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DETERMINATION OF HYDROPHOBIC PARAMETERS FOR PYRIDAZINONE HERBICIDES BY LIQUID–LIQUID PARTITION AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The retention behaviour of eight herbicidal pyridazinones in a reversed-phase high-performance liquid chromatographic (RP-HPLC) system has been examined. Using methanol–water as the mobile phase, a linear relationship between the volume fraction of the organic modifier and the logarithm of the capacity factor (log k') over a limited range was established for every solute. A comparison of the resulting curves showed that the separation system is selective with respect to the lipophilic trifluoromethyl substituent with otherwise the same structure. The influence of such a selective effect on the correlation between log k' and the partition coefficient, P, obtained using the standard *n*-octanol–water system, is demonstrated. This effect can be eliminated if log P is related to the extrapolated k' value with pure water as eluent. The resulting curve accommodates the two sets of data ($r = 0.992$). It is concluded that the organic modifier in RP-HPLC exerts subtle effects on the retention behaviour of pyridazinones, a discriminative feature which may also be important in biological membranes.

INTRODUCTION

Since the development of the quantitative structure–activity relationship (QSAR) by Hansch and co-workers[1,2], it has become apparent that the biological activity of a given class of chemicals is in many instances predominantly a function of their lipophilic behaviour. 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[2,3]. The calculations have limitations, however, and there are innumerable compounds for which log P values have to be determined. The conventional shaking flask method has limited application range up to log $P = 4$ (ref. 3) and is a laborious and time-consuming procedure, often complicated by instability in aqueous media, impurities and the tendency for the compound to dissociate.

In recent years attempts have been made to introduce chromatographic

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techniques for the determination of the lipophilicity of different chemicals, especially thin-layer chromatography[4–6] and reversed-phase high-performance liquid chromatography (RP-HPLC). The latter technique has attracted much interest because it produces very efficiently high-precision data with respect to retention, which is believed to be a measure of the partition behaviour between the non-polar bonded stationary phase and the more polar eluent.

The few reports published so far[7–14] deal with the correlation between log P and the logarithm of the capacity factor, k', mainly obtained with octadecylsilica as the bonded stationary phase. k' is given by

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where t_R and t_0 are the retention times of a retained and an unretained solute, respectively, in a given system. The most direct approach was made by Mirrlees *et al.*[7], who covered trimethylchlorosilane-treated silica *in situ* with a thin layer of *n*-octanol and used *n*-octanol-saturated water as the eluent. This seems to be a true analogy to liquid–liquid partitioning in octanol–water, and therefore the correlation between log k' and log P is excellent with a slope of the regression curve very close to 1.0. The same is perhaps true for buffers[8] or a very small amount of organic modifier in water[9] when used together with octadecylsilica. Here the slope also has a value near 1.0, although the correlation in the case of buffer is much poorer.

Several workers have used methanol–water and acetone–water as eluents to extend the applicability of the method to more hydrophobic compounds. They also found good correlations between log k' and log P[10–14] but varying slopes on regression analysis.

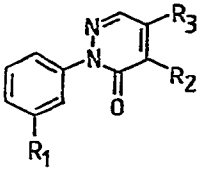
It has been emphasized[9] that free silanol groups at the accessible surface of the stationary phase can influence to some extent the retention behaviour of the solutes, so that vigorous silylation of the silica is recommended. However, several commercially available packings already have a very high surface coverage and can be used without further treatment[14].

On the basis of the above experience, RP-HPLC seems to be a convenient technique with respect to accuracy, sensitivity and application range for the determination of hydrophobicity data for chemical groups beyond the few tested so far. As we are interested in the QSAR for pyridazinone herbicides[15], the partition data for which have not yet been published and which have a complicated structure with respect to hydrophilic and hydrophobic substituents (Fig. 1), a study of the retention and partition behaviour should give a good basis for testing the validity of RP-HPLC for the determination of these pharmacologically important physico-chemical parameters.

