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ANOMALIES IN THE REVERSED-PHASE THIN-LAYER CHROMATOGRAPHIC BEHAVIOUR OF SOME HETEROCYCLIC QUATERNARY AMMONIUM SALTS

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SUMMARY

The determination of the lipophilicity of 3,3,5-trimethyl-1-azocycloheptane- and 1,3,3-trimethyl-6-azobicyclo[3.2.1]octane derivatives by reversed-phase thin-layer chromatography shows marked anomalies. In ion-free eluent systems the R_M values show an extremely small dependence on the proportion of organic solvent in the mobile phase. A logarithmic relationship was found between the R_M value and the pH of the eluent system. The effect of ion concentration can be modelled by a polynomial function. These anomalies can be explained by the change in the ion-pair formation of the very hydrophilic quaternary ammonium group and by the change in the solvate shell around the very lipophilic organic part of the molecule caused by different pH values and by different ion concentrations.

INTRODUCTION

Lipophilicity is one of the most important molecular parameters applied in QSAR studies because the biological activity of a molecule is generally correlated with its ability to penetrate the different hydrophobic barriers (membranes), that is, with its lipophilicity[1,2]. In addition to the classical partition method[3], lipophilicity can be determined by reversed-phase thin-layer chromatography (TLC)[4,5] and high-performance liquid chromatography[6,7]. The lipophilicity of some quaternary ammonium salts was determined in 0.1 M hydrochloric acid solution and it showed marked anomalies[8,9].

For QSAR studies we determined the lipophilicity of some 3,3,5-trimethyl-1-azocycloheptane and 1,3,3-trimethyl-6-azobicyclo[3.2.1]octane derivatives and studied the effect of different conditions (water to organic solvent ratio, pH, ion concentration) on the R_M value.

EXPERIMENTAL

The compounds investigated are shown in Fig. 1.

In the reversed-phase TLC method used pre-coated TLC sheets (DC Alufolien Kieselgel 60; Merck, Darmstadt, G.F.R.) were impregnated with 5 % paraffin oil in *n*-
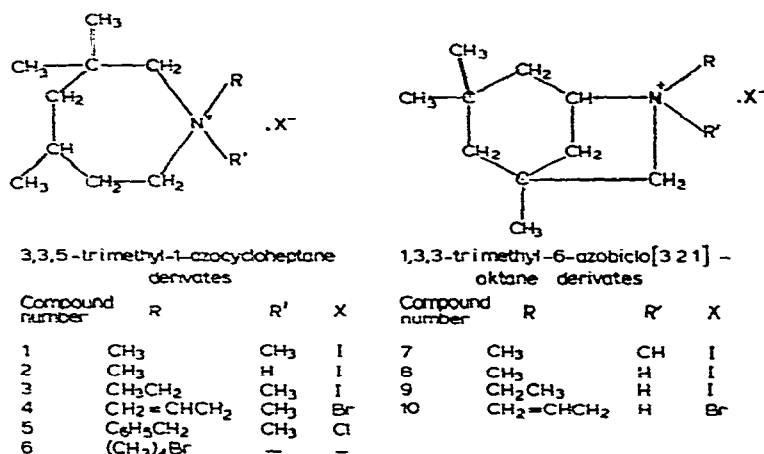
3,3,5-trimethyl-1-azocycloheptane derivates

1,3,3-trimethyl-6-azobiclo[3 2 1] – oktane derivates

Compound number	R	R'	X	Compound number	R	R''	X
1	CH₃	CH₃	I	7	CH₃	CH	I
2	CH₃	H	I	8	CH₃	H	I
3	CH₃CH₂	CH₃	I	9	CH₂CH₃	H	I
4	CH₂=CHCH₂	CH₃	Br	10	CH₂=CHCH₂	H	Br
5	C₆H₅CH₂	CH₃	Cl				
6	(CH₂)₄Br	—	—				

Fig. 1. Structures of compounds investigated.

hexane and dried at room temperature. The compounds (2 μl of solutions of 10 mg of heterocyclic ammonium salt in 1 ml of methanol) were spotted on the sheets. The effect of pH, ion concentration and quality and amount of the organic phase on the R_M values of compounds 1, 4 and 5 was studied in different eluent systems (Table I). The influence of the ionic environment and pH on the order of lipophilicity of all ten compounds was also determined. We measured further the effect of low ion concentrations on the R_M value of compound 10 (for eluent systems see Table II). After development the sheets were dried at 105°C. The spots were revealed with iodine vapour.

