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Isotope shifts in polycyclic aromatic hydrocarbon anions

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Isotope shifts are a well established tool for structural analysis by NMR. The substitution of a proton with a deuterium is the most widely studied of these effects. We have synthesized all three monodeuterated anthracenes and shown that their ^{13}C spectra provide the same information that can be obtained from perdeuterated anthracene. The isotope shifts change when the PAH is reduced by an alkali metal. For a planar molecule such as anthracene, the isotope shifts change in approximate proportion to the change in charge density. However, when there is steric hindrance, such as in chrysene, reduction weakens the framework, allowing conformational change that substantially alters the isotopic shifts.

The characterization of polycyclic aromatic hydrocarbons (PAHs) is critical to the understanding of their reactivity. Interest in PAHs ranges from synthetic[1–3] to environmental and biological chemistry. In organic chemistry, PAHs are models for graphite, fullerenes and other carbon rich compounds. In the environment, PAHs are pollutants produced by the combustion of fuels and other organic materials.[4] In biology, they bind to DNA *via* enzymatic bay region activation causing mutations and cancer.[5] The steric crowding of the bay region hinders detoxification and increases the mutagenic and carcinogenic activity.

Isotopic substitution causes a perturbation of the charge distribution and characterizes small changes in molecular structure in a manner analogous to the way a derivative characterizes small changes in a mathematical function. Isotope shifts have been studied and used since quite early in the development of NMR. They have been used for structural and bonding studies, assignment and testing theories of chemical shift.[6–9] The form of isotopic substitution most commonly studied is the exchange of a protium with a deuterium. The doubling of the atomic weight causes the largest isotope effect of any substitution (excluding substituting protium with tritium).

Isotope shifts in ^{13}C NMR caused by deuteration have predictable magnitudes and can be used for the assignment of the spectrum.[10–13] 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, *i.e.*, $\Delta\mathrm{C(D)} = \delta_\mathrm{C}(\mathrm{D}) - \delta_\mathrm{C}(\mathrm{H})$.[8,9] α substitution causes a one bond isotope shift, $^{1}\Delta\mathrm{C(D)}$, of approximately 280 ppb, β substitution causes a two bond isotope shift, $^{2}\Delta\mathrm{C(D)}$, between 80 and 120 ppb and γ substitution causes a three bond effect, $^{3}\Delta\mathrm{C(D)}$, between 0 and 70 ppb. Magnitudes of $^{4}\Delta\mathrm{C(D)}$ isotope shifts are less than 10 ppb in aliphatic systems but $^{4}\Delta\mathrm{C(D)}$ and $^{5}\Delta\mathrm{C(D)}$ isotope shifts may be larger in conjugated systems.

The heavier isotope is larger and more massive which leads to slight changes in bond lengths and angles arising from vibrational changes.[6,14,15] This effect usually causes shielding, *i.e.*, a decrease in chemical shift, of the neighboring nucleus. For aromatic compounds, it has been shown that the one and two bond deuterium isotope effects on carbon can be directly related to C–H and C–C bond length and charge density.[16,17] The isotope shift is a measure of chemical shift change with perturbation and can be predicted with some accuracy. This, despite the fact that the absolute value of the chemical shift pertaining to the substituted compound cannot be determined that accurately. Indeed, the accuracy of most modeled molecular parameters is often less than the size of the isotope effects being observed. Isotopic substitution is therefore a useful tool in molecular modeling.

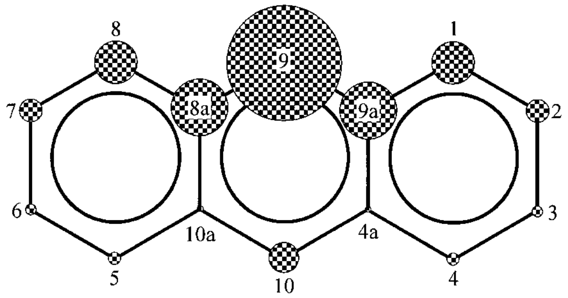

Fig. 1 Isotope shifts in 9-deuteroanthracene. Area of circle indicates the size of the isotope effect.

