Journal of Chromatography, 261 (1983) 329–343
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,747

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR HERBICIDES

REVERSED-PHASE LIQUID CHROMATOGRAPHIC RETENTION PARAMETER, log k_w, *versus* LIQUID–LIQUID PARTITION COEFFICIENT AS A MODEL OF THE HYDROPHOBICITY OF PHENYLUREAS, *s*-TRIAZINES AND PHENOXYCARBONIC ACID DERIVATIVES

THOMAS BRAUMANN*, GERT WEBER and L. HORST GRIMME
Universität Bremen, Fachbereich Biologie/Chemie, Postfach 330440, D-2800 Bremen 33 (G.F.R.)
(First received December 20th, 1982; revised manuscript received February 4th, 1983)

SUMMARY

The retention behaviour of phenylureas, *s*-triazines and phenoxycarbonic acid derivatives in a reversed-phase high-performance liquid chromatographic (RPLC) system has been examined. Using methanol–water or acetonitrile–water as the mobile phase, a linear relationship between the volume fraction of the organic modifier, φ, and the logarithm of the capacity factor, log k', is established for each solute. The different correlation curves for each compound indicate selective effects upon retention due to solute–solvent and solute–stationary phase interactions. It is shown that log k_w, a theoretical capacity factor obtained by extrapolation of retention data in binary solvent systems to pure aqueous eluent, is suitable for eliminating the selective effects and thereby for quantitatively describing the hydrophobic nature of solutes in a way which is strongly related to the partition coefficient, log P, of the standard *n*-octanol–water partitioning system. The dependence of log k_w on the nature of the organic modifier and an analysis of functional group behaviour in different eluents reveal that log P and log k_w are not completely interchangeable, because certain substituents, *i.e.*, methylthio and trifluoromethyl groups, behave differently in RPLC and a true liquid–liquid partitioning system. The consequences of this non-polar group selectivity in RPLC on the quality of quantitative structure–activity relationships for electron transport-inhibiting herbicides are demonstrated. The results suggest that log k_w might be a better model for the assessment of the hydrophobicity of drugs in biological systems.

INTRODUCTION

The aim of studies on quantitative structure–activity relationships (QSARs), introduced by Hansch and co-workers[1,2], is the correlation of biological activity with chemical structure for a congeneric series of compounds. Most of these studies, in-

0021-9673/83/$03.00

cluding those with herbicides[3–5], have shown that the inhibitory action of drugs is predominantly a function of their hydrophobic nature. The use of partition coefficients, P, obtained from an n-octanol–water partitioning system has become a standard method[2] for modelling biological membranes and thereby quantifying the hydrophobicity of a given compound as: $\log P = \log C_{oct} - \log C_{water}$

Log P is either determined experimentally or calculated[2,6]. The conventional shaking flask method is laborious and time.consuming, often complicated by instability in aqueous media, impurities and the tendency for a compound to dissociate. Furthermore, this procedure only has a limited application range up to $\log P = 4$[6]. Calculation of $\log P$ using known Hansch π values is often only successful when the molecular framework of a given series of compounds exhibits roughly the same physico-chemical properties as those from which the π values were derived. Thus, there are innumerable compounds for which $\log P$ values have to be determined.

Alternatively, chromatographic techniques can be used for the determination of the hydrophobic nature of drugs, especially thin-layer chromatography (TLC)[7] and reversed-phase high-performance liquid chromatography (RPLC) (see refs. 8–10 for extensive references). The latter technique has been shown to produce very efficiently high-precision data with respect to retention which are believed to be a measure of the partition behaviour between the non-polar bonded stationary phase and the more polar eluent. The capacity factor, k', is given by

$$k' = (t_R - t_0)/t_0 \tag{1}$$

where t_R and t_0 are the retention times of a retained and an unretained solute, respectively. k' is directly related to the Gibbs free energy attributed to the retention process, ΔG_0, according to

$$\log k' = \log \varphi - \Delta G_0/2.3\ RT \tag{2}$$

where R, T and φ are the gas constant, temperature and phase ratio of mobile and stationary phases, respectively. Log k' is therefore equivalent to log P and can also be used to obtain extrathermodynamic substituent constants[8,11].

It has been shown[10] that within a group of compounds of comparable size, shape and polarity, good correlations between log k' and log P are observed. However, with octadecyl-silica as the stationary phase and water–organic mixtures as eluents, polar group selectivity[12] and non-polar group selectivity[10,13] appear which depend both on solute structure and mobile phase composition. To eliminate these selective effects, we have introduced $\log k_w$ as a measure of hydrophobicity in RPLC and as a better analogue to log P[10]. This approach is equivalent to the R_m^0 values, introduced for TLC[14], and has recently found further application in studies of the hydrophobic nature of phenols[15], the prediction of solubility in water[16] and in ion-pair RPLC[17].

In this study, we investigate in detail the relation between the reversed-phase retention behaviour and bulk liquid–liquid partition coefficients of several economically important herbicidal groups. The results indicate that $\log k_w$ can be used instead of log P as a hydrophobicity parameter for phenylureas and phenoxycarbonic acid derivatives. However, the two parameters are not completely interchangeable because

specific substituents behave differently in RPLC and a true liquid–liquid partitioning system. In the case of *s*-triazines, only RPLC provides hydrophobicity parameters which are strongly related to the inhibitory action of these compounds on photosynthetic electron transport.

