

Assignment of ^1H NMR Spectra of Polycyclic Aromatic Hydrocarbons by Multiple Quantum Filtration

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It is shown that multiple quantum filtration may be used to rapidly assign the ^1H NMR spectra of polycyclic aromatic hydrocarbons. This technique is faster than two-dimensional chemical shift correlation spectroscopy and gives very similar information. In addition, since multiple quantum filtration simplifies spectra by elimination of some resonances, it can be invaluable for assignment of resonances that are unresolved due to inadequate field strength. Application of this technique to assignment of the ^1H NMR spectra of benzo[*a*]pyrene-7,10-dione, 10-methylbenzo[*a*]pyrene, and 6-acetoxybenzo[*a*]pyrene is presented.

Assignment of ^1H spectra of polycyclic aromatic hydrocarbons (PAH) has classically relied on empirical rules¹ that designate different chemical shifts for protons bound to certain carbon atoms (e.g., α - or β -naphthalenic, *meso*-anthracenic, etc.). Support in the assignments has been offered by double-resonance experiments and deuterium substitution. Recently, an elegant method was published describing an approach to complete assignment of ^1H and ^{13}C NMR spectra of PAH using the INAPT (insensitive nuclei assigned by polarization transfer) pulse sequence.^{2,3} This methodology takes advantage of the fact that 3-bond carbon-hydrogen coupling constants, $^3J_{\text{HC}}$, in aromatic systems are always about 5 Hz and $^2J_{\text{HC}}$ and $^4J_{\text{HC}}$ are smaller. It is important to note that two-dimensional (2-D) chemical shift correlation spectroscopy (COSY)^{4,5} is often required for assignment of at least some of the proton resonances before the application of the INAPT sequence. We have been involved in the synthesis and structural determination of several mono- and disubstituted derivatives of benzo[*a*]pyrene (BP), and several of these compounds have presented problems in the assignment of their ^1H NMR spectra. At 300 MHz, the field strength used in our studies, the overlapping resonances in the observed spectra often lead to ambiguous COSY data unless a large number of increments in t_1 are used to obtain the required resolution. This usually results in lengthy experiments, and it is for this reason that we propose the application of one-dimensional (1-D) multiple quantum filtration (MQF)⁶⁻⁸ as an alternative to COSY for assignment of ^1H resonances in the spectra of PAH.

Recently, MQF has been used for simplification of 1-D and 2-D ^1H spectra of biomacromolecules.⁹⁻¹¹ Double quantum correlation spectroscopy (2-D INADEQUATE)¹² has become a powerful tool for elucidation of carbon backbones in organic molecules.¹³ Few reports, however, have appeared regarding application of ^1H MQF for structural elucidation in small molecules. PAH are ideally suited for application of MQF since their simple spin topologies lead to easily predicted results. When used as a 1-D experiment, this technique allows a more rapid assignment of ^1H spectra of these molecules than the COSY technique (minutes vs. hours). In addition, overlapping resonances many times cannot be unambiguously assigned utilizing the COSY experiment, regardless of the degree of resolution obtained. This is especially important at the field strengths used in most laboratories (≤ 300 MHz).

An MQF experiment is a pulse sequence that simplifies spectra by eliminating signals from spin systems consisting

of too few spins. For instance, a triple quantum filtered (TQF) ^1H spectrum will contain only resonances arising from spin systems with three or more scalar coupled spins (ignoring strong coupling effects). Once coupling networks have been identified by their presence or absence in the filtered spectrum, assignment of specific resonances is accomplished by application of well-established empirical rules,¹ and/or application of INAPT or other long-range heteronuclear correlation experiments.^{4,5}

Theory

Many methods have been proposed for MQF experiments.⁶⁻⁸ The pulse sequence used in our experiments is the offset-independent method of excitation shown in Figure 1. Following the first nonselective 90° pulse, the spins evolve during the delay $2T$ under the influence of their homonuclear scalar coupling (chemical shift and heteronuclear couplings are removed by the 180° pulse). The evolution of the spins under scalar coupling allows the second nonselective 90° pulse to generate multiple quantum coherence among all coupled spins.¹⁴ Figure 2 shows an energy level diagram illustrating the three possible types of coherence for an AX system. For instance, in a 2-spin AX system, zero and double quantum coherences are generated by the second 90° pulse (single quantum coherence already exists due to the first 90° pulse). A third nonselective 90° pulse is required to observe the signals. The resulting free induction decay (fid) contains signals

