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Note

^{1}H NMR isotope shifts arising from the substitution of ^{12}C by ^{13}C: application to polycyclic aromatic hydrocarbon anions

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Isotope shifts are a well-established tool for structural analysis by NMR. The substitution of a protium with a deuterium is the most widely studied of these effects. However, such studies call for specific deuteration that requires complex synthetic techniques owing to the low natural abundance of deuterium. ^{13}C occurs at a higher natural abundance and couples strongly with its attached proton. We have developed and refined a method to reliably observe these much smaller shifts without needing to resort to chemical synthesis. We show that carbon induced isotope shifts reflect structural features. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; isotope shifts; polycyclic aromatic hydrocarbon anions

INTRODUCTION

NMR assignment is an important method for determining structure and reactivity. Chemical shifts can provide a direct measure of charge distribution and aromaticity. Isotopic substitution causes a perturbation of the charge distribution and measures the influence of small changes on molecular structure.

Isotope shifts have been studied and used since early in the development of NMR. They have been used for structural and bonding studies, assignment and testing theories of chemical shift.[1–4] The form of isotopic substitution most commonly studied is the exchange of a protium for a deuterium. This often requires complex and expensive synthesis. We propose an alternative: to study the effect of substitution of ^{12}C with ^{13}C on the ^{1}H NMR spectrum, utilizing a gradient-enhanced quantum filter in a novel manner. No special synthesis is required because nature provides us with a 1% random distribution of ^{13}C. All that is required is to find a way to measure these tiny isotope shifts reliably. A different quantum filter for the measurement of other (deuterium and ^{18}O isotope effects on ^{13}C and ^{31}P) isotope shifts has been reported.[5] In this case, the signal suppression was much less than the 100 : 1 demanded by ^{13}C so the filter could be applied successfully without gradient enhancement.

We chose to develop and apply the method to the study of polycyclic aromatic hydrocarbon (PAH) anions (scheme 1), partly because we wanted to compare the alternative isotope effects with the results of our previous deuteration study[6] and also because of the importance of PAHs and their reactivity. The characterization of PAHs is critical to the understanding of their reactivity. Interest in PAHs ranges from synthetic to environmental and biological chemistry.[7–11]

Isotope shifts in ^{13}C NMR caused by deuteration have predictable magnitudes and can be used for the assignment of the spectrum.[12–15] The isotope effects are small, so it is customary to measure them in ppb rather than ppm. The isotope shift is by convention the chemical shift of the nucleus substituted by the heavier isotope minus that substituted by the lighter isotope, e.g. $\Delta C(D) = \delta(^{13}C)(D) - \delta(^{13}C)(H)$.[3] The heavier isotope is larger and more massive, which leads to slight changes in bond lengths and angles arising from vibrational changes.[1,16,17] This effect usually causes shielding, i.e. a decrease in chemical shift, of the neighboring nucleus.

The effects of ^{13}C on ^{1}H are much smaller, a one-bond shift, $^{1}\Delta H(^{13}C)$, typically being 2 ppb. Such effects have been observed previously while analyzing the carbon satellites in order to determine ^{1}H–^{13}C coupling constants. The isotope shifts were included in the analysis to improve parameter fits but little further attention was paid to them.[2,18] This form of analysis requires high sensitivity, a good model of the spin system and excellent homogeneity. It is usually only practical for widely separated singlets.

METHOD

In order to make the isotope shift arising from ^{13}C observable, we used a gradient quantum filter to extract the ^{13}C attached signals from the interference of the main ^{12}C attached signals. The pulse sequence (Fig. 1) chosen selects single

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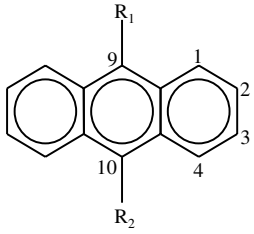
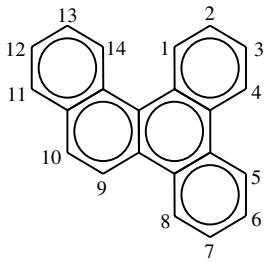
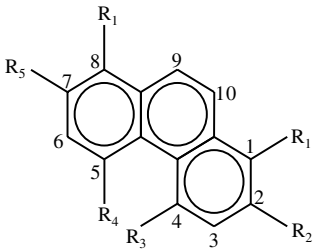