Results and discussion

There are a number of practical procedures for measuring isotope shifts arising from deuteration. Of these, the only way to be sure of the assignment of individual isotope shifts is to specifically monodeuterate the compound and compare it with the regular (protiated) molecule (Fig. 1). This is synthetically difficult even for a simple molecule such as anthracene. We synthesized the three isotopomers (1, 2 and 9) of monodeuterated anthracene and compared their ^{13}C NMR spectra with those of their dilithium salts.

The isotope shifts in the ^{13}C spectra of monodeuterated anthracene allow the resolution of individual isotope effects. However, anthracene's symmetry makes the assignment of each effect ambiguous. For example, a mixture of regular anthracene and 2-deuteroanthracene yields five carbon signals where C4a is expected. The identity of the isotope effects on C4a, C8a, C9a and C10a can only be surmised by comparison with isotope effects *via* a similar number of bonds in similar molecules (Tables 1 and 2—Estimated shifts) and assuming that shorter-range effects are larger.

We compared the results with an alternative method: to use a mixture of the perdeuterated compound and the regular (protiated) compound.[18,19] In the ^{13}C spectrum, two sets of signals appear (Fig. 2). The signals of the protiated compound

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appear as singlets while the perdeuterated compound yields triplets with a coupling constant to deuterium of about 24 Hz. The perdeuterated signals appear much weaker because they are not NOE enhanced and are split by deuterium coupling. In principle, it is possible to use a deuterium lock switch and a triple resonance probe to decouple the deuterium during acquisition while maintaining lock. This would increase sensitivity by a factor of three for the perdeuterated signals. The difference in chemical shift is typically 400 ppb corresponding to the sum of all the isotope effects on the carbon. While some estimate of the individual isotope shifts is possible from perdeuteration, the information obtainable is limited.

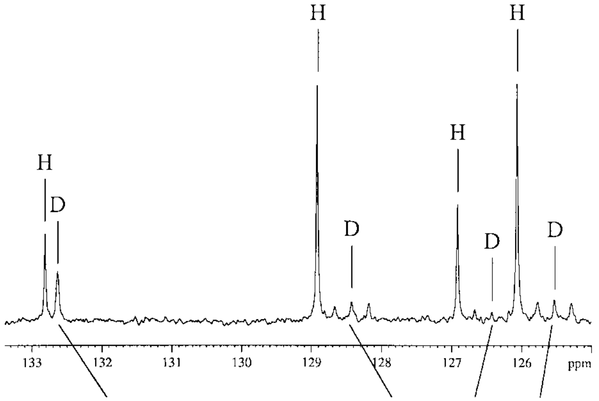


Fig. 2 ^{13}C NMR spectrum of anthracene and anthracene-d_{10} mixture.

Table 1 Total isotope shifts on each carbon of anthracene

Carbon	Isotope pattern	Estimated shift[a]	From individual[b]	Observed shift[c]
1	$^1\Delta + {}^2\Delta + 2{}^3\Delta$	460	502	494
2	$^1\Delta + 2{}^2\Delta + {}^3\Delta$	520	527	541
4a	$2{}^2\Delta + 2{}^3\Delta$	240	147	178
9	$^1\Delta + 2{}^3\Delta$	360	480	488

[a] Estimated as described in the text. [b] From Table 3. [c] Accurate to ±20 ppb.

Table 2 Total isotope shifts on each carbon of anthryllithium

Carbon	Isotope pattern	Estimated shift[a]	From individual[b]	Observed shift[c]
1	$^1\Delta + {}^2\Delta + 2{}^3\Delta$	460	591	589
2	$^1\Delta + 2{}^2\Delta + {}^3\Delta$	520	640	628
4a	$2{}^2\Delta + 2{}^3\Delta$	240	237	198
9	$^1\Delta + 2{}^3\Delta$	360	510	577

[a] Estimated as described in the text. [b] From Table 4. [c] Accurate to ±20 ppb.