EXPERIMENTAL

Materials

All herbicides obtained from Riedel-de Haen (Hannover, G.F.R.) were of the highest purity available. In Table I are collected the common and chemical names of the compounds. Distilled water was prepared with an all-glass double distillation unit (Heraeus-Schott, Mainz, G.F.R.). All other reagents were of analytical reagent grade and used without further treatment. The column (25 cm × 4.6 mm I.D.) (E. Merck, Darmstadt, G.F.R.) was self-packed by the slurry technique using tetrachloroethylene as the suspending medium. The suspension was introduced into the column

TABLE I

COMMON NAMES AND CHEMICAL NAMES OF THE HERBICIDAL COMPOUNDS

Common name	*Chemical name*
Phenylureas	
Chloroxuron	3-[4-(4-Chlorophenoxy)phenyl]-1,1-dimethylurea
Chlortoluron	3-(3-Chloro-*p*-tolyl)-1,1-dimethylurea
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Fenuron	1,1-Dimethyl-3-phenylurea
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
Metobromuron	3-(4-Bromophenyl)-1-methoxy-1-methylurea
Metoxuron	3-(3-Chloro-4-methoxyphenyl)-1,1-dimethylurea
Monolinuron	3-(4-Chlorophenyl)-1-methoxy-1-methylurea
Monuron	3-(4-Chlorophenyl)-1,1-dimethylurea
Neburon	1-Butyl-3-(3,4-dichlorophenyl)-1-methylurea
s-Triazines	
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
Propazine	2-Chloro-4,6-di(isopropylamino)-1,3,5-triazine
Simazine	2-Chloro-4,6-di(ethylamino)-1,3,5-triazine
Prometryn	2,4-Di(isopropylamino)-6-methylthio-1,3,5-triazine
Desmetryn	2-Isopropylamino-4-methylamino-6-methylthio-1,3,5-triazine
Terbutryn	2-*tert*.-Butylamino-4-ethylamino-6-methylthio-1,3,5-triazine
Phenoxycarbonic acids	
2,4-D	2,4-Dichlorophenoxyacetic acid
Dichlorprop	2-(2,4-Dichlorophenoxy)propionic acid
Fenoprop	2-(2,4,5-Trichlorophenoxy)propionic acid
MCPA	4-Chloro-2-methylphenoxyacetic acid
MCPB	4-(4-Chloro-2-methylphenoxy)butyric acid
Mecoprop	2-(4-Chloro-2-methylphenoxy)propionic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
Phenoxycarbonic acid methyl esters	
All methyl esters of phenoxycarbonic acids listed above	

with the aid of a Haskel pump at 500 bar. The stationary phase was 10-μm Li-Chrosorb RP-18 (Merck, batch No. VV 1106). The column was used without further treatment in all experiments.

Chromatography

The liquid chromatograph consisted of a Series 2/2 reciprocating pump (Perkin-Elmer, Norwalk, CT, U.S.A.), a Model LC-55 variable wavelength UV–visible detector (Perkin-Elmer) and a Servogor Model S pen recorder (Metrawatt, Nürnberg, G.F.R.). The detector was set at 230 nm (*s*-triazines), 250 nm (phenylureas) and 280 nm (phenoxycarbonic acid derivatives).

A volume of 5 μl of a $2.5 \cdot 10^{-4}$ *M* sample solution was injected by means of a 10-μl precision syringe via a Rheodyne Model 7105 sample valve (Rheodyne, Berkeley, CA, U.S.A.), and the retention times were measured with a stop-watch. The reproducibility of the retention times was better than 1%, so that in all experiments two independent runs were carried out.

The mobile phase consisted of different volume fractions of methanol and acetonitrile in water, respectively, prepared with the gradient former of the chromatograph. Instead of water, the phenoxycarbonic acids were analysed in a 0.5 *M* acetate buffer (pH 2.9) as the aqueous phase. The flow-rate was 1.7 ml/min at room temperature. The column dead time was determined by the injection of acetone dissolved in methanol or acetonitrile with 100% methanol or acetonitrile, respectively, as the mobile phase. We have checked the reliability of t_0 obtained by this procedure by comparison with values derived from the linearization of the net retention time for homologous alkylbenzenes[18]. The t_0 values were identical within the experimental error. The standard error of log k' determinations was less than ± 0.005.

Measurement of the Hill reaction

Broken spinach chloroplasts were prepared according to a standard procedure[19]. The reaction medium contained 7 m*M* phosphate buffer (pH 6.4), 0.25 *M* sucrose, 0.1 m*M* dichlorophenolindophenol and 50 μg chlorophyll per ml and was illuminated for 45 sec with 50,000 lux by means of a projector lamp. The decrease in absorption was measured at 590 nm. At least four different herbicide concentrations were used for the determination of pI_{50}, the logarithm of the concentration of a herbicide which causes 50% inhibition of the Hill reaction.

RESULTS AND DISCUSSION

When the reversed-phase packing of octadecyl groups attached to a silica surface does not contain unreacted silanol groups which contribute to the retention of a solute, ligand–solute interaction will be weak and non-selective[20]. Genieser *et al.*[21] estimated the ligand content of the stationary phase used in this study and found a very high surface coverage of about 22%. Furthermore, determination of the dead volume with acetone gave the same values as derived from the linearization of the net retention time for homologous alkylbenzenes, which is very unlikely in the case of free silanol groups. It is therefore reasonable to assume that there are no accessible hydroxyl groups to contribute to retention. According to the solvophobic theory[20,22], retention will then be a function of solution behaviour in the mobile phase.

