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Lipophilicity and Chromatographic Behaviour of Benzoic Acid Derivatives

Margarita PAGOU* and Antonios KOUTSELINIS

Department of Forensic Medicine and Toxicology, School of Medicine, University of Athens, Athens 115 27, Greece.
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The *Rm* values of 32 compounds, derivatives of benzoic acid, were determined by reversed-phase thin layer chromatography (RP-TLC). The logarithm of the capacity factor, log *k'*, of the same compounds was studied with reversed-phase high performance liquid chromatography (RP-HPLC). The lipophilicity was calculated according to the fragmental methods of Rekker and of Leo–Hansch and was compared with the experimental values of *Rm* and the log *k'*. The comparison shows that the Rekker method gives a better description of lipophilicity than that of the Leo–Hansch method.

Keywords quantitative structure–activity relationship; lipophilicity; key number (*kn*); benzoic acid derivative; TLC; HPLC

The chromatographic behaviour of a range of aromatic acids has been studied elsewhere.[1−5] In this experimental work we also have turned our attention to the choice of benzoic acid derivatives which constitute the classical basic structure of a great number of drugs. It is well known that the octanol–water partition coefficients, log *P*, are probably the most suitable as direct criteria of lipophilicity if they can be derived.[6−13] The aim of the present work was to investigate the lipophilic character and account for chromatographic techniques which show indirectly the lipophilic behaviour in quantitative structure–activity relationship (QSAR) studies. The advantages of RP-TLC and RP-HPLC over the "shake-flask" technique, which is the most widely used for experimental measurements of partition coefficients, are that they are rapid, accurate, without objection to the use of impure substances, nor in demand of adequate laboratory experience, and that they seem to be more suitable for obtaining high-quality correlations. The disadvantages are the limited lipophilicity range that can be covered by one elution composition and the frequently overlooked demand for structural congenericity in an investigated series of compounds.[9,14−16]

Experimental

Chemicals The investigated compounds (Table I) were of the highest available purity. Eighteen of these compounds (No. **1**—**7**, **9**, **10**, **14**—**17**, **19**, **21**, **24**, **28**, and **30**) were obtained from Fluka. Three (**8**, **11**, **12**) were synthesised by acetylation and nine (**18**, **20**, **22**, **23**, **25**—**27**, **29**, and **31**) by esterification of benzoic acid in the laboratory of Medicinal Chemistry, University of Athens. Two others (**13** and **32**) were kindly donated by the laboratory of Organic Chemistry, University of Athens.

Determination of *Rm* Values The pH values of the solution which was used for the RP-TLC were obtained from Orion Research model 601 I/digital Ionalyser pH meter at room temperature. The graduation and the control of the pH meter before and after the measurements was obtained with buffers pH 4 and 7. As mobile phases we used acetone (Ferac)–chloride buffer (25 ml 0.2 M KCl + 67 ml 0.2 M HCl, pH 1) methanol (Ferac)–HCl solution (0.01 M pH 2.50). The *Rm* values were calculated from *Rf* values using the following equation[17]:

$$Rm = \log(1/Rf - 1) \tag{1}$$

where *Rf* is the ratio of distance moved by a compound and the solvent front in a given time.

The experiments were performed on DC Fertigplatten Silica gel 60F 254, type E, Merck. Impregnation was carried out for 90 s in a solution of 5% paraffin oil in dry petroleum ether 40 : 60 (v/v) at room temperature. The plates were dried afterwards at 40 °C and were developed the same day in closed chromatography tanks saturated with the solvent at room temperature. The mobile phase was acetone–chloride buffer pH 1 for the study of all the acids and esters **19**, **21**, and **30**. The measurements were obtained by the above mentioned mobile phase which consisted of acetone 25%, 30%, 35%, 40%, 45%, 50%, 55%, and 60%. At each acetone concentration 6 to 20 measurements were obtained. The *Rm* values of esters of benzoic acid derivatives were also determined on the same stationary phase with mobile phase methanol–HCl pH 2.50. The measurements were obtained in a concentration range of methanol between 30 and 90%. At each methanol concentration 7—11 measurements were obtained. The spots appeared in a chromatography chamber with J_2 gas.

