Contribution to the study of carcinogenicity of polycyclic aromatic hydrocarbons and their azaanalogues Carcinogenesis in the excited triplet state

J. LEŠKA, G. KRČULOVÁ, and D. LOOS

Department of Organic Chemistry, Faculty of Natural Sciences, Komenský University, CS-842 15 Bratislava

Received 14 January 1985

The assumption that polycyclic aromatic hydrocarbons occurring in the excited triplet states, which are generated by sensitization due to the triplet carbonyl compounds, are able to exhibit carcinogenic effect was investigated by constructing the correlation of experimental indices of carcinogenicity of polycyclic aromatic hydrocarbons with the energy of their lowest triplet and the component of the electron transition moment in the direction of the largest dimension of a molecule. The statistical characteristics as well as pattern recognition show that the carcinogenesis by polycyclic aromatic hydrocarbons molecules in the excited triplet states is probable.

Предположение, что полициклические ароматические углеводороды в возбужденном триплетном состоянии, генерированном посредством сенсибилизирования триплетными карбонильными соединениями, могут обладать канцерогенными свойствами, было проверено посредством построения корреляции экспериментальных показателей канцерогенности PAH с величинами энергии их наинизшего триплета и с составляющей электронного переходного момента в направлении наибольшего размера молекулы. Статистические характеристики, а также pattern recognition свидетельствуют о том, что канцерогенез молекулами PAH в возбужденных триплетных состояниях вполне вероятен.

There are several phases which may be observed in the development of the theory of carcinogenicity of polycyclic aromatic hydrocarbons (PAH). In the first phase, the extraordinary reactivity of certain positions in these compounds expressed by quantum chemical indices [1—3] was considered to be cause of their carcinogenic incidence. A more precise criterion for estimating the carcinogenicity of PAH was given by the *A.* and *B. Pullman*'s theory of the K and L regions [4]. According to this theory, the K regions of the type with 9 and 10 position in phenanthrene by means of which the carcinogen is joined to cell components are a cause of the carcinogenic effect. On the other hand, the L regions of the type with 9 and 10 positions in anthracene produce a detoxification and thus reduce the

carcinogenic effect. The values of *ortho*- and *para*-localization energy are a quantitative measure for appreciating these contrary effects. The further phase is characterized by the knowledge that PAH must be metabolically activated for getting mutagenic, toxic, and carcinogenic. Now we know on the basis of investigation of a strongly effective carcinogen such as benzo[*a*]pyrene (BP) [5] that PAH are transformed by an enzyme into diolepoxide which is able to form the carbonium ion and thus may be bonded to the DNA base. Owing to these facts, the position of the oxiran ring and hydroxyl groups in the six-membered ring which has become saturated by that biochemical transformation is decisive for mutagenic as well as carcinogenic incidence. The experiments and calculations of stability of the carbonium ions arising from epoxides [6] show that those isomers are effective in which the oxiran ring and the remainder of the molecule form "bay region", *i.e.* exhibit a form of bay. However, the presence of bay region is not a sufficient condition for carcinogenic incidence. Some PAH or their azaanalogues have no carcinogenic effect in spite of the fact that they exhibit bay region. The cause consists in the circumstance that the four-step metabolic cycle giving rise to carcinogenicity may be interrupted by a reaction of other kind which is due to the reactivity of other positions. In this case, the theory of the K and L regions which assumes the detoxificating effect of the L regions obtains its satisfaction.

The mentioned reactivity of the molecules of PAH and the enzyme-catalyzed formation of metabolites concern the ground state of these PAH molecules. For estimating the carcinogenicity of PAH, the lowest excited states of PAH [7, 8] were also taken into consideration. A comparison of the excitation energies of PAH and amino acid tryptophan as possible bioreceptor, which ought to be (because of the greatest interaction) as close as possible, was taken as a criterion of incidence. It was found that the carcinogenic PAH should have the excitation energy in the interval of (3.24 ± 0.11) eV. However, this criterion appeared to be insufficient for explanation of all cases. It was disclosed that the consideration of a single property of carcinogen could not give reliable information. For this reason, a multi-parameter correlation [9, 10] was calculated. In this case, the reactivities of the K and L regions, excitation energies of the α- and p-bands as well as their difference and intensity of the β-band are put into the correlation relationship. A highly significant correlation was thus obtained for the investigated set of PAH molecules. Recently [11], the significance of parameters of this correlation was interpreted by means of perturbation expansion of the interaction energy of PAH molecule with a molecule of bioreceptor.

