Autocorrelated ^{13}C-^{13}C Double Quantum Coherence Two-Dimensional NMR Spectroscopy: Utilization of a Modified Version of the Technique as an Adjunct in the Total Assignment of the ^{1}H- and ^{13}C-NMR Spectra of the Mutagen Phenanthro[3,4-*b*]thiophene

M. J. Musmar, M. Robert Willcott, III and Gary E. Martin [1]

Department of Medicinal Chemistry, College of Pharmacy,
University of Houston, Houston, Texas 77004

Robert T. Gampe, Jr.

Department of Biochemistry and Molecular Biology, University of Texas
Health Science Center, Houston, Texas 77030

Masatomo Iwao [2] and Milton L. Lee

Department of Chemistry, Brigham Young University,
Provo, Utah 84601

Ralph E. Hurd and LeRoy F. Johnson

Nicolet Magnetics Corporation, 255 Fourier Avenue,
Fremont, California 94539

Raymond N. Castle

Department of Chemistry, University of South Florida,
Tampa, Florida 33620

Received May 31, 1983

Development of successively higher field nmr spectrometers has facilitated the study of increasingly more complex molecules, although smaller molecules such as phenanthro[3,4-*b*]thiophene still offer very substantial assignment problems because of the highly congested nature of their ^{1}H- and ^{13}C-nmr spectra. Assignments of such spectra, if they are to be unequivocal, frequently require the utilization of two-dimensional nmr spectroscopic techniques. Total assignments of the ^{1}H- and ^{13}C-nmr spectra of phenanthro[3,4-*b*]thiophene are reported. Assignments were based on a conventional high resolution 500 MHz ^{1}H-nmr spectrum, autocorrelated two-dimensional ^{1}H-nmr spectra (COSY), two-dimensional ^{1}H-^{13}C chemical shift correlation spectra and a modified version of autocorrelated ^{13}C-^{13}C double quantum coherence two-dimensional nmr spectroscopy. From NOE measurements, a separation of 1.99 Å between H1 and H11 was computed, suggesting that phenanthro[3,4-*b*]thiophene has a pronounced helical conformation in solution.

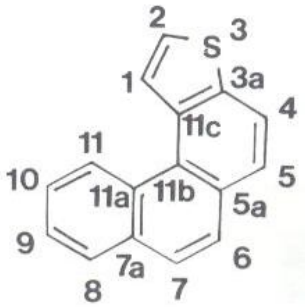
J. Heterocyclic Chem., **20**, 1661 (1983).

Introduction.

Developments in the area of superconductive magnet technology have made available nmr spectrometers which operate at higher and higher observation frequencies. As a direct consequence of the availability of such instruments, studies are now beginning to appear which deal with large biomolecules which could not be undertaken otherwise. Despite advances with large molecules, polycyclic aromatic hydrocarbons (PAH) and their fused ring heterocyclic analogs still offer a very substantial assignment problem because of the highly congested nature of their ^{1}H- and ^{13}C-nmr spectra. Thus, the second frequency domain offered by two-dimensional nmr experiments offers a means of simplifying these spectra to a point at which they may be unequivocally assigned if sufficient care is taken in the selection of appropriate two-dimensional experiments. Recently, we have reported the total assignment of the ^{1}H- and ^{13}C-nmr spectra of phenanthro[1,2-*b*]thiophene (**2**) [3] and the assignment of the ^{1}H-nmr spectrum of benzo[2,3]-phenanthro[4,5-*bcd*]thiophene (**3**) [4], both relying on the concerted utilization of two-dimensional nmr experiments.

Recent advances in two-dimensional nmr techniques have led to the development of modifications of the ^{13}C-^{13}C double quantum coherence (DQC) experiment first reported for one dimension (INADEQUATE [5-7]) and subsequently modified for two dimensions [8,9]. Specifically, Turner has described a further modification of the pulse sequence which takes advantage of properties of the DQC to provide an autocorrelated two-dimensional spectrum with responses correlated about the diagonal, $F_1 = F_2$ [10,11] rather than about the axis $F_1 = 2F_2$ as in earlier two-dimensional DQC experiments [8,9]. More recently, Mareci and Freeman have described a modification of conventional 2D-DQC experiments which relies on a 135° read pulse rather than the usual 90° pulse [12], thus providing a means of determining the sign of the DQC. We would now like to report the incorporation of the 135° pulse of Mareci and Freeman [12] into the autocorrelated

pulse sequence of Turner [10,11] and the utilization of the modified experiment in the total assignment of the ^{13}C-nmr spectrum of the mutagen phenanthro[3,4-*b*]thiophene (**1**) [13].



1

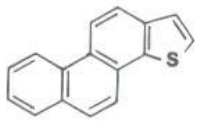
Assignment Strategy.

Numerous useful strategies are possible for the assignment of the proton and carbon spectra of complex heterocycles which permit the flexibility of adopting a new tact midway through the assignment should an unanticipated difficulty be encountered. Despite the flexibility inherent in these assignment strategies, it is still useful to outline an initial approach to any complex assignment which may be utilized as a point of departure. In the specific case of phenanthro[3,4-*b*]thiophene (**1**), we may use to some advantage a knowledge of the spectral assignments of the related phenanthro[1,2-*b*] (**2**) ring system [3].

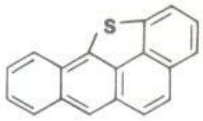
As a starting point for the assignment of the proton nmr spectrum of **1**, it is reasonable to assume that the H1 and H11 resonances will be observed furthest downfield, H1 and H11 analogous to the H10 and H9 resonances of **2** respectively. Furthermore, the H8-H11 spin system would be expected to constitute either an ABMX or an ABXY spin system based on the assignments of the corresponding four spin systems of **2** and **3** [3,4]. The H1-H2 spin system of **1**, in contrast, would be expected to appear as an AX rather than as an AB spin system because of the 3,4-fusion of the thiophene ring to the phenanthrene nucleus. The remaining two spin systems, H4-H5 and H6-H7, would both be expected to appear as AB spin systems. Thus, a key requirement of any successful assignment strategy will be the ability of the approach to correctly orient the two AB spin systems relative to the other two spin systems. In part, it is reasonable to expect that to some extent it will be possible to accomplish this goal by resorting to long range coupling constants as in the case of **3** [4]. Alternatively, as is discussed below, it may be preferable to establish carbon assignments *via* the autocorrelated ^{13}C-^{13}C 2D-DQC experiment and to utilize these assignments to either make or confirm the proton assignments through the two-dimensional proton-carbon chemical shift correlation experiment (CSCM).