EXPERIMENTAL

Materials

SAN 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone], SAN 9789 [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone; Norflurazon], SAN 9774 [5-amino-4-chloro-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone], SAN 9785 [4-chloro-5-(dimethylamino)-2-phenyl-3(2H)-pyrida-



No.	Compound	Substituents
1	SAN 6706	$R_1=CF_3, R_2=Cl, R_3=N(CH_3)_2$
2	BAS 44521	$R_1=CF_3, R_2=Cl, R_3=OCH_3$
3	SAN 9789	$R_1=CF_3, R_2=Cl, R_3=NHCH_3$
4	SAN 9774	$R_1=CF_3, R_2=Cl, R_3=NH_2$
5	SAN 9785	$R_1=H, R_2=Cl, R_3=N(CH_3)_2$
6	BAS 33650	$R_1=H, R_2=Br, R_3=OCH_3$
7	SAN133-410	$R_1=H, R_2=Cl, R_3=NHCH_3$
8	BAS13033	$R_1=H, R_2=Cl, R_3=NH_2$

Fig. 1. Chemical structures of pyridazinone derivatives.

zinone] and SAN 133-410 H [4-chloro-5-(methylamino-2-phenyl-3(2H)-pyridazinone] were gifts from Sandoz (Basle, Switzerland). BAS 44521 [4-chloro-5-methoxy-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone], BAS 33650 (4-bromo-5-methoxy-2-phenyl-3(2H)-pyridazinone], and BAS 13033 [5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone; Pyrazon) were kindly supplied by BASF (Ludwigshafen, G.F.R.). Some of these compounds were technical products and recrystallized twice prior to use. Distilled water was prepared with an all-glass double distilling unit (Heraeus-Schott, Mainz, G.F.R.). All other reagents were of analytical-reagent grade (Merck, Darmstadt, G.F.R.). A 10-μm LiChrosorb RP-18 column (25 cm $\times$ 4.6 mm I.D.) (Merck) was used without further treatment in all experiments.

Partition coefficient measurements

Previously water-saturated *n*-octanol and *n*-octanol-saturated water were used as the liquid phases. Two different amounts of the herbicides (10 and 30 mg) were introduced into 25-ml glass flasks equipped with glass stoppers and dissolved in 5 ml of *n*-octanol. A few drops of methanol improve dissolution without a measurable effect on the partition coefficients. Then 5 ml of water were added and the flasks were shaken mechanically with a Model TR 1 shaker (Infors, Basle, Switzerland) at a frequency of 300 min^{-1} for 1 h. All experiments were performed at room temperature (20–23°C). The contents of the flaks were decanted into centrifuge tubes and the two phases were allowed to separate. The upper *n*-octanol phase was carefully removed and the remaining aqueous phase was centrifuged for 1 h at 5000 rpm in a Labofuge III (Heraus-Christ, Osterode, G.F.R.) to remove *n*-octanol droplets. Subsequently the absorbance at the wavelength maximum was measured in a Gilford Model 250 spectrophotometer (Gilford, Oberlin, OH., U.S.A.).

Quantitative evaluation was performed with molar absorption coefficients which were determined by dissolving two different amounts of the herbicides in *n*-octanol-saturated water and measuring the absorbance as described above. The samples were diluted, if necessary, to yield an absorbance of 0.2–0.5.

The partition coefficients represent the means of six independent measurements and the standard error of log *P* was better than ± 0.05.

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 (Perking-Elmer) set at 280 nm, and a Servogor Model S pen recorder (Metrawatt, Nürnberg, G.F.R.).

A volume of 5 μl of a 10^{-4} M sample solution was injected with a 10-μl precision syringe and the retention times were measured with a stop-watch. The mobile phase consisted of different volume fractions of methanol in water, prepared with the gradient former of the chromatograph. The flow-rate was 1.7 ml/min at room temperature. The reproducibility of the retention times was checked by making a series of six injections under fixed conditions and was found to be better than 1%, so that in all other experiments two independent runs were carried out. The column dead time was determined by the injection of a small amount of acetone dissolved in water. Acetone has a log P of -0.29 (ref. 3) and was expected not to be retained in our system. The retention times were indeed the same at volume fractions of 0.55 and 0.80, so that retention due to interactions with the stationary phase could be excluded. The standard error of log k' determinations was better than ± 0.005.

