

QUANTITATIVE RELATIONSHIPS BETWEEN INHIBITION OF PHOTOSYNTHESIS AND LIPOPHILICITY OF PIPERIDINOETHYL ALKOXYPHENYL CARBAMATES

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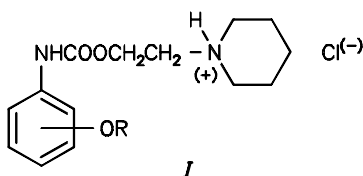
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Piperidinoethyl esters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids (PAPC) inhibit photosynthetic processes in alga *Chlorella vulgaris* and spinach chloroplasts. These inhibitory activities of PAPC were correlated with chromatographically determined lipophilicity characteristics $\log k'$ (from HPLC) and Kovats indices K_{10} (from gas chromatography) using parabolic and bilinear model. Both theoretical models are suitable for description of studied correlations, however from the viewpoint of statistical analysis Kubinyi's bilinear model gives better results.

Piperidinoethyl esters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids (PAPC) I belong to membrane-active amphiphilic compounds with a wide spectrum of biological, e.g. local anaesthetic¹, antimicrobial² and algicidal³ activity.



Recently it was found that PAPC inhibit photosynthetic processes in plant chloroplasts causing destruction of photosystem II with subsequent release of Mn^{2+} ions from the manganese-protein complex³. The intensity of the biological effects of PAPC depends on the alkyl chain length as well as on the position of the alkoxy substituent on the benzene ring of the effector¹⁻³. The highest local anaesthetic activity was shown by 2-alkoxy substituted PAPC derivatives¹ but as antimicrobial and algicidal agents were

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the most effective 4-substituted derivatives^{2,3}. The quasi-parabolic dependence of the above mentioned biological activities of amphiphilic PAPC compounds on the lipophilicity of the effectors can be explained by the free volume theory^{4,5}. According to this theory, short chain molecules incorporated in the membrane create relatively large free volume below their chain, however the number of these molecule in the membrane is low due to their low lipophilicity. On the other hand, the high lipophilicity of long chain derivatives enables incorporation of numerous molecules into the membrane with a relatively small free volume created bellow their chains. However, the resulting inhibitory effects in both above cases are relatively low in comparison to those of the molecules having sufficiently high hydrophobicity, but simultaneously creating sufficiently large free volume in the membrane⁵.

This paper presents correlations between biological activity of PAPC concerning photosynthesis inhibition in photosynthesizing organisms (*Chlorella vulgaris* and spinach chloroplasts) and chromatographically determined lipophilicity characteris $\log k'$ (from HPLC) and Kovats indices K_{10} (from gas chromatography). For the description of above correlations two theoretical models – the parabolic model of Hansch⁶ and Kubinyi's bilinear model⁷ have been used.

EXPERIMENTAL

Piperidinoethyl esters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids (alkyl = methyl – n-decyl) (PAPC) were synthesized according to Cizmarik et al.¹. Effect of PAPC on chlorophyll production of stationary cultured alga *Chlorella vulgaris* (7 days, photoperiod 16 h light/8 h dark) was investigated using method described by Kralova et al.⁸. Their effect on oxygen evolution rate in spinach chloroplasts was studied spectrophotometrically (Specord UV-VIS Zeiss, Jena, Germany) in the presence of electron acceptor 2,6-dichlorophenolindophenol³. The inhibitory activity of PAPC on photosynthesizing organisms has been expressed by minimum inhibitory concentration MIC (for algae) and IC₅₀-value (for chloroplast systems), i.e. by concentration of the effector causing total or 50% inhibition of the studied parameter.

Chromatographic retention factors $\log k'$ were determined⁹ by HPLC (Separon[®] SGXC 18 column 150 × 3.2 mm, particle size 5 μm, flow-rate 0.6 ml/min). The compounds were dissolved in methanol (1 mg/1 ml). For preparation of the mobile phase, 90% methanol and 3.4 g CH₃COONa was added to 500 ml. A Waters diode detector model 990 was used in the range 210 – 290 nm. The Kovats indices K_{10} were determined by gas chromatography (apparatus Chrom 4 with FID, packed glass column 1 200 mm, diameter 4 mm, with OV 17 on Chromosorb W HP 80 – 100 mesh; temperature of injectors 300 °C; N₂ gas carrier inlet pressure flow rate of 0.1 MPa; H₂ 40 ml/min; flow rate of air 0.5 l/min) using methanolic solutions of corresponding PAPC derivatives¹⁰. The correlation indices between $\log k'$ and K_{10} or the number of carbon atoms m in the alkoxy substituent of PAPC and between K_{10} and m were in the range of 0.989 – 0.999.

