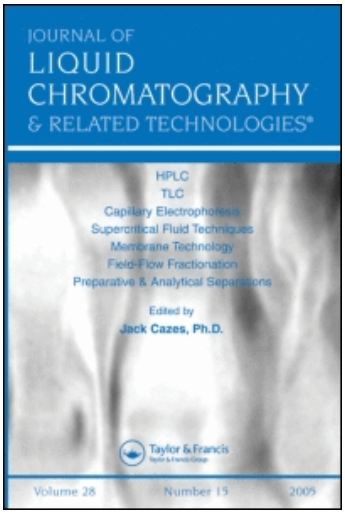

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RP-HPLC Retention Data of New 2-Amino-2-Oxazolines. An Approach of Their Lipophilic Properties

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RP-HPLC RETENTION DATA OF NEW 2-AMINO-2-OXAZOLINES. AN APPROACH OF THEIR LIPOPHILIC PROPERTIES

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ABSTRACT

A RP-HPLC procedure has been developed for measuring the capacity factor k' of a series of new 5-substituted-2-amino-2-oxazolines. The chromatographic behaviour measured on a μBondapack C_{18} column with methanol-aqueous buffer as mobile phase was related to the volume fraction of methanol Φ. The log k' value extrapolated to 0% organic modifier in the eluent (log k'_w) was chosen as a measure of the solute lipophilicity. A good correlation was found between the slope S and the intercept value (log k'_w) of the log k' *versus* Φ straight lines for all these structurally related 2-amino-2-oxazolines.

Some parameters related either to chromatographic conditions or to chemical structure were shown to influence the capacity factor. A study made at different pH values indicated that log k'_w increased with the basicity of the mobile phase.

The influence of the eluent pH on the capacity factor of 2-amino-2-oxazolines was related to their pKa's values determined by a potentiometric method.

Since log k' is considered as a valuable indice of the lipophilicity, the determined values will be used for quantitative structure-activity relationship studies of these new class of structurally related compounds

INTRODUCTION

In search of bioactive compounds we prepared and tested a series of 2-amino-2-oxazolines bearing different amino substituents (1, 2). The preliminary pharmacokinetic results of one compound developed for its antidepressant activity were recently published (3). As part of our program of work related to quantitative structure-activity relationship studies (QSAR), the purpose of the present paper was a contribution to the lipophilicity study of an homogenous series of 5-dialkylaminomethyl-2-amino-2-oxazolines by means of a reversed-phase HPLC technique.

One of the most important parameter in quantitative structure-activity relationships (QSAR) seems to be the partition coefficient between water and an organic phase, log P (4). The partition coefficient measured in the n-octanol/water system by a shake-flask method (log $P_{o/w}$) is widely used because of the ability of n-octanol to simulate biological membranes. This technique has to be considered as the reference one but it is time consuming and requires relatively large amounts of pure stable compounds (5). This has led to search new methods for determining the lipophilicity, especially chromatographic ones (6,7,8,9)

For a number of years, RP-HPLC has been proposed as a method for the measurement of lipophilicity. An interrelationship between the partition coefficient (P) and the chromatographic column capacity factor (k') in RP-HPLC has been established (10,11,12,13) in a Collander-type equation : log P= a log k'+ b. The capacity factor k' is measured for each compound at different volume fractions of organic modifier in the mobile phase (Φ). Generally, methanol is considered as the most suitable organic solvant (10). For analytical studies, some authors used only the capacity factors determined at a given Φ value as lipophilic indices (14,15). Some others (10,12,16,17) proposed to extrapolate series of log k' values (measured at various volume fractions of the organic modifier) to log k' values which correspond to 0% organic modifier. These extrapolated retention data (log k'_w) can serve as excellent measures of solute lipophilicity, since log k'_w is more closely related to log $P_{o/w}$ than isocratic factors.

The aim of our study was to describe the retention behaviour of seventeen 5-dialkylaminomethyl-2-amino-2-oxazolines on a C_{18} reversed-phase column as a function of the volume fraction of methanol in the mobile phase.

