424 Notes *Chem. Pharm. Bull.* **44**(2) 424—428 (1996) Vol. 44, No. 2

Relationship between the ^{13}C-NMR Chemical Shift and the Carcinogenic Activity of Benz[*a*]anthracenes

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A thorough NMR spectroscopic investigation was made on 7,12-dimethylbenz[*a*]anthracene (1) and the ^{1}H- and ^{13}C-NMR spectra were completely assigned. We compared the ^{13}C-NMR chemical shifts (δ) of three benz[*a*]anthracenes, including compound 1, whose carcinogenicities vary largely, and investigated the relationship of the δ and carcinogenicity. It was found that the δ values at the C1, C2, and C6 positions differed significantly between the carcinogenic and noncarcinogenic compounds.

Key words 7,12-dimethylbenz[*a*]anthracene; ^{13}C-NMR; chemical shift; proton HOHAHA; ^{13}C–^{1}H HOHAHA; carcinogenicity

It is known that there are significant differences in carcinogenic activities among polycyclic aromatic hydrocarbons (PAHs),[1-3] and that the differences depend strongly on the electronic states of the parent PAHs[4,5] and their metabolites.[6-9] To investigate such electronic states, ^{13}C-NMR is very useful, because ^{13}C-NMR chemical shifts reflect the electronic states of all carbon atoms of PAHs.

Thus we have investigated the structure–carcinogenic activity correlation on the basis of ^{13}C-NMR chemical shifts of PAHs, and indeed found a correlation between the average chemical shifts (δ_{av}) and carcinogenicity.[10-12] However, there still remains uncertainty as to the chemical significance of the δ_{av}-carcinogenicity correlation. In order to clarify the uncertainty, it is necessary to focus on the individual chemical shifts of PAHs. As sample compounds, benz[*a*]anthracenes (BAs) were chosen, because their carcinogenicity varies largely with the number and position of the methyl substituent. In spite of their same fundamental structure, 7,12-dimethylbenz[*a*]anthracene (**1**) and 7-methylbenz[*a*]anthracene (**2**) are carcinogenic, but 1-methylbenz[*a*]anthracene (**3**) is noncarcinogenic. The ^{1}H- and ^{13}C-NMR chemical shifts of compounds **1**—**3** were only partially assigned[13,14] and several ambiguities remain. In the present study, we paid special attention to compound **1** because of its extremely strong carcinogenicity and assigned the ^{1}H- and ^{13}C-NMR spectra completely. On the basis of the data, we investigated the relationship between δ and carcinogenicity for the BAs. It was found that the δ is considerably different at several carbon atoms between the carcinogenic and noncarcinogenic BAs.

Experimental

7,12-Dimethylbenz[*a*]anthracene was purchased from Tokyo Kasei Kogyo Co., Ltd., and used without further purification.

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL α 500 spectrometer, operating at 500 MHz at 27 °C and 30 °C by using a deuterium lock system. $CDCl_3$ was used as a solvent. The concentration of the sample was 30 mg ml^{-1}. The chemical shifts were measured relative to tetramethylsilane. The experimental conditions were as follows: number of accumulations, 128—2048; data point, 64 k or 128 k; spectral width, 6000 Hz or 24000 Hz; flip angle, 45° or 30°; interval between pulses, 8s or 2s.

We used double quantum filtered correlated spectroscopy (DQF-COSY) and measured absolute value two-dimensional spectra, proton homonuclear Hartmann–Hahn (HOHAHA) spectra, and ^{13}C–^{1}H HOHAHA spectra. A total of 128 increments of 2048 data points for ^{13}C–^{1}H spectra were collected; these were zero-filled to 512 data points in the F1 dimension, giving 0.97 and 1.22 Hz per point in the F2 and F1 dimensions, respectively. A sine-bell window function was applied in both dimensions before Fourier transformation. We also measured complete proton noise decoupling, ^{13}C–^{1}H heteronuclear correlated spectroscopy (^{13}C–^{1}H COSY), and correlation spectroscopy *via* long-range coupling (COLOC).

