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APPLICATION OF A GEOMETRIC PARAMETER DEFINING MOLECULAR SHAPE, FOR THE QUANTITATION OF INTERACTION OF POLYCYCLIC AROMATIC HYDROCARBONS WITH ENZYME SYSTEMS

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Abstract—Quantitative relationships between the ability of polycyclic aromatic hydrocarbons to induce and repress various enzyme systems and a geometric parameter expressing their molecular shape have been described. In contrast, no statistically significant relationship was found when quantum-chemical parameters characterizing the reactivity and donor-acceptor properties of these compounds were correlated with their biological activity.

In our previous report [1], we established a correlation between the ability of PAH* to repress dimethylnitrosamine-demethylase and their shape parameter, η, defined as the ratio of the longer to the shorter side of the minimum rectangular envelope around the structure, drawn proportionally to atomic dimensions. The shape parameter extends the work of Arcos *et al.* [2] on the role of steric effects in the biological activity of PAH.

A drawback precluding extensive verification of the hypothesis is that graphically the shape parameter can be determined for ideally coplanar molecules only. However in another work [3], we reported a correlation between η and the experimental g.l.c. retention indices on both nematic (I_N) and isotropic non-polar (I_I) phases

$$\eta = 0.00281\ I_N - 0.00529\ I_I + 3.20. \quad (1)$$

The number of compounds considered has been $n = 14$, standard deviation $s = 0.1370$, and correlation coefficient $r = 0.9133$. Thus by means of equation (1) we can determine the shape parameter for both co-planar and non-planar molecules. The 'experimental' shape parameter, calculated using equation (1) is designated here as ω.

The objective of this work is to investigate the possible correlation between biological activity of PAH and the 'experimental' shape parameter ω.

PARAMETERS OF BIOLOGICAL ACTIVITY

The data on the induction of azo dye *N*-demethylase by PAH were taken from the report by Arcos *et al.* [4] and designated here as A_1. The parameter, A_1 represents the percentage of increase of *N*-demethylase activity. The same report [4] yielded the data on the shortened duration of zoxazolamine paralysis in rats pretreated with PAH; these data are designated here A_2. The parameter represents the percentage decrease in the duration of paralysis.

The data obtained by Hradec *et al.* [5] on the charging of isolated tRNA with L-methionine are designated here as A_3. The tRNA isolated from postmitochondrial supernatant following hydrocarbon pretreatment [5] was supplemented with aminoacyl-tRNA synthetase from *E. coli*, and tested for charging of initiator tRNA with L-methionine. The results are expressed as the ratio of pmole of methionine to nmole of tRNA. According to Hradec *et al.* [5] tRNA has been modified by the action of active intermediates resulting from the metabolic activation of PAH by microsomal enzymes.

The Ames mutagenicity test [6] requires prior enzymic conversion of hydrocarbons to biologically active species by the *S*-9 fraction added to the system. The data expressing this activity were taken from the paper by McCann *et al.* [7] and are designated as A_4.

All bioactivity of PAH are summarized in Table 1.

Table 1. Biological data used in regression equations

Compound	log A_1	log A_2	log A_3	log A_4
Naphthalene	0.95	1.60		−1.04
Anthracene	0.78	1.74	0.29	−2.00
Phenanthrene	0.78		0.37	−0.60
Acenaphthene	0.78	−2.00		
Fluorene	0.95	1.46		−2.00
Fluoranthene			0.31	
Pyrene	0.78		0.31	−1.70
Benz(*a*)anthracene	2.18		0.57	1.04
Chrysene	2.28		0.39	1.58
Benzo(*e*)pyrene			0.26	−0.22
Benzo(*a*)pyrene	2.34		0.72	2.08
Perylene	1.96			
3-Methylcholanthrene	2.38	1.95	0.73	1.76
Anthanthrene	2.35	1.97		
Dibenz(*ah*)anthracene	2.39	1.97	0.71	1.04
Coronene	0.95	−2.00		

For explanation of symbols and references see text.