Variation of the capacity factor with mobile phase composition

The analytical tool to assess selective effects of specific molecular structures is the variation of the k' of the sample with the organic modifier content of the mobile phase[23]:

$$\log k' = \log k_w + S\varphi \tag{3}$$

where φ is the volume fraction of organic solvent in the water–organic solvent mixture, k_w represents the capacity factor of a solute with pure water as mobile phase (usually obtained by extrapolation to the intercept of the ordinate) and S, the slope of the regression curve, should be related to the solvent strength of the pure organic solvent[23]. Table II shows the retention data of herbicides at different volume fractions of methanol, φ_M, in water. Table III collects the data from regression analysis of the relation between log k' and φ_M, and includes the partition coefficients, log P.

From Table III, it is seen that eqn. 3 describes the variation of sample k' with φ_M. The proposal of Snyder *et al.*[23] that S depends only on the solvent strength of the pure organic modifier and should therefore be constant for different kinds of solutes is obviously not correct. Values of S can vary over the range -3.0 to -6.0, which indicates that a given increment in organic modifier concentration causes large differences in retention. The same behaviour has been found for benzene derivatives[16] and for ion-pair RPLC of benzenoic acids[17]. Within the different groups of herbicides, functional group selectivity clearly exists, as indicated by a large variation of the free energy change of the sorption process with a given change in φ_M of the mobile phase. The capacity factor, k', is therefore not a good parameter to describe the hydrophobic nature of a solute since compounds with the same k' at a given φ_M do not necessarily exhibit the same retention mechanism due to different values of S. We have therefore suggested[10] that log k_w is a better measure of the hydrophobicity of a solute because selective effects are eliminated owing to the extrapolation to $\varphi_M = 0$. That S as well as log k_w is largely dependent on the hydrophobic surface area of the solute is demonstrated by the close correlation of these variables:

$$S = -0.719 \log k_w - 1.980 \; (n = 30, r = 0.952) \tag{4}$$

The fact that the herbicides belong to quite different chemical classes essentially does not influence the relationship between S and log k_w. However, the different herbicidal groups show a small but significant deviation from the overall relation of eqn. 8 (Table IV).

If S and log k_w depend on the same properties of the solute, the slope of the regression curve will be -1.0. The smallest deviation from -1.0 is found for the *s*-triazines which possess only hydrophobic substituents symmetrically arranged around the heterocyclus. It is therefore reasonable to assume that the solvophobic effect is the dominant factor controlling retention. The phenoxycarbonic acids on the other hand are partly ionized at the pH of the mobile phase so that also electronic contributions play a considerable rôle with a concomitant by low slope of -0.458. The other herbicidal groups contain both hydrophilic and hydrophobic substituents and show a behaviour between the two extremes.

TABLE II

ISOCRATIC *log k'* VALUES OF HERBICIDES FOR DIFFERENT VOLUME FRACTIONS OF METHANOL, φ_M, IN WATER

M = Methyl ester.

Common name	*Volume fraction, φ_M*								
	0.90	*0.85*	*0.80*	*0.75*	*0.70*	*0.65*	*0.60*	*0.55*	*0.50*
Fenuron					−0.699	−0.538	−0.347	−0.184	0.036
Metoxuron				−0.734	−0.459	−0.292	0.131	0.064	0.238
Monuron			−0.620	−0.420	−0.244	−0.060	0.100	0.279	0.459
Monolinuron			−0.456	−0.292	−0.102	0.076	0.253	0.446	0.637
Chlortoluron			−0.398	−0.299	−0.027	0.158	0.344	0.543	0.743
Metobromuron			−0.377	−0.215	−0.022	0.158	0.344	0.544	0.743
Diuron			−0.292	−0.102	0.090	0.276	0.476	0.686	
Linuron			−0.187	0.017	0.207	0.410	0.618	0.840	
Chloroxuron			−0.097	0.117	0.352	0.583	0.829		
Neburon			0.021	0.262	0.496	0.725	1.010		
Simazine	−0.796	−0.638	−0.469	−0.284					
Atrazine	−0.699	−0.509	−0.310	−0.125					
Propazine	−0.585	−0.377	−0.180	0.053					
Prometryn	−0.252	0.009	0.283	0.581					
Desmetryn	−0.208	0.061	0.336	0.619					
Terbutryn	−0.131	0.140	0.430	0.758					
2,4-D					−0.509	−0.337	−0.180	−0.018	0.127
MCPA					−0.420	−0.252	−0.092	0.083	0.230
2,4,5-T					−0.276	−0.097	0.072	0.233	0.407
Dichlorprop					−0.260	−0.092	0.076	0.248	0.394
Mecoprop					−0.194	−0.018	0.155	0.322	0.483
Fenoprop					−0.066	0.117	0.301	0.483	0.650
MCPB					0.127	0.292	0.484	0.642	0.816
2,4-D-M	−0.523	−0.301	−0.102	0.086	0.312				
MCPA-M	−0.481	−0.268	−0.060	0.137	0.360				
Dichlorprop-M	−0.377	−0.161	0.053	0.265	0.502				
Mecoprop-M	−0.357	−0.143	0.072	0.286	0.529				
2,4,5-T-M	−0.310	−0.092	0.117	0.330	0.569				
MCPB-M	−0.180	0.041	0.260	0.502	0.753				
Fenoprop-M	−0.187	0.053	0.274	0.509	0.770				

