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Scientific paper

CHROMATOGRAPHIC BEHAVIOR AND LIPOPHILICITY OF N-(4-PHENYLSUBSTITUTED)-2,3-DIPHENYLPROPANAMIDES

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Abstract

Retention behavior of some N-(4-phenylsubstituted)-2,3-diphenylpropanamides was investigated on reversed phase thin layer chromatography (RP-C_{18}). Retention constants of investigated compounds were determined in the following solvent systems: water-ethanol, water-n-propanol and water-i-propanol. Linear relationships were obtained between retention, R_M and volume fraction of organic solvent, φ. As a measure of lipophilicity of tested propanamides, Hansch`s lipophilicity parameter, $\pi$, were calculated. Chromatography retention constants $R_M^0$ were correlated with Hansch`s lipophilicity parameter, and good linear relationships were obtained. These results confirm, that retention constants, (R_M^0), determined in reversed phase thin-layer chromatography (RP-TCL) can be used, as criteria of lipophilicity.

Key words: *Chromatography, Compound, Structure.*

1. INTRODUCTION

Hydrophobic or lipophilic character is important physicochemical parameter of a molecule, which affected the activity of bioactive compounds. Lipophilicity plays an important role in the transport of compounds through a biological system and it may also influence the formation of complex between a compound and receptor or biomacromolecule.

There are different ways of expression lipophilicity. In the most cases the lipophilicity can be quantitatively characterized as log *P* (the logarithm of the ratio of the concentrations of solute in a saturated 1-octanol-water system) and as Hansch`s lipophilicity parameter, $\pi$ [1-3]. The Hansch`s lipophilicity parameter, π, measures the free energy change caused by a substituent and usually used to characterized the effect of a particular substituent to the lipophilic nature of a molecule [1,2].

Processes of drug absorption, distribution and excretion in the pharmacokinetic phase of drug action, as well as drug–receptor interactions in the pharmacodynamic phase, are dynamic in nature as are the analyte's distribution processes in chromatography. As consequence, the chromatography can be used as powerful techniques for estimating physico-chemical parameters and biological activities [4-13]. That is the reason why reversed phase liquid chromatography (RPLC) has received considerable attention to predict the pharmacological and pharmacokinetic properties of drugs in the early stages of the drug discovery phase. Owing to its simplicity, as well as efficiency, reversed-phase thin-layer chromatography was selected for this investigation.

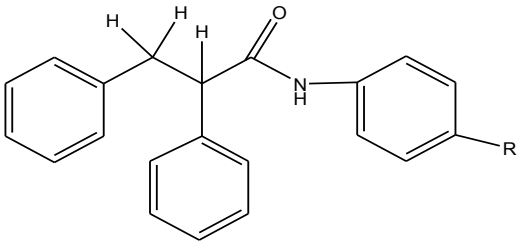
Studying hydrophobicity of phenylpropanamide derivatives is a very interesting problem because several derivatives of phenylamides exhibit different biological activities. Some phenylamide derivatives displayed potent analgesic [14-16], anticonvulsant [17,18], antileishmanial [19], antimicrobial, antifungal[20-22] and cytotoxic activity [23,24]. Some of them can inhibit measle virus [25] and the enzyme aldoze reductase [26]. In the most of case, strength of activities and spectrum of activity varied markedly depending upon the substituents on the phenyl ring [22, 23, 27].

Aim of this study was to investigate the chromatographic behavior and lipophilicity of a series of newly synthesized derivatives of phenylpropanamide, which differ in the nature of the substituents in *para* position on phenyl ring.

2. EXPERIMENTAL

The structures of the investigated compounds are presented in Table 1. Solutions (2 mg mL^{-1}) for chromatographic investigations were prepared by dissolving of compounds in ethanol. These solutions (0.2μL) were spotted on RP TLC C_{18}/UV_{254} plates (Macherey-Nagel). The plates were developed in unsaturated chambers by ascending technique with aqueous solutions of three organic modifiers: ethanol (φ= 0.55-0.75, v/v), n-propanol (φ = 0.45-0.65, v/v) and i-propanol (φ = 0.45-0.65,v/v). The measurements temperature was equal to 25^0C. After development the dried plates were examined in UV light at λ=254 nm as dark spots. At least three chromatograms were developed for each solute-solvent combination and R_f values averaged. R_M values were calculated from $R_M = log\ (1/R_f - 1)$. All calculations were carried out using the computer program Origin, version 6.1.

