Assignments of the ^{1}H and ^{13}C NMR Spectra of Pseudo-Symmetric Heterocyles Using the HMQC-TOCSY Experiment to Differentiate Overlapping Spin Systems

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

Received June 30, 1992

The ^{1}H nmr spectra of phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline, benzo[*f*]phenanthro-[9',10':4,5]thieno[2,3-*c*]quinoline and benzo[*h*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline are highly congested. For each compound, all protons abide in an aromatic environment complicated by pseudo-symmetric regions which result in multiple overlap of the different spin systems these molecules contain. We illustrate here the utility of the HMQC-TOCSY experiment to identify spin systems when the proton spectrum is highly congested. To complete the assignment of the ^{1}H and ^{13}C nmr spectra of each compound the HMBC experiment is used to assign the quaternary carbons.

J. Heterocyclic Chem., **29**, 1805 (1992).

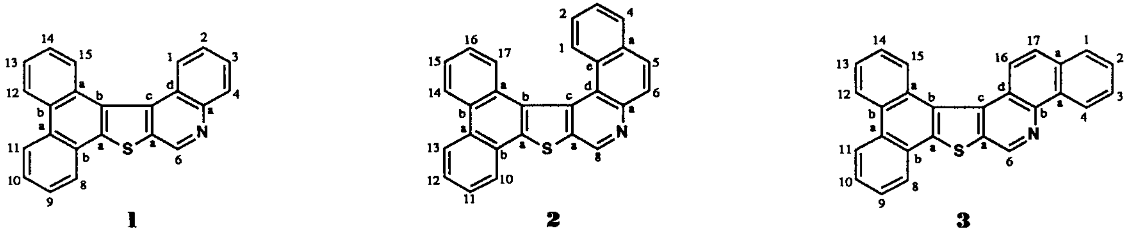
Introduction.

In this paper, we will describe the complete chemical shift assignment of the ^{1}H and ^{13}C nmr spectra of phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline (**1**), benzo-[*f*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline (**2**) and benzo[*h*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline (**3**) (Figure 1). In this series of polynuclear aromatic heterocycles, the pseudo-symmetric nature of these compounds results in extreme crowding of their proton and carbon spectra. Each of these compounds contains three four-spin systems and in most cases some of the resonances are overlapped with members of symmetrically opposed spin systems. This makes the differentiation of spin systems by homonuclear experiments such as the COSY and DQF-COSY (Double Quantum Filtered-COSY) ambiguous at best.

To identify spin systems we will use the HMQC-TOCSY (Heteronuclear Multiple Quantum Coherence TOtal Correlation SpectroscopY) experiment where proton-proton connectivities can be easily obtained because the responses are resolved as a function of their ^{13}C chemical shifts [1]. For each compound a series of four HMQC-TOCSY experiments were performed with mixing times of 12, 24, 36 and 48 msec, respectively. At 12 msec relayed responses are only observed between proton pairs that are *ortho* to one another. At 24 msec relayed responses between both *ortho* and *meta* proton pairs are observed and at 36 msec relayed responses between *para* proton pairs are observed as well. Finally at 48 msec some of the direct responses have disappeared. The spectrum generated from the 12 msec mixing time is the most straightforward because indirect responses are only observed between vicinal proton pairs, hence, we will always start with this spectrum and only refer to the other spectra, obtained using longer mixing times, for verification if the results at 12 msec are ambiguous.

As an experiment, we also acquired HMQC-TOCSY spectra of **2** with 64, 78 and 96 msec mixing times to determine whether relayed responses between *peri* protons (*i.e.*, adjacent protons separated by one quaternary carbon atom) would be observed. These

Figure 1



experiments did not show any relayed responses between *peri* protons.

With the spin systems identified, the HMBC (Heteronuclear Multiple Bond Correlation) experiment gives entry into each spin system identifying the individual members as well as all of the quaternary carbons. For each of these compounds the singlet farthest downfield corresponds to the proton bonded to the carbon adjacent to the nitrogen atom. Using long-range proton-carbon correlations, observed on the HMBC spectrum, this singlet then provides an entry point for the chemical shift assignments of each molecule. With this approach, we will describe an unequivocal assignment of all the proton and carbon resonances of **1**, **2**, and **3**.

Results and Discussion.

Compound 1.

We will begin by assigning the singlet at 9.35 to H6. The HMBC spectrum (Figure 2) shows long-range correlations between H6 and three carbon resonances. Examining the structure of **1** we can identify the resonances corresponding to the quaternary carbons C4a, C15c, and C6a. The correlation with C6a is attributed to $^2J_{CH}$. We have precedent for assigning C6a based on $^2J_{CH}$ because we have observed the coupling on other ring systems containing the thieno[2,3-*c*]quinoline moiety [2]. The resonances corresponding to C4a and C15c both long-range correlate to the proton resonance at 8.76 identifying H1, and C4a also correlates with the resonance at 7.74 identifying H3 (Figure 3).

With the resonances corresponding to H1 and H3 identified we now have entry into this four-spin system and can use the HMQC-TOCSY spectra (Figures 4A and 4B) to complete the assignment of the protons and protonated carbons in this spin system. A comparison of the HMQC (Heteronuclear Multiple Quantum Coherence) spectrum, which was acquired but is not shown, and HMQC-TOCSY spectra allows us to differentiate the direct responses from the relayed responses, both of which appear on the HMQC-TOCSY spectrum. With this approach we can easily assign the protons and carbons at positions 1, 2, 3 and

