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R_M VALUES FROM REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AS PARAMETERS OF LIPOPHILICITY IN QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS IN FOUR SERIES OF ARYLALIPHATIC ACIDS

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SUMMARY

Series of β-aryl-*n*-butyric (I), arylacetic (II), α-methyl-β-arylpropionic (III) and cinnamic (IV) acids were subjected to reversed-phase thin-layer chromatography on silica gel impregnated with silicone oil. In the series of acids I, II, and IV, characterized by a broad range of lipophilicity of the aromatic substituents, a non-linear course of the relationship between R_M values and π parameters was found. The influence was studied of these non-linear relationships on the usability of R_M values for the characterization of lipophilicity in quantitative structure–activity relationships. A change from a linear form of activity–π dependence to a parabolic form of activity–R_M dependence was found solely in instances in which the statistical significance of the linear dependence was characterized by a high correlation coefficient, approaching 0.99. In such exceptional instances, requiring the highly accurate determination of the biological activity concerned, a fallacious quadratic activity–lipophilicity relationship may thus appear. In most of the regression equations studied, however, the use of R_M values instead of π parameters did not influence the activity–lipophilicity relationships.

With the aid of values obtained from partition chromatography, the lipophilicity of some disubstituted derivatives of acids I–IV was characterized, in which the addiditivy of the π parameters could be expected to fail as a result of the action of the *ortho*-effect of their substituents. For this purpose either the R_M values were used directly, or π parameters calculated from the relationship between π and R_M values were used.

INTRODUCTION

Recently we investigated[1] the lipophilicities of β-aryl-*n*-butyric and arylacetic acids with the aid of partition thin-layer chromatography (TLC). We used silica gel impregnated with silicone oil, which is frequently used[2–5] for the evaluation of lipophilicity in biological correlations. Applying the method proposed by Hulshoff and Perrin[6,7], we found that in the chromatographic separation of both series of acids an adsorption mechanism participated, in addition to the partition mechanism. The

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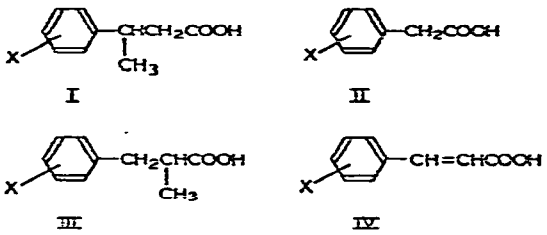
dichotomy of the mechanism manifests itself in deviations from linearity in the correlations between the tabulated π parameters and the experimental R_M values. Within a broader range of lipophilicity, the non-linear correlation between the π and R_M values can be substituted[1] either by a quadratic dependence or by a pair of straight lines with different regions of lipophilicity of the substituents.



In this study we applied the R_M values to correlations of certain biological activities in series of β-aryl-*n*-butyric (I), arylacetic (II), α-methyl-β-arylpropionic (III) and cinnamic (IV) acids. We aimed to establish the way in which the non-linear correlation between π and R_M modified the dependences of biological activities on R_M values. We studied first the inhibition of the heat denaturation of serum albumin and the activation of fibrinolysis, phenomena which depend solely on the lipophilicity, as ascertained in previous studies[8–12]. We also performed, for the series of acids I, regression analyses of erythrocyte membrane stabilization against hypotonic haemolysis and of the anti-inflammatory activity in the test for inhibition of kaolin-induced oedema. These processes are dependent on both the lipophilicity and the polar effects of aromatic substituents[13].

EXPERIMENTAL

Chromatography

The stationary phase was prepared by shaking 25 g of silica gel GF_{254} for 90 sec with a mixture of x% (v/v) of silicone oil with 6 ml of acetone and diluting with dioxane to 50 ml. The glass plates (10 × 20 cm) were covered with a 0.25-mm layer of a slurry of the support using standard equipment. The volatile components of the impregnating solutions were evaporated within 16 h at 20°C.

Solutions (1%) of acids I–IV in methanol were prepared, and 5-μl samples were applied to the plate 3 cm from the lower edge. After evaporating the methanol at 20°C, ascending, one-dimensional TLC was carried out using 50% acetone containing a citrate buffer (pH 3.4) as the mobile phase. A chromatographic chamber was equilibrated for 16 h with the mobile phase at 20°C. After migration for 15 cm the plates were removed and, after evaporating the remaining mobile phase, the acids were detected under UV light (λ = 254 nm). Each chromatogram contained six compounds, two acids serving as reference samples. In the individual chromatograms the R_F values of the standards did not differ by more than 0.02.