The spectrum of the perdeuterated/regular anthracene mixture (Fig. 2) shows two sets of signals. If we assume that the individual isotope shifts are similar to those previously reported:[9] $^1\Delta C(D)$, 280; $^2\Delta C(D)$, 100; $^3\Delta C_{quaternary}(D)$, 20 and $^3\Delta C_{proton\ attached}(D)$, 40 then it is possible to estimate the isotope shifts. Table 1 shows that the predicted magnitudes are in the correct order but are not very accurate. The discrepancies are due to the magnitudes being taken mostly from aliphatic compounds and longer-range effects not having been taken into account.

The sum of the individual isotope shifts (Table 3) on each carbon should be the same as the observed overall isotope shift for the perdeuterated compound (Table 1). (This assumes that second order summation effects are negligible as has been shown many times in the literature.[10]) The discrepancies that arise are due mainly to unresolved shifts and inaccuracies in measuring small shifts.

The question arises as to whether the total of deuterium isotope shifts on a particular carbon, the total shifts arising from a particular deuterium, or an individual isotope shift better characterizes the physical properties of the molecule. Reduction with alkali metal causes large changes in the charge densities at the carbons that are known to correlate with the change in ^{13}C chemical shift ($\Delta\delta_C$).[20] The effect of perdeuteration on reduction with alkali metals is to slightly localize the charge density and slightly accentuate steric effects. In the case of anthracene, there is little steric hindrance and the change in total isotope effect on each carbon upon reduction follows the same pattern[16,17] as the change in charge density[21] (Table 2 for reduction with lithium and Fig. 3 for reduction with sodium). We found that the change in total isotope effect, $\Delta\Sigma[\Delta C(D)]$, on a particular carbon and the change in one bond effects, $\Delta^1\Delta C(D)$, correlate better with change in charge density than the total effect arising from a particular deuterium. In fact, the total isotope shifts arising from each hydrogen do not follow any obvious pattern (Table 4). This indicates that the use of perdeuteration provides enough isotope shift information to measure the effects of charge density and molecular structure and that specific deuteration only reinforces the findings by providing one bond isotope shifts.

Another method for measuring isotope shifts is to randomly partially deuteriate.[10,11,13] This is achieved by exchanging the hydrogens in a partially deuterated solvent and obtaining a

Table 3 Individual isotope shifts of anthracene[a]

	H-1	2	3	4	5	6	7	8	9	10
C-1	318	121	[b]	11	[b]	[b]	[b]	[b]	48	4
2	129	283	92	3	[b]	[b]	3	[b]	14	3
3	3	92	283	129	[b]	3	[b]	[b]	3	14
4	11	[b]	121	318	[b]	[b]	[b]	[b]	4	48
4a	4	4	−10	64	[b]	[b]	[b]	[b]	1	84
5	[b]	[b]	[b]	[b]	318	121	[b]	11	4	48
6	[b]	[b]	3	[b]	129	283	92	3	3	14
7	[b]	3	[b]	[b]	3	92	283	129	14	3
8	[b]	[b]	[b]	[b]	11	0	121	318	48	4
8a	[b]	[b]	[b]	[b]	4	−10	4	64	84	1
9	50	3	[b]	[b]	[b]	3	[b]	50	350	24
9a	64	−10	4	4	[b]	[b]	[b]	[b]	84	1
10	[b]	[b]	3	50	50	[b]	3	[b]	24	350
10a	[b]	[b]	[b]	[b]	64	4	−10	4	1	84
Total	579	496	496	579	579	496	496	579	682	682

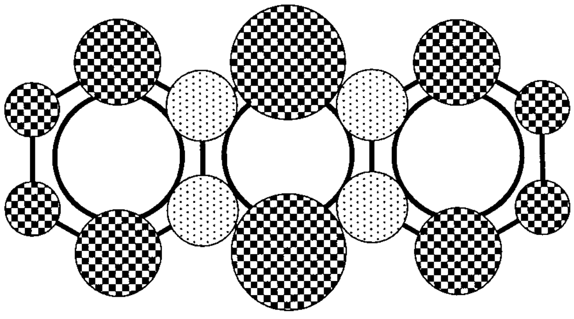
[a] One bond isotope shifts are accurate to ±20 ppb, other shifts generally accurate to ±3 ppb unless compromised by signal overlap. [b] Isotope shift too small to be resolved.

