Study of Local Anaesthetics, Part 156: Some Physicochemical and Lipophilic Properties of Pyrrolidinoethyl Esters of *o*-, *m*-, *p*-Alkoxy-Substituted Phenylcarbamic Acid

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In the series of pyrrolidinoethyl esters of *o*-, *m*-, *p*-alkoxy-substituted phenylcarbamic acid the values of lipophilic parameters (R_M, log P_{app}, log k) increase with lengthening of alkoxy chain on benzene ring, each methylene group increases the lipophilicity. Comparing advantages and disadvantages of different methods for a drug's lipophilicity determination, as well as experiences from the laboratory, the easiest and best method seems to be HPLC. According to the results, the highest local anaesthetic activity can be assumed by the compounds with 6—8 carbon atoms in the side chain.

Lipophilicity of a substance is one of the parameters which influences its biological activity and is well-known as a prime physicochemical descriptor of xenobiotics with relevance to their biological properties. The hydrophobic interactions of drugs with their receptors, the pharmacokinetic behaviour of drug molecules, and toxicological properties as well as pharmaceutical aspects like solubility are examples of a steadily increasing number of topics in which lipophilicity plays an important role [1].

The lipophilicity of a compound is often considered as an important design factor since it is related to processes such as absorption, brain uptake, and protein binding. Lipophilicity is usually measured by the partition coefficient of the organic compound between a nonpolar phase and water [2]. The octanol—water system is often taken as a reference or standard for the partition coefficients. It is the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase system at equilibrium. Since measured values range from 10^{-4} to 10^{8} (at least three orders of magnitude), the logarithm (log P) is commonly used to characterize its value. Many studies have shown that log P is useful for correlating a drug's transport processes and its interaction with receptors [3].

The determination of the partition coefficient by direct measurement using the shake-flask equilibriation method faces problems such as poor reproducibility, length of time for experiment and it needs a reasonable quantity of compound. These difficulties can be overcome by using reversed phase chromatography. The liquid chromatographic method has the advantage of speed of determination, better reproducibility and the purity of the sample is not a necessary condition. The retention parameter can be log k for HPLC or R_M ($R_M = \log[(1/R_F) - 1]$ for RP-TLC). The retention factor k is given as $k = (t_r - t_0)/t_0$, where t_r is the retention time of a sample and t_0 is column dead time. The retardation factor R_F is given as $R_F = b/a$, where b is the distance travelled by the centre of the spot and a is the distance simultaneously travelled by the mobile phase [2].

Dissociation constant pK_a together with pH has an influence on drug absorption from the gastrointestinal tract. Studies of this influence resulted in the development of pH partition hypothesis. According to this hypothesis, ionizable compounds diffuse through biological membranes primarily in the unionized forms. Therefore, the extent of absorption of compounds across lipid membranes depends on the degree of ionization. The pH and pK_a of compounds control the fraction of the unionized species. Thus, for acid, pH = pK_a + log (ionized c/unionized c) and for bases pH = pK_a + log (unionized c/ionized c).

The unionized form is assumed to be sufficiently lipophilic to traverse membranes [4]. Compounds used in this study are the series of 30 newly synthesized basic esters of phenylcarbamic acid. The chemical structure of basic esters of phenylcarbamic acid can be modified in all parts of molecule: in the lipophilic part, in the hydrophilic part, and in the connective chain. Modifications on base structure influence physicochemical and biological properties of the compound. The homologous series of pyrrolidinoethyl esters of *o*-, *m*-, *p*-alkoxy-substituted phenylcarbamic

acid studied in this work represent a group of local anaesthetics.

For the compounds that have common parent structure and differ only in the alkyl chain length, a linear change in physicochemical properties is expected. The purpose of the present study was to correlate some physicochemical parameters ($\log P_{app}$, $\log k$, $R_{M,}$ pK_a) of homologous series with the alkyl chain, as well as investigating the lipophilic behaviour of these compounds in order to predict their biological activity. This paper will also point out advantages and disadvantages of different methods for the determination of a drug's lipophilicity.

In many studies very low activity of *p*-substituted phenylcarbamic acid was determined. Biological activities of these compounds were determined and our assumption was confirmed. It can be said that knowing the physicochemical parameters helps predicting the biological activity.

EXPERIMENTAL

The derivatives of phenylcarbamic acid were prepared according to the literature [5].