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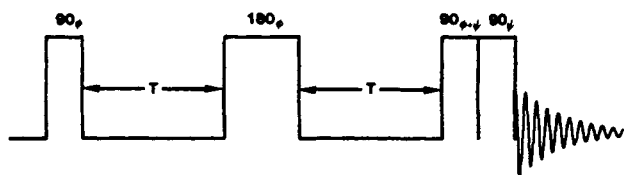


Figure 1. Pulse sequence for MQF. Proper choice of the fixed delay T is critical and depends on the type of spin system being examined; e.g. see ref 7, 8, and 11. The cycling of ϕ and the receiver phase is described in the text. For even-order filters ($n = 0, 2, 4, \dots$) $\Psi = X$ and for odd order filters $\Psi = Y$.

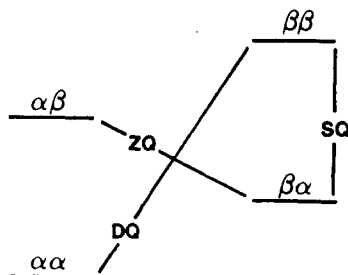


Figure 2. Energy level diagram for an AX 2-spin system. The lines connecting energy levels represent the three possible types of coherence: zero quantum (ZQ), double quantum (DQ), single quantum (SQ).

which, following Fourier transformation, will appear at their normal positions in the spectrum. If the phase of the excitation pulses is now incremented by 90° and another transient acquired, the double quantum coherences generated will be increased in phase by $2 \times 90^\circ = 180^\circ$. It is well-known that an n quantum coherence changes phase by $n\phi$ when the rf phase of the pulses in the excitation period are phase shifted by ϕ .¹⁴ Analogous to the double quantum coherences, the single quantum and zero quantum coherences will be incremented in phase by $1 \times 90^\circ$ and $0 \times 90^\circ$, respectively. If the receiver phase is incremented by 270° to follow the double quantum signal, the addition of this fid to the previous one will result in an additive contribution from the signals passing through double quantum coherence, but a differential contribution from the signals passing through zero and single quantum coherence. It is easily seen that if two more transients are acquired incrementing ϕ by 90° and the receiver by $(n+1)\phi$ each time, the zero and single quantum coherences will cancel out. The resulting double quantum filtered spectrum will only contain resonances from spin systems with two or more coupled spins. It is important to note that the coupling between all spins actively involved in the transition must be resolved for maximum sensitivity.

In general, an n quantum filtered spectrum is obtained by performing a multiple of $2n$ transients^{7,8} and incrementing ϕ according to

$$\phi = \frac{k \times 180^\circ}{n} \quad k = 0, 1, 2, \dots, 2n - 1$$

Since multiple quantum filtration of order $n > 2$ requires rf phase shifts of less than 90° , which are not available on most commercial spectrometers, either a digital or transmitter frequency-based phase shifter must be obtained. We have utilized the latter "phase-pulse" technique as described by Weitekamp¹⁵ and Guittet et al.¹⁶ This methodology is easily implemented on any spectrometer that allows changing of transmitter frequency during pulse

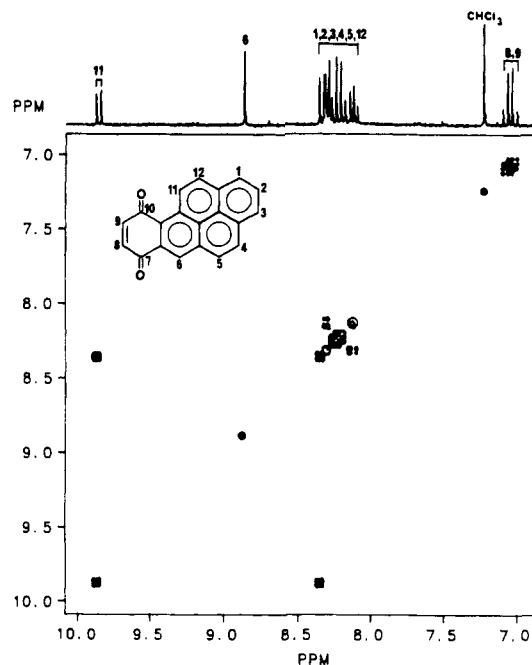


Figure 3. Two-dimensional contour map of a magnitude COSY spectrum of BP-7,10-dione. The normal ^1H spectrum is shown across the top. Symmetrical Gaussian pseudo echo weighting was applied prior to transformation of 1024×1024 points; 512 increments in t_1 were acquired.

