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Application of chiral chromatographic parameters in quantitative structure–activity relationship analysis of homologous malathion derivatives

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Abstract

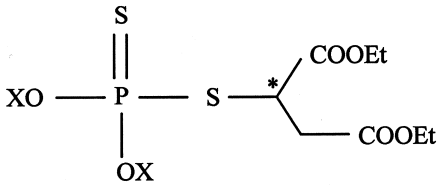
The conditions of the chiral resolution of the racemic malathion *O,O*-di-*n*-alkyl derivatives on cellulose tris(3,5-dimethylphenylcarbamate) are described. Quantitative relationships between chromatographic parameters obtained on chiral and achiral stationary phases and acute toxicity of the compounds towards house fly are derived and discussed.

Keywords: Quantitative structure–activity relationship; Chiral resolution; Malathion

1. Introduction

Malathion (*O,O*-dimethyl-*S*-1,2-di(ethoxycarbonyl)ethyl dithiophosphate) (Fig. 1) is one of the most widely used acaroinsecticides. It has been applied as a racemic mixture although it is known that (*R*)-enantiomer of the *O,O*-diethyl analogue of malathion shows a higher biological activity than (*S*)-isomer [1]. The first total synthesis of malathion enantiomers was published by Berkman and Thompson in 1992 [2]. Recently, the synthesis and insecticidal activity evaluation (as LD_{50} towards the house fly – *Musca domestica* L.) of racemic and homochiral *O,O*-di-*n*-alkyl malathion derivatives (Fig. 1) were reported (Połeć et al., private communication). The enantiomeric purity of the homochiral compounds was determined with the aid of high performance liquid chromatography on cellulose tris(3,5-dimethylphenylcarbamate) stationary phase.

It is known that the biological activity of compounds may correlate with some molecular parame-



Compd.	1	2	3	4	5	6	7
X	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_6H_{13}	C_7H_{15}

$X = CH_3$ - malathion

Fig. 1. Chemical structures of malathion derivatives.

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ters such as lipophilicity, molecular refraction, etc. [3–7]. It is also known that chromatographic capacity factors k may be correlated with the classical shake-flask log P values (octanol–water partition coefficient) according to the Collander equation [8]:

$$\log P = a \log k + b \quad (1)$$

2. Experimental

2.1. Materials

The malathion and its analogues were obtained from the Institute of Industrial Organic Chemistry, Warsaw, Poland.

The solvents were HPLC-grade from J.T. Baker (via Witko-Eurocolor, Łódź, Poland).

Chiral column: Cellulose tris(3,5-methylphenylcarbamate) (Chiralcel OD-H),), 25 cm×4.6 mm I.D., 5 μm mean particle diameter was purchased from Daicel (via Witko-Eurocolor, Łódź, Poland). Achiral column: Chemically bound octadecylsilane (Kromasil C18), 25 cm×4.6 mm I.D., 5 μm particle size was a gift from Eka Nobel, Bohus, Sweden.

2.2. Methods

Log P values were calculated with the aid of ProLogP 5.0 program from CompuDrug, Budapest, Hungary.

A HPLC system Varian 5000 consisting of a gradient pump, a heating chamber, a UV/VIS variable wavelength detector and a Valco valve injector with a 10-μl loop was used. The system worked under LC Star Workstation program version 4.0. Flow rate was 1 ml/min, temperature 26°C, $\lambda = 220$ nm. The following mobile phases were employed:

Chiral chromatography: hexane–propanol-2 (250:1, v/v).

Achiral chromatography: methanol–water (50, 25, 20, and 15% of methanol).

3. Results and Discussion

In order to determine the enantiomeric purity of (*R*)- and (*S*)-enantiomers of the compounds high-performance liquid chromatography on chiral supports was employed. Very good resolution ($\alpha = 1.25$–1.31, $R_S = 1.77$–2.96) for all the compounds was obtained on cellulose tris(3,5-dimethylphenylcarbamate). (*R*)-enantiomers of the compounds were the first-eluted isomers (Table 1). An exemplary chromatogram is shown in Fig. 2. Capacity factors of (*R*)- and (*S*)-enantiomers plotted against their log P values gave almost parallel descending lines (Fig. 3) and selectivities ($\alpha_{S/R}$) were almost constant for the whole series. That would mean that an alkyl substituent (X) does not participate in enantiodifferentiating interactions with chiral sites on the stationary phase. That was different from an observation made for a series of homologous hypnotic–sedative compounds where selectivity changed with the elongation of an aliphatic substituent [9]. What we observe here could probably be attributed to a long distance between the asymmetry centre and the alkyl substituent in malathion derivatives. The shape of capacity factors curves (positive curvature) could be interpreted in terms of a growing repulsive interaction between the cellulose stationary phase and the compounds with the elongation of the aliphatic chains of X-substituents [10,11].

