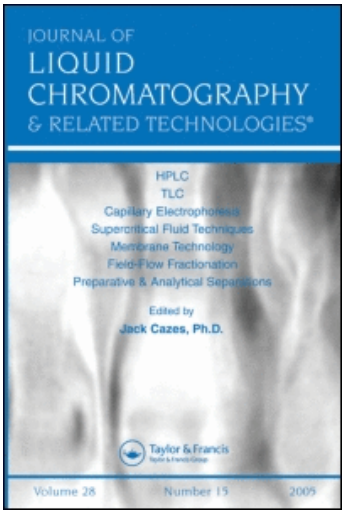

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF NEW POTENTIAL ANXIOLYTIC DRUGS AND RELATED BENZODIAZEPINES: A COMPARATIVE STUDY OF HYDROPHOBICITY

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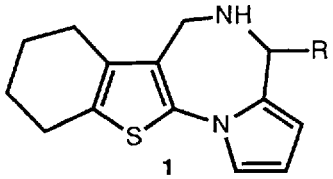
ABSTRACT

A comparative study of hydrophobicity in diazepam (**5**) chlordizepoxide (**6**), buspirone (**7**) and four related pyrrolothieno-1,4-dizepine systems (**1-4**) has been carried out, using HPLC (RPC) technique. The capacity factors (K') of compounds studied have been measured in five different elution conditions (mobile phase in which the percentage of methanol varies). The capacity factors (K'_o) corresponding to the absence of organic solvent in the mobile phase have been calculated. Correlations among hydrophobic parameters of series **1, 2, 3,** and **4** have been established and K'_o of compounds not yet synthesized have been calculated.

INTRODUCTION

Since the introduction of 1,4-benzodiazepines as anxiolytic drugs many efforts have been carried out to find new active related compounds.[1] The addition of a ring to the "classical" ben-



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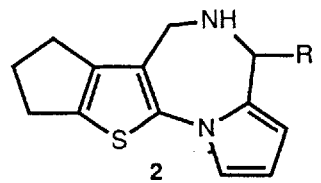

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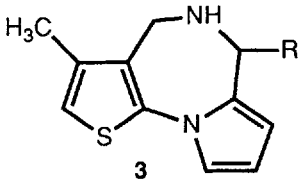

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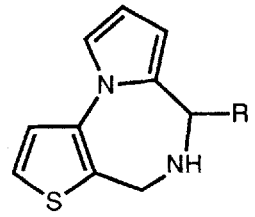
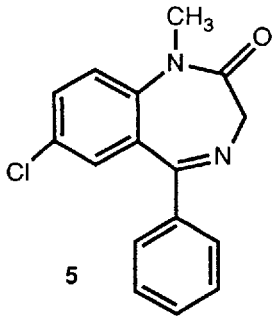


4

a R= C_6H_5
b R= C_6H_4-Cl-*o*
c R= C_6H_4-Cl-*m*
d R= C_6H_4-Cl-*p*
e R= C_6H_4-NO_2-*p*
f R= C_6H_4-OCH_3-*m*
g R= C_6H_4-OCH_3-*p*
h R= C_6H_3-$(OCH_3)_2$ (3,4)
i R= C_6H_3-OCH_2O- (3,4)
j R= C_6H_4-$NHCOCH_3$-*p*
k R= C_6H_4-$N(CH_3)_2$-*p*



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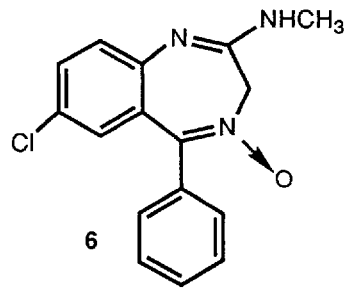

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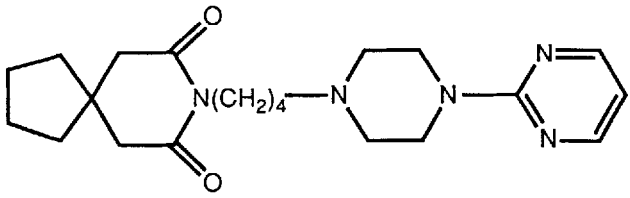

7

SCHEME

zodiazepine system and the replacement of the benzene ring by an heterocycle are found among the useful structural manipulations carried out.[2] Following these guidelines, we have synthesized[3,4] and tested as anxiolytic agents four series of compounds **1**, **2**, **3**, **4** belonging to two pyrrolothieno-1,4-diazepine systems.