Table 1. Structures of the investigated compounds



Compound	***-R***	*Compound*	***-R***
1	*-$COCH_3$*	**7**	*-OH*
2	*-NO_2*	**8**	*-CH_3*
3	*-$N(CH_3)_2$*	**9**	*-Br*
4	*-Cl*	**10**	*-I*
5	*-H*	**11**	-OCH_3
6	*-COOH*		

3. RESULTS AND DISCUSSION

The chromatographic retention mechanism is dependent basically on the solute size and its hydrogen bonding capability. The solute size depends on the molecular structure of the parent molecule and also of the substituents existing in the molecule. The chromatographic retention behavior of the newly synthesized N-(4-phenylsubstituted)-2,3-diphenylpropanamide derivatives in reversed-phase thin layer chromatography are presented in Table 2, from which is evident that their retention behavior were affected by the presence of the substituent in the *para* position of a molecule. The retention data obtained for separation on the C-18 bonded silica gel in all three mobile phases are generally typical of reversed phase chromatographic behavior: less polar solute are more strongly retained. For example, halogens generally increase the retention and also the hydrophobicity of molecule in the order: Cl < Br < I. This is possibly a consequence of differences between the London dispersion interactions between halogen atom and the non-polar stationary phase [28, 29]. Also presence of non-polar alkyl substituents CH_3 and $N(CH_3)_2$ results in an increase in RP retention. The retention of compound with polar substituents, (except OH group), decrease mainly in the order $COCH_3 < NO_2 < OCH_3 <$ COOH. Unusual retention behavior of compound with OH group could be explained by ionization of OH group. The charge formed upon ionization can be stabilized through resonance delocalization. The keto form of the molecule occurs as a result. That form is less polar than enol form and has much longer retention than excepted.

Table 2. The chromatographic retention behavior of diphenylpropanamides on RP-TCL C_{18} in different modifier (φ = 0.65,v/v)

Compound	***Rf***			
	Substituents	n-propanol	i-propanol	ethanol
1.	$COCH_3$	0.65	0.56	0.30
2.	NO_2	0.70	0.59	0.33
3.	$N(CH_3)_2$	0.60	0.68	0.35
4.	Cl	0.67	0.53	0.27
5.	H	0.69	0.59	0.39
6.	COOH	0.81	0.78	0.54
7.	OH	0.51	0.39	0.10
8.	CH_3	0.63	0.57	0.36
9.	Br	0.59	0.51	0.25
10.	I	0.58	0.50	0.18
11.	OCH_3	0.73	0.65	0.43

Information about the effect of mobile phase modifiers on chromatographic retention behavior of N-(4-phenylsubstituted)-2,3-diphenylpropanamides was investigated by changing the amount of organic solvent in mobile phase. Determined R_M values by TLC is usually in linear relationship on the organic modifier volume fraction (φ) in the mobile phase:

$$R_M = R_M^0 + m\varphi$$

Where φ is the volume fractions of the organic solvent in the mobile phase and m (slope of TLC equation) is the change in R_M caused by unit change of organic modifier volume fraction in the mobile phase and R_M^0 (intercept) is the retention constant. The equations of these straight lines are given in Table 3. The relationships were characterized by high correlation coefficients.

In reversed phase chromatography, in which the solvatation effect plays a very important role the retention depends on the molecular structure of the solute so obtained R_M^0 values are different for each compounds. But it is also known that R_M^0 depends on the nature of organic modifier of the binary aqueous eluents employed in the RPC [30]. As a consequence, different R_M^0 values were obtained for ethanol, n-propanol and i-propanol as modifiers. The R_M^0 values from water- ethanol system are larger than corresponding data determined for water-i-propanol and water– n-propanol systems. This difference can be explained by larger polarity of ethanol than i-propanol and n-propanol. The compounds were more mobile in the modifier with n-propanol than with ethanol. N-propanol as less polar of the tested solvents interacts strongly with non-polar solutes than more polar solvents as i-propanol and ethanol.