In the case of phenanthro[3,4-*b*]thiophene (**1**), the assignment of resonances in the ^{1}H-nmr spectrum was accomplished by using an autocorrelated two-dimensional ^{1}H-nmr spectrum (COSY) and long range couplings which were obtained from a resolution enhanced 500 MHz ^{1}H-nmr spectrum. It should be noted that the autocorrelated or COSY proton spectrum is particularly useful in establishing the identity of components of the various spin systems, especially when one of the involved spins may be assigned on some basis, *e.g.*, chemical shift.

Assignment of the ^{13}C-nmr spectrum of **1** presents a substantial challenge despite the empirical nature of the ^{13}C-chemical shift. In particular, assignments are complicated by the fact the ten protonated carbons of the molecule resonate within a region less than 10 ppm wide, seven resonances of this group tightly clustered within a region of less than 1.5 ppm. Under such conditions, unequivocal assignments dictate the utilization of two-dimensional proton-carbon chemical shift correlation or two-dimensional DQC spectra.



2



3

Autocorrelated Two-Dimensional ^{1}H-NMR Spectra COSY.

Having established a plausible assignment strategy for phenanthro[3,4-*b*]thiophene (**1**), a 500 MHz autocorrelated two-dimensional ^{1}H-nmr (COSY) spectrum [14-16] was first obtained (Figure 1) to facilitate the segregation of the individual resonance among the four spin systems described above. Based on this spectrum, the H1-H2 AX spin system was found to be comprised of the resonances at δ = 8.653 and 7.750; the H4-H5 and H6-H7 spin systems consisted of the resonances at δ = 8.086 and 7.849 and δ = 7.883 and 7.834 respectively; the H8-H11 four spin system, as expected, was an ABMX spin system comprised of the resonances observed at δ 9.161, 8.006, 7.736 and 7.656. No individual assignments were possible within the AB spin systems solely on the basis of the COSY connectivities. Within the ABMX spin system, only the H8 and H11 resonances could be assigned to the resonances at δ = 8.006 and 9.161 respectively.

High Resolution One-Dimensional 500 MHz ^{1}H-NMR.

Having established the identities of the individual proton resonances comprising the four spin systems of **1**, we next undertook the specific assignment of the individual resonances. In each case, the ortho coupling ($^3J_{HH}$) verified the pairings made on the basis of the COSY spectrum (Table I). Beyond this, the high resolution 500 MHz

Table I

^{1}H Chemical Shifts and Coupling Constants of Phenanthro[3,4-*b*]thiophene (**1**) in Deuteriochloroform at 500 MHz

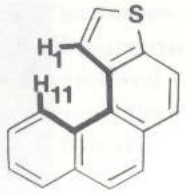
Position	Spin Label	^{1}H	$^3J_{HH}$ (*ortho*)	$^4J_{HH}$ (*meta* or *peri*)	$^5J_{HH}$ (*para*, *epi* long range)	$^6J_{HH}$ (long range)	$^7J_{HH}$ (long range)
H1	X	8.653	$^3J_{H_1H_2}$ = 5.62	—	$^5J_{H_1H_4}$ = 0.90 [a]	$^6J_{H_1H_{11}}$ = 0.55 [a]	—
H2	A	7.750	$^3J_{H_2H_1}$ = 5.61	—	—	—	$^7J_{H_2H_5}$ = 0.55
H4	A	8.086	$^3J_{H_4H_5}$ = 8.45	—	$^5J_{H_4H_1}$ = 0.88 [a]	—	—
H5	B	7.849	$^3J_{H_5H_4}$ = 8.37	$^4J_{H_5H_6}$ = 0.52 [b]	—	—	$^7J_{H_5H_2}$ = 0.52
H6	A	7.883	$^3J_{H_6H_7}$ = 8.62	NO OTHER COUPLINGS RESOLVED			
H7	B	7.834	$^3J_{H_7H_6}$ = 8.64	$^4J_{H_7H_8}$ = 0.62	—	—	—
H8	M	8.006	$^3J_{H_8H_9}$ = 7.89	$^4J_{H_8H_{10}}$ = 1.55	$^5J_{H_8H_{11}}$ = 0.67	—	—
	—	—	—	$^4J_{H_8H_7}$ = 0.67	$^5J_{H_8H_6}$ = 0.22 [c]	—	—
H9	B	7.656	$^3J_{H_9H_8}$ = 7.90	$^4J_{H_9H_{11}}$ = 1.18	—	—	—
	—	—	$^3J_{H_9H_{10}}$ = 6.91	—	—	—	—
H10	A	7.736	$^3J_{H_{10}H_9}$ = 6.92	$^4J_{H_{10}H_8}$ = 1.57	—	—	$^7J_{H_{10}H_6}$ = 0.24 [d]
	—	—	$^3J_{H_{10}H_{11}}$ = 8.49	—	—	—	—
H11	X	9.161	$^3J_{H_{11}H_{10}}$ = 8.50	$^4J_{H_{11}H_9}$ = 1.27	$^5J_{H_{11}H_8}$ = 0.63	$^6J_{H_{11}H_1}$ = 0.63 [a]	—

