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Use of reversed-phase high-performance liquid chromatography in lipophilicity studies of 9*H*-xanthene and 9*H*-thioxanthene derivatives containing an aminoalkanamide or a nitrosoureido group

Comparison between capacity factors and calculated octanol–water partition coefficients

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ABSTRACT

The lipophilicity of 9*H*-xanthene and 9*H*-thioxanthene derivatives, containing either a basic alkanamide or a nitrosoureido group, was studied by means of reversed-phase high-performance liquid chromatography using an octadecylsilane stationary phase, methanol as organic modifier and *n*-decylamine as a masking agent. Correlation of the extrapolated capacity factors with log *P* values calculated according to Rekker's fragmental system showed an excellent parallelism between HPLC and the octanol–water partition system and permitted the generation of a hydrophobic fragmental constant for the nitrosoureido group. Tetrahydrofuran was also tried as an organic modifier but without satisfactory results.

INTRODUCTION

The lipophilic character of drugs is a parameter of major importance in quantitative structure–activity (QSAR) studies and has attracted considerable interest [1–3]. Octanol–water partition coefficients, the most widely accepted lipophilicity index, are measured mainly by means of the shaking flask method, which presents a number of practical disadvantages and is limited to compounds with a log *P* range between −2 and +4 [3,4]. Calculation procedures based on the additive–constitutive character of the logarithm of partition coefficients have also been developed [5,6]. However, hydrophobic fragmental constants are not available for every structural characteristic, so log *P* predictions are not always possible. In recent years, partition chromatography, especially RP-HPLC, has become a popular alternative for measuring lipophilicity. Under suitable conditions the logarithms of the capacity factors show good linear correlations with log *P* [7,8]. Moreover, extrapolated log k_w values which correspond to a mobile phase consisting of pure water are in

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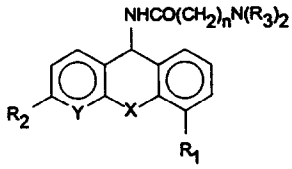
many instances and under suitable chromatographic conditions very close to octanol–water partition data [9]. Thus, Collander-type equations ($\log P = a \log k_w + b$) with a regression coefficient close to 1 and an intercept close to zero have often been reported [10–12].

Theoretically, $\log k_w$ values should be independent of the organic modifier that usually must be added to the mobile phase in order to facilitate the elution of solutes. However, it has been shown that the effect of the organic modifier is not fully suppressed by the extrapolation and this is reflected in the significant deviations that may be observed among $\log k_w$ values obtained using different solvents. Bechalany *et al.* [10], who studied the effects of three different organic modifiers on $\log k_w$, suggested that methanol should be the solvent of choice. Moreover, when an ODS column is used as the stationary phase, *n*-decylamine should be added in order to suppress the silanophilic interactions that arise between the solutes and the free silanol groups [11,13]. Tetrahydrofuran (THF), which has been used for the analysis of more lipophilic solutes in order to reduce retention times [14], may also be suitable for the determination of the lipophilicity of compounds containing polar functional groups, especially strong hydrogen bond acceptors, provided that the measurements are restricted only to volume fractions rich in water. In this case the addition of a masking agent is not necessary [10]. However, such studies concern model series that include compounds with low or moderate lipophilicity. For highly lipophilic compounds, limitations in the performance of the experiments impede analogous investigations.

The aim of this study was to apply HPLC in order to determine lipophilicity indices for some novel highly lipophilic 9*H*-xanthene and 9*H*-thioxanthene derivatives and to compare them with calculated octanol–water partition coefficients. The compounds under study were designed and synthesized as potential anticancer agents and include derivatives with a side-chain that bears either an aminoalkanamide group (type I) or a nitrosoureido moiety (type II) (Tables I and II). For the latter a hydrophobic fragmental constant in the octanol–water system

TABLE I

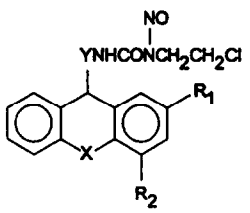
AMINOALKANAMIDES I.1–I.11



Compound	R_1	R_2	R_3	X	Y	*n*
I.1	H	H	C_2H_5	O	CH	1
I.2	CH_3	H	CH_3	O	CH	1
I.3	CH_3	H	C_2H_5	O	CH	1
I.4	CH_3	H	CH_3	O	CH	2
I.5	CH_3	H	C_2H_5	O	CH	2
I.6	CH_3	Cl	CH_3	O	CH	1
I.7	CH_3	H	CH_3	S	CH	1
I.8	CH_3	H	C_2H_5	S	CH	1
I.9	CH_3	H	CH_3	S	CH	2
I.10	CH_3	H	C_2H_5	S	CH	2
I.11	CH_3	H	CH_3	O	N	1