The influence of hydrophobicity of drugs on their "in vivo" pharmacological activities is well known.[5,6] For this reason we have carried out a comparative study of the hydrophobic character of compounds of the series **1-4**, using as standard anxiolytic drugs diazepam (**5**), chlordiazepoxide (**6**) and buspirone (**7**) (see scheme).

Reversed-phase HPLC is a useful technique to determine hydrophobicity of drugs[7] and the retention time parameter log K' has been correlated with the classical octanol-water partition coefficient log P.[8,9] Retention in HPLC has also been correlated directly with biological activity.[8,10,11] It has been suggested hydrophobic parameters in HPLC are correlated better than log P with biological activity due to the similarity between HPLC process and the behaviour of a solute through bilipid layer membranes.

In this work we have measured the capacity factors (K') of series **1-4** and standards at different concentrations of organic solvent, using reversed-phase HPLC and calculated the capacity factors (K'_o) in absence of organic solvent.

MATERIAL AND METHODS

Materials

High performance liquid chromatography grade methanol was obtained from E.M. Merck Co. and reagent grade water was generated by a Millipore Milli-Q Water purification system. Diazepam, chlordiazepoxide and buspirone were obtained from Prodes, Roche and Bristol Mayers, S.A.E. companies, respectively.

Compounds of series **1**[3] and **4**[4] were synthesized by reported methods and compounds of series **2** and **3** were prepared by synthetic methods which will be described elsewhere.

Chromatography

HPLC was performed on a Waters HPLC system consisting of a 6000A solvent delivery system and a U6K injector. The compounds studied on this system were detected by a Waters 440 absorbance spectrophotometric detector at 254 nm. Retention data were collected by a Waters 730 Data module at a rate of 0.5 cm./min.

For analysis a Hibare μ-Bondapack C-18 column at 25°C was used. Mobile phase consisted of mixtures of MeOH/H_2O buffer solutions at 65%, 70%, 75%, 80% and 85% (v/v), at a flow rate of 0.9 ml/min.

Phosphate buffer has been reported to cause the least deviation from octanol-water values.[12] The mobile phase solutions were used with a 0.025 M NaH_2PO_4 buffer that had been adjusted to an apparent pH of 7.0 after the MeOH/H_2O had been added.

The capacity factors (K') of compounds studied were determined from the observed retention time (Tr) and the retention time (T_o) of uracile, as unretained compound,

$$K' = \frac{Tr - T_o}{T_o} \qquad [1]$$

Methods

Compounds were dissolved in methanol at a concentration of 250 μg/ml. Then they were injected separate from each other. The experiments were carried out four times and the mean value of the retention time was obtained for each compound.

RESULTS AND DISCUSSION

Test compounds and standards were chromatographed under a variety of conditions in which the percentage of methanol in the

mobile phase varied from 85% to 65% and the apparent pH was always kept at physiologic value 7.0. Experiments with lower percentage of methanol than 65% afforded experimental retention times too long to be measured, mainly in series **1**.

Standards are weak bases (pKa of **5** = 3.50,[13] pKa of **6** = 4.76[13] and pKas of **7** = 4.12 and 7.32[14]). Thus, at pH = 7 the percentage of unionized form is higher than 99% for **5** and **6** and ~50% for **7** and sufficient amount of neutral form can be eluted. Althoug pKa values of compounds of series **1-4** were not measured, they are probably weak bases too, since they were easily eluted at pH 7. The measured retention times (Tr) and capacity factors (K') of test compounds and standards are gathered in Tables 1-4.

The K' values of the four series and standards decrease linearly with increasing methanol percentage of mobile phase (figures 1-4). The data of correlation equations found for log K' vs % MeOH (least squares) are shown in Tables 5-8. Intercepts represent the corresponding capacity factors in absence of organic solvent, log K'_o, which could be correlated with biological activity.

By comparing the capacity factors of the four series, the following increasing order of lipophilic character was found: **1** > **2** > **3** > **4**. The influence of hydrogenated hexa- and pentacycles in series **1** and **2** accounts for their high lipophilicity.

"A priori" compounds of series **4** might be better anxiolytic agents than those of series **1**, **2** and **3**, because they are as lipophilic as standards. However, lipophilicity is not the only factor in biological activity.