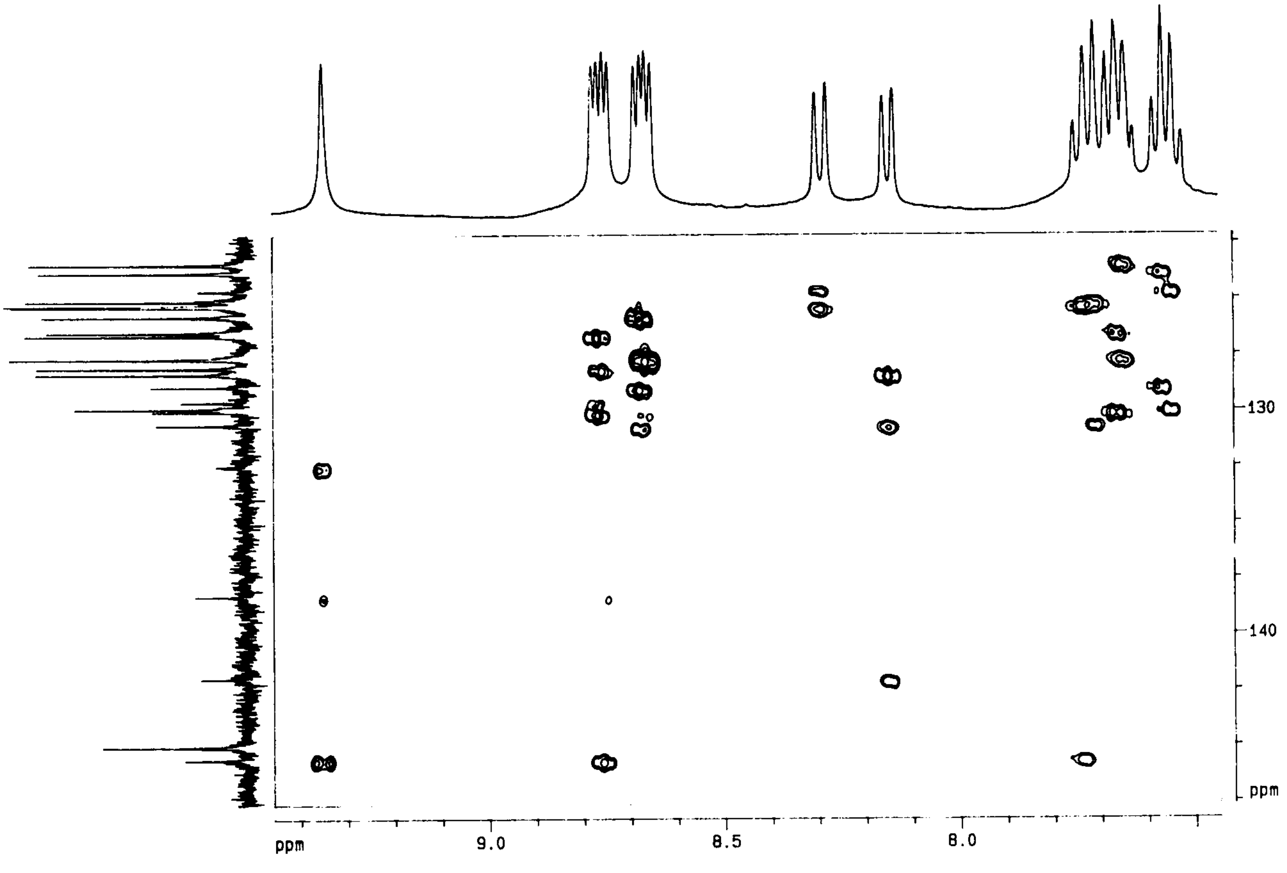


Figure 2. The HMBC spectrum of **1**.

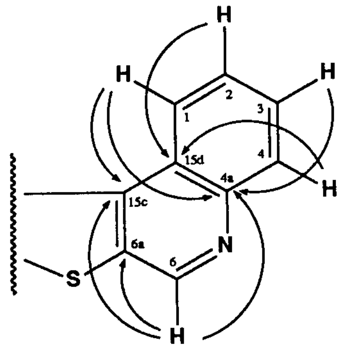


Figure 3. Diagram of long-range correlations observed on the HMBC spectrum for the thieno[2,3-c]quinoline moiety of **1**.

4. The only difficulty is with positions 1 and 2 because the resonances corresponding to C1 and C2 are only separated by 0.05 ppm causing the relayed responses to overlap with the direct responses. The ambiguity of overlapping responses is overcome at longer mixing times. For example, at 24 msec we observe relayed responses between the proton pairs 1/3 and 2/4 and with 36 msec we observe a relayed response between 1/4 as well.

H6 is ineffective as an entry point for the phenanthrene moiety because none of the quaternary carbons have long-range correlations with protons on both sides of the molecule. Because of the pseudo-symmetric nature of **1** those protons close enough in space for the transfer of nuclear Overhauser enhancement (nOe) have nearly identical chemical shifts thus, negating the possibility of determining their identity by homo-nuclear nOe experiments. To gain entry into the phenanthrene moiety of **1** an HMQC-NOESY was acquired without heteronuclear decoupling during acquisition [3]. From this experiment nOe cross peaks are observed 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 tied directly back to the singlet corresponding to H6 (Figure 5).

Compound 2.

The structure of **2** is similar to **1** except that it contains another benzene ring fused to the quinoline

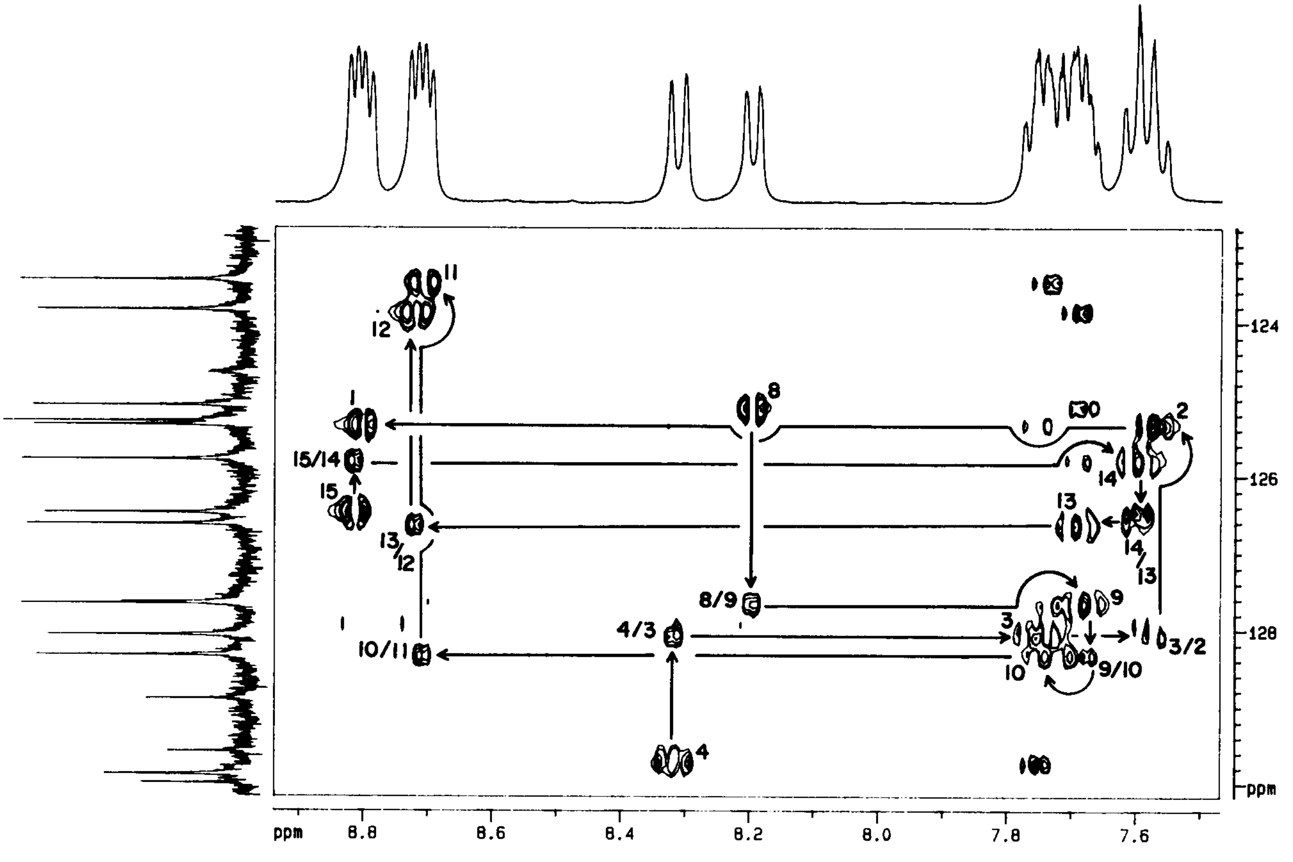


Figure 4A. The HMQC-TOCSY spectrum of **1** acquired with a mixing time of 12 msec. Note that the direct responses from the proton carbon pairs at positions 1 and 2 are overlapped with the relayed responses between the proton pair at those positions.