Sample preparation

β-Aryl-*n*-butyric acids (I) were prepared by the method proposed by Asano *et al.*[14] and described in detail elsewhere[8,15]. Arylacetic acids (II) were prepared by

the Wilgerodt reaction; alkoxy derivatives were obtained[16] by alkylation of the methyl esters of the corresponding 4-hydroxyarylacetic acids and subsequent hydrolysis. α-Methyl-β-arylpropionic acids (III) were obtained[10] either by hydrogenation of substituted α-methylcinnamic acids or by hydrolysis and decarboxylation of the corresponding α-benzyl-α-methylmalonates. Cinnamic acids (IV) were prepared by the Wittig reaction of substituted benzaldehydes with ethoxycarbonylmethylene phosphorane[17].

Biochemical testing

Inhibition of the heat denaturation of bovine serum albumin was determined according to Mizushima[18] as described in ref. 8. The efficiency was expressed by the molar concentration, C^I, causing 50% inhibition.

Activation of fibrinolysis was measured by the "hanging clot" method[19]. The efficiency was expressed by the minimum molar concentration, C^F, that dissolved the coagulum after incubation for 24 h at 37°C.

Stabilization of erythrocyte membranes against hypotonic haemolysis was determined by the method proposed by Kalbhen *et al.*[20], modified by using whole rat blood[21]. The activity was expressed as the concentration, C^{St}, in moles per litre, bringing about 50% inhibition of haemolysis.

Kaolin oedema inhibition was measured according to Hillebrecht[22] as described in detail in ref. 23. The effects of the compounds tested were expressed in terms of the percentage inhibition of inflammation, and the activity index, I^K, was calculated as the ratio of the effects of the compound tested and of the standard; the standard was 3-chloro-4-benzyloxyphenylacetic acid[23].

Calculations

In the regression analysis, the π parameters derived[24] for arylacetic (in the series I–III) and benzoic (in the series IV) acids were used. The π parameters for alkoxy and for higher alkyl groups were calculated from the value for the methoxy group, or the methyl group, and from the following increments[25]: $\Delta\pi = 0.5$ for aliphatic CH_2, 0.41 for cyclic CH_2, −0.2 for branching and −0.3 for a double bond. For the calculation of $\Sigma\pi$ for disubstituted derivatives, the difference between the lipophilicity[26] of remaining aromatic parts, $C_6H_4<$ and $C_6H_3<$, was taken into consideration, so that the value 0.23, corresponding[27] to 0.5 log P of hydrogen, was subtracted from the sum of both substituents.

The coefficients in the regression equations were calculated from experimental results by multiple regression analysis using the least-squares method on a Hewlett-Packard 9820 computer. The statistical significances of the regression equations were tested by the standard deviation, s, the coefficient of multiple correlation, r, and the Fischer–Snedecor criterion, F. Individual parameters were evaluated statistically by Student's t-test at the minimal significance level $\alpha = 0.001$; the exceptions are noted in the text.

RESULTS AND DISCUSSION

β-Aryl-n-butyric acids

In the series of acids Ia–In the R_M values were determined with two concen-

TABLE I

CHROMATOGRAPHIC BEHAVIOUR AND BIOLOGICAL ACTIVITIES OF β-ARYL-*n*-BUTYRIC ACIDS (I)

