Journal of Chromatography, 160 (1978) 37–47

CHROM. 11,010

RELATIONSHIPS BETWEEN CHROMATOGRAPHIC PROPERTIES, PARTITION DATA AND CHEMICAL STRUCTURE OF O-ALKYL-O-ARYL-PHENYLPHOSPHONOTHIOATES

W. STEURBAUT

Institute for Chemical Research, Ministry of Agriculture, Tervuren (Belgium)

and

W. DEJONCKHEERE and R. H. KIPS

Department of Crop Protection Chemistry, State University of Ghent, Ghent (Belgium)

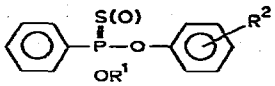
(First received December 15th, 1977; revised manuscript received March 13th, 1978)

SUMMARY

A series of O-alkyl-O-arylphenylphosphonothioates were synthesized and their partition coefficients and the corresponding R_F values were determined by thin-layer chromatography (TLC) with different solvent systems and by reversed-phase and polyamide layer techniques. Gas–liquid chromatographic (GLC) relative retention times were determined on different stationary phases with increasing polarity. Partition between *n*-octanol and water was determined using a GLC detection technique. The partition data and chromatographic properties were compared and correlated with physico-chemical linear free energy parameters for electronic, hydrophobic and steric forces. GLC partitioning is influenced by electronic and steric forces, whereas TLC partitioning is influenced by electronic and hydrophobic forces, depending on the polarity of the mobile phase.

INTRODUCTION

The relationship between the chemical structures and the chromatographic behaviour of chemicals has been discussed in several papers[1–3]. This study deals with the thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) of O-alkyl-O-arylphenylphosphonothioates[4] (Fig. 1). These properties must be considered



R^1: $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$.

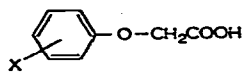
R^2: $-H$, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-F$, $-Cl$, $-Br$, $-I$, $-OCH_3$, $-NO_2$, $-CN$.

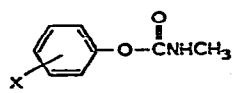
Fig. 1. Chemical structure of O-alkyl-O-arylphenylphosphonothioates.

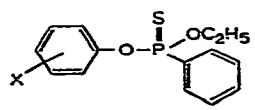
in the context of a study of structure–activity relationships of these insecticidal compounds[5]. In particular, those aspects which are important for biological activity, such as hydrophobicity, have been studied. These hydrophobic forces are difficult to determine exactly owing to their complexity. Meyer and Hemmi[6] and Overton[7], however, showed a strong relationship between some biological processes and the partition of a compound between an organic phase and water, expressed as the partition coefficient. This is the fraction of a dissolved compound that is transferred to the non-polar phase of equal volumes of immiscible solvents. The partition coefficient is expressed as k/l, where k is the fraction in the non-polar phase and l the fraction in the polar phase. Fujita *et al.*[8] introduced a substituent constant, π, derived from partition coefficients:

$$\pi = \log\left[\frac{(k/l)_X}{(k/l)_H}\right]$$

where X and H are a substituted and the unsubstituted molecule with analogous structures, respectively. Various series of partition coefficients were determined by Fujita *et al.*[8], *e.g.*, on substituted benzene derivatives, phenoxyacetic acids, benzoic acids and phenols. The π values determined for the individual substituents of these "standard" series have since been frequently used as approximations for π values in other series. Hansch and Deutsch[9] used the π values of the phenoxyacetic acid series for correlation of a methylcarbamate series because of their analogous molecular structures:

phenoxyacetic acids 

methylcarbamates 

phosphonothioates 

For similar reasons, it is possible to use these π values in correlations with the phosphonothioates investigated in this work.

Direct determinations of partition coefficients are often inaccurate, especially for compounds that are only slightly miscible with one of the two phases. To obviate this difficulty, Boyce and Milborrow[3] used chromatographic R_M values, which were introduced by Bate-Smith and Westfall[10] as log $[(1/R_F) - 1]$. It was shown[11] that the R_F values in liquid–liquid chromatography were related to partition coefficients according to the equation

$$k/l = K \log [(1/R_F) - 1]$$

Analogous to the π values, ΔR_M can be defined as:

$$\Delta R_M = R_M(X) - R_M(H)$$

where X and H are a substituted and the unsubstituted molecule of an analogous series, respectively.