In general, in the four series, chlorinated derivatives (**d**, **b**, **c**, in this order) behaved as the most lipophilic compounds, being **e**, **h** and **j** derivatives the least ones.

As usual, the substituent position dependence (o, m, p) in lipophilicity has smaller effect than the nature of substituent

Table 1. Tabulated π values for R and HPLC measured parameters of serie 1 and standards

	65% MeOH		70% MeOH		75% MeOH		80% MeOH		85% MeOH		
Comp.	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'	π(R)
1a	16.78	0.665	12.76	0.511	6.25	0.045	5.62	-0.058	4.12	-0.428	1.96
1b	20.20	0.761	11.55	0.454	7.75	0.208	6.19	0.026	4.26	-0.376	2.67
1c	22.61	0.818	12.53	0.501	7.84	0.216	6.32	0.044	4.35	-0.346	2.67
1d	23.51	0.838	13.63	0.549	7.68	0.202	6.55	0.073	4.38	-0.337	2.67
1e	14.23	0.576	9.60	0.313	6.36	0.059	5.21	-0.132	3.96	-0.494	1.68
1f	15.65	0.628	9.70	0.348	6.00	0.115	5.61	-0.060	4.15	-0.416	1.94
1g	15.71	0.630	9.80	0.354	5.76	-0.024	5.70	-0.045	4.21	-0.394	1.94
1h	12.06	0.483	9.72	0.350	5.44	-0.077	4.98	-0.180	3.96	-0.494	1.92
1i	15.48	0.622	12.34	0.492	6.05	0.018	5.58	-0.065	4.06	-0.452	1.91
1j	8.98	0.303	6.34	-0.045	4.68	-0.235	4.20	-0.397	3.71	-0.625	0.99
1k	19.60	0.746	12.14	0.482	6.13	0.029	5.95	-0.007	4.60	-0.273	2.14
Uracil	2.98	-	3.00	-	2.96	-	3.00	-	3.00	-	log P
5	4.93	-0.173	4.21	-0.394	3.80	-0.547	3.53	-0.753	3.35	-0.933	2.80
6	4.61	-0.262	4.04	-0.460	3.71	-0.596	3.50	-0.778	3.36	-0.921	2.44
7	5.91	-0.007	4.82	-0.221	4.16	-0.392	3.83	-0.558	3.58	-0.714	3.43

Table 2. HPLC measured parameters of serie **2** and standards

	65% MeOH		70% MeOH		75% MeOH		80% MeOH		85% MeOH	
Comp.	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'
2a	10.42	0.375	6.56	0.058	5.43	-0.101	4.61	-0.275	4.05	-0.456
2b	12.25	0.472	7.32	0.144	6.07	0.001	4.65	-0.264	4.07	-0.448
2c	13.40	0.523	7.80	0.190	6.34	0.038	4.96	-0.189	4.14	-0.420
2d	13.52	0.528	7.96	0.204	6.30	0.033	4.98	-0.184	4.17	-0.409
2e	8.97	0.279	6.23	0.015	5.21	-0.143	4.31	-0.366	3.81	-0.569
2f	9.52	0.318	6.51	0.052	5.47	-0.094	4.56	-0.289	4.02	-0.468
2g	9.61	0.330	6.60	0.063	5.42	-0.103	4.66	-0.261	4.10	-0.436
2h	8.08	0.208	5.76	-0.054	4.93	-0.203	4.33	-0.358	3.97	-0.491
2i	9.28	0.302	6.46	0.046	5.37	-0.112	4.50	-0.305	4.00	-0.477
5	4.55	-0.326	4.20	-0.490	3.78	-0.606	3.51	-0.788	3.35	-0.933
6	4.36	-0.386	4.05	-0.216	3.73	-0.636	3.50	-0.529	3.36	-0.921
7	5.36	-0.134	4.92	-0.217	4.24	-0.399	3.90	-0.530	3.58	-0.714
Uracil	3.09	-	3.06	-	3.03	-	3.01	-	3.00	-

Table 3. HPLC measured parameters of serie 3[a]