* Abbreviation used: PAH, polycyclic aromatic hydrocarbons.

Table 2. Physicochemical parameters used in regression equations

Compound	ω	$^1\chi$	C	K_{HOMO}	$\Delta E_{1,1}$	K_{reg}	$\Delta E_{deloc/\beta}$	R
Naphthalene	1.473	3.405	10	0.616	1.236	3.56	0.488	−23.0
Anthracene	1.500	4.809	14	0.414	0.829	3.53	0.544	−23.9
Phenanthrene	1.356	4.815	14	0.605	1.210	3.36	0.658	−22.0
Acenaphthene	1.073	4.444	12					
Fluorene	1.399	4.611	13					−20.4
Fluoranthene	1.142	5.565	16					
Pyrene	1.161	5.559	16	0.445	0.890	3.33	0.664	
Benz(*a*)anthracene	1.605	6.220	18	0.425	0.905	3.29	0.766	−21.6
Chrysene	1.634	6.226	18	0.520	1.040	3.38	0.640	−22.4
Benzo(*e*)pyrene	1.147	6.975	20				0.714	−21.4
Benzo(*a*)pyrene	1.602	6.975	20	0.371	0.742	3.23	0.794	−22.4
Perylene	1.238	6.970	20	0.347	0.695			
3-Methylcholanthrene	1.406	7.685	21		1.460			
Anthanthrene	1.477	7.714	22		0.582	3.20		
Dibenz(*ah*)anthracene	1.568	7.631	22		0.947	3.30	0.738	−21.3
Coronene	1.053	8.464	24	0.539	1.078			

PHYSICOCHEMICAL PARAMETERS

The 'experimental' shape parameter ω, provides a significant improvement in describing the biological activity of polycyclic aromatic hydrocarbons. Numerical values of ω were taken from our earlier work [3]. Other parameters considered are related to the size and geometry of the PAH molecules. These include the connectivity index $^1\chi$ devised by Randič [8], and the number of carbon atoms in the PAH molecule, C, which are most readily computable. The connectivity indices were calculated after Kier and Hall [9]. An illustrative example of the calculations can be found elsewhere [10]. It should be noted that both the parameters related to the molecular size, and physicochemical parameters such as molar refractivity, water solubility and binding constants to human serum albumin, are linearly related to the number of carbon atoms in the molecule.

In view of suggestions that both the induction of aryl hydrocarbon hydroxylase (AHH) [11] and carcinogenicity of PAH [12] are due to electron transfer from protein to an unoccupied orbital of hydrocarbon, variables have been introduced into regression equations, which characterize acceptor–donor properties of PAH. The variables include absolute values of the highest occupied molecular orbital, k_{HOMO}; and the first electronic transition energy, $\Delta E_{1,1}$. The numerical values have been taken from the monograph of Kier [13].

The biological data on the mutagenicity of PAH have also been correlated with physicochemical parameters of the PAH derived from the *K*-region [14] and bay-region [15] hypotheses. The data characterizing the *K*-region have been obtained from Pullmans' report [14]. This is a complex index formed by a sum of bond localization energies (B. L. E.) and the smallest of the two possible carbon localization energies (C. L. $E._{min}$). This sum is referred to as K_{reg}. Values $\Delta E_{deloc/\beta}$ for carbonium ion formation at bay-region benzylic position were obtained from the article by Jerina and Lehr [14].

The bay-region characteristics are represnted by Loew *et al.* [16] describing reactivity of parent compounds to distal bay-region epoxidation expressed in units of thousandths of electron charge per eV. These are designated here as *R*.

All physicochemical data used in the regression equations are presented in Table 2.

REGRESSION EQUATIONS

Induction of azo dye N-*demethylase* (A_1)

The data used for deriving the regression equations are listed in Tables 1 and 2.