No.	*X*	π*	R_F**		R_M**		*Log (1/C^I)****	*Log (1/C^P)****	*Log (1/CSt)*†	*Log(I^K)*†
			A	*B*	*A*	*B*				
Ia	H	0	0.65	0.58	−0.27	−0.14	3.046	0.922	3.088	0.51
Ib	4-CH_3O	0.01	0.655	0.61	−0.28	−0.19	3.097	1.046	3.079	0.48
Ic	4-CH_3	0.45	0.60	0.525	−0.18	−0.04	3.444	1.097	3.127	0.47
Id	4-Cl	0.70	0.59	0.495	−0.16	0.01	3.495	1.398	3.288	0.27
Ie	4-*iso*-C_3H_7O	0.81	0.58	0.45	−0.14	0.09	3.514	1.398	−††	−††
If	4-C_2H_5	0.90	0.56	0.45	−0.10	0.09	3.770	1.456	3.191	−0.39
Ig	4-Br	0.90	0.57	0.46	−0.12	0.07	3.602	1.523	3.258	−0.24
Ih	3-Br	0.91	0.565	0.46	−0.11	0.07	3.569	1.523	3.352	−0.24
Ii	4-*iso*-C_3H_7	1.40	0.52	0.39	−0.03	0.19	3.863	1.699	3.281	−0.28
Ij	4-*iso*-C_4H_9	1.90	0.435	0.30	0.11	0.37	4.125	2.155	3.414	−0.16
Ik	4-*iso*-C_5H_{11}	2.25	0.335	0.225	0.30	0.54	4.377	2.222	3.430	−0.15
Il	4-*n*-C_5H_{11}	2.45	0.32	0.20	0.33	0.60	4.398	−†††	3.513	−0.04
Im	4-*n*-$C_6H_{13}O$	2.51	0.30	0.195	0.37	0.62	4.280	−†††	−†††	−††
In	4-2′-ethylhexyl	3.90	0.14	0.06	0.80	1.19	−†††	−†††	3.895	−0.25
Io	3-Br-4-*iso*-C_3H_7	2.08 (1.70†)	0.435	0.305	0.11	0.36	4.046	2.097	3.470	−0.13
Ip	3-Br-4-*iso*-C_4H_9	2.58 (2.20†)	0.355	0.23	0.26	0.52	4.301	2.301	3.650	0.04
Ir	3-Br-4-*n*-C_5H_{11}	3.28 (2.77†)	0.275	0.16	0.42	0.73	4.456	−†††	−††	−††
Is	4-$C_6H_5CH_2$	2.34 (1.96†)	0.40	0.295	0.18	0.38	−††	−††	3.421	−0.56

* π parameters taken from refs. 24 and 25.

** Chromatography was performed on silica gel containing (A) 3.5% and (B) 7.5% of silicone oil.

*** Activities taken from refs. 8 and 15.

† Activities taken from ref. 13.

†† Not established.

††† Insoluble under the conditions of the test.

‡ Average values calculated from R_M values using eqns. 1 and 2; these values were used in correlation analysis of biological activities (eqns. 3, 6, 9 and 12).

trations of silicone oil in the silica gel (see Table I). The correlations between the π parameters and the R_M values are expressed by eqn. 1 for the 3.5% concentration and eqn. 2 for the 7.5% concentration.

	n	r	s	F	
$\pi = 4.030\,R_{M(3.5)} - 1.138\,[R_{M(3.5)}]^2 + 1.316$	14	0.994	0.131	460	(1)
$\pi = 3.481\,R_{M(7.5)} - 0.625\,[R_{M(7.5)}]^2 + 0.622$	14	0.998	0.082	1168	(2)

From these equations, with experimental R_M values inserted, the $\Sigma\pi$ values of substituents were calculated for the acids Io–Ir. The values obtained are lower than would correspond to the tabulated figures (see Table I), probably owing to a hydrophobic interaction between the *ortho*-substituents. For the acids Ia–Ir, regression analysis of the results of the test for the inhibition of heat denaturation of serum albumin led to eqn. 3. If in this series of acids the lipophilicity is characterized by the experimental R_M values, then the dependence of the inhibitory activity on lipophilicity is expressed by eqns. 4 and 5.

	n	r	s	F	
$\log(1/C^I) = 0.504\,\pi + 3.148$	16	0.986	0.080	493	(3)
$\log(1/C^I) = 2.243\,R_{M(3.5)} - 2.575\,[R_{M(3.5)}]^2 + 3.905$	16	0.993	0.060	446	(4)
$\log(1/C^I) = 2.118\,R_{M(7.5)} - 1.050\,[R_{M(7.5)}]^2 + 3.468$	16	0.988	0.076	275	(5)

In eqns. 4 and 5, the $R_M{}^2$ terms are statistically significant at the $\alpha = 0.005$ level. This apparent parabolic activity–lipophilicity relationship evidently results from the quadratic relationships in eqns. 1 and 2, respectively.