Table 3. Extrapolated R_M^0 values, slope of TCL equations, m, and correlation coefficients, r, of TCL equations $R_M = R_M^0 + m\varphi$

Comp.	***Water-ethanol***			***Water-n-propanol***			***Water-i-propanol***		
	R_M^0	*m*	*r*	R_M^0	*m*	*r*	R_M^0	*m*	*r*
1.	**2.530**	**-3.764**	**0.999**	**1.448**	**-2.619**	**0.978**	**2.391**	**-3.847**	**0.999**
2.	**2.994**	**-4.113**	**0.994**	**1.715**	**-3.185**	**0.995**	**2.441**	**-4.017**	0.998
3.	**1.972**	**-2.713**	**0.981**	**2.119**	**-3.578**	**0.998**	**1.888**	**-3.532**	0.981
4.	**3.267**	**-4.382**	**0.996**	**1.968**	**-3.463**	0.990	2.595	-4.077	0.997
5.	**2.595**	**-3.700**	0.993	1.532	-2.894	0.984	2.261	-3.774	0.995
6.	**2.285**	**-3.657**	0.993	1.198	-2.849	0.986	2.148	-3.762	0.998
7.	5.925	-7.571	0.999	2.710	-4.187	0.991	3.010	-4.242	0.980
8.	2.789	-4.002	0.999	1.618	-2.862	0.990	2.471	-3.978	0.998
9.	3.572	-4.755	0.993	1.564	-2.656	0.995	3.048	-4.072	0.999
10.	3.937	-4.934	0.989	1.938	-3.208	0.986	2.997	-4.549	0.998
11.	2.415	-3.507	0.999	1.441	-2.852	0.993	2.312	-3.999	0.999

Obtained values of slope, *m,* are not the same for all investigated substances. For the examined group of compounds, *m* depends not only on the solvent applied as a component of the mobile phase, but also on a considerable extent of specific interaction between solutes, stacionary and mobile phase. It is apparent from the data in Table 3. that the obtained R_M^0 values and absolute value of *m* increase with increasing hydrophobicity of the N-(4-phenylsubstituted)-2,3-diphenylpropanamides. Because of that there is a linear relationship between these two constants, with good correlation coefficients *r* (Table 4).

Table 4. Relationship between intercept R_M^0 and slope m of TCL equations

Modifier	Equation	r	sd
Ethanol	$R_M^0 = -0.601 - 0.868\,m$	0.990	0.160
n-propanol	$R_M^0 = -0.670 - 0.786\,m$	0.969	0.104
i-propanol	$R_M^0 = -2.392 - 1.229\,m$	0.874	0.193

Obtained linear equation indicates that both, R_M^0 values and *m* seem to be related to the same physico-chemical factors and therefore they are intercorrelated. From that reason some authors have suggested that the slope, *m,* may be used as another criteria for estimating the lipophilicity of compounds. [31, 32].

The lipophilicity of a substance is one of the parameters that influence its biological activity. Lipohilicity is usually measured by the partition coefficient of the organic compound between a non-polar phase and water ($\log P$) [33]. Hansch`s lipophilicity parameter, π,

specifically address the effect of a substituent on the partitioning of a molecule between two solvents. Values of π measure the free energy change caused by a particular substituent and relate to biological activity. The difference between the substitute and unsubstituted log *P* values gives the π value for that particular substituent. By definition, the π value for hydrogen is zero. The partition coefficients, log *P,* of tested propanamides were calculated using the computer software Chem.Office 7.0. Found data were used to obtain Hansch`s lipophilicity parameter, π for investigated diphenyl propanamides.

Hansch`s parameters π, for individual substituents when they are in position 4 for benzene ring [1] and π related to the same substituents in position 4 in the investigated diphenyl propanamide derivatives are presented in Table 5.

Table5. Hansch`s lipophilicity parameter, π

Compound	*Substituents*	*π according to Hansch of benzene system [1]*	*Calculated π to diphenylpropan amides*
1.	$COCH_3$	-0.55	-0.09
2.	NO_2	-0.28	-0.18
3.	$N(CH_3)_2$	+0.18	+0.16
4.	Cl	+0.71	+0.97
5.	H	0	0
6.	COOH	-0.28	-0.06
7.	OH	–0.67	-0.67
8.	CH_3	+0.56	+0.50
9.	Br	+0.86	+1.12
10.	I	+1.26	+1.38
11.	OCH_3	-0.02	-0.06

Results in Table 5 show that the values of the parameter π, obtained for investigated propanamide derivatives follow the trend of the influence of substituents on lipophilicity. Obtained results (Table 5) show that presence of $N(CH_3)_2$ group, non polar alkyl substituents CH_3, and halogenides (Cl < Br < I) cause, as expected an increase (positive *π)*, whereas polar substituents (COOH < OCH_3 < $COCH_3$ < NO_2 < OH) cause a decrease (negative *π)* of lipophilic character of molecule, in relation to unsubstituted (compound 5).

