Relations between the Averaged ^{13}C Nuclear Magnetic Resonance Chemical Shift and the Carcinogenic Activity of Polycyclic Aromatic Hydrocarbons

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^{13}C Nuclear magnetic resonance (^{13}C-NMR) spectra were measured for polycyclic aromatic hydrocarbons (PAH) to examine the correlation between their chemical shift and carcinogenicity. We confirmed our previous proposition that the carcinogenicity of PAH molecules can be predicted from the value of the averaged ^{13}C-NMR chemical shift. It was also found, using the averaged chemical shift as a parameter, that several quantum chemical indices for the intermediate states of the metabolic transformation are correlated with the carcinogenic activity of PAH. This indicates that the averaged chemical shift can be applied to investigate the metabolic transformation of carcinogenicity.

Keywords averaged chemical shift; ^{13}C-NMR; carcinogenicity; polycyclic aromatic hydrocarbons; index range; dihydrodiol epoxide; carbocation; metabolic transformation; molecular orbital calculation

Introduction

There is wide variety in the carcinogenicity of polycyclic aromatic hydrocarbons (PAH). As is well known, 3,4-benzopyrene has very strong carcinogenic activity, whereas its geometrical isomer, 1,2-benzopyrene, is non-carcinogenic. Various approaches have been taken to investigate the carcinogenicity of PAH,[1-3] many of them based on quantum chemical calculations. The molecular orbital theory has been applied[4,5] to examine the carcinogenic activity and metabolic transformation of PAH. In these studies, several parameters derived from the molecular orbital theory such as the charge density and the superdelocalizability were used as an index to predict whether the molecule in question has carcinogenic activity, or to ascertain whether a reaction scheme presumed for the metabolic transformation is valid.

Relatively few attempts have been made to develop an experimental index for estimating carcinogenic activity. For such a purpose, we have focused on the data of nuclear magnetic resonance (NMR). ^{13}C-NMR chemical shifts of aromatic compounds are determined by the electronic environments of individual carbon atoms, which are closely related to quantum chemical quantities. According to a theoretical consideration of the shielding of ^{13}C in aromatic hydrocarbons,[6] the chemical shift for each carbon atom can be expressed as $\delta \propto (1/\Delta E) \cdot Q$, where ΔE is excitation energy and Q is a quantity representing the electronic states. ΔE and Q are fundamental quantities for calculating quantum chemical indices related to carcinogenicity. Therefore, it is expected that we can indirectly get information on the carcinogenicity of the aromatic compounds through the δ_{av} values in their ^{13}C-NMR spectra.

In our previous papers,[7,8] we have indeed shown that there is a correlation between the carcinogenic activity of PAH and their averaged ^{13}C-NMR chemical shifts, δ_{av}, for all the carbon atoms in the rings of each molecule: The averaged chemical shifts of known carcinogenic PAH molecules are within the range $\delta_{av} = 126.80$ to 127.87 ppm.

In this paper, we summarized and extended the previous work to clarify the δ_{av}–carcinogenicity relationship.[7,8] Eighty-one PAH molecules in total were examined. The result provided strong support for our proposition that the carcinogenic activity of PAH molecules correlates with the averaged ^{13}C-NMR chemical shift. Moreover, to study the metabolic transformation of carcinogenicity of PAH, their carcinogenic activities were plotted against δ_{av} and quantum chemical indices for the intermediate states of the transformation which are taken from the Hückel molecular orbital calculations. Fairly good correlation was found between the activities and the indices for an appropriate region of δ_{av}. This suggests that δ_{av} can be used as a parameter to investigate the carcinogenic activity of PAH.

Results and Discussion

Averaged ^{13}C-NMR Chemical Shift (δ_{av}) and Carcinogenicity The 81 PAH molecules examined are tabulated in Table I with the values of their averaged chemical shift (δ_{av}) and their carcinogenicity. The $\pm$ signs denote an estimation of the carcinogenic activity taken from the literature of animal experiments.[1,9-18] The activity increases in the order of $-$, $\pm$, $+$, $++$, $+++$, $++++$.