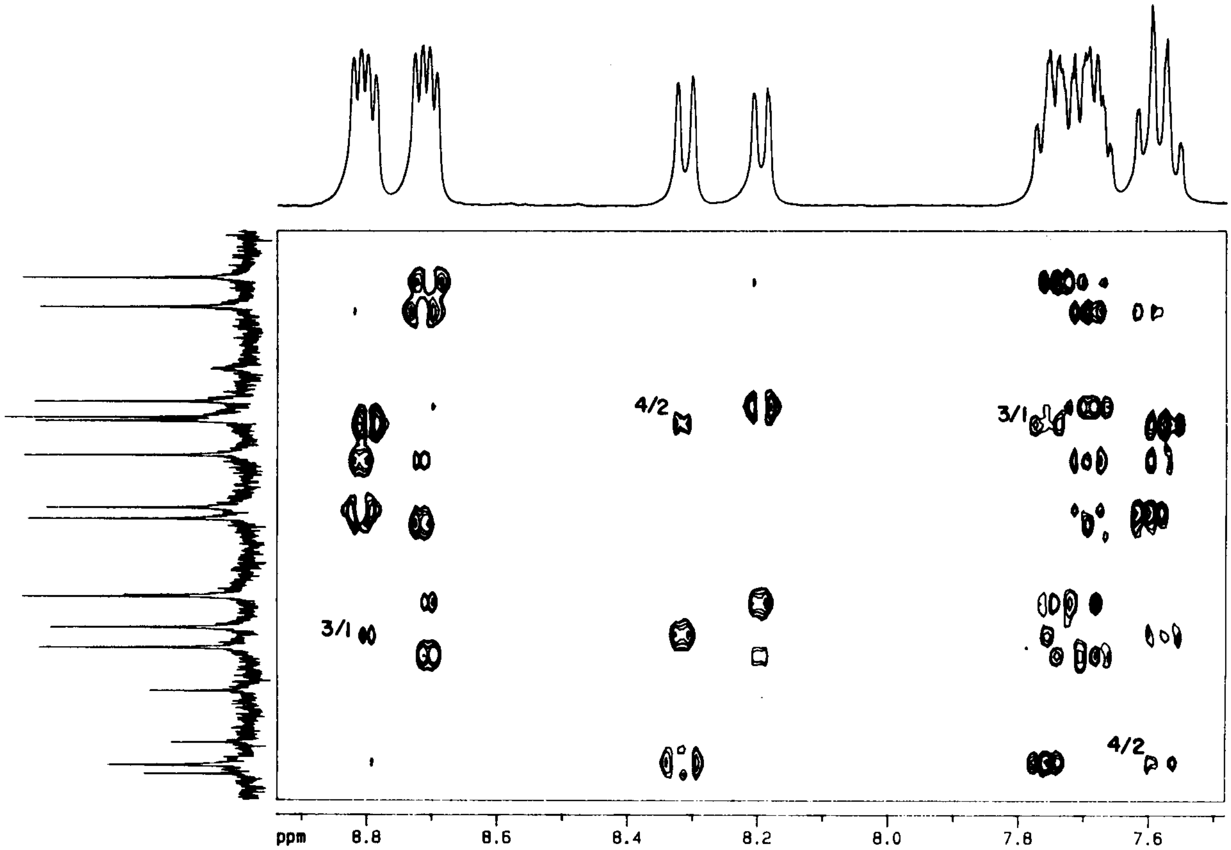


Figure 4B. HMQC-TOCSY spectrum of **1** acquired with a mixing time of 24 msec. Note that the relayed responses between the proton pairs 1/3 and 4/2 are now present confirming the assignment of positions 1 and 2.

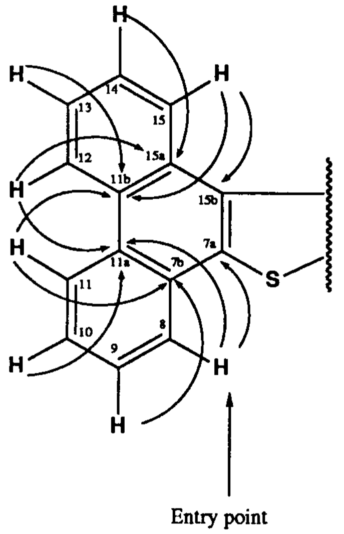


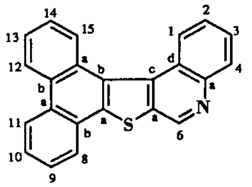
Figure 5. Diagram of long-range correlations observed on the HMBC spectrum for the phenanthrene moiety of **1**.

moiety of the molecule such that the bay region is expanded and the helical nature of the molecule is increased. The addition of the extra ring decreases the symmetrical nature of **2** relative to **1**. A comparison of the proton spectra of **1** and **2** reflect the level of symmetry of each respective compound. The spectrum of **1** shows only three proton resonances that are completely resolved while for **2** nearly all of the resonances are resolved. In fact for **2** most of the "symmetry" is localized in the phenanthrene moiety.

Because the proton spectrum is so well resolved for **2** we can differentiate one spin system from another with relative ease using the HMQC-TOCSY spectrum (Figure 6). Hence, all that is needed is to tie the spin systems together from long-range correlations observed in the HMBC spectrum (Figure 7). The singlet corresponding to H8 provides a suitable entry point to assign the quaternary carbons and identify the spin systems for the benzo[*f*][2,3-*c*]quinoline moiety of **2** as is illustrated in Figure 8.

Table 1

^{1}H and ^{13}C Chemical Shifts of Phenanthro-[9',10':4,5]thieno[2,3-*c*]quinoline **1**



1

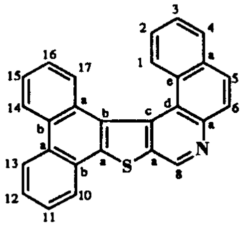
Position	Chemical shifts δ^1H	Carbon(s) to which long-range correlations were observed	Chemical shifts δ^{13}C
1	8.76, d	C3, C4a, C15c	125.33
2	7.55 [a]	C4, C15d	125.29
3	7.74 [a]	C1, C4a	128.07
4	8.29, d	C2, C15d	129.88
4a			145.61
6	9.35, s	C4a, C6a, C15c	145.02
6a			132.46
7a			141.98
7b			127.69
8	8.15, d	C10, C11a, C7a	125.08
9	7.65 [a]	C11, C7b	127.66
10	7.73 [a]	C8, C11a	128.34
11	8.67, d	C9, C7b, C11b	123.44
11a			130.60
11b			130.00
12	8.68, d	C14, C11a, C15a	123.83
13	7.67 [a]	C15, C11b	126.48
14	7.56 [a]	C12, C15a	125.79
15	8.77, d	C13, C11b, C15b	126.63
15a			128.90
15b			129.58
15c			138.30
15d			124.65

[a] These chemical shifts were taken from one-dimensional slices of the HMQC spectrum.