There is a significant correlation between induction of azo dye *N*-demethylase and parameters determining the shape and size of PAH molecules (ω and $^1\chi$ as well as ω and C)

$$\log A_1 = 2.24(\pm 1.09)\omega + 0.31(\pm 0.13)^1\chi - 3.42(\pm 1.71)$$
$$n = 14,\ s = 0.3625,\ r = 0.9044,$$
$$F_{1,11} = 21.39,\ F_{1,11p\leq 0.01} = 9.65, \quad (2)$$

$$\log A_1 = 2.17(\pm 1.21)\omega + 0.11(\pm 0.05)C - 3.39(\pm 1.91)$$
$$n = 14,\ s = 0.3949,\ r = 0.8854. \quad (3)$$

$F_{1,11}$ is sequential *F*-test value calculated after ω variable has been included into the equation. In parenthesis the *F*-test value is given for appropriate significance level. For all the equations presented in this paper, *F*-test values are given which are calculated after introducing the ω variable into equations describing the activity as a function of the bulk parameters C or $^1\chi$.

From statistical point of view it is also important to establish whether there is significant correlation

Table 3. Squared correlation matrix for equation (2)

	$\log A_1$	$^1\chi$	ω
$\log A_1$	1.0	0.3756	0.4638
$^1\chi$	–	1.0	0.0002
ω	–	–	1.0

between the independent variables used in a given regression equation. If the equation variables highly correlate with one another, then chance correlation may result. In Table 3 the square correlation matrix is given for the variables used in equation (2). As it may be seen from Table 3, ω and $^1\chi$ are practically independent.

The linear relations between log A_1 and parameters ω, $^1\chi$ and C are represented by equations (4)–(6).

$$\log A_1 = 2.24(\pm 1.09)\omega + 1.66$$
$$n = 14,\ s = 0.6399,\ r = 0.6129, \quad (4)$$
$$\log A_1 = 0.12\ C - 0.46$$
$$n = 14,\ s = 0.5993,\ r = 0.6276, \quad (5)$$
$$\log A_1 = 0.31\ ^1\chi - 0.33$$
$$n = 14,\ s = 0.5931,\ r = 0.6810. \quad (6)$$

Parameters determining acceptor–donor properties appear to be inessential for correlation with biological activity

$$\log A_1 = -2.48\ k_{HOMO} + 0.19\ ^1\chi + 1.57$$
$$n = 10,\ s = 0.7364,\ r = 0.5834, \quad (7)$$
$$\log A_1 = 0.30\ \Delta E_{1,1} + 0.29\ ^1\chi + 0.12$$
$$n = 12,\ s = 0.6735,\ r = 0.6177. \quad (8)$$

Effect of PAH on duration of zoxazolamine paralysis (A_2)

This activity correlates significantly with the shape parameter ω, as demonstrated in the linear equation (9)

$$\log A_2 = 8.71(\pm 2.27)\ \omega - 11.08(\pm 3.13)$$
$$n = 8,\ s = 0.5103,\ r = 0.9695. \quad (9)$$

On the other hand, parameters describing molecular size do not yield significant correlations

$$\log A_2 = -0.02\ C + 0.87$$
$$n = 8,\ s = 2.0836,\ r = 0.0063, \quad (10)$$
$$\log A_2 = -0.03\ ^1\chi + 1.02$$
$$n = 8,\ s = 2.0824,\ r = 0.0344. \quad (11)$$

Charging tRNA with L-methionine (A_3)

The best equation relating biological data to ω and $^1\chi$ is given below

$$\log A_3 = 0.45(\pm 0.50)\omega + 0.11(\pm 0.09)\ ^1\chi - 0.87(\pm 0.83)$$
$$n = 10,\ s = 0.1244,\ r = 0.8537,$$
$$F_{1,7} = 5.88,\ F_{1,7,p\leq 0.05} = 5.59. \quad (12)$$

Linear relations between log A_3 and the variables ω, $^1\chi$ and $\Delta E_{1,1}$ are described by equation (13–15), respectively.

$$\log A_3 = 0.59\omega - 0.36$$
$$n = 10,\ s = 0.1759,\ r = 0.6060, \quad (13)$$
$$\log A_3 = 0.15\ ^1\chi - 0.37$$
$$n = 10,\ s = 0.1517,\ r = 0.7275, \quad (14)$$
$$\log A_3 = 0.15\ \Delta E_{1,1} + 0.36$$
$$n = 10,\ s = 0.2237,\ r = 0.1798. \quad (15)$$

As the number of data was limited, multiparameter equation relating log A_3 to both $\Delta E_{1,1}$ and $^1\chi$ was not derived. From equation (15) it may be seen, however, that parameter $\Delta E_{1,1}$ is of little significance.