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

MATERIALS AND METHODS

Apparatus and Chromatographic Conditions.

A Waters Assoc. liquid chromatograph was used. The instrument was equipped with a Model M45 pump, a Lambda-Max Model 480 ultraviolet detector operating at 208nm and an U_{6K} manuel injector. The retention times of the chemical compounds were recorded with a Model 730 Data Module. The column was a 300 x 3.9 mm I.D. stainless-steel column packed with μBondapack C18 of particule size 10 μm. The flow rate was 1.5 ml/mn. The mobile phase composition ranged from 20 to 80% (v/v) methanol with 0.06M phosphate buffer at various pH (6.6 ; 7.4 ; 7.8 ; 9). At pH 9, a pre-column (20mm x 4mm I.D.) filled with a reversed-phase material (C_{18} Corasil, 35-50 μm ; Waters) was placed just before the injector in order to protect the analytical column.

Standards and Reagents

The structures of all tested 2-amino-2-oxazolines were established by IR and NMR spectral data. Their purity was checked by elemental C H N analysis. Stock solutions containing 1mg/ml of each drug were prepared in methanol and stored at -20°C.

HPLC-grade methanol (Prolabo) was used to prepare the mobile phase. Water was glass-distilled deionized. To prepare the phosphate buffer solutions of different pH, potassium dihydrogenphosphate and dipotassium hydrogenphosphate trihydrate of proanalysis quality (Merck, Darmstadt, F.R.G.) were used. The buffer solution and the organic solvant were filtered through a 0.45 μm membrane filter before they were used for HPLC.

Measurement of logk'

The dead volume of the system was measured as the first distortion of the baseline after injection of pure water. The stock solutions of tested compounds were diluted with water to the final injected concentrations (50 µg/ml). A 15-µl injection was made in triplicate. According to their chromatographic behaviour, the retention times of the solutes were determined at six different methanol-phosphate buffer mixtures ranged from 20 to 80%. At each mobile phase composition, the measurement consisted in determining the capacity factor k' calculated according to $k' = (t_r - t_o) / t_o$, where t_r and t_o were the retention times of the analyte and of the non-retained compound, respectively. The log k'_w values (log k' at 100% aqueous mobile phase) were obtained from the y-intercepts of plots log k' *versus* percent of methanol in the mobile phase.
Correlation studies were performed using a statistical program on a Vectra computer (Hewlett Packard).

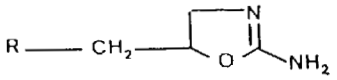
Measurement of pKa

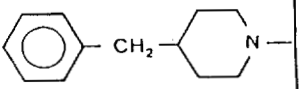
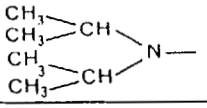
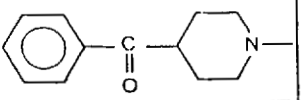
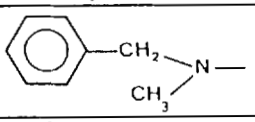
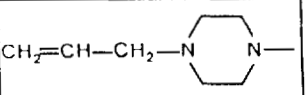
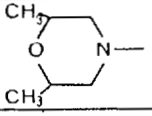
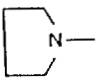
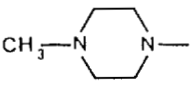
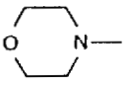
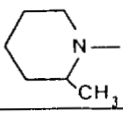
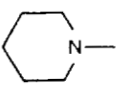
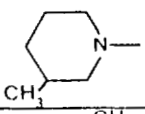
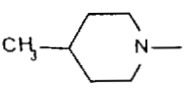
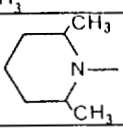
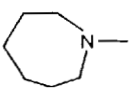
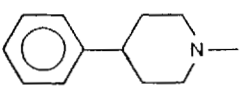
The pKa's determinations were performed using a potentiometric method.
For water-soluble compounds, 2.5×10^{-4} mole of drug was dissolved in 100ml of freshly twice-distilled water and titrated by a 0.05N hydrochloric acid solution.
For non water-soluble compounds, 2.5×10^{-4} mole was dissolved in 100ml of 0.01 N hydrochloric acid. For the titration by a 0.05 N sodium hydroxyde solution, 80ml of freshly twice-distilled water were added to 20 ml of the acidic solution.
In both cases, the ionic strength of the solution was kept 0.15M by adding sodium chloride.
For each volume of reagent added, the pH was measured and a pKa's value was obtained and a mean-value of pKa was calculated from a series of almost constant values.