	65% MeOH		70% MeOH		75% MeOH		80% MeOH		85% MeOH	
Comp.	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'
3a	6.34	0.022	5.30	-0.135	4.46	-0.327	3.92	-0.520	3.60	-0.699
3b	7.20	0.124	5.82	-0.045	4.63	-0.277	3.97	-0.497	3.63	-0.678
3c	7.90	0.192	6.10	-0.003	4.86	-0.220	4.08	-0.450	3.68	-0.646
3d	8.08	0.208	6.27	0.021	4.91	-0.208	4.12	-0.434	3.70	-0.633
3e	6.00	-0.026	4.93	-0.214	4.20	-0.413	3.75	-0.611	3.48	-0.796
3f	6.34	0.022	5.24	-0.147	4.41	-0.342	3.90	-0.530	3.60	-0.699
3g	6.46	0.037	5.40	-0.117	4.49	-0.318	4.03	-0.470	3.65	-0.665
3h	5.60	-0.090	4.80	-0.246	4.18	-0.421	3.84	-0.561	3.54	-0.745
3i	6.20	0.003	5.18	-0.160	4.35	-0.361	3.92	-0.520	3.55	-0.738

[a] Tr of uracile as in Table 2.

Table 4. HPLC measured parameters of serie **4**[a]

	65% MeOH		70% MeOH		75% MeOH		80% MeOH		85% MeOH	
Comp.	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'	Tr	log K'
4a	4.98	-0.213	4.36	-0.373	3.86	-0.564	3.63	-0.688	3.49	-0.788
4b	5.35	-0.136	4.61	-0.296	3.98	-0.504	3.65	-0.674	3.45	-0.824
4c	5.86	-0.048	4.98	-0.203	4.11	-0.448	3.75	-0.611	3.50	-0.780
4d	5.96	-0.032	4.94	-0.212	4.14	-0.436	3.77	-0.598	3.52	-0.762
4e	4.76	-0.268	4.21	-0.426	3.75	-0.625	3.50	-0.790	3.36	-0.921
4f	4.90	-0.233	4.34	-0.379	3.82	-0.585	3.58	-0.723	3.46	-0.815
4g	5.10	-0.187	4.40	-0.359	3.83	-0.578	3.63	-0.688	3.55	-0.737
4h	4.51	-0.338	4.12	-0.461	3.70	-0.656	3.53	-0.764	3.51	-0.769
4i	4.86	-0.243	4.32	-0.386	3.81	-0.590	3.56	-0.740	3.50	-0.780

[a] Tr of uracile as in Table 2.

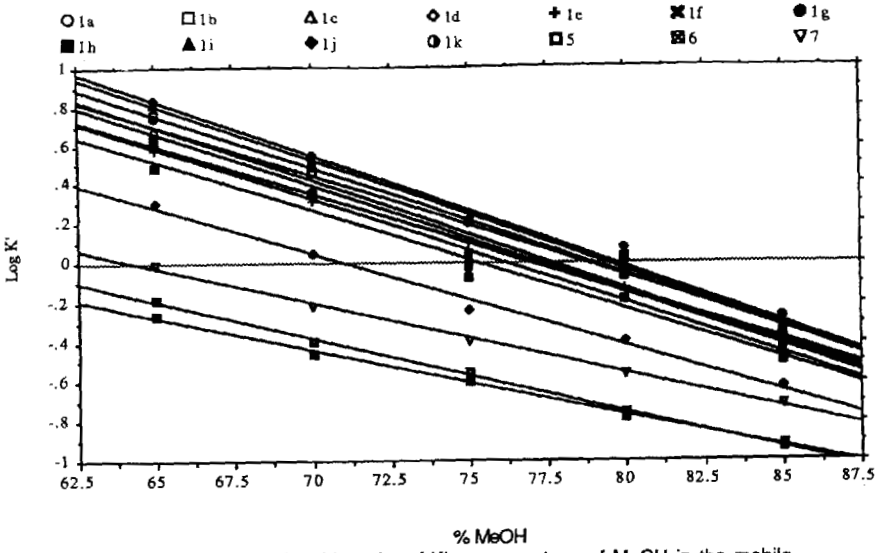

Figure 1. Semi-log plot of K' vs percentage of MeOH in the mobile phase for serie 1 and standards.