This contribution results from the assumption that PAH can exhibit a carcinogenic effect even in the excited triplet state, the formation of which from the ground state singlet is forbidden but it may be generated by interactions of the molecules of PAH with heavy atoms that are always present in great number in biological medium. Provided that a triplet state arises, it has much greater length of

life than a singlet one owing to which the probability of its effect increases. It is worth noticing that a possible effect of PAH in a triplet state arising from an excited singlet was suggested earlier [11]. Recently [12], it was evidenced that the triplet carbonyl molecules can be generated in great yield in biological medium during the oxidation of convenient substrates catalyzed by peroxidase. They function as sensibilizers and are able to introduce PAH molecules into an excited triplet state even without irradiation. The excited molecules of PAH can then induce cancer by interaction with the molecules of bioreceptor.

Our approach to the problem is fairly statistical. We correlated the experimental indices of carcinogenicity of a set of PAH molecules with the energy of their first triplet as well as with its intensity which was calculated by a quantum chemical method.

Results and discussion

The quantitative so-called Iball indices of carcinogenicity are available only for a small set of 10 molecules (Table 1). There are qualitative data for a number of other molecules. Besides the mentioned 10 molecules, we took into consideration 16 PAH molecules and their azaanalogues with qualitative data about carcinogenicity as stated in Table 1.

We calculated the electronic spectra of the above molecules by the PPP method. In order to include the activity in the ground state into the investigated carcinogenicity, we took into account the parameters according to the theory of K and L region. *Herndon* [13] used the Pauling bond orders which are in good correlation with localization energies according to his data [13] for the characterization of K and L regions. We used the Coulson bond orders calculated by the PPP method. For the ground state, we also took into consideration the parameter $\Delta = |n - 24|$ (n is the number of carbon atoms in a molecule of PAH) which proved to be significant in the calculations performed by Herndon. Altogether we took into account the parameters E_T, $|Q_T|$, p_K, p_L, and Δ. The symbols E_T, Q_T, and p_K, p_L stand for the excitation energy (in eV) of the first triplet with nonzero intensity (formally calculated by the PPP method), the absolute value of the electron transition moment (in relative dimensionless units), and the Coulson bond orders of K and L regions of the corresponding molecule, respectively. First of all, we investigated correlations with all possible combinations of the above parameters and linear relationships among them. However, it has been revealed that the parameter $|Q_T|$ is insignificant. On the other hand, it has appeared that the projection of electron transition moment in the direction of the largest dimension of molecule $|Q_{T,x}| = |Q_T| \cos \alpha$ is significant. The dimension is measured along the straight line which is either parallel or perpendicular to the CC or CN bond. As five

Table 1

Characteristics of polycyclic aromatic hydrocarbons and their azaanalogues

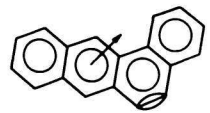
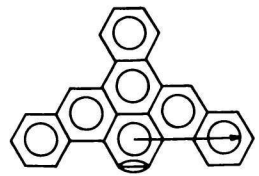
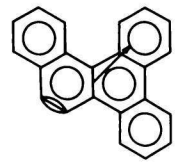
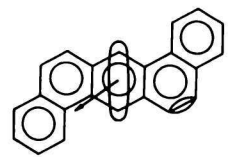
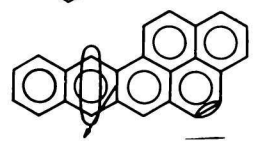
Number	Formula	E_T/eV[b]	$\lvert Q_{T,x}\rvert$	p_K	p_L	$\lvert\Delta\rvert$	I_c (exp.)	Iball qualit.
1		1.577	0.884	0.843	−0.345	6	5	–
2		2.042	4.282	0.765	−0.221	4	17	–
3		2.244	1.723	0.782	−0.221	2	18	+
4		1.956	2.200	0.835	−0.349	2	26	−[a]
5		1.239	2.397	0.854	−0.377	0	27	–