Apparent Partition Coefficient Determination

The octanol-phosphate buffer (pH = 7.3) partition coefficients of studied compounds were evaluated. Each compound was dissolved in methanol (0.002 g, 10 cm^{-3}) and to 1 cm^3 of this solution 9 cm^3 of buffer were added. The absorbance was measured at 246 nm using a UV spectrophotometer (8452A, Diode Array Spectrophotometer, Hewlett—Packard) (A_1). To the solution in Erlenmeyer flask 0.1 cm^3 of octanol was added and the mixture was shaken at room temperature for 1 h so that equilibrium partitioning could be achieved. After another hour the absorbance of buffer phase was measured at 246 nm (A_2). The partition coefficients were calculated by the equation

$$P = [(1000 \cdot m) - (a \cdot c_{H_2O} \cdot M)]/(b \cdot c_{H_2O} \cdot M)$$

where m is the mass of drug (0.0002 g), a is the amount of aqueous phase (10 cm^3), b is the amount of octanol (0.1 cm^3), M is the molar mass of drug (g mol^{-1}), c_{H_2O} is the concentration of drug in the aqueous phase ($c_{H_2O} = A_2/\varepsilon$, $\varepsilon = A_1/c$, c is the concentration of solution used for measurement of A_1).

TLC Analysis

Plates Silufol® UV_{254}, 150 mm × 150 mm (Kavalier, Czechoslovakia) were used for the measurements by thin-layer chromatography. Each plate was impregnated with 5 % silicone in heptane. The samples (1 % solution in methanol) were applied as spots on the plates using calibrated micropipettes, 1.5 cm from the bottom edge, and the migration distance was 12 cm in all cases. The plates were developed in normal chambers, previously saturated for 1 h, the mobile phase was a mixture of 1 M-HCl:acetone (φ_r = 1:1). After development, the plates were dried at the room temperature and examined in UV light at 254 nm. R_F value was used for R_M calculation.

HPLC Analysis

The chromatographic system consisted of a pump Deltachrom ^{TM}SDS 030 (Watrex®) with injection valve (Rheodyne) and UV detector. The analytical chromatography column was a Separon SGX C18 (250 mm × 4 mm, particle size 7 μm). The mobile phase was a mixture of CH_3OH and sodium acetate solution (6.8 g dm^{-3}) (φ_r = 95:5), the pH was adjusted with acetic acid to pH = 6. The flow rate of the mobile phase was 0.6 cm^3 min^{-1}. The chromatograms were scanned at 246 nm. The injection volume was 20 mm^3. The concentration of analyzed drug solutions was 1 mg cm^{-3}. A solution of $NaNO_2$ (c = 1 mg cm^{-3}) was used for determination of t_0.

Potentiometric Determination of pK_a

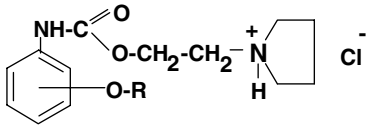
The solutions of compounds (50 cm^3) in concentration $c = 10^{-3}$ mol dm^{-3} were potentiometrically titrated with 0.1 M-NaOH. The exact amount of 1 M-NaOH was calculated by the Hahn method. $pK_a = pH_{1/2}$, where $pH_{1/2}$ is the pH of the solution in the middle of the titration.

RESULTS AND DISCUSSION

The physicochemical properties of pyrrolidinoethyl esters of *o*-, *m*-, and *p*-alkoxy-substituted phenylcarbamic acid determined in the experiment are listed in Table 1. Partition coefficient log P was determined in octanol-phosphate buffer (pH = 7.3). Because high lipophilicity by all the compounds was assumed, the amount of organic solvent was small (0.1 cm^3). High lipophilicity of the compounds was confirmed according to the results in Table 1, as all compounds are lipophilic and easily traverse to the nonpolar phase (octanol). Partition coefficient, as well as the value of its logarithm increases by lengthening of the side chain (Fig. 1) and by compounds *V-Z*, *VIII-Z*, *XI-Z*, *XIV-Z* this acquisition is regular and has the value of 0.41—0.42. Rekker's mathematical model predicts a rise of lipophilicity ($\log P$) by lengthening of side chain by a value of 0.519 for each methylene group [6].