TABLE III

REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN THE VOLUME FRACTION OF METHANOL, φ_M, AND log k': log k' = log k_w − $S\varphi_M$

Partition coefficients, log P, were calculated with π values given by Norrington *et al.*[24]. Diuron (log P = 2.68) was the reference for the dimethylureas, linuron (log P = 2.76) for the methylmethoxyureas. Log P values of *s*-triazines were calculated with simazine (log P = 1.51) as the standard compound. The log P values of the reference compounds were taken from ref. 25. Log P values of the phenoxycarbonic acids and their methyl esters were calculated with acetic acid (log P = −0.24), propionic acid (log P = 0.29) and butyric acid (log P = 0.70) as the parent compound, respectively. M = Methyl ester.

Common name	*log* k_w	$-S$	r	*log P*
Fenuron	1.838	3.642	0.9984	1.18
Metoxuron	2.185	3.859	0.9977	1.98
Monuron	2.239	3.556	0.9997	1.91
Monolinuron	2.453	3.650	0.9998	1.99
Chlortoluron	2.640	3.813	0.9998	2.55
Metobromuron	2.603	3.746	0.9996	2.37
Diuron	2.816	3.891	0.9998	2.68
Linuron	3.072	4.081	0.9997	2.76
Chloroxuron	3.602	4.636	0.9997	3.65
Neburon	3.920	4.882	0.9992	4.31
Simazine	2.267	3.410	0.9994	1.51
Atrazine	2.759	3.842	0.9999	2.05
Propazine	3.211	4.222	0.9994	2.59
Prometryn	4.731	5.546	0.9995	1.91
Desmetryn	4.749	5.512	0.9999	2.46
Terbutryn	5.178	5.914	0.9991	2.56
2,4-D	1.726	3.182	0.9996	2.22
MCPA	1.872	3.270	0.9997	2.30
2,4,5-T	2.103	3.392	0.9998	2.99
Dichlorprop	2.051	3.296	0.9996	2.75
Mecoprop	2.182	3.388	0.9998	2.83
Fenoprop	2.498	3.662	0.9999	3.52
MCPB	2.546	3.456	0.9996	3.53
2,4-D-M	3.186	4.114	0.9995	2.64
MCPA-M	3.277	4.174	0.9998	2.72
Dichlorprop-M	3.551	4.368	0.9998	3.17
Mecoprop-M	3.599	4.402	0.9997	3.25
2,4,5-T-M	3.611	4.360	0.9997	3.41
MCPB-M	3.998	4.654	0.9995	3.95
Fenoprop-M	4.076	4.740	0.9996	3.94

Influence of the nature of the organic modifier on log k_w

Log k_w was defined before as the capacity factor with pure water as the mobile phase and should therefore be independent of the nature of the organic modifier. Additionally, log k_w should be an intrinsic property of the solute, indicating the non-polar surface area[17,26]. In order to verify these assumptions, we have estimated the k' values of the phenylurea herbicides with acetonitrile–water mixtures as the mobile phase. Table V collects the resulting values for S and log k_w and compares them to those obtained from the methanol–water system.

TABLE IV

REGRESSION ANALYSIS OF THE RELATIONSHIP $S = a \log k_w + b$

Herbicides	$-a$	$-b$	n	r
s-Triazines	0.857	1.471	6	0.999
Phenylureas	0.643	2.219	10	0.932
Phenoxyacetic acid methyl esters	0.688	1.916	7	0.996
Phenoxyacetic acids	0.458	2.398	7	0.893
Pyridazinones*	0.792	2.096	8	0.969

* Data taken from refs. 10.

Again, we find a linear relationship between the capacity factor and the volume fraction of acetonitrile, φ_A, in water. However, $\log k_{w(A)}$ and $S_{(A)}$ are quite different from $\log k_{w(M)}$ and $S_{(M)}$. A regression analysis yields:

$$\log k_{w(M)} = 1.435 \log k_{w(A)} + 0.279 \; (n = 10, r = 0.939) \tag{5}$$

The moderate correlation coefficient indicates that $\log k_w$ indeed reflects basically the same molecular properties of the solute in both solvents, but these properties contribute differently to retention. This is also seen in the poor correlation of $S_{(A)}$ to $\log k_{w(A)}$ in contrast to the good correlation of $S_{(M)}$ to $\log k_{w(M)}$:

$$S_{(A)} = -0.203 \log k_{w(A)} - 2.749 \; (n = 10, r = 0.410) \tag{6}$$

Most probably, these differences arise from the chemical nature of the two organic modifiers. Methanol exhibits both hydrogen donor and acceptor abilities and will therefore easily be incorporated into the network of water molecules, whereas aceto-

TABLE V

REGRESSION ANALYSIS OF THE RELATIONSHIP $\log k' = \log k_{w(A)} - S_{(A)}\varphi_A$

The corresponding values of $S_{(M)}$ and $\log k_{w(M)}$ are included.