TABLE II

NITROSOUREAS II.1–II.13



Compound	R_1	R_2	X	Y
II.1	H	H	O	–
II.2	CH_3	H	O	–
II.3	H	CH_3	O	–
II.4	CH_3	CH_3	O	–
II.5	OCH_3	H	O	–
II.6	OC_2H_5	H	O	–
II.7	H	OCH_3	O	–
II.8	H	H	S	–
II.9	CH_3	H	S	–
II.10	H	CH_3	S	–
II.11	H	H	O	SCH_2CH_2
II.12	H	H	S	SCH_2CH_2
II.13	H	H	O	CH_2

is not available and part of this study was focused on evaluating the contribution of that fragment to the overall lipophilicity. The choice of the organic modifier with respect to the practical limitations and the establishment of suitable partitioning conditions is also discussed.

EXPERIMENTAL

All compounds (Tables I and II) were synthesized in our laboratory and identifier by ^{1}H NMR spectroscopy and elemental analysis, the results of which have been reported elsewhere [15,16].

Capacity factors were determined using a Waters HPLC instrument equipped with a UV detector operating at 254 nm. An ODS column (25 cm × 4 mm I.D.) prepacked with LiChrosorb RP-18 (particle size 10 μm) stationary phase was used. Mobile phases were made up volumetrically using different concentrations of methanol with 0.02 *M* 3-morpholinopropanesulphonic acid buffer (pH 7.4) in the presence of *n*-decylamine (0.2%) as a masking agent. Compounds of type II were measured also using different mixtures of THF and water as the mobile phase in the absence of a masking agent. All solutions were purified and degassed by filtration using a Millipore Milli-Q system. Retention times were measured at ambient temperature. The flow-rate was adjusted at 2.5 ml/min and the column dead time was determined using the organic modifier as the non-retained compound. Isocratic capacity factors, log k_i, defined as $\log[(t_r - t_0)/t_0]$ were determined at three different proportions of methanol in the range 70–60% and five different proportions of THF in the range 60–40% and extrapolated to 100% water as the mobile phase to yield log k_w and log k_{wTHF} values, respectively.

RESULTS AND DISCUSSION

Use of methanol as organic modifier

The use of methanol in the mobile phase led to long retention times so that measurements were limited to a small range of organic modifier concentrations. However, the excellent linearity observed between capacity factors and volume fractions of methanol permitted the derivation of reliable extrapolated capacity factors, log k_w (Table III). For compounds of type I suitable corrections for ionization were applied using the equation

$$\log k_w = \log k_w(\text{pH } 7.4) + \log(1 + 10^{pK_a - pH}) \quad (1)$$

pK_a values were calculated as suggested by Perrin [17], starting from the pK_a value of a tertiary amine and taking into account the presence of the amide group attached one or two carbon atoms away from the basic centre and also the number of methyl groups directly attached to the basic centre. According to this procedure, pK_a values around 7.30 and 7.70 were assigned to the dimethylamino and diethylamino derivatives, respectively, when $n = 1$. When $n = 2$ the base-weakening effect of the amide group is lower and the corresponding pK_a values were calculated to be around 8.40 and 8.80, respectively. The corrected log k_w values are presented in Table IV.

In a preliminary study [18], a relationship was established between the chromatographic data and calculated octanol–water log *P* values, expressed by the equation

$$\log P = 0.768(\pm 0.021) \log k_w + 2.115(\pm 0.115) I_N + 0.415(\pm 0.095) I_S \quad (2)$$

