^{13}C and ^1H NMR Analysis of Isotopically Labeled Benzo[*a*]pyrenes

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Abstract: ^{13}C and ^1H NMR spectra of each of the 12 isotopomers of benzo[*a*]pyrene specifically labeled with carbon-13 at a methine position have been obtained. These studies lead to reassignment of 11 of the 20 ^{13}C resonances of benzo[*a*]pyrene, and to unambiguous ^1H assignments. $^1J_{CC}$ values were found to follow Hansen's rule, although the specific parametrization varied significantly depending on the source of the bond-length data. The optimum fit for the methine carbons was determined to be $^1J_{CC} = -143l + 257$; for coupling between methine and quaternary carbons, $^1J_{CC} = -119l + 227$. Vicinal couplings between ^{13}C atoms do not show a significant correlation with the bond order of the central bond in the vicinal coupling pathway; however, coupling between vicinal carbons which are trans along the periphery of the ring system (class I) have a mean of 5.45 Hz, compared with the vicinal trans coupling through the ring (class II) with a mean of 3.06 Hz.

I. Introduction

Interest in benzo[*a*]pyrene and related polynuclear aromatic hydrocarbons derives primarily from the associated carcinogenic activity of these compounds. Both ^1H and ^{13}C NMR studies[1,2] have been carried out in order to provide background data for analytical applications and to yield NMR parameters which can be related to the electronic structure of the molecule. Several theories relating electronic structure to microsomal activation and carcinogenic activity have been proposed.[3-5] Previous ^{13}C assignments have been based on double-resonance experiments, the use of model compounds, and the use of deuterium isotope effects of deuterated analogues.[2] It is well established that the first two approaches can lead to equivocal and occasionally contradictory results.[6-8] Further, the present studies indicate that three of the five resonances corresponding to carbons β to deuterium atoms in [1,3,6-$^2H_3$]benzo[*a*]pyrene were assigned incorrectly.

As part of a more general synthetic program, procedures have been developed for labeling each of the 12 protonated carbons of benzo[*a*]pyrene with ^{13}C. Details of the syntheses are reported elsewhere.[9] These isotopically labeled compounds have provided us with a unique opportunity to determine the spectral properties of benzo[*a*]pyrene. In addition to providing direct information on the assignments of the protonated carbons, the large one-bond carbon-hydrogen and carbon-carbon coupling constants allow unequivocal assignments of the protons and quaternary carbons to be made. Coupling constant data can also be related to electronic structure.

II. Materials and Methods

The synthesis of benzo[*a*]pyrene singly labeled at any of the 12 protonated carbon atoms has been completed recently.[9] Synthetic routes to the unambiguous incorporation of the label at C-4, C-5, C-11, or C-12,[9a] C-6,[9b] C-1, C-2, or C-3,[9c] and C-7, C-8, C-9, or C-10[9d] are reported in detail elsewhere. High-resolution ^1H and ^{13}C NMR spectra were obtained on a Bruker wide-bore WM 300 NMR spectrometer operating in the Fourier transform mode; 300-MHz ^1H NMR spectra were obtained at 25 °C with a spectral width of 1500 Hz and a 16K data table using a 90° pulse. Samples (1 mg/mL of $^2H_2CCl_2$) were degassed and sealed under an atmosphere of argon. Chemical shifts are reported in ppm downfield from tetramethylsilane (Me_4Si). The central resonance of $^2H_2CCl_2$ was used as an internal reference. Proton-decoupled ^{13}C NMR spectra (75.4 MHz) were obtained at 35 °C with an 8K spectral width and 32 K data table using a 33° pulse. Samples were 25 mg/2.5 mL of chloroform-*d*. Chemical shifts are reported in ppm downfield from Me_4Si using the center resonance of chloroform-*d* as an internal reference.

III. Results and Discussion

^1H and ^{13}C NMR spectra were obtained for the unlabeled and ^{13}C-labeled benzo[*a*]pyrene analogues at 300 and 75.4 MHz, respectively. As an illustrative example, the proton-decoupled ^{13}C spectra corresponding to unlabeled and to [12-^{13}C]benzo[*a*]pyrene (90 mol % ^{13}C) are shown in Figures 1A and B. In addition to the direct assignment of C-12 from these spectra,

Table I. ^{13}C and ^1H Chemical Shift Assignments of Benzo[*a*]pyrene

position[a]	^{13}C shift[b]	^1H shift[c]
1	125.53	8.260
2	126.06	8.007
3	124.82	8.110
3a	131.63	
4	127.83	7.944
5	128.15	8.014
5a	129.90	
6	124.75	8.543
6a	131.41	
7	128.92	8.310
8	126.04	7.794
9	125.94	7.848
10	123.02	9.066
10a	128.29	
10b	127.42	
11	122.18	9.078
12	127.51	8.356
12a	131.45	
12b	125.47	
12c	123.84	

[a] Carbon atom designations as illustrated in Figure 1. [b] In ppm from Me_4Si using the center resonance of the $^2HCCl_3$ as an internal reference. [c] In ppm from Me_4Si using the center resonance of the $^2H_2CCl_2$ as an internal reference.