Table 1 (Continued)

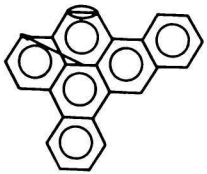
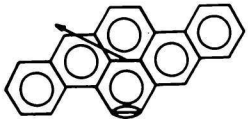
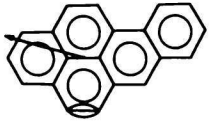
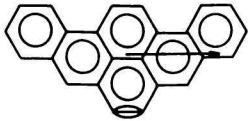
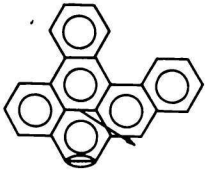
Number	Formula	E_T/eV[b]	$\lvert Q_{T,x}\rvert$	p_K	p_L	$\lvert\Delta\rvert$	I_c (exp.)	Iball qualit.
6		1.814	3.213	0.743	−0.221	0	50	+
7		1.188	3.902	0.824	−0.221	0	68	+
8		1.420	3.870	0.850	−0.221	4	72	+
9		1.531	4.418	0.861	−0.221	0	74	+
10		1.667	2.888	0.846	−0.221	0	82	+
11		1.550	0	0.756	−0.221	14		−

Table 1 (Continued)

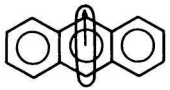
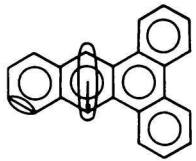
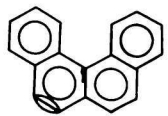
Number	Formula	E_T/eV[b]	$\lvert Q_{T,x}\rvert$	p_K	p_L	$\lvert\Delta\rvert$	I_c (exp.)	Iball qualit.
12		1.791	2.011	0.826	−0.221	10		−
13		1.092	0	0.785	−0.386	10		−
14		1.223	0	0.785	−0.357	10		−
15		1.257	0	0.790	−0.334	10		−
16		0.830	0	0.769	−0.369	6		−
17		1.988	0	0.764	−0.349	2		−
18		2.031	0	0.803	−0.221	6		+

Table 1 (Continued)

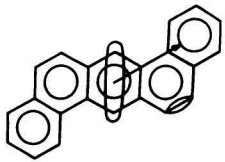
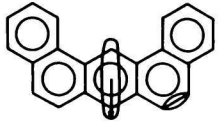
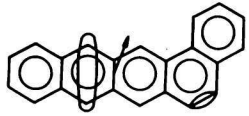
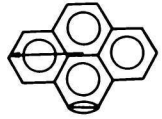
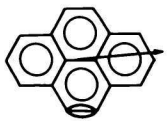
Number	Formula	E_T/eV[b]	$\lvert Q_{T,x}\rvert$	p_K	p_L	$\lvert\Delta\rvert$	I_c (exp.)	Iball qualit.
19		2.075	2.517	0.835	−0.324	2		+
20		1.914	0	0.836	−0.355	2		+
21		2.272	0	0.836	−0.320	2		+
22		1.152	0.428	0.849	−0.390	2		−
23		1.484	3.524	0.835	−0.221	8		−

Table 1 (Continued)

Number	Formula	E_T/eV[b]	$\lvert Q_{T,x}\rvert$	p_K	p_L	$\lvert\Delta\rvert$	I_c (exp.)	Iball qualit.
24		1.655	3.892	0.806	−0.221	8		–
25		1.941	0	0.830	−0.221	4		–
26		1.044	3.667	0.851	−0.221	2		–

a) There is an ambiguous datum about carcinogenicity in literature [14]. We have chosen the sign − according to value of the Iball index.
b) The meaning of symbols is explained in the text.

parameters represent a too great number for the sets of 10 and 26 molecules [14, 15], we put forward two correlations

$$I_c(E_T, |Q_{T,x}|) = a_0 + a_1 E_T + a_2 |Q_{T,x}| \quad (1)$$

$$I_c(p_K, p_L, \Delta) = b_0 + b_1 p_K + b_2 p_L + b_3 \Delta \quad (2)$$

The parameter Δ has not been included in correlation (*1*) because the influence of the size of molecule manifests itself already in the excitation energy E_T.