GLC is also a partition process between two phases. The retention time of a substituted molecule X (t_X) relative to that of the unsubstituted molecule H (t_H) of an analogous series of compounds is, in fact, the ratio of the partition of the two molecules between the gas phase and the stationary phase:

$$R_{F_{X,H}} = \frac{t_X}{t_H} = \frac{\text{partition of X between gas and stationary phase}}{\text{partition of H between gas and stationary phase}}$$

By analogy with the π values of Fujita *et al.*[8], a π_{GLC} value[12] can be formulated as

$$\pi_{GLC} = \log R_{F_{X,H}}$$

EXPERIMENTAL

Determination of the partition coefficient, k/l

A 0.5-ml volume of a 100 ppm insecticide stock solution is pipetted into a graduated test-tube (20 ml). The solvent (acetone) is evaporated and *n*-octanol (5 ml, previously saturated with distilled water) is added. After shaking for 1 min, 2 μl (A) are injected into a gas chromatograph and the peak height is measured. Subsequently another 5 ml of *n*-octanol are added and, after shaking, a further 2 μl (B) are injected. The two peak heights obtained permit a calibration graph to be constructed (Fig. 2). Distilled water (10 ml, previously saturated with *n*-octanol) is added and the tube is shaken for 1 min. After centrifugation (5 min at 2000 *g*), 2 μl (C) of the organic layer are injected and the concentration is determined by means of the calibration graph (Fig. 2). The partition coefficient is calculated as $k/l = x/(1 - x)$, where x is the percentage remaining in the organic phase.

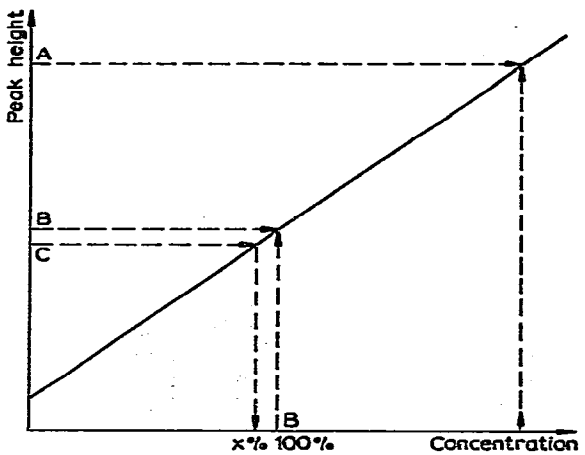


Fig. 2. Calibration graph for the determination of partition coefficients.

Determination of ΔR_M values

Determination of ΔR_M values by reversed-phase thin-layer chromatography. TLC aluminium sheets pre-coated with silica gel 60 F_{254} (Merck, Darmstadt, G.F.R.), with a layer thickness of 0.2 mm, are impregnated with a 5% (v/v) solution of a mineral oil (liquid paraffin B.P.) in *n*-hexane and the solvent is evaporated at 40°. The samples (30 μg) are spotted on a line 2 cm from the bottom edge. To avoid any systematic error, the samples are spotted according to a random allocation. The unsubstituted reference compound is applied on each plate as a standard. The plates are then eluted in a developing chamber by the ascending technique, the solvent front being allowed to travel a distance of 15 cm from the starting line. The plates are air dried and the compounds localized by the appearance of dark spots in transmitted UV light (254 nm). The different R_F, R_M and ΔR_M values are calculated with reference to the unsubstituted compound.

Determination of ΔR_M values by polyamide layer chromatography. TLC aluminium sheets, pre-coated with Polyamide 11 F_{254} (Merck), with a layer thickness of 0.2 mm, are spotted in the same way as for reversed-phase TLC and R_F, R_M and ΔR_M values are calculated in an analogous manner.

Determination of π_{GLC} values

A standard series (5 ml of a 10 ppm solution) of the O-alkyl-O-arylphenylphosphonothioates were injected into a Varian 2700 gas chromatograph equipped with a Melpar flame-photometric detector in the phosphorus mode. Three columns (Pyrex glass, 3 m × 2 mm I.D.), filled with 3% OV-101, 3% OV-225 and 3% DEGS on Gas-Chrom Q, were used.