RESULTS AND DISCUSSION

The chemical formulae of all tested compounds are given in Table 1.

Table 1. Structural Formulae of Compounds



n°	Structural formula of R	n°	Structural formula of R
1		10	
2		11	
3		12	
4		13	
5		14	
6		15	
7		16	
8		17	
9			

Effect of Volume Fraction of Organic Modifier

All compounds were chromatographied under a wide variety of conditions in which the volume fraction of methanol in the mobile phase Φ varied from 0.2 to 0.8. The retention times of investigated solutes decreased with increasing methanol content in the mobile phase.

According to its chromatographic behaviour, the capacity ratio of each compound was measured at six different eluent compositions.

Over the whole volume fraction range, the relationship between the solute retention and the composition of the mobile phase was described by the equation :

$\log k' = \log k'_W + A\Phi^2 - S\Phi$ (9,11). A regression analysis of the observed linear portion of the curve (Φ in the range 0.2-0.8) allowed to obtain the values of the slope (S) and of the intercept ($\log k'_W$).

Determination of log k'w and S

Retention parameters obtained from HPLC (log k') measurements could be calculated in two different ways.

The first approach was to use isocratic data measured at a certain eluent composition X ($\log k'_X$) (14,15). This monocratic method has been criticized (12) considering that the tested compounds did not eluted in the same way under the selected chromatographic conditions. Some solutes were unretained or retained too long at the chosen volume fraction of methanol. This approach often led to the impossibility to directly compare log k' results and to a poor correlation between log k' and log $P_{o/w}$.

To overcome these problems, an alternative method has been developed (polycratic method). This approach consists in measuring the chromatographic data extrapolated to 0% of the organic modifier in the mobile phase. According to some authors (10,12), the latter appeared to be more reliable and allowed more adequate evaluation of the hydrophobic nature of the solute. The principal advantage of this technique was to provide a scale of lipophilic parameters normalized to one set of conditions ($\Phi = 0$) more closely related to $\log P_{o/w}$.

This polycratic method was retained in this paper.

Plots of the volume fraction of methanol in the mobile phase *versus* log k' were linear for each compound (correlation coefficient >0.98) (Table II).

The plot of the slope S and the intercept value (log k'_w) were investigated at the four pH levels. A good linear correlation was observed at each pH : at pH 7.4, r = 0.9555. This good correlation may be a consequence of the structural similarity of all studied 2-amino-2-oxazolines in regard to their partition behaviour in RP-HPLC (19).

Effect of the pH Eluent on log k'w

Table 2 shows the variations of log k'_w values with the pH of the buffer solution ($pH_{mob.}$). The retention times increased with increasing the pH. In order to explain the variations of log k'_w in terms of ionization, the pKa's determinations of 2-amino-2-oxazolines have been performed.

These basic molecules show two pKa values (Table 3). The first value pKa_1 is near to 9 (range 8.81 - 9.75) and the second value pKa_2 is below 7.5 (range 4.66 - 7.5). At pH 6.6, the basic 2-amino-2-oxazolines were more ionized than at pH 9 ; as a consequence they showed more hydrophilic properties and the capacity factor k' was minimum.

By comparison of Tables 2 and 3, it was possible to class these 17 compounds in two groups. For the first one (compounds 1,3,4,9,11,14), the retention was not greatly affected by change of the $pH_{mob.}$ in the range 6.6 - 7.4. This fact was explained by considering their pKa_2 values. The other compounds with a pKa_2 value superior to 6.6 showed an increase of log k' factor when the $pH_{mob.}$ varied from 6.6 to 7.4.