RESULTS AND DISCUSSION

Several studies have been reported on the variation of sample k' values with the volume fraction, Φ, of organic solvent in water–organic solvent mixtures[16–18]. Snyder *et al.*[16] give this relationship as

$$\log k' = \log k_W - S\,\Phi_B \qquad (2)$$

where k_W represents the k' value of a compound with pure water as the mobile phase (usually an extrapolation where the intercept on the ordinate is taken as log k_W) and S is related to the solvent strength of pure solvent B. For methanol–water and for other polar organic solvents S should therefore be constant for a given column and different types of solutes. Snyder *et al.* gave average values of S for methanol (3.0), acetonitrile (3.1), tetrahydrofuran (4.4) and other organic solvents. If this suggestion is true, the retention mechanism for different solutes in a particular system should be the same, which is a prerequisite for a strong relationship between retention data in RP-HPLC and partition coefficients. Table I shows the k' values for pyridazinones over a range of 0.55–0.80 for Φ_M, the volume fraction of methanol in the mobile phase.

As can be seen from Table II, eqn. 2 describes appropriately the behaviour of the different solutes with a small standard error of fit and a high linear correlation coefficient. From these data we conclude, in accordance with Snyder *et al.*[16], that the relationship between Φ_M and log k' over a limited range of Φ_M is perfectly linear and that there is not need to introduce a parabolic function, as has been previously suggested[19]. However, with respect to S the solutes have to be divided into two different groups as the values of S are significantly different. The pyridazinones with a trifluoromethyl substituent and different polar groups at the C-5 position have high values of 4.45–4.68 for S, whereas the same pyridazinones without this lipophilic substituent have values of only 3.42–3.72. The RP-HPLC system can clearly discriminate between solutes with and without a trifluoromethyl substituent due to different retention mechanisms, which probably depend on electronic or steric contributions in addition to the lipophilicity of this group. As a result, S depends not only on the solvent strength

TABLE I

ISOCRATIC k' VALUES FOR DIFFERENT VOLUME FRACTIONS OF METHANOL IN WATER, Φ_M, AND PARTITION COEFFICIENTS IN *n*-OCTANOL–WATER (LOG *P*)

*Compound No.**	*Volume fraction, Φ_M*					*log P*
	0.55	*0.60*	*0.65*	*0.70*	*0.80*	
1	6.21	3.57	2.12	1.23	0.40	2.67
2	3.89	2.32	1.53	0.81	0.25	2.39
3	3.77	2.21	1.43	0.79	0.25	2.30
4	2.55	1.52	1.00	0.55	0.17	2.41
5	1.50	0.98	0.67	0.41	—	1.55
6	1.07	0.71	0.47	0.29	—	1.42
7	0.78	0.51	0.37	0.22	—	1.19
8	0.47	0.30	0.21	—	—	1.14

* See Fig. 1.

TABLE II

REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN THE VOLUME FRACTION OF METHANOL Φ_M AND LOG k': LOG k' = LOG $k_W - S\Phi_M$

*Compound No.**	*Log k_W*	*S*	*r***	*s****
1	3.340	4.642	0.9999	0.003
2	3.042	4.452	0.9959	0.007
3	3.134	4.636	0.9992	0.005
4	3.003	4.684	0.9972	0.016
5	2.164	3.619	0.9976	0.006
6	2.081	3.721	0.9987	0.005
7	1.768	3.422	0.9925	0.012
8	1.614	3.552	0.9954	0.002

* See Fig. 1.
** Linear correlation coefficient.
*** Standard error of fit ($\alpha = 0.001$).

of the mobile phase but also to a considerable extent on specific interactions between solutes, stationary phase and mobile phase. Tanaka *et al.*[18] have shown that the composition of the mobile phase can have such an effect on hydrophobic group selectivity and especially polar group selectivity. Pyridazinones offer several possibilities for selective interactions as both hydrophilic and hydrophobic substituents are available, whereas only the trifluoromethyl substituent can realize this property in our particular separation system.

The relationship between log *P* and log k' is shown in Fig. 2, from which the following linear equation is obtained:

$$\log P = 1.537 \log k' + 1.473 \quad (3)$$

with $n = 8$, $r = 0.964$ and $s = 0.664$ ($\alpha = 0.001$). The correlation is not as good as reported earlier for other types of solutes (see Table IV), as expected from the results