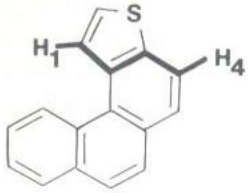
[a] Confirmed by homonuclear decoupling. [b] The corresponding coupling for the H6 resonance was unresolved. Typical *peri* couplings in coumarin based systems range from 0.35-0.40 Hz (M. W. Jarvis and A. G. Moritz, *Aust. J. Chem.*, **21**, 2445 (1968)); *peri* couplings in a variety of polycyclic aromatic hydrocarbons ranged from 0.20-0.50 Hz (K. D. Bartle, D. W. Jones and R. S. Matthews, *Rev. Pure Appl. Chem.*, **19**, 191 (1969)), which are in excellent agreement with the 0.52 Hz coupling observed in the case at hand. [c] The corresponding coupling for the H6 resonance was unresolved. Analogous five bond couplings in coumarin based systems ranged from 0.30-0.45 Hz (M. W. Jarvis and A. G. Moritz, *Aust. J. Chem.*, **21**, 2445 (1968)); the corresponding J_{17} coupling of napthalene is reported as 0.21 Hz (M. W. Jarvis and A. J. Moritz, *Aust. J. Chem.*, **24**, 89 (1971)), which is in excellent agreement with the observed coupling of **1**. [d] The corresponding coupling for the H6 resonance was unresolved. The corresponding J_{26} coupling in napthalene was observed to be 0.28 Hz (M. W. Jarvis and A. G. Moritz, *Aust. J. Chem.*, **24**, 89 (1971)), which agrees quite well with the observed coupling of 0.24 Hz between H10 and H6 in **1**.

spectrum of **1** also provided a number of interesting long range couplings which were verifiable by homonuclear decoupling.

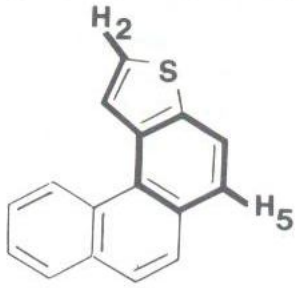
Perhaps the most interesting long range couplings contained in the proton spectrum of **1** were those arising from the H1-H2 AX spin system. In particular, two long range couplings of 0.90 and 0.55 Hz were observed for the H1 resonance. The larger of these couplings was identified by decoupling the resonance observed at δ = 8.086, which is ultimately assigned as H4. The observed couplings thus an epi zig zag coupling and, as such, is well within the range of values observed for other polycyclic aromatics of 0.6-1.1 Hz reported in the review of Bartle, Jones and Matthews [17]. The smaller and perhaps more interesting coupling was also readily identified on decoupling of the H11 resonance δ = 9.161. This coupling is noteworthy in that it is quite similar to a "bay" coupling of the type frequently encountered in phenanthrene systems [17], but with the difference that this coupling is through six rather than five bonds. For purposes of comparison, other six bond couplings are known, for example, that between H4 an H7 in coumarin systems [18] and that between H1 and H6 in napthalene [19], both of which are in the range of 0.15 Hz. The basis for the unexpectedly large coupling between the H1 and H11 resonances, however, remains to be accounted for.

Interestingly, the multiplet for H2 which resonates at δ = 7.750 also exhibited a long range coupling of 0.55 Hz.





On the basis of homonuclear decouplings, this coupling was attributed to a coupling between the H2 and H5 resonances which may be either through six bonds *via* the sulfur or alternatively through seven bonds. In the latter case, this coupling is analogous to the extended epi zig zag couplings of benzo[*c*]phenanthrene [17], which range from 0.4-1.0 Hz. We favor assigning this as a seven bond coupling as shown, although arguments for the six bond coupling pathway have also been made [31].



Other than the relatively unusual couplings associated with the H1-H2 AX spin system, the balance of the observed long range couplings were unremarkable (Table I). The

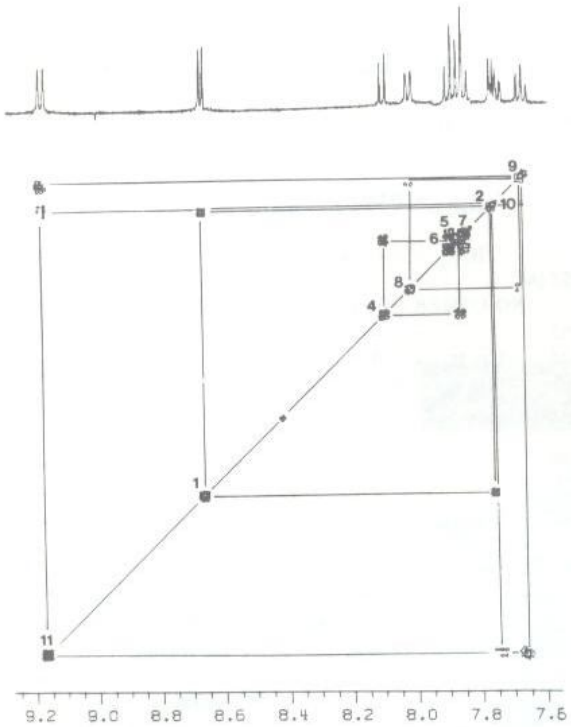


Figure 1. Four level contour plot of the autocorrelated two-dimensional proton (COSY) nmr spectrum of **1** in deuteriochloroform at 500 MHz. The spectrum shown is a plot of the symmetrized $S(F_2,F_1)$ data matrix consisting of 512 × 512 points. Off-diagonal responses correlating H8 with H10 were observable when more contour levels were plotted. A 300 MHz COSY spectrum (not shown) also contained several of the long range couplings discussed in the text.

observation of peri couplings, $^4J_{H_5H_6}$ and $^4J_{H_7H_8}$, did, however, serve to firmly establish the orientation of the two AB spin systems relative to the H1-H2 AX and H8-H11 ABMX spin systems, thus permitting an unequivocal assignment of the ^{1}H-nmr spectrum of **1**.

Two-Dimensional Proton-Carbon Chemical Shift Correlation (CSCM).

Following the completion of the assignment of the proton nmr spectrum of **1**, we next undertook the assignment of the ^{13}C-nmr spectrum of the molecule at 75 MHz *via* a two-dimensional proton-carbon chemical shift correlation (CSCM) spectrum [20-22]. Rather unexpectedly, the ^{1}H chemical shifts of the resonances of **1** were found to be very sensitive to changes in concentration, this effect encountered when additional material was added to the sample employed for the COSY spectrum to speed up data acquisition. This potential interchange of resonances reintroduces the potential for ambiguous assignments of the protonated carbon resonances which is dealt with below.