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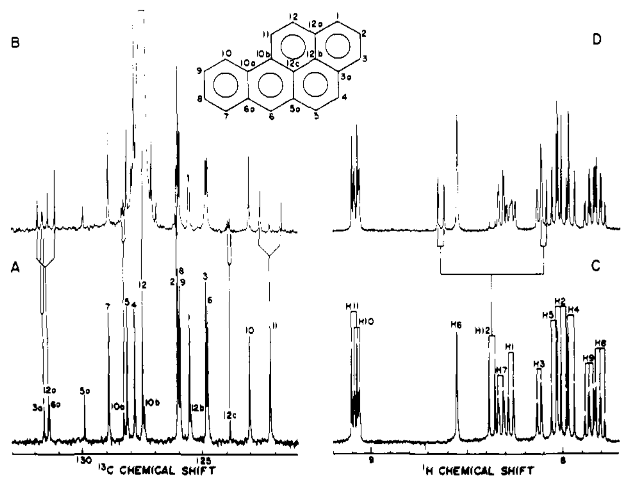

Figure 1. (A) Proton-decoupled ^{13}C NMR spectrum of natural abundance benzo[*a*]pyrene dissolved in chloroform-*d*; (B) proton-decoupled ^{13}C NMR spectrum of [12-^{13}C]benzo[*a*]pyrene (90 mol% ^{13}C); (C) ^1H NMR spectrum of unlabeled benzo[*a*]pyrene dissolved in $^2H_2CCl_2$; (D) ^1H NMR spectrum of [12-^{13}C]benzo[*a*]pyrene. Other conditions are as described in the Materials and Methods section.

Table II. $^1J_{CC}$ Values for Benzo[*a*]pyrene

coupled nuclei	$^1J_{CC}$ (Hz)	coupled nuclei	$^1J_{CC}$ (Hz)
C-1, C-2	58.1	C-3, C-3a	60.1
C-2, C-3	56.0	C-3a, C-4	53.2
C-4, C-5	*a*	C-5, C-5a	54.1
C-7, C-8	60.0	C-5a, C-6	63.0
C-8, C-9	*a*	C-6, C-6a	57.1
C-9, C-10	60.0	C-6a, C-7	56.1
C-11, C-12	62.0	C-10, C-10a	58.1
		C-10b, C-11	*a*
		C-12, C-12a	54.1
		C-12a, C-1	58.8

[a] Couplings not well resolved.

resonances corresponding to C-11 and C-12a are readily identified on the basis of the large $^1J_{CC}$ values. A marked increase in the intensity of the C-12a resonance in the C-12 labeled compound is also apparent, reflecting the contribution of the relatively significant $^{13}C-^{13}C$ dipolar interaction for the quaternary C-12a carbon.[10] Assignment of C-12b and C-12c which are at least two bonds removed from any of the protonated (labeled) carbons requires an interpretation based on remote couplings, as is discussed below. The 300-MHz ^1H spectra corresponding to the unlabeled and C-12 labeled benzo[*a*]pyrenes are shown in Figures 1C and D. The observed coupling allows unequivocal identification of H-12. A summary of the ^{13}C and ^1H resonance assignments is given in Table I.

The present labeling studies lead to reassignments of 11 of the 20 ^{13}C resonances relative to those made by Buchanan and Ozubko,[2] and to the unequivocal assignments of H-8 and H-9 which had not been made previously.[1] In general, these reassignments involve interchange of pairs of assignments: 3a and 6a, 10a and 10b, 2 and 9, and 6 and 1, as well as reassignment within the group containing the C-4, -5, and -12 peaks. These extensive reassignments reflect some of the difficulties inherent in standard assignment strategies, including double-resonance methods, the use of model compounds, and the use of remote deuterium isotope effects. Problems with the use of the double-resonance approach to assign ^{13}C resonances based on the ^1H spectrum presumably reflect the large magnitude of the $^1J_{CH}$ couplings. This approach was previously found to lead to a number of incorrect assignments in carbohydrates,[6,7] and in the example of benzo[*a*]pyrene led to an incorrect assignment of C-6, although C-10 and C-11 were correctly assigned on the basis of the H-10 and H-11 decoupling experiments. The use of γ deuterium isotope effects, as were invoked for the assignment of carbons 10a and 10b, is also not supported by the present studies. Finally, conclusions regarding the lack of additivity of the β deuterium isotope shifts were based on an incorrect assignment of C-2.[2]

