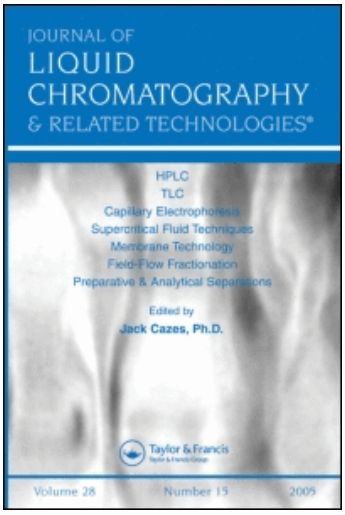

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DETERMINATION OF PARTITION COEFFICIENTS OF 2-AMINO-2-OXAZOLINES BY RP-HPLC: APPLICATION TO HYDROPHOBICITY STUDIES

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ABSTRACT

A comparative study of the hydrophobicity for a series of bioactive 5-aryloxy-methyl-2-amino-2-oxazolines is reported. Octanol-water partition coefficients were measured using the classical shake-flask method. For two lipophilic compounds, high-performance liquid chromatography (HPLC) was used as a detector for the shake-flask method. The capacity factors (log k') were determined by an HPLC method using methanol-water as a mobile phase and a C_{18} column as stationary phase. The influence of the mobile phase composition was examined, allowing the determination of log k'_w values through extrapolation to 100% water from capacity factors data. The relationship between the pH eluent and the capacity factor was studied by working at different pH values.

INTRODUCTION

The 1-octanol/water partition coefficient ($P_{o/w}$) and its related properties continue to provide a major means of studying drug activities.[1,2] For a number of years, the chromatographic methods, especially RP-HPLC, have been proposed to determine the lipophilicities of drugs.[3-6] An interrelationship between the partition coefficient P and the chromatographic column capacity factor k' in RP-HPLC has been established in a Collander-type equation.[7]

We recently developed such a chromatographic approach of the lipophilicity, applied to series of potentially active 2-amino-2-oxazolines.[8] The aim of the present work was to study the lipophilicities of 16 new 5-aryloxymethyl-2-amino-2-oxazolines by mean of a RP-HPLC technique. First, we measured log $P_{o/w}$ using the classical shake-flask method. For two lipophilic compounds, the log $P_{o/w}$ measurement was made using HPLC as a detector. The capacity factors (log k') were determined by a high-performance liquid chromatography method using methanol-water as a mobile phase and a C_{18} column as stationary phase. The reliability of the chromatographic methodology was checked by the correlation of the k'_w data, obtained through extrapolation to 100% water from capacity factors data, with the 1-octanol/water partition coefficient.

The influence of the pH eluent on the capacity factor was studied by working at three different pH values. The observed variations were discussed in relation to the pKa's of the 5-aryloxymethyl-2-amino-2-oxazolines, determined by a potentiometric method.

MATERIALS AND METHODS

Apparatus and Chromatographic Conditions

For the capacity factor determination, chromatography was performed with a Waters Assoc. (Milford, MA, USA) apparatus equipped with a Model 501 pump, a Model 455 ultraviolet detector operating at 254 nm and a U6K manual injector. The compounds were chromatographied on a C_{18} Novapak column, 3.9 mm x 150 mm, 4 μm particle size, (Waters).

The mobile phase composition ranged from 30 to 50 % (v/v) methanol with 0.06 M phosphate buffer at various pH's (5.4; 6.4 ; 7.4). The flow rate was 1.5 mL/min. The detector output was recorded with a Model 746 Data Module integrator.

A specific procedure was developed for the log $P_{o/w}$ determination of the lipophilic compounds **15** and **16**. In this way, HPLC was used as the detector. The same apparatus, equipped with the C_{18} Novapak column was used, but the mobile phase was a mixture of acetonitrile/water with 0.06 M phosphate buffer at pH 7.4. The flow rate was 1.5 mL/min.

Standards and Reagents

New 5-aryloxymethyl-2-amino-2-oxazolines were synthesized by a previously described method.[9] Their structures were supported by elemental analysis, IR, ^{1}H and ^{13}C NMR spectral data. Stock solutions, containing 1mg/mL of each drug, were prepared in methanol and stored at -20°C.

HPLC-grade methanol and acetonitrile (Prolabo) were used without further purification to prepare the mobile phases. Water was deionized and double distilled. To prepare the phosphate buffer solutions, potassium dihydrogen phosphate and dipotassium hydrogen phosphate trihydrate (Merck) were used. The mobile phases were filtered through a 0.45 μm membrane filter.