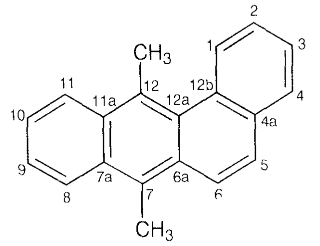

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Fig. 1. Molecular Formula of 7,12-Dimethylbenz[*a*]anthracene

Table 1. ^{1}H and ^{13}C Chemical Shifts of Compounds **1**—**3**

This work **1**				**2**[15]	**3**[15]
H1	8.46	C1	130.29	123.6	136.0
H2	7.53	C2	124.79	126.8	131.6
H3	7.55	C3	126.42	126.8	126.3*,***
H4	7.80	C4	127.47	128.3	127.4**
		C4a	133.16	131.7*	133.7****
H5	7.56	C5	126.46	126.9	126.4***
H6	8.01	C6	123.85	123.1	126.4***
		C6a	129.07	131.5*	132.1****
		C7	127.82	131.0	127.4
		C7a	130.48	129.8	131.1
H8	8.33	C8	124.93	124.4	128.9**
H9	7.60	C9	125.20	125.2	125.4*
H10	7.62	C10	125.39	125.7	126.0*
H11	8.36	C11	125.56	129.3	128.0**
		C11a	132.23	128.7	128.0?
		C12	129.17	120.3	127.2
		C12a	129.00	130.9	131.4
		C12b	131.32	130.5	130.2
7-Me	3.05	7-Me	14.30	—	—
12-Me	3.34	12-Me	21.18	—	—

*, **, *** and **** indicate groups of resonances where specific assignments cannot be made due to proximity.

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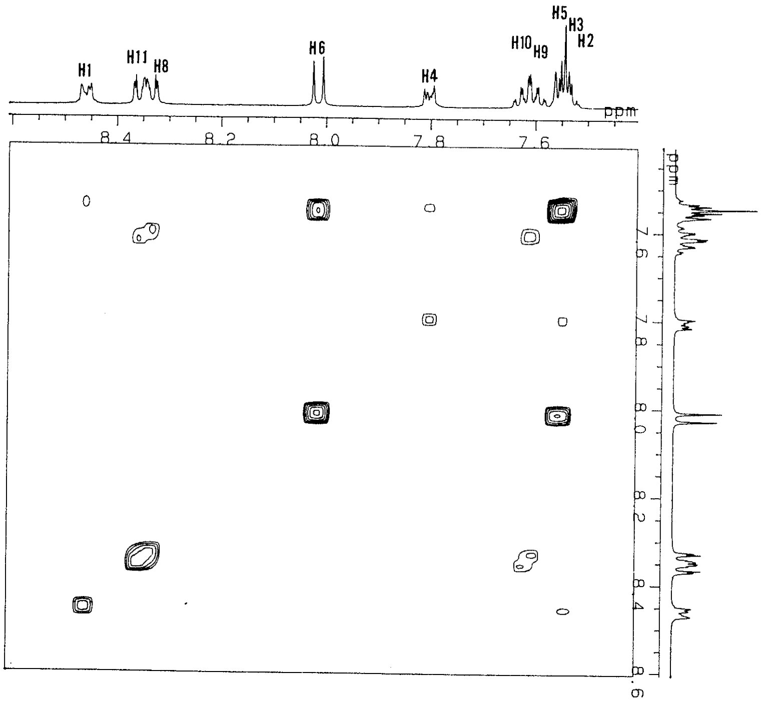

Fig. 2. DQF-COSY Spectra of 7,12-Dimethylbenz[*a*]anthracene
In the aromatic region.