Special care was taken with ionizable compounds. The *Rm* value can be determined at a certain pH even when the compound is partially ionized, through the ionized form of the molecule is taken as not to participate at the distribution.[14] The corrected migration rate, $Rf^{\text{corr.}}$, the apparent migration rate, $Rf^{\text{app.}}$, and the fraction of the compound at the non-ionized form, $1-a$, are connected as follows:

$$\frac{1}{Rf^{\text{corr.}}} - 1 = \frac{1}{Rf^{\text{app.}}} - 1/(1-a) \tag{2}$$

The corrected *Rm* value, $Rm^{\text{corr.}}$ and the apparent *Rm* value, $Rm^{\text{app.}}$ are defined as follows:

$$\log\left(\frac{1}{Rf^{\text{corr.}}} - 1\right) = Rm^{\text{corr.}} \tag{3}$$

$$\log\left(\frac{1}{Rf^{\text{app.}}} - 1\right) = Rm^{\text{app.}} \tag{4}$$

The calculation of $1-a$ is given by Eq. 4[14]:

$$1 - a = \frac{1}{1 + k_a/[\mathrm{H}^+]} = \frac{1}{10^{\mathrm{pH}-\mathrm{p}K_a} + 1} \tag{5}$$

Combining Eqs. 2, 3, 4, and 5, we get the following expression used in our experiments:

$$Rm^{\text{corr.}} = R^{\text{app.}} + \log(10^{\mathrm{pH}-\mathrm{p}K_a} + 1) \tag{6}$$

We used this equation in order to determine the $Rm^{\text{corr.}}$ values.

Determination of log *k'* Values The graduation and the control of the pH meter was obtained with buffers pH 5 and 6. Methanol (Merk) and a water solution of acetic acid pH 2.90 were used as the mobile phase. The water solution of acetic acid was prepared and stored at 6 °C when it was not in use and was allowed to equilibrate to room temperature before use. The mobile phase (always freshly mixed) was deaerated for 20 min.

The retention times were determined in a HPLC (Varian 5000) apparatus equipped with a UV (254 nm) absorbance detector. The data were then transferred to an integrator (Hewlett-Packard 3390A) and then to an OmniScribe recorder. The column used throughout was RPtype (μBondapak C_{18}; Waters Assoc.). Sample sizes were chosen so as to give a good detection signal. Retention times were expressed in terms of log (capacity factors, *k'*) by[18]

$$\log k' = \log[(t_R - t_o)/t_o] \tag{7}$$

where t_R represents the retention time of the compound and t_o the elution time of an unretained peak generated by formamide in absolute methanol.

The corrected $\log k'$, $\log k^{\mathrm{corr.}}$, is also expressed by:

$$\log k^{\mathrm{corr.}} = \log k^{\mathrm{app.}} + \log(10^{\mathrm{pH}-\mathrm{p}K_a} + 1) \tag{8}$$

where $\log k^{\mathrm{app.}}$ represents the apparent $\log k'$. The measurements were obtained in a concentration range of methanol of 20—80%. At each methanol concentration we obtained 2—3, measurements.

Results and Discussion

Table I summarizes log *P* values,[19] both Σf_R and $\Sigma f_{L/H}$ values,[13,20] which are the sum of hydrophobic fragmental constants according to the methods of Rekker and Leo–Hansch, respectively, and include p*K* values as they were reported in the bibliography or calculated according to the Hammet equation for benzoic acid and aniline, respectively[21]:

$$\mathrm{p}K_a = 4.20—1.00\,\Sigma\sigma \tag{9}$$

$$\mathrm{p}K = 4.58 - 2.90\,\Sigma\sigma \tag{10}$$

where σ is a constant for the particular substituent for the Hammet equation.

Table II shows the measurements of *Rf* values of benzoic acid derivatives. The chromatographic experiments exhibit that all acids migrated too close to the front of the solvent. In Fig. 1 is plotted the corrected *Rm* values as a function of acetone concentration. The figure shows a non-linear relationship between $Rm^{\mathrm{corr.}}$ values and acetone concentration (%) which is in discrepancy with Martin's equation.[22,23]

The observed distinct non-linearity for the substituted benzoic acids could be a consequence of the degree of impregnation of silica gel by paraffin oil.

TABLE I. $\log P$, Σf_R, $\Sigma f_{L/H}$, $\mathrm{p}K_a$ and $\mathrm{p}K$ Values