For description of correlations between biological activity and chromatographic characteristics of PAPC reflecting lipophilicity of the effector two theoretical models have been used – the parabolic model of Hansch⁶ (1) and Kubinyi's bilinear model⁷ (2a and 2b):

$$\log (1/Y) = AX + BX^2 + C \quad (1)$$

$$\log (1/Y) = A \log X - B \log (\beta X + 1) + C \quad (2a)$$

$$\log (1/Y) = AX - B \log (\beta \cdot 10^X + 1) + C, \quad (2b)$$

where Y is the corresponding biological (MIC or IC_{50} value) and X the corresponding lipophilicity characteristics ($\log k'$ and K_{10}). The study was completed with correlations between inhibitory activity of PAPC against photosynthesizing organisms and the number of carbon atoms m in the alkoxy substituent of the effector.

RESULTS AND DISCUSSION

The biological ($\log 1/MIC$ and $\log 1/IC_{50}$) and physico-chemical characteristics ($\log k'$ and K_{10}) are summarized in Table I. From the Table I the quasi-parabolic course of the dependences of $\log 1/MIC$ (or $\log 1/IC_{50}$) = $f(\log k')$ or $f(K_{10})$ or $f(m)$ is evident. The regression coefficients of regression equations as well as the corresponding parameters of the statistical analysis obtained by both theoretical models (parabolic (P) and bilinear (B) respectively) are shown in Table II.

Table II shows that both applied theoretical models – P and B – are suitable for description of above mentioned correlations. From comparison of the corresponding parameters of statistical evaluation it can be concluded that the bilinear model gave better results (higher correlation indices r , higher F -test values (F) as well as positive values of the partial F -test (pF) determined according to Kubinyi⁷). Better results of statistical evaluation were obtained for chloroplast systems. This is manifested by the values of correlation indices r and of the F -test which are in the range of 0.966 – 0.996 and 42.0 – 394.6 respectively in comparison with the range of the corresponding parameters obtained for algal systems, i.e. $r = 0.840 - 0.979$, $F = 7.2 - 68.3$. This is in good accordance with results obtained for correlations of photosynthesis inhibiting activity of structurally similar morpholinoethyl esters¹¹ and quaternary ammonium salts of heptacain¹² and the lipophilicity of these effectors.

By application of the bilinear model more effective improvement of the statistical parameters of parabolic equations was obtained with the algal systems. The best improvement of statistical parameters (pF in the range of 2.8 – 25.8) was found for relationships $\log (1/MIC) = f(m)$ and $\log (1/IC_{50}) = f(m)$.

In general it can be concluded that the calculated alkyl chain length corresponding to the derivative showing the highest biological activity was heptyl – nonyl for chloroplast systems and pentyl – octyl for algal systems. 4-Alkoxy substituted derivatives reach maximum of activity at longer alkyl chains than the 2- and 3-substituted ones. Nine members in the set ($n = 9$) can be regarded as insufficient for the more-parameter equations, however the calculated statistical parameters (r , s , F) and the confidence intervals of the regression coefficients ($p < 0.01$) show that the obtained results are significant.

TABLE I
Biological and physico-chemical characteristics of piperidinoethyl esters of alkoxyphenylcarbamic acids

Substituent position	<i>m</i>	log (1/IC ₅₀)	log (1/MIC)	log <i>k'</i>	<i>K</i> ₁₀
	1	1.220	3.497	0.0969	2.341
	2	1.782	4.003	0.1207	2.468
	3	2.114	4.103	0.1761	2.566
	4	2.591	4.282	0.2540	2.622
2	5	3.262	–	0.3319	2.694
	6	3.431	4.846	0.4409	2.782
	7	3.485	4.971	0.5187	2.924
	8	–	4.878	0.6110	3.022
	9	3.252	3.459	0.7175	3.088
	10	3.002	2.804	0.8342	3.188
	2	2.388	4.263	0.0717	2.564
	3	2.581	4.399	0.1514	2.653
	4	3.117	4.833	0.2398	2.750
	5	3.563	4.962	0.3079	2.857
3	6	3.965	5.000	0.4239	2.973
	7	4.048	5.041	0.5279	3.061
	8	4.351	4.937	0.5836	3.104
	9	3.875	4.205	0.7342	3.247
	10	4.020	3.902	0.8691	3.354
	1	1.477	4.239	0.0111	2.552
	2	2.204	4.395	0.0717	2.653
	3	2.609	4.701	0.1532	2.709
	4	3.262	4.904	0.2540	2.847
4	5	3.184	4.845	0.3446	2.919
	6	3.720	5.091	0.4464	3.029
	8	4.712	5.104	0.6612	3.207
	9	4.079	4.947	0.7744	3.293
	10	3.876	3.858	0.9020	3.424

