Journal of Chromatography, 445 (1988) 317–326
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 490

HYDROPHOBICITY STUDIES ON DIADAMANTANYL DERIVATIVES

COMPARISON BETWEEN CALCULATED PARTITION COEFFICIENTS AND EXPERIMENTAL LIPOPHILICITY INDICES DETERMINED BY REVERSED-PHASE HIGH-PERFORMANCE CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY

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(Received February 8th, 1988)

SUMMARY

The hydrophobic behaviour of a series of diadamantanyl derivatives with antimicrobial activity was studied by means of reversed-phase high-performance liquid chromatography and thin-layer chromatography. Octanol–water log P values were calculated using Rekker's fragmental procedure. The comparison of all the three data sets led to the conclusions that differences between the three partition systems do exist concerning the proximity effect, the *ortho* effect, the aromatic oxygen fragment and the phenyl ring. These differences were quantified as multiples of Rekker's magic constant and very good correlations were obtained between the chromatographic data and the calculated log P values. The correlation with biological data was in favour of the calculated log P values, indicating that the octanol–water system simulates better the biological processes, in the case of the compounds studied.

INTRODUCTION

Lipophilicity is a very important property in studies of quantitative structure–activity relationships (QSARs). The most widely used lipophilic index, the octanol–water partition coefficient, is however associated with difficulties concerning its measurement. The conventional shaking-flask method is tedious and time-consuming and is limited to compounds for which log P ranges between -2 and $+4$. Thus, efforts have been made to predict the log P values by calculation using the hydrophobic substituent constants, π, or the fragmental constants, f[1–3].

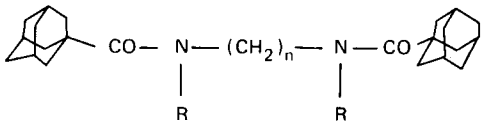
On the other hand, partition chromatographic data, log k and R_M values, are increasingly used as alternative hydrophobic indices[4–10]. Such data may correlate directly with biological activity or other biological processes in QSAR studies, or may be transformed to log P values via Collander type equations.

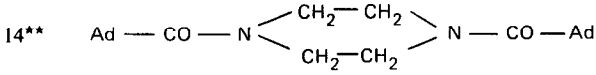
Although, very good correlations between log P and log k or R_M data may be found in the literature, certain discrepancies in the parallelism between the

0021-9673/88/$03.50

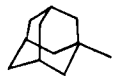
TABLE I

THE SERIES OF DIADAMANTYL DERIVATIVES STUDIED AND THEIR CALCULATED PARTITION COEFFICIENTS



No.	*R*	$(CH_2)_n$	Σf	*kn**
1	$-CH_2C_6H_5$	$(CH_2)_2$	9.060	2
2	$-CH_2C_6H_5$	$(CH_2)_3$	9.001	0
3	$-CH_2C_6H_5$	$(CH_2)_6$	10.558	0
4	$-CH_2C_6H_5$	$(CH_2)_{10}$	12.634	0
5	$-CH_2C_6H_4$-4-OCH_3	$(CH_2)_3$	9.161	0
6	$-CH_2C_6H_4$-4-OCH_3	$(CH_2)_6$	10.718	0
7	$-CH_2C_6H_2$-3,4,5-$(OCH_3)_3$	$(CH_2)_8$	12.076	0
8	$-CH_2C_6H_2$-2,3,4-$(OCH_3)_3$	$(CH_2)_8$	12.076	0
9	$-CH_2C_6H_4$-2-OCH_3	$(CH_2)_8$	11.756	0
10	-H	$(CH_2)_2$	5.284	2
11	-H	$(CH_2)_5$	6.263	0
12	-H	$(CH_2)_6$	6.782	0
13	-H	$(CH_2)_{10}$	8.858	0
14**			5.958	4

* *kn* = Key-number, which indicates how many times the correction factor $C_M = 0.289$ has been applied.

** Ad = 

octanol–water and the chromatographic systems have been reported recently, mostly associated with the presence of polar groups in the molecule[11–13,21].