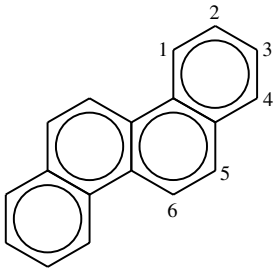
1: Anthracene: $R_1 = R_2 = H$
2: 9-Phenylanthracene: $R_1 = C_6H_5$, $R_2 = H$
3: 9,10-Diphenylanthracene: $R_1 = R_2 = C_6H_5$



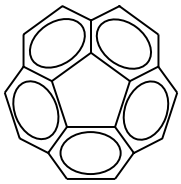
4: Benzo[g]chrysene



5: Phenanthrene: $R_1 = R_2 = R_3 = R_4 = R_5 = H$
6: 2,4-Dibutyl-5,7-dimethylphenanthrene: $R_1 = H$, $R_2 = R_3 = {}^t\text{Bu}$, $R_4 = R_5 = Me$
7: 1,4,5,8-Tetramethylphenanthrene: $R_1 = R_3 = R_4 = Me$, $R_2 = R_5 = H$



8: Chrysene



9: Corannulene

Scheme 1. Compounds studied.

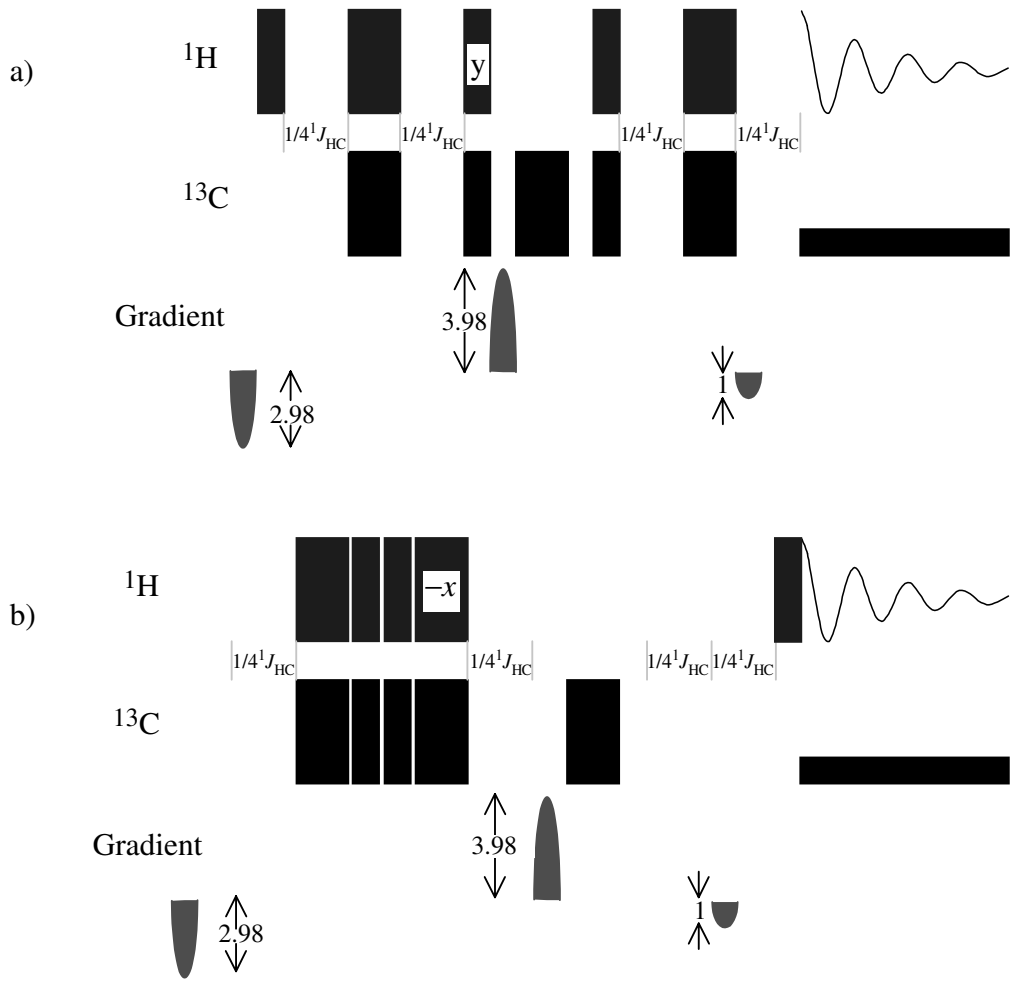


Figure 1. The pulse sequences: (a) the single quantum carbon-edited sequence and (b) the dummy sequence that yields a regular proton spectrum having irradiated the sample with the same amount of heat as sequence (a).

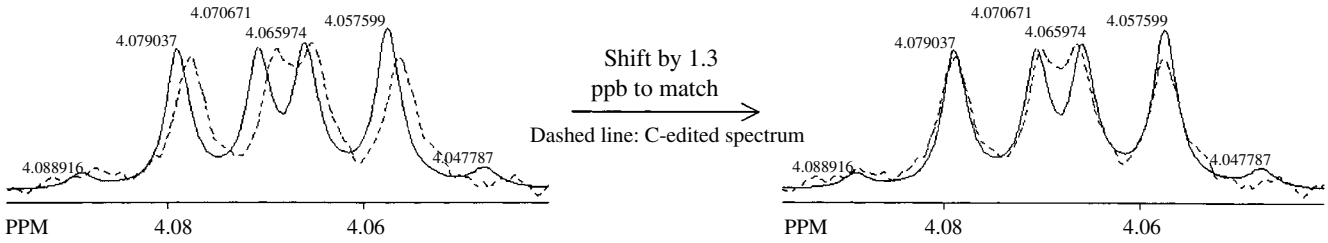


Figure 2. Comparison of carbon-edited and regular spectrum: H-2 of 9,10-diphenylanthracene dianion with sodium.

quantum (SQ) transfer, optimized for one-bond coupling constants. The selection of one-bond couplings is not perfect, however. A small fraction of the signal arises from long-range couplings. For a neutral PAH, this contribution is typically 3%. An alternative method is to use multiple quantum (MQ) transfer. The MQ signal strength is less dependent on the optimized coupling constant and therefore yields stronger signals than SQ if the coupling constant is misadjusted or if there is a relatively wide range of coupling constants. In the present case, this is a disadvantage because the reduction in selectivity increases the contribution of long-range couplings to typically 5% for a neutral PAH. We avoided the use of parallel excitation of two orthogonal pathways for sensitivity improvement because NOE effects between the protons increase the contribution of long-range signals and change the isotope shift measurements. In summary, the method of choice was an SQ transfer without sensitivity improvement.