Table III. Linear Regression Analysis of $^1J_{CC}$ as a Function of Bond Length

$\Delta J/\Delta l$[a]	intercept	r^2	ref
I. Methine-Quaternary Couplings			
−201	342	0.96	15
−119	227	0.95	16
−119	227	0.89	17
II. Methine-Methine Couplings			
−217	360	0.99	15
−143	257	0.99	16
−182	309	0.74	17

[a] Expressed in Hz/Å.

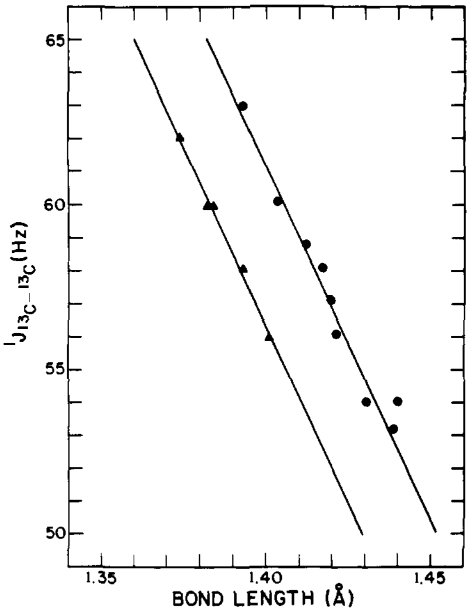

Figure 2. Plot of $^1J_{CC}$ values corresponding to couplings between pairs of methine carbons (▲) and between methine and quaternary carbons (●) as a function of theoretical bond lengths for benzo[*a*]pyrene given by Vasudevan and Laidlaw.[15]

In addition to the assignment information, a large amount of coupling constant data is obtained from these studies. The one-bond J_{CC} values are summarized in Table II. Relationships between J_{CC} values and π bond orders or bond lengths have been suggested by a number of groups.[11-14] Hansen has noted that a plot of $^1J_{CC}$ values as a function of bond order or bond length for a series of polycyclic aromatic hydrocarbons leads to two parallel lines, one corresponding to couplings between methine and quaternary carbons, and the second to couplings involving only methine carbons.[14] However, the parameters corresponding to these curves have not been reported. We have carried out this analysis using two sets of theoretical bond-length values, as well as lengths derived from an X-ray crystallographic study which have been reported for benzo[*a*]pyrene.[15-17] In each case, the results are qualitatively as described by Hansen, although the

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Table IV. Vicinal $^{3}J_{CC}$ Coupling Constants in Benzo[*a*]pyrene[a]

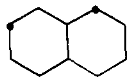
coupled nuclei	$^{3}J_{CC}$	coupled nuclei	$^{3}J_{CC}$
Class I (trans)		Class III (cis)	
C-2, C-4	4.9	C-10, C-11	4.9
C-3, C-5	5.9	C-7, C-10	8.3
C-4, C-6	6.9	C-1, C-3a	7.8
C-6, C-8	5.4	C-3, C-12a	6.9
C-1, C-11	5.4	C-4, C-12c	4.9
C-2, C-12	5.9	C-5, C-12b	2.9
C-5, C-6a	5.9	C-9, C-6a	3.9
C-7, C-5a	5.4	C-12, C-12c	5.9
C-9, C-10b	4.9	C-6, C-10b	6.9
C-10a, C-12	3.9		
Class II (trans)			
C-6, C-10	2.9	C-4, C-12a	3.9
C-7, C-10b	2.9	C-3, C-12c	3.0
C-1, C-12c	2.9	C-5a, C-11	2.9
C-12, C-3a	2.9		

[a] Vicinal couplings not reported in the table correspond to resonances which were obscured by additional peaks.