No.	Compound	$\log P$	Σf_R	$\Sigma f_{L/H}$	$\mathrm{p}K_a$	$\mathrm{p}K$
1	Ar-COOH	1.87	1.769	1.87	4.20	
2	Ar-COOH,2-OH	2.24	1.273	2.35	2.98	
3	Ar-COOH,3-OH	1.50	1.273	1.32	4.08	
4	Ar-COOH,4-OH	1.58	1.273	1.32	4.58	
5	Ar-COOH,2-Cl	1.98	2.511	2.58	2.92	
6	Ar-COOH,4-Cl	2.65	2.511	2.58	3.99	
7	Ar-COOH,2-NO_2	1.46	1.534	1.73	2.17	
8	Ar-COOH,3-$NHCOCH_3$	1.14	0.998	0.90	4.07	
9	Ar-COOH,3-CH_3	2.37	2.288	2.53	4.29	
10	Ar-COOH,2,5-OH	1.74	0.777	1.80	2.88	
11	Ar-COOH,2-OH,4-$NHCOCH_3$	—	0.502	1.38	2.98	
12	Ar-COOH,2-OH,5-$NHCOCH_3$	—	0.502	1.38	2.77	
13	Ar-COOH,2,3-OCH_3	1.94	1.929	2.21	3.94	
14	Ar-COOH,2-OH,4-Cl	—	2.015	3.06	2.75	
15	Ar-COOH,2-OH,5-Br	3.23	2.207	3.21	2.62	
16	Ar-$COOCH_3$	2.30	2.157	2.11		
17	Ar-$COOC_2H_5$	2.68	2.676	2.65		
18	Ar-$COOC_3H_7$	3.23	3.195	3.19		
19	Ar-$COOCH_3$,2-OH	2.55	1.661	2.81		
20	Ar-$COOCH_3$,3-OH	1.89	1.661	1.44		
21	Ar-$COOCH_3$,4-OH	1.96	1.661	1.56		
22	Ar-$COOCH_3$,2-Cl	2.42	2.899	3.02		
23	Ar-$COOCH_3$,4-Cl	2.87	2.899	2.82		
24	Ar-$COOC_2H_5$,2-OH	2.54	2.180	3.35		
25	Ar-$COOC_2H_5$,3-OH	2.47	2.180	2.10		
26	Ar-$COOC_2H_5$,4-OH	2.35	2.180	2.10		
27	Ar-$COOC_2H_5$,2-Cl	2.96	3.418	3.56		
28	Ar-$COOC_2H_5$,4-NH_2	1.86	1.652	2.19		2.49
29	Ar-$COOC_3H_7$,2-OH	—	2.699	3.89		
30	Ar-$COOC_3H_7$,4-OH	2.71	2.699	2.64		
31	Ar-$COOC_3H_7$,2-Cl	3.45	3.937	4.10		
32	Ar-$COOC_6H_5$,2-OH	—	2.800	4.19		

For mixtures with methanol, Table III gives the *Rf* values of 17 derivatives and Fig. 2 shows the *Rm* values as a function of methanol concentration (%) of 7 derivatives for better observation according to the Eq. 1.

For each compound there is a linear relationship between *Rm* and the composition of the eluents. A theoretical explanation of this type of behavior has been presented by several authors.[24-26] The application of regular solution theory[27] indeed gives retention as a linear function of eluent composition, expressed as a volume fraction. Extrapolations to pure water result in Rm_0. Only the Rm_0 value of Ar-$COOC_2H_5$, 4-NH_2 is corrected because of ionization.

The key number, *kn*, and the Rm_0 values of the investigated compounds are summarized in Table IV. *kn* indicates the number of times the C_M factor (or magic constant = 0.289) should be applied in order to correct a given abnormality. In other words, *kn* has a corrective role in the final fit and is connected with resonance, steric and systemic peculiarities of the structure under consideration. Although there is no

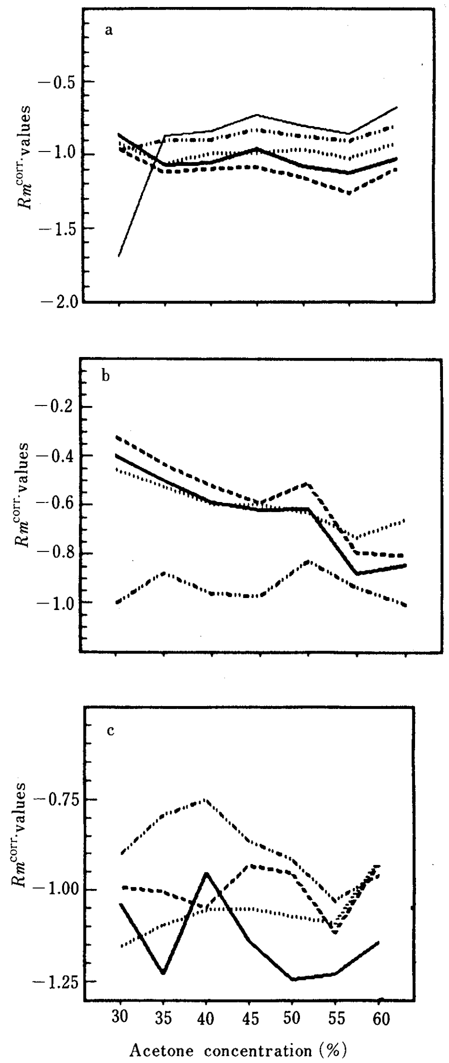

Fig. 1. Relationship between $Rm^{\mathrm{corr.}}$ Values of Benzoic Acid Derivatives and Acetone Concentration in the Mobile Phase

