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THE LIPOPHILICITY DETERMINATION FOR SOME AMIDOESTERS OF ETHANOLAMINE WITH POTENTIAL BIOLOGICAL ACTIVITY BY REVERSED PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT. The lipophilicity of 13 derivatives, amidoesters of ethanolamine, with potential biologic activity, was studied by reversed phase liquid chromatography, on a LiChrosorb RP-18 column by using methanol-water and methanol-phosphate buffer (pH 7.0) as mobile phase. The extrapolated $\log k_W$ values, to 100% water as eluent was correlated with the calculated LogP values obtained from Pomona College database or by using Rekker's revised fragmental constant system. High correlation coefficients were obtained for the dependence $\log k = f(\varphi)$, where φ is the methanol concentration in the mobile phase, and for the relationships $\log k_W = f(\mathrm{CLogP})$ or $\log k_W = f(\mathrm{LogP}_{Rekker})$.

INTRODUCTION

The partition coefficient (log P) of a substance in a two-phase system consisting of n-octanol/water is often used to predict or to correlate its biological activity. Fujita et al. has proposed the n-octanol-water partition coefficient ($P_{O/W}$) as a standard measure of hydrophobicity of substances [1]. The determination of log P values of substances with potential biological activity by reversed-phase liquid chromatography (RPLC) can, in principal, overcome the difficulties of the conventional "shake-flask" method. The direct measurement of $P_{O/W}$ values by the latter method faces different problems as the necessary high purity of substances, which must be available in reasonable quantities and the fact that the method is not applicable to very hydrophilic or very hydrophobic compounds. In addition, this method is rather time consuming too. Since the first research of Meyer [2] and Overton [3], several techniques have been developed to determine substance

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lipophylicity experimentally, by the "shake-flask" method and by alternative approaches like e.g. chromatographic methods [4, 5]. In a recent review these techniques were thoroughly reviewed [6].

For some time RPLC is proposed as an alternative method for log $P_{o/w}$ determination, showing distinct advantages as speed of determination and better reproducibility compared to conventional methods. Furthermore, only small amounts of even contaminated samples are sufficient to use RPLC for this purpose [7-12]. Many researchers have discussed the use of RPLC as an attractive alternative method for the determination of log $P_{o/w}$ values using correlations between chromatographic data and the corresponding log $P_{o/w}$ values from other sources, e.g. shake-flask experiments or calculations. At present a substantial number of papers can be found in literature reporting the use of RPLC to establish octanol-water partition coefficients, with correlation coefficients between 0.5-0.999, depending on the applied columns and compounds under investigation [13-19]. Still there is debate on and to what extend the various parameters in RPLC, like e.g. nature of the reversed phase (RP) stationary phase and composition of the eluent, are of influence on log $P_{o/w}$ determination by RPLC.

13 amidoesters of ethanolamine were studied by RPLC. Logk_w measurements were performed on LiChrosorb RP-18 column. LogP data were obtained from Pomona College and by using Rekker's revised fragmental constant system too [20-22]. Logk_w values were obtained from the RPLC measurements by extrapolation to 100% water or buffer. The correlations between the experimentally obtained logk_w values, and the log P data are discussed.

EXPERIMENTAL

The structures of the studied amidoesters of ethanolamine are shown in Figure 1. These are new compounds synthesized at Faculty of Chemistry and Chemical Engineering, and have plant growth stimulating activity, according to the Moewus test on *Lepidium Sativum* [23, 24]. The samples were prepared as methanolic solutions, 0.1 mg/ml.

All solvents were of analytical purity. Methanol was purchased from Merck (Darmstadt, Germany). Deionized water was obtained by a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). Sodium phosphate ($NaH_2PO_4 \cdot H_2O$) was obtained from Vel (Leuven, Belgium) and was used for buffer solutions (pH 7.0) adjusted with NaOH 0.01M. Tricine (N-tris[hydroxymethyl]methylglycine) from Sigma (St. Louis, MO, SUA) was used as organic buffer.

Instrumentation

The instrumentation included a Beckman pump Model 100A (Beckman, Fullerton, CA, USA), a Merck-Hitachi AS-2000A autosampler (Merck, Darmstadt, Germany), an UV-VIS Philips detector (ATI Unicam, Cambridge, UK) and a PE Nelson 900 Interface (Perkin Elmer, San Jose, CA, USA). The detection wavelength was 254 nm, the flow rate 1mL/min and the amount of sample solution was 10 µl/injection. The logk values were measured on a LiChrosorb® RP-18 column (5µm, L=119 mm, i.d. = 4 mm) (Merck, Darmstadt, Germany). The dead time, t_0, was measured for each experimental condition, using uracil as unretained compound.