$n = 24$; $r = 0.985$; $s = 0.260$; $F = 302$

Log *P* values were calculated according to Rekker's system [6] as a summation of fragmental constants and correction terms expressed as multiples of the magic constant ($c_M = 0.289$). For the –NHCOCH$_2$N– group the fragmental value −2.729 proposed by Le Therizien *et al.* [19] was used. For compounds of type II the expression log $P - f_{[-NHCON(NO)-]}$ was considered in place of log *P*, as a fragmental constant for the –NHCON(NO)– group is not available. The presence of the –NHCON(NO)– fragment was expressed by the indicator parameter I_N on the right-hand side of eqn. 2. The regression coefficient of I_N with opposite sign then represents the contribution of that fragment to the lipophilicity. In eqn. 2, a second indicator I_S was necessary to account for the presence or absence of a sulphur

TABLE III

ISOCRATIC AND EXTRAPOLATED CAPACITY FACTORS DETERMINED USING METHANOL AS ORGANIC MODIFIER AND *n*-DECYLAMINE AS A MASKING AGENT

Compound	Log k_{60}	Log k_{65}	Log k_{70}	Log k_w [a]	S	r
I.1	0.87	0.60	0.43	3.49(±0.38)	4.4	0.992
I.2	0.95	0.72	0.52	3.53(±0.11)	4.3	0.999
I.3	1.15	0.85	0.62	4.32(±0.26)	5.3	0.997
I.4	0.85	0.77	0.56	2.61(±0.49)	2.9	0.968
I.5	0.71	0.56	0.34	2.94(±0.26)	3.7	0.994
I.6	1.47	1.24	1.00	4.29(±0.04)	4.7	1.000
I.7	0.95	0.72	0.52	3.53(±0.11)	4.3	0.999
I.8	1.20	0.89	0.69	4.24(±0.41)	5.1	0.992
I.9	0.83	0.78	0.53	2.66(±0.75)	3.0	0.933
I.10	0.71	0.58	0.36	2.83(±0.34)	3.5	0.989
I.11	0.39	0.25	0.09	2.19(±0.07)	3.0	0.999
II.1	1.10	0.89	0.59	4.17(±0.34)	5.1	0.995
II.2	1.34	1.09	0.79	4.65(±0.19)	5.5	0.999
II.3	1.40	1.16	0.84	4.77(±0.30)	5.6	0.997
II.4	1.64	1.38	1.05	5.19(±0.26)	5.9	0.998
II.5	1.18	0.91	0.63	4.48(±0.04)	5.5	1.000
II.6	1.38	1.09	0.80	4.86(±0.01)	5.8	1.000
II.7	0.92	0.70	0.46	3.68(±0.07)	4.6	0.999
II.8	1.27	1.04	0.72	4.59(±0.34)	5.5	0.996
II.9	1.52	1.25	0.94	5.01(±0.15)	5.8	0.999
II.10	1.54	1.28	0.96	5.03(±0.23)	5.8	0.998
II.11	1.34	1.04	0.73	5.00(±0.04)	6.1	1.000
II.12	1.31	1.02	0.70	4.98(±0.11)	6.1	0.999
II.13	1.12	0.89	0.62	4.13(±0.15)	5.0	0.999

[a] Capacity factors linearly extrapolated to 100% water according to the equation $\log k = \log k_w - S\varphi$ (φ = fraction of methanol).

atom in the molecule. All data used in eqn. 2 are reported in Table IV.

Eqn. 2 however, although highly significant, represents a rough analysis of the data and further investigation is necessary in order to draw final conclusions. A drawback of eqn. 2 is the low regression coefficient of log k_w. Under analogous chromatographic conditions a regression coefficient of log k_w close to 1 and an intercept close to zero has been reported [10,12]. Closer examination of the data reveals that compounds **II.7** and **II.13**, although not outliers, show deviating behaviour, their lipophilicity being much lower than predicted. For compound **II.7** the reason may lie in the steric *ortho* effect of the methoxy group with respect to the ring oxygen. For compound **II.13** the decrease in lipophilicity can be attributed to the presence of a methylene bridge between the xanthene moiety and the polar nitrosoureido function. Omission of compounds **II.7** and **II.13** from the statistical analysis leads to the following equation with a higher correlation coefficient and a regression coefficient of log k_w almost equal to 1 but with a large negative intercept:

$$\log P = 1.012(\pm 0.071) \log k_w + 1.819(\pm 0.087) I_N + 0.433(\pm 0.060) I_S - 0.983(\pm 0.284) \quad (3)$$