Table 4 Individual isotope shifts of anthryllithium[a]

	H-1	2	3	4	5	6	7	8	9	10
C-1	269	82	45	43	[b]	[b]	28	[b]	96	28
2	104	270	188	24	[b]	[b]	30	[b]	39	−15
3	24	188	270	104	[b]	30	[b]	[b]	−15	39
4	43	45	82	269	[b]	28	[b]	[b]	28	96
4a	26	13	20	109	[b]	−18	3	[b]	23	61
5	[b]	[b]	28	[b]	269	82	45	43	28	96
6	[b]	[b]	30	[b]	104	270	188	24	−15	39
7	[b]	30	[b]	[b]	24	188	270	104	39	−15
8	[b]	28	[b]	[b]	43	45	82	269	96	28
8a	[b]	−18	3	[b]	26	13	20	109	61	23
9	96	46	−31	15	15	−31	46	96	290	−32
9a	109	20	13	26	[b]	3	−18	[a]	61	23
10	15	−31	46	96	96	46	1	15	−32	290
10a	[b]	3	−18	[b]	109	20	13	26	23	61
Total	686	676	676	686	686	676	676	686	722	722

[a] One bond isotope shifts are accurate to ±20 ppb, other shifts generally accurate to ±5 ppb unless compromised by signal overlap. [b] Isotope shift too small to be resolved.

Chemical shift change on charging



Isotope effect change on charging

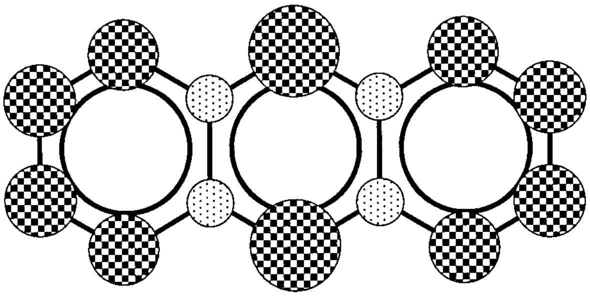


Fig. 3 Effect on anion Anthracene^{2-} 2Na$^+$. Area of circle indicates the size of the change: positive checked and negative spotted.

spectrum under conditions of slow or no exchange. The technique is known as secondary isotope multiplets of partially labeled entities or SIMPLE NMR.[10] For PAHs this can be achieved by refluxing for several hours in partially deuterated TFA, cooling and then quenching with water. Once the acid has been removed, the sample retains a stable isotopic ratio. The result is a mixture of a wide variety of isotopomers each yielding different carbon signals. From such a spectrum, it is theoretically possible to isolate all the individual isotope effects although assigning them correctly remains a problem. However, for aromatic systems, the isotope shifts can easily be observed over more than three bonds. This leads to a very large number of unresolvable signals. Our preliminary studies[22] with phenanthrene showed that very concentrated solutions were required in order to obtain a spectrum. The result was very complicated and only a partial interpretation of the spectrum was possible.

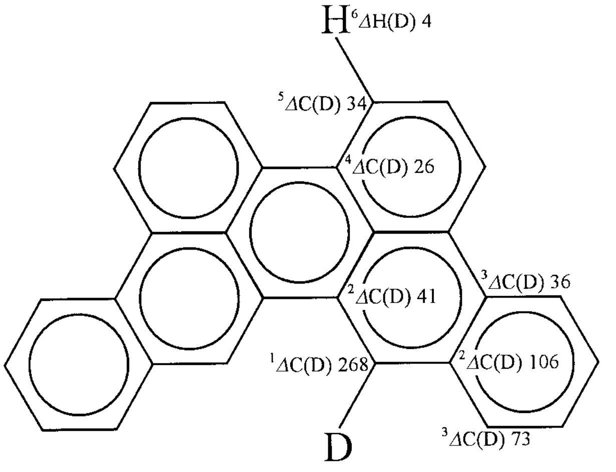


Fig. 4 Long-range isotope shifts (ppb) in dibenzoperylene.

