Journal of Chromatography, 105 (1975) 33–41

CHROM. 7966

RELATIONSHIPS BETWEEN HANSCH'S π PARAMETERS AND R_M VALUES DETERMINED ON POLYAMIDE THIN LAYERS

DAVID BROWN and DAVID WOODCOCK
University of Bristol, Long Ashton Research Station, Bristol BS18 9AF (Great Britain)
(Received September 25th, 1974)

SUMMARY

R_M values of two groups of N-(1-substituted-2,2,2-trichloroethyl)formamides have been measured using chromatography on polyamide thin layers.

Substituent ΔR_M values correlated poorly with Hansch's π parameters, unless the electronic effect of the substituent was taken into account. R_M values correlated with a linear combination of π and certain electronic parameters, including the Hammett σ function and specific quantum mechanical indices. Consequently, the relative influence of electronically determined solvation forces is thought to be different in polyamide TLC from that in Hansch's octanol–water partitioning system, and in Hansch-type regression equations employing parameters determined on polyamide layers some fraction of the electronic term will compensate for this effect.

The mechanism of chromatography on polyamide thin layers is considered, and the possibility raised that, with certain compounds, charge-transfer complexing might be an important influence.

INTRODUCTION

Since the pioneering work of Overton[1], Meyer[2] and Collander[3] it has been established that the lipophilic nature of a substance is often an important factor in controlling its interaction with biological systems. Hansch and Fujita[4] proposed a general mathematical relationship between the penetration of a molecule into cells and a substituent constant π which they defined by the free energy relationship

$$\pi = \log P_X - \log P_H \tag{1}$$

where P_H and P_X are the octanol/water partition coefficients of the parent compound and a derivative, respectively. π has proved to be of great utility in correlating structure with biological activity[4].

To avoid the practical difficulties often associated with the direct determination of partition coefficients, Boyce and Milborrow[5] exploited the theoretical relationship between the partition coefficient and R_M values from liquid–liquid partition chromatography:

$$R_M = a \log P + b \tag{2}$$

They pointed out that the value of R_M for a substituent (ΔR_M) is a free-energy-based constant analogous to π used by Hansch and Fujita, and consequently it should be possible to correlate the penetration of substances with either their R_M or ΔR_M values. This premise has been validated by authors using a variety of reversed-phase systems, including silica gel plates treated with paraffin oil[5] or silicone DC-200[6], and cellulose plates impregnated with ethyl oleate[7] or *n*-octanol[8].

Recently Draber *et al.*[9] have shown that commercial polyamide plates behave as a hydrophobic phase without further treatment, and Verloop[10] has suggested that this polyamide plate method might give better constants for hydrophobic bonding, because Hansch and Helmer[11] have shown that the mechanism of such bonding is the same in both synthetic and natural polymers.

We show in this paper that in a series of N-(1-substituted-2,2,2-trichloroethyl)-formamides prepared for an assessment of their antifungal properties[12,13], R_M values determined on polyamide thin layers do not directly correlate with Hansch's π constants.

EXPERIMENTAL

Preparation of compounds

The N-(1-substituted phenoxy-2,2,2-trichloroethyl)formamides and 1-substituted anilino-2,2,2-trichloroethyl)formamides were prepared by reaction of the appropriate phenol or aniline with N-(1,2,2,2-tetrachloroethyl)formamide, as described elsewhere[12,13].

Thin-layer chromatography

Polyamide powder (Woelm 8.5 g) and absolute ethanol (50 ml) were homogenised for 2 min in a Waring blendor with cellulose powder (Macherey, Nagel & Co.), (Düren, G.F.R.) MN 300: 1.5 g) added for its binding properties[14]. The inclusion of cellulose produced no detectable change in R_F values. The resulting slurry was spread onto two 40 × 20 cm glass plates, giving a layer 0.3 mm thick. The air-dried layers were marked by a line 15 cm from and parallel to the bottom edge, then aliquots (1 μl, 10^{-2} *M*) of the compounds in acetone were applied to the layers at 1.5-cm intervals in a line 2.5 cm from the bottom edge, 5 cm of the layer at each side of the plates being left free in order to minimise edge effects. Two spots of each compound and the parent (unsubstituted) compound of the congeneric series were applied to randomised positions on each of four plates, which were then developed simultaneously by the ascending technique in a paper-lined chromatography tank previously equilibrated for 72 h. The distance migrated by the leading edge of each spot in a ethanol–water (1:1) mobile phase was determined after air-drying the plates, spraying with a silver nitrate–hydrogen peroxide reagent[14], and irradiating with UV light (254 nm) for 1–2 h.