Measurement of Log k'

The column dead-time of the system (t_o) was measured as the time from injection to the first distortion of the baseline after injection of pure water. Consequently, the stock solutions of tested compounds were diluted with water to the final injected concentrations of 50 μg/mL.

According to their chromatographic behaviour, the retention time (t_r) of each compound was determined at five different methanol-phosphate buffer mixtures ranged from 30 to 50%. The compounds were injected separately from each other three times and the mean value of the retention time was retained.

The log k'_w value for each compound was obtained by regression analysis of log k' data, expressed from the retention times t_r, through the formula: $k' = (t_r - t_o) / t_o$, and extrapolation to 0% methanol content.

The correlation/regression analyses were carried out with a statistical program on a Vectra computer (Hewlett Packard).

Measurement of pKa

Except for compounds **15** and **16**, which are not soluble enough in aqueous solution, the pKa determinations were performed using a classical potentiometric method described elsewhere.[10]

Determination of Log P

For all compounds, the octanol-water partition coefficients were determined by the classical "shake-flask" technique using a conventional methodology. Samples in a weight range of 5-10 mg were partitioned between 5 mL of 1-octanol saturated with water and 50 mL of water saturated with n-octanol. The pH of the water phase was adjusted at 11, ensuring that all compounds were more than 99% un-ionized.

For the two compounds **15** and **16**, $P_{o/w}$ was determined using HPLC as the detector, from the ratio of peak areas in the octanol and buffer phases, respectively.[11] Four independent measurements were performed for each sample.

RESULTS AND DISCUSSION

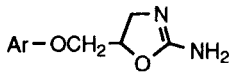
The chemical formulae of the tested 5-aryloxymethyl-2-amino-2-oxazolines are given in Figure 1.

Determination of Log k'_w and S (Slope of the regression analysis)

In this study, we have chosen to measure the log k' value extrapolated to 0% of the organic modifier in the mobile phase, log k'_w (polycratic method). For all compounds, linear relationships ($r > 0.98$) were proven to exist between the log k' values and methanol concentrations, allowing the calculation of log k'_w and S through extrapolation (Table 1). The slopes, S, for the equations were mostly constant, which is related to the structural similarity of the molecules.

Effect of the pH of the Eluent on Log k'_w

The influence of the solute ionization on RP-HPLC determination of capacity factors has often been discussed.[12-14] In general, for the determination



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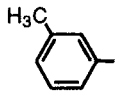
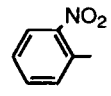
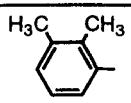
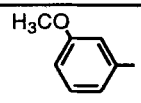
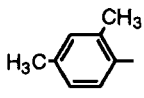
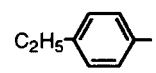
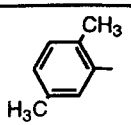
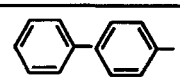
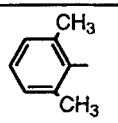
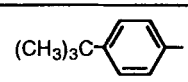
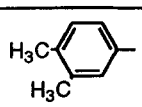
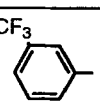
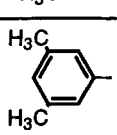
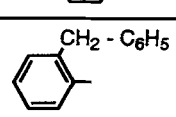
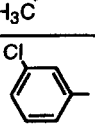
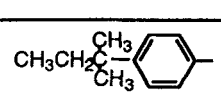
1		9	
2		10	
3		11	
4		12	
5		13	
6		14	
7		15	
8		16	

Figure 1. Structural formulae of 5-aryloxymethyl-2-amino-2-oxazolines.

of hydrophobicity, the unionized form of the solutes is taken as the reference state.[1,4] For basic compounds, it is necessary to work at high pH. As aqueous mobile phases above pH 8 often cause premature column failure, this can be a limiting factor for the HPLC applications.[15]