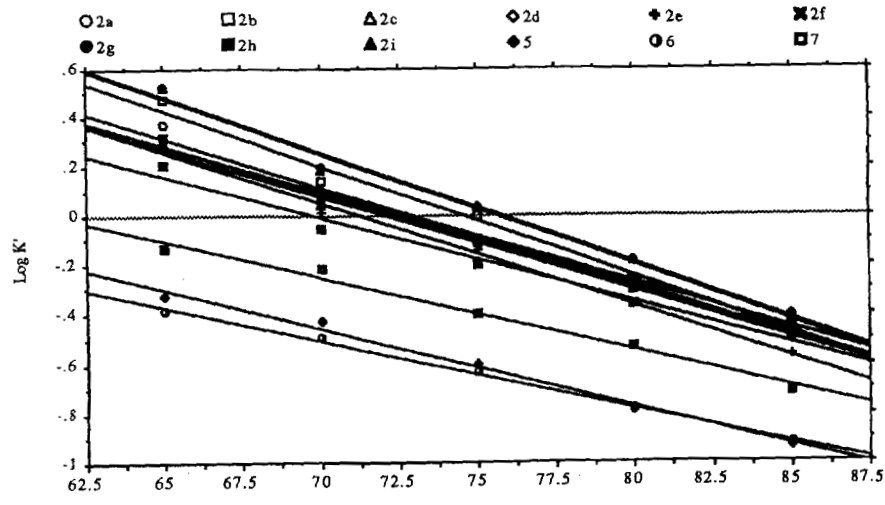

Figure 2. Semi-log plot of K' vs percentage of MeOH in the mobile phase for serie 2 and standards.

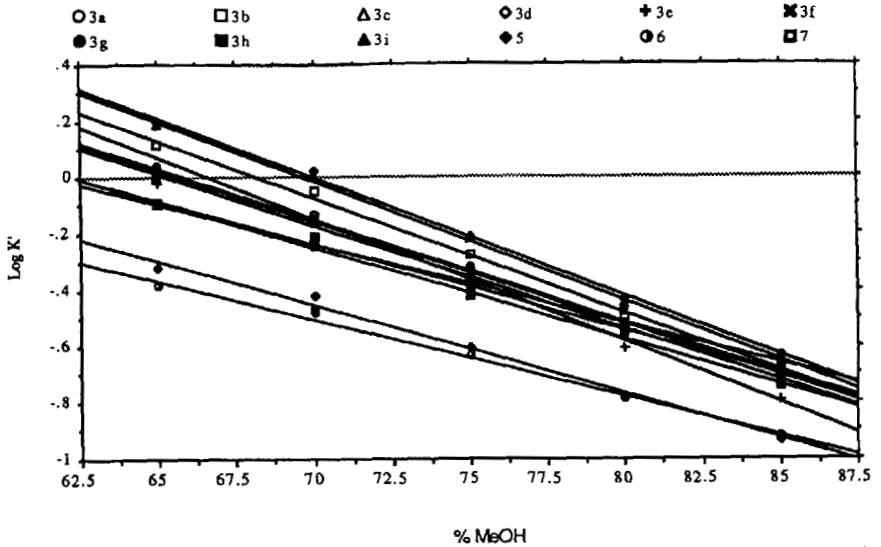

Figure 3. Semi-log plot of K' vs percentage of MeOH in the mobile phase for serie 3 and standards.

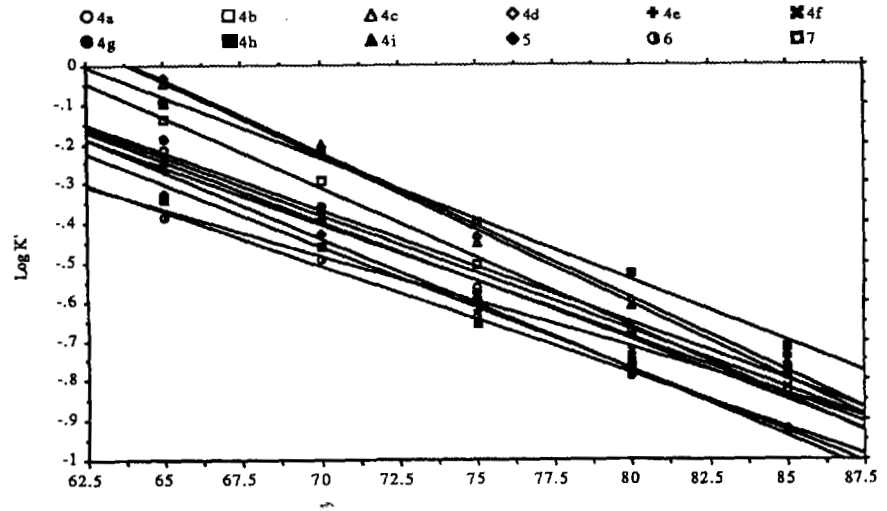

Figure 4. Semi-log plot of K' vs percentage of MeOH in the mobile phase for serie 4 and standards.