The actual forms of the correlations with Iball indices of the first ten molecules (Table 1) are

$$I_c(E_T, |Q_{T,x}|) = 49.392 - 27.508\ \{E_T\} + 13.561\ |Q_{T,x}| \quad (3)$$

$$I_c(p_K, p_L, \Delta) = -208.044 + 418.447 p_K + 315.005 p_L - 5.167\ \Delta \quad (4)$$

The *t* test gives 95 % significance for the parameters E_T and $|Q_{T,x}|$ and 99 %, 99.5 %, and 97.5 % significance for the parameters p_K, p_L, and Δ, respectively. It results from the *F* test that the overall significance of correlation (*3*) and correlation (*4*) is equal to 92 % and 99 %, respectively. The values of correlation coefficient *r* for eqns (*3*) and (*4*) are equal to 0.716 and 0.922 while the corresponding standard deviations are 22.530 and 13.510, respectively. A comparison of the overall significances of both correlations displays that the carcinogenic activity of the molecules of PAH in their ground state is more probable than in the excited triplet state. Nevertheless, the probability for carcinogenesis produced by the molecules of PAH in the excited triplet state is rather high as results from the 95 % significance of the parameters E_T and $|Q_{T,x}|$. A comparison of the statistical characteristics of correlation (*4*) with the data given by *Herndon* [13] who used the Pauling bond orders as parameters p_K and p_L shows resemblance, though the data by Herndon are somewhat better. The significance of the parameter Δ in our correlation is high, which means that the size of molecule is important for carcinogenesis. That is evidently due to geometrical conditions of the interaction between PAH molecules and bioreceptor molecules. According to *Sung* [10], the parameter Δ proved to be insignificant. It is, however, comprehensible, if we take into consideration that he used the first excitation energy calculated by the Hückel method as a parameter in his correlation. As known, this quantity is sensitive to structural alterations and depends on the size of molecule, too. Thus the parameter Δ is included in the excitation energy *E*. If other structural properties on which *E* is also dependent are much more significant than Δ, this parameter need not manifest itself as significant.

Fig. 1 represents the correlation of the experimental Iball indices with the characteristics $r = 0.719$ and $s = 20.976$ calculated according to eqn (*3*), the significance according to *F* test being 97.5 %. It can be seen that the correlation

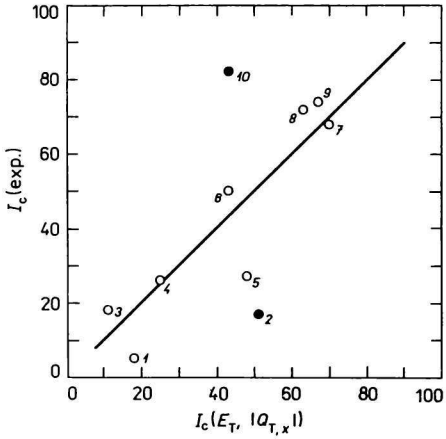


Fig. 1. Correlation between experimental and theoretical index of carcinogenicity as calculated according to eqn (*3*).

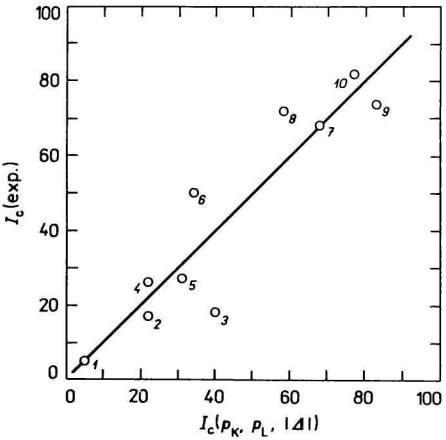


Fig. 2. Correlation between experimental and theoretical index of carcinogenicity as calculated according to eqn (*4*).

trend is evident. However, two points (tribenzo[*a*,*e*,*i*] pyrene and dibenzo[*a*,*l*]-pyrene) considerably deviate from the regression curve, which deteriorates the correlation. If we omit these points, the correlation is much improved (significance = 99.5 %, $r = 0.916$, $s = 11.689$).