According to the literature [7 , 8] the value of experimental log P is limited to the range $-3 < \log P < 3$. Literature [8] mentions that practical difficulties arise by determination of $\log P$ of polar and highly lipophilic drugs, if $\log P > 4$. This can be confirmed with our experiments. By the compounds *XXII-Z*—

Table 1. Some Physicochemical Properties of Studied Compounds



Label	R	R_M	R_F	log P_{app}	log k	pK_a
I-Z	2-CH_3	−0.33 ± 0.026	0.68 ± 0.013	1.51 ± 0.035	–[b]	8.28
IV-Z	2-C_2H_5	−0.25 ± 0.067	0.64 ± 0.036	1.55 ± 0.007	0.14 ± 0.006	8.62
VII-Z	2-C_3H_7	−0.21 ± 0.048	0.62 ± 0.027	1.66 ± 0.099	0.17 ± 0.006	8.41
X-Z	2-C_4H_9	−0.16 ± 0.072	0.59 ± 0.039	2.16 ± 0.092	0.24 ± 0.003	8.41
XIII-Z	2-C_5H_{11}	−0.14 ± 0.072	0.58 ± 0.040	2.19 ± 0.035	0.29 ± 0.006	8.58
XVI-Z	2-C_6H_{13}	0.00 ± 0.134	0.50 ± 0.077	2.82 ± 0.169	0.37 ± 0.005	8.33
XIX-Z	2-C_7H_{15}	0.16 ± 0.223	0.41 ± 0.118	3.29 ± 0.354	0.45 ± 0.005	8.30
XXII-Z	2-C_8H_{17}	0.16 ± 0.317	0.41 ± 0.163	–[a]	–[b]	8.45
XXV-Z	2-C_9H_{19}	0.29 ± 0.278	0.34 ± 0.131	–[a]	–[b]	8.74
XXVIII-Z	2-$C_{10}H_{21}$	0.48 ± 0.332	0.25 ± 0.119	–[a]	0.70 ± 0.006	8.65
II-Z	3-CH_3	−0.52 ± 0.051	0.77 ± 0.021	1.19 ± 0.092	0.02 ± 0.005	8.01
V-Z	3-C_2H_5	−0.41 ± 0.015	0.72 ± 0.007	1.51 ± 0.049	0.08 ± 0.016	7.99
VIII-Z	3-C_3H_7	−0.21 ± 0.031	0.63 ± 0.017	1.92 ± 0.064	0.14 ± 0.002	8.65
XI-Z	3-C_4H_9	−0.10 ± 0.017	0.56 ± 0.010	2.33 ± 0.057	0.20 ± 0.003	8.51
XIV-Z	3-C_5H_{11}	0.00 ± 0.029	0.50 ± 0.017	2.75 ± 0.021	0.26 ± 0.003	8.37
XVII-Z	3-C_6H_{13}	0.16 ± 0.027	0.39 ± 0.015	3.29 ± 0.230	0.37 ± 0.002	8.38
XX-Z	3-C_7H_{15}	0.35 ± 0.038	0.34 ± 0.019	3.35 ± 0.021	0.45 ± 0.001	8.42
XXIII-Z	3-C_8H_{17}	0.50 ± 0.038	0.27 ± 0.024	3.50 ± 0.212	0.53 ± 0.003	8.53
XXVI-Z	3-C_9H_{19}	0.69 ± 0.050	0.19 ± 0.016	3.74 ± 0.283	0.62 ± 0.001	7.99
XXIX-Z	3-$C_{10}H_{21}$	0.91 ± 0.044	0.12 ± 0.006	4.71 ± 0.000	0.68 ± 0.001	8.03
III-Z	4-CH_3	−0.35 ± 0.052	0.69 ± 0.025	0.89 ± 0.226	0.09 ± 0.002	7.98
VI-Z	4-C_2H_5	−0.29 ± 0.029	0.66 ± 0.015	1.58 ± 0.141	0.15 ± 0.002	8.48
IX-Z	4-C_3H_7	−0.21 ± 0.038	0.62 ± 0.021	2.04 ± 0.064	0.23 ± 0.001	8.70
XII-Z	4-C_4H_9	−0.02 ± 0.030	0.51 ± 0.017	2.32 ± 0.007	0.32 ± 0.012	8.52
XV-Z	4-C_5H_{11}	0.10 ± 0.037	0.44 ± 0.021	2.53 ± 0.021	0.42 ± 0.002	8.48
XVIII-Z	4-C_6H_{13}	0.29 ± 0.121	0.34 ± 0.061	2.88 ± 0.014	0.49 ± 0.002	8.51
XXI-Z	4-C_7H_{15}	0.50 ± 0.057	0.24 ± 0.023	3.58 ± 0.276	0.59 ± 0.001	8.47
XXIV-Z	4-C_8H_{17}	0.75 ± 0.054	0.15 ± 0.015	–[a]	0.66 ± 0.001	8.60
XXVII-Z	4-C_9H_{19}	0.86 ± 0.066	0.12 ± 0.015	–[a]	0.77 ± 0.001	8.88
XXX-Z	4-$C_{10}H_{21}$	1.12 ± 0.112	0.07 ± 0.015	–[a]	0.80 ± 0.001	8.72

a) Not determined, *b*) missing amount of compounds.