Common name	*Acetonitrile*				*Methanol*	
	$\log k_{w(A)}$	$-S_{(A)}$	n	r	$\log k_{w(M)}$	$-S_{(M)}$
Fenuron	0.910	2.902	7	0.9995	1.838	3.642
Metoxuron	1.493	3.518	7	0.9975	2.185	3.859
Monuron	1.428	3.086	7	0.9984	2.239	3.556
Monolinuron	1.732	3.035	7	0.9989	2.453	3.650
Chlortoluron	1.524	2.837	7	0.9981	2.640	3.813
Metobromuron	1.789	3.024	7	0.9997	2.603	3.746
Diuron	1.673	2.929	7	0.9979	2.816	3.891
Linuron	2.025	3.064	7	0.9985	3.072	4.081
Chloroxuron	2.132	3.217	7	0.9985	3.602	4.636
Neburon	2.427	3.302	7	0.9979	3.920	4.882

TABLE VI

REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN THE VOLUME FRACTION OF DIFFERENT ORGANIC MODIFIERS AND log k'

Retention data were taken from the compilation of ref. 27. Included are measured log P values, mostly published by the Hansch group[1,2].

Compound	*Methanol*		*Acetonitrile*		*Tetrahydrofuran*		*log P*
	$-S_{(M)}$	$\log k_{w(M)}$	$-S_{(A)}$	$\log k_{w(A)}$	$-S_{(T)}$	$\log k_{w(T)}$	
Acetophenone	2.73	1.92	2.28	1.42	2.71	1.21	1.66
Aniline	2.06	1.21	–	–	2.62	1.27	1.10
Anisole	2.66	2.15	2.62	1.86	3.21	1.81	2.11
Benzaldehyde	2.65	1.80	2.22	1.36	2.62	1.20	1.48
Benzene	2.56	2.16	2.57	1.86	3.08	1.85	2.13
Benzonitrile	2.63	1.77	2.44	1.54	3.01	1.45	1.56
Benzophenone	3.72	3.15	2.99	2.34	3.96	2.25	3.18
Benzyl alcohol	2.52	1.47	1.86	0.80	2.65	0.93	1.10
Biphenyl	4.23	3.89	3.25	2.88	3.99	2.54	4.02
n-Butylbenzene	4.54	4.32	3.34	3.03	–	–	4.26
Chlorobenzene	3.27	2.80	3.01	2.33	3.81	2.33	2.81
p-Chlorophenol	3.00	2.15	2.79	1.67	–	–	2.39
p-Chlorotoluene	3.76	3.37	3.01	2.50	–	–	3.33
o-Cresol	2.65	1.81	2.51	1.46	3.61	1.88	1.96
o-Dichlorobenzene	3.62	3.26	2.93	2.44	–	–	3.38
Diethyl phthalate	3.70	2.90	3.22	2.30	3.52	1.80	3.15
2,4-Dimethylphenol	3.09	2.31	2.74	1.77	3.94	2.15	2.30
Dimethyl phthalate	3.22	2.21	2.61	1.63	3.01	1.23	2.11
m-Dinitrobenzene	2.62	1.91	2.87	1.86	3.68	2.06	1.49
o-Dinitrobenzene	2.90	2.02	3.07	1.97	–	–	1.58
p-Dinitrobenzene	2.62	1.81	2.91	1.89	–	–	1.46
2,4-Dinitrotoluene	3.00	2.37	3.21	2.21	–	–	1.98
Diphenyl ether	4.34	3.91	3.52	2.91	4.00	2.54	4.20
Ethylbenzene	3.52	3.18	3.37	2.64	3.57	2.33	3.15
m-Fluoronitrobenzene	2.73	2.13	2.80	1.93	–	–	1.99
p-Fluoronitrobenzene	2.73	2.01	2.78	1.87	–	–	1.99
p-Fluorophenol	2.70	1.62	2.50	1.28	–	–	1.77
p-Hydroxybenzaldehyde	2.59	1.38	2.25	0.84	–	–	1.35
p-Methoxybenzaldehyde	2.79	1.97	2.35	1.42	–	–	1.68
p-Methylbenzaldehyde	2.92	2.15	2.54	1.73	–	–	2.04
Methyl benzoate	2.87	2.28	2.61	1.82	–	–	2.12
Naphthalene	3.58	3.22	3.01	2.46	4.13	2.54	3.37
p-Nitroacetophenone	2.77	1.95	2.65	1.68	3.28	1.68	1.53
p-Nitrobenzaldehyde	2.69	1.72	2.53	1.54	–	–	1.20
Nitrobenzene	2.70	2.03	2.66	1.80	3.36	1.80	1.85
m-Nitrophenol	2.72	1.80	2.68	1.46	4.10	2.10	2.00
o-Nitrophenol	2.54	1.90	2.50	1.61	–	–	1.79
p-Nitrophenol	2.79	1.77	2.81	1.49	–	–	1.91
Phenol	2.35	1.34	2.19	1.06	3.19	1.50	1.46
2-Phenylethanol	2.81	1.80	2.08	1.04	3.04	1.17	1.36
p-Phenylphenol	3.67	2.96	3.52	2.40	–	–	3.20
3-Phenylpropanol	3.06	2.19	2.43	1.41	–	–	1.88
n-Propylbenzene	4.15	3.82	3.29	2.83	–	–	3.68
Toluene	3.15	2.72	2.94	2.28	3.63	2.28	2.69
2,4,5-Trichlorotoluene	4.19	3.84	3.41	2.92	–	–	2.92

nitrile can serve only as an hydrogen acceptor and will change the structure of the mobile phase more drastically. The free energy change for the retention process will thus be dependent on the different molecular properties of the solute. In order to gain more insight into functional group selectivity in different organic modifiers, we have analysed the retention data of Schoenmakers *et al.*[27] with respect to S and $\log k_w$ (Table VI) and included measured $\log P$ values reported in the literature.