This paper describes and discusses differences observed in the partition behaviour of the series of diadamantanyl derivatives presented in Table I. These compounds had been previously synthesized in our laboratory as antimicrobial agents[14]. The rôle of lipophilicity in antimicrobial activity had been demonstrated in the first QSAR papers[15,16]. So, we considered it to be worthwhile to investigate the hydrophobic behaviour of these compounds. For this purpose, experimental data, obtained by means of high-performance liquid chromatography (HPLC) and reversed-phase thin-layer chromatography (TLC), were compared with calculated partition coefficients, since the high values of the latter would not permit reliable direct measurements in the octanol–water system.

MATERIALS AND METHODS

The physical constants of the compounds investigated (Table I) are reported in ref. 14. Compounds 8 and 9 have been newly synthesized and identified by NMR spectroscopy. Their melting points are 128–130°C (decomp.) and 155–157°C respectively.

Analytical grade solvents and distilled water were used throughout the chromatographic experiments.

HPLC experiments were performed for compounds 1–9 using a Waters Associates HPLC system with an UV detector at 237 nm and a Lichrosorb C_{18} column (25 cm × 4.6 mm I.D.). Acetonitrile–water mixtures (85:15 and 90:10, v/v) were used as the mobile phases with or without the addition of 0.2% (v/v) *n*-decylamine as a masking agent. The mobile phase was filtered through a Millipore system. The flow-rate was 2.5 ml/min and potassium dichromate was used to determine the dead time.

Retention times, t_R, were converted into the logarithms of the capacity factors via the equation:

$$\log k = \log\left(\frac{t_R - t_0}{t_0}\right)$$

The two sets of log k values obtained in the absence of a masking agent are related with a correlation coefficient, $r = 0.9999$. The addition of *n*-decylamine decreases the retention times, as expected, since silanophilic interactions are eliminated[17,18] without, however, altering the partition system. Thus, log k_D values, determined at 85% acetonitrile, are related with the corresponding log k_{85} values with a correlation coefficient, $r = 0.9994$.

The reversed-phase TLC experiments were performed for compounds 2–14 on precoated silica gel 60 F_{254} TLC plates (Merck), which were impregnated with paraffin oil and eluted with acetone–water mixtures (65:35 and 70:30, v/v)[7]. The plates were developed in a closed chromatographic tank, dried at *ca.* 75°C and the spots were located under UV light or iodine vapours. The R_F values were averaged from six to eight determinations with standard deviations between 0.03 and 0.06, and they were converted into R_M values via the relationship:

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

The two sets of R_M values are related with a correlation coefficient, $r = 0.994$.

Log P values were calculated in Rekker's fragmental system[3] and expressed as Σf. Proximity effect corrections were incorporated when necessary (Table I). It is a drawback of the fragmental system that it does not include any corrections for the distinction of isomers, so the same value is assigned to both compounds 7 and 8.

RESULTS AND DISCUSSION

Straightforward correlations between the chromatographic data and Σf values are presented in Table II. Their quality is very poor and compound 7 is a distinct outlier. Its exclusion gives significantly improved results, which however remain unsatisfactory since correlation levels with $r > 0.99$ have been reported[7]. Moreover, the correlation between capacity factors and R_M values is also low.

TABLE II

REGRESSION EQUATIONS BETWEEN CAPACITY FACTORS, R_M VALUES AND CALCULATED OCTANOL–WATER PARTITION COEFFICIENTS

System	*Regression equations*	*n*	*r*	*s*	*Eqn.*
HPLC:					
Acetonitrile–water (90:10)	$\Sigma f = (4.424 \pm 2.863)\log k + (5.779 \pm 3.314)$	9	0.802	0.918	1
	$\Sigma f = (5.078 \pm 1.534)\log k + (4.780 \pm 1.808)$	8*	0.954	0.468	2
HPLC:					
Acetonitrile–water (85:15)	$\Sigma f = (4.232 \pm 2.585)\log k_D + (6.123 \pm 2.925)$	9	0.818	0.884	3
+0.2% *n*-decylamine	$\Sigma f = (4.754 \pm 1.400)\log k_D + (5.300 \pm 1.615)$	8*	0.958	0.450	4
RPTLC:	$\Sigma f = (5.982 \pm 2.845)R_M + (9.875 \pm 0.987)$	13	0.809	1.579	5
Acetone–water (65:35)	$\Sigma f = (5.823 \pm 2.735)R_M + (9.677 \pm 0.988)$	12*	0.829	1.492	6
HPLC:					
Acetonitrile–water (90:10)	$\log k = (0.753 \pm 0.690)R_M + (1.071 \pm 0.186)$	8	0.736	0.196	7
TLC:					
Acetone–water (65:35)					

* Compound 7 excluded.