Isotope shifts arising from ^{13}C are very small and often less than the linewidth. With this in mind, the reliability of the measurement becomes a major issue and a number of steps must be taken to ensure accuracy. A small change in experimental conditions may cause a change in chemical shift comparable to the isotope shift. Therefore, great care has to be taken to ensure that both the carbon-edited and regular spectrum are acquired under identical conditions.

We tested the temperature stability of the system using H-1 of disodium 9,10-diphenylanthracene (**3**) in THF-d_8. At 220 K, the temperature dependence of this signal is -4.6 ppb K^{-1}. Two effects were investigated: the effect of radiative heating (mainly carbon decoupling) on the sample from the pulse sequence and the effect of switching from air cooling to a liquid nitrogen evaporator. The radiative heating effect increased the sample temperature by 0.19 K with a time constant of 0.6 min. To achieve a chemical shift stable to within 0.05 ppb, it was necessary to acquire dummy scans for 3 min.

The initial drift when switching from air to evaporated nitrogen is nearly 15 K h^{-1} with a time constant of about 20 min. To yield a chemical shift stable to 0.05 ppb h^{-1}, we waited for at least 2 h 20 min after changing to or from evaporated nitrogen before starting our experiments. The sample first overcools to compensate the thermocouple from thermal radiation from the probe, then slowly warms to the desired temperature.