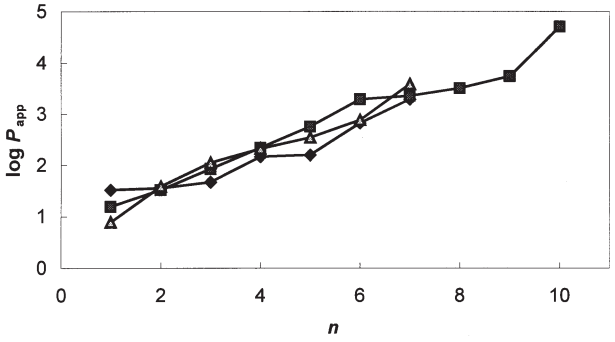


Fig. 1. Relationship between log P_{app} and number (n) of carbon atoms in the alkoxy chain on benzene ring for *o*- (♦), *m*- (■), *p*-substituted (△) basic esters.

XXX-Z, the determination of log P was very difficult and even though log P of *XXIII-Z*, *XXVI-Z*, and *XXIX-Z* were determined, taking the course of the determination into account, these values are not reproducible. There is a possibility to increase the amount of the sample, but this is inconsistent with the demand to work with the lowest amount of the analyte in order to prevent molecules from association in any phase. Another limitation is the requirement on highly pure compounds, because even small impurities might remarkably change the experimental value, especially if sensitive analytical methods are used.

Parameters obtained from TLC (R_M, R_F) can be used for the definition of drug's lipophilicity. Advantages of TLC methods are: studied compounds do not have to be 100 % pure, better economy (especially comparing with HPLC), because plate costs and solvent consumption per sample are low, off-line process (a number of samples are chromatographed simultaneously, side by side), no analytical methods for quantity determination are needed (spots on the plate can be detected with any specific or nonspecific method), study of great variety of both lipophilic and hydrophilic compounds can be performed.

These advantages were a big help in the experimental part of this study, but one should not forget that R_M and R_F values determined under these circumstances can be used only for rough characterization of

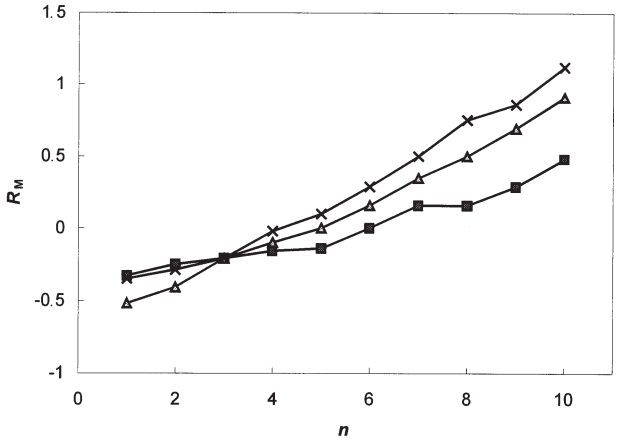


Fig. 2. Dependence of R_M and number (n) of carbon atoms in the alkoxy chain on benzene ring for *o*- (×), *m*- (■), *p*-substituted (△) basic esters.

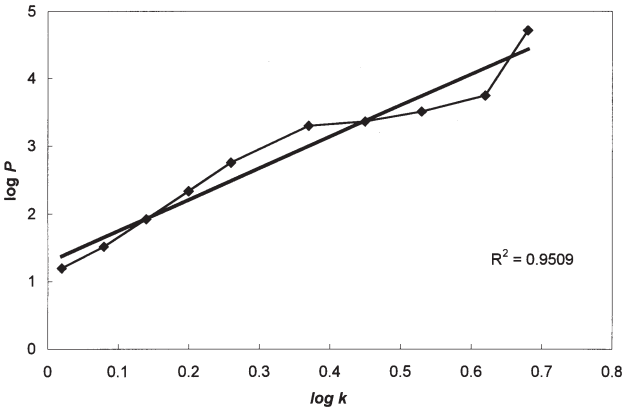


Fig. 3. The linear relationship between $\log P$ and $\log k$ for *m*-substituted basic esters.