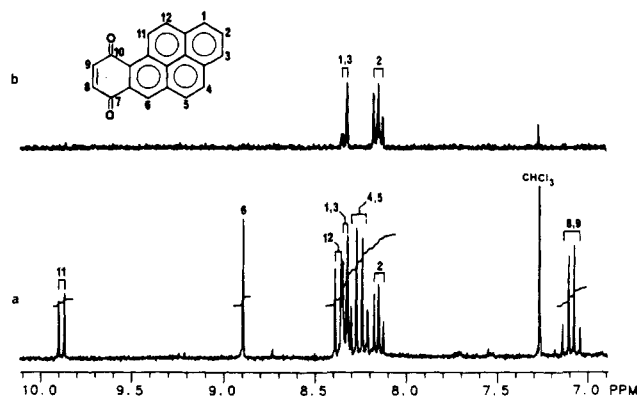


Figure 4. (a) Conventional ^1H spectrum of BP-7,10-dione; 24 transients accumulated. (b) Triple quantum filtered spectrum of same sample, $T = 0.035$; 48 transients accumulated with a recycle time of 15 s. Absolute value display was used, and no weighting was applied; 1024 complex points were zero filled twice before Fourier transformation. The solvent peak is not totally suppressed since the recycle time was only $\sim 2.5T_1$ for CHCl_3 in this sample.

sequence execution. Since the elimination of unwanted coherences requires their self-cancellation through phase cycling, a constant equilibrium magnetization must be established. This is accomplished by waiting $5 \times T_1$ between transients, where T_1 is the longest ^1H spin-lattice relaxation time among the spins to be suppressed. Alternatively, if a large number of transients are required due to low sample concentration, it is advantageous to establish a steady state and recycle at a higher rate.

Results and Discussion

Figure 3 shows a 2-D COSY of BP-7,10-dione. The complex region between 8.1 and 8.4 ppm is difficult to interpret, because the overlapped multiplets show extensive coupling. In particular, the 3-spin $\text{H}_1\text{H}_2\text{H}_3$ system is difficult to assign. This 2-D COSY experiment required 512 increments in t_1 for a total of slightly more than 9-h

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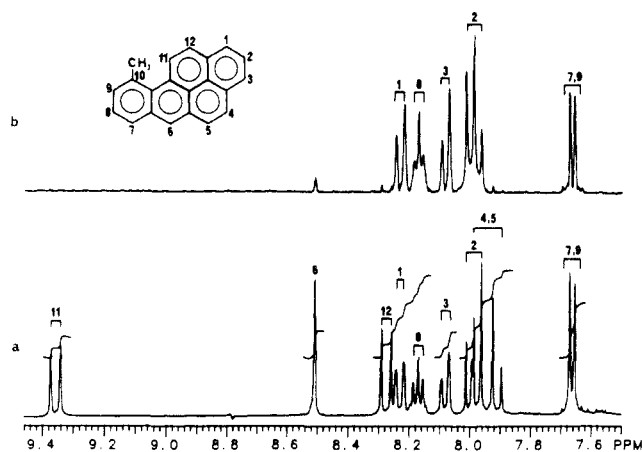


Figure 5. (a) Conventional ^1H spectrum of 10-Me-BP; 24 transients accumulated. (b) Triple quantum filtered spectrum of the same sample, $T = 0.035$; 48 transients accumulated with a recycle time of 15 s. Absolute value display is used, and no weighting was applied; 1024 complex points were zero filled twice before Fourier transformation.

acquisition, and interpretation is still rather difficult. By judicious use of quadrature aliasing,¹⁷ an approximate doubling of resolution could be obtained, but interpretation would remain ambiguous due to overlapping resonances.

Figure 4a shows a conventional ^1H spectrum of BP-7,10-dione, and Figure 4b shows the corresponding TQF ^1H spectrum. Line-shape distortions are due to absolute value display mode. Since the only proton system on the molecule with three or more spins is $\text{H}_1\text{H}_2\text{H}_3$, the peaks that appear in the TQF spectrum may be assigned to these spins. Acquisition of the TQF spectrum required only 48 transients over 12 min, much less time than that required for 2-D COSY acquisition. The overlap of H_1 and H_3 would complicate their assignment by 2-D COSY even at higher field strength, whereas the TQF spectrum allows immediate recognition of the $\text{H}_1\text{H}_2\text{H}_3$ system.

Assignment of H_{11} and H_{12} is made by their absence in the TQF spectrum and through application of empirical rules.¹ The strongly coupled pairs H_4H_5 and H_8H_9 cannot be assigned specifically with the TQF spectrum, but application of empirical rules does allow their assignment to specific pairs of doublets in the spectrum. Performance of a COSY experiment would give no additional information.