Table 5. Correlation equations log K' vs % MeOH data of serie **1** and standards

Comp.	Intercept log K'_o	Slope	r^2
1a	4.285	-0.055	0.968
1b	4.273	-0.054	0.986
1c	4.430	-0.056	0.989
1d	4.506	-0.057	0.983
1e	3.952	-0.052	0.992
1f	3.852	-0.050	0.974
1g	3.781	-0.049	0.957
1h	3.749	-0.050	0.969
1i	4.185	-0.054	0.960
1j	3.274	-0.046	0.992
1k	3.991	-0.051	0.949
5	2.222	-0.037	0.998
6	1.850	-0.036	0.997
7	2.253	-0.035	0.997

Table 6. Correlation equations log K' vs % MeOH data of serie **2** and standards

Comp.	Intercept log K'_o	Slope	r^2
2a	2.913	-0.040	0.981
2b	3.351	-0.045	0.987
2c	3.427	-0.045	0.988
2d	3.429	-0.045	0.990
2e	2.958	-0.042	0.996
2f	2.775	-0.038	0.992
2g	2.684	-0.037	0.989
2h	2.371	-0.034	0.981
2i	2.754	-0.038	0.994
2j[a]	2.028	-	-
2k[a]	2.827	-	-
5	1.734	-0.031	0.994
6	1.407	-0.027	0.996
7	1.928	-0.031	0.995

[a] Calculated from equation 2.

Table 7. Correlation equations log K' vs % MeOH data of serie **3**

Comp.	Intercept log K'$_o$	Slope	r^2
3a	2.407	-0.037	0.999
3b	2.808	-0.041	0.997
3c	2.955	-0.042	0.999
3d	2.993	-0.043	0.999
3e	2.491	-0.039	1.000
3f	2.397	-0.036	0.999
3g	2.330	-0.035	0.998
3h	2.023	-0.032	0.999
3i	2.404	-0.037	0.998
3j[a]	1.730	-	-
3k[a]	2.417	-	-

[a] Calculated from equation 3.

Table 8. Correlation equations log K' vs % MeOH data of serie **4**

Comp.	Intercept log K'$_o$	Slope	r^2
4a	1.668	-0.029	0.986
4b	2.143	-0.035	0.997
4c	2.385	-0.037	0.994
4d	2.359	-0.037	0.996
4e	1.905	-0.033	0.996
4f	1.716	-0.030	0.984
4g	1.632	-0.029	0.950
4h	1.152	-0.023	0.928
4i	1.591	-0.029	0.963
4j[a]	0.914	-	-
4k[a]	1.705	-	-

[a] Calculated from equation 4.

(compare log K'_o of **b, c, d** and of **c, f**) because the field effect of substituents is more important than the resonance effects.[15a]

If the tabulated π values[15b] corresponding to R substituents are compared with log K' values of compounds, **h** derivatives present an anomalous elution order in the four series, showing less lipophilic character, in all the conditions studied, than expected.

The dependence of interactions among compounds and silanol groups of stationary phase upon the nature of the mobile phase is shown by the fact that the slopes corresponding to the plots log K' vs % MeOH are not the same for all compounds in each series; therefore on changing the nature of the mobile phase the elution order of a series of compounds can vary in some cases.

Correlations have been established between log K'_o of series **1** and those of series **2, 3** and **4** (equations 2, 3 and 4).

$$\log K'_o\ (\mathbf{2}) = 1.114 \log K'_o\ (\mathbf{1}) - 1.620, \quad n = 9,\ r = 0.86 \quad [2]$$

$$\log K'_o\ (\mathbf{3}) = 0.958 \log K'_o\ (\mathbf{1}) - 1.405, \quad n = 9,\ r = 0.85 \quad [3]$$

$$\log K'_o\ (\mathbf{4}) = 1.103 \log K'_o\ (\mathbf{1}) - 2.696, \quad n = 9,\ r = 0.78 \quad [4]$$

Log K'_o of **j** and **k** derivatives of series **2, 3** and **4**, not yet synthesized, have been calculated from equations 2, 3 and 4, respectively.

On the other hand, the elution order found for the three standard compounds employed are in agreement with their values of log P in the literature.[13]

ACKNOWLEDGMENTS

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