Due to the helical nature of this molecule H1 is close enough to H17 for the observation of nOe, and the resonances are resolved allowing the use of nOe-difference experiments. Irradiation of the doublet at 7.85, corresponding to H1 transferred nOe to the doublet at 7.44 identifying this resonance as H17. The reverse experiment, irradiation of the doublet at 7.44 transferred nOe to the doublet at 7.85, which corresponds to H1, verifying the results of the previous experiment. The nOe signals were used to calculate the distance between H1 and H17 giving 2.79 Å, compared with a calculated distance of 2.87 Å. The identification of H17 then gives entry to the phenanthrene moiety and allows us to tie every assignment back to the singlet corresponding to H8, which is the entry point. From H17 we can assign the protons and carbons of this spin system directly from the HMQC-TOCSY spectrum and using the HMBC spectrum link the two spin system together *via* long-range correlations (Figure 9) thereby completing the assignments of all the protons and carbons of **2** (Table 2).

Table 2

^{1}H and ^{13}C Chemical Shift Assignments of Benzo[*f*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline **2**



2

Position	Chemical shift δ^1H	Carbon(s) to which long-range correlations were observed	Chemical shift δ^{13}C
1	7.85, d	C3, C4a, C17d	129.93
2	7.03 [a]	C4, C17e	124.86
3	7.50 [a]	C1, C4a	126.87
4	7.99, d	C2, C17e, C5	127.47
4a			131.66
5	8.07, d	C4, C6a, C17e	129.34
6	8.21, d	C4a, C17d	127.78
6a			145.65
8	9.44, s	C6a, C8a, C17c	143.80
8a			136.25
9a			142.50
9b			127.84
10	8.25, d	C12, C13a, C9a	125.32
11	7.70, t	C13, C9b	127.67
12	7.76, t	C10, C13a	128.55
13	8.73, d	C11, C9b, C13b	123.55
13a			131.06
13b			129.12
14	8.65, d	C16, C13a, C17a	123.27
15	7.49 [a]	C17, C13b	126.16
16	7.00 [a]	C14, C17a	125.42
17	7.44, d	C15, C13b, C17b	128.24
17a			128.37
17b			130.02
17c			133.83
17d			122.23
17e			128.35

[a] These chemical shifts were taken from one-dimensional slices of the HMQC spectrum.

Compound 3.

This structure again is similar to **1** except that this time the extra benzene ring is fused to the quinoline moiety such that another small bay region is formed. This molecule also has less symmetrical character than

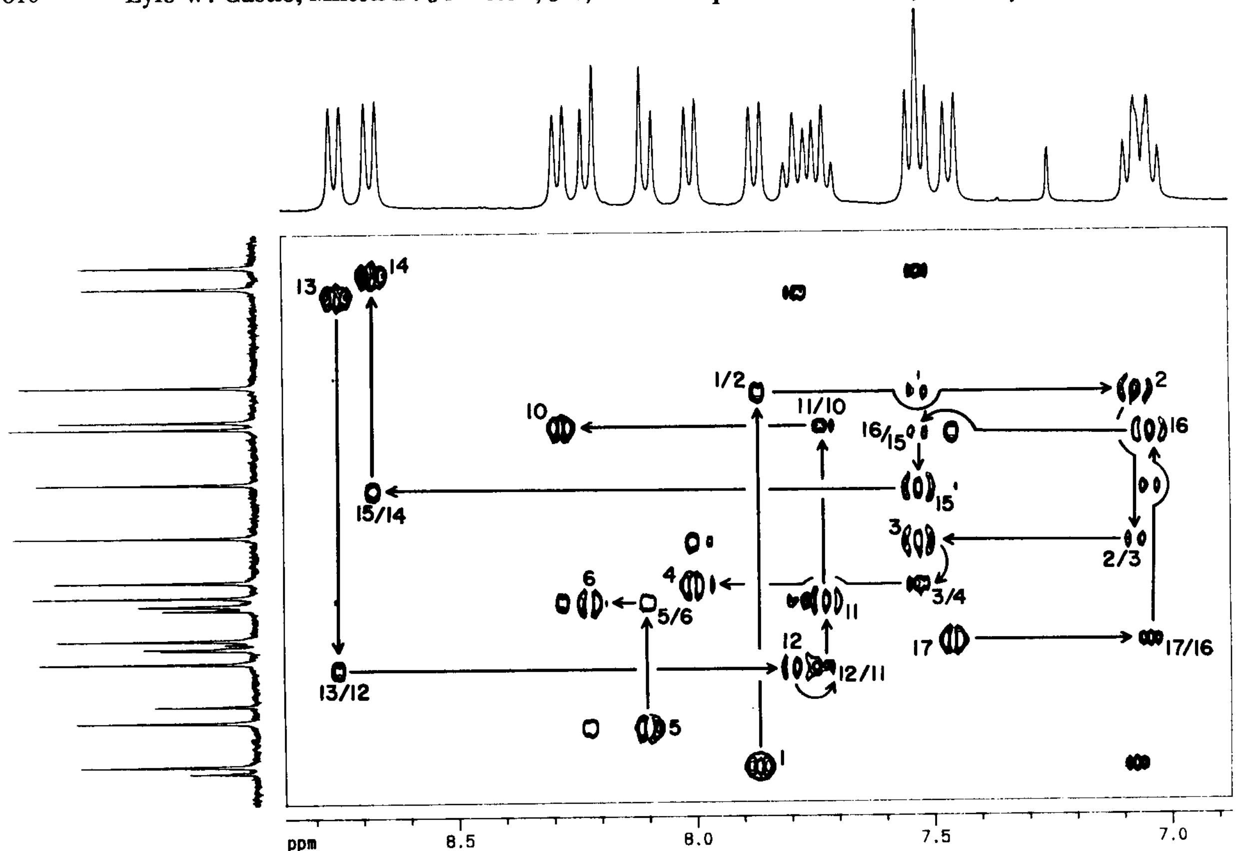


Figure 6. The HMQC-TOCSY spectrum of **2** acquired with a mixing time of 12 msec.