Figure 1 shows a plot of the carcinogenic activities of the PAH molecules listed in Table I against δ_{av}. It can be clearly seen that many of the compounds with δ_{av} values falling in the range of 126.80—127.87 ppm exhibit strong carcinogenic activity. Though a few compounds, such as **5**, **6**, **10** and **48**, which have been reported to be noncarcinogenic, are located in this δ_{av} range, the fact that strongly carcinogenic compounds give almost the same δ_{av} value is clear. This confirms that there is a significant correlation between the carcinogenicity and the averaged chemical shift. Moreover, the finding leads to expectation that the carcinogenic activity of PAH molecules can be predicted by using their δ_{av} values. The δ_{av} range, 126.80—127.87 ppm, can be regarded as an index which discerns the carcinogenicity of PAH; it is referred to as the "index range". Compounds with the $\pm$ sign in Fig. 1, for which carcinogenic activity has not been determined, are predicted to be carcinogenic if their δ_{av} values are within this index range.

In general, the carcinogenic phenomenon is very complicated; the parent molecules undergo verious metabolic transformations so that the properties of all metabolic intermediates are taken into account as well as those of the parent molecules in order to predict the carcinogenic activity. From this widely accepted view, the present finding that the δ_{av} values of strongly carcinogenic com-

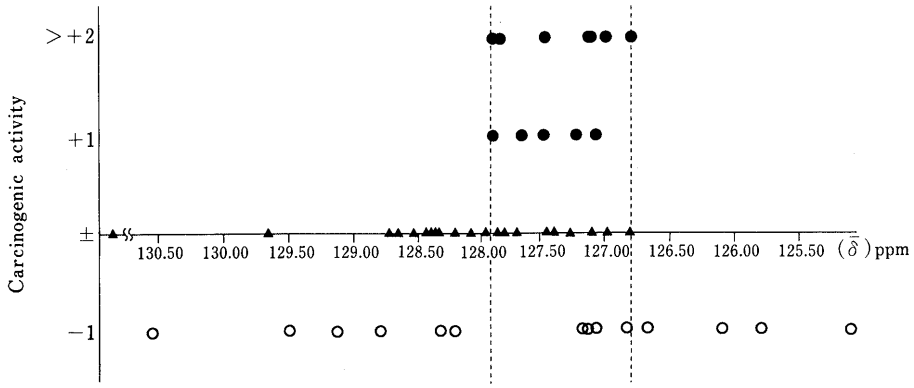

Fig. 1. A Plot of the Carcinogenic Activity Taken from the Literature on Animal Experiments *vs.* the Averaged ^{13}C-NMR Chemical Shift (δ_{av}) Showing the Position of Compounds.

TABLE I. Averaged ^{13}C-NMR Chemical Shifts over All the Ring Carbons of Polycyclic Aromatic Hydrocarbons and Their Carcinogenicities