$n = 22$; $r = 0.995$; $s = 0.159$; $F = 636$

Separate analysis of the aminoalkanamide derivatives leads to the following equation, in good agreement with eqn. 3:

$$\log P = 1.004(\pm 0.092) \log k_w + 0.536(\pm 0.121) I_S - 0.989(\pm 0.370) \quad (4)$$

$n = 11$; $r = 0.968$; $s = 0.191$; $F = 77$

The large negative intercept generated by

TABLE IV

DATA USED IN REGRESSION EQNS. 2–10

Compound	Log k_w [a]	Log P [b]	Log P_c [c]	Log P_{rev} [d]	I_N	I_S
I.1	3.97	3.214	4.081	4.385	0	0
I.2	3.78	2.695	3.562	3.866	0	0
I.3	4.80	3.733	4.600	4.904	0	0
I.4	3.65	2.509	3.376	3.728	0	0
I.5	4.36	3.547	4.414	4.766	0	0
I.6	4.54	3.437	4.304	4.595	0	0
I.7	3.78	3.244	4.111	3.980	0	1
I.8	4.72	4.282	5.149	5.018	0	1
I.9	3.70	3.058	3.925	3.842	0	1
I.10	4.24	4.096	4.963	4.880	0	1
I.11	2.44	1.557	2.424	2.497	0	0
II.1	4.17	5.176	6.043	6.325	1	0
II.2	4.65	5.695	6.562	6.844	1	0
II.3	4.77	5.695	6.562	6.844	1	0
II.4	5.19	6.214	7.081	7.363	1	0
II.5	4.48	5.256	6.123	6.394	1	0
II.6	4.86	5.775	6.642	6.913	1	0
II.7	3.68	5.256	5.545	5.737	1	0
II.8	4.59	5.725	6.592	6.437	1	1
II.9	5.01	6.244	7.111	6.956	1	1
II.10	5.03	6.244	7.111	6.956	1	1
II.11	5.00	6.282	6.860	7.026	1	1(0[e])
II.12	4.98	6.831	7.409	7.140	1	2(1[e])
II.13	4.13	5.695	5.695	6.187	1	0

[a] For compounds of type I the log k_w values are corrected for ionization according to eqn. 1.
[b] For compounds of type II the values in this column correspond to log $P - f_{[NHCON(NO)]}$.
[c] For compounds of type II the values in this column correspond to log $P_c - f_{[NHCON(NO)]}$.
[d] For compounds of type II the values in this column correspond to log $P_{rev} - f_{[NHCON(NO)]}$.
[e] The values in parentheses correspond to those used in eqn. 8 (only ring sulphur is taken into account).

eqns. 3 and 4 reflects an underestimation of lipophilicity, indicating that special attention should be paid in the calculation procedure to the direct attachment of polar groups to the bulky tricyclic system. Such a direct attachment may lead to a decrease in the hydration of the polar groups and consequently to an increase in lipophilicity. This assumption is further supported by the bad fit of compound **II.13**, already mentioned. In this respect, and as evaluated from the magnitude of the intercept in eqn. 4, a correction equal to 3 c_M should be considered for the direct attachment of the –NHCO– group to the ring system. Correlation of the corrected log P_c of the aminoalkanamide derivatives and the corresponding log k_w values leads to the equation

$$\log P_c = 1.004(\pm 0.092) \log k_w + 0.536(\pm 0.120) I_S - 0.122(\pm 0.370) \quad (5)$$

$n = 11$; $r = 0.968$; $s = 0.191$; $F = 77$

In eqn. 5, the intercept is no longer significant and after forcing through the origin the following equation is obtained:

$$\log P_c = 0.974(\pm 0.017) \log k_w + 0.536(\pm 0.114) I_S \quad (5')$$

Eqn. 5′ can be further refined by omission of compound **I.9**. Compound **I.9** has an inacurrate log k_w value with a standard error that substantially exceeds twice the standard deviation of eqn. 5 (Table III). Eqn. 6′, derived from eqn. 6

after forcing through the origin, includes only good-quality data and may serve as a reference equation.

$$\log P_c = 0.976(\pm 0.091)\log k_w + 0.612(\pm 0.129)I_S - 0.015(\pm 0.363) \quad (6)$$

