. The traces presented beneath the reference spectrum arose from the selective irradiation of the H13 resonance. Mixing times were 15, 30 and 60 msec as indicated on the spectra.

Relayed coherence transfer 1D-HOHAHA, recently reported by Bax and co-workers [11] provides an interesting expansion to the capabilities of the 1D-HOHAHA experi-

ment. This feature, as noted by Bax, is intended to bridge potential gaps in proton connectivity networks associated with small vicinal couplings. Alternatively, the relay step may also be used to provide the means of transferring coherence between adjacent spin systems *via* long range coupling pathways, *e.g. peri, epi-zig-zag* and extended *epi-zig-zag* coupling pathways which are common in the spectra of polynuclear aromatic compounds. Recently, we have exploited this property of the two-dimensional HOHAHA experiment with polynuclear aromatics in a similar fashion [14].

To demonstrate the utility of the relay portion of the pulse sequence shown in Figure 1 (lower pulse sequence pathway), consider the coupling possibilities for the H1 proton of **1**. Plausible long range couplings include five bond coupling to H5; seven bond coupling between H1 and H8, which is not quite a classical extended *epi-zig-zag* orientation; and seven bond coupling to H13 opposite H1 across the "bay". These are shown by **2**.



Application of the selective 180° pulse to the H1 doublet resonating at 9.01 ppm followed by isotropic mixing periods with durations from 15-60 msec failed to give any information about potential long range couplings to H8 and/or H13. In contrast, when a 45 msec isotropic mixing interval was coupled with a relayed coherence transfer step with the fixed delay, τ , set to 25 or 50 msec, we observed the long-range coupling responses sought. A very prominent response for H8 was observed in the 50 msec relay experiment that was only weakly apparent in the 25 msec relay experiment (see Figure 4). Responses to H5 and H13 were weaker, and it might be argued that they were perhaps only marginally more intense than the residual signals from protons that were incompletely canceled. Hence, it is clear that long range coupling responses, such as those recorded here, must be interpreted with care. However, when the facility of obtaining the spectra shown in Figure 4 is considered, it is quite reasonable to explore a wide range of mixing and relay times to determine if responses such as those to H5 and H13 are real or artifactual.

Conclusions.

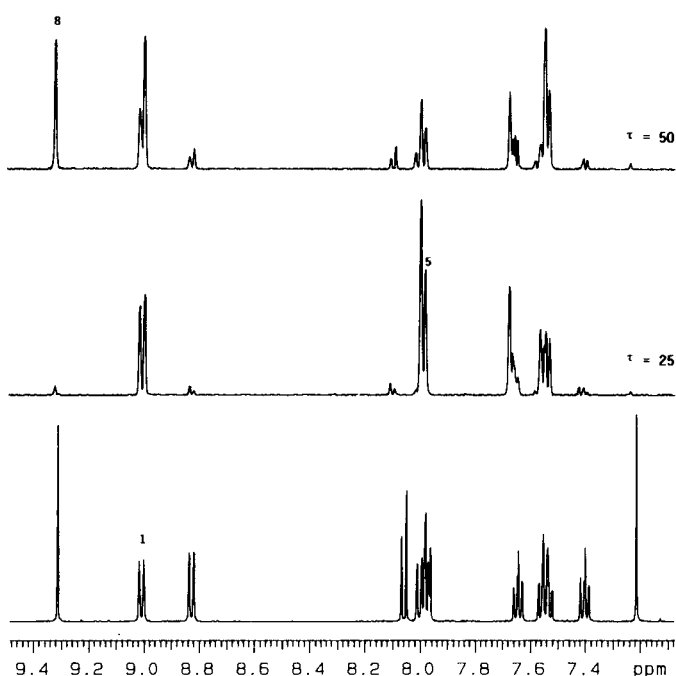


Figure 4. Selective 1D-HOHAHA spectra obtained by selective irradiation of the H1 doublet (9.01 ppm), isotropic mixing with a 45 msec mixing time followed by a relay step (see Figure 1, lower pulse sequence pathway) with the fixed delay, τ , of the relay interval optimized for 25 and 50 msec as indicated on the spectra. It will be noted in the 25 msec trace that there is minimal intensity for the H8 resonance which is coupled to H1 *via* a seven bond coupling pathway. In contrast, there is excellent signal intensity for the H8 resonance in the 50 msec mixing interval spectrum. Responses for long range coupling of H1 to H5 and H13 are of lower intensity and did not improve substantially with relay times (τ) of as long as 125 msec when combined with a 45 msec isotropic mixing interval. It is conceivable however, that responses with greater intensity could be observed for these resonances using some other combination of isotropic mixing and relay times.

With the continuing refinement of nmr pulse sequences, we now have recourse to very sophisticated one-dimensional nmr experiments that can provide spectral correlation information in a relatively short period of time; this information was formerly available only from complex two-dimensional nmr experiments. As additional one-dimensional analogs of two-dimensional nmr experiments are developed, prospects are very bright for further applications in the area of polynuclear aromatic and heteroaromatic compounds.

EXPERIMENTAL

The synthesis and total assignment of the proton and carbon nmr spectra of benzo[fl]benzothieno[2,3-c]quinoline (**1**) is reported separately [14].

The COSY spectrum (Figure 2) was recorded using a Nicolet NT-300 spectrometer operating at 300.068 MHz and controlled by a model 293-C pulse programmer. The spectrum was recorded

using the decoupler coil of a 5 mm dual-tuned $^1\text{H}/^{13}\text{C}$ probe as 256 x 1K data points and was processed using sinusoidal multiplication prior to both Fourier transformations and zero filling prior to the second to afford a final matrix consisting of 512 x 512 points which was symmetrized [15] prior to plotting.

Table 1. Phase Cycling for the 1D-HOHAHA/Relay Pulse Sequence Shown in Figure 1

Selective 180°	00000000	22222222	11111111	33333333
90° Observe	00112233	22330011	11223300	33001122
Trim Pulses	00000000	22222222	11111111	33333333
MLEV-17	11223300	33001122	22330011	00112233
Receiver (+odd; -even)	02132031	20310213	13023102	31021320
180° Relay	11223300	33001122	22330011	00112233
90° Relay	00112233	22330011	11223300	33001122

The 1D-HOHAHA spectra were recorded using a Varian VXR-500 operating at 499.84 MHz, controlled by Varian's Unix-based VXR-5000S software package running on a Sun 3/260. The spectra were recorded using the pulse sequence shown in Figure 1. The phase cycling was modified from that described by Bax [11] with phase alternation of the receiver reference phase to yield directly the difference spectrum (see Table 1). Copies of the pulse program code employed are available from the authors upon request. Pulses were calibrated using the model compound giving a 60 msec duration for the selective 180° pulse corresponding to an effective excitation bandwidth of 8.3 Hz. Traces shown in Figure 3 are the average of 32 transients.

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