TABLE I

ELUENT SYSTEMS USED TO STUDY THE BEHAVIOUR OF COMPOUNDS 1, 4 AND 5 IN REVERSED-PHASE TLC

Organic phase	Proportion (%)	Water proportion (%)	Remarks
Tetrahydrofuran	25–95	75–5	
n-Propanol	25–95	75–5	
Methanol	25–95	75–5	
Methanol	50	50	Britton–Robinson buffers of pH 1.81, 2.21, 3.29, 4.56, 5.72, 6.80 and 11.98
Methanol	50	50	Saturated with LiCl to 0, 25, 50, 75 and 100%

RESULTS AND DISCUSSION

The R_M values measured are summarized in Table III. The R_M values characterizing the molecular lipophilicity have an extremely small dependence on the amount and quality of the organic phase. No significant difference between the organic phases was observed. The R_M values decrease linearly with increasing concentration of organic phase at a significance level of 95%, but the slopes do not differ

TABLE II

ELUENT SYSTEMS USED TO MEASURE THE ORDER OF LIPOPHILICITY OF COMPOUNDS 1–10 IN REVERSED-PHASE TLC

Organic phase: 50% methanol.

Eluent No.	Aqueous phase	Eluent No.	Aqueous phase
1	Non-buffered	8	as 3, LiCl added to 9.8 N
2	0.1 N buffer, pH = 3.30	9	as 3, LiCl added to 5.0 N
3	0.1 N buffer, pH = 6.00	10	as 3, LiCl added to 2.5 N
4	0.1 N NaOH	11	as 3, LiCl added to 1.25 N
5	as 2, LiCl added to 9.8 N	12	as 3, LiCl added to 0.7 N
6	as 4, saturated with LiCl	13	as 1, NaCl added to 0.05–0.1 N
7	as 3, LiCl added to 19.6 N		

significantly from zero (Table IV). This phenomenon was not observed with other types of compounds and it can be explained by the fact that each molecule contains a very hydrophilic and a very hydrophobic part. With different proportions of organic solvent the solvation of the hydrophilic and hydrophobic parts changes in a different manner and the effects almost counterbalance each other. The effect of lipophilic substituents acts in the reverse direction, as would be expected from the general rule. This finding is in accordance with the observation of Leo *et al.*[8].

With different pH values the anions of the buffers can form ion pairs of different bonding strengths with the quaternary ammonium groups, thus influencing the lipophilicity of the molecule. The effect is more marked at low pH and is negligible between the pH 6.80 and 11.98. The correlation between the pH and R_M values can be described by a logarithmic function at a significance level of 99% (Table V).

A polynomial function fits well the data describing the dependence of R_M

TABLE III

R_M VALUES OF COMPOUNDS 1–10 IN DIFFERENT REVERSED-PHASE TLC SYSTEMS

For symbols see Fig. 1 and Table II.