The GLC conditions were as follows: injector temperature, 225°; oven temperature, 220°; carrier gas (nitrogen) flow-rate, 60 ml/min; oxygen flow-rate, 12 ml/min; hydrogen flow-rate, 100 ml/min; and air flow-rate, 30 ml/min.

Relative retention times compared with the unsubstituted compound were calculated and π_{GLC} values were obtained by taking the logarithmic value.

RESULTS

Results are given in Tables I, II and III for the partition coefficients, ΔR_M values and π_{GLC} values, respectively.

TABLE I

PARTITION COEFFICIENTS AND π VALUES

Substituent	*k*					*k (average)*	*k/l*	π	π *(phenoxyacetic acid)*
	a	*b*	*c*	*d*	*e*				
H	0.99	0.98	0.98	0.98	0.99	0.984	61.50	0.000	0.000
4-CH_3	1.00	1.00	0.99	0.99	1.00	0.996	166.00	0.421	0.520
4-CN	0.97	0.97	0.97	0.98	0.97	0.972	34.72	−0.248	−0.320
4-NO_2	0.99	0.99	1.00	0.99	0.98	0.990	99.00	0.207	0.240

TABLE II

R_F, R_M AND ΔR_M VALUES OBTAINED USING THREE DIFFERENT TLC SYSTEMS

R^2*	R^1*	*Reversed-phase TLC, acetone–water (6:4, v/v)*			*Polyamide TLC, n-hexane–acetic acid (95:5, v/v)*			*Polyamide TLC, acetone–water (6:4, v/v)*		
		R_F	R_M	ΔR_M	R_F	R_M	ΔR_M	R_F	R_M	ΔR_M
H	C_2H_5	0.467	0.059	0.000	0.761	−0.503	0.000	0.355	0.259	0.000
2-CH_3	C_2H_5	0.397	0.182	0.123	0.799	−0.599	−0.096	0.324	0.319	0.060
3-CH_3	C_2H_5	0.414	0.151	0.092	0.770	−0.525	−0.220	0.321	0.325	0.066
4-CH_3	C_2H_5	0.412	0.154	0.096	0.777	−0.547	−0.039	0.294	0.380	0.181
2-OCH_3	C_2H_5	0.515	−0.026	−0.085	0.638	−0.244	0.257	0.414	0.151	−0.109
3-OCH_3	C_2H_5	0.481	0.033	−0.026	0.684	−0.335	0.168	0.391	0.193	−0.067
4-OCH_3	C_2H_5	0.489	0.019	−0.040	0.658	−0.284	0.219	0.370	0.231	−0.028
2-Cl	C_2H_5	0.411	0.156	0.097	0.717	−0.404	0.099	0.295	0.378	0.197
3-Cl	C_2H_5	0.348	0.273	0.214	0.769	−0.523	−0.019	0.260	0.454	0.195
4-Cl	C_2H_5	0.355	0.259	0.201	0.770	−0.525	−0.022	0.244	0.491	0.232
2-CN	C_2H_5	0.559	−0.103	−0.162	0.502	−0.003	0.499	0.446	0.094	−0.165
3-CN	C_2H_5	0.508	−0.014	−0.073	0.484	0.028	0.531	0.412	0.154	−0.105
4-CN	C_2H_5	0.523	−0.040	−0.099	0.429	0.124	0.627	0.373	0.226	0.034
2-NO_2	C_2H_5	0.525	−0.043	−0.102	0.496	0.007	0.510	0.408	0.162	−0.098
3-NO_2	C_2H_5	0.444	0.098	0.039	0.517	−0.030	0.473	0.324	0.319	0.060
4-NO_2	C_2H_5	0.428	0.126	0.067	0.486	0.024	0.527	0.282	0.406	0.147
4-F	C_2H_5	0.441	0.103	0.044	0.733	−0.439	0.064	0.345	0.279	0.019
4-Br	C_2H_5	0.331	0.306	0.247	0.763	−0.508	−0.049	0.208	0.581	0.327
4-I	C_2H_5	0.299	0.370	0.317	0.707	−0.383	0.120	0.174	0.676	0.417
4-C_2H_5	C_2H_5	0.337	0.294	0.235	0.807	−0.621	−0.118	0.246	0.486	0.227
4-*n*-C_3H_7	C_2H_5	0.270	0.432	0.373	0.835	−0.704	−0.201	0.198	0.608	0.348
2,5-di-Cl-4-Br	C_2H_5	0.215	0.562	0.504	0.751	−0.479	0.024	0.114	0.891	0.631
4-CN	CH_3	0.567	−0.117	0.176	0.287	2.374	0.877	0.409	0.160	−0.099
4-CN	C_3H_7	0.446	0.094	0.035	0.498	0.003	0.506	0.321	0.325	0.066
2,5-di-Cl-4-Br (oxygen analogue)	C_2H_5	0.146	0.767	0.708	0.907	−0.987	−0.484	0.096	0.974	0.715