a) ——, Ar-COOH; —·—, Ar-COOH, 2,3-OCH_3; ------, Ar-COOH, 3-$NHCOCH_3$; ---, Ar-COOH, 2-OH, 4-$NHCOCH_3$; ——, Ar-COOH, 2-OH, 5-$NHCOCH_3$. *b*) —·—, Ar-COOH, 2-Cl; ------, Ar-COOH, 4-Cl; ---, Ar-COOH, 2-OH, 4-Cl; ——, Ar-COOH, 2-OH, 5-Br. *c*) —·—, Ar-COOH, 2-OH; ------, Ar-COOH, 3-OH; ---, Ar-COOH, 4-OH; ——, Ar-COOH, 2,5-OH.

Table II. *Rf* Values for Benzoic Acid Derivatives

Compound	Acetone concentration (%)						
	30	35	40	45	50	55	60
Ar-COOH	0.980±0.042	0.883±0.007	0.874±0.010	0.843±0.018	0.865±0.021	0.878±0.010	0.827±0.33
Ar-COOH,2-OH	0.890±0.019	0.863±0.030	0.851±0.016	0.881±0.008	0.893±0.031	0.916±0.019	0.902±0.017
Ar-COOH,3-OH	0.935±0.010	0.926±0.012	0.919±0.017	0.883±0.018	0.922±0.018	0.925±0.009	0.894±0.038
Ar-COOH,4-OH	0.908±0.021	0.910±0.034	0.918±0.006	0.896±0.006	0.900±0.017	0.929±0.014	0.896±0.015
Ar-COOH,2-Cl	0.911±0.030	0.884±0.020	0.903±0.005	0.905±0.004	0.872±0.008	0.897±0.003	0.911±0.007
Ar-COOH,4-Cl	0.740±0.043	0.771±0.018	0.799±0.013	0.801±0.013	0.812±0.036	0.844±0.012	0.820±0.028
Ar-COOH,3-NHCOCH$_3$	0.894±0.018	0.920±0.012	0.909±0.007	0.907±0.005	0.902±0.025	0.914±0.011	0.894±0.013
Ar-COOH,2,5-OH	0.917±0.011	0.945±0.015	0.901±0.023	0.933±0.005	0.947±0.016	0.945±0.008	0.934±0.012
Ar-COOH,2-OH,4-NHCOCH$_3$	0.902±0.031	0.931±0.015	0.927±0.006	0.925±0.007	0.936±0.017	0.949±0.015	0.927±0.013
Ar-COOH,2-OH,5-NHCOCH$_3$	0.881±0.019	0.923±0.008	0.920±0.007	0.902±0.019	0.924±0.014	0.931±0.014	0.915±0.023
Ar-COOH,2,3-OCH$_3$	0.904±0.029	0.890±0.020	0.888±0.008	0.873±0.009	0.884±0.015	0.890±0.010	0.864±0.022
Ar-COOH,2-OH,4-Cl	0.678±0.020	0.735±0.022	0.773±0.015	0.799±0.007	0.768±0.047	0.863±0.017	0.867±0.047
Ar-COOH,2-OH,5-Br	0.719±0.038	0.764±0.018	0.799±0.010	0.811±0.020	0.809±0.041	0.885±0.022	0.877±0.038

Each *Rf* value represents the mean±S.D. of 6—19 determinations.