Figure 5a shows the ^1H spectrum of 10-Me-BP, a molecule containing two 3-spin systems: $\text{H}_1\text{H}_2\text{H}_3$ and $\text{H}_7\text{H}_8\text{H}_9$. Figure 5b shows the TQF spectrum of the same sample. It is obvious that the two 3-spin systems appear in the TQF spectrum. Again, only 48 transients were acquired for 12-min total acquisition time. Application of empirical rules allows assignment of the spectrum since H_1 , H_2 , and H_3 are in their usual relative positions for derivatives of benzo[a]pyrene. Assignment of H_1 and H_3 is made following application of the calculations of Haigh and Mallion.¹⁸ The observed chemical shifts of H_7 and H_9 are in accord with the expected electronic effect of methyl substitution at the 10-position. One selective decoupling experiment (irradiation of H_8) confirmed the assignment of the $\text{H}_7\text{H}_8\text{H}_9$ coupling network. This molecule illustrates the fact that even if more than one 3-spin system exists, application of the empirical chemical shift rules generally allows assignments to be made, and any ambiguities may

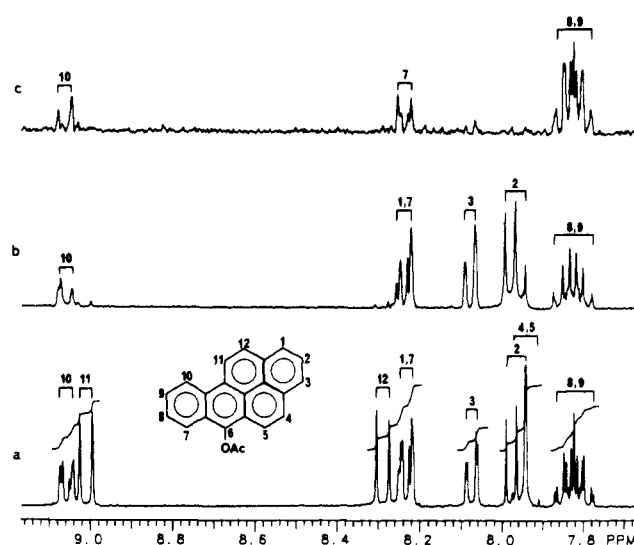


Figure 6. (a) Conventional ^1H spectrum of 6-acetoxy-BP; 24 transients accumulated. (b) Triple quantum filtered spectrum of same sample, $T = 0.035$; 48 transients accumulated with a recycle time of 15 s. Absolute value display was used, and no weighting was applied; 1024 complex points were zero filled twice before Fourier transformation. (c) Quadruple quantum filter of same sample; 32 transients accumulated, $T = 0.15$ s; all other processing and acquisition parameters are identical with those in (b).

be resolved by selective decoupling.

Figure 6a shows a ^1H spectrum of 6-acetoxy-BP, and Figure 6b shows the corresponding TQF spectrum. This spectrum required 48 transients or 12-min total acquisition. It is apparent that both the 3-spin $\text{H}_1\text{H}_2\text{H}_3$ system and the 4-spin $\text{H}_7\text{H}_8\text{H}_9\text{H}_{10}$ system are present since seven multiplets exist in the spectrum. A logical progression leads us to application of a quadruple quantum filtered (QQF) experiment for assignment of the 4-spin system and, hence, the 3-spin system by its absence. A QQF spectrum of the same sample is shown in Figure 6c. This spectrum required 32 transients or 8-min total acquisition time. The $\text{H}_7\text{H}_8\text{H}_9\text{H}_{10}$ system is easily assigned from the QQF spectrum since it is the only 4-spin system in 6-acetoxy-BP. As mentioned earlier, all couplings among the spins involved in a multiple quantum transition must be resolved for maximum sensitivity. This is due to the fact that the components of a multiplet that have passed through multiple quantum coherence are antiphase with respect to one another. Therefore, if a coupling is not resolved, the antiphase nature of the spectral multiplets will lead to their self-cancellation. Inspection of Figure 6a shows that the $^5J_{7,10}$ is not well resolved, implying that H_7 and H_{10} should be attenuated in the QQF spectrum of Figure 6c. Indeed, the relative intensity of H_7 and H_{10} with respect to H_8 and H_9 is decreased slightly when compared to that in Figure 6a, as expected. However, this spectrum does illustrate the fact that even when couplings are not completely resolved, adequate sensitivity may still be obtained in an MQF experiment. Finally, since scalar coupling constants are field independent, performance of high order multiple quantum filtration is not limited by field strength.