The biological activity of the compounds (Połeć et al., private communication) was correlated with chromatographic capacity factors which could, in general, be considered as a measure of lipophilicity. In the present work the lipophilicity of racemic malathion derivatives was estimated by means of partition chromatography on octadecylsilica stationary phase (RP-18) and for structure–activity study log k values were used.

Table 1
Chromatographic parameters obtained on chiral stationary phase for compounds **1**–**7**[a]

Compound	$\log(1/LD_{50})$	$\log k_R$	$\log k_S$	$\alpha_{S/R}$	R_s
1	0.28	0.94	1.04	1.26	2.63
2	0.72	0.64	0.76	1.32	2.96
3	0.33	0.53	0.63	1.26	2.69
4	−0.48	0.41	0.51	1.25	2.36
5	−1.22	0.31	0.42	1.28	1.99
6	−1.60	0.28	0.40	1.31	1.96
7	−1.75	0.27	0.38	1.28	1.77

[a] Column, Chiralcel OD-H, 25 cm×4.6 mm I.D., 5 μm; flow rate, 1 ml/min, temperature 26°C, $\lambda = 220$ nm; mobile phase, hexane–propanol-2 (250:1, v/v).

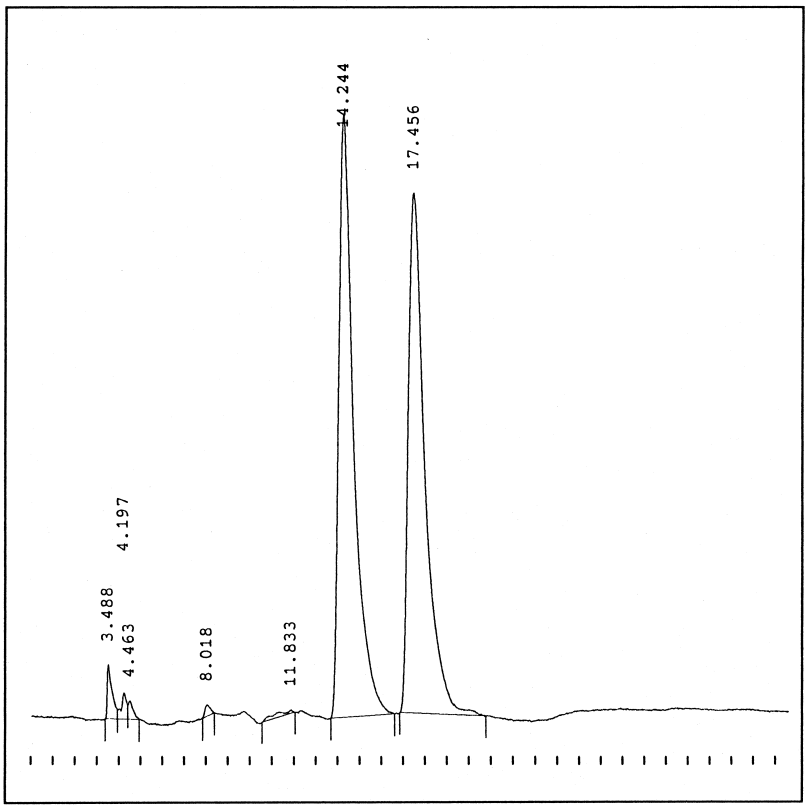

Fig. 2. An exemplary chromatogram of malathion derivatives on cellulose tris(3,5-dimethylphenylcarbamate). Compound, **3**; column, Chiralcel OD-H, 25 cm×4.6 mm I.D., 5 μm; flow-rate, 1 ml/min; temperature, 26°C; $\lambda = 220$ nm. Mobile phase: hexane–propanol-2 (250:1, v/v).

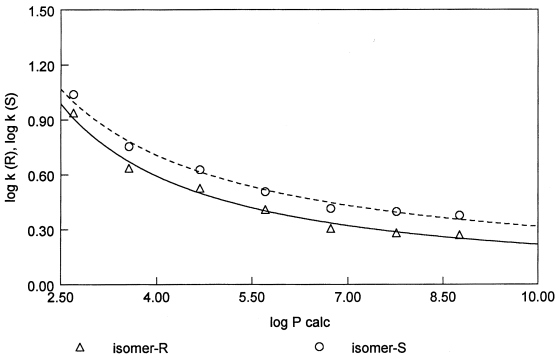

Fig. 3. Relationship between calculated lipophilicity log P_{calcd} and capacity factors for compounds **1**–**7**. Column, Chiralcel OD-H, 25 cm×4.6 mm I.D., 5 μm; flow-rate, 1 ml/min; temperature, 26°C; $\lambda = 220$ nm. Mobile phase: hexane–propanol-2 (250:1, v/v).