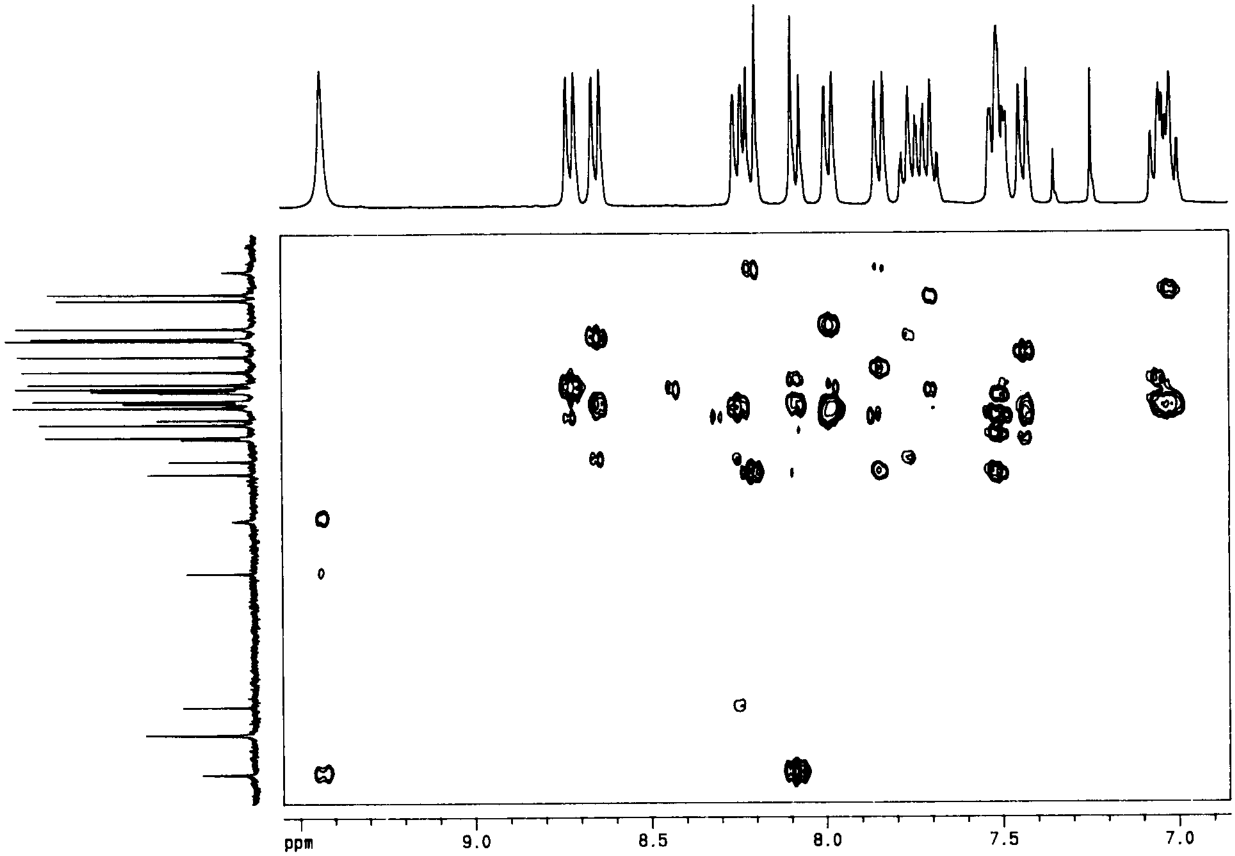


Figure 7. The HMBC spectrum of **2**.

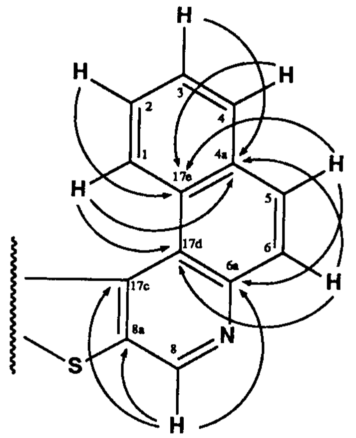


Figure 8. Diagram of long-range correlations observed on the HMBC spectrum for the benzo[*f*]thieno[2,3-*c*]quinoline moiety of **2**.

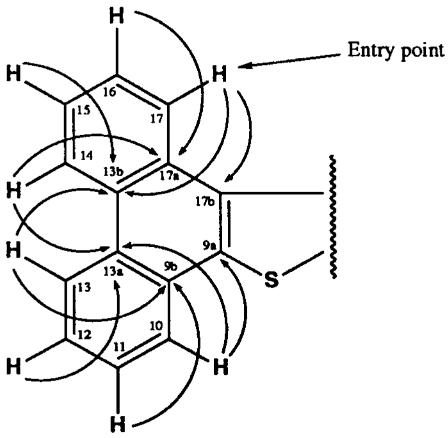


Figure 9. Diagram of long-range correlations observed on the HMBC spectrum for the phenanthrene moiety of **2**.

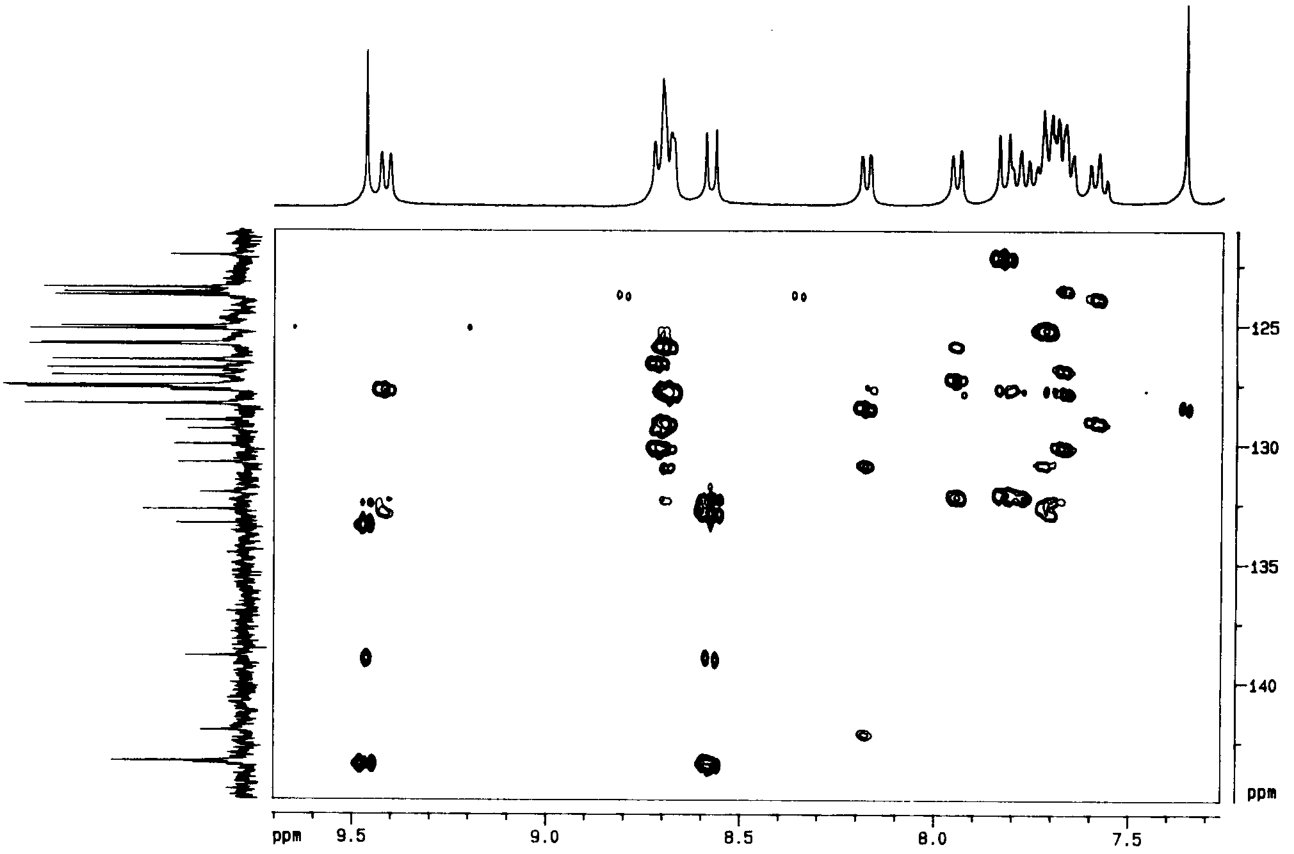


Figure 10. The HMBC spectrum of **3**.