Compound	Averaged chemical shift (ppm)	Predicted carcinogenicity[a]	Carcinogenicity literature[b]
Naphthalene (**1**)	128.28		− (1, 9, 10)
Anthracene (**2**)	128.17		− (1, 9, 11)
Phenanthrene (**3**)	128.68	−	± (9, 10, 11)
Benz[*a*]anthracene (**4**)	127.86		+ (9, 10, 11)
Pyrene (**5**)	127.16	+	− (9, 11)
Triphenylene (**6**)	126.82	+	− (1, 9, .18)
Dibenz[*a,c*]anthracene (**7**)	127.21		+ (1, 9, 11)
Dibenz[*a,h*]anthracene (**8**)	127.83		+ + (1, 9, 11)
Benzo[*a*]pyrene (**9**)	127.11		+ + + (9, 10, 11)
9,10-Dimethylanthracene (**10**)	127.06	+	− (18)
7-Methylbenz[*a*]anthracene (**11**)	127.47		+ (18)
7,12-Dimethylbenz[*a*]-anthracene (**12**)	127.87		+ + + (9, 18)
1-Methylnaphthalene (**13**)	128.08[19]	−	
2-Methylnaphthalene (**14**)	128.75[19]	−	
1,2-Dimethylnaphthalene (**15**)	128.45[19]	−	
1,3-Dimethylnaphthalene (**16**)	128.82[19]	−	
1,4-Dimethylnaphthalene (**17**)	128.02[19]	−	
1,5-Dimethylnaphthalene (**18**)	128.10[19]	−	
1,6-Dimethylnaphthalene (**19**)	128.76[19]	−	
1,7-Dimethylnaphthalene (**20**)	128.83[19]	−	
1,8-Dimethylnaphthalene (**21**)	130.87[19]	−	
2,3-Dimethylnaphthalene (**22**)	129.26[19]	−	
2,6-Dimethylnaphthalene (**23**)	129.52[19]	−	
2,7-Dimethylnaphthalene (**24**)	129.73[19]	−	
2,3,5-Trimethylnaphthalene (**25**)	129.32[19]	−	
2,3,6-Trimethylnaphthalene (**26**)	129.96[19]	−	
1,6,7-Trimethylnaphthalene (**27**)	129.26[19]	−	
1,5,8-Trimethylnaphthalene (**28**)	130.82[19]	−	
1-Methylanthracene (**29**)	127.96[20]	−	
2-Methylanthracene (**30**)	128.61[20]	−	
9-Methylanthracene (**31**)	127.57[20]	+	
1,4-Dimethylanthracene (**32**)	127.97[20]	−	
1,8-Dimethylanthracene (**33**)	127.99[20]	−	
1,4,9-Trimethylanthracene (**34**)	128.59[20]	−	
2,7,9-Trimethylanthracene (**35**)	128.33[20]	−	
1,4,5,9-Tetramethylanthracene (**36**)	127.94[20]	+	
1,4,5,8-Tetramethylanthracene (**37**)	127.94[20]	+	
1,4,5,8,9-Pentamethylanthracene (**38**)	129.83[20]	−	
Dibenzo[*a,l*]pyrene (**39**)	127.47	+	+ + (11)
Phenanthro[4,5-*bcd*]thiophene (**40**)	127.64	+	+ (11)
Dibenzo[*a,i*]pyrene (**41**)	126.99	+	+ + + + (11)
Dibenzo[*a,h*]pyrene (**42**)	126.80	+	+ + + + (11)
Benzo[*e*]pyrene (**43**)	126.66	−	− (11)
Naphtho[1,2,3,4-*def*]chrysene (**44**)	127.47	+	+ + + (11)
Picene (**45**)	126.08	−	− (1, 12)
Coronene (**46**)	125.78	−	− (1, 12)
1-Methylbenz[*a*]anthracene (**47**)	129.47	−	− (11)
Benzo[*b*]chrysene (**48**)	127.12	+	− (11)
Benzo[*g*]chrysene (**49**)	127.12	+	+ + (1, 13)
Benzo[*c*]phenanthrene (**50**)	128.40	−	± (11)
Anthanthrene (**51**)	126.97	+	± (11)
Benzo[*ghi*]perylene (**52**)	126.79	+	± (11)
Cyclopenta[*cd*]pyrene (**53**)	128.29	−	± (11)
Chrysene (**54**)	127.10	+	± (11)
Benzo[*c*]chrysene (**55**)	127.92	−	± (11)
Indeno[1,2,3-*cd*]pyrene (**56**)	128.17	−	± (13)
Perylene (**57**)	128.32	−	± (13)
Rubrene (**58**)	130.57[21]	−	
Dibenzo[*a,j*]anthracene (**59**)	127.06	+	+ (11)
9-Phenylanthracene (**60**)	129.83[22]	−	
9,10-Diphenylanthracene (**61**)	130.64[22]	−	
9,10-Dihydroanthracene (**62**)	129.94[23]	−	
Acenaphthylene (**63**)	129.15[24]	−	
Acenaphthene (**64**)	130.96[24]	−	
1,2,2a,3,4,5-Hexahydro-acenaphthene (**65**)	132.36[25]	−	
1-Methylphenanthrene (**66**)	127.66	+	± (13)
2-Methylphenanthrene (**67**)	128.23[26]	−	
3-Methylphenanthrene (**68**)	128.20[19]	−	
3,6-Dimethylphenanthrene (**69**)	128.68[27]	−	
1,2,3,4-Tetrahydrophenanthrene (**70**)	128.56[28]	−	
1,2,3,4,5,6,7,8-Octahydrophenanthrene (**71**)	131.94[28]	−	
1-Methylchrysene (**72**)	127.44	+	± (11)
2-Methylchrysene (**73**)	128.49	−	± (11)
3-Methylchrysene (**74**)	127.76	+	± (11)
4-Methylchrysene (**75**)	128.61	−	± (11)
5-Methylchrysene (**76**)	128.34	−	± (11)
6-Methylchrysene (**77**)	127.38	+	± (11)
Benzo[*b*]naphtho[1,2-*d*]-thiophene (**78**)	129.62	−	± (11)
Benzo[*b*]naphtho[2,1-*d*]-thiophene (**79**)	131.53	−	± (11)
Benzo[*b*]naphtho[2,3-*d*]-thiophene (**80**)	128.04	−	± (11)
Benzo[2,3]phenanthro[4,5-*bcd*]-thiophene (**81**)	127.83	+	± (11)