In a further evaluation of the data, the following factors were considered as the possible sources of the discrepancies between the partition systems: (1) substitution in the phenyl ring, the effect of the aromatic –O– fragment; (2) deviations from additivity due to proximity and *ortho* effects.

Following the concept of the hydrophobic fragmental constants, *f*, Rekker proposed a Collander variant, in which differentiations between the octanol–water and any other partition system A may be expressed as multiples of the magic constant, C_M, which is equal to 0.289:

$$\log P_{oct} = \alpha \log P_A \pm kn \cdot C_M + b$$

The value of *kn* is strongly dependent on the hydrophobic behaviour of the functional groups, being equal to 0 for obviously lipophilic groups independent of the solvent systems compared. Moreover, it may be a measure of the similarity between the partition system A and octanol–water.

In the present study this approach is extended also to other structural features, such as the position of the functional groups in the molecule, and is applied to the evaluation of chromatographic systems.

Analysis of the HPLC data

The aromatic fragment –O–. Tables I and III show that, the substituent $-OCH_3$ contributes positively to the lipophilicity of the molecule in the octanol–water system, while the contrary is valid for the HPLC system. This decrease in lipophilicity may be attributed to the lack of hydrogen donating ability of acetonitrile. As postulated[19], the organic modifier is enriched in the stationary phase as a result of hydrophobic expulsion from the aqueous mobile phase. Thus, while in the octanol–water system the organic solvent may compete with water to form an hydrogen bond with the

TABLE III

HPLC DATA INCLUDING THE KEY-NUMBER PARAMETER APPLIED IN THE COLLANDER VARIANT

n.m. = Not measured.

*Compound**	*log* k_{90}**	*log* k_{85}***	*log* k_D§	*kn*
1	0.942	1.120	0.906	+1
2	0.905	1.065	0.839	+1
3	1.136	1.333	1.105	0
4	1.576	1.817	1.567	0
5	0.818	0.979	0.763	−1
6	1.064	1.248	1.032	−2
7	0.978	1.157	0.956	−8
8	1.310	n.m.	1.300	−2
9	1.449	n.m.	1.440	0

* Compounds numbered as in Table I.

** Capacity factors obtained using acetonitrile–water (90:10) as the mobile phase.

*** Capacity factors obtained using acetonitrile–water (85:15) as the mobile phase.

§ Capacity factors obtained using acetonitrile–water (85:15) + 0.2% (v/v) *n*-decylamine as the mobile phase.

TABLE IV

COMPARISON BETWEEN ADDITIVE AND OBSERVED CAPACITY FACTORS

Compound	*log* k_{add}*	*log* k**	Δ
1	0.696	0.942	0.246
2	0.806	0.905	0.099
5	0.734	0.818	0.084
9	1.284	1.449	0.165
8	1.140	1.310	0.170
7	1.140	0.978	−0.162

* Additive capacity factors, obtained via the equation $\log k_{add} = \log k'_{uns} + \Sigma f'$, where $\log k'_{uns} = \log k'$ values of the "unsuspected" structures 3 and 6.

** Capacity factors obtained at acetonitrile–water (90:10).

oxygen of the $-OCH_3$ group, such an interaction is impossible with acetonitrile in the stationary phase.

Deviations from additivity. The high precision of the HPLC technique permits the establishment of HPLC fragment values, f', for $-CH_2$ and $-OCH_2$ using the data for the "unsuspected" compounds 3, 4 and 6: $f'_{CH_2} = 0.110$, $f'_{OCH_2} = -0.036$.