For fibrinolysis activation, in this series of acids we calculated the regression eqn. 6. Owing to insolubility of the more strongly lipophilic derivatives II, m, n and r under the conditions of the test, the lipophilicity range is narrower in this instance. Consequently, when the R_M values are used, a linear relationship between the activation of fibrinolysis and lipophilicity remains preserved, as is evident from eqns. 7 and 8. Additional insertion of $R_M{}^2$ terms into these equations lowers their statistical significance.

	n	r	s	F	
$\log(1/C^F) = 0.608\,\pi + 0.942$	13	0.987	0.077	428	(6)
$\log(1/C^F) = 2.395\,R_{M(3.5)} + 1.715$	13	0.966	0.124	156	(7)
$\log(1/C^F) = 1.932\,R_{M(7.5)} + 1.314$	13	0.978	0.100	245	(8)

For the stabilization of erythrocyte membranes against hypotonic haemolysis, the dependence on the lipophilicity and polar effects of substituents was calculated, expressed by eqn. 9. In this equation the partial correlation coefficients have the value $r(\pi) = 0.951$ and $r(\sigma) = 0.124$. By using the R_M values for the characterization of lipophilicity, the linear dependence on lipophilicity did not change, as is evident from eqns. 10 and 11.

	n	r	s	F	
$\log(1/C^{St}) = 0.206\,\pi + 0.246\,\sigma + 3.068$	15	0.981	0.045	158	(9)
$\log(1/C^{St}) = 0.757\,R_{M(3.5)} + 0.290\,\sigma + 3.322$	15	0.988	0.037	246	(10)
$\log(1/C^{St}) = 0.608\,R_{M(7.5)} + 0.261\,\sigma + 3.201$	15	0.988	0.037	241	(11)

We assume that the statistical significance of R_M^2 values in the regression equations are influenced by the statistical significance of the lipophilicity–activity linear relationship.

An analogous result was obtained in assessments of the dependence of anti-inflammatory activity on the lipophilicity parameters π and R_M. In this series of acids I we found[13] that kaolin oedema inhibition had a quadratic dependence on lipophilicity and a linear dependence on the polar constants σ of aromatic substituents (eqn. 12), with partial correlation coefficients $r(\pi) = 0.71$, $r(\pi^2) = 0.46$ and $r(\sigma) = 0.31$. In this instance also the original quadratic dependence of activity on lipophilicity underwent no change when the R_M values were used for characterizing the lipophilicity (see eqns. 13 and 14).

	n	r	s	F	
$\log I^K = 0.302\pi - 0.056\pi^2 + 0.175\sigma - 0.515$	15	0.952	0.052	35.5	(12)
$\log I^K = 0.718\,R_{M(3.5)} - 0.873\,[R_{M(3.5)}]^2 + 0.209\sigma - 0.223$	15	0.967	0.044	52.1	(13)
$\log I^K = 0.764\,R_{M(7.5)} - 0.534\,[R_{M(7.5)}]^2 + 0.182\sigma - 0.356$	15	0.957	0.049	40.4	(14)

Arylacetic acids

In the series of acids IIa–IIs the relationships between the π parameters and the R_M values, determined on silica gel impregnated with 3% silicone oil, are expressed by eqns. 15 and 16.

	n	r	s	F	
$\pi = 3.571\,R_M + 2.236$	18	0.995	0.119	1824	(15)
$\pi = 3.513\,R_M - 0.787\,R_M^2 + 2.322$	18	0.998	0.076	2254	(16)

In eqn. 16 the quadratic term is statistically significant at the $\alpha = 0.001$ level. On insertion of the experimental R_M value into eqn. 16, $\Sigma\pi$ for the substituents of derivative IIt was calculated (see Table II); the decrease in lipophilicity against the assumed sum of the tabulated π values is evidently a consequence of the *ortho*-effect of the two alkoxy groups.

In the series of arylacetic acids the dependence of the inhibition of the heat denaturation of serum albumin on lipophilicity is expressed by a linear correlation (eqn. 17). When characterizing the lipophilicity by R_M values, we obtain eqn. 18, in which the quadratic term is statistically significant at the $\alpha = 0.005$ level.

	n	r	s	F	
$\log (1/C^I) = 0.590\,\pi + 2.615$	16	0.988	0.083	585	(17)
$\log (1/C^I) = 1.759\,R_M - 1.406\,R_M^2 + 4.003$	16	0.991	0.077	337	(18)

This result corresponds to an analogous dependence expressed by eqr.. 3 or 4 for the series of acids I. For fibrinolysis activation a linear dependence on lipophilicity was found, expressed by eqn. 19. When lipophilicity was characterized by R_M values, the linear correlation remained preserved (eqn. 20), probably again as a

TABLE II

CHROMATOGRAPHIC BEHAVIOUR AND BIOLOGICAL ACTIVITIES OF ARYLACETIC ACIDS (II)