Electronic parameters

Hammett σ values for the ring substituents are from the compilation of Jaffé[15].

Electronic properties of the π-electron system in the phenoxy and anilino portions of the molecules were computed by the Hückel molecular orbital method[16,17]. The quantum mechanical values computed were the π-electron density (q) at each atom, and the energies in β units of the highest occupied molecular orbital (E_{HOMO}) and the lowest empty molecular orbital (E_{LEMO}) in each molecule. Details of the procedure and parameters used are given elsewhere[12,18].

Regression analysis

Correlations were determined by multiple regression analysis using the "Genstat" statistical program package on an I.C.L. 4/70 computer system. Results are expressed in terms of the equation for the best-fitting straight line, together with the multiple correlation coefficient r, standard deviation s, and Students t test. n is the number of data points included in the regression.

RESULTS AND DISCUSSION

Mean R_M values for the compounds and substituent ΔR_M values, together with Hansch's π parameters calculated according to the additivity rule[19,20], are given in Table I. Also included are those electronic parameters significant to the ensuing discussion. A complete list of the calculated atomic charge densities for the substituted anilines and phenols is recorded elsewhere[12].

Since they were determined in different "partitioning" systems, ΔR_M and π differ quantitatively, but ΔR_M should vary directly with π if eqn. 2 holds. The plots in Figs. 1a and 1b indicate that this is not so, and the corresponding regression equations for the two sets of compounds (eqns. 3 and 4, respectively) have low correlation coefficients and relatively high standard deviations. In Fig. 1a there is no correlation for the complete set of compounds:

$$\pi = \underset{(1.01)^{*}}{1.629}\Delta R_M + 0.687 \tag{3}$$

$$n = 12,\ r = 0.042,\ s = 0.473$$

Line 1 in Fig. 1b includes all compounds and obeys the regression equation:

$$\pi = \underset{(3.82)}{3.303}\Delta R_M + 0.481 \tag{4}$$

$$n = 16,\ r = 0.715,\ s = 0.422$$

A visual survey of the plots suggested that better correlations would be obtained in both series if the data points were divided into two groups, one containing compounds with only alkyl substituents in the aromatic ring (eqns. 5 and 6, relating to lines 1 and 2 of Figs. 1a and 1b, respectively)

$$\pi = \underset{(5.04)}{11.892}\Delta R_M + 0.101 \tag{5}$$

$$n = 5,\ r = 0.946,\ s = 0.153$$

$$\pi = \underset{(5.05)}{11.592}\Delta R_M + 0.077 \tag{6}$$

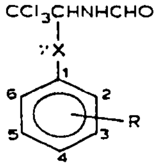
$$n = 5,\ r = 0.946,\ s = 0.162$$

---

[*] The figure in parentheses is the Student's t test for the significance of the variable above.

TABLE I

CHROMATOGRAPHIC, PARTITIONING AND ELECTRONIC DATA FOR N-(1-SUBSTITUTED PHENOXY-2,2,2-TRICHLOROETHYL)FORMAMIDES AND 1-SUBSTITUTED ANILINO-2,2,2-TRICHLOROETHYL)FORMAMIDES