$n = 10$; $r = 0.974$; $s = 0.182$; $F = 84$

$$\log P_c = 0.973(\pm 0.016)\log k_w + 0.613(\pm 0.120)I_S \quad (6')$$

For the direct attachment of the nitrosoureido and sulphur group, corrections of 3 and 2 c_M, respectively, were incorporated in the expression $\log P - f_{[-NHCON(NO)-]}$. The following equation includes all compounds except **I.9** and **II.7**:

$$\log P_c = 1.016(\pm 0.077)\log k_w + 1.749(\pm 0.091)I_N + 0.377(\pm 0.068)I_S - 0.104(\pm 0.308) \quad (7)$$

$n = 22$; $r = 0.993$; $s = 0.173$; $F = 476$

After forcing through the origin, the following, equation is obtained

$$\log P_c = 0.990(\pm 0.014)\log k_w + 1.763(\pm 0.079)I_N + 0.382(\pm 0.064)I_S \quad (7')$$

If the indicator I_S is assigned only to the sulphur present in the ring, eqn. 8′ is obtained, which does not differ in statistics but with a higher regressor of I_S, which is in better accordance with the value generated by eqn. 6′.

$$\log P_c = 1.030(\pm 0.074)\log k_w + 1.799(\pm 0.089)I_N + 0.461(\pm 0.080)I_S - 0.186(\pm 0.298) \quad (8)$$

$n = 22$; $r = 0.993$; $s = 0.169$; $F = 509$

After forcing through the origin, the following equation is obtained:

$$\log P_c = 0.985(\pm 0.014)\log k_w + 1.825(\pm 0.078)I_D + 0.470(\pm 0.078)I_S \quad (8')$$

Using the value −1.825 for the nitrosoureido fragment, the partition coefficients of compounds of type II were calculated and are reported in Table V. Log P values estimated according to reference eqn. 6′ are also reported. For compound **II.7** $\log P_{est} = 3.580$. Comparison of that value with the value of 4.298 obtained by the calculation procedure reveals a correction of $-2\ c_M$ to be necessary for the *ortho* steric effect.

Calculation of partition coefficients according to Rekker's revised system

Rekker recently revised his fragmental system [20]. In the revised system, much attention has been paid to heteroaromatics. Heteroaromatics should not be further fragmented and the revised system includes several fragments concerning whole structures. Molecules such as diphenyl ether and diphenyl sulphide, although they do not strictly belong to that class of compounds, need extra correction terms which are equal to 4 and 2 times the magic constant c_M (in the revised system $c_M = 0.219$), respectively. Hence both structures are more lipophilic than expected and at the same time sulphur and oxygen appear to be almost isolipophilic. This behaviour is in accordance with the lack of differentiation between the xanthene and thioxanthene derivatives observed also in the HPLC partition system.

Partition coefficients were therefore recalculated using the revised fragmental constants and considering the diphenyl ether or diphenyl sulphide moieties as the starting structures. For the $-NHCOCH_2N-$ group a fragmental constant was calculated for the revised system using Le Therizien's data and procedure [19]. A value of −2.895 is proposed which includes 5 c_M for the proximity effect. Moreover, corrections of 3, 3 and 2 c_M were considered for the direct attachment of –NHCO–, –NHCON(NO) and –S– to the ring system, respectively. Log P_{rev} values (Table IV) show an excellent parallelism with the corresponding log k_w data, in accordance with the results obtained so far by studying model series of compounds [10,12]. As revealed by eqns. 9 and 10, the introduction of an indicator variable for the presence of sulphur in the ring system is not necessary, while the slope and intercept remain close to 1 and 0, respectively.

TABLE V

CALCULATED AND ESTIMATED PARTITION COEFFICIENTS OF COMPOUNDS OF TYPE II

Compound	Log P [a]	Log P_{est} [b]	Log P_{rev} [c]	Log P_{est} [d]
II.1	4.218	4.057	4.557	4.411
II.2	4.737	4.524	5.076	4.920
II.3	4.737	4.641	5.076	5.047
II.4	5.256	5.050	5.595	5.491
II.5	4.298	4.359	4.626	4.740
II.6	4.817	4.728	5.145	5.142
II.7	3.431[e]	3.580	3.969[f]	3.893
II.8	4.767	5.079	4.669	4.856
II.9	5.286	5.487	5.188	5.300
II.10	5.286	5.506	5.188	5.321
II.11	5.035	4.865	5.258	5.290
II.12	5.584	5.457	5.372	5.267
II.13	3.870	4.018	4.419	5.369