Table III. *Rf* Values for Derivatives of Benzoic Acid Esters

Compound	Methanol concentration (%)												
	30	35	40	45	50	55	60	65	70	75	80	85	90
Ar-COOCH$_3$						0.288±0.057	0.336±0.079	0.495±0.049	0.667±0.044	0.710±0.042	0.834±0.038		
Ar-COOC$_2$H$_5$						0.248±0.038	0.271±0.060	0.451±0.064	0.553±0.046	0.639±0.066	0.711±0.044	0.766±0.039	0.818±0.022
Ar-COOC$_3$H$_7$							0.186±0.063	0.256±0.066	0.403±0.068	0.576±0.049	0.611±0.043	0.705±0.046	0.764±0.025
Ar-COOCH$_3$,2-OH						0.359±0.041	0.417±0.042	0.468±0.035					
Ar-COOCH$_3$,3-OH	0.370±0.027	0.462±0.039	0.505±0.043	0.608±0.051	0.638±0.035	0.641±0.032	0.755±0.025	0.737±0.047					
Ar-COOCH$_3$,4-OH	0.366±0.016	0.429±0.024	0.509±0.047	0.637±0.042	0.667±0.038	0.663±0.027	0.776±0.037	0.748±0.047					
Ar-COOCH$_3$,2-Cl						0.274±0.052	0.350±0.058	0.460±0.059	0.630±0.049	0.681±0.044	0.801±0.042		
Ar-COOCH$_3$,4-Cl						0.281±0.029	0.362±0.045	0.441±0.033	0.520±0.034	0.659±0.039	0.735±0.022		
Ar-COOC$_2$H$_5$,2-OH									0.299±0.030	0.359±0.047	0.524±0.082	0.668±0.040	0.602±0.134
Ar-COOC$_2$H$_5$,3-OH		0.306±0.032	0.356±0.052	0.451±0.056	0.546±0.028	0.549±0.034	0.691±0.026	0.618±0.036	0.818±0.037				
Ar-COOC$_2$H$_5$,4-OH	0.215±0.016	0.312±0.026	0.387±0.033	0.477±0.052	0.549±0.031	0.548±0.033	0.700±0.040	0.684±0.059	0.813±0.039				
Ar-COOC$_2$H$_5$,2-Cl						0.229±0.036	0.312±0.051	0.391±0.065	0.566±0.029	0.677±0.043	0.757±0.030		
Ar-COOC$_2$H$_5$,4-NH$_2$	0.267±0.028	0.360±0.031	0.456±0.058	0.533±0.037	0.585±0.018	0.618±0.042	0.695±0.028	0.714±0.061					
Ar-COOC$_3$H$_7$,2-OH									0.214±0.020	0.335±0.046	0.425±0.041	0.522±0.045	0.610±0.043
Ar-COOC$_3$H$_7$,4-OH			0.232±0.032	0.310±0.040	0.402±0.030	0.490±0.061	0.599±0.063	0.589±0.055	0.784±0.051				
Ar-COOC$_3$H$_7$,2-Cl								0.254±0.030	0.414±0.007	0.510±0.039	0.590±0.059	0.675±0.044	0.724±0.045
Ar-COOC$_6$H$_5$,2-OH							0.196±0.023	0.304±0.036	0.432±0.039	0.559±0.054	0.685±0.033	0.678±0.048	0.738±0.016

Each *Rf* value represents the mean±S.D. of 6—10 determinations.

physical interpretation available for C_M, it has been demonstrated that the anomalies are nearly always multiples of C_M.[28,29)]

The *Rm* value and the $\log k'$ analogous to it are directly proportional to the logarithm of the partition coefficient according to the equations (Collander-type equations)[29-31)]:

$$\log P = aR_m + b \tag{11}$$

$$\log P = a' \log k' + b' \tag{12}$$

The following two equations express the relationship of Rm_0 and *kn* as a function of Σf_R, and Rm_0 as a function of $\Sigma f_{L/H}$ of compounds **19—21**, **24—26**, **28—30**, and **32**.

$$\Sigma f_R = 0.8838 + 0.9877 Rm_0 - 0.2753 kn \tag{13}$$

$n=10 \quad r=0.9873 \quad s=0.0849 \quad F=134.686$

where *n* is the number of observations
r is the correlation coefficient
F is the *F*-value