* See Fig. 1.

TABLE III

GLC R_F AND π_{GLC} VALUES ON THREE DIFFERENT STATIONARY PHASES

R^{2*}	R^{1*}	*OV-101*		*OV-225*		*DEGS*	
		$R_{F_{R,H}}$	π_{GLC}	$R_{F_{R,H}}$	π_{GLC}	$R_{F_{R,H}}$	π_{GLC}
H	C_2H_5	1.00	0.000	1.00	0.000	1.00	0.000
2-CH_3	C_2H_5	1.29	0.111	1.15	0.061	1.00	0.000
3-CH_3	C_2H_5	1.29	0.111	1.27	0.104	1.12	0.049
4-CH_3	C_2H_5	1.43	0.155	1.44	0.158	1.31	0.117
2-OCH_3	C_2H_5	1.76	0.246	2.42	0.384	2.39	0.378
3-OCH_3	C_2H_5	2.02	0.306	2.67	0.427	2.75	0.439
4-OCH_3	C_2H_5	2.31	0.364	3.24	0.511	3.39	0.530
2-Cl	C_2H_5	1.62	0.210	1.71	0.233	1.64	0.215
3-Cl	C_2H_5	1.70	0.230	1.68	0.225	1.55	0.190
4-Cl	C_2H_5	1.82	0.260	1.90	0.279	1.85	0.267
2-CN	C_2H_5	2.05	0.312	3.75	0.574	3.88	0.589
3-CN	C_2H_5	2.33	0.367	4.61	0.664	4.82	0.683
4-CN	C_2H_5	2.70	0.431	5.80	0.763	6.70	0.826
2-NO_2	C_2H_5	2.64	0.422	5.15	0.712	5.61	0.749
3-NO_2	C_2H_5	3.36	0.526	6.29	0.799	6.55	0.816
4-NO_2	C_2H_5	4.04	0.606	8.29	0.919	9.21	0.964
4-F	C_2H_5	0.93	−0.032	0.87	−0.061	0.91	−0.041
4-Br	C_2H_5	2.57	0.410	2.88	0.459	2.85	0.455
4-I	C_2H_5	3.73	0.572	5.00	0.699	4.94	0.694
4-C_2H_5	C_2H_5	1.95	0.290	1.86	0.270	1.55	0.190
4-*n*-C_3H_7	C_2H_5	2.62	0.418	2.37	0.375	1.76	0.246
2,4-di-Cl	C_2H_5	2.71	0.433	2.58	0.412	2.36	0.373
2,5-di-Cl-4-Br	C_2H_5	5.45	0.736	5.79	0.763	5.21	0.717
4-CN	CH_3	2.75	0.439	5.71	0.757	7.47	0.873
4-CN	C_3H_7	3.53	0.548	7.12	0.852	6.97	0.843

* See Fig. 1.

DISCUSSION

Partition coefficients

The determination of partition coefficients is fairly simple[13]. Essentially all of the methods described are analogous and only the instrumental detection techniques vary with the compounds under investigation. The results depend on the accuracy and the sensitivity of the detection methods and the mutual solubility of the solvents. To minimize the influence of the latter, both solvents are previously saturated with each other. The two most important detection techniques used are GLC[14] and radiotracer methods[15]. Although tracer techniques have a greater sensitivity, they have some disadvantages compared with GLC: certain impurities and hydrolysis products can cause erroneous results, whereas the GLC technique separates the compounds under investigation from these impurities; and the GLC determination is simpler and no radioactively labelled compounds are required.