Determination of logk$_W$ values

The retention factors were determined by RP-HPLC, using the following binary solvent systems as mobile phases: methanol-water, methanol-phosphate buffer (20 mM, pH 7.0) and methanol – Tricine buffer (20 mM, pH 7.0). The measurements were performed starting with 50% (v/v) methanol, gradually decreasing with increments of 10% to 30-40% organic modifier in the mobile phase. The compounds have a high degree of hydrophobicity, so that 3, maximum 4 measurements could be performed. In order to increase the reliability, every measurement was performed in triplicate and the mean value was used for calculations. The $\log k_W$ values were obtained by extrapolation to 100% water as mobile phase, using the linear relationship logk = f(φ), where φ is the methanol concentration in the mobile phase.

RESULTS AND DISCUSSION

The calculated LogP values, CLogP, obtained from Pomona College database (Claremont, CA, USA), and $\text{LogP}_{\text{Rekker}}$, calculated by using Rekker's revised fragmental constant system [20, 21] are shown in Table 1.

A linear relationship is established between the calculated LogP values by using the two methods (equation 1).

$$\text{LogP}_{\text{Rekker}} = 0.083\ (\pm 0.624) + 1.078\ (\pm 0.246)\ \text{CLogP} \qquad (1)$$
$$s_{a0} = 0.083,\ s_{a1} = 0.078,\ s = 0.183,\ F = 93,\ r = 0.946,\ n = 13$$

where s_{a0}, sa1 are the standard errors for the intercept and the slope, s is the fit standard error, F is the statistic parameter for the F test, n is the number of compounds, and r is the correlation coefficient for 95% confidence limits.

The high values of correlation coefficients show that both values can be used in lipophilicity calculations. The $\text{LogP}_{\text{Rekker}}$ values have been calculated by the addition of the corresponding fragmental constants and a number of corrections, C_M=0.219, the so-called "magic constant", which were applied following Rekker's rules: 4 C_M for polar groups separated by an aliphatic carbon, 2 or 3 C_M for polar groups separated by two aliphatic carbons, 1 C_M for ortho substituents, and 1 C_M for extra chain-branching. The substitution of the pyridil fragment in para or meta position was not taken into account when calculation CLogP or $\text{LogP}_{\text{Rekker}}$ values, so that the pairs A1 – A9, A3 – A10, A4 – A11, A6 – A13 and A8 – A14 have the same LogP values.

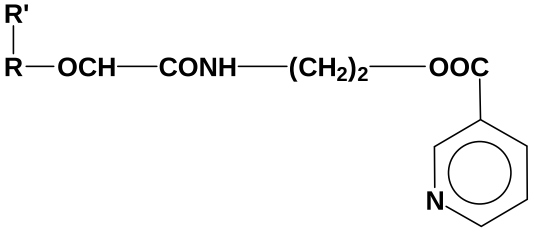
Table 1. The calculated LogP values by using Rekker's revised fragmental constant system ($\text{LogP}_{\text{Rekker}}$), or obtained from Pomona College database (CLogP) for compounds A1-A13.

Compound	CLogP	$\text{logP}_{\text{Rekker}}$
A1	1.746	1.879
A2	2.920	3.167
A3	3.194	3.555
A4	2.599	2.607
A5	2.055	2.399
A6	2.584	3.137
A7	2.744	2.198
A8	2.245	2.617
A9	1.746	1.879
A10	3.194	3.555
A11	2.599	2.607
A12	2.584	3.137
A13	2.245	2.617

The degree of dissociation of residual silanol groups in the non-popular stationary phase, or the degree of (de)protonation of polar or ionic compounds cannot be controlled very well when using non-buffered eluents. The chromatographic behaviour of the studied amidoesters of ethanolamine was checked in buffered and in non-buffered eluents.