No.	*X*	π*	R_F**	R_M	*Log* $(1/C^I)$***	*Log* $(1/C^F)$***
IIa	H	0	0.795	−0.59	—§	—§§
IIb	4-CH_3O	0.01	0.795	−0.59	2.523	—§§
IIc	4-Cl	0.70	0.725	−0.42	3.000	—§§
IId	4-*iso*-C_3H_7O	0.81	0.705	−0.38	3.056	1.000
IIe	4-C_2H_5	0.90	0.70	−0.37	3.056	1.222
IIf	3-Cl-4-CH_2=$CHCH_2O$	1.16	0.675	−0.32	3.377	1.398
IIg	3-Cl-4-*iso*-C_3H_7O	1.26	0.64	−0.25	3.511	1.398
IIh	4-*iso*-C_3H_7	1.40	0.645	−0.26	3.488	1.398
IIi	4-*tert.*-C_4H_9	1.68	0.59	−0.16	3.678	1.699
IIj	4-*iso*-C_4H_9	1.90	0.57	−0.12	3.724	1.699
IIk	4-cyclo-C_6H_{11}	2.46	0.50	0	4.125	2.222
IIl	4-cyclo-$C_6H_{11}CH_2O$	2.47	0.46	0.07	4.046	2.301
IIm	3-Cl-4-cyclo-$C_6H_{11}O$	2.51	0.49	0.02	4.131	2.222
IIn	4-*n*-$C_6H_{13}O$	2.51	0.46	0.07	4.034	2.222
IIo	3-Cl-4-cyclo-$C_6H_{11}CH_2O$	2.92	0.40	0.18	4.347	2.398
IIp	3-Cl-4-*n*-$C_6H_{13}O$	2.96	0.40	0.18	4.201	2.398
IIr	4-2'-ethylhexyl	3.90	0.265	0.47	—§§§	—§§§
IIs	3-Cl-4-*n*-$C_8H_{17}O$	3.96	0.215	0.56	—§§§	—§§
IIt	3-CH_3O-4-cyclo-$C_6H_{11}CH_2O$	2.28 (1.91†)	0.56	−0.10	3.801	2.000

* π parameters taken from refs. 24 and 25.

** Chromatography was performed on silica gel containing 2.5% of silicone oil.

*** Activities taken from ref. 16.

§ Not established.

§§ Inactive.

§§§ Insoluble under the conditions of the test.

† Average value calculated from R_M value using eqns. 15 and 16; this value was used in correlation analysis of biological activities (eqns. 17 and 19).

consequence of the lower statistical significance of the linear dependence of fibrinolysis activation on lipophilicity.

	n	*r*	*s*	*F*	
$\log(1/C^F) = 0.645\,\pi + 0.590$	14	0.982	0.094	328	(19)
$\log(1/C^F) = 2.419\,R_M + 2.076$	14	0.977	0.107	248	(20)

α-Methyl-β-arylpropionic acids

In this series of acids (IIIa–IIIm), the linear correlation between the tabulated π parameters and R_M values is expressed by eqn. 21.

	n	*r*	*s*	*F*	
$\pi = 2.827\,R_M + 0.625$	13	0.993	0.084	754	(21)

Owing to a narrower range of lipophilicity of the aromatic substituents, $(0 < \pi < 1.9)$, the parabolic π *versus* R_M relationship is statistically less significant. $\Sigma\pi$ values were calculated for the 3,4-disubstituted derivatives IIIn–IIIp (see Table III) by inserting experimental R_M values into eqn. 21.

TABLE III

CHROMATOGRAPHIC BEHAVIOUR AND BIOLOGICAL ACTIVITIES OF CYCLOHEXYLAMMONIUM SALTS OF α-METHYL-β-ARYLPROPIONIC ACIDS (III)