R	*Partitioning data*				*Electronic parameters****				
	R_m*	ΔR_M	*Hansch's π value*	σ	E_{LEMO}	E_{HOMO}	q_1	q_6	q_7
Phenoxy compounds ($X = O$)									
H	−0.17±0.01	0.00	0.00	0.00	−1.0000	0.8413	1.0171	1.0208	1.9255
3-OCH_3	−0.16±0.01	0.01	0.12	0.08	−1.0112	0.7830	1.0165	1.0379	1.9255
4-F	−0.11±0.01	0.06	0.15	0.06	−0.9659	0.8605	1.0035	1.0224	1.9230
4-Cl	−0.05±0.02	0.22	0.70	0.23	−0.9755	0.8575	1.0050	1.0222	1.9233
4-Br	0.10±0.01	0.27	1.02	0.23	−0.9859	0.8447	1.0079	1.0220	1.9239
4-NO_2	−0.27±0.03	−0.10	0.24	0.78	−1.0000	0.5988	0.9213	1.0147	1.8984
3,4-$(CH_3)_2$	−0.08±0.00	0.09	1.03	−0.30	−1.0124	0.7615	1.0287	1.0326	1.9272
3,5-$(CH_3)_2$	−0.08±0.01	0.09	1.02	−0.14	−1.0281	0.8180	1.0152	1.0542	1.9254
2-CH_3-4-Cl	0.10±0.01	0.27	1.38	0.06	−0.9779	0.8200	1.0232	1.0217	1.9252
3-CH_3-4-Cl	0.07±0.00	0.24	1.21	0.17	−0.9790	0.8422	1.0042	1.0353	1.9234
3,4-Cl_2	0.16±0.01	0.33	1.46	0.53	−0.9397	0.8598	1.0063	1.0100	1.9235
2,4-Cl_2	0.14±0.01	0.31	1.29	0.46	−0.9466	0.8636	0.9860	1.0232	1.9207
3-CH_3-4-C_2H_5	−0.08±0.00	0.09	1.50	−0.32	−1.0124	0.7615	1.0287	1.0326	1.9272
2,3,5-$(CH_3)_3$	−0.02±0.00	0.15	1.70	−0.34	−1.0309	0.7730	1.0336	1.0532	1.9272
2,4,5-Cl_3	0.68±0.01	0.85	2.05	0.76	−0.8982	0.8652	0.9872	1.0019	1.9208
4-Cl-3,5-$(CH_3)_2$	0.08±0.01	0.25	1.72	0.10	0.9873	0.8373	1.0034	1.0556	1.9234
Anilino compounds ($X = NH$)									
H	−0.04±0.00	0.00	0.00	0.00	−1.0000	0.5539	0.9812	1.0614	1.8493
3-Cl	0.21±0.01	0.25	0.76	0.37	−0.9598	0.5516	0.9822	1.0493	1.8493
4-CH_3	−0.01±0.00	0.03	0.52	−0.17	−1.0000	0.5183	0.9921	1.0599	1.8531
4-Cl	0.18±0.01	0.22	0.70	0.23	−1.0000	0.5701	0.9703	1.0625	1.8441
4-*n*-C_4H_9	0.12±0.00	0.16	1.90	−0.16	−1.0000	0.5183	0.9921	1.0599	1.8531
3,4-$(CH_3)_2$	0.06±0.01	0.10	1.03	−0.30	−1.0136	0.5135	0.9912	1.0725	1.8531
3,4-Cl_2	0.28±0.02	0.32	1.46	0.53	−0.9563	0.5710	0.9713	1.0508	1.8441
3-Cl-4-CH_3	0.23±0.01	0.27	1.28	0.24	−0.9606	0.5204	0.9933	1.0475	1.8533
3-I-4-CH_3	0.33±0.00	0.37	1.67	0.18	−0.9759	0.5190	0.9929	1.0524	1.8532
3-NO_2-4-Cl	0.27±0.01	0.31	0.81	0.94	−0.9553	0.5761	0.9719	0.9648	1.8463
2,5-Cl_2	0.27±0.01	0.31	1.46	0.53	−0.9612	0.5813	0.9533	1.0624	1.8388
2,4,5-$(CH_3)_3$	0.06±0.01	0.10	1.71	−0.47	−1.0257	0.4865	1.0066	1.0812	1.8559

* Mean of eight observations ± standard error of the mean.