The linear correlations 2 for methanolwater and methanol-phosphate buffer, as well as the statistical data for 95% confidence limits are shown in Tables 2 and 3.

$$\log k = a_0 + a_1\varphi \qquad (2)$$



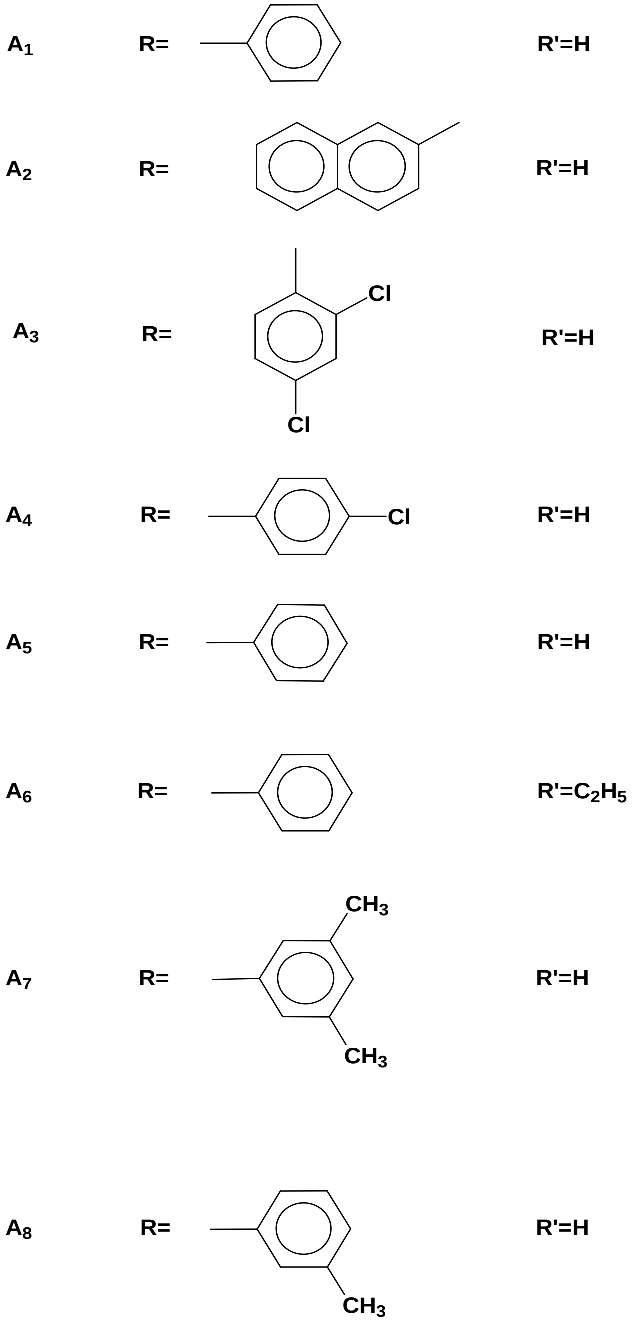


Figure 1. The structures of the studied amidoesters of ethanolamine

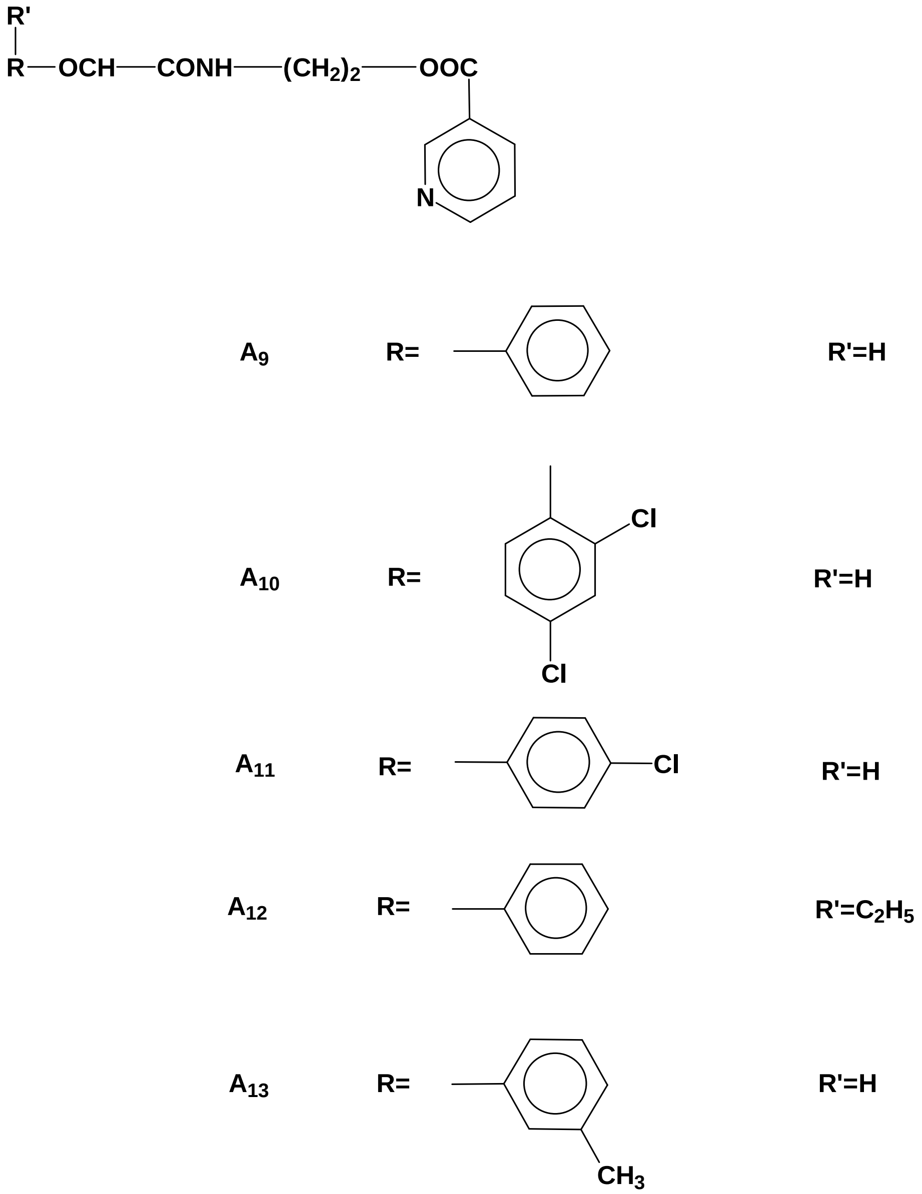


Figure 1. The structures of the studied amidoesters of ethanolamine

In order to check if the nature of buffering substance, inorganic or organic, influences the partition equilibrium of compounds between the stationary and the mobile phases, several experiments were performed with Tricine buffer (N-tris[hydroxymethyl]methylglycine], pH 7.0. The results (not shown) were in the experimental error limits, so that the organic buffer does not influence the chromatographic behaviour of the studied compounds. This observation can be very important for compounds, which need high values of pH in order to be maintained in the neutral form. The RPLC experiments at pH>7.0 can be performed on silica-based stationary phases, only if the eluent is buffered with an organic substance.

Table 2. The $\log k_w$ values obtained on LiChrosorb®RP-18 with methanol-water as mobile phase.

$a_0 = \log k_w$ = the intercept of equation 2, a_1 = the slope (eq. 2)
s_{a0}, s_{a1} = standard errors for the intercept and the slope; s = fit standard error, F = the statistic parameter for F test, r = correlation coefficient, n = 13 (number of compounds); confidence limits 95%.