The geometry of the entire molecule may be changed by isotopic substitution when there is steric hindrance, reflecting change of conformation with perturbation. An example of this is dibenzoperylene that yields a $^5\Delta$C(D) effect of 34 ppb and a $^6\Delta$H(D) effect of 4 ppb (Fig. 4). These effects occur on the opposite side of the molecule due to twisting as a result of steric hindrance across the bay region.[23]

Chrysene is a PAH that includes two bay regions and therefore suffers considerable steric hindrance. This steric effect, combined with weakening of the carbon framework causes its anion to be twisted as has been shown to be the case for phenanthrene.[24] By contrast, anthracene is an example of a PAH with no bay region and little steric hindrance. As a result, anthracene's dianion is almost planar.[25]

Chrysene's alkali metal salts combine the phenomena of changes in charge density with changes in conformation. Consequently, the pattern of change in the isotope shifts upon formation of a dianion is very different from that of the charge density (Fig. 5) and is due to conformational change. The isotope shifts at the center of the molecule are very dependent on the type of metal cation (Table 5). This shows that the proximity of the cations is linked to the twisting of the anion. This indicates that the cations (with the possible exception of lithium) are in close contact with the center of the molecule. The result is some very unusual isotope shifts. The isotope shifts on carbon caused by deuteration reported in the literature are mostly positive.[10] Reported negative shifts are very small and usually less than 30 ppb. C4a of the chrysene dianion considerably exceeds this for the salts of sodium, potassium, rubidium and caesium.

Conclusions

Deuterium isotope shifts in the ^{13}C NMR spectra of alkali metal salts of PAHs are a good indicator of charge density and

Table 5 Isotope shifts on neutral and reduced chrysene[a]

Carbon	Calculated	Neutral	Li	Na	K	Rb	Cs
1	420	501	411	[c]	492	500	505
2	520	534	540	536	511	470	511
3	520	531	559	536	539	600	576
4	460	389	463	386[c]	392	[c]	311
4a	240	84	39	−43	−99	−133	−76
4b	180	161	17	90[c]	17	[b]	[b]
5	420	400	459	386	382	400	346
6	400	515	805	703	666	650	690
6a	160	83	[b]	90[c]	17	[b]	[b]

[a] One bond effects accurate to ±20 ppb, other shifts generally accurate to ±5 ppb unless compromised by signal overlap. [b] Isotope shift too small to be resolved. [c] Value of isotope shift uncertain due to signal overlap.

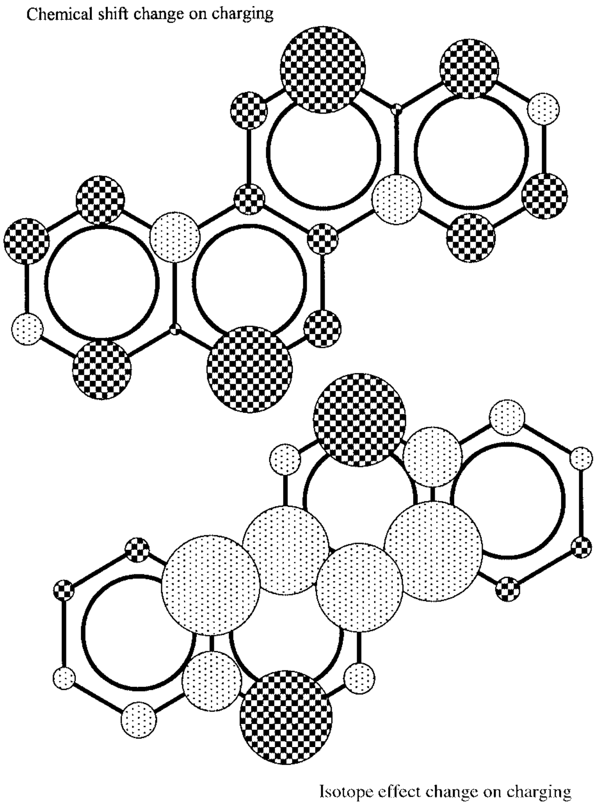


Fig. 5 Chrysene^{2-} 2K^{+}. Area of circle indicates the size of the change: positive checked and negative spotted.

conformation. From the charge density and conformation, it is possible to draw conclusions about the nature of the ionic bonding effects. While specific deuteration may reconfirm results, enough information may be obtained by comparing the perdeuterated compound with the regular (protiated) compound.