Although the gradient amplifier was blanked during acquisition, we kept the lock disabled during the gradient pulse sequence and acquisition because the reactivation of the lock at the start of acquisition causes serious distortion of the lineshape.

Self-shielding gradient probes are now standard hardware. However, sufficient distortion of the lineshape arising from eddy currents was observed, enough to influence the results. The eddy currents were suppressed by inserting a negative gradient prior to the pulse sequence whose intensity matched the sum of the gradients in the pulse sequence. The effect on the lock signal is obvious. Instead of a long decay back to the original lock level, the lock signal was immediately returned close to its full intensity. Slight adjustment of the intensity of the preparation gradient can bring the lock signal exactly to its correct position but this adjustment is unnecessary, as it does not lead to a discernible improvement in lineshape. The gradient pulses were sine-bell shaped so that pre-emphasis was not necessary. Indeed, the use of pre-emphasis only served to reduce sensitivity.

To achieve the desired accuracy, the carbon-edited spectrum was sandwiched between two regular spectra and, when sensitivity allowed, the experiment was repeated several times. Each spectrum of the sequence was acquired with enough dummy scans to reach a thermal steady state. The regular spectrum was not acquired using the normal pulse sequence but rather a carbon-edited sequence that had been doctored to yield a regular spectrum while irradiating the sample with the same amount of heat.

The spectra were compared by varying the relative intensity and chemical shift for the best least-squares fit for each multiplet (Fig. 2). The fit was carried out by bracketing and multisection. Although this may not be the most computationally efficient method, it almost always reaches a global minimum.

Self-consistency and comparison with satellite signal analysis (chloroform, acetone-d_5, TMS and corannulene tetraanion[19]) show conclusively that our method is reliable with a standard deviation of error no more than 4% of the linewidth provided that the signal-to-noise ratio exceeds 20 : 1 in the carbon-edited spectrum.

RESULTS AND DISCUSSION

We chose to concentrate our study on PAHs because of their very different spatial properties, their importance in synthetic,[7,8,9] environmental[10] and biological[11] chemistry and in order to compare our results with published deuterium isotope shifts.[6] Chrysene and phenanthrene are examples of PAHs that include bay regions and therefore considerable steric hindrance. This steric effect, combined with weakening of the carbon framework, causes its anion to be twisted,

MRC

Table 1. $^{13/12}$C isotope shifts on attached protons[a]

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-9	H-10	H-11	H-12	H-13	H-14	H-2′	H-3′	H-4′
1	1.3	1.2							1.0								
+2Li	0.8	1.2							1.7								
+2Na	1.4	1.6							2.3								
2	1.2	1.6	1.9	1.7						1.4					2.1	2.0	1.4
+2Na	−0.4	1.2	1.3	0.2						1.2					1.9	1.0	0.7
3	1.4	1.1													1.4	1.0	1.6
+2Na	0.2	1.2													1.2	1.7	1.7
4	1.4	2.0	1.8	1.8	1.6	1.8	0.9	1.6	1.6	1.6	1.5	1.9	1.8	1.9			
+2Li	2.1	2.2	2.2	1.4	0.8	0.8	1.4	0.6	2.0	2.6	1.1	1.6	1.0	1.8			
5	1.8	1.7	1.6	1.7					1.5								
+2Li	0.4	1.1	1.2	0.1					1.4								
6	1.1	1.4[c]	1.4	1.3[c]	1.5[b]	1.5	1.3[b]	[d]	1.0	1.0							
+2Li	0.4	1.4[c]	1.2	1.4[c]	1.9[b]	1.1	1.7[b]	0.9	0.6	1.4							
7	1.4[b]	0.6	1.9	1.5[b]					1.4								
+2Li	2.0[b]	1.9	1.2	2.0[b]					1.2								
8	1.9	1.6	1.8	1.8	1.4	1.4											
+2Li	−0.7	1.3	0.2	0.7	−0.4	0.8											
+2Na	−2.0	0.6	−0.4	−1.4	−0.4	0.4											
+2K	0.4	3.3	1.4	1.1	2.1	3.2											
+2Rb	0.0	0.6	0.6	−1.2	0.9	1.3											
+2Cs	2.1	1.8	1.3	0.8	1.8	1.6											
9	1.9																
+4Li	−0.1																
Chloroform	2.7																
TMS	1.4																
Acetone-d_5	1.9																