TABLE II

Regression coefficients of parabolic (1) and bilinear (2a and 2b) regression equations (A, B, C) and corresponding parameters of statistical analysis (nine members in all sets, s standard deviation, r correlation index, β non linear parameter of bilinear equation, F F -test value, X_0 theoretical value of X corresponding to the highest activity, $Y = \text{MIC}$ for *Chlorella vulgaris* and IC_{50} for spinach chloroplasts)

Substituent position	X	Equation	A	B	C	$\log \beta$	r	s	F	$\log X_0$ or X_0	pF
Spinach chloroplasts											
2	$\log k'$	1	$1.121 \cdot 10^1$ (± 0.932)	-9.886 (± 1.003)	0.425 (± 0.170)	-	0.986	0.154	107.7	0.567	
2	k'	2a	$6.359 \cdot 10^5$ ($\pm 4.066 \cdot 10^4$)	$6.359 \cdot 10^5$ ($\pm 4.066 \cdot 10^4$)	$2.820 \cdot 106$ ($\pm 1.803 \cdot 10^5$)	4.434	0.994	0.099	265.9	0.537	7.1
2	K_{10}	1	$3.880 \cdot 10^1$ (± 6.425)	-6.581 (± 1.156)	$-5.375 \cdot 10^1$ (± 8.864)	-	0.969	0.230	46.7	2.948	
2	K_{10}	2b	8.702 (± 0.993)	$2.319 \cdot 10^2$ ($\pm 3.453 \cdot 10^1$)	$-1.835 \cdot 10^1$ (± 2.317)	-4.367	0.977	0.199	63.1	2.958	1.7
2	m	1	0.846 (± 0.076)	-0.057 (± 0.007)	0.306 (± 0.181)	-	0.987	0.152	110.6	7.386	
2	m	2b	0.486 (± 0.019)	0.647 (± 0.039)	0.736 (± 0.075)	-6.054	0.996	0.081	394.6	6.531	12.5
3	$\log k'$	1	7.414 (± 0.960)	-5.670 (± 1.005)	1.757 (± 0.191)	-	0.975	0.177	58.7	0.654	

TABLE II
(Continued)

Substituent position	X	Equation	A	B	C	log β	r	s	F	log X_0 or X_0	pF
3	k'	2a	$1.239 \cdot 10^1$ (± 1.793)	$2.046 \cdot 10^1$ (± 3.557)	4.401 (± 0.332)	-0.463	0.976	0.174	60.7	0.649	0.2
3	K ₁₀	I	$3.183 \cdot 10^1$ (± 5.898)	-5.006 (± 0.998)	$-4.646 \cdot 10^1$ (± 8.666)	-	0.976	0.173	61.3	3.179	
3	K ₁₀	2b	7.476 (± 0.990)	$3.566 \cdot 10^1$ (± 6.607)	$-1.592 \cdot 10^1$ (± 2.456)	-3.751	0.979	0.163	69.5	3.174	0.6
3	m	I	0.792 (± 0.126)	-0.047 (± 0.010)	0.818 (± 0.339)	-	0.974	0.181	56.1	8.348	
3	m	2b	0.408 (± 0.035)	0.526 (± 0.084)	1.488 (± 0.165)	-6.923	0.985	0.140	95.8	7.461	3.4
4	log k'	I	7.923 (± 1.227)	-5.728 (± 1.314)	1.490 (± 0.215)	-	0.970	0.281	48.3	0.692	
4	k'	2a	8.022 (± 1.135)	$8.193 \cdot 10^{13}$ ($\pm 1.706 \cdot 10^{13}$)	2.296 (± 0.149)	-13.699	0.975	0.260	56.6	0.690	0.8
4	K ₁₀	I	$3.440 \cdot 10^1$ (± 8.434)	-5.262 (± 1.411)	$-5.205 \cdot 10^1$ ($\pm 1.251 \cdot 10^1$)	-	0.969	0.289	45.5	3.268	
4	K ₁₀	2b	7.839 (± 1.179)	$1.273 \cdot 10^8$ ($\pm 2.974 \cdot 10^7$)	$-1.778 \cdot 10^1$ (± 3.000)	-10.456	0.974	0.261	56.3	3.246	1.1

TABLE II
(Continued)