Mutagenicity of PAH (A_4)

The mutagenic activity of PAH has been most adequately described by equation (16)

$$\log A_4 = 4.29(\pm 3.58)\omega + 0.81(\pm 0.44)\ ^1\chi - 10.95(\pm 5.57)$$
$$n = 11,\ s = 0.8635,\ r = 0.8871,$$
$$F_{1,8} = 7.93,\ F_{1,8,p\leq 0.05} = 5.32. \quad (16)$$

The squared correlation matrix for equation (16) is given in Table 4.

In view of two-step nature of the Ames test (enzymic conversion and mutagenic effect of metabolites) it is interesting to compare equation (16) with similar equations in which the variable ω is replaced by one characterizing acceptor–donor properties of the molecule, k_{HOMO} and $\Delta E_{1,1}$. These parameters failed to correlate with mutagenicity

$$\log A_4 = 3.14(\pm 16.15)k_{HOMO} + 0.86(\pm 1.02)\ ^1\chi - 6.56(\pm 12.27)$$
$$n = 9,\ s = 1.3389,\ r = 0.6961,$$
$$\log A_4 = 0.98(\pm 4.43)\Delta E_{1,1} + 0.87(\pm 0.72)\ ^1\chi - 5.94(\pm 6.50) \quad (17)$$
$$n = 9,\ s = 1.2213,\ r = 0.7912. \quad (18)$$

In spite of the limited number of data for the sake of comparison with equation (16), the equations are given below for the same group of 9 data points in which the ω variable is replaced with quantum-chemically calculated data K_{reg}, $\Delta E_{deloc/\beta}$ and R. The pairs of equations obtained are as follows

$$\log A_4 = -5.31(\pm 16.83)\ K_{reg} + 0.41(\pm 1.38)\ ^1\chi + 15.55(\pm 63.50)$$
$$n = 9,\ s = 1.2931,\ r = 0.7200,$$
$$\log A_4 = 4.32(\pm 4.24)\omega + 0.68(\pm 0.60)\ ^1\chi - 10.22(\pm 6.74) \quad (19)$$
$$n = 9,\ s = 0.9122,\ r = 0.8720. \quad (19a)$$

Table 4. Squared correlation matrix for equation (16)

	log A_4	$^1\chi$	ω
log A_4	1.0	0.5756	0.2833
$^1\chi$	–	1.0	0.0097
ω	–	–	1.0

Table 5. Squared correlation matrix for equations (19) and (19a)

	$\log A_4$	$^1\chi$	ω	K_{reg}
$\log A_4$	1.0	0.4613	0.3908	0.4677
$^1\chi$	–	1.0	0.0147	0.6277
ω	–	–	1.0	0.0088
K_{reg}	–	–	–	1.0

Table 7. Squared correlation matrix for equations (21) and (21a)

	$\log A_4$	$^1\chi$	ω	$\Delta E_{deloc/\beta}$
$\log A_4$	1.0	0.4613	0.3895	0.4894
$^1\chi$	–	1.0	0.0144	0.7417
ω	–	–	1.0	0.0100
$\Delta E_{deloc/\beta}$	–	–	–	1.0

The square correlation matrix for equations (19) and (19a) is given in Table 5.