[a] Calculated according to Rekker's original system.
[b] Estimated according to eqn. 6'.
[c] Calculated according to Rekker's revised system.
[d] Estimated according to eqn. 9'.
[e] An extra correction equal to $-2 \cdot 0.289$ is included to account for the *ortho* effect.
[f] An extra correction equal to $-3 \cdot 0.219$ is included to account for the *ortho* effect.

$$\log P_{rev} = 1.087(\pm 0.096) \log k_w - 0.116(+0.393) \quad (9)$$

$$n = 10;\ r = 0.966;\ s = 0.199;\ F = 124$$

$$\log P_{rev} = 1.066(\pm 0.067) \log k_w + 1.763(\pm 0.082) I_N - 0.036(\pm 0.273) \quad (10)$$

$$n = 22;\ r = 0.994;\ s = 0.157;\ F = 825.86$$

Eqns. 9 and 10 do not include compound **I.9** and compounds **I.9** and **II.13**, respectively, for the same reasons as already mentioned for eqns. 6 and 7.

After forcing through the origin, the following equations are obtained

$$\log P_{rev} = 1.058(\pm 0.014) \log k_w \quad (9')$$

$$\log P_{rev} = 1.058(\pm 0.012) \log k_w + 1.768(\pm 0.071) I_N \quad (10')$$

According to eqn. 10', the value −1.768 represents the hydrophobic fragmental constant of –NHCON(NO)– for the revised system and was used to calculate the partition coefficients of the nitrosoureido derivatives. Calculated log P values and log P values obtained using the reference eqn. 9' are reported in Table V. For compound **II.7** log $P_{est} = 3.969$. This value indicates that in the revised system a correction of $-3\ c_M$ is required to account for the *ortho* effect.

Use of tetrahydrofuran as organic modifier

In efforts to overcome the experimental limitations that arise from the use of methanol, measurements for compounds of type II were also performed using THF as organic modifier. THF is a more hydrophobic solvent and leads to shorter retention times. A second reason for the choice of THF was to check its merits when a polar group such as –NHCON(NO)– is attached to a highly lipophilic skeleton. As already mentioned, studies on model series suggest that this solvent may be suitable for the assessment of the lipophilicity of compounds that contain polar groups and especially hydrogen bond acceptors [10]. Moreover, this solvent drags enough water on to the stationary phase to mask the free

silanol groups, so the addition of a hydrophobic amine as a masking agent may not be necessary. On the other hand, THF disturbs the water network to a greater extent than methanol and may therefore exhibit a selective effect on solute retention [10,21]. For this reason, large percentages of THF in the mobile phase should be avoided. A mobile phase containing 60% THF was considered to be the upper limit of the concentration range used in the experiments while a lower limit of 40% THF could be reached. This concentration range, although wider than that achieved with methanol, is not satisfactory because a quadratic relationship is usually expected between isocratic capacity factors and the percentage of THF [10]. Therefore, more data should be available for the extrapolation procedure. If only the linear part of the relationship is considered, then data restricted to those obtained with mobile phases rich in water should be used. In the concentration range used in this study, linear extrapolation was possible but as the percentage of THF was fairly high, its effect is reflected in the log k_{wTHF} values, which are much lower than those derived from methanol (Table VI). Straightforward correlation between the two sets of log k_w values leads to the following equation with poor statistics:

$$\log k_w = 1.274(\pm 0.245) \log k_{wTHF} + 0.396(\pm 0.818) \quad (11)$$

$n = 12$; $r = 0.838$; $s = 0.245$; $F = 26$

The regression equation is improved when an indicator parameter I_S for the presence or absence of sulphur in the molecule is introduced:

$$\log k_w = 1.276(\pm 0.120) \log k_{wTHF} + 0.419(\pm 0.074) I_S + 0.247(\pm 0.403) \quad (12)$$