[a] Isotope shifts in ppb to an accuracy of ±0.2 ppb.
[b] Of the methyl substituent.
[c] Of the *tert*-butyl substituent.
[d] Not measured owing to overlap.

as reported previously.[20,21] By contrast, anthracene and tetracene are examples of PAHs with no bay region and little steric hindrance. As a result, their anions are almost planar.[22]

Deuterium isotope shifts are known to depend on electron density, as has been demonstrated for the cyclopentadienyl, benzene, tropylium series.[23,24] An attempt to correlate the carbon isotope shift (Table 1) change with calculated electronic properties and chemical shift change upon reduction was made, owing to the dependence of the chemical shifts on electron density. However, the observed correlation was very weak. The systems studied are polycyclic and many are strained unlike the above series. We previously reported that the bay region may produce different isotope shifts because the effect of reduction on the conformation of a strained system is greater.[25] When the change in isotope shift for lithium and sodium salts is plotted against a specific linear combination [$12.5\Delta\delta(^1\mathrm{H}) - \Delta\delta(^{13}\mathrm{C})$, a principle component] of the change in proton and carbon chemical shifts, the phenanthrene bay region values mostly fall in a different region from the other values (Fig. 3 and Table 2). There is an overlap of only about 30% between the regions. A much greater overlap (>80%) would be expected if the difference was random. This shows that the isotope shift does correlate with a structural feature but that further work is required to find a useful application.

CONCLUSIONS

We have shown for the first time that ^{13}C-induced isotope effects on protons can be measured reliably using a gradient-enhanced quantum filter. These effects have an advantage over deuterium isotope shifts on ^{13}C because complex synthesis is not required. We have shown that carbon-induced isotope shifts correlate with structural features of PAH anions. The exact nature of this information requires further research.

EXPERIMENTAL

Compounds **1**, **8**, **5**, **2** and **3** were commercial samples (Aldrich) and **6**,[20] **7**[20], **9**[19] and **4**[26,27] were prepared by published methods.

NMR experiments for neutral molecules were carried out using a Bruker DRX-400 spectrometer equipped with a BGUII *z*-gradient. Anions were measured at 220 K owing to their instability and/or broad signals in the NMR spectrum at room temperature. The neutral molecules were not soluble