The contribution of a substituent to retention can be defined[8,11] as

$$\tau = \log (k'_j/k'_i) \tag{7}$$

where k' is the capacity factor of solutes j and i which differ by a substituent. When transformed to $\log k_w$, eqn. 7 becomes:

$$\tau_w = \log k_{w(j)} - \log k_{w(i)} \tag{8}$$

We have examined τ_w for the different monosubstituted benzenes of Table VI and with three different organic modifiers (Table VII).

In general, the methanol–water system is the most discriminating eluent with respect to substituent effects on retention. These differences are most pronounced for completely non-polar groups (Table VII). If we examine polar substituents, significant differences in polar group selectivity are found within the different mobile phases. Short alcoholic groups (as in 2-phenylethanol) produce polar contributions to

TABLE VII

FUNCTIONAL GROUP VALUES, τ_w, DERIVED FROM MONOSUBSTITUTED BENZENES OF TABLE VI

Log k_w for benzene is taken as reference. π values are taken from ref. 2.

Substituent	*Methanol–water* τ_M	*Acetonitrile–water* τ_A	*Tetrahydrofuran–water* τ_T	π
CH_3	+0.56	+0.42	+0.41	+0.56
CH_3CH_2	+1.02	+0.78	+0.48	+1.02
n-Propyl	+1.66	+0.97	–	+1.55
n-Butyl	+2.16	+1.17	–	+2.13
Phenyl	+1.73	+1.02	+0.73	+1.96
OH	−0.82	−0.80	−0.35	−0.67
CH_2OH	−0.69	−1.06	−0.92	−1.03
CH_2CH_2OH	−0.36	−0.82	−0.68	−0.77
$CH_2CH_2CH_2OH$	+0.03	−0.45	–	−0.25
Cl	+0.64	+0.47	+0.48	+0.71
NO_2	−0.13	−0.06	−0.05	−0.28
NH_2	−0.95	–	−0.58	−1.23
CN	−0.39	−0.32	−0.40	−0.57
CHO	−0.36	−0.50	−0.65	−0.65
$COCH_3$	−0.24	−0.44	−0.64	−0.55
OCH_3	−0.01	0.00	−0.04	−0.02

the benzene ring in the acetonitrile (AN) and tetrahydrofuran (THF) systems which are almost twice those in the methanol system. This selectivity difference has also been observed by Tanaka *et al.*[12] for different *n*-alcohols. These authors also showed that phenol is specifically retarded in the THF system, in accordance with our results (Table VII). Acetophenone and benzaldehyde also possess a greater polarity in THF and AN, but the functional group values in this case follow the order $\tau_M < \tau_A < \tau_T$. On the other hand, nitro and amino groups attached to the benzene ring result in a stronger retention in AN and THF (*i.e.*, τ_A and τ_T are smaller than τ_M). The dependence of τ for non-electrolytes on the nature of the organic modifier may be the result of electronic interactions between the solute and solvent, especially in the cases of polar group selectivity observed in the different phase systems.

Relationship between log k_w and the partition coefficient, log P, of the n-octanol–water system

The relation between log k_w and log P can be considered as a special case of the Collander equation[28] which relates the partition coefficients of different solvent systems:

$$\log P_2 = a \log P_1 + b \tag{9}$$

Using the values of log k_w and log P from Table VI, we obtain the following regression curves for the methanol system

$$\log k_{w(M)} = 0.870 \log P + 0.401 \; (n = 45, r = 0.958) \tag{10}$$

for the acetonitrile system

$$\log k_{w(A)} = 0.601 \log P + 0.525 \; (n = 44, r = 0.894) \tag{11}$$

and for the THF system:

$$\log k_{w(T)} = 0.460 \log P + 0.799 \; (n = 24, r = 0.827) \tag{12}$$

If a solute is equally distributed between octanol and water, *i.e.*, log $P = 0$, the intercept of the Collander equation gives a measure of the hydrophobicity of the non-aqueous phase in relation to the hydrophobicity of *n*-octanol; a positive value indicates that the solvent is less hydrophobic than octanol. The positive intercept in our case can be explained by recent findings[13,29] that the organic modifier is extracted into the stationary phase to solvate the octadecyl ligands and thereby decreases the hydrophobicity of the "effective" stationary phase. Furthermore, the mobile phase consists of water–organic mixtures which leads to a decrease in the free energy change of expulsion from the eluent relative to pure water. The overall effect is as if the non-polar phase is more hydrophilic than octanol.

The slope of the regression equation is a measure of the solvent system's sensitivity to changes in the hydrophobicity of solutes. Methanol again proves to be the most discriminating modifier and is in this respect quite similar to the octanol–water system. For THF, changes in solute structure will usually result in only half the

differences when compared to methanol or the octanol–water system. The magnitude of the correlation coefficient observed is only acceptable for the linear relation between log $k_{w(M)}$ and log P. We can therefore conclude from the different correlation coefficients of eqns. 9–11 that only methanol–water as the mobile phase produces retention data which are strongly related to log P.