Fig. 2 reproduces the correlation of the experimental Iball indices with the characteristics $r = 0.921$ and $s = 11.78$ calculated according to eqn (*4*), the significance according to F test being 99.5 %.

Since the set of 10 molecules as a statistical set is too small, we extended it by other 16 molecules of PAH and their azaanalogues for which only a qualitative statement of carcinogenicity, usually denoted by the sign + or −, is known. We used the data given in paper [16] which originated from other sources than the Iball indices. We have quantitatively expressed these two properties by the values 100 and 0.

The actual forms of correlation are

$$I_c(E_T, |Q_{T,x}|) = -55.640 + 50.576\,\{E_T\} + 7.066\,|Q_{T,x}| \qquad (5)$$

$$I_c(p_K, p_L, \Delta) = 172.542 - 50.583\,p_K + 220\,p_L - 6.825\,\Delta \qquad (6)$$

Irrespective of the fact that these correlations are only vague, the F test gives 95 % significance for eqn (*5*) and 99 % significance for eqn (*6*). However, the correlation coefficient is considerably decreased for both correlations while the standard deviation is increased. Thus the values of r and s are 0.505 and 43.503 for eqn (*5*) and 0.617 and 40.551 for eqn (*6*). The roughness of correlation manifests

itself by a change in significance of the parameters. The significance rose to 97.5 % for parameter E_T and dropped to 90 % for parameter $|Q_{T,x}|$. The significance increased to 99.5 % for parameter Δ, but decreased to 90 % for parameter p_L and for parameter p_K still more. The attempt to join all five parameters into one correlation results in a considerable decrease in significance of all parameters. It is obviously a consequence of a relatively small magnitude of the set of molecules. It is seen from these results that the parameter E_T in this set appears to be highly significant. The parameter $|Q_{T,x}|$ is also fairly significant. The fact that the absolute value of the electron transition moment $|Q_T|$ appears as insignificant parameter whereas its projection in the direction of the largest dimension of molecule is significant is evidently due to vigour of the interaction between PAH molecule and bioreceptor molecule. According to logical assumption, the greater is the interaction the tighter is the contact between molecules. The tightest contact must arise if the molecules touch one another in the directions of their largest dimension. Some structural formulae of molecules for which the electron transition moments are also outlined are represented in Table 1. The molecules are so positioned that their largest dimension (perpendicular or parallel to CC or CN bonds) is orientated in the direction of the coordinate axis x. According to the correlation (*6*), the parameter Δ is very significant for the reason stated earlier. Correlations (*5*) and (*6*) indicate that the carcinogenesis by the molecules of PAH and their azaanalogues in the ground state is more probable.

The roughness of the last correlations and consequent low significance of some parameters at the limit of acceptability as well as low values of correlation coefficient, to a certain extent, deteriorates the resulting conclusions. For this reason, we used the method of pattern recognition [17, 18]. The normalized distances d_{ij} of the points calculated in five-dimensional space for five parameters E_T, $|Q_{T,x}|$, p_K, p_L, $|\Delta|$ of 26 studied molecules form two aggregations. It is seen in Fig. 3 where the distances d of individual points from the centres of gravity of

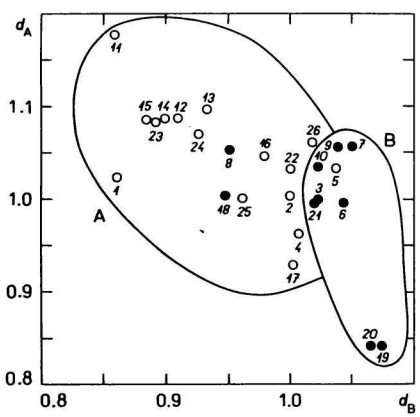


Fig. 3. Diagram of d_A against d_B according to the method of pattern recognition (○ noncarcinogenic; ● carcinogenic).

aggregations [19] corresponding to carcinogenic and noncarcinogenic molecules are plotted. The set of points in Fig. 3 is distinctly separated in two sub-sets A, B of which A contains the points of prevailingly noncarcinogenic and B the points of carcinogenic molecules.