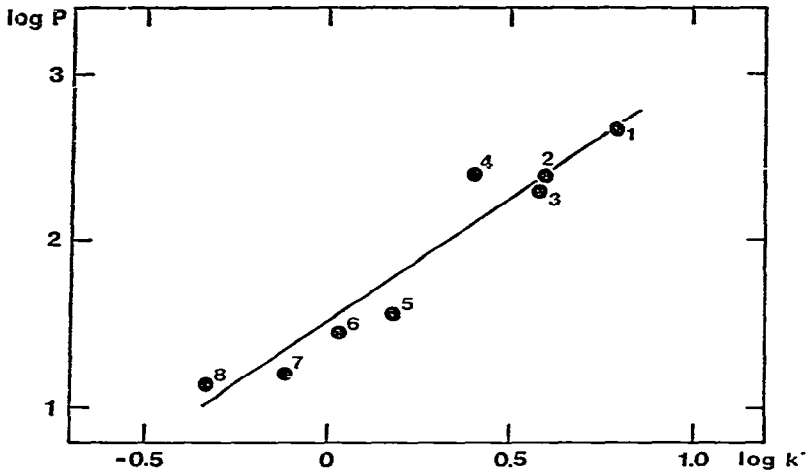


Fig. 2. Log k' *versus* log P for eight pyridazinones (numbered as in Fig. 1). k' was measured at a volume fraction of methanol in water of $\Phi = 0.55$.

described above. However, the selective effects in Table II do not distort the relationship. For comparison we performed the same experiments with a few benzene derivatives for which literature data for partition coefficients were related to the capacity factor at $\Phi_M = 0.80$, and obtained the equation

$$\log P = 2.746 \log k' + 3.021 \quad (4)$$

with $n = 4$ and $r = 0.994$. Table III shows the relevant data for the benzenes.

TABLE III

CAPACITY FACTOR, k', AND PARTITION COEFFICIENTS FOR SOME BENZENE DERIVATIVES

Mobile phase, 80% methanol–water; flow-rate, 1.7 ml/min; column, 10-μm LiChrosorb RP-18.

Compound	k'	*Log k'*	*Log P**
Benzene	0.47	−0.33	2.14
Toluene	0.81	−0.09	2.69
1,3-Dichlorobenzene	1.48	0.17	3.60
1,3,4-Trichlorobenzene	2.24	0.35	3.93

* Data taken from ref. 3.

A similar regression curve was reported earlier[14] for over 30 benzenes, toluenes and anilines. The slope was nearly twice that for the pyridazinones, which indicates different retention mechanisms for the two types of solutes. What has been shown before within the class of heterocyclic pyridazinones now finds its counterpart in the different log P *versus* log k' relationships for different chemical classes. Together with some previously published data the following picture emerges (Table IV). When water and buffers consisting of water with a small amount of an organic modifier (*e.g.*, 1%

TABLE IV

COMPARISON OF LITERATURE REGRESSION DATA FOR THE RELATIONSHIP LOG $P = a$ LOG $k' + b$

In some instances not all of the relevant data were included in the work reported. In these instances we have carried out the regression analysis from the published k' and log P, not knowing the accuracy of the data, so that minor errors have to be taken into account.

Class of compounds	*a*	*b*	*r*	*Reference*	*Separation system**
Sulphonamides	0.98	−8.03	0.937	7	ODS/buffer
Miscellaneous	1.086	1.14	0.982	8	ODS/1% TEA in water
Miscellaneous	1.006	−0.622	0.999	6	*n*-Octanol-coated silica/water
Phenols	1.907	1.922	0.961	9	ODS/acetone–water
Anilines	2.240	1.438	0.968	9	ODS/acetone–water
Miscellaneous	1.701	1.293	0.988	19	ODS/methanol–water
Miscellaneous	1.451	1.016	0.983	19	ODS/acetonitrile–water
Benzenes, toluenes, anilines	2.50	2.12	0.989	13	ODS/methanol–water

* ODS = octadecylsilica; TEA = triethylamine.

of triethylamine[9]) are used as eluents in RP-HPLC, the resulting k' values of a variety of substances are based on comparable mechanisms, indicated by the same slope of nearly 1.0 in all instances. Organic–water mixtures, on the other hand, produce selective effects that depend on both solute type and mobile phase composition.