The CSCM spectrum of **1** is shown in Figure 2A, an expansion of the region 125-128 ppm in F_2 shown in Figure 2B. As will be noted from Figure 2B, the two triplets associated with the C9 and C10 proton multiplet structures are now overlapped rather than one of these resonances being furthest upfield as in the 500 MHz spectrum (compare Figure 1). Despite this unfortunate circumstance, a number of resonance assignments are still possible from this spectra *via* the reconfirmation of some of the proton resonance identities with the 300 MHz COSY spectrum (not shown).

Thus, the C11 resonance was assignable to the doublet response (proton multiplet structure - see Figure 2A) at δ = 126.24; Cl was assigned to the doublet response at δ = 125.42; C4 was assigned to the doublet response at δ = 121.29; C8 was assigned to the doublet response at δ = 126.68; and finally, C2 was assigned to the doublet response at δ = 126.11. Beyond these unequivocal protonated carbon assignments, the balance of the protonated carbon resonances may be subgrouped and no more. Thus, the triplet responses (Figure 2B) observed at δ = 125.92 and 162.20 may be attributed to H9/H10; the three remaining doublet responses were observed at δ = 127.30, 126.31 and 125.56 may be attributed to C5/C6/C7.

Autocorrelated Two-Dimensional ^{13}C-^{13}C Double Quantum Coherence (DQC) Spectroscopy.

Assignments of the six quaternary carbons and the five remaining unassigned protonated carbon resonances of **1** were made *via* carbon-carbon connectivities. The carbon-carbon connections were established using a modified two-dimensional autocorrelated double quantum coherence (DQC) ^{13}C-nmr experiment [10,11]. The experiment that we actually utilized is a combination of two different two-dimensional double quantum coherence (DQC) pulse sequences, one described by Turner [10,11] and the other by Mareci and Freeman [12]. Both sequences create double quantum coherence using the same sequence ($90° - \tau - 180° - \tau - 90°$) as was employed in the original two-dimensional double quantum coherence experiment [8], the DQC was allowed to evolve for the period of time = t_1 before being converted back into observable transverse magnetization with final 90° read pulse (see Figure 3A). Turner's modification [10,11] delays detection of the observable transverse magnetization until time = $2t_1$, where the DQC has been allowed to evolve for an additional period of time, t_1 (Figure 3B). By simply delaying the acquisition in this fashion, a direct correlation of the coupled spins about the diagonal $F_1 = F_2$ is obtained.

Although Turner [11] anticipated the modification of this autocorrelated DQC experiment, for example in a fashion analogous to the INEPT-INADEQUATE experiment [23], the work of Mareci and Freeman [12] leads to a modification which is somewhat more subtle and no more