These fragments are further used to calculate the additive log k values of the remaining six compounds (Table IV), which, when compared to the corresponding experimental data, lead to the following remarks. In HPLC the proximity effect is enhanced, and is also evident for a 3-C separation. A negative *ortho* effect, due to steric factors, is observed for compound 7, in accordance with previous findings for the CH_3O/CH_3O *ortho* substituent pair in HPLC[20]. Unexpectedly, its isomer, compound 8, as well as compound 9 show higher experimental values than the corresponding additive ones. ^{13}C NMR studies (Table V) for compounds[6–9] show an higher deshielding of the carbonyl carbon atom in the derivatives which bear $-OCH_3$ groups *ortho* to the $O{=}\overset{|}{C}{-}\overset{|}{N}{-}CH_2$ chain. The difference in the chemical shift is higher be-

TABLE V

^{13}C NMR CHEMICAL SHIFTS AND THE KEY-NUMBERS APPLIED FOR THE POSITIVE *ORTHO*-EFFECT IN HPLC AND TLC

Compound	δ*	Δ**	*kn (HPLC)*	*kn (TLC)*
3	176.880			
6	176.751	−0.194		
7	176.686	−0.129		
8	177.517	+0.637	+6	+6
9	177.015	+0.135	+2	0

* Measured in deuterochloroform in ppm downfield from tetramethylsilane, with an accuracy of ±0.03 ppm.

** The difference from the chemical shift of the unsubstituted compound 3, in ppm.

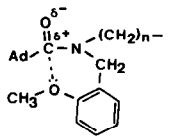


Fig. 1. Postulated intramolecular electrostatic interaction.

tween compounds 7 and 8. For the latter the increase in lipophilicity is also more evident. It may be postulated that this increase in lipophilicity is due to an intramolecular electrostatic interaction between the oxygen of the *ortho* methoxy group and the electron-poor carbonyl carbon atom of the chain (Fig. 1). Another assumption is that compounds 8 and 9 have such a conformation that the oxygen of the methoxy groups is "hidden" by the highly lipophilic surroundings, its hydration thus being hindered.

Final equation for the HPLC data. Taking into account all the factors described and applying the Collander variant, the following regression equation is obtained:

$$\Sigma f = (4.936 \pm 0.450)\log k - (0.310 \pm 0.042)kn + (4.820 \pm 0.531) \quad (8)$$

$$n = 9, \quad r = 0.996, \quad s = 0.143, \quad F = 457.6$$

The values of the key-number parameter, *kn*, are presented in Table III. Similar results are obtained when the capacity factors determined in the presence of *n*-decylamine are used:

$$\Sigma f = (4.606 \pm 0.457)\log k_D - (0.297 \pm 0.044)kn + (5.348 \pm 0.528) \quad (9)$$

$$n = 9, \quad r = 0.996, \quad s = 0.156, \quad F = 383.6$$

In both equations the regressor of *kn* is, within statistical limits, in accordance with the value of Rekker's magic constant, $C_M = 0.289$.

Analysis of the RP-TLC data. The factors already studied for the HPLC data influence also the relationship between R_M and Σf values.

(a) The enhancement in proximity effect is the same as in the HPLC system. (b) The decrease in lipophilicity of the aromatic –O– fragment is more evident, reaching a key-number of 3. This decrease is also attributed to the absence of an hydrogen-bond interaction with the stationary phase in RP-TLC. (c) No steric effect is observed for the *ortho* CH_3O/CH_3O pair. (d) The trimethoxy compound 8 shows higher lipophilicity than its isomer, compound 7. However such an increase in lipophilicity is not evident for the *ortho*-monomethoxy compound 9.

Moreover: (e) aromatic compounds should be treated separately from aliphatic ones. The phenyl ring contributes more than expected to the lipophilicity of the molecule when all the compounds are treated together. (f) Ring closure increases significantly the lipophilicity of the molecule. Thus compound 14 needs an extra key-number equal to 3.

If all these factors are taken into account, the following Collander variant is obtained for the aromatic compounds:

$$\Sigma f = (6.640 \pm 0.729)R_M - (0.282 \pm 0.028)kn + (8.641 \pm 0.265) \quad (10)$$

$$n = 8, \quad r = 0.996, \quad s = 0.150, \quad F = 341.8$$

TABLE VI

RPTLC DATA AND THE KEY-NUMBER PARAMETERS APPLIED IN THE COLLANDER VARIANT

n.m. = Not measured.