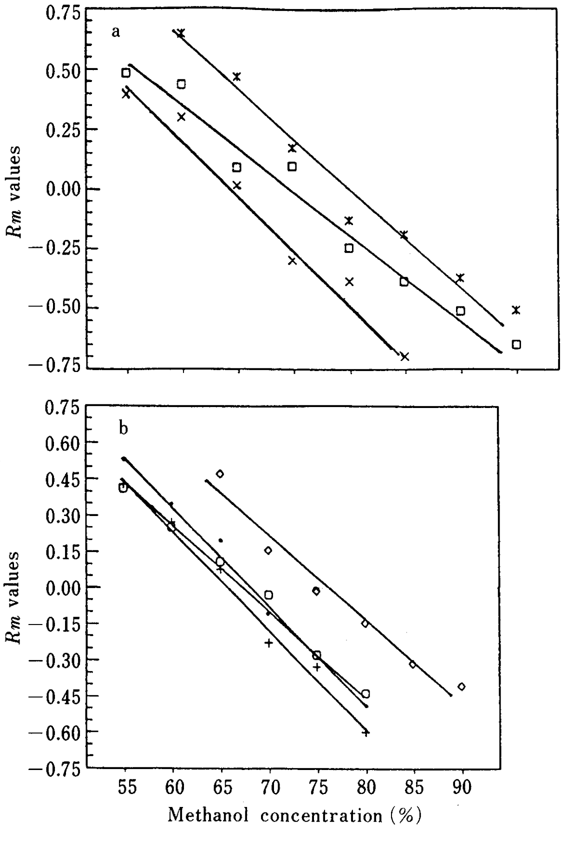

Fig. 2. Relationship between *Rm* Values of Derivatives of Benzoic Acid Esters and Methanol Concentration in the Mobile Phase

$$\Sigma f_{L/H} = 0.6934 + 1.1460 Rm_0 \tag{14}$$

$n=10 \quad r=0.9525 \quad s=0.3024 \quad F=78.267$

The relationship of $\log P$ and Rm_0 of compounds 19—21, 24—26, and 29—30 results:

$$\log P = 1.6645 + 0.4511 Rm_0 \tag{15}$$

$n=8 \quad r=0.6795 \quad s=0.2672 \quad F=5.1476$

The best separation of compounds **16—18**, **22**, **23**, **27**, and **31** (Fig. 2) was observed with 70% methanol. $\log P$, Σf_R, *kn*, $\Sigma f_{L/H}$ and Rm_{70} of these compounds are correlated as follows

$$\log P = 2.9877 + 2.2137 Rm_{70} \tag{16}$$

$n=7 \quad r=0.9491 \quad s=0.1433 \quad F=45.434$

$$\Sigma f_R = 2.8715 + 2.1463 Rm_{70} - 0.2569 kn \tag{17}$$

$n=7 \quad r=0.9953 \quad s=0.0673 \quad F=210.332$

$$\Sigma f_{L/H} = 3.2235 + 2.4553 Rm_{70} \tag{18}$$

$n=7 \quad r=0.6794 \quad s=0.5166 \quad F=4.286$

Compounds **19**, **21**, and **30** were studied in both chromatographic systems with mobile phase acetone and methanol, respectively. Table V shows the measured *Rf* and calculated Rm_0 values with acetone–chloride buffer (pH 1) as the mobile phase.

In comparison, Tables IV and V show that there is a disagreement between the calculated Rm_0 values for both chromatographic systems. The Rm_0 values should be the property of the solute and independent of the kind of organic co-solvent of the mobile phase. Between the Rm_0 $(CH_3)_2$-

TABLE IV. Rm_0 and *kn* Values of Esters of Benzoic Acid

Compound	Rm_0	*kn*
$Ar\text{-}COOCH_3$	2.886	0
$Ar\text{-}COOC_2H_5$	2.333	0
$Ar\text{-}COOC_3H_7$	2.949	0
$Ar\text{-}COOCH_3$,2-OH	1.347	2
$Ar\text{-}COOCH_3$,3-OH	0.773	0
$Ar\text{-}COOCH_3$,4-OH	0.851	0
$Ar\text{-}COOCH_3$,2-Cl	2.720	−2
$Ar\text{-}COOCH_3$,4-Cl	2.270	0
$Ar\text{-}COOC_2H_5$,2-OH	2.350	4
$Ar\text{-}COOC_2H_5$,3-OH	1.245	0
$Ar\text{-}COOC_2H_5$,4-OH	1.351	0
$Ar\text{-}COOC_2H_5$,2-Cl	2.869	−3
$Ar\text{-}COOC_2H_5$,4-NH_2	1.373	2
$Ar\text{-}COOC_3H_7$,2-OH	3.096	4
$Ar\text{-}COOC_3H_7$,4-OH	1.825	0
$Ar\text{-}COOC_3H_7$,2-Cl	2.607	−3
$Ar\text{-}COOC_6H_5$,2-OH	2.661	3

TABLE V. *Rf* Values and Rm_0 Values of Benzoic Acid Esters

Compound	*Rf* Acetone concentraion (%)							Rm_0
	30	35	40	45	50	55	60	
$Ar\text{-}COOCH_3$,2-OH			0.193±0.019	0.268±0.055	0.374±0.068	0.55 ±0.079	0.627±0.006	0.938
$Ar\text{-}COOCH_3$,4-OH	0.490±0.032	0.565±0.021	0.640±0.029	0.669±0.021	0.724±0.032	0.773±0.028	0.810±0.040	0.727
$Ar\text{-}COOC_3H_7$,4-OH		0.284±0.032	0.375±0.047	0.455±0.014	0.539±0.044	0.635±0.028	0.708±0.070	1.721

Each *Rf* value represents the mean±S.D. of 7—14 determinations.