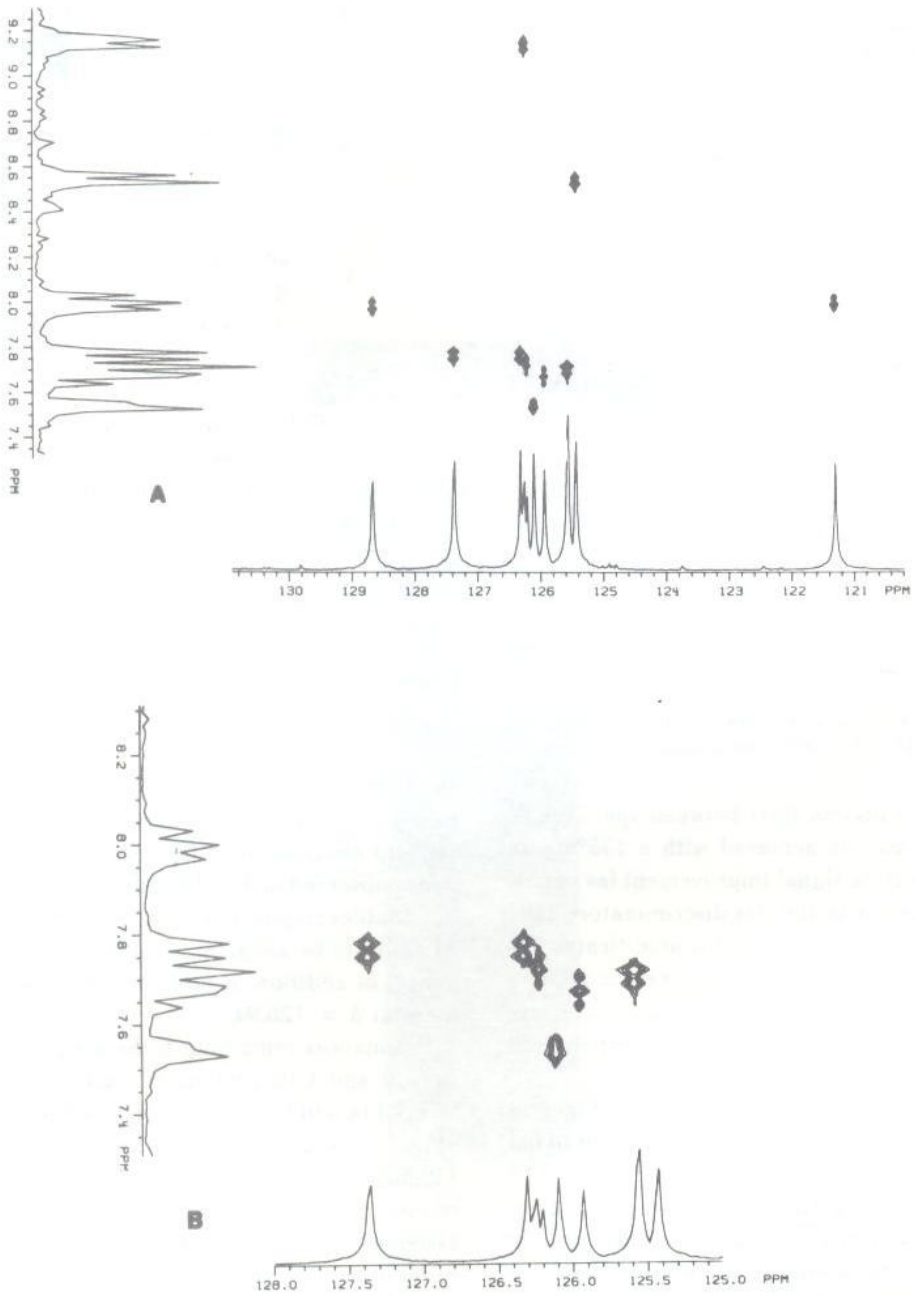


Figure 2A. Contour plot of the two-dimensional proton-carbon chemical shift correlation (CSCM) spectrum of **1** in deuteriochloroform at 75 MHz. The spectrum plotted was the $S(F_2,F_1)$ matrix which consisted of 512 × 256 data points. Four contour levels were plotted; B) Expansion of the region from 125-128 ppm on F_2.

complicated than the initial experiment. Generally, a 90° setting for the final read pulse (see Figures 3A and 3B) transfers none of the real component of the DQC and thus the detected signal is a pure amplitude modification. Mareci and Freeman [12], however, observed that when the final read pulse is set to a value of $<90°$ or $>90°$, the observed signal contains components of both the real and imaginary parts of the double quantum coherence, thus introducing a phase modulation and consequently permitting the determination of the sign of the DQC. Maximal peak heights are obtained for the "antiecho" and for the "echo" components respectively when read pulses of 60° and 120° are employed, both leading to a 30% increase in signal intensity relative to the normal 90° read pulse [12,33]. Detection of the "echo" being preferable, the optimum flip angle of the read pulse is thus 120°, although a

Table II

Calculated *vs.* Observed ^{13}C-nmr Chemical Shifts of Phenthro[3,4-*b*]thiophene (**1**) in Deuteriochloroform at 75 MHz

Position	δ ^{13}C calcd. [a]	δ ^{13}C observed	
1	127.2	125.42	−1.8
2	125.4	126.11	+0.7
3a	137.8	140.56	+2.8
4	120.5	121.29	+0.8
5	124.3	125.56	+1.3
5a	127.7	130.86	+3.2
6	126.9	127.30	+0.4
7	126.9	126.31	−0.6
7a	132.0	133.38	+1.4
8	128.5	128.68	+0.2
9	126.5	125.92	−0.6
10	126.5	126.20	−0.3
11	122.6	126.24	+3.6
11a	130.3	130.78	+0.5
11b	125.5	126.94	+1.4
11c	133.8	135.31	+1.5

[a] ^{13}C-nmr chemical shifts of **1** were calculated by incrementing the known chemical shifts of phenanthrene for the [3,4-*b*] ring fusion of the thiophene ring to the phenanthrene nucleus. Chemical shift additivities for thie type of ring fusion were derived by calculating differences between the observed chemical shifts of benzo[*b*]thiophene [P. Geneste, J.-L. Olive, S. N. Ung, M. E. A. El Faghi, J. W. Easton, H. Bierbeck and J. K. Saunders, *J. Org. Chem.*, **44**, 2887 (1979)] and benzene.

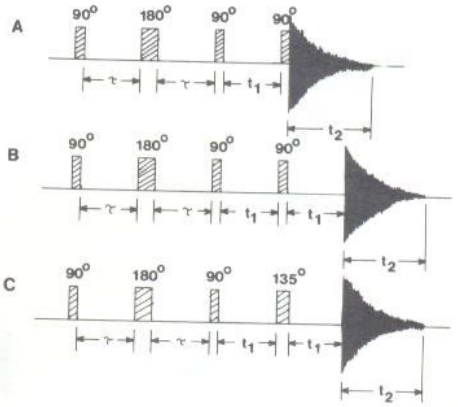


Figure 3. Pulse sequences for two-dimensional ^{13}C-^{13}C double quantum coherence nmr spectroscopy: A) conventional pulse sequence - multiplets are correlated about a diagonal axis $F_1 = F_2$; B) Turner autocorrelated experiment - introduction of the second period, t_1, produces an autocorrelation, responses are correlated about a true diagonal $F_1 = F_2$; C) Turner autocorrelated experiment modified to include a 135° read pulse to optimize sensitivity and discrimination between the echo and antiecho.

higher level of discrimination (6:1) between the "echo" and the "antiecho" may be achieved with a 135° pulse while still retaining a 20% signal improvement (as opposed to 30% enhancement with the less discriminatory 120° read pulse [33]). The incorporation of this modification into the Turner [10,11] autocorrelated two-dimensional double quantum coherence pulse sequence (Figure 3C) provides us with a simple, direct version of the experiment with enhanced sensitivity [12,32].

The autocorrelated DQC experiment shown in Figure 4 was performed on a sample of 65 mg of **1** dissolved in 0.4 ml of deuteriochloroform in a 5 mm sample tube. The spectrum was obtained using 4K data points in t_2, with only 22 blocks of data collected in t_1 and zero filled to 256 points prior to processing. A total of 256 acquisitions were collected/block of data, with an interpulse delay of 8 seconds to allow quaternary relaxation between acquisitions. Total performance time for the experiment using the pulse sequence shown in Figure 3C was 18.5 hours.

As in the proton COSY spectrum (Figure 1), the autocorrelated 2D-DQC spectrum shown in Figure 4 is correlated about a diagonal. Beginning with the C4 resonance observed at $\delta = 121.29$, we observe that it exhibits, as expected, two correlations, one to a protonated carbon resonance and the other to a quaternary carbon. The resonances which appear at $\delta = 140.56$ and 125.56 are assignable as C3a and C5 respectively. Utilizing the assignment of the C5 resonance, the C5a resonance may next be assigned as the downfield member ($\delta = 130.86$) of the group of two nearly overlapped quaternary carbons. From C5a, the subsequent assignment of C6 may also be made to the resonance observed at $\delta = 127.30$, thus permitting the remaining doublet response from the CSCM experiment described above to be assigned to C7 ($\delta = 126.31$). The C5a resonance, in addition, is also correlated to C11b which resonates at $\delta = 126.94$.