It is supposed that the best estimation of lipophilicities may be obtained for capacity factors extrapolated to pure water (log k_w) [12]. Therefore, the capacity factors for different contents of methanol were measured and log k_w values (Table 2) were calculated according to the Soczewiński–Wachtmeister equation [13]:

$$\log k = \log k_w - S\Phi \quad (2)$$

Φ, volume fraction of methanol in eluent.

The very good linearity observed between log k values at different concentrations of methanol (Table 2) permitted the extrapolation to 0% methanol. The obtained log k_w values often referred to as 'hydrophobic index' [14] correlated very well (linear regression coefficient $r = 0.998$) with the calculated lipophilicities (log P_{calc}).

Table 2
Isocratic and extrapolated capacity factors determined using methanol as an organic modifier

Compound	$\log k_{85}$	$\log k_{80}$	$\log k_{75}$	$\log k_{50}$	$\log k_w$	S	r[a]
1	0.04	0.19	0.36	1.52	3.89	4.62	0.998
2	0.22	0.42	0.64	2.01	4.85	5.53	0.998
3	0.47	0.72	0.98	2.56	5.89	6.47	0.999
4	0.73	1.03	1.34	–	6.25	6.52	1.000
5	1.00	1.35	1.73	–	7.50	7.69	1.000
6	1.24	1.68	2.11	–	9.02	9.18	1.000
7	1.59	2.02	2.50	–	9.78	9.69	1.000

[a] Linear correlation coefficient.

$$\log P_{calc} = 1.02(\pm 0.06) \log k_w - 1.18(\pm 0.41)$$
$$n = 7,\ r = 0.992,\ s = 0.3079,\ F = 303.88,$$
$$P < 0.001 \qquad (3)$$

n, the number of compounds; r, correlation coefficient; s, standard error of estimate; F, the F-test of significance; P, significance level.

The 'hydrophobic index' exhibited a nonlinear correlation (Fig. 4a) with the acute toxicities of the compounds (*R*)-enantiomers (Table 3). The bell-shaped curve could not be described by the Hansch parabolic model proposed for lipophilicity–biological activity relationships [15–17].

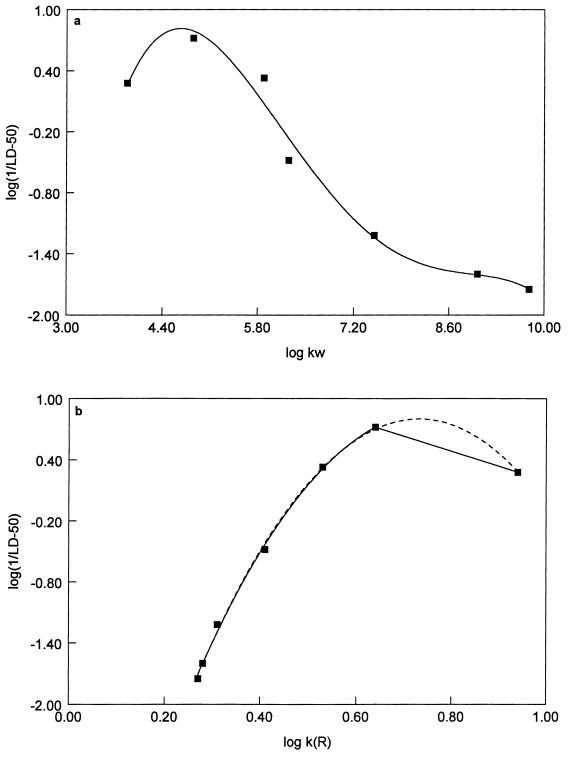

Fig. 4. Relationship between reciprocal of acute toxicity and achiral (a) and chiral (b) capacity factors for compounds **1**–**7**.