a) −: For noncarcinogenic, and +: for carcinogenic. *b*) Carcinogenicity reported by reference. Most of the data are from biochemical experiments. −: noncarcinogenic, +: carcinogenic, ±: noncarcinogenic or carcinogenic, + +, + + +, + + + +: increasing carcinogenic activity.

pounds concentrate in the "index range", is very interesting, because the NMR measurements were performed only for the parent molecules in our study. This suggests that, in a sense, properties of the parent molecules predetermine properties of the later metabolic transformation. In the following section, we will discuss the chemical significance of the averaged chemical shift in the metabolic transformation processes of carcinogenicity.

Relation of the Averaged ^{13}C-NMR Chemical Shift (δ_{av}) and the Quantum Chemical Indices with Respect to Carcinogenicity According to the "bay-region theory" of carcinogenicity, generally the metabolic transformations of PAH consist of the following four steps[4]: (1) a PAH molecule is activated via cellular monooxygenase to form an epoxide, (2) the epoxide converts to a dihydrodiol with the assistance of epoxide hydrase, (3) the dihydrodiol is transformed to dihydrodiol epoxide, and (4) the dihydrodiol epoxide converts spontaneously to the triol carbocation. These transformations are illustrated in Fig. 2 using the example of 3,4-benzopyrene. Carbonium ions produced in the final step are thought to act as ultimate carcinogens *via* electrophilic attack on critical cellular nucleophiles, *e.g.*, deoxyribonucleic acid.

The processes described above have been partially verified experimentally, but ambiguities remain. Since it is difficult to provide actual proof of all the processes, an alternative means is molecular orbital calculations. In such a study, the energy change in each step or the reactivity of each carbon atom in the molecule is chosen as a quantitative measure to evaluate carcinogenic activity, and a correlation is sought between the computational results and the experimental estimation of carcinogenicity.

It has been found experimentally that the formation of dihydrodiol epoxide and the subsequent formation of epoxide carbocation of a PAH molecule are important in the carcinogenic process; the easier the formation of carbocation is, the larger the carcinogenic activity becomes. The feasibility of carbocation formation can be estimated by calculating the change in the delocalization energy in step (4), $\Delta E(3)$: $\Delta E(3) = E_{de} - E_{cc}$, where E_{de} and E_{cc} are the delocalization energies of the diol epoxide and benzylic carbocation, respectively. As $\Delta E(3)$ increases, the formation of carbocation becomes easier, which may result in strong carcinogenic activity.