When only a small amount of the compound is soluble in one of the two phases, the k and l values obtained are merely approximations, owing to the great influence of the experimental error. A difference of 0.01 in an extreme k or l value (*e.g.*, $k = 0.99$ or 0.98) can give very different k/l values (99 or 49). Therefore, very high and very low partition coefficients are often unreliable. By taking the average

of five determinations this difficulty can be partly avoided. The results obtained (Table I) with four compounds with diverging lipophilic substituents are in general agreement with the π values of substituted phenoxyacetic acids[8]. As a result of this analogy and the inaccuracy of the method, no further experimental π values were determined and the π values of phenoxyacetic acid substituents were subsequently used in this study.

ΔR_M values

The determination of ΔR_M values has some important advantages over the determination of partition coefficients[16].

Liquid–liquid partition chromatography is best applied with the method of reversed-phase TLC. The carrier material (silica gel) is impregnated with a lipid phase (such as silicone oil or liquid paraffin) and the spotted compounds are chromatographed with a hydrophilic solvent (water, acetone or acetic acid). The choice of both phases is arbitrary so that a set of solvents can be used with optimal R_F values for the whole series of compounds.

Draber *et al.*[17] found a very interesting relationship between R_M values using polyamide TLC and reversed-phase TLC. The separation with polyamide TLC depends essentially on the hydrogen bonding forces between hydroxyl groups in phenolic compounds and the –CONH– groups in the polyamide molecules[18], but specific interactions between aromatic nitro groups and polyamide molecules can play an important role[19]. The chemical structure of organophosphorus pesticides includes many "bridges" such as –O–, –S–, P=S and P=O. Each oxygen or sulphur atom in these systems can in some way be involved in the hydrogen bonding with the polyamide molecule. Hence it could be possible that the polyamide TLC ΔR_M values obtained give interesting data on these hydrophobic forces which influence the biological activity of these compounds.

In this study, liquid paraffin was taken as the lipid phase and acetone–water (6:4, v/v) as the hydrophilic phase. Polyamide TLC was carried out with acetone–water (6:4, v/v) and *n*-hexane–acetic acid (95:5, v/v) in order to give a wide range of polarities.

Table II shows the difference in polarity between the solvents. Table IV illustrates the analogy between the three different TLC systems.

TABLE IV

COMPARISON OF THE R_F VALUES OBTAINED USING THREE DIFFERENT TLC SYSTEMS

Substituent	*R_F values*		
	Polyamide TLC		*Reversed-phase TLC, acetone–water (6:4, v/v)*
	n-Hexane–acetic acid (95:5, v/v)	*Acetone–water (6:4, v/v)*	
R^1 = Me, Et, *n*-C_3H_7	Me < Et < *n*-C_3H_7	*n*-C_3H_7 < Et < Me	*n*-C_3H_7 < Et < Me
R^2 = H, Me, Et, *n*-C_3H_7	H < Me < Et < *n*-C_3H_7	*n*-C_3H_7 < Et < Me < H	*n*-C_3H_7 < Et < Me < H
P = S or P = O	P = S < P = O	P = O < P = S	P = O < P = S
R^2 = *o*-, *m*-, *p*-subst.	Varies but *p*- ⩽ *o*- ⩽ *m*-		Varies but *m*- ⩽ *p*- < *o*-
R^2 = halogen	I < F < Br < Cl	I < Br < Cl < F	I < Br < Cl < F

π_{GLC} values

The GLC stationary phases can be classified according to their polarity using the table of McReynolds[20]. Three frequently used stationary phases with different polarities were taken, viz., DEGS (polar), OV-101 (non-polar) and OV-225 (intermediate polarity); 3% (w/w) of these stationary phases were applied on Gas-Chrom Q. The three stationary phases had only a slight influence on the chromatographic elution sequence of the compounds (Fig. 3); only the absolute retentions were different. The retention times of the *p*-nitro compound, under analogous experimental conditions, were 30 min for OV-225, 25 min for DEGS and 18 min for OV-101.