Except for three compounds (5,13,14), we have observed a maximum value of log k'_w at pH 9. The results obtained for compounds 5,13,14 could not be explained only by a protonation modification occurring on the basic 2-amino-2-oxazolines and as a consequence by hydrophobic effects. As it was already described (15), a change in the degree of protonation of the residual silanol groups of the chromatographic column may be taken into account. By increasing the pH of the buffer solution, more residual silanol groups were

TABLE 2. The Slope (S) and The Intercept Values (log k'_w)*

	pH 6.6		pH 7.4		pH 7.8		pH 9	
n°	log k'w	S	log k'w	S	log k'w	S	log k'w	S
1	0.939	-0.016	0.829	-0.012	1.308	-0.018	1.819	-0.031
2	1.122	-0.011	1.529	-0.020	1.746	-0.020	2.348	-0.032
3	1.442	-0.02	1.721	-0.024	1.607	-0.022	3.502	-.0026
4	0.564	-0.015	0.330	-0.007	0.674	-0.012	1.266	-0.025
5	0.009	-0.020	0.840	-0.016	1.699	-0.026	1.487	-0.021
6	0.871	-0.012	1.486	-0.019	1.721	-0.022	2.335	-0.034
7	0.802	-0.010	1.288	-0.015	1.645	-0.021	2.091	-0.030
8	0.799	-0.012	1.138	-0.014	1.721	-0.023	2.278	-0.034
9	2.484	-0.032	2.334	-0.028	2.444	-0.033	3.302	-0.046
10	2.826	-0.031	3.166	-0.038	2.912	-0.035	4.839	-0.068
11	1.887	-0.026	1.834	-0.025	1.696	-0.025	2.398	-0.037
12	1.122	-0.013	1.121	-0.013	1.367	-0.018	2.108	-0.034
13	0.794	-0.008	1.266	-0.018	1.866	-0.021	1.764	-0.025
14	0.402	-0.012	0.432	-0.014	0.658	-0.016	0.456	-0.014
15	0.740	-0.005	1.306	-0.016	2.182	-0.028	2.657	-0.042
16	1.179	-0.015	1.721	-0.021	1.928	-0.025	2.131	-0.030
17	0.786	-0.011	1.650	-0.020	2.320	-0.028	2.495	-0.035

* In all cases, the calculated correlation coefficient was found superior to 0.98

TABLE 3. pKa Values of the Compounds

Compound n°	pKa_1	pKa_2
1	9.12	6.40
2	9.75	7.40
3	9.05	6.11
4	8.95	4.83
5	9.04	7.50
6	9.40	7.29
7	9.23	7.13
8	9.48	7.28
9	9.00	6.13
10	8.93	7.02
11	8.81	6.13
13	9.38	7.30
14	8.87	4.66
15	9.27	7.18
16	9.22	7.13
17	9.48	7.21

negatively charged and, in parallel, the protonation of compounds 5,13 and 14 was suppressed. These two effects led to a decrease in retention strength. At high pH, the silanol groups would play the role of a weak cation exchanger. Finally, these results showed that retention phenomena were also affected by the interaction with residual groups of the packing material. In order to minimize these internal interactions we chose a double bonded μBondapack column for this study.

CONCLUSION

In order to approach their lipophilic properties, a series of potentially pharmacological 5-dialkylaminomethyl-2-amino-2-oxazolines was studied by RP-HPLC. The capacity factors (log k') were measured at different compositions of the eluent. The value corresponding to 0% of methanol ($logk'_w$) was obtained by extrapolation of the linear portion of the curve log k'

versus volume fraction of methanol. This parameter was retained as a valuable indice of lipophilicity and was used in further QSAR studies.

The pH of the mobile phase greatly affected the chromatographic behaviour of the studied compounds. These variations have been related to the basicity of 2-amino-2-oxazolines illustrated by their pKa's values determined by a potentiometric method.

However other molecular effects such as steric ones should be taken into account. Thus, somewhat different capacity factors have been measured for the 3 isomeric 5-[(methylpiperidino)methyl]-2-amino-2-oxazolines.

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