*Compound**	R_{M70}**	R_{M65}***	*kn*
2	n.m.	0.122	1
3	−0.07	0.288	0
4	n.m.	0.602	0
5	−0.397	−0.158	− 5
6	−0.261	0.070	− 6
7	−0.550	−0.231	−18
8	−0.339	0.004	−12
9	−0.098	0.191	− 6
10	−0.908	−0.584	3
11	−0.835	−0.525	0
12	−0.770	−0.454	0
13	−0.468	−0.177	0
14	n.m.	−0.359	5

* Compounds numbered as in Table I.
** R_M values obtained using acetone–water (70:30) as the mobile phase.
*** R_M values obtained using acetone–water (65:35) as the mobile phase.

For the aliphatic compounds the parameter *kn* should be incorporated in the f summation since not enough compounds are available. The following equation is obtained:

$$\Sigma f = (7.805 \pm 0.334) R_M + (10.252 \pm 0.148) \qquad (11)$$

$$n = 5, \quad r = 0.997, \quad s = 0.107$$

TABLE VII

DIFFERENCES IN THE LIPOPHILIC BEHAVIOUR OF THE DIADAMANTYL DERIVATIVES BETWEEN THE TWO CHROMATOGRAPHIC SYSTEMS AND OCTANOL–WATER, EXPRESSED IN KEY-NUMBERS

Type of effect	*kn*		
	Octanol–water	*HPLC*	*TLC*
(1) Proximity effect			
(a) 2-C separation	2	3	3
(b) 3-C separation	0	1	1
(2) *Ortho* effect			
(a) CH_3O/CH_3O	0	−1	0
(b) $CH_3O/CH_2-N-C=O$			
for monomethoxy (compound 9)	?	2	0
for trimethoxy (compound 8)	?	6	6
(3) Aromatic –O– fragment*	–	−1	−3
(4) Ring closure (compound 14)	0	–	3
(5) Phenyl effect*	–	–	3

* Taking as reference the octanol–water system.

TABLE VIII

COMPARISON BETWEEN LIPOPHILICITY DATA AND ANTIMICROBIAL ACTIVITY

Compound	Σf	R_M	*log % Inh**
3	10.558	0.288	1.30
10	5.284	−0.584	1.78
12	6.782	−0.454	1.48
13	8.858	−0.177	1.48
14	5.958	−0.359	1.70

* Inhibition against *Staphylococcus aureus* using Carbenicillin as the reference substance[14].

The two equations may be combined if a key-number equal to 3 is assigned to each phenyl ring:

$$\Sigma f = (7.495 \pm 0.359)R_M - (0.306 \pm 0.024)kn + (10.204 \pm 0.119) \quad (12)$$

$$n = 13, \quad r = 0.998, \quad s = 0.192, \quad F = 1165.4$$

The key-numbers used in eqn. 10 and 12 are presented in Table VI.

CONCLUSIONS

The differences in the lipophilic behaviour of the diadamantanyl derivatives in the chromatographic and octanol–water systems, expressed in key-numbers, are summarized in Table VII. Since experimental log P values are not available and in Rekker's fragmental system no distinction between isomers is considered, the true lipophilic behaviour of compounds 7–9 in octanol–water remains uncertain.

On the other hand, the question arises, which of the partition systems simulates better the biological processes. A preliminary evaluation of some available microbiological data of the diadamantanyl derivatives[14] (Table VIII) results in favour of Σf over R_M values leading to the following equations:

$$\log \% \text{ Inh} = -(0.082 \pm 0.061)\Sigma f + (2.159 \pm 0.474) \quad (13)$$

$$n = 5, \quad r = 0.926, \quad s = 0.084$$

$$\log \% \text{ Inh} = -(0.476 \pm 0.566)R_M + (1.430 \pm 0.225) \quad (14)$$

$$n = 5, \quad r = 0.839, \quad s = 0.121$$

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Professor R. F. Rekker for his helpful and stimulating discussion.

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