Eluent No.	Compound No.									
	1	2	3	4	5	6	7	8	9	10
1	0.93	0.86	0.99	0.92	0.89	0.86	1.02	0.88	0.92	0.82
2	0.15	0.09	0.13	0.15	0.18	0.19	−0.02	0.10	0.02	0.07
3	0.42	0.47	0.50	0.38	0.31	0.51	0.41	0.44	0.37	0.25
4	0.66	0.71	0.56	0.59	0.55	0.68	0.61	0.61	0.53	0.66
5	0.17	0.12	0.24	0.23	0.44	0.23	0.14	0.15	0.14	0.33
6	0.32	0.29	0.36	0.40	0.63	0.38	0.33	0.32	0.28	0.49
7	0.38	0.35	0.44	0.43	0.75	0.36	0.41	0.33	0.33	0.58
8	0.32	0.26	0.37	0.38	0.66	0.39	0.39	0.32	0.28	0.53
9	0.02	−0.01	0.12	0.08	0.22	0.05	0.05	0.01	−0.04	0.08
10	0.10	0.00	0.08	0.08	0.20	0.09	−0.01	0.00	−0.02	0.04
11	0.25	0.22	0.30	0.25	0.36	0.26	0.18	0.23	0.15	0.22
12	0.21	0.20	0.24	0.16	0.18	0.14	0.15	0.14	0.08	0.11

TABLE IV

PARAMETERS OF LINEAR CORRELATIONS BETWEEN THE R_M VALUES (y) OF COMPOUNDS 1, 4 AND 5 AND THE PROPORTION OF THE ORGANIC PHASE (x %)

$y = a + bx$; $n = 14$; $r_{95\%} = 0.5324$.

Organic phase	Compound No.	a	$b \cdot 10^2$	r	$s_b \cdot 10^2$
Tetra-	1	1.76	-2.8	0.5319	1.7
hydro-	4	1.63	-2.3	0.5760	1.4
furan	5	1.01	-3.1	0.5415	1.8
n-Propa-	1	1.31	-2.7	0.5477	1.4
nol	4	1.17	-3.6	0.5719	2.3
	5	1.06	-1.9	0.5865	0.9
Methanol	1	1.38	-3.9	0.5455	2.1
	4	1.17	-1.1	0.5743	0.7
	5	1.15	-3.7	0.5913	1.9

values on ion saturation. The R_M values decrease with increasing ion concentration in the lower saturation range but they increase with increasing ion concentration above 50% saturation (Table VI). This phenomenon is a result of two effects: the ions, as mentioned above, can form ion pairs with the quaternary ammonium groups (this effect dominates at lower saturations), and at the same time they decrease the neutralizing solvate shell around the molecule (at higher ion saturation the ion pair formation is quasi-completed and this second effect prevails).

From the data concerning the order of lipophilicity of all ten derivatives we established that their mobility in an ion-free mobile phase is very low, and these R_M values do not correlate with the R_M values obtained in any ionic systems at neutral pH (Table VII, functions 20 and 21), that is, ion concentrations as low as 0.1 N modify in different manners the R_M values of compounds.

We could not find any correlation between the orders of lipophilicity measured at different pH values (at the same ion concentration); the pH dependence of the molecules differ from each other. This means that the substituents influence differently the ion-pair forming ability of the quaternary ammonium group.

There is no correlation between the orders of lipophilicity determined at low and high ion concentrations in basic and acidic mobile phase (Table VII, functions 1

TABLE V

PARAMETERS OF LOGARITHMIC FUNCTIONS BETWEEN TNE R_M VALUES (y) OF COMPOUNDS 1, 4 AND 5 AND THE pH (x) OF THE ELUENT SYSTEM

$y = a + b\log x$. $n = 10$; $r_{99\%} = 0.7636$.

Compound No.	$a \cdot 10^2$	$b \cdot 10$	$s_b \cdot 10$	r
1	7.58	8.65	1.92	0.7886
4	3.20	7.07	1.79	0.8496
5	0.20	5.82	1.79	0.7991

TABLE VI

PARAMETERS OF POLYNOMIAL FUNCTIONS BETWEEN THE R_M VALUES (y) OF COMPOUNDS 1, 4 AND 5 AND THE ION SATURATION ($x\%$) OF THE MOBILE PHASE

$y = a + b_1 x + b_2 x^2$. $n = 14$; $F_{95\%} = 3.88$; $t_{95\%} = 2.20$.