$$\text{Log } A_4 = -0.26(\pm 1.11)R + 0.88(\pm 0.82)\ ^1\chi - 10.78(\pm 26.38)$$
$$n = 9,\ s = 1.2404,\ r = 0.7639, \quad (20)$$
$$\log A_4 = 4.18(\pm 5.55)\omega + 0.75(\pm 0.62)\ ^1\chi - 10.47(\pm 8.49)$$
$$n = 9,\ s = 0.9884,\ r = 0.8577. \quad (20a)$$

The square correlation matrix for equations (20) and (20a) is presented in Table 6.

$$\text{Log } A_4 = 6.49(\pm 22.0)\ \Delta E_{deloc/\beta} + 0.33(\pm 1.66)\ ^1\chi - 6.23(\pm 8.08)$$
$$n = 9,\ s = 1.3014,\ r = 0.7157, \quad (21)$$
$$\log A_4 = 4.32(\pm 4.23)\ \omega + 0.68(\pm 0.60)\ ^1\chi - 10.21(\pm 6.73)$$
$$n = 9,\ s = 0.9125,\ r = 0.8719. \quad (21a)$$

The square correlation matrix for equations (21) and (21a) is given in Table 7.

DISCUSSION

The relations here obtained illustrate the significance of the 'experimental' shape parameter, ω, for characterizing the biological activity of PAH both as enzyme inducers (activity scales A_1 and A_2) and toward activating enzyme systems transforming the parent hydrocarbons into active metabolites (activity scales A_3 and A_4).

In contrast to ω, parameters $\Delta E_{1,1}$ and k_{HOMO} related to the acceptor–donor properties of PAH do not provide a quantitative description of biological activity expressed either as the ability to induce microsomal mixed-function oxidases [equations (7) and (8)] or their mutagenicity in the Salmonella test [equations (17) and (18)]. As it has been shown in the case of mutagenicity ω is also more significant for a quantitive relationship between structure and activity than the appropriate parameters connected with PAH reactivity (namely, K_{reg}, $\Delta E_{deloc/\beta}$ and R).

Table 6. Squared correlation matrix for equations (20) and (20a)

	$\log A_4$	$^1\chi$	ω	R
$\log A_4$	1.0	0.5559	0.2764	0.0089
$^1\chi$	–	1.0	0.0202	0.1134
ω	–	–	1.0	0.0786
R	–	–	–	1.0

As is evident from the correlation matrices for the parameters studied (Tables 3–7), ω is an original, independent parameter unrelated either to acceptor–donor properties determining the chemical reactivity of a particular part of the PAH molecule or the size of the molecule. Parameter ω is related to molecular shape, calculated from experimental data obtained in a chromatographic procedure. The rational explanation of its suitability for the description of bioactivity can be suggested assuming parameter ω probably reflects steric hindrance controlling the transport of PAH to a receptor or the fitting of the compound to a receptor site.

Satisfactory description of activity of PAH is obtained when ω is applied together with one of commonly used additive-constitutive parameters related to molecular size; the best equations resulted when connectivity index $^1\chi$ was applied. The importance of the size parameter may be related to its possible role in nonspecific interactions between the PAH and a receptor site (hydrophobic or Van der Waals). This suggestion is supported by the data of Franke [11] on the binding constants k_B, of the binding of PAH to human serum albumin

$$\log k_B = 0.18\ C + 2.93$$
$$n = 14,\ s = 0.2619,\ r = 0.9214. \quad (22)$$

Other size related parameters in the literature describe quantitatively or semiquantitatively the bioactivity of PAH: among these Hansch's hydrophobicity parameter [17] and the incumbrance area of Arcos *et al.* [2] are the best known. The connectivity index $^1\chi$ chosen here as a size parameter is a topological parameter reflecting to some extent differences among isomers. As observed here $^1\chi$ correlates significantly with $\Delta E_{deloc/\beta}$ [15] for carbonium ion formation at bay region benzylic position. This significant correlation cannot be considered, however, as conflicting with bay-region theory. Indeed, complex biological processes such as mutagenicity and carcinogenicity cannot be adequately described by reactivity alone. However, reactivity parameters provide substantial information to regression analysis involving molecular shape and size.

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