Experimental

Anthracene, anthracene-d_{10}, chrysene and chrysene-d_{12} were commercial samples (Aldrich). NMR experiments for neutral molecules were carried out at 298.5 ± 0.5 K using a Bruker DRX-400 spectrometer equipped with a BGUII z-gradient. Anions were measured at 220 K due to their instability at room temperature. The neutral molecules were not soluble enough at 220 K to obtain good ^{13}C spectra in a reasonable time. However, tests revealed that the temperature difference had no discernable effect on the isotope shifts. The materials (2 to 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_H THF-d_8 3.575 (298.5 K), 3.572 (220 K); δ_C THF-d_8 67.397 (298.5 K), 67.368 (220 K)].

Solvents

THF-d_8 was dried and degassed as previously reported.[26] 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, 5:1 alloy. This was then sonicated until dry or for several minutes until the solvent turned light blue.

Preparation of anions

The alkali metal was placed in the upper part of the NMR tube containing the PAH (2 to 4 mg to yield 4 to 8 mM solutions). Lithium was inserted into the tube under argon while the other alkali metals were distilled into the tube. Dry THF-d_8 was vacuum transferred into the tube, which was then sealed under vacuum.[27] 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.

1-Deuteroanthracene

1-Aminoanthracene (0.50 g, 2.59 mmol) was introduced into D_2O (6 mL) and DCl (0.75 mL, 20%), preheated to boiling under an inert atmosphere. The mixture was cooled to −15 °C and allowed to warm to ~5 °C. An additional portion of DCl (1 mL, 20%) was added followed by a solution of $NaNO_2$ (0.36 g, 5.22 mmol) in D_2O (0.4 mL) over 15 min, giving a slightly turbid crimson solution. A cooled solution of D_3PO_2 (1.1 mL, 1.49 g, 21.6 mmol) in D_2O (2.3 mL) was added and the solution was allowed to react for 22 h at −2 ± 2 °C and 28 h at room temperature. The reaction mixture was then diluted with H_2O (250 mL), extracted with benzene, then with NaOH (2.5%) and once again with benzene. The combined organic layers were washed with NaOH (5%) to give the crude product after standard work-up. TLC showed the presence of anthracene and 1-deuteroanthracene. Further purification by column chromatography (eluent: ethanol) and by vacuum sublimation at 5×10^{-5} mbar/75–84 °C over 14 days afforded white crystals (3.3 mg, 18.41 μmol, 0.71%, mp 210 °C).

2-Deuteroanthracene

2-Aminoanthracene (0.66 g, 3.41 mmol) was introduced into D_2O (8 ml) and DCl (1 mL, 20%), preheated to boiling under an inert atmosphere. The mixture was cooled to −15 °C and allowed to warm to ~5 °C. An additional portion of DCl (1.5 mL, 20%) was added followed by a solution of $NaNO_2$ (0.48 g, 6.95 mmol) in D_2O (0.6 mL) over 15 min, giving a crimson solution. A cooled solution of D_3PO_2 (7.4 mL, 10.00 g, 0.14 mol) in D_2O (5 mL) was added and the solution was allowed to react for 24 h at 0 °C and 44 h at room temperature. The reaction mixture was then diluted with H_2O (250 mL), extracted with benzene, then with NaOH (2.5%) and once again with benzene. The combined organic layers were washed with NaOH (5%) to give the crude product after standard work-up. Further purification by column chromatography (eluent: ethanol) gave the product (220 mg, 1.23 mmol, 36.1%, red solid). Additional purification conducted on 150 mg of the product by vacuum sublimation at 2×10^{-4} mbar/82–83 °C over 7 days afforded white crystals (6.4 mg, 35.71 μmol, 1.1%, mp 209 °C).