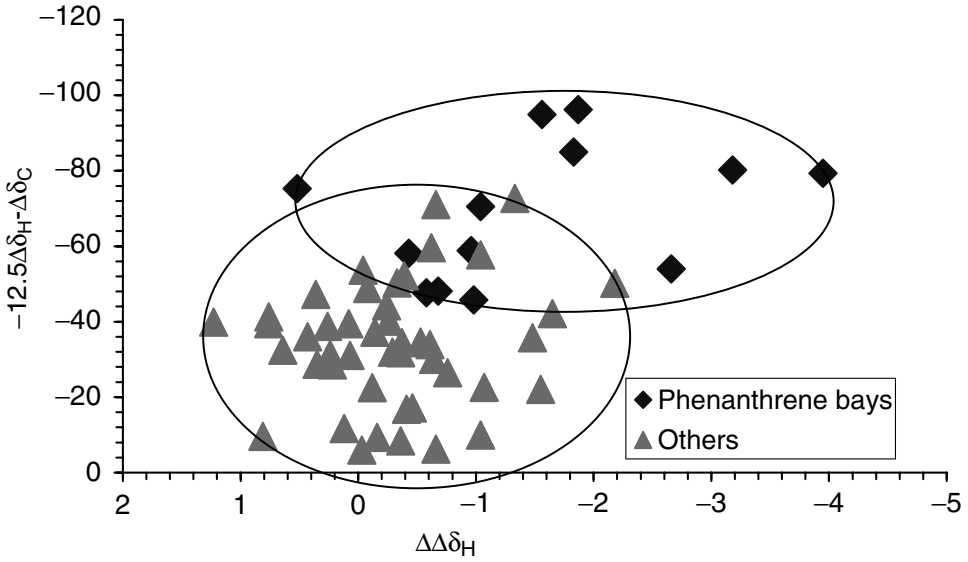


Figure 3. Dependence of carbon isotope shift change upon reduction on a linear combination of proton and carbon chemical shift changes for phenanthrene bay regions as compared with other protons.

Table 2. Changes in $^{13/12}$C isotope shifts on attached protons upon reduction[a]

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-9	H-10	H-11	H-12	H-13	H-14	H-2′	H-3′	H-4′
1 + 2Li	−0.4	0.1							0.5								
+2Na	0.3	0.4							0.1								
2 + 2Na	−1.6	−0.4	−0.6	−1.5						−0.1					−0.4	−1.0	−0.7
3 + 2Na	−1.1	0.2													−0.2	0.8	0.0
4 + 2Li	0.8	0.4	0.4	−0.4	−0.7	−0.6	0.6	−1.0	0.5	0.8	−0.2	−0.3	−0.3	−0.1			
5 + 2Li	−1.3	−0.7	−0.4	−1.6					0.0								
6 + 2Li	−0.8	0.0[c]	−0.1	0.1[c]	0.4[b]	−0.4	0.4[b]	—[d]	−0.5	0.1							
7 + 2Li	0.6[b]	1.2	−0.6	0.5[b]					0.2								
8 + 2Li	−2.7	−0.3	−1.6	−1.0	−1.8	−0.6											
+2Na	−4.0	−1.0	−2.2	−3.2	−1.9	−1.0											
+2K	−1.5	1.6	−0.4	−0.7	0.6	1.7											
+2Rb	−1.9	−1.1	−1.2	3.0	−0.5	−0.1											
+2Cs	0.2	0.1	−0.5	−1.0	0.3	0.2											
9 + 4Li	−2.0																

[a] Isotope shifts in ppb to an accuracy of ±0.3 ppb.
[b] Of the methyl substituent.
[c] Of the *tert*-butyl substituent.
[d] Not measured owing to overlap.

enough at 220 K to obtain good spectra in a reasonable time and so were measured at 298.5 ± 0.5 K. However, tests revealed that the temperature difference had no discernible effect on the isotope shifts. The materials (2–4 mg) were dissolved in THF-d_8 and degassed under vacuum using the freeze–pump–thaw method. Chemical shifts were calibrated to the downfield solvent signal [δ(^{1}H) THF-d_8 3.575 (298.5 K), 3.572 (220 K); δ(^{13}C) THF-d_8 67.397 (298.5 K), 67.368 (220 K)].

At least 32 scans were acquired for each spectrum. For the carbon-edited spectrum, more scans were usually required (up to 8192) in order to achieve the necessary signal-to-noise ratio (20 : 1 for the weakest multiplet). The digital resolution was typically 0.15 Hz, the spectrum was zero filled twice and further interpolated using a spline fit. The FID was oversampled and digitally filtered to yield a spectral width that was sufficient to include the spectrum of interest.

Solvents

THF-d_8 was dried and degassed as reported previously.[28] The solvent was placed in a flask and connected to a vacuum line, then degassed with several freeze–pump–thaw cycles before being vacuum transferred into a flask containing distilled Na–K (1 : 5) alloy. This was then shaken for several minutes until the solvent turned light blue, indicating dryness.

Preparation of anions

The alkali metal was placed into the upper part of the tube containing the PAH (2–4 mg to yield 4–8 mM solutions).

Lithium was inserted into the tube under argon while the other alkali metals were distilled into the tube under vacuum. Dry THF-d_8 was vacuum transferred into the tube, which was then sealed.[29] Repeated inversion of the tube at −78 °C brought the solution into contact with the metal. ^{1}H NMR spectroscopy detected the formation of the anions.

Acknowledgement

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