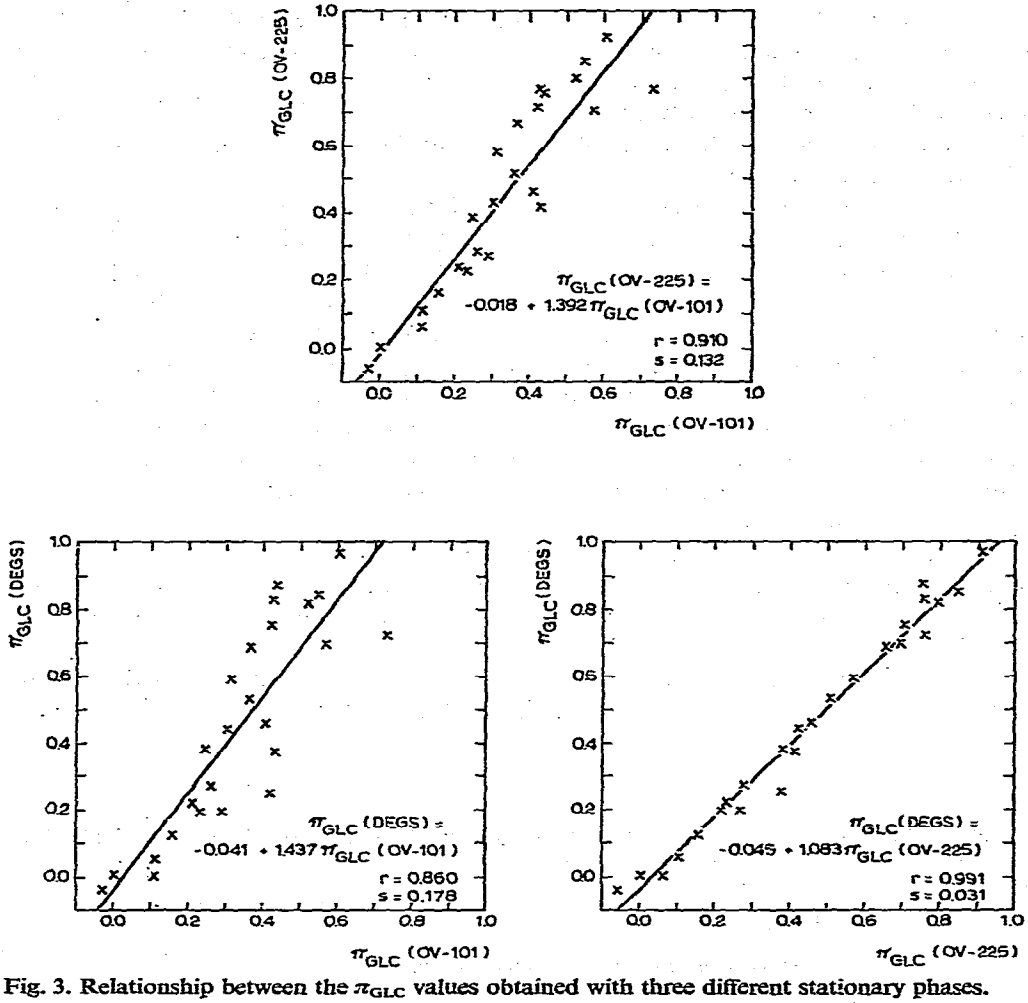


Fig. 3. Relationship between the π_{GLC} values obtained with three different stationary phases.

Relationships between chromatographic properties and linear free energy parameters

Investigations of the correlation between chemical structure and biological activity[21] make use of linear free energy substituent parameters to define electronic hydrophobic and steric influences. Frequently used parameters are σ (ref. 22) for

electronic factors, π (ref. 8) for hydrophobic factors and E_S (ref. 23) for steric factors. Correlations of these linear free energy parameters with the experimental chromatographic parameters can provide valuable information.

Experimental π_{GLC} values of the *para*-substituted compounds on a non-polar stationary phase (OV-101) show some relationship with steric factors:

$$\pi_{GLC(OV\text{-}101)} = 0.39 - 0.43\,E_S\ (n = 11,\ s = 0.516,\ r = 0.700,\ F = 8.65^{*})$$

where n is the number of points used in the regression, s is the standard deviation, r is the correlation coefficient and F is the calculated F value, which can exceed the level of 95%* or 99%** significance. These equations are computed by regression analysis using the least-squares method[24].