Table 6. Equations of relationships between R_M^0 and Hansch`s lipophilicity parameter, π

	Positive π		*Negative π*	
Modifier	Equation	r	Equation	r
Ethanol	$\pi = -1.156 + 0.638\ R_M^0$	0.989	$\pi = 0.338 - 0.170\ R_M^0$	0.999
n-propanol	-	-	$\pi = 0.526 - 0.434\ R_M^0$	0.985
i-propanol	$\pi = -1.730 + 0.983\ R_M^0$	0.941	$\pi = 1.694 - 0.775 R_M^0$	0.971

We correlated chromatography retention constants R_M^0 and obtained Hansch`s lipophilicity parameter, π, separately for substituents with negative and substituents with positive π values, because R_M^0 are often used to assess the lipophilicity (biological activity) of various molecular species. Figure 1 shows the dependence of these parameters in ethanol as modifier.

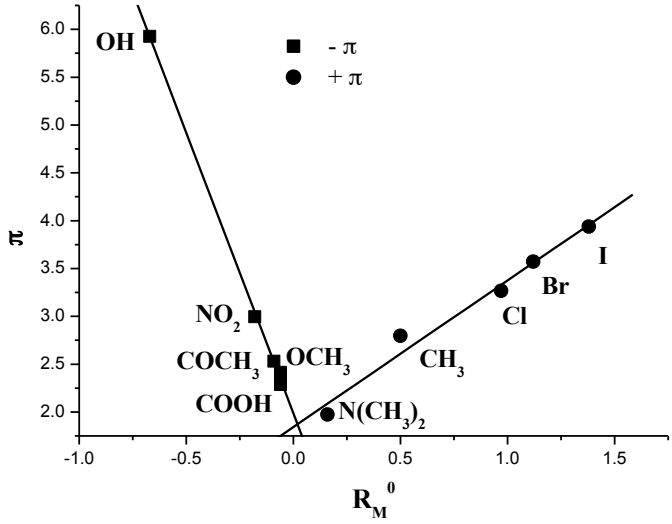


Figure 1. Relationship between retention R_M^0 and Hansch`s parameter, π, for ethanol as modifier

Good linear relationships (Table 6) were obtained in both of case (except + π values in n-propanol) in all modifier. This points to the fact that retention constant of a molecule (R_M^0) in reversed phase thin-layer chromatography (RP-TCL) can be used, as criteria of its lipophilicity and potential biological activity prediction.

4. CONCLUSION

The chromatographic behavior of N-(4-phenylsubstituted)-2,3-diphenylpropanamides was investigated using reversed phase thin-layer chromatography. The mixture of water-ethanol, water-n-propanol and water-i-propanol were used as solvent systems. The effect of solvents and the nature of substituents on the retention behavior of diphenylpropanamides were investigated. Halogenides, $N(CH_3)_2$ group and non-polar alkyl substituent, CH_3, increase, the polar substituents ($COCH_3$, OCH_3, COOH) caused decrease retention. Chromatography retention constant, R_M^0 was calculated. The R_M^0 values from water-ethanol system are larger than corresponding data determined for water-i-propanol and water–n-propanol systems. Hansch`s lipophilicity parameter, π, as measure of lipophilicity of tested propanamides was calculated. Presence of $N(CH_3)_2$, nonpolar alkyl substituents CH_3 and halogenides (Cl < Br < I) cause an increase, while polar substituents (COOH < OCH_3 < $COCH_3$< NO_2 < OH) cause a decrease in π

value, in relation to unsubstituted molecule. Chromatography retention constants R_M^0 were correlated with obtained Hansch`s lipophilicity parameter, and good linear relationships were obtained. The results show that retention constant of molecule (R_M^0), determined in reversed phase thin-layer chromatography (RP-TCL) can be used, as criteria of its lipophilicity and potential biological activity prediction.

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