We will now examine the validity of these results for the more complicated structures of the herbicides. Since these compounds contain mostly hydrophobic substituents, it is expected that at least log $k_{w(M)}$ will be closely related to log P. Fig. 1 depicts this relationship for phenylureas and phenoxycarbonic acid derivatives. Indeed, the two hydrophobicity parameters may be regarded as equivalent in this instance. The poor correlation of log k_w to log P ($r = 0.923$, $n = 10$), when determined in acetonitrile–water, also fits into the framework established by the analysis of the simple benzene derivatives.

Interestingly, *s*-triazines show a completely different behaviour in the two systems:

$$\log P = 0.196 \log k_{w(M)} + 1.432 \quad (n = 6, r = 0.555) \tag{13}$$

The lack of correlation between log k_w and log P is not the result of polar group selectivity as observed for the benzene derivatives, but arises from the unusual behaviour of the non-polar methylthio substituent in RPLC. When *s*-triazines contain this functional group instead of a chloro-substituent, a small decrease in hydrophobicity should occur according to the π values of Cl (+0.71) and SCH_3 (+0.67)[2]. What is actually observed is a large increment in retention with a corresponding Δlog k_M of 1.5, which is equivalent to an additional propyl group attached to the heterocy-

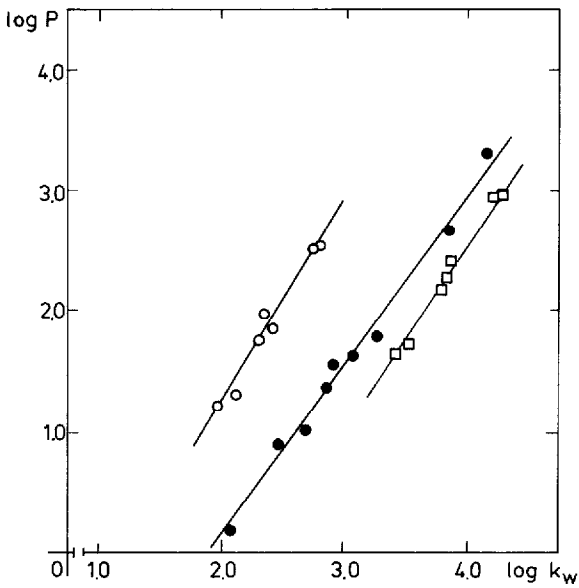

Fig. 1. Relationship between log P and log $k_{w(M)}$ for phenylureas (●), phenoxycarbonic acids (○) and phenoxycarbonic acid methyl esters (□).

clus. We have shown[10] that a trifluoromethyl group also gives an unusually large increase in hydrophobicity when compared to the π value ($\Delta\log k_w$ of about 1.5). Both results demonstrate that specific non-polar groups exert selective effects which are not found in liquid–liquid partitioning systems. As a consequence, we have measured the inhibitory activity of *s*-triazines to examine which hydrophobicity parameter is better suited for modelling the hydrophobicity of these herbicides.

Structure–activity relationships

Phenylureas and *s*-triazines inhibit the photosynthetic electron transport of chloroplasts. Since both herbicidal groups are known to bind to a specific protein near photosystem II[30], the ability to inhibit electron flow upon illumination is strongly dependent on the hydrophobic nature of these herbicides[5]. Therefore, QSAR with $\log k_w$ and $\log P$ is a good test of whether polar and non-polar group selectivity influences the quality of the relation between the biological response and the hydrophobicity of these compounds.

Using the I_{50} values for the inhibition of the Hill reaction from Table VIII, for phenylureas:

$$pI_{50} = 0.945 \log k_{w(M)} + 3.583 \quad (n = 10,\ r = 0.841) \tag{14}$$

This equation is equivalent to those reported previously[4,5] and is also valid if $\log P$

TABLE VIII

pI_{50} VALUES FOR THE INHIBITION OF THE HILL REACTION BY PHENYLUREAS AND *s*-TRIAZINES

The reaction medium contained 7 m*M* phosphate buffer (pH 6.4), 0.25 *M* sucrose, 0.1 m*M* dichlorophenolindophenol and 50 μg chlorophyll per ml and was illuminated for 45 sec with 50,000 lux. The decrease in absorption was measured at 590 nm.

Compound	pI_{50}	
	Measured	*Reported*[5]
Fenuron	4.9	4.6–5.5
Metoxuron	6.0	6.6
Monuron	5.5	5.6–6.8
Monolinuron	5.8	5.6–6.1
Chlortoluron	6.4	7.0
Metobromuron	5.6	6.0
Diuron	7.0	6.7–7.5
Linuron	6.7	6.7–7.0
Chloroxuron	6.7	6.8–7.3
Neburon	7.1	6.7–6.9
Simazine	5.7	5.4–6.4
Atrazine	6.0	6.1–6.6
Propazine	5.7	5.4–6.3
Prometryn	6.8	7.0
Desmetryn	6.4	–
Terbutryn	7.1	–

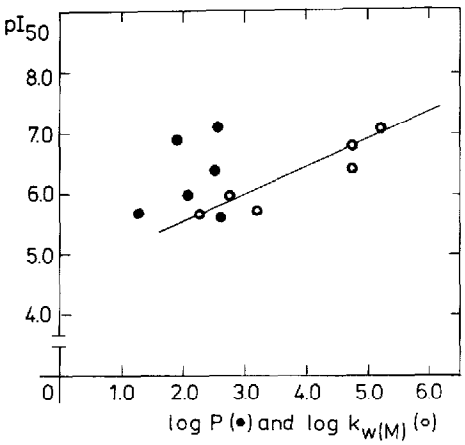

Fig. 2. Relationship between the inhibition of the photosynthetic electron transport by *s*-triazines and their log $k_{w(M)}$ and log P values, respectively. The inhibitory activity is given as pI_{50}, the logarithm of the concentration which causes 50% inhibition of the Hill reaction with dichlorophenolindophenol as electron acceptor.

values are used instead of log k_w. Obviously, log k_w can fully replace log P as a hydrophobicity parameter. This is also true for the phenoxycarbonic acids and their methyl esters and for a variety of compounds, *i.e.*, pyridazinones[10], phenols[15] and alkylbenzamides[31].