Because the application of the achiral capacity factors did not result in a significant correlation with the biological activity of the compounds we used the capacity factors obtained for (*R*)-enantiomers on cellulose tris(3,5-dimethylphenylcarbamate). Chiral capacity factors for (*R*)-enantiomers plotted as $\log k_R$ against biological activity (expressed as $\log 1/LD_{50}$) (Fig. 4b) exhibited a very good fitting to the Hansch parabolic model (Eq. (4)).

$$\log (1/LD_{50}) = -11.83(\pm 0.44)(\log k_R)^2 + 17.28(\pm 0.53)(\log k_R) - 5.52(\pm 0.13);\ n = 7,\ r = 0.998,\ s = 0.0532,\ F = 1069.92,\ P < 0.001 \qquad (4)$$

Table 3
Hydrophobic index, calculated lipophilicities and acute toxicity for malathion analogues **1**–**7**

Compound	LD_{50} (μg/individual)[a]	$\log P$	$\log k_w$[b]
1	0.53	2.70	3.89
2	0.19	3.56	4.85
3	0.47	4.68	5.89
4	2.99	5.71	6.25
5	16.76	6.73	7.50
6	39.74	7.76	9.02
7	56.20	8.76	9.78

[a] Acute toxicities of more active (*R*)-enantiomers against house fly (*Musca domestica* L.). Data taken from reference (Poleć et al., private communication) with the authors' permission.

[b] Column, Kromasil C18, 25 cm×4.6 mm I.D., 5 μm – a gift from Eka Nobel, Bohus, Sweden.

The curve exhibited maximum for $\log k_R = 0.73$ what corresponded to the predicted acute toxicity value of 0.16 μg/individual. The calculated value was not much different from the experimentally found (0.19 μg/individual) for diethylmalathion derivative **2**. The data suggested that the optimum of biological activity was almost reached in the tested series of compounds.

The plot (Fig. 4b) could also be described by the Franke's mixed linear-parabolic model [18] (quadratic Eq. (5) for ascending and linear Eq. (6) for descending part of the plot) with critical $\log k_{Rx} = 0.64$.[1]

$$\log (1/\mathrm{LD}_{50}) = -10.95(\pm 1.86)(\log k_R)^2 + 16.52(\pm 1.67)(\log k_R) - 5.37(\pm 0.34) \quad (5)$$

for $\log k_R \leq \log k_{Rx}$

$n = 6, r = 0.998, s = 0.0591, F = 755.81,$
$P < 0.001$

$$\log (1/\mathrm{LD}_{50}) = -1.47 (\log k_R) + 1.66 \quad (6)$$

for $\log k_R \geq \log k_{Rx}$
$(n = 2)$.

The relationships between chiral biological interactions (acute toxicity of (*R*)-isomers) and chiral chromatographic parameters may be well explained by the simple parabolic or mixed linear-parabolic model. Similar relationships for achiral capacity factors would require more complicated models.

References

[1] A. Hassan, W.C. Dauterman, Biochem. Pharmacol. 17 (1968) 1431.
[2] C.E. Berkman, C.M. Thompson, Tetrahedron Lett. 33 (1992) 1415.
[3] R. Kaliszan, in: J.D. Wineforder (Ed.), Quantitative Structure – Chromatographic Retention Relationships, ch. 12, John Wiley & Sons, New York, 1987, p. 279.
[4] R.M. Wolf, E. Francotte, D. Lohmann, J. Chem. Soc. Perkin Trans. II (1988) 893.
[5] R. Kaliszan, T. A. G. Noctor, I. W. Wainer, Mol. Pharmacol. 42 (1992) 512.
[6] G. Hansson, M. Ahnoff, J. Chromatogr. A 666 (1994) 505.
[7] R. Kaliszan, T. A. G. Noctor, I. W. Wainer, Chromatographia 33 (1992) 546.
[8] J. M. McCall, J. Med. Chem. 18 (1975) 549.
[9] Z. Chilmonczyk, H. Ksycińska, M. Mazgajska, J. Cybulski, R. Kaliszan, J. Chromatogr. A 788 (1997) 81.
[10] C. Roussel, C. Suteu, J. Chromatogr. A 761 (1997) 129.
[11] C. Roussel, C. Popescu, Chirality 6 (1994) 251.
[12] M. Kuchar, M. Jelinková, in: M. Kuchar, (Ed.), QSAR in Design of Bioactive Compounds, J.R. Prous Science Publishers, S.A., Barcelona. 1992, p. 65.
[13] E. Soczewiński, C.A. Wachtmeister, J. Chromatogr. 7 (1962) 311.
[14] N. Chen, Y. Zhang, P. Lu, J. Chromatogr. A 633 (1993) 31.
[15] C. Hansch, Acc. Chem. Res. 2 (1967) 232.
[16] C. Hansch, in: E.J. Ariëns (Ed.), Drug Design, vol. I., Academic Press, New York, 1971, p. 271.
[17] C. Hansch, M. Clayton, J. Pharm. Sci. 62 (1973) 1.
[18] R. Franke, W. Schmidt, Acta Biol. Med. Germ. 31 (1973) 273.

[1] Corresponding to critical $\log P_x$ value [18], where the linear relationship changes into a nonlinear one.