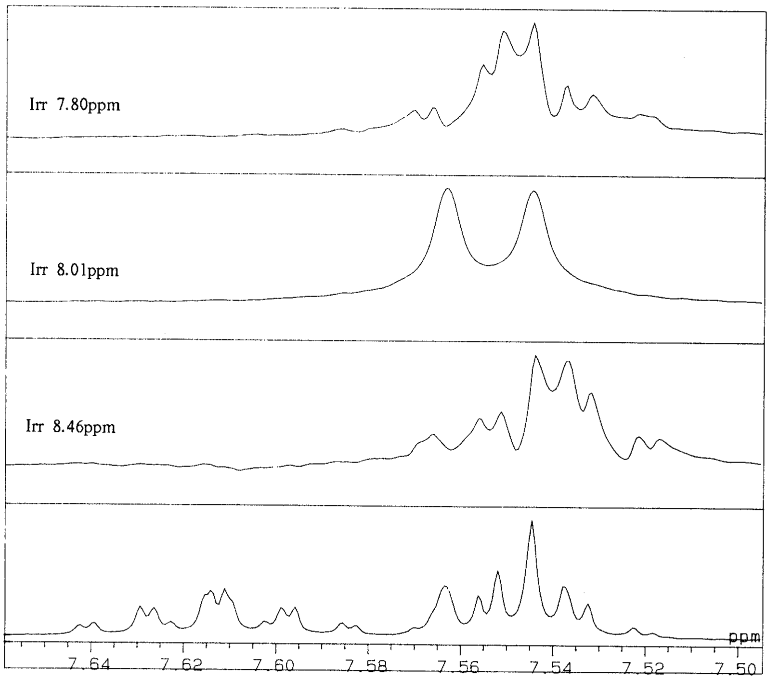

Fig. 3. Proton HOHAHA Spectra of 7,12-Dimethylbenz[*a*]anthracene
In the aromatic region.

Results and Discussion

Complete Assignment of 7,12-Dimethylbenz[*a*]anthracene (1) The structural numbering scheme for compound (1) is presented in Fig. 1 and the pertinent ^{1}H and ^{13}C chemical shifts (δ's) are summarized in Table 1. A detailed explanation of the assignment is as follows. Figure 2 shows the ^{1}H-NMR spectrum. The most downfield signal at $\delta = 8.46$ can be assigned to H1 explicitly.

In order to resolve the overlapping lines around $\delta = 7.5$, DQF-COSY, proton HOHAHA measurements were performed at 500.0 MHz in $CDCl_3$ solution. On irradiation of H1 and the protons at $\delta = 8.01$ and 7.80, the signals at $\delta = 7.53$, 7.56 and 7.55 were enhanced, respectively, as shown in Fig. 3.

This reveals that connectivity exists between H1 and the proton at $\delta = 7.53$, the protons at $\delta = 7.55$ and 7.80, and the protons at $\delta = 7.56$ and 8.01. From these findings, the signals at $\delta = 7.53$, 7.55, 7.80, 7.56, and 8.01 are straightforwardly assigned to H2, H3, H4, H5, and H6. Correlation can also be recognized between the signals at $\delta = 8.33$ and 7.60 and between the signals at $\delta = 8.36$ and 7.62 in the DQF-COSY spectrum. These correlations

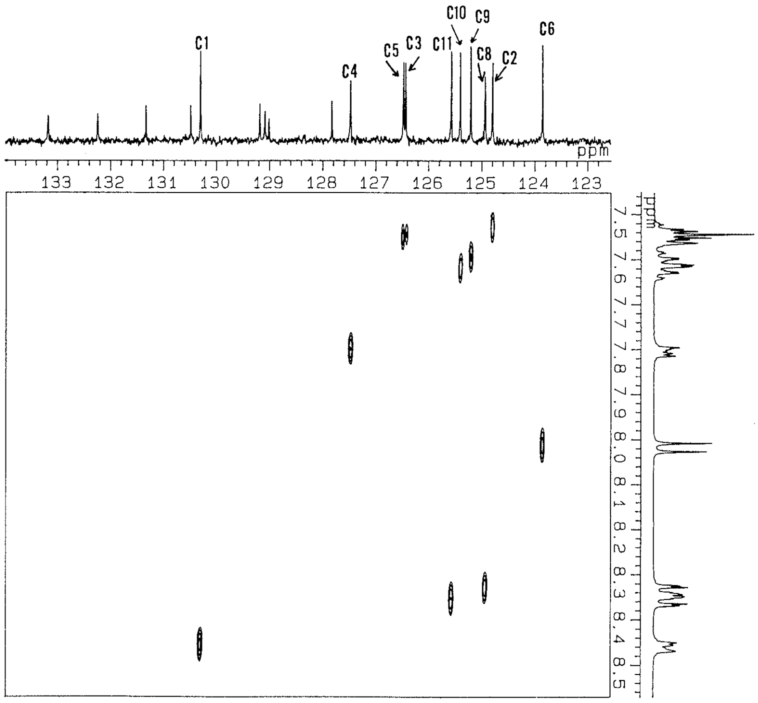

Fig. 4. ^{13}C–^{1}H COSY Spectra of 7,12-Dimethylbenz[*a*]anthracene
In the aromatic region.