In fact, positive correlations have been found between $\Delta E(3)$ and the carcinogenic activity of PAH molecules.[29,30] Besides $\Delta E(3)$, many quantum chemical indices have been examined with respect to their carcinogenic activity; some of them are the delocalization energy differences in steps (1) and (3) ($\Delta E(1)$ and $\Delta E(2)$), the B-region π-electron bond order of the A-region dihydrodiol (P'_B), and the electron density (Q_b), superdelocalizability (S_b) and free valence (F_b) at the benzylic carbon position of the carbocation.[4]

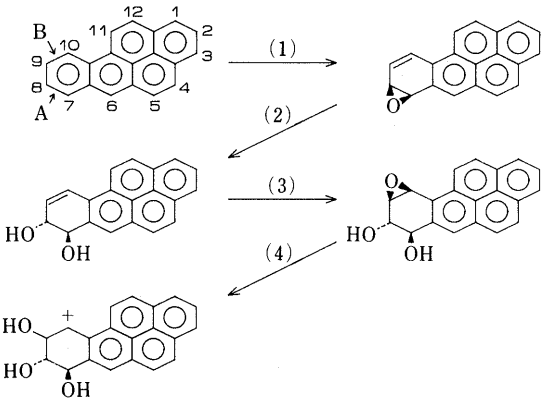

Fig. 2. Metabolic Transformation of 3,4-Benzo[*a*]pyrene[4]

Although such quantum chemical indices are easily obtained, their reliability is always a serious problem. There are well-known limitations in the molecular orbital calculation; for example, the simple Hückel molecular orbital (HMO) method is most appropriate for calculating properties of planar molecules but is less reliable for non-planar molecules. Furthermore, it cannot be difinitely specified which quantum chemical index is "best" to use for predicting the carcinogenic activity, or which intermediate in the metabolic transformation determines the carcinogenicity. An experimental index would be very useful in overcoming such difficult points. For this reason, we have chosen δ_{av} of parent PAH molecules as such an index.

We investigate the relationship between quantum chemical indices and carcinogenicity taking into account the correlation between the carcinogenicity and δ_{av} described in the previous section. Twenty four PAH are chosen because their theoretical indices were available in the literature.[4] Those indices were, however, calculated by the HMO method and the validity of its use must be checked. As seen in Fig. 2, only the terminal ring of the bay region is successively changed in the metabolic transformation. Moreover, though the ring is partly saturated, the motion of its component atoms is restricted since the ring structure remains still. Insofar as the geometrical planarity is concerned then, the intermediates illustrated in the figure deviate only slightly from the planar parent molecule. This supports the validity of applying the HMO method to the metabolic transformation based on the "bay region theory", and we can continue our discussion.

To survey correlations among the three quantities, we draw three dimensional plots of the carcinogenicity taken to be in the Z direction against δ_{av} and theoretical indices to be in the X and Y direction, respectively. First, we note the delocalization–energy changes in steps (1), (3), and (4), *i.e.*, $\Delta E(1)$, $\Delta E(2)$, and $\Delta E(3)$. Figure 3a—c shows their three dimensional plots.

The plots show no correlation between δ_{av} and each theoretical index; the data points projection from the Z direction are randomly scattered in the X–Y plane. $\Delta E(2)$ and $\Delta E(3)$ correlate fairly well to the carcinogenic activity, but $\Delta E(1)$ does not. Then we take into account δ_{av}. For a certain region of δ_{av}, the activity correlates more significantly with the quantum chemical indices, becoming larger as $\Delta E(2)$ decreases and $\Delta E(3)$ increases. Step (3) is predicted to advance with decreasing $\Delta E(2)$, because this process is the one in which delocalization energy of the π-electrons is reduced. On the other hand, step (4) should proceed as $\Delta E(3)$ increases because of an increase in delocalization of the π-electrons. Such predictions are seen in Fig. 3b and c.