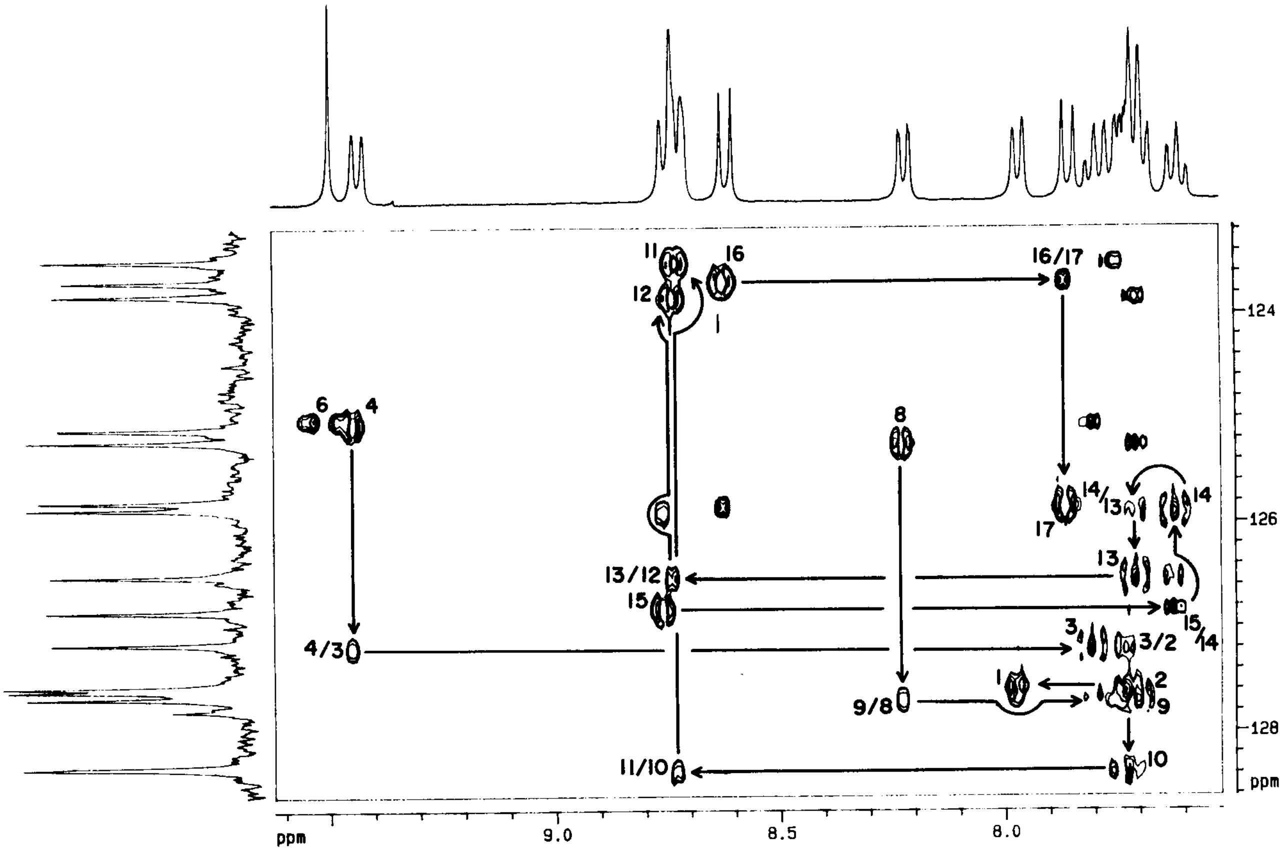


Figure 11A. The HMQC-TOCSY spectrum of **3** acquired with a mixing time of 12 msec. Due to the ^{13}C chemical shift of C1 and C2 the direct responses from the proton carbon pairs are overlapping with the relayed responses between the proton pair 1/2 at those positions. The ^{1}H chemical shift of H9 and H10 are overlapping causing their direct responses to overlap with the relayed responses between the proton pair 9/10.

1 as is reflected by the complete resolution of five resonances in the proton spectrum. It should be noted that the proton spectrum of **3** shows more overlapping resonances than that of **2**.

To initiate chemical shift assignments we will assign H6 to the singlet at 9.46 and use this as the entry point. From the HMBC spectrum (Figure 10) we identify C4b and in turn from C4b we identify H4 and H16. Using the HMQC-TOCSY spectra (Figures 11A and 11B) we can identify the doublet at 9.42 as H16 because it is a member of a two spin system and likewise the doublet at 8.58 as H4 because it is a member of a four-spin system. With the spin systems already identified from the HMQC-TOCSY spectrum the protons and carbons on the benzoquinoline moiety are easily assigned (Figure 12). The only area where difficulty is encountered is at positions 1 and 2. Because C1 and C2 are only separated by 0.11 ppm their relayed responses are overlapped with their direct responses. The ambiguity imposed by overlapping responses is overcome at longer mixing times by the following relayed responses: at 24 msec (Figure 11B) we observe relayed responses of the proton pairs 4/2 and 3/1, and at 36 msec the proton pair 4/1 is also observed.

Irradiation of the doublet at 8.58, which is H16, transferred nOe to the doublet at 8.72 identifying H15 and providing an entry point into the phenanthrene moiety. The nOe signals were used to calculate the distance between H16 and H15 giving 2.35 Å, compared with the calculated distance of 2.29 Å. Because the spin systems are identified, all that is necessary is to link the two four-spin systems through long-range correlations (Figure 13) observed on the HMBC spectrum and thereby complete the assignment of **3** (Table 3).