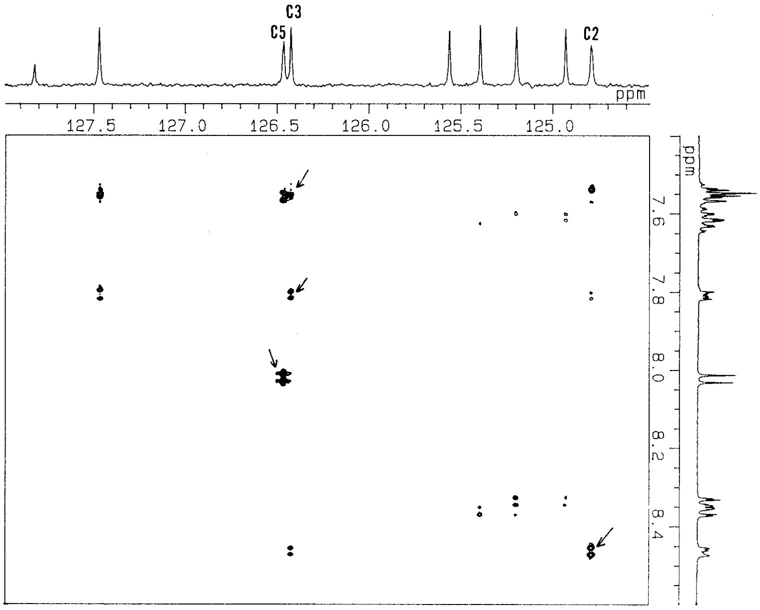

Fig. 5. ^{13}C–^{1}H HOHAHA Spectra of 7,12-Dimethylbenz[*a*]anthracene
In the aromatic region.

are considered to arise from the interaction between the H8 and H9 protons, and the H11 and H10 protons. Signal enhancement of the H8 proton by irradiation of the C7-CH_3 protons was observed, and also that of the H11 proton by irradiation of the C12-CH_3 protons was observed.

Heteronuclear correlation between the ^{13}C–^{1}H δ can be seen in Fig. 4.

The carbon resonances at $\delta=125.24$, 125.39, 126.42, 126.46, 124.93, 125.56 and 130.29 correlate with the proton resonances at $\delta=7.60$, 7.62, 7.55 (H3), 7.56 (H5), 8.33, 8.36 and 8.46 (H1), respectively. Therefore, the signals at $\delta=126.42$, 126.46, and 130.29 are assigned to C3, C5, and C1, respectively. Two-dimensional ^{13}C–^{1}H COSY spectra permitted distinction between C9 ($\delta=125.39$) and C10 ($\delta=125.24$), C3 ($\delta=126.42$) and C5 ($\delta=126.46$), and C11 ($\delta=125.56$) and C8 ($\delta=124.93$).

From the ^{13}C–^{1}H HOHAHA spectrum (Fig. 5), correlations were observed between the signals of C2 ($\delta=124.79$) and H1, of C3 ($\delta=126.42$) and H2, and of C5 ($\delta=126.46$) and H6. Thus, these findings give additional support to the assignment of H2, H3, H5, C3, and C5. Quaternary carbon atoms (C4a, C6a, C7, C7a, C11a, C12, C12a, and C12b) were assigned on the basis of the ^{13}C–^{1}H COLOC measurements. A long-range heteronuclear correlation between ^{13}C–^{1}H δ was shown