Figure 3d—g shows three dimensional plots of the activity with respect to P'_B of the dihydrodiol, and Q_b, S_b, and F_b of the carbocation. A feature similar to that in $\Delta E(2)$ and $\Delta E(3)$ can also be observed in the plots: correlations between the carcinogenicity and the indices are revealed more clearly, by taking δ_{av} into account. The activity increases as P'_B is reduced. This is understandable because P'_B is related to activation of the B-region bond of the dihydrodiol and hence to the progress of the succeeding transformation processes; a decrease in P'_B frees the localization of the π-electrons of the B-region bond, which permits easy formation of the epoxide. The activity also becomes higher with an increase

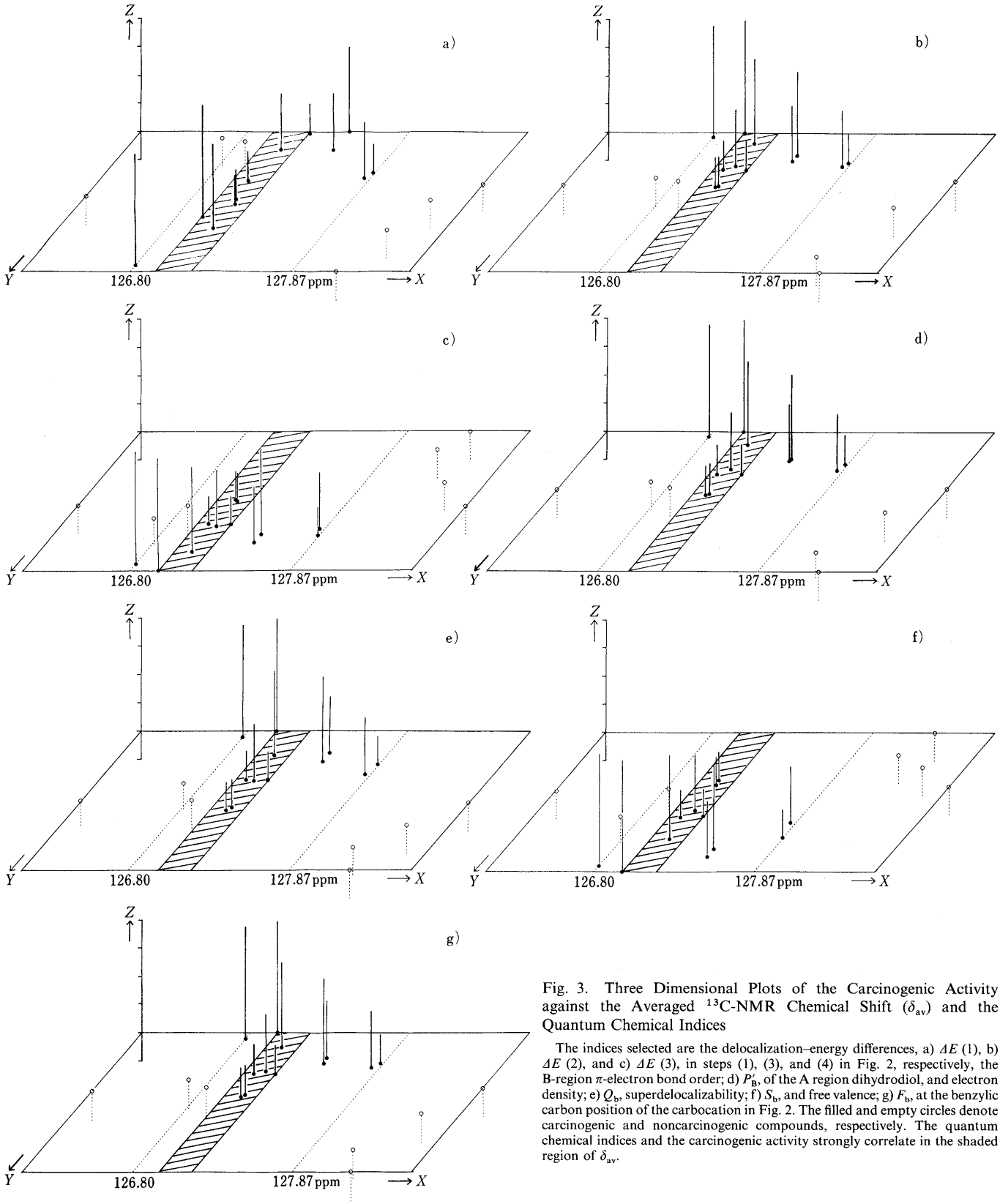

Fig. 3. Three Dimensional Plots of the Carcinogenic Activity against the Averaged ^{13}C-NMR Chemical Shift (δ_{av}) and the Quantum Chemical Indices