** From octanol–water partitioning experiments[4].

*** A complete list of the electronic parameters included in the regressions is given elsewhere[12].

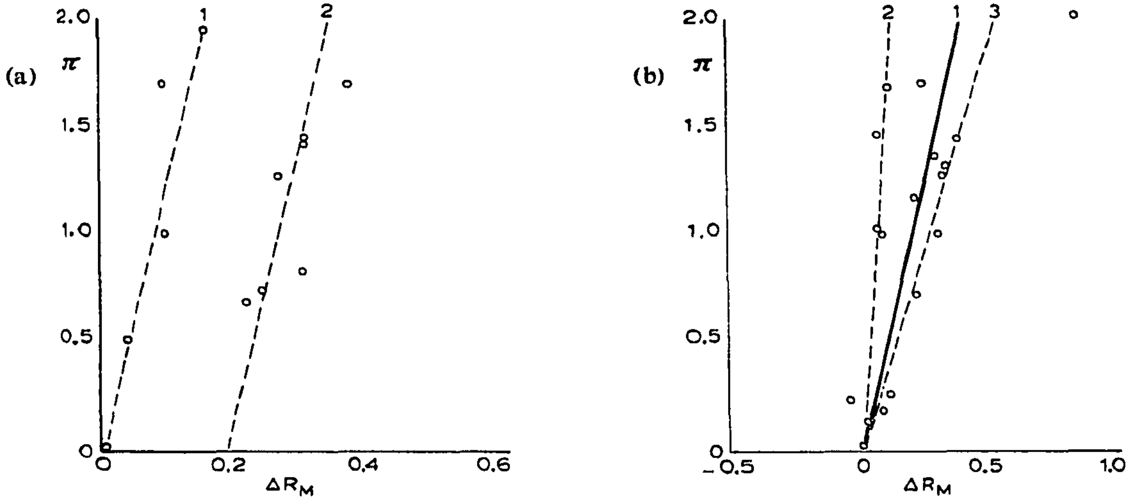


Fig. 1. Correlations between Hansch's π values and ΔR_M values determined experimentally on polyamide thin layers. (a) N-(1-Substituted anilino-2,2,2-trichloroethyl)formamides. (b) N-(1-Substituted phenoxy-2,2,2-trichloroethyl)formamides.

and the other including compounds with halogeno substituents in the aromatic ring (eqns. 7 and 8, relating to lines 2 and 3 of Figs. 1a and 1b, respectively)

$$\pi = 6.072\Delta R_M - 0.652 \quad (7)$$
$$(2.64)$$
$$n = 7,\ r = 0.797,\ s = 0.234$$

$$\pi = 4.859\Delta R_M - 0.078 \quad (8)$$
$$(3.97)$$
$$n = 10,\ r = 0.832,\ s = 0.211$$

It is unlikely that the low correlations between π and ΔR_M are due to errors in either the experimental determination of R_M values or the calculation of π by additivity methods[21], because good correlations were obtained between π and ΔR_M values determined by a similar technique on cellulose thin layers impregnated with ethyl oleate[12,18]. The low correlations appear to indicate that the chromatographic process on polyamide thin layers is not analogous to octanol–water partitioning, or indeed to reversed-phase thin-layer chromatography (TLC) on ethyl oleate-impregnated cellulose layers. Draber *et al.*[9] also suggested that chromatography on untreated polyamide plates is not a pure partitioning process, and speculated that hydrogen bonding and charge-transfer complexing may be important. If this is so, the low correlation between substituent π and ΔR_M values may be due to the electronic effects of the substituents.

To test this hypothesis, and to see whether the nature of any differing electronic influences in the two systems could be deduced, the π and ΔR_M values were subjected to linear regression analysis with either Hammett σ constants or certain molecular orbital indices chosen as a measure of specific weak electronic interactions (hydrogen bonding and charge-transfer complexing) which may influence the chromatographic and partitioning process. The significant regressions that resulted from this treatment are given in Tables II and III.

In both series of compounds, inclusion of the electronic term σ in the regression led to a significant improvement in the equations correlating π and ΔR_M (eqns. 14 and 22), and the positive coefficient of σ indicates that ΔR_M values of electron-withdrawing substituents are larger than predicted from Hansch's octanol–water partition data.