Substituent position	X	Equation	A	B	C	$\log \beta$	r	s	F	$\log X_0$ or X_0	pF
4	m	1	0.796 (± 0.155)	-0.046 (± 0.014)	0.703 (± 0.362)	-	0.966	0.300	42.0	8.672	
4	m	2b	0.445 (± 0.040)	0.931 (± 0.181)	1.201 (± 0.180)	-8.028	0.982	0.220	80.6	7.990	4.3
<i>Chlorella vulgaris</i>											
2	$\log k'$	1	$1.107 \cdot 10^1$ (± 1.692)	$-1.306 \cdot 10^1$ (± 1.868)	2.600 (± 0.301)	-	0.946	0.279	25.3	0.424	
2	k'	2a	$1.362 \cdot 10^1$ (± 1.941)	$7.049 \cdot 10^1$ (± 9.549)	5.400 (± 0.227)	-1.068	0.951	0.266	28.2	0.447	0.5
2	K_{10}	1	$5.238 \cdot 10^1$ ($\pm 1.345 \cdot 10^1$)	-9.490 (± 2.425)	$-6.745 \cdot 10^1$ ($\pm 1.849 \cdot 10^1$)	-	0.849	0.454	7.7	2.760	
2	K_{10}	2b	8.932 (± 1.751)	$1.321 \cdot 10^0$ ($\pm 2.459 \cdot 10^0$)	$-1.633 \cdot 10^1$ (± 4.077)	-10.979	0.911	0.354	14.6	2.809	3.2
2	m	1	0.930 (± 0.208)	-0.087 (± 0.019)	2.401 (± 0.475)	-	0.887	0.397	11.0	5.356	
2	m	2b	0.259 (± 0.031)	1.334 (± 0.115)	3.326 (± 0.144)	-7.582	0.979	0.176	68.3	6.963	20.4

TABLE II
(Continued)

Substituent position	X	Equation	A	B	C	$\log \beta$	r	s	F	$\log X_0$ or X_0	pF
3	$\log k'$	I	5.444 (± 0.692)	-6.335 (± 0.724)	3.851 (± 0.138)	-	0.966	0.127	41.6	0.430	
3	k'	2a	$1.568 \cdot 10^1$ (± 1.762)	$2.502 \cdot 10^1$ (± 2.725)	9.180 (± 0.484)	-0.193	0.969	0.122	45.7	0.418	0.5
3	K_{10}	I	$3.854 \cdot 10^1$ (± 4.086)	-6.577 (± 0.691)	$-5.141 \cdot 10^1$ (± 6.003)	-	0.970	0.120	47.2	2.929	
3	K_{10}	2b	8.268 (± 0.862)	$2.716 \cdot 10^1$ (± 2.685)	$-1.504 \cdot 10^1$ (± 2.083)	-3.305	0.973	0.113	53.4	2.946	0.6
3	m	I	0.742 (± 0.101)	-0.064 (± 0.008)	2.905 (± 0.272)	-	0.955	0.145	31.3	5.774	
3	m	2b	0.206 (± 0.028)	0.697 (± 0.071)	3.869 (± 0.135)	-7.015	0.972	0.116	50.6	6.639	2.8
4	$\log k'$	I	4.613 (± 0.939)	-5.063 (± 1.006)	4.094 (± 0.164)	-	0.899	0.215	12.7	0.456	
4	k'	2a	4.860 (± 0.657)	$4.340 \cdot 10^8$ ($\pm 5.727 \cdot 10^7$)	4.812 (± 0.087)	-8.462	0.952	0.151	28.8	0.511	5.1

TABLE II
(Continued)

Substituent position	X	Equation	A	B	C	log β	r	s	F	log X_0 or X_0	pF
4	K_{10}	<i>I</i>	$3.095 \cdot 10^1$ (± 6.808)	-5.165 (± 1.139)	$-4.126 \cdot 10^1$ ($\pm 1.010 \cdot 10^1$)	—	0.880	0.233	10.4	2.996	
4	K_{10}	<i>2b</i>	5.021 (± 0.750)	$1.409 \cdot 10^0$ ($\pm 2.074 \cdot 10^8$)	-7.977 (± 1.909)	-11.496	0.941	0.166	23.2	2.923	4.8
4	<i>m</i>	<i>I</i>	0.519 (± 0.138)	-0.046 (± 0.012)	3.607 (± 0.322)	—	0.840	0.267	7.2	5.701	
4	<i>m</i>	<i>2b</i>	0.141 (± 0.017)	1.525 (± 0.140)	4.184 (± 0.083)	-8.896	0.976	0.107	59.7	7.905	25.8

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