The indices selected are the delocalization–energy differences, a) ΔE (1), b) ΔE (2), and c) ΔE (3), in steps (1), (3), and (4) in Fig. 2, respectively, the B-region π-electron bond order; d) P'_B, of the A region dihydrodiol, and electron density; e) Q_b, superdelocalizability; f) S_b, and free valence; g) F_b, at the benzylic carbon position of the carbocation in Fig. 2. The filled and empty circles denote carcinogenic and noncarcinogenic compounds, respectively. The quantum chemical indices and the carcinogenic activity strongly correlate in the shaded region of δ_{av}.

in S_b and with an decrease in Q_b and F_b. These correlations are consistent with the "bay region theory" which predicts that the carbocation is more stabilized with an increase in S_b and with an decrease in Q_b and F_b.

The finding that the carcinogenic activity changes correlatively with the quantum chemical indices can be explained as follows: PAH molecules with almost the same δ_{av} value are considered to be in similar electronic states, because δ_{av} strongly depends on excitation energy and electronic states, as stated in the Introduction. This means that the initial state in the metabolic transformation of carcinogenicity resembles all the molecules with the same

chemical shift; as a result, the degree of carcinogenicity is mainly determined by the intermediate states in the transformation. In the present case, the quantum chemical indices represent the properties of the intermediate states, *i.e.*, delocalization energy, reactivity, and electron density. These properties dominate the reactions leading to carcinogenesis. Therefore, the carcinogenicity depends on the indices which specify the properties of the intermediate states, and it correlatively changes with the indices. Conversely, such correlative changes provide evidence that the metabolism scheme assumed and the indices chosen are adequate. Moreover, such changes enable prediction of the carcinogenic activity quantitatively on the basis of δ_{av} for the parent molecules and the indices for their metabolites.

The compounds situated outside the δ_{av} region described above are considered to be different from those inside the region in the initial state of the metabolic transformation. Consequently, they should be treated separately; insufficient data prevents detailed discussion at this stage.

Conclusion

We have shown that it is possible to use the "index range" of the averaged ^{13}C-NMR chemical shift to classify PAH compounds as carcinogenic or noncarcinogenic. Detailed experimental data are needed because the carcinogenic activity varies a good deal depending on the selection of experimental animals, the method of application, experimental conditions, *etc.* From this point of view, the data of ^{13}C δ_{av} are very useful for carcinogenic examination and for furthering the knowledge of carcinogenicity. We have also shown that several quantum chemical indices which are related to the metabolic transformation of carcinogenicity are correlated with the carcinogenic activity of the PAH molecules, using ^{13}C δ_{av} as a parameter. This finding shows that the averaged chemical shift is applicable for study of the metabolic transformation. In addition, NMR measurements of the parent molecules will open a new path in prediction of the degree of carcinogenicity.

Experimental

We measured ^{13}C-NMR spectra of twelve compounds **1**—**12** among eighty-one commercial products.

The ^{13}C-NMR spectra were recorded with Fourier transform spectrometers, Hitachi R-900 FT and JEOL GX-400 FT, operating at 22.6 and 100.4 MHz, respectively, at 35 °C and at 27 °C, using an external lock system and a deuterated lock system. Deuteriochloroform was used as a solvent. The concentration of each sample solution was in the range 25—62% (w/v); the chemical shift was almost independent of the concentration within this range.

The chemical shifts were expressed in ppm relative to tetramethylsilane. Other experimental conditions were as follows: accumulation times, about 400—14000; data points, 16 k or 32 k; spectral width, 18000 Hz; flip angle 25°, and interval between pulses, 3 s.

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