slopes of the lines were generally not identical. Slopes, intercepts, and r^2 values obtained from a linear regression analysis are summarized in Table III, and the plot with highest r^2 values is given in Figure 2. Although the bond-length values given by Vasudevan and Laidlaw correspond to maximium r^2 values, the theoretical bond lengths of Dewar and Trinajstic corresponded to lines which were in closer agreement with the X-ray data, and therefore must be preferred. These results are qualitatively consistent with the conclusion that the size of the coupling is related to the s character of the bond, and hence with the dominance of the Fermi contact mechanism. The curves obtained appear to apply generally to the class of polycyclic aromatic hydrocarbons. Coupling data corresponding to naphthalene,[18] anthracene[19], phenanthrene,[13,20] and pyrene[18] can be plotted as a function of bond length with similar results. In each case, the best agreement with the benzo[*a*]pyrene curves is obtained if the bond lengths are derived from the same (theoretical) source.[15,21]

Despite the variation reflected in Table III, the relationship between $^{1}J_{CC}$ values and bond lengths appears to be a useful basis for predicting the latter in systems for which crystallographic data may not be available. For example, the reported ^{1}J(C-10,C-11) value of 53.8 Hz in phenanthrene would predict a bond length of 1.45 Å using the second (preferred) curve of Table III. This bond length is considerably longer than the value of 1.39 Å reported in an early crystallographic study[22] but is in much better agreement with the values of 1.450 and 1.455 Å obtained more recently from X-ray and neutron diffraction studies.[23] For the case of benzo[*a*]pyrene, the plot of $^{1}J_{CC}$ as a function of the bond lengths determined by X-ray crystallography was characterized by significantly smaller r^2 values than the plot using the theoretical Dewar–Trinajstic values. This result may reflect larger bond-length errors in the crystallographic analysis.

Vicinal carbon–carbon coupling constants were observed in all cases for which the resonances were not obscured because of overlap and are summarized in Table IV. Following the analysis given by Hansen et al.,[13] these can be divided into three classes, corresponding to trans arrangements along the periphery of the ring system (class I), trans arrangements going through the ring system (class II), and cis arrangements (class III). The data given in Table IV indicate that this is a useful division since significant differences in magnitude are observed between the different classes. Of course, these observations do not imply that the three-bond coupling pathway is the only or even the predominant pathway involved; however, in general, the vicinal couplings are larger than other remote couplings which can be resolved, suggesting the importance of the three-bond pathway. Attempts to plot the vicinal couplings as a function of the bond length of the central bond in the coupling pathway as suggested by Hansen et al.[13] produced some indication of a correlation, although many of the points show significant scatter. For example, $^{3}J_{CC}$ values for the class II couplings are all significantly smaller than those of class I; however, the mean length of the central bonds is longer for class I, implying a lower bond order for this class. $^{3}J_{CC}$ values corresponding to class III arrangements exhibit more scatter. This result is presumably related to the fact that, with the exception of the C-10–C-11 coupling, there are two vicinal coupling pathways in each case. This class includes both the smallest and largest of the vicinal couplings, and in general the correlation between magnitude and length of the central bond is poor.



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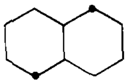

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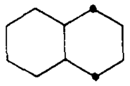

III

The use of remote ^{13}C–^{13}C couplings for assignment purposes would greatly extend the value of the ^{13}C labeling technique. To the extent that these couplings can be predicted, fewer labeled analogues need to be synthesized, and carbons such as C-12b and C-12c in benzo[*a*]pyrene which are not adjacent to a readily labeled position could be readily assigned. The data presented here suggest that reasonable predictions and hence feasible assignments can be made for carbons vicinal to a ^{13}C label which fall into class I or II relative to the labeled position. This distinction for polycyclic systems appears to be general. In addition to the other polycyclic aromatic hydrocarbons studied by Hansen and co-workers, a similar trend can be noted in the indole side chain of tryptophan, for which the ^{3}J(C-3,C-5), a class I coupling, is significantly greater than ^{3}J(C-3,C-7), a class II coupling.[8]

Summary

Carbon-13 isotopic substitutions have been carried out for all protonated carbons of benzo[*a*]pyrene and lead to extensive reassignments of the ^{13}C resonances as well as further unequivocal assignments of the ^{1}H resonances. One-bond carbon–carbon scalar coupling constants are closely related to bond length and fall into two clases corresponding to pairs of methine carbons and to methine–quaternary carbon interactions. The relationships apply to all of the polycyclic aromatic hydrocarbons which have been investigated to date and are of predictive value for the determination of accurate bond lengths. This approach will be further strengthened as more accurate bond lengths for the molecules for which coupling data are available become known to higher accuracy. Vicinal coupling interactions in which the coupled atoms are trans appear to be sufficiently predictable so that these can be used for resonance assignments, and labeling strategies can be designed to incorporate this feature.

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Registry No. Benzo[*a*]pyrene, 50-32-8.

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