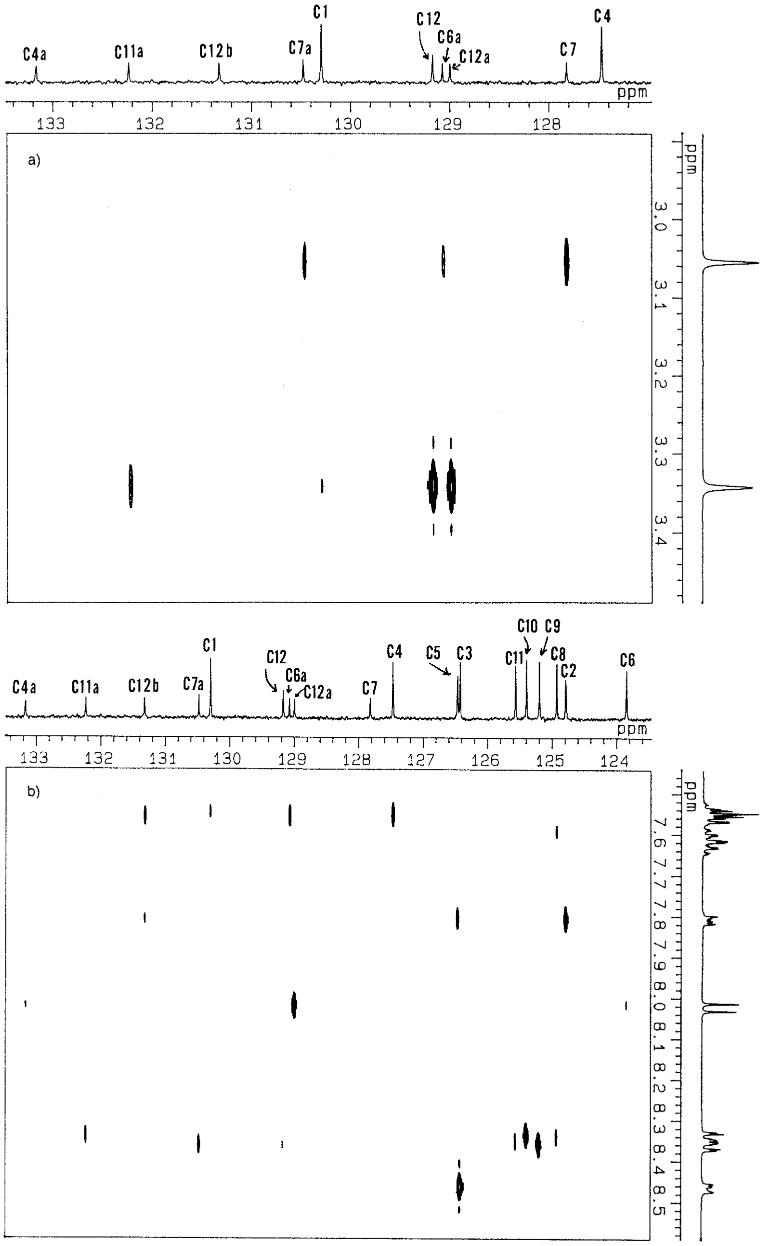

Fig. 6. ^{13}C–^{1}H COLOC Spectra of 7,12-Dimethylbenz[*a*]anthracene
a) in the methyl region; b) in the aromatic region.

by the ^{13}C–^{1}H COLOC measurement (Fig. 6 a, b). In this spectrum, correlations of both signals $\delta = 132.23$ and 129.00 with 12-CH_3 protons, and of both signals $\delta = 130.48$ and 129.07 with 7-CH_3 protons are observed (Fig. 6a). Correlations of C9 with H11, C10 with H8, the carbon at $\delta = 133.16$ with H6, the carbon at $\delta = 131.32$ with H4, and the carbon at $\delta = 131.32$ with H2 are also observed (Fig. 6b).

Relations of ^{13}C-NMR Chemical Shift (δ) and the Carcinogenicity of Benz[*a*]anthracenes Table 1 shows the δ's of compounds **1**—**3** (compounds **1** and **2** are carcinogens and compound **3** is a noncarcinogen). The δ values of the carbon atoms, C1, C2, C6 and C8, differ by greater than 2 ppm between the carcinogenic and noncarcinogenic compounds. The positions of C4 and C8 are possibly interchanged in compound **3**. Even in that case, the δ value for the C8 atom of compound **3** is larger by more than 2.5 ppm than those for compound **1** and compound **2**. In particular, the δ values for C1 and C2 of **1** (very strongly carcinogenic) are much larger than those of compound **3** (noncarcinogenic); it is noted that the large δ value for the C1 atom for compound **3** can be partially attributed to a downfield shift due to steric effects from the methyl group. The C1 and C2 positions are in the so-called B region, *i.e.*, the site of final epoxidation on the terminal ring of the bay region.[16] Moreover, the C6 position corresponds to the K-region. This suggests that the correlation between δ and carcinogenicity for BAs is originated from the notion that the δ values reflect the degree of activation of the B- and K-regions.

As described in our introduction, the chemical shifts should correlate to the electronic states of all carbon atoms of each PAH. To examine the relationship of the chemical shifts and the electronic states with respect to carcinogenicity, we are now performing theoretical calculations for the three BAs.

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