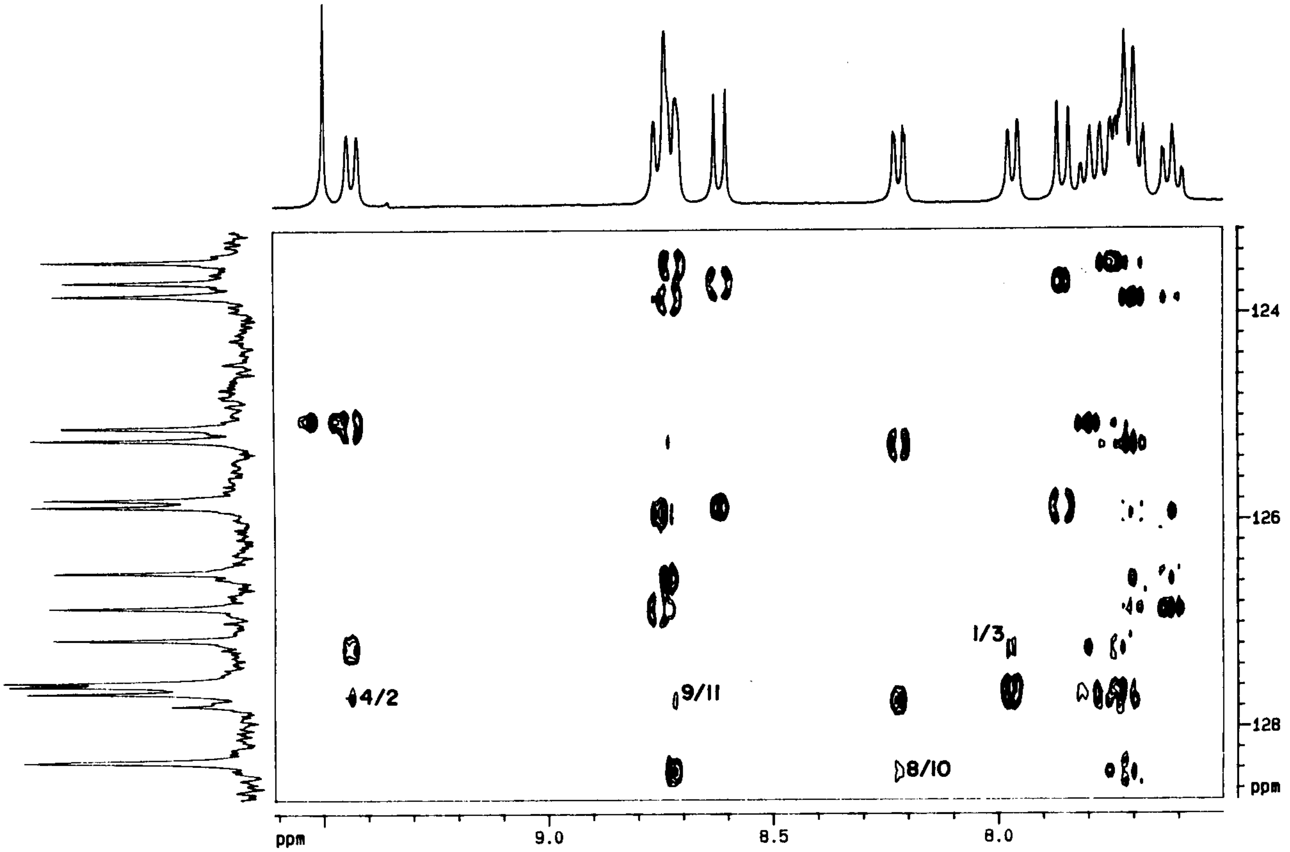


Figure 11B. HMQC-TOCSY spectrum of **3** acquired with a mixing time of 24 msec. Note that the relayed responses between the proton pairs 1/3 and 4/2 are now present confirming the assignments at positions 1 and 2 and those of 8/10 and 9/11 confirm the assignments of positions 9 and 10.

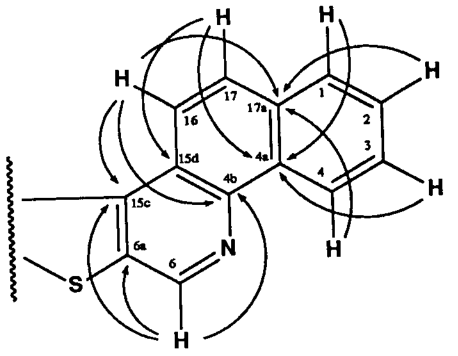


Figure 12. Diagram of long-range correlations observed on the HMBC spectrum for the benzo[*h*]thieno[2,3-*c*]quinoline moiety of **3**.

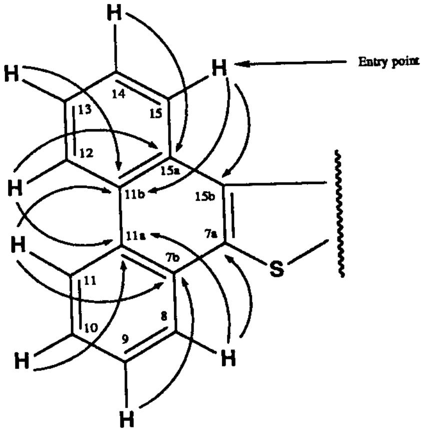
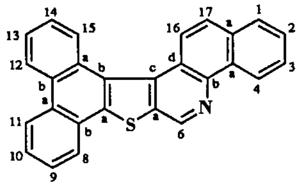


Figure 13. Diagram of long-range correlations observed on the HMBC spectrum for the phenanthrene moiety of **3**.

In spite of the break in the symmetry of **3** versus **1** the proton spectrum is still quite congested with a number of overlapping resonances and each spin system has resonances in the highly congested region between 7.60 and 7.80 ppm. To further complicate the spectrum the resonances corresponding to H15, H12 and H11 all have nearly the same chemical shift and each of these resonances correlates with other resonances in the highly congested region between 7.60 and 7.70 ppm. These complications negate the possibility of identifying the spin systems based on

Table 3

^{1}H and ^{13}C Chemical Shifts of Benzo[*h*]phenanthro[9',10':4,5]thieno[2,3-*c*]quinoline **3**



3

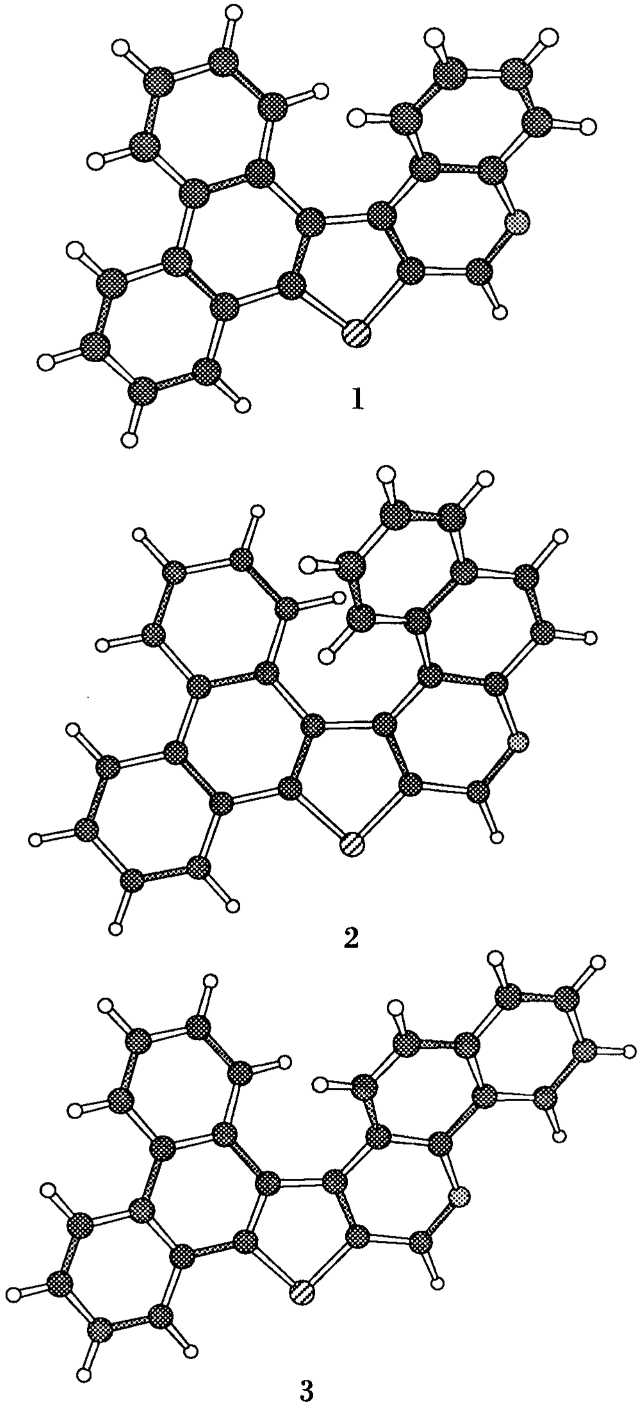
Position	Chemical shift δ^1H	Carbon(s) to which long-range correlations were observed	Chemical shift δ^{13}C
1	7.95, d	C3, C17, C4a	127.56
2	7.68 [a]	C4, C17a	127.66
3	7.77 [a]	C1, C4a	127.15
4	9.42, d	C2,C17a, C4b	125.10
4a			132.06
4b			143.42
6	9.46, s	C4b, C6a, C15c	143.35
6a			133.36
7a			142.05
7b			127.79
8	8.18, d	C10, C7a, C11a	125.21
9	7.66 [a]	C11, C7b	127.59
10	7.70 [a]	C8, C11a	128.33
11	8.69 [a]	C9, C7b, C11b	123.48
11a			130.79
11b			130.03
12	8.69 [a]	C14, C11a, C15a	123.81
13	7.66 [a]	C15, C11b	126.50
14	7.58, t	C12, C15a	125.86
15	8.72 [a]	C13, C11b, C15b	126.84
15a			129.02
15b			129.38
15c			138.92
15d			122.10
16	8.58, d	C4b, C15c, C17a	123.68
17	7.82, d	C1, C4a, C15d	125.79
17a			132.78