Resonances remaining to be assigned at this point are the C9 and C10 protonated carbon resonances and the C7a, C11a and C11c quaternary carbon resonances. Returning to the assignment of the C3a resonance at $\delta = 140.56$, a connectivity to an additional quaternary carbon resonance at $\delta = 135.31$ is also observed, this resonance assigned to C11c. Of the two remaining quaternary carbon resonances, the resonance at 133.38 exhibits a connectivity to the C8 resonance at $\delta = 128.68$, thus permitting the assignment of the former as C7a. As a consequence of the two preceding assignments, the remaining quaternary carbon resonance at $\delta = 130.78$ may be assigned as C11a, this assignment coroborated *via* a C7a-C11a connectivity. Finally, in addition to its connectivity to C7a, the C8 resonance also exhibits a connectivity to a protonated carbon resonance which can only be C9. Thus, C9 is assigned to the signal observed at $\delta = 125.92$ with the remaining resonance at $\delta = 126.20$ assigned to C10, thus completing the assignment of the ^{13}C-nmr spectrum of **1**. Complete calculated *vs.* observed ^{13}C-chemical shifts of **1** are summarized in Table II.

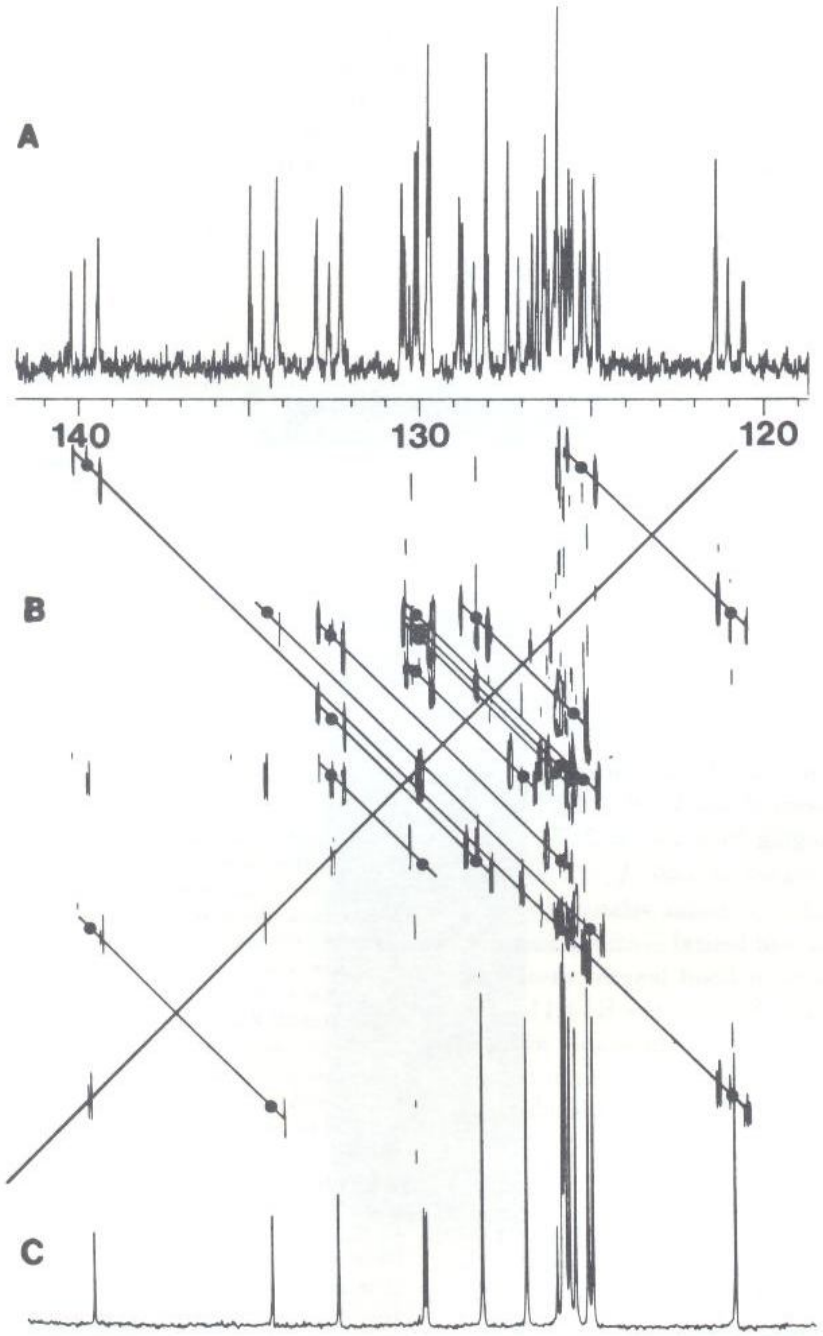


Figure 4. Experimental autocorrelated double quantum coherence spectrum of **1** obtained using the pulse sequence shown in Figure 3C. The spectrum consisted of 4K data points in t_2 and 22 blocks of data in t_1 which was zero filled to 256 points during the data processing: A) 0° projection of the $S(F_1,F_2)$ data matrix to show ^{13}C-^{13}C couplings, residual responses at the chemical shift of the resonances are incompletely canceled signals due to isolated ^{13}C nuclides; B) Four level contour plot of the $S(F_1,F_2)$ data matrix illustrating the diagonal and the correlation of resonances about the diagonal. Less than the total number of possible correlations was obtained, those missing are absent due to strong coupling (AB spin systems rather than AX for which the experiment was optimized); C) Conventional ^{13}C-nmr spectrum of **1**.

Helical Structure of Phenanthro[3,4-*b*]thiophene.