$n = 12$; $r = 0.963$; $s = 0.120$; $F = 68$

After forcing through the origin, the following equation is obtained:

$$\log k_w = 1.349(\pm 0.013) \log k_{wTHF} + 0.426(\pm 0.073) I_S \quad (12')$$

Similar results, expressed by eqns. 13 and 14, are obtained when log k_{60} and log k_{40THF} values, which represent the limits of the organic modifier concentration range, are compared:

$$\log k_{60} = 1.349(\pm 0.242) \log k_{40THF} - 0.371(\pm 0.303) \quad (13)$$

$n = 12$; $r = 0.855$; $s = 0.107$; $F = 31$

TABLE VI

ISOCRATIC AND EXTRAPOLATED CAPACITY FACTORS DETERMINED USING TETRAHYDROFURAN AS ORGANIC MODIFIER

Compound	Log k_{60}	Log k_{55}	Log k_{50}	Log k_{45}	Log k_{40}	Log k_{wTHF} [a]	S	r
II.1	0.18	0.39	0.65	0.81	1.22	3.15(±0.22)	5.0	0.9897
II.2	0.23	0.50	0.73	0.97	1.32	3.40(±0.12)	5.3	0.997
II.3	0.24	0.48	0.76	0.99	1.36	3.51(±0.13)	5.5	0.996
II.4	0.19	0.52	0.85	1.04	1.50	3.96(±0.21)	6.3	0.999
II.5	0.11	0.39	0.69	0.80	1.18	3.18(±0.21)	5.1	0.990
II.6	0.20	0.43	0.71	0.95	1.32	3.48(±0.14)	5.5	0.996
II.7	0.00	0.22	0.44	0.62	0.95	2.75(±0.17)	4.6	0.995
II.8	0.19	0.39	0.61	0.81	1.18	3.04(±0.19)	4.8	0.988
II.9	0.18	0.45	0.68	0.92	1.27	3.35(±0.12)	5.3	0.997
II.10	0.16	0.41	0.67	0.93	1.30	3.49(±0.13)	5.6	0.997
II.11	0.11	0.37	0.63	0.93	1.21	3.41(±0.05)	5.5	0.999
II.13	0.11	0.37	0.59	0.80	1.17	3.16(±0.16)	5.1	0.994

[a] Capacity factors linearly extrapolated to 100% water according to the equation $\log k = \log k_{wTHF} - S\varphi$ (φ = fraction of THF).

$$\log k_{60} = 1.379(\pm 0.138) \log k_{40\text{THF}} + 0.174(\pm 0.037) I_S - 0.467(\pm 0.174) \quad (14)$$

$n = 12$; $r = 0.956$; $s = 0.061$; $F = 59$

Correlation between the calculated $\log P$ values and $\log k_{\text{wTHF}}$ leads to eqn. 15, which, after forcing through the origin, gives eqn. 15′:

$$\log P_{\text{rev}} = 1.398(\pm 0.107) \log k_{\text{wTHF}} + 0.271(\pm 0.065) I_S + 0.157(\pm 0.356) \quad (15)$$

$n = 12$; $r = 0.972$; $s = 0.106$; $F = 87$

$$\log P_{\text{rev}} = 1.445(\pm 0.010) \log k_{\text{wTHF}} + 0.273(\pm 0.062) I_S \quad (15')$$

The regressors of $\log k_{\text{wTHF}}$ in eqns. 12′ and 15′ indicate a hypodiscriminative power of the HPLC partition system obtained with THF as organic modifier compared with the corresponding system obtained with methanol and with the octanol–water system. Moreover, with THF as organic modifier, the sulphur appears to be less lipophilic than in the two other systems.

CONCLUSIONS

Despite the experimental limitations, and HPLC partition system formed using an ODS stationary phase, methanol as organic modifier and *n*-decylamine as a masking agent, proved to be suitable for the assessment of the partitioning behaviour of compounds with log *P* values above 4. It may be concluded that if good linear relationships are found between capacity factors and the percentage of methanol, the extrapolated capacity factors can be regarded as reliable substitutes for octanol–water log *P* values even if the concentration range of the organic modifier is narrow and limited data points are available for the extrapolation procedure. The good parallelism between octanol–water and the HPLC system described above, as revealed by the proper description of the structural characteristics in the calculated partition coefficients, permitted the generation of a hydrophobic fragmental constant for the –NHCON(NO)– group, which may be used for the calculation of the log *P* values of other compounds containing a nitrosoureido function. In this respect, the advantage of Rekker's revised system mainly consists in the better description of oxygen and sulphur in the 9*H*-xanthene and 9*H*-thioxanthene moieties.

The use of THF for the study of highly lipophilic compounds is not recommended as its merits (lower retention times) are offset by restrictions in the extrapolation procedure and by its higher selective effect towards special structural characteristics (the presence of sulphur in the case studied).

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