Table VI. $\log k'$, $\log k'_w$ and kn Values of Benzoic Acid Derivatives

Compound	$\log k'$ Methanol concentration (%) 20	30	40	45	50	55	60	70	80	$\log k'_w$	kn
Ar-COOH		0.66	0.42	0.25	0.11	−0.18	−0.20	−0.48		1.587	0
Ar-COOH,2-OH		0.72	0.41	0.27	0.08	−0.07	−0.17	−0.48		1.871	2
Ar-COOH,3-OH	0.50	0.24	−0.04	−0.21	−0.39	−0.68	−0.70	−1.16		1.261	0
Ar-COOH,4-OH	0.36	0.10	−0.13	−0.29	−0.47	−0.76	−0.78	−1.36		1.178	0
Ar-COOH,2-Cl	0.97	0.69	0.38	0.24	0.03	−0.16	−0.30	−0.59		1.928	−2
Ar-COOH,4-Cl			0.91	0.77	0.59	0.26	0.22	−0.16		2.411	0
Ar-COOH,2-NO$_2$	0.25	−0.07	−0.38	−0.56	−1.06	−1.28				2.024	2
Ar-COOH,3-NHCOCH$_3$	0.67	0.34	−0.09	−0.15	−0.35	−0.57	−0.70	−1.16		1.434	2
Ar-COOH,3-CH$_3$		1.10	0.69	0.59	0.43	0.10	0.06	−0.34		2.181	0
Ar-COOH,2,5-OH	0.18	−0.04	−0.38	−0.54	−0.72	−0.86	−1.11			1.191	2
Ar-COOH,2-OH,4-NHCOCH$_3$	0.64	0.33	−0.04	−0.22	−0.38	−0.56	−0.74	−1.28		1.707	4
Ar-COOH,2-OH,5-NHCOCH$_3$	0.29	0.00	−0.12	−0.53	−0.83	−0.95	−1.28			1.565	4
Ar-COOH,2,3-OCH$_3$	0.99	0.64	0.19	0.02	−0.09	−0.29	−0.45	−0.81		1.722	0
Ar-COOH,2-OH,4-Cl			0.75	0.66	0.52	0.39	0.19	−0.17		2.432	2
Ar-COOH,2-OH,5-Br			0.86	0.58	0.46	0.33	0.18	−0.15		2.539	2
Ar-COOCH$_3$			0.81	0.70	0.52	0.33	0.16	−0.14		1	0
Ar-COOC$_2$H$_5$				0.99	0.79	0.58	0.38	0.04	−0.33	2.657	0
Ar-COOC$_3$H$_7$					1.09	0.84	0.61	0.24	−0.16	3.105	0
Ar-COOCH$_3$,2-OH			1.01	0.88	0.73	0.49	0.39	0.08	−0.28	2.317	2
Ar-COOCH$_3$,3-OH		0.73	0.34	0.28	0.06	−0.13	−0.31	−0.63		1.751	0
Ar-COOCH$_3$,4-OH		0.70	0.37	0.24	0.07	−0.15	−0.24	−0.57		1.653	0
Ar-COOCH$_3$,2-Cl			0.96	0.81	0.64	0.42	0.26	−0.12	−0.48	2.441	−2
Ar-COOCH$_3$,4-Cl					1.00	0.77	0.53	0.19	−0.13	2.821	0
Ar-COOC$_2$H$_5$,2-OH					0.96	0.74	0.60	0.24	−0.18	2.816	2
Ar-COOC$_2$H$_5$,3-OH			0.65	0.52	0.33	0.11	−0.007	−0.41		2.100	0
At-COOC$_2$H$_5$,4-OH			0.64	0.52	0.33	0.10	−0.04	−0.41		2.101	0
Ar-COOC$_2$H$_5$,2-Cl				1.08	0.86	0.63	0.42	0.06	−0.33	2.850	−2
Ar-COOC$_2$H$_5$,4-NH$_2$		0.96	0.63	0.51	0.33	0.12	−0.01	−0.32		2.487	3
Ar-COOC$_3$H$_7$,2-OH						1.07	0.85	0.45	0.03	3.344	2
Ar-COOC$_3$H$_7$,4-OH			1.04	0.87	0.68	0.37	0.25	−0.16	−0.62	2.721	0
Ar-COOC$_3$H$_7$,2-Cl					1.16	0.90	0.67	0.27	−0.15	3.280	−2
Ar-COOC$_6$H$_5$,2-OH						1.12	0.87	0.44		3.585	3

Each $\log k'$ value represents the mean of 2—3 determinations.