Depending on the polarity of the stationary phase, steric influences (E_S) decrease in favour of electronic influences (σ):

$$\begin{aligned}\pi_{GLC(OV\text{-}225)} &= 0.54 - 0.80\,E_S\ (n = 11,\ s = 0.590,\ r = 0.577,\ F = 4.49)\\ &= 0.21 + 1.33\,\sigma\ (n = 11,\ s = 0.262,\ r = 0.683,\ F = 7.86^{*}).\\ \pi_{GLC(DEGS)} &= 0.56 - 0.97\,E_S\ (n = 11,\ s = 0.630,\ r = 0.490,\ F = 2.84)\\ &= 0.22 + 1.27\,\sigma\ (n = 11,\ s = 0.235,\ r = 0.756,\ F = 11.98^{**}).\end{aligned}$$

There is a striking lack of any correlation between π_{GLC} values and the hydrophobic parameter π, which indicates that the π_{GLC} values are not parameters related to hydrophobic forces:

$$\begin{aligned}\pi_{GLC(OV\text{-}101)} &= -0.28 + 1.11\,\pi\ (n = 11,\ s = 0.573,\ r = 0.328,\ F = 1.08);\\ \pi_{GLC(OV\text{-}225)} &= -35.33 + 66.66\,\pi\ (n = 11,\ s = 0.606,\ r = 0.008,\ F = 0.01);\\ \pi_{GLC(DEGS)} &= 2.62 - 4.16\,\pi\ (n = 11,\ s = 0.601,\ r = 0.136,\ F = 0.17).\end{aligned}$$

ΔR_M values obtained on polyamide TLC with *n*-hexane–acetic acid (95:5) as eluent showed correlations with electronic and hydrophobic parameters:

$$\begin{aligned}\Delta R_M &= -0.04 + 1.00\,\sigma\ (n = 11,\ s = 0.230,\ r = 0.766,\ F = 12.81^{**})\\ &= 0.47 + 0.68\,\pi\ (n = 11,\ s = 0.449,\ r = 0.672,\ F = 7.42^{*}).\end{aligned}$$

On the other hand, polyamide ΔR_M values with acetone–water (6:4) as the eluent give a very good correlation with hydrophobic forces but not with electronic influences:

$$\begin{aligned}\Delta R_M &= 0.73 + 6.67\,\sigma\ (n = 11,\ s = 0.358\ \ r = 0.069,\ F = 0.04)\\ &= 0.08 + 0.19\,\pi\ (n = 11,\ s = 0.043,\ r = 0.823,\ F = 37.61^{**}).\end{aligned}$$

The relationship between ΔR_M on polyamide using acetone–water and π is illustrated in Fig. 4.

An analogous behaviour can be deduced from the ΔR_M values in reversed-phase TLC with the same solvent pair:

$$\begin{aligned}\Delta R_M &= 0.43 - 2.18\,\sigma\ (n = 11,\ s = 0.351,\ r = 0.207,\ F = 0.40)\\ &= -0.01 + 0.26\,\pi\ (n = 11,\ s = 0.070,\ r = 0.993,\ F = 659.36^{**}).\end{aligned}$$

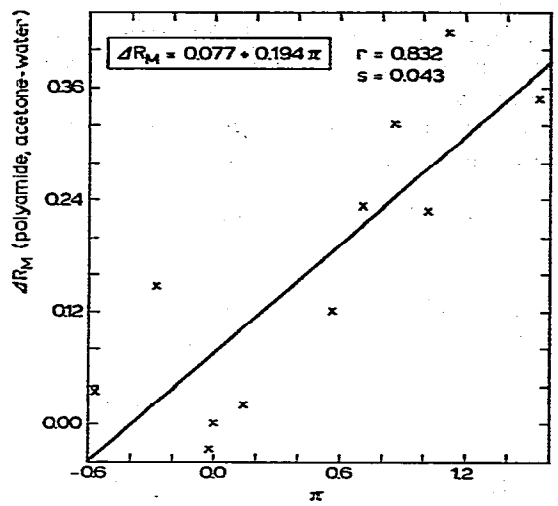


Fig. 4. Relationship between ΔR_M (polyamide, acetone–water) and π.

Fig. 5 demonstrates the relationship between these ΔR_M values and π. These results show that the solvent systems must be sufficiently polar in order to obtain ΔR_M values that are representative of hydrophobic forces and that give good partitioning between the mobile and stationary phases.