A prediction of carcinogenicity of some PAH or azaanalogue which may be obtained from correlations (*2—6*) is evidently only probable and connected with possible occurrence of fluctuations. This probability may also be affected by the fact that the quantum chemical parameters have been calculated by the PPP method which cannot describe the triplet $n \rightarrow \pi^*$ transitions. As these transitions may occur in azaanalogues, the information put into correlations is not complete.

The results of this study lead to the conclusion that the constructed correlations between the parameters of ground and excited state and the qualitative and quantitative indices of carcinogenicity exhibit fairly high significance. However, only the values of some correlation coefficients are acceptable with respect to character of the problem. That impairs the possibility of unambiguous prediction of carcinogenesis not only by molecules in the ground state but also in the first excited state. But a fairly good separation of molecules in carcinogenic and noncarcinogenic ones by the method of pattern recognition by using equal parameters as in correlations upholds the assumption of possible carcinogenesis by PAH molecules in the ground state as well as in the first excited triplet state.

The calculations of molecule parameters were carried out by the quantum chemical PPP method with variable β-approximation [20] by means of a computer Siemens 4004/151 in the Institute of Computing Technique of Universities in Bratislava.

References

1. Pullman, A., *Ann. Chim.* (Paris) *2*, 5 (1947).
2. Greenwood, H. H., *Brit. J. Cancer 5*, 441 (1951).
3. Chalvet, O., Daudel, R., and Moser, C., *Acta Unio Int. contro Cancero 15*, 595 (1959).
4. Pullman, A. and Pullman, B., *Advan. Cancer Res. 3*, 117 (1955).
5. Levin, W., Wood, A. W., Wislocki, P. G., Chang, R. L., Kapitulnik, J., Mah, H. D., Yagi, H., Jerina, D. M., and Conney, A. H. in *Polycyclic Hydrocarbons and Cancer Chemistry, Molecular Biology and Environment.* (Gelboin, H. V. and P. O. P. T'so, Editors.) Academic Press, New York, 1978.
6. Lehr, R. E., Levin, W., Wood, A. W., Conney, A. H., Yagi, H., and Jerina, D. M., *Carcinog.-Compr. Surv. 3*, 129 (1978).
7. Birks, J. B., *Nature 190*, 232 (1961).
8. Mason, R., *Nature 181*, 820 (1958).
9. Sung, S. S., *Int. J. Quantum Chem. XII, Suppl. 1*, 387 (1977).
10. Sung, S. S., *Arch. Geschwulstforsch. 47*, 109 (1977).
11. Ulmer, W., *Theor. Chim. Acta 56*, 133 (1980).
12. Cilento, G., *Pure Appl. Chem. 56*, 1179 (1984).

13. Herndon, W. C., *Int. J. Quantum Chem., Quantum Biology Symp. 1*, 123 (1974).
14. Kuchař, M. and Rejholec, V., *Kvantitativní vztahy mezi strukturou a biologickou aktivitou.* (Quantitative Relationships between Structure and Biological Activity.) P. 77. Academia, Prague, 1980.
15. Topliss, J. G. and Castello, R. J., *J. Med. Chem. 15*, 1066 (1972).
16. Podaný, V., Vachálková, A., Miertuš, S., and Bahna, L., *Neoplasma 22*, 5 (1975).
17. Kowalski, B. R. and Bender, C. F., *J. Amer. Chem. Soc. 94*, 5632 (1972).
18. Hansch, C., Unger, S. H., and Forsythe, A. B., *J. Med. Chem. 16*, 1217 (1973).
19. Kowalski, B. R. and Bender, C. F., *J. Amer. Chem. Soc. 96*, 916 (1974).
20. Nishimoto, K. and Forster, L. S., *Theor. Chim. Acta 4*, 155 (1966).

Translated by R. Domanský