Compound	% (v/v) Methanol	a_0 ($\log k_w$)	a_1	s_{a0}	s_{a1}	s	F	r
A1	60-30	2.882 ±0.495	-0.047 ±0.011	0.115	0.002	0.055	360	0.997
A2	60-40	3.662 ±1.932	-0.053 ±0.038	0.152	0.003	0.042	314	0.998
A3	60-40	3.989 ±1.691	-0.056 ±0.033	0.133	0.003	0.037	452	0.999
A4	60-30	3.519 ±0.417	-0.053 ±0.009	0.097	0.002	0.047	653	0.998
A5	60-30	3.051 ±0.480	-0.049 ±0.010	0.112	0.002	0.054	416	0.998
A6	60-30	3.550 ±0.459	-0.054 ±0.010	0.107	0.002	0.051	558	0.998
A7	60-40	3.615 ±1.914	-0.052 ±0.038	0.151	0.003	0.042	306	0.998
A8	60-30	3.564 ±0.509	-0.054 ±0.011	0.118	0.002	0.057	454	0.998
A9	60-30	2.965 ±0.484	-0.048 ±0.010	0.112	0.002	0.054	399	0.997
A10	60-40	3.988 ±1.486	-0.056 ±0.029	0.117	0.002	0.033	580	0.999
A11	60-30	3.590 ±0.462	-0.055 ±0.010	0.107	0.002	0.052	555	0.998
A12	60-40	3.574 ±0.465	-0.054 ±0.010	0.108	0.002	0.052	546	0.998
A13	60-40	3.557 ±0.479	-0.054 ±0.010	0.111	0.002	0.054	507	0.998

The correlation coefficients in Tables 2 and 3 are very good. Usually, the logk values for the compounds, which have the heteroatomic nitrogen in "para" regarding the substituent, are a little higher than the corresponding values for "meta" position. Almost all logk data obtained in the buffered system are higher than the logk values obtained in methanol-water. This fact can be attributed to the neutral form of the molecule of studied compounds, which leads to a stronger retention. The small differences between the logk values obtained in buffered and non-buffered eluents shows the almost neutral character of the studied compounds.