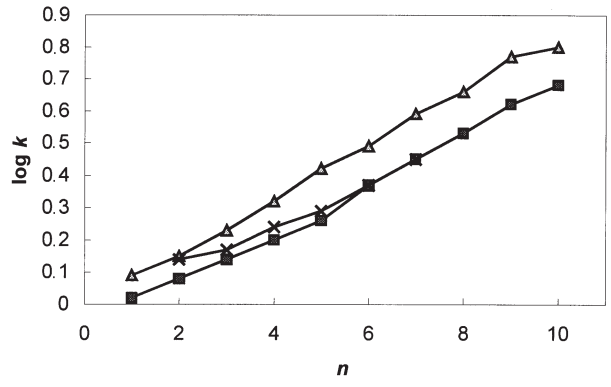


Fig. 4. Relationship between $\log k$ and number (n) of carbon atoms in the alkoxy chain on benzene ring for *o*- (×), *m*- (■), *p*-substituted (△) basic esters.

a drug's lipophilicity. With the increase of alkyl chain the R_M values increased (Fig. 2) and R_F decreased, so the influence of the methylene group on a drug's lipophilicity is evident. Taking into account that accuracy and reproducibility of chromatographic R_M and R_F is closely linked with the application of standard conditions (control of the room temperature and humidity, use of starting markers and densitometric control of chromatogram evaluation) log k from HPLC became a very important lipophilic parameter. Values of log k correlate with log P values (Fig. 3) and once the HPLC has been successfully set in the laboratory, it becomes a method of choice for the determination of drug's lipophilicity [8].

Retention times of studied compounds increase with increasing number of carbon atoms in the alkoxy chain and this causes the increase of log k values (Fig. 4). More studies [9, 10] have shown that log k values of esters of phenylcarbamic acids, determined under the same conditions, can be appropriate parameters for determination of local anaesthetic activity and optimal values of log k for local anaesthetics of phenylcarbamate type are ranging between 0.3—0.6. Into this range fall values of all compounds with greater number of carbons in the alkoxy substituent than 6, but also values of compounds *XII-Z* and *XV-Z*.

Local anaesthetics generally used are compounds of basic type, which can exist both in the form of unionized base B or in the form of ionized cation BH^+. Both forms of the compound can exist in the environment with physiological pH, if their pK_a range between 8.0—9.5. The pK_a values of local anaesthetics, mostly used in the practice, are between 7.6—8.9 [11]. Into this range fall all pK_a values determined in this study. If only this parameter was taken into consideration, local anaesthetic activity would be predicted by all studied compounds. The influence of the alkoxy chain on pK_a values was not established, pK_a in homologous series have neither decreasing nor increasing character. The progress of the curve is nonlinear (Fig. 5).

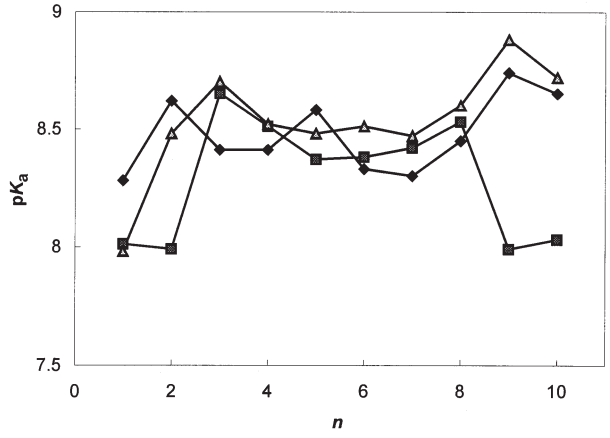


Fig. 5. Relationship between pK_a and number (n) of carbon atoms in the alkoxy chain on benzene ring for *o*- (♦), *m*- (■), *p*-substituted (△) basic esters.

Table 2. Correlation between Lipophilic Parameters for *o*-Substituted Derivatives

	R_M	R_F	log P	log k	n
R_M	1	0.9995	0.98	0.9911	0.9751
R_F		1	0.9824	0.9909	0.9774
log P			1	0.9915	0.9571
log k				1	0.9926
n					1

Table 3. Correlation between Lipophilic Parameters for *m*-Substituted Derivatives

	R_M	R_F	log P	log k	n
R_M	1	0.9949	0.9802	0.9963	0.9958
R_F		1	0.9856	0.9969	0.9988
log P			1	0.9751	0.9843
log k				1	0.9967
n					1

Table 4. Correlation between Lipophilic Parameters for *p*-Substituted Derivatives

	R_M	R_F	log P	log k	n
R_M	1	0.9898	0.961	0.9872	0.9898
R_F		1	0.9577	0.9944	0.9922
log P			1	0.977	0.9843
log k				1	0.9981
n					1

The values of physicochemical parameters were correlated between each other and also with the length of alkoxy substituent. By all parameters (except pK_a) linear influences were confirmed, and the best correlations were obtained for the homologous series with the alkoxy substituent in *meta* position (Tables 2—4).

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