[a] These chemical shifts were taken from one-dimensional slices of the HMQC spectrum.

homonuclear techniques such as the COSY or DQF-COSY spectra. Hence the HMQC-TOCSY experiment was essential for the differentiation of the spin systems in **3**. Without the HMQC-TOCSY spectrum we would have been forced to flip back and forth between the HMBC, HMQC and COSY-type spectra to make these assignments.

Conclusion.

These assignments illustrate the utility of using the HMQC-TOCSY for identifying spin systems on compounds with psuedo-symmetric character where



3

Figure 14. The structures of **1**, **2** and **3** produced from MOPAC calculations. These structures show the twist associated with helical compounds of this nature. For **1** the calculated distance between H1 and H15 is 2.22 Å. For **3** the calculated distance between H16 and H15 is 2.29 Å whereas the nOe derived distance is 2.35 Å. For **2** the calculated distance between H1 and H17 is 2.87 Å whereas the nOe derived distance is 2.79 Å. Also, for **2** the calculated distances between H17 and C1 is 2.67 Å and between H1 and C17 is 2.77 Å illustrating that H1 and H17 are interacting with the π-cloud at the opposite side of the molecule and that this interaction shifts these two resonances upfield. Because of the geometry of **2** the interaction is more pronounced for H17 than for H1 as is born out by their chemical shifts.

the spin systems overlap in their proton spectrum. Examination of the ^1H and ^{13}C spectra of these three ring systems show that for compounds **1** and **3** there are more overlapping resonances than that of **2**. This indicates that the addition of the extra fused ring perturbed the symmetry of **2** to a greater extent than that of **3** relative to **1**. That postulate is in fact correct. Because of the helical nature of **2**, compared with that of **3**, the chemical environment of the rings at each end of the bay region are perturbed by the π-cloud of the opposing rings. Because of the geometry of **2** the atoms at the phenanthrene end of the bay region are imbedded into the π-cloud of the opposing ring making the change in the chemical environment greater on this side of the molecule than that of the opposing interaction (*i.e.*, the interaction between the benzo[*f*]-ring with the π-cloud of the phenanthrene moiety). This is illustrated by the chemical shifts of H17 at 7.44 and H1 at 7.85 in **2**, and both of these resonances are shifted upfield relative to those at each end of the bay region of **1** and **3**. This interaction in **2** and the lack of it in **1** and **3** can be seen very clearly from their structures which were generated from MOPAC calculations (Figure 14).

EXPERIMENTAL

All experiments were performed on a Bruker AMX 360 MHz NMR spectrometer operating at an observation frequency of 360.13 MHz for ^1H and 90.56 for ^{13}C. All experiments were performed using an inverse-geometry 5-mm broad band probe. Both the ^1H and ^{13}C 90° pulses were calibrated, and the values obtained were 7.20 and 14.50 µsec, respectively.

The proton spectra were recorded with 16384 data points and all the chemical shifts were referenced to TMS. The carbon spectra were recorded with 64K data points and were 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.

For the HMQC experiment we used the Bruker pulse program (*invbdgtp*) using the BIRD sequence optimized for short-range couplings (132 Hz $^1J_{CH}$) [4]. For the HMBC experiment we used the Bruker pulse program (*inv4lplrnd*) [5] optimized for 9 Hz $^3J_{CH}$ couplings and a delay for one bond suppression of 3 msec. Both the HMQC and HMBC spectra were acquired at a digital resolution of 1024 points for F_2 and 256 for F_1 and upon processing the F_1 dimension was zero-filled to achieve a real data matrix of 512 by 512 points. For both the HMQC and HMBC spectra F_1 and F_2 were subjected to cosine squared multiplication prior to transformation of each dimension.

The HMQC-TOCSY spectra were acquired using the standard Bruker pulse program (*invbmltp*) [6]. Four experiments were run on each compound at mixing times of 12, 24, 36 and 48 msec. The spectra were acquired at a digital resolution of 512 points for F_2 and 128 for F_1. The sweep width for F_1 (carbon) was 1340 Hz for **1**, 1339 Hz for **2** and 831 Hz for **3**. A relaxation delay of 1 sec was used between scans. The total instrument time used for each experiment was about three hours. The data was zero-filled to achieve a real data matrix of 512 by 256 points and processed with Gaussian multiplication prior to the first Fourier transformation (F_2) and cosine multiplication prior to the second (F_1).

The structures of **1**, **2** and **3** were computed using SYBYL on a SUN 4/330 computer. The structures were first minimized using the forcefield provided by the SYBYL program set at the default settings, and the structure generated was then used as a starting structure for a MOPAC geometry minimization. The MOPAC calculation was set to PM3 parameters [7] for a singlet state molecule.

The internuclear distances obtained from the MOPAC calculations were compared with distances obtained from the nOe experiments performed on compounds **2** and **3** (*cf.* Figure 14). For **2**, irradiation of H1 transferred 4.5% nOe to H17 and 9.1% nOe to H2. For **3**, irradiation of H16 transferred 16% nOe to H15 and 13% nOe to H17. The distances were calculated from the percent nOe using the three spins linear approximation, where the center spin was irradiated [8]. An assumed vicinal proton-proton distance of 2.79 Å was used for the calculation.

Acknowledgment.

The authors wish to thank the National Science Foundation (CHE-8813620) for providing the funds for the acquisition and operation of the Bruker AMX360 NMR spectrometer used for this work.

REFERENCES AND NOTES

[1] G. E. Martin and R. C. Crouch, *J. Nat. Prod.*, **54**, 1 (1990).

[2] L. W. Castle, A. S. Zektzer and M. D. Johnston, Jr., *Magn. Reson. Chem.*, **30**, 779 (1992); J.-K. Luo, A. S. Zektzer and R. N. Castle, *J. Heterocyclic Chem.*, **28**, 737 (1991); J.-K. Luo and R. N. Castle, *J. Heterocyclic Chem.*, **28**, 1825 (1991).

[3] L. W. Castle, M. D. Johnston, Jr., Ch. L. Camoutsis and R. N. Castle, *J. Heterocyclic Chem.*, **29**, 0000 (1992).

[4] A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

[5] A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).

[6] L. Lerner and A. Bax *J. Magn. Reson.*, **69**, 375 (1986).

[7] J. P. P. Stewart, *J. Comput. Chem.*, **10**, 221 (1989).

[8] J. H. Noggle and R. E. Schirmer, The Nuclear Overhauser Effect, Academic Press, New York, 1971, p 58.