Conclusions

We have shown that MQF is a valuable tool in rapid unambiguous assignment of ^1H spectra of PAH. The same type of information is obtained with MQF as with COSY, but with much less acquisition time. More important, perhaps, is the ability of the MQF experiments to simplify

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interpretation of overlapping multiplets at conventional field strengths.

Experimental Section

All spectra were recorded on a Varian XL-300 spectrometer utilizing a Varian 5-mm broad-band switchable probe at 25.0 °C controlled to ± 0.2 °C by the standard variable-temperature hardware. All samples were 80 ± 10 mM in 0.5 mL of CDCl_3 . No weighting functions were applied to any of the spectra presented. Benzo[a]pyrene-7,10-dione was prepared by electrochemical oxidation of 7-hydroxybenzo[a]pyrene provided by the National Cancer Institute (NCI) Repository. The 10-methylbenzo[a]pyrene was also provided by the NCI Repository, and the 6-acetoxybenzo[a]pyrene was prepared by manganic acetate oxidation of benzo[a]pyrene.¹⁹

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Thermolysis of 3,4-Cyclic 1,2-Dioxetanes: Effect of 6-Ring Conformation on the Activation Parameters

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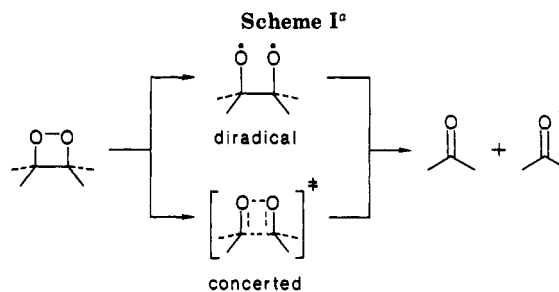
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2a,3,8,8a-Tetrahydronaphtho[2,3-c]-1,2-dioxete (1) and 2a,3,4,8b-tetrahydronaphtho[1,2-c]-1,2-dioxete (2) were synthesized by the Kopecky method. The dioxetanes, purified by low-temperature chromatography, were characterized by ¹H NMR spectroscopy and by analysis of their thermolysis products. The activation parameters for the thermolysis of 1 and 2 in xylenes were determined by the chemiluminescence method [1: $E_a = 27.4$ kcal/mol, $\log A = 14.4$, $k_{60^\circ} = 2.8 \times 10^{-4} \text{ s}^{-1}$. 2: $E_a = 24.5$ kcal/mol, $\log A = 12.8$, $k_{60^\circ} = 5.1 \times 10^{-4} \text{ s}^{-1}$]. The thermal decomposition of both dioxetanes produced (directly) much higher yields of excited triplet carbonyl products than excited singlet products. The total yields of excited states were 1% for both dioxetanes, much less than normal for disubstituted dioxetanes. Molecular mechanics (MM2) calculations were carried out. The most stable conformation of 1 was predicted to be a boat with a O-C-C-O torsion angle of 0°. 3,4-Tetramethylene-1,2-dioxetane (3) was predicted to favor a "twist-boat" conformation with a dioxetane ring torsion angle of 10°. The conformation of 2 was calculated to be intermediate with a dioxetane ring torsion angle of 4°. The relative stabilities of the dioxetanes seem to correlate with ring conformation and dioxetane (O-C-C-O) torsion angle.

The thermal decomposition of 1,2-dioxetanes² results in the quantitative cleavage of the 4-membered ring peroxide into two carbonyl fragments, one of which may be produced in an excited state. The thermolysis of dioxetanes that contain "easily oxidized" groups has been shown to directly produce high yields of excited singlet carbonyls and appears to be well described³ by the CIEEL or electron-transfer type mechanism.² On the other hand, the mechanism of thermolysis of simple (alkyl, aryl, alkoxy) dioxetanes (direct production of high yields of excited triplet products) remains controversial and of high theoretical interest. Historically, two mechanistic extremes have been postulated to explain this unique process: (a) diradical; (b) concerted (Scheme I).

Most of the experimental evidence has been interpreted⁴



^a Asterisk denotes excited state.

to be consistent with a diradical-like process. However, in recent years the distinctions between the two mechanisms have become blurred and the concept of a merged mechanism has arisen.⁵ Results for dioxetanes with cyclic substituents⁶ were interpreted to be indicative of a twisting

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