Construction of Dreiding model of **1**, assuming as a starting point a planar conformation, shows the H1 and H11 resonances to be within 1.3 Å of one another. Clearly, such a close intramolecular contact would be unfavored and it is thus logical to speculate that to avoid such a con-

tact that the molecule would adopt a helical rather than a planar conformation, with corresponding bond length deformations analogous to those observed for the parent phenanthrene nucleus [24]. Similar helical structures have also been reported for a number of thiophene containing heterohelicenes [25,26]. To probe the potentially helical structure of **1**, interatomic distances between two key pairs of hydrogens, H1-H2 and H10-H11, were computed at 2.42 and 2.27 Å respectively, the atom coordinates of 1,16-dihydroindeno[5,4-*c*:4′,5′-*g*]phenanthrene [27] providing the basis for the computation. Given these interatomic distances, the distance between H1 and H11 may be computed once NOE measurements are made between H1 and H11 and between either H1 or H11 and the counterpart atom a known distance away [28].

A series of four independent NOE difference measurements were made by irradiating the H1 and H11 resonances for periods of 3 and 5 seconds. To provide a reference spectrum, equivalent irradations were performed in the empty spectral region between the H1 and H11 resonances. From this series of irradiations, NOE's were obtained between H10-H11/H11-H1 and between H2-H1/H1-H11. Given a set of two NOE values and one known interatomic distance, four separate estimates of the H1-H11 interatomic distance were obtained, ranging from 1.93 to 2.07 Å with an average interatomic distance of 1.99 Å. To accomodate this interatomic distance, it seems relatively certain that **1** must adopt a pronounced helical conformation with the corresponding deviations in bond lengths associated with helical molecules [24-27]. Further, the H1-H11 interatomic distance of 1.99 Å agrees very favorably with the H8-H8′ distance of 2.06 Å observed in the case of trithia-(5)heterohelicene [26], suggesting that such molecules may share a common helical shape.

Conclusion.

Total assignments for the ^{1}H- and ^{13}C-nmr spectra of phenanthro[3,4-*b*]thiophene (**1**) have been described. The proton assignments were established through a 500 MHz COSY spectrum and through homonuclear decouplngs which also served to confirm the identities of several long range coupling constants. The ^{13}C-nmr spectrum of **1** was partially assigned through the CSCM spectrum, while the balance of the assignments were made through the autocorrelated 2D-DQC spectrum obtained using the pulse sequence shown in Figure 3C. That these assignments could be completed with the acquisition of only 22 blocks of data in t_1 on a relatively modest quantity of material in a 5 mm sample tube clearly demonstrates both the viability and the utility of this experiment.

Finally, the nmr study of **1** has also yielded a prediction of the solution structure of the molecule with the H1 and H11 resonances separated by a calculated distance of 1.99 Å. Assuming that such a helical conformation will result in deformations of bond lengths about the "bay" region of the molecule as is the case with other helicenes [24-27,29], this could account for the potential of this region of the molecule to undergo epoxidation and subsequent ring opening reactions, thereby accounting for the mutagenicity of **1** [30].

EXPERIMENTAL

The sample of phenanthro[3,4-*b*]thiophene (**1**) utilized in this study was prepared as previously described [13]. The sample for the 500 MHz high resolution and COSY spectra was prepared by dissolving approximately 2 mg of **1** in 0.4 ml of 100% deuteriochloroform. The sample utilized in the two-dimensional proton-carbon chemical shift correlation (CSCM) experiments and the two-dimensional ^{13}C-^{13}C double quantum coherence experiment was prepared by dissolving 65 mg of **1** in approximately 0.5 ml of deuteriochloroform.

The 500 MHz high resolution ^{1}H-nmr spectrum was obtained on a Nicolet NIC-500 spectrometer operating at 500.0517 MHz. A pulse width of 7 μsec (90° pulse = 10 μsec) and an interpulse delay of 2 seconds were employed with a sweep width of ±824.22 Hz digitized with 32K data poipts to give an acquisition time of 6.56 seconds. A total of 64 acquisitions were accumulated.

The autocorrelated proton (COSY) spectrum was obtained using the standard 90°-t_1-90°-acquire pulse sequence, the last pulse phase cycled to give the equivalent of quadrature data in the second dimension. The spectrum was collected as 512 × 1K blocks of data ($S(t_1,t_2)$) and was processed using sinusoidal multiplication in each dimension followed by symmetrization of the final data matrix. Total performance time for the experiment was approximately 1 hour. The plot shown in Figure 1 is a six level contour plot of the final symmetrized data matrix.

The two-dimensional proton-carbon chemical shift correlation (CSCM) experiment was obtained using the standard pulse sequence with ^{1}H-decoupling in F_2 and ^{13}C-decoupling in F_1. The experiment was performed on a NIC-300WB spectrometer operating at 75.4613 MHz for ^{13}C with F_2 = 300.0691 proton in the second dimension. The spectrum was acquired using a sample prepared as described above, with 256 × 1K data points ($S(t_1,t_2)$) with a total of 4 acquisitions per block. A sweep width of ±405.19 Hz was employed in F_2 with a dwell time of 617 μsec for F_1. The data was processed by using a phase shifted sine bell in F_2 and sinusoidal multiplication in F_1. Total acquisition time for the experiment was 30 minutes. The contour plots shown in Figures 2A and 2B were plotted using four contour levels. It should be noted that a substantial shift of the H2 resonance, from δ = 7.750 to approximately δ = 7.52, was observed on increasing the concentration for the acquisition of the ^{13}C spectral data. It would be ideal to obtain a spectra at identical concentrations on the same instrument although the practical considerations of performance time requirements and spectral dispersion will in many cases preclude this approach. Thus, care must be taken to avoid incorrect resonance assignments which could arise when concentrations are altered to minimize data acquisition times.

The two-dimensional ^{13}C-^{13}C double quantum coherence (2D-DQC) spectrum was acquired using the same sample as in the CSCM experiment described above. The pulse sequence utilized is that shown in Figure 3C. Phase cycling employed was that of Turner [10,11]. The experiment was performed on a NIC-300WB spectrometer operating at 75.4613 MHz with 22 × 4K data points ($S(t_1,t_2)$) with a total of 256 acquisitions per block and an interpulse delay of 8 seconds. Data files were processed using a 1 Hz exponential broadening in F_2 and no digital weighting in F_1, although the second dimension was zero filled to 256 points prior to the second Fourier transform. The spectrum shown in Figure 4 is a four level contour plot (center) accompanied by a 0° projection (top) and a standard ^{13}C reference spectrum (bottom).