9-Deuteroanthracene

9-Bromoanthracene (2.5 g, 9.72 mmol) in THF (50 mL) was cooled to −78°, then treated with 2.5 M BuLi in hexane (4.5 mL, 11.2 mmol) over 15 min. The colored solution was stirred while the mixture was allowed to warm to 25 °C (45 min). At room temperature the solution was reacted with D_2O (10 mL), and mixed over 10 min. The mixture was then extracted with three 75 mL portions of CH_2Cl_2, giving a white solid after standard work-up. Recrystallization from MeOH–hexane afforded 1.52 g of white solid. The NMR spectrum indicated that the mixture contained both fully protiated anthracene and 9-deuteroanthracene in nearly 1 : 1 ratio, showing that 9-deuteroanthracene was obtained in 43.6% yield (4.24 mmol).

Acknowledgements

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References

1 G. Balanikas, N. Hussain, S. Amin and S. S. Hecht, *J. Org. Chem.*, 1988. **53**, 1007.
2 R. E. Lehr, P. L. Kole, M. Singh and K. D. Tschappat, *J. Org. Chem.*, 1989, **54**, 850.
3 A. S. Kiselyov, H. Lee and R. G. Harvey, *J. Org. Chem.*, 1995, **60**, 6123.
4 R. Sangaiah, A. Gold, K. O. Newcomb and L. M. Ball, *J. Med. Chem.*, 1991, **34**, 546.
5 D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin and A. H. Conney, in *In vitro metabolic activation in mutagenesis testing: proceedings*, eds. F. J. De Serres, J. R. Fouts, J. R. Bend and R. M. Philpot, Elsevier, Amsterdam, 1976, p. 159.
6 H. Batiz-Hernandez and R. A. Bernheim, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1967, **3**, 63.
7 P. E. Hansen, *Annu. Rep. NMR Spectrosc.*, 1983, **15**, 105.
8 P. E. Hansen, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1988, **20**, 207.
9 D. B. Davies, J. C. Christofides and R. E. Hoffman, *Isotopes: Essential Chemistry & Applications II*, ed. J. R. Jones, Royal Society of Chemistry, London, 1988.
10 J. C. Christofides and D. B. Davies, *J. Am. Chem. Soc.*, 1983, **105**, 5099.
11 J. Reuben, *J. Am. Chem. Soc.*, 1983, **105**, 3711.
12 J. Reuben, *J. Am. Chem. Soc.*, 1984, **106**, 6180.
13 J. C. Christofides, PhD Thesis, University of London, 1985.
14 K. L. Servis and F.-F. Shue, *J. Am. Chem. Soc.*, 1980, **102**, 7233.
15 C. J. Jameson and H. J. Osten, *Annu. Rep. NMR Spectrosc.*, 1986, **17**, 1.
16 Y. Nakashima, H. Kanada, M. Fukunaga, K. Suzuki and K. Takahashi, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 2894.
17 Y. Nakashima, T. Teranishi, T. Suzuki, T. Sone and K. Takahashi, *Magn. Reson. Chem.*, 1994, **32**, 578.
18 S.-C. Ho, H. J. Koch and R. S. Stuart, *Carbohydr. Res.*, 1978, **64**, 251.
19 P. E. Pfeffer, K. M. Valentine and F. W. Parrish, *J. Am. Chem. Soc.*, 1979, **101**, 1265.
20 D. H. O'Brien, *Comprehensive Carbanion Chemistry*, ed. E. Buncel and T. Durst, Elsevier, New York, 1980, Part A, Ch. 6.
21 Y. Cohen, J. Klein and M. Rabinovitz, *J. Am. Chem. Soc.*, 1988, **110**, 4634.
22 Unpublished results from our laboratory.
23 R. Benshafrut, M. Rabinovitz, R. E. Hoffman, N. Ben-Mergui, K. Müllen and V. S. Iyer, *Eur. J. Org. Chem.*, 1999, 37.
24 A. Ioffe, A. Ayalon and M. Rabinovitz, *J. Chem. Soc.*, *Perkin Trans. 2*, 1994, 1115.
25 W. E. Rhine, J. Davis and G. Stucky, *J. Am. Chem. Soc.*, 1975, **97**, 2079.
26 R. E. Hoffman, M. Nir, I. O. Shapiro and M. Rabinovitz, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 1225.
27 I. O. Shapiro, M. Nir, R. E. Hoffman and M. Rabinovitz, *J. Chem. Soc.*, *Perkin Trans. 2*, 1994, 1519.