TABLE II

CORRELATIONS BETWEEN ΔR_M, π AND VARIOUS ELECTRONIC PROPERTIES OF N-(1-SUBSTITUTED PHENOXY-2,2,2-TRICHLOROETHYL)FORMAMIDES ($n = 16$)

Correlations and properties	*Equation*
$\Delta R_M = 0.303\pi - 0.146$ (3.86) $r = 0.719$, $s = 0.428$	9
$\Delta R_M = 4.954\ E_{LEMO} + 5.073$ (5.07) $r = 0.805$, $s = 0.132$	10
$\Delta R_M = 0.173\pi + 3.927\ E_{LEMO} + 3.881$ (5.03) (6.29) $r = 0.938$ and $s = 0.079$	11
$\Delta R_M = 0.208\pi + 1.257\ E_{HOMO} - 1.041$ (3.68) (2.38) $r = 0.815$, $s = 0.134$	12
$\Delta R_M = 0.263\pi - 6.655q_6 + 6.770$ (5.54) (3.47) $r = 0.866$, $s = 0.115$	13
$\Delta R_M = 0.239\pi + 0.275\sigma - 0.095$ (4.86) (3.16) $r = 0.852$, $s = 0.120$	14

The successful correlations incorporating molecular orbital indices may be indicative of the differing electronic influence of substituents during polyamide TLC and octanol–water partitioning. Within the two congeneric series ΔR_M values are higher than expected from their π octanol–water values in compounds with:

(a) less negative E_{LEMO} values —*i.e.* greater ability to accept electrons in charge-transfer reactions;

(b) more positive E_{HOMO} values —*i.e.* lower ability to donate electrons in charge-transfer reactions; and

(c) lower pi-electron densities (q_6 or q_1, q_6 and q_7) over the –O–C=C– or –N–C=C– part of the phenolic or anilino ring, respectively —*i.e.* compounds with less tendency to interact electrostatically at these positions with positively charged moieties.

The intercorrelation between the electronic parameters in eqns. 11–14 and 17–22, as shown by the correlation matrices in Table IV, makes it impossible to say

TABLE III

CORRELATIONS BETWEEN ΔR_M, π AND VARIOUS ELECTRONIC PROPERTIES OF N-(1-SUBSTITUTED ANILINO-2,2,2-TRICHLOROETHYL)FORMAMIDES ($n = 12$)

Correlations and properties	*Equation*
$\Delta R_M = 0.614\pi - 0.422$ (1.01) $r = 0.042, s = 0.473$	15
$\Delta R_M = 3.826\ E_{LEMO} + 3.968$ (3.99) $r = 0.784, s = 0.253$	16
$\Delta R_M = 0.096\pi + 3.721\ E_{LEMO} + 3.759$ (3.02) (5.24) $r = 0.900, s = 0.177$	17
$\Delta R_M = 0.174\pi + 3.205\ E_{HOMO} - 1.712$ (3.44) (3.20) $r = 0.807, s = 0.081$	18
$\Delta R_M = 0.164\pi - 7.556q_1 + 7.467$ (3.06) (2.79) $r = 0.773, s = 0.088$	19
$\Delta R_M = 0.115\pi - 2.34q_6 + 2.536$ (2.24) (2.40) $r = 0.733, s = 0.094$	20
$\Delta R_M = 0.146\pi - 19.001q_7 + 35.197$ (2.67) (2.43) $r = 0.737, s = 0.093$	21
$\Delta R_M = 0.127\pi + 0.252\sigma + 0.025$ (4.44) (6.10) $r = 0.927, s = 0.052$	22

TABLE IV

CORRELATION MATRICES OF ELECTRONIC DATA FOR SUBSTITUTED PHENOLS AND ANILINES

Phenols						
σ	1.0000					
E_{LEMO}	−0.8597	1.0000				
E_{HOMO}	0.1822	−0.2174	1.0000			
q_1	−0.0782	−0.1985	−0.0679	1.0000		
q_6	−0.5654	0.7194	−0.2344	−0.1098	1.0000	
q_7	−0.2590	0.0439	0.1849	−0.1247	−0.2383	1.0000
	σ	E_{LEMO}	E_{HOMO}	q_1	q_6	q_7
Anilines						
σ	1.0000					
E_{LEMO}	−0.7487	1.0000				
E_{HOMO}	−0.7666	0.4917	1.0000			
q_1	−0.6946	0.4401	0.9807	1.0000		
q_6	−0.8279	0.9807	0.5043	0.4286	1.0000	
q_7	−0.6662	0.3397	0.9615	0.9901	0.3407	1.0000
	σ	E_{LEMO}	E_{HOMO}	q_1	q_6	q_7

with any certainty which of the electronic effects described by these parameters is the more important. Indeed, the evidence against all these factors is largely statistical and statistical association is not proof of cause and effect. It is clear, however, that for these series of compounds the relative influence of electronically determined solvation forces differs in the polyamide TLC and octanol–water partitioning systems, and that in Hansch-type regression equations[4] employing parameters determined on polyamide layers, some fraction of the electronic term will compensate for this effect.