Measurement of the nuclear Overhauser enhancements (NOE) utilized in the prediction of the helical structure of **1** was conducted at an observation frequency of 300.069 MHz. NOE values were measured by difference, an enhanced spectrum produced by irradiation of either the H1 or H11 resonance multiplet for a period of 3 or 5 seconds. A reference spectrum acquired by irradiation for an equivalent period in the region between the H1 and H11 resonances was then subtracted from the enhanced spectrum. For a 3 second irradiation of H1, H2 exhibited a 2.5% NOE while H11 exhibited a 9.2% NOE; 5 second irradiation of H1 gave NOE values fo 3.7% and 11.4% for H2 and H11 respectively. Correspondingly, a 3 second irradiation of H11 gave a 4.2% NOE for H10 and a 7.4% NOE for H1; a 5 second irradiation of H11 gave NOE values of 4.6% and 12.0% respectively for H10 and H1.

Acknowledgements.

Two of the authors, G. E. M. and M. R. W., would like to acknowledge the generous support of the Robert A. Welch Foundation through Grant Nos. E-792 and E-183 which also provided a post doctoral fellowship for M. J. M. We would also like to acknowledge the support of the Department of Energy through Contract No. DE-AC0279EV10237 to R. N. C. and M. L. L. which provided the sample of phenanthro[3,4-*b*]thiophene (**1**) utilized in this study.

Note Added in Proof.

A modification of the original Turner two-dimensional DQC experiment [10,11] has been reported [33] since the initial submission of this manuscript for publication, the modified version of the experiment employing a 120° read pulse to improve sensitivity. In addition, the original version of the experiment [10,11], was also recently criticized for its sensitivity relative to the earlier 2D-INADEQUATE experiments [8,9] by Bax and Mareci in a recent report [32], a problem which is solved by both Turner's modification [33] and that described in this work.

REFERENCES AND NOTES

[1] To whom inquiries should be addressed.

[2] Present address: Nagasaki University, Nagasaki, Japan.

[3] G. E. Martin, S. L. Smith, W. J. Layton, M. R. Willcott, III, M. Iwao, M. L. Lee and R. N. Castle, *J. Heterocyclic Chem.*, **20**, 1367 (1983).

[4] M. J. Musmar, R. T. Gampe, Jr., G. E. Martin, W. J. Layton, S. L. Smith, R. D. Thompson, M. Iwao, M. L. Lee and R. N. Castle, *ibid.*, submitted.

[5] A. Bax, R. Freeman and S. P. Kempsell, *J. Am. Chem. Soc.*, **102**, 4849 (1980).

[6] A. Bax, R. Freeman and S. P. Kempsell, *J. Magn. Reson.*, **41**, 349 (1980).

[7] A. Bax and R. Freeman, *ibid.*, **41**, 507 (1980).

[8] A. Bax, R. Freeman, T. A. Frenkiel and M. H. Levitt, *ibid.*, **43**, 478 (1981).

[9] A. Bax, R. Freeman and T. A. Frenkiel, *J. Am. Chem. Soc.*, **103**, 2102 (1981).

[10] D. L. Turner, *Molec. Phys.*, **44**, 1051 (1981).

[11] D. L. Turner, *J. Magn. Reson.*, **49**, 175 (1982).

[12] T. H. Mareci and R. Freeman, *ibid.*, **48**, 158 (1982).

[13] M. Iwao, M. L. Lee and R. N. Castle, *J. Heterocyclic Chem.*, **17**, 1259 (1980).

[14] W. P. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).

[15] K. Nagayama, A. Kumar, K. Wuthrich and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980).

[16] A. Bax, R. Freeman and G. Morris, *ibid.*, **42**, 164 (1981).

[17] K. D. Bartle, D. W. Jones and R. S. Matthews, *Rev. Pure Appl. Chem.*, **19**, 191 (1969).

[18] M. W. Jarvis and A. G. Moritz, *Aust. J. Chem.*, **21**, 2445 (1969).

[19] M. W. Jarvis and A. G. Moritz, *ibid.*, **24**, 89 (1971).

[20] A. A. Maudsley and R. R. Ernst, *Chem. Phys. Letters*, **50**, 369 (1977).

[21] G. Bodenhausen and R. Freeman, *J. Magn. Reson.*, **28**, 471 (1977).

[22] A. Bax and G. A. Morris, *ibid.*, **42**, 501 (1981).

[23] O. W. Sorensen, R. Freeman, T. Frenkiel, T. J. Mareci and R. Schuck, *ibid.*, **46**, 180 (1982).

[24] J. Trotter, *Acta Cryst.*, **16**, 605 (1963).

[25] M. B. Groen and H. Wynberg, *J. Am. Chem. Soc.*, **93**, 2968 (1971).

[26] M. Konno, Y. Saito, K. Yamada and H. Kawazura, *Acta Cryst.*, **B36**, 1680 (1980).

[27] J. C. Dewar, *ibid.*, **B37**, 1421 (1981).

[28] J. H. Noggle and R. E. Schiemer, "The Nuclear Overhauser Effect", Academic Press, New York, 1971.

[29] J. Navaza, G. Tsoucaris, G. le Bas, A. Navaza and C. de Rango, *Bull. Soc. Chim. Belg.*, **88**, 863 (1979).

[30] R. A. Pelroy, D. L. Stewart, M. L. Lee, M. Iwao, R. D. Thompson, R. Pratap, Y. Tominaga and R. N. Castle, *Mutat. Res.*, in press.

[31] I. M. Al-Najjar, H. B. Amin and S. S. Al-Showiman, *J. Chem. Soc., Pak.*, **4**, 155 (1982); T. Kanda, F. Shoji and Y. Matsuki, *Bull. Chem. Soc. Japan*, **38**, 508 (1965).

[32] A. Bax and T. H. Mareci, *J. Magn. Reson.*, **53**, 360 (1983).

[33] D. L. Turner, *ibid.*, **53**, 259 (1983).