The linear correlations between the extrapolated $\log k_w$ values (to 100% water as eluent) and the calculated LogP values (CLogP and $\mathrm{LogP}_{\mathrm{Rekker}}$) are shown in equations 3-6.
methanol-water:

$$\log k_w = 1.804(\pm 0.415) + 0.680(\pm 0.327)\ \mathrm{CLogP} \qquad (3)$$
$$s_{a0} = 0.189,\ s_{a1} = 0.074,\ s = 0.122,\ F = 84,\ r = 0.940,\ n = 13$$

$$\log k_w = 1.877(\pm 0.459) + 0.585(\pm 0.162)\mathrm{LogP}_{\mathrm{Rekker}} \qquad (4)$$
$$s_{a0} = 0.208,\ s_{a1} = 0.074,\ s = 0.138,\ F = 63,\ r = 0.922,\ n = 13$$

The statistical parameters in equations 3 - 6 have the same significance as in the legend of Table 2.

Table 3. The $\log k_w$ values obtained on LiChrosorb® RP-18 with methanol-phosphate buffer (20 mM, pH 7.0) as mobile phase.
$a_0 = \log k_w$ = the intercept of equation 2, a_1 = the slope (eq. 2)
s_{a0}, s_{a1} = standard errors for the intercept and the slope; s = fit standard error, F = the statistic parameter for F test, r = correlation coefficient, n = 13 (number of compounds); confidence limits 95%.

Compound	% (v/v) Methanol	a_0 ($\log k_w$)	a_1	s_{a0}	s_{a1}	s	F	r
A1	60-30	2.804 ±0.397	-0.045 ±0.008	0.092	0.002	0.044	514	0.998
A2	60-40	3.591 ±1.654	-0.051 ±0.033	0.130	0.002	0.036	398	0.999
A3	60-40	3.931 ±1.375	-0.054 ±0.027	0.108	0.002	0.030	639	0.999
A4	60-30	3.364 ±0.331	-0.050 ±0.007	0.077	0.002	0.037	895	0.999
A5	60-30	2.919 ±0.331	0.046 ±0.007	0.077	0.002	0.037	769	0.999
A6	60-30	3.431 ±0.338	-0.051 ±0.007	0.079	0.002	0.038	922	0.999
A7	60-40	3.588 ±0.855	-0.051 ±0.017	0.067	0.001	0.019	1458	0.999
A8	60-30	3.461 ±0.367	-0.052 ±0.008	0.085	0.002	0.041	791	0.999

A9	60-30	2.868 ±0.352	-0.046 ±0.007	0.082	0.002	0.039	682	0.998
A10	60-40	3.926 ±1.654	-0.054 ±0.033	0.130	0.003	0.036	437	0.999
A11	60-30	3.401 ±1.282	-0.050 ±0.025	0.101	0.002	0.028	639	0.999
A12	60-40	3.513 ±0.313	-0.053 ±0.007	0.073	0.002	0.035	1130	0.999
A13	60-40	3.507 ±0.310	-0.052 ±0.007	0.072	0.001	0.035	1138	0.999

methanol-phosphate buffer (pH 7.0):

$$\log k_w = 1.659(\pm 0.464) + 0.701(\pm 0.183)\mathrm{CLogP} \quad (5)$$
$$s_{a0} = 0.211,\ s_{a1} = 0.083,\ s = 0.136,\ F = 71,\ r - 0.931,\ n = 13$$

$$\log k_w = 1.707(\pm 0.461) + 0.613(\pm 0.163)\mathrm{LogP}_{Rekker} \quad (6)$$
$$s_{a0} = 0.209,\ s_{a1} = 0.074,\ s = 0.139,\ F = 68,\ r = 0.928,\ n = 13$$

The differences between the linear correlations 3-6 for buffered and non-buffered eluent are smaller when LogP_{Rekker} was included in the calculations, compared with CLogP. The data in Tables 2 and 3 shows that there are no significant differences between the retention parameters, logk, obtained in buffered or non-buffered mobile phases.

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