Parameter	Compound No.		
	1	4	5
$b_1 \cdot 10^2$	− 3.3	− 3.3	− 2.9
$b_2 \cdot 10^4$	2.6	2.8	2.6
a	1.04	0.98	0.92
r	0.8513	0.8624	0.9228
$b_1'(\%)$	54.90	53.04	51.24
$b_2'(\%)$	45.10	46.96	48.976
s	0.30	0.27	0.16
$s_1 \cdot 10^2$	1.4	1.2	0.7
$s_2 \cdot 10^4$	1.2	1.1	0.7
F	3.95	4.35	8.61
t_1	2.54	2.86	4.15
t_2	2.09	2.53	3.95

TABLE VII

PARAMETERS OF LINEAR FUNCTIONS BETWEEN THE R_M VALUES (y AND x) DETERMINED IN DIFFERENT REVERSED-PHASE TLC SYSTEMS

$y = a + bx$. $n = 10$; $r_{95\%} = 0.6319$; $r_{99.9\%} = 0.8721$.

Eluent No. (see Table II)		a	b	r	Function No.
y	x				
4	6	0.70	−0.24	0.4201	1
3	4	−0.08	0.80	0.5794	2
3	2	0.38	0.25	0.2091	3
4	2	0.60	0.11	0.1210	4
3	8	0.39	−0.41	0.6356	5
6	8	0.04	0.87	0.9755	6
2	5	0.03	0.34	0.5020	7
8	5	0.14	1.13	0.9482	8
6	5	0.15	1.04	0.9795	9
8	7	0.01	0.88	0.9622	10
3	7	0.59	−0.42	0.6744	11
3	9	0.43	−0.34	0.3067	12
7	9	0.31	1.41	0.8715	13
3	10	0.42	−0.22	0.1808	14
7	10	0.36	1.42	0.7270	15
3	11	0.40	0.03	0.0224	16
7	11	0.07	1.51	0.6547	17
3	12	0.27	0.86	0.4913	18
7	12	0.41	0.19	0.0690	19
1	3	0.82	0.23	0.3139	20
1	7	0.94	−0.08	0.1687	21

and 7). An opposite order of lipophilicity can be observed at low and high ion concentrations in a neutral mobile phase (Table VII, functions 5 and 11), that is, the effect of the ionic environment is higher with more hydrophobic molecules. This is in good accordance with our other investigations. This opposite effect decreases with decreasing ion concentration (Table VII, functions 10 and 13–19).

The differentiating effect of pH is insignificant at high ion concentrations, and the orders of lipophilicity show good correlations independent of the pH of the mobile phase (Table VII, functions 6, 8 and 9). At low ion concentrations (0.005–0.1 N) a Freundlich isotherm describes well the dependence of the R_M values on the ion concentration, which indicates that in this range the ions exert a greater effect on the ion-pair formation than on the solvate shell of the hydrophobic organic part of the compound (Fig. 2).
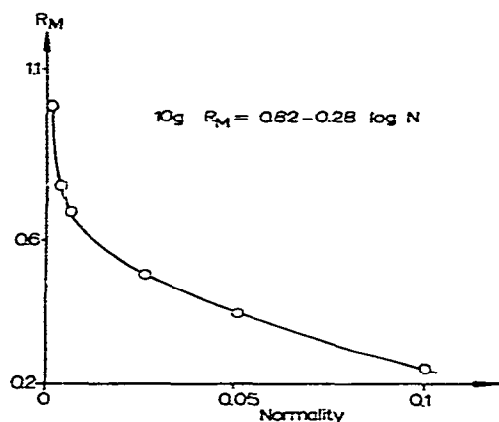

Fig. 2. Dependence of R_M value of compound 10 on ion concentration at low saturation.

Because the order of lipophilicity depends considerably on the ion concentration and on pH, it is highly questionable which of the orders of lipophilicity measured at different pH values and ion concentrations can be used in QSAR studies. Therefore, we strongly advocate that the effects of ion concentration and pH should be determined in similar cases, as the bioactive molecules exert their effect at a definite pH and ion concentration.

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