The view that the solutes are partitioning between the hydrocarbonaceous surface layer of the non-polar stationary phase and the mobile phase is too simple an analogy in this instance and does not account for the differences between RP-HPLC with organic–water eluents and a true liquid partitioning system such as *n*-octanol–water. The bonded phase may be only a monolayer thick and the bonded molecules have fewer translational and rotational degrees of freedom than those comprising a true liquid. The behaviour of the bonded phase further depends on its type and surface coverage. A theoretical treatment of the binding process has shown[20] that the capacity factor of a given solute depends on the volume ratio of the stationary and mobile phases, the free energy change for binding of the solute to the bonded phase, the free energy change for cavity formation in the mobile phase and Van der Waals and electrostatic contributions that result from the interaction between the solvent and solute. Every term, except the volume ratio, is a function of the molecular structures of the solutes and eluents, so that it is expected that k' will be sensitive to small variations in the physical properties of the structures concerned. Within a group of compounds of comparable size, shape and polarity, good correlations between log k' and log P can always be obtained, but a relationship derived for a particular groups cannot be generalized to other solutes and separation systems. This conclusion restricts the value of such relationships and offers little help for the case of unknown samples.

An alternative is provided by the measurement of k' values at different Φ_M as described above. The regression analysis results in an extrapolated log k_W, the capacity factor with pure water as eluent. If these theoretical k' values are correlated with the partition coefficients of the solutes, a much improved relationship is obtained:

$$\log P = 0.901 \log k_W - 0.384 \qquad (5)$$

with $n = 8$, $r = 0.992$ and $s = 0.207$ ($\alpha = 0.001$). The selective effects of the mobile phase are eliminated as the slope is close to 1.0 and the linear correlation coefficient is much better than before. Schoenmaker *et al.*[19] have shown that the relationship between log k' and Φ is not really linear over the whole range of Φ, especially at the upper and lower end of the range. They suggested a function of the following type:

$$\log P = A\,\Phi^2 + B\,\Phi + C \tag{6}$$

The simple linear extrapolation to $\Phi_M = 0$ nevertheless yields lipophilicity data that are strongly related to the partition coefficients of the *n*-octanol–water system. The validity of this approach for other chemical classes however, has not yet been demonstrated but eqn. 2 certainly implies such a relationship.

The determination of log k_w requires detailed studies of the elution of the solute in question under different isocratic conditions and is necessary only if one has to correlate partition and retention data of more complex molecules. This is generally of theoretical interest for gaining an insight into retention mechanisms in RP-HPLC. In QSAR the question is whether the model for the behaviour of chemicals during the passage through biological membranes is adequate. It is now well established that these membrane structures are highly compartmentalized with respect to their lipid and protein moieties. Therefore, membranes are expected to be far more discriminative than is indicated by the gross lipophilic behaviour in *n*-octanol–water partitioning. Secondly, biomembranes will not behave like bulky liquids because their components are asymmetrically arranged with fewer translational and rotational degrees of freedom. Most of the membranes should therefore be able to distinguish between minor steric and electronic variations in molecular structure, a feature that is also common in RP-HPLC. We have shown that in our system pyridazinones with and without a trifluoromethyl substituent possess different retention mechanisms. Preliminary results, obtained in this laboratory, indicate that biomembranes of green plants can also discriminate between these two types of structures so that the question arises of whether the capacity factor in RP-HPLC could be a better alternative for modelling the hydrophobicity of reagents in QSAR.

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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 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
4 C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, 208 (1967) 2275.
5 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
6 W. Steurbaut, W. Dejonckheere and R. H. Kips, *J. Chromatogr.*, 160 (1978) 37.

7 M. S. Mirrlees, S. J. Moulton, C. T. Murphy and P. J. Taylor, *J. Med. Chem.*, 19 (1976) 615.
8 D. Henry, J. H. Block, J. L. Anderson and G. R. Carlson, *J. Med. Chem.*, 19 (1976) 619.
9 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
10 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
11. I. Molnár and Cs. Horváth, *J. Chromatogr.*, 142 (1977) 623.
12 T. Hanai, *Chromatographia*, 12 (1979) 77.
13 S. H. Unger and T. F. Feuerman, *J. Chromatogr.*, 176 (1979) 426.
14 H. Könemann, R. Zelle, F. Busser and W. E. Hammers, *J. Chromatogr.*, 178 (1979) 559.
15 H. W. Kümmel and L. H. Grimme, *Z. Naturforsch., C*, 30 (1975) 333.
16 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
17 J. W. Dolan, J. R. Gant, and L. R. Snyder, *J. Chromatogr.*, 165 (1979) 31.
18 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
19 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
20 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.