Table 1

Analytical Data for 5-Aryloxymethyl-2-amino-2-oxazolines

Cpd No.	Log k'_w pH = 5.4	Slope	Log k'_w pH = 6.4	Slope	Log k'_w pH = 7.4	Slope	pK_a	Log $P_{o/w}$
1	1.793	-0.04	1.799	-0.04	1.309	-0.02	8.48	1.75
2	2.202	-0.04	2.264	-0.04	1.710	-0.02	8.81	2.25
3	2.494	-0.05	2.397	-0.04	2.105	-0.03	8.23	2.23
4	2.267	-0.04	2.298	-0.04	1.531	-0.02	8.92	2.29
5	2.115	-0.04	2.015	-0.04	2.136	-0.04	8.44	2
6	1.992	-0.03	2.207	-0.04	1.720	-0.02	8.61	2.1
7	2.342	-0.04	2.664	-0.05	1.818	-0.03	8.80	2.25
8	1.969	-0.04	2.139	-0.02	1.296	-0.02	8.32	2.09
9	0.937	-0.03	1.343	-0.04	0.839	-0.03	8.86	0.8
10	1.427	-0.04	1.449	-0.04	1.176.	-0.02	8.86	1.34
11	2.331	-0.04	2.442	-0.04	2.012	-0.03	8.51	2.27
12	3.014	-0.05	3.234	-0.05	2.781	-0.04	9.14	2.8
13	3.364	-0.05	3.175	-0.05	2.793	-0.03	8.46	2.84
14	2.053	-0.04	2.703	-0.05	1.924	-0.03	8.88	2.35
15	3.723	-0.06	3.415	-0.05	3.174	-0.04	---	3.05*
16	4.346	-0.06	3.924	-0.06	3.562	-0.04	---	3.69*

* HPLC determination.

In order to study the variation of log k'_w in terms of ionization, the pKa determinations of compounds **1-14** have been performed (Table 1). As the 5-aryloxymethyl-2-amino-2-oxazolines are basic molecules (pKa = 8.67 ± 0.27, variation coefficient 3.1%), the ionization percentage is near 100% at pH 5.4, 99% at pH 6.4 and 89% at pH 7.4. So, at pH 7.4, besides the cation form, the neutral (uncharged) species are present.

Table 1 shows the variations of the log k'_w values with the pH of the eluent. For most compounds, the capacity factors are almost comparable at pH 5.4 and 6.4, which is related to similar ionization effects. But, the log k'_w values are very often minimum at pH 7.4. This last result seems paradoxical, because it takes no account of the contribution of the less polar species in developping the final retention of the solute.[13]

On the other hand, as a function of the mobile-phase apparent pH, the properties and the capacities of the modified stationary phase can, therefore, vary.[14]

Correlation Between Lipophilic Indices

For all compounds, the log k'_w values correlated with log $P_{o/w}$ according to the equations **1, 2, and 3** for the experimental data listed in Table I (where *n* is the number of data, *r* is the correlation coefficient, *s* is the standard error of the estimate, *F* is a measure of the significance of the correlation, and *p* is the probability level).

$$pH = 5.4 \log k'_w = 1.235\ (\pm 0.09) \log P - 0.389\ (\pm 0.211) \quad (1)$$
$$(n = 16,\ r = 0.965,\ s = 0.232,\ F = 189.021,\ p < 0.0001)$$

$$pH = 6.4 \log k'_w = 1.021\ (\pm 0.07) \log P + 0.163\ (\pm 0.164) \quad (2)$$
$$(n = 16,\ r = 0.969,\ s = 0.181,\ F = 214.617,\ p < 0.0001)$$

$$pH = 7.4 \log k'_w = 1.044\ (\pm 0.116) \log P - 0.362\ (\pm 0.271) \quad (3)$$
$$(n = 16,\ r = 0.924,\ s = 0.298,\ F = 81.602,\ p < 0.0001)$$

If the log $P_{o/w}$ values of compounds **15** and **16** are omitted from the analysis, then the correlation coefficient *r* always decreases (0.947, 0.945 and 0.863 at pH 5.4, 6.4 and 7.4 respectively).

As judged from the regression analysis, a significant correlation was established among the studied hydrophobic parameters, especially at pH 5.4 and 6.4, when the ionization percentage of all compounds is almost comparable.

This confirms the choice of log k'_w as a lipophilic index. However, at pH 7.4, near the pKa values, the correlation decreases slightly. Based on the knowledge of the pKa, the apparent partition coefficient *D* has been calculated at pH 7.4 from the log $P_{o/w}$ value.[16] Nevertheless, the substitution of log D instead of log $P_{o/w}$ did not improve the correlation.

Finally, we would like to develop the determination of log $P_{o/w}$ using HPLC as a detector, for lipophilic compounds. It is hoped that this technique will find widespread application due to its simplicity and reliability.

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