The equations may also be indicative of the mechanism of the chromatographic process on polyamide thin layers. The principle adsorption forces in polyamide chromatography are thought to involve hydrogen bonding between protons of the absorbate and amide groups of the absorbent[22]. Oxygen atoms found in either an ether linkage or a carbonyl group may also partially bond with the hydrogen of the amide group. Bark and Graham[23] envisaged that the chromatographic process involves breaking the solute–polyamide bonds through displacement of the solute by an eluent capable of hydrogen bonding with the polyamide surface.

Any of these interactions could occur with the compounds in this investigation, and the higher R_M values of compounds containing halogeno substituents might be attributed to their stronger adsorption to the stationary phase through hydrogen bonds involving the halogeno lone-pair electrons. However, the striking correlation in both series of compounds between R_M and E_{LEMO} (eqns. 10 and 16) raises the question of whether the ability of the compounds to accept electrons in charge-transfer interactions might be particularly influential in the chromatographic process. This type of interaction might be of general importance in those aromatic compounds that are unable to form strong hydrogen bonds with the polyamide layer, and a re-examination of relevant published data for such compounds may be rewarding.

ACKNOWLEDGEMENTS

We are indebted to Mr. P. Brain for performing the regression analyses, and to Dr. G. L. Caldow of the Theoretical Chemistry Department, Bristol University, for the computer program used for molecular orbital calculations. One of us (D.B.) thanks the Agricultural Research Council for a postgraduate studentship.

REFERENCES

1 E. Overton, *Studien über die Narkose*, Gustav Fischer, Jena, 1901.
2 H. Meyer, *Arch. Exp. Pathol. Pharmakol.*, 46 (1901) 338.
3 R. Collander, *Physiol. Plant.*, 7 (1854) 420.
4 C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
5 C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, 208 (1965) 537.
6 G. L. Biagi, A. M. Barbaro, M. F. Gamba and M. C. Guerra, *J. Chromatogr.*, 41 (1969) 371.
7 L. S. Bark and R. J. T. Graham, *J. Chromatogr.*, 23 (1966) 417.
8 A. E. Bird and A. C. Marshall, *J. Chromatogr.*, 63 (1971) 313.
9 W. Draber, K. H. Büchel and K. Dickoré, in A. S. Tahori (Editor), *Pesticide Chemistry*, Vol. 5, Gordon and Breach, New York, 1972, p. 153.
10 A. Verloop, in E. J. Ariens (Editor), *Drug Design*, Vol. 5, Academic Press, New York, 1972, p. 389.
11 C. Hansch and F. Helmer, *J. Polym. Sci.*, 6 (1968) 3295.
12 D. Brown, *Ph. D. Thesis*, University of Bristol, Bristol, 1974.
13 D. Brown and D. Woodcock, *Pestic. Sci.*, in press.

14 E. Stahl, *Thin-layer Chromatography*, Academic Press, New York, 1965.
15 H. H. Jaffé, *Chem. Rev.*, 53 (1953) 191.
16 A. Streitwieser, *Molecular Orbital Theory for Organic Chemists*, Wiley, New York, 1961.
17 B. Pullman and A. Pullman, *Quantum Biochemistry*, Wiley-Interscience, New York, 1963.
18 D. Brown and D. Woodcock, *Pestic. Sci.*, in press.
19 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
20 C. Hansch, J. E. Quinlan and G. Lawrence, *J. Org. Chem.*, 33 (1968) 347.
21 C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani and E. J. Lien, *J. Med. Chem.*, 16 (1973) 1207.
22 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
23 L. S. Bark and J. T. Graham, *J. Chromatogr.*, 27 (1967) 109.