CO and Rm_0 CH_3OH values is the following expression:

$$Rm_0(CH_3)_2CO = -0.2348 + 1.0168 Rm_0 CH_3OH \qquad (19)$$

$$n=3 \quad r=0.9455 \quad s=0.2409 \quad F=8.444$$

In spite of the different extrapolated Rm values, Eq. 19 shows a high correlation coefficient. The disagreement of Rm_0 values seems to come possibly from the different chemical structures of methanol (proton donor and acceptor) and acetone (proton acceptor).

Table VI shows $\log k'$ values as measured by RP-HPLC as well as extrapolated to pure aqueous eluent $\log k'$, $\log k'_w$ and kn. The calculated values of $\log k'_w$ for compounds **1**—**15** were corrected because of ionization. In this case the Collander type equations are valid.

$$\log P = 0.6806 + 0.7588 \log k'_w \qquad (20)$$

$$n=27 \quad r=0.8124 \quad s=0.3466 \quad F=48.559$$

$$\Sigma f_R = 0.1044 + 0.9745 \log k'_w - 0.2837 kn \qquad (21)$$

$$n=32 \quad r=0.9942 \quad s=0.0912 \quad F=1239.559$$

$$\Sigma f_{L/H} = 0.2939 + 1.2267 \log k'_w \qquad (22)$$

$$n=32 \quad r=0.9153 \quad s=0.3518 \quad F=154.991$$

When we use key numbers as an extra parameter in regression Eqs. 13, 17, 21 we observe that the quantified correction, kn, is accompanied by a regressor which does not appreciably differ from the factor C_M.

The above equations show that the Rekker fragmental method gives the best results because of the higher r and F values and the lower s value as the Leo/Hansch method and the octanol–water partition coefficients.

According to Rekker's theory in the present investigation, the 2-OH group with $kn=2$ for hydrogen bonding and $kn=1$ for cross-conjugations results in increased lipophilicity for RP-HPLC determinations. This proposal is effective on the RP-HPLC system and not on the RP-TLC system for both the extrapolated and non-extrapolated values. The observed abnormality on the RP-TLC system probably is due to hydration differences of functional groups.

In the case of the -Cl group, which is a sufficiently bulky substituent in the *ortho*-position to the functional group, because of the resonance interaction it abandons it's coplanarity with the aromatic ring-system. That has as a result a decrease in lipophilicity. These resonance interactions by -Cl cause the subtraction of $2C_M$ (RP-HPLC system) and $2C_M - 3C_M$ (RP-TLC system).

In the case of the 2-NO_2 group, it has to be considered as a resonance-increasing group.

For the 3-OH, 3-CH_3 and 2,3-OCH_3 groups the use of C_M is not necessary for the approach between the experimental and the theoretical values of lipophilicity.

The presence of the 3-$NHCOCH_3$ substituent makes necessary a totally different hydration pattern for -COOH and as an unexpected result the lipophilicity may change in quantifiable steps of $2C_M$.

For the 4-OH and 4-Cl groups it is concluded that the use of C_M is not necessary for both the RP-TLC and the RP-HPLC measurements. Furthermore, for compounds which do not need the corrected value of 0.289 we also do not have to use the extrapolated values.

The resonance effect results in the use of $2C_M$ (RP-TLC) and $3C_M$ (RP-HPLC) for the 4-NH_2 groups.

In conclusion, the Σf value (index of lipophilicity) was determined by RP-TLC and RP-HPLC indirectly and the experimental values were compared to those theoretically obtained by both the fragmental method of Rekker and the Leo/Hansch fragmental system, respectively.

The statistical evaluations were more reliable in the Rekker system. The presence of 2-OH, 2-NO_2, 3-$NHCOCH_3$ and 4-NH_2 groups resulted in the increase of lipophilicity and its quantitative value depended on the specific chromatographic technique. The presence of the 2-Cl group resulted in the decrease of lipophilicity and it also seemed that the quantitative value of this decrease depended on the technique.

The substitutes 3-OH, 3-CH_3, 2,3-OCH_3, 4-Cl and 4-OH did not have any influence on lipophilicity. There was no statistically significant correlation between the Σf values of the organic acids which were determined by RP-TLC.

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