The *s*-triazines with SCH_3 substituents show non-polar group selectivity in RPLC so that log k_w and log P are only poorly correlated (eqn. 13). Interestingly, log k_w can be used to model the behaviour of *s*-triazines in thylakoid membranes whereas log P cannot (Fig. 2). The parallelism between the increase in activity and the increase in retention upon methylthio substitution cannot be interpreted at the moment. For that purpose, more QSAR studies with congeneric series would have to be performed where τ_w or log k_w of those substituents which show polar and non-polar group selectivity in RPLC should be related to their biological activity. Preliminary results with trifluoromethyl-substituted pyridazinones are promising in this respect.

CONCLUSIONS

Extrapolated log k_w values of different herbicides and of benzene derivatives can provide hydrophobicity parameters which are strongly related to log P in the standard octanol–water system. However, polar group selectivity and non-polar group selectivity is observed in RPLC for certain substituents, among them OH, SCH_3 and CF_3, which indicates that log k_w is basically an expression of the solvophobic effect but also includes additional information about the physico-chemical properties of a compound. The few QSAR studies which have employed retention parameters all show that log k' or log k_w can fully replace log P as a measure of the hydrophobicity of drugs. We have shown here that in the specific case of *s*-triazines with methylthio substituents only log k_w can be used for this purpose.

Biomembranes are highly compartmentalized structures with respect to their lipid and protein moieties, and are thus not homogeneous within their non-polar nor

polar regions. Thus, membranes should be better able to discriminate between minor structural differences than is indicated by the gross hydrophobic behaviour in liquid–liquid systems. This may be especially true for the protein-rich functional membranes of chloroplasts and mitochondria which are the preferred targets for herbicides. Furthermore, the lipids and proteins of the biomembranes are asymmetrically arranged and cannot move freely, laterally or transversely, so that steric and electronic requirements of the molecular structure come into play which do not operate in bulk liquids. Probably, the dynamic chromatographic process —the exclusion of a solute from the mobile phase into a more or less ordered stationary phase— is a better model of the behaviour of drugs during their passage through biomembranes.

ACKNOWLEDGEMENTS

We are indebted to the Senat der Freien Hansestadt Bremen for financial support during part of the work. Thanks are due to Dr. T. L. Hafkenscheid (University of Amsterdam) for his critical evaluation of the manuscript, and to Professor Kasche (University of Bremen) for helpful discussions.

REFERENCES

1 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
2 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
3 C. Hansch, in H. Metzner (Editor), *Progress in Photosynthesis Research*, Vol. 3, Tübingen, 1969, pp. 1685–1692.
4 K. H. Büchel, *Pestic. Sci.*, 3 (1972) 89.
5 C. Fedtke, *Biochemistry and Physiology of Herbicide Action*, Springer, Berlin, 1982.
6 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
7 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
8 E. Tomlinson, H. Poppe and J. C. Kraak, *Int. J. Pharmaceutics*, 7 (1981) 225.
9 T. Hanai, C. Tran and J. Hubert, *H. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 454.
10 Th. Braumann and L. H. Grimme, *J. Chromatogr.*, 206 (1981) 7.
11 B.-K. Chen and Cs. Horváth, *J. Chromatogr.*, 171 (1979) 15.
12 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
13 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.
14 G. L. Biagi, A. M. Barbaro, M. F. Gamba and M. C. Guerra, *J. Chromatogr.*, 41 (1969) 371.
15 W. Butte, C. Fooken, R. Klussmann and D. Schuller, *J. Chromatogr.*, 214 (1981) 59.
16 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 218 (1981) 409.
17 C. M. Riley, E. Tomlinson and T. L. Hafkenscheid, *J. Chromatogr.*, 218 (1981) 427.
18 G. E. Berendsen, P. J. Schoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony and J. Inczédy, *J. Liquid Chromatogr.*, 3 (1980) 1669.
19 H. Y. Nakatani and J. Barber, *Biochim. Biophys. Acta*, 461 (1977) 510.
20 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
21 H.-G. Genieser, D. Gabel and B. Jastorff, *J. Chromatogr.*, 244 (1982) 368.
22 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
23 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
24 F. E. Norrington, R. M. Hyde, S. G. Williams and R. Wootton, *J. Med. Chem.*, 18 (1975) 604.
25 G. G. Briggs, *J. Agr. Food Chem.*, 29 (1981) 1050.
26 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
27 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 261.
28 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.
29 R. M. McCormick and B. L. Karger, *J. Chromatogr.*, 199 (1980) 259.
30 K. Pfister and C. J. Arntzen, *Z. Naturforsch., C*, 34 (1979) 996.
31 M. J. M. Wells, C. R. Clark and R. M. Patterson, *J. Chromatogr.*, 235 (1982) 43.