It can be concluded that ΔR_M parameters obtained from polyamide TLC and reversed-phase TLC are interesting indices of hydrophobicity for inclusion in quantitative structure–activity relationships. Good correlations are obtained with the hydrophobic substituent parameter π as reviewed recently by Tomlinson[16].

In contrast with the study of Clifford and Watkins[12], the π_{GLC} values derived from gas chromatographic retention times, are not related with hydrophobic free-energy related parameters but with electronic and steric substituent parameters.

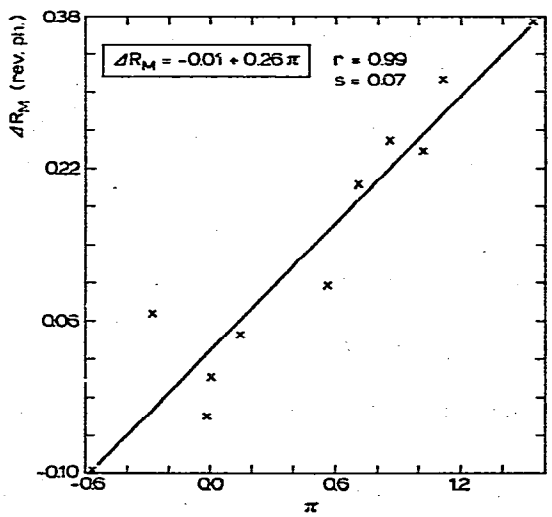


Fig. 5. Relationship between ΔR_M (reversed-phase, water–acetic acid) and π.

REFERENCES

1 S. Marcinkiewicz, J. Green and D. McHale, *J. Chromatogr.*, 10 (1963) 42.
2 G. L. Biagi, M. C. Guerra, A. M. Barbaro and M. J. Gamba, *J. Med. Chem.*, 13 (1970) 511.
3 C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, 208 (1965) 537.
4 W. Steurbaut, N. De Kimpe, L. Schreyen and W. Dejonckheere, *Bull. Soc. Chim. Belge*, 84 (1975) 751.
5 W. Steurbaut, W. Dejonckheere and R. H. Kips, in N. R. McFarlane (Editor), *Crop Protection Agents —Their Biological Evaluation*, Academic Press, London, 1976, p. 79.
6 K. H. Meyer and H. Hemmi, *Biochem. Z.*, 277 (1935) 39.
7 E. Overton, *Z. Phys. Chem.*, 22 (1897) 189.
8 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
9 C. Hansch and E. W. Deutsch, *Biochim. Biophys. Acta*, 126 (1966) 117.
10 E. C. Bate-Smith and R. G. Westfall, *Biochim. Biophys. Acta*, 4 (1950) 427.
11 A. J. P. Martin, *Biochem. Soc. Symp.*, 3 (1949) 4.
12 D. R. Clifford and D. A. M. Watkins, *Pestic. Sci.*, 2 (1971) 41.
13 M. Beroza, M. N. Inscoe and M. C. Bowman, *Residue Rev.*, 30 (1969) 1.
14 P. S. Jaglan and F. A. Gunther, *Analyst (London)*, 95 (1970) 763.
15 R. M. Hollingworth, *Ph.D. Thesis*, University of California, Riverside, Calif., 1966.
16 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
17 W. Draber, K. H. Buchel and K. Dickore, *Proc. 2nd Int. Congr. Pestic. Chem., 1971*, Vol. 5, 1972, p. 153.
18 H. Endres and H. Hormann, *Angew. Chem.*, 75 (1963) 288.
19 H. Endres, *Z. Anal. Chem.*, 181 (1961) 391.
20 W. O. McReynolds, *J. Chromatogr. Sci.*, 8 (1970) 214.
21 C. Hansch, in E. J. Ariens (Editor), *Drug Design*, Vol. 1, Academic Press, New York, 1974, pp. 271–342.
22 L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill, New York, 1950.
23 R. W. Taft, in M. S. Newman (Editor), *Steric Effects in Organic Chemistry*, Wiley, New York, 1956, pp. 556–675.
24 N. R. Draper and H. Smith, *Applied Regression Analysis*, Wiley, New York, 1966.