No.	*X*	π*	R_F**	R_M	*Log* $(1/C^I)$***	*Log* $(1/C^F)$***
IIIa	H	0	0.60	−0.18	3.192	0.921
IIIb	4-CH_3O	0.01	0.61	−0.19	3.169	0.921
IIIc	3-CH_3O	0.04	0.615	−0.20	3.169	—‡
IIId	3-Cl-4-CH_3O	0.46	0.535	−0.06	3.327	—‡‡
IIIe	3-Cl	0.68	0.49	0.02	3.498	1.222
IIIf	4-Cl	0.70	0.50	0	3.524	1.222
IIIg	4-*iso*-C_3H_7O	0.81	0.47	0.05	3.607	1.347
IIIh	4-Br	0.90	0.48	0.03	3.620	1.398
IIIi	3-Cl-4-*iso*-C_3H_7O	1.26	0.38	0.21	3.896	—‡‡‡
IIIj	4-*iso*-C_3H_7	1.40	0.345	0.28	3.982	1.824
IIIk	4-*tert.*-C_4H_9	1.68	0.28	0.41	4.071	2.000
IIIl	3-Cl-4-*iso*-C_4H_9O	1.76	0.28	0.41	4.201	—‡‡‡
IIIm	4-*iso*-C_4H_9	1.90	0.26	0.45	4.292	2.222
IIIn	3-CH_3O-4-*iso*-C_3H_7O	0.62 (0.41†)	0.535	−0.06	3.327	1.097
IIIo	3-CH_3O-4-*n*-$C_5H_{11}O$	2.32 (1.80†)	0.275	0.42	4.171	2.097
IIIp	3-Br-4-*iso*-C_3H_7	2.08 (1.73†)	0.29	0.39	4.071	2.000

* π parameters taken from refs. 24 and 25.
** Chromatography was performed on silica gel containing 7.5% of silicone oil.
*** Activities taken from ref. 10.
‡ Inactive.
‡‡ Not established.
‡‡‡ Insoluble under the conditions of the test.
† These values, calculated from R_M values using eqn. 21, were used in correlation analysis of biological activities (eqns. 22 and 23).

In the series of acids III the dependences of the inhibition of denaturation of serum albumin and of fibrinolysis activation on lipophilicity are expressed by eqns. 22 and 23.

	n	*r*	*s*	*F*	
$\log(1/C^I) = 0.569\,\pi + 3.135$	16	0.993	0.047	1069	(22)
$\log(1/C^F) = 0.679\,\pi + 0.830$	12	0.990	0.070	493	(23)

In line with eqn. 21, in this instance substitution of π parameters by experimental R_M values led to the linear correlations in eqns. 24 and 25, equivalent to the preceding equations.

	n	*r*	*s*	*F*	
$\log(1/C^I) = 1.634\,R_M + 3.483$	16	0.989	0.061	621	(24)
$\log(1/C^F) = 1.960\,R_M + 1.247$	12	0.992	0.063	623	(25)

Cinnamic acids

In the series of acids IVa–IVl the correlations between the π parameters and the R_M values are expressed by the quadratic dependence in eqn. 26.

	n	*r*	*s*	*F*	
$\pi = 3.660\,R_M - 1.232\,R_M^2 + 1.130$	12	0.997	0.077	724	(26)

from which the values of $\Sigma\pi$ were calculated for derivatives IVm and IVn (see Table IV). Owing to the poor solubility of cinnamic acids under the conditions of both tests, it was possible to assess only the inhibition of denaturation of serum albumin in

TABLE IV

CHROMATOGRAPHIC BEHAVIOUR AND BIOLOGICAL ACTIVITY OF CINNAMIC ACIDS (IV)

No.	*X*	π*	R_F**	R_M	*Log* $(1/C^I)$***
IVa	H	0	0.655	−0.28	3.252
IVb	4-CH_2=$CHCH_2O$	0.78	0.69	−0.35	3.495
IVc	3-Cl	0.83	0.55	−0.09	3.509
IVd	4-Cl	0.87	0.53	−0.05	3.569
IVe	4-*iso*-C_3H_7O	0.88	0.54	−0.07	3.479
IVf	3-Cl-4-CH_2=$CHCH_2O$	1.38	0.47	0.05	3.757
IVg	4-*iso*-C_3H_7	1.40	0.44	0.10	3.801
IVh	3-Cl-4-*iso*-C_3H_7O	1.58	0.44	0.10	3.792
IVi	4-*iso*-C_4H_9	1.90	0.385	0.20	4.149
IVj	4-cyclo-$C_6H_{11}O$	2.18	0.315	0.34	—§
IVk	4-*n*-$C_6H_{13}O$	2.58	0.24	0.51	—§
IVl	3-Cl-4-*n*-$C_6H_{13}O$	3.18	0.16	0.72	—§
IVm	3-CH_3-O-4-*iso*-C_3H_7O	0.79 (0.52§§)	0.595	−0.17	3.306
IVn	3-CH_3O-4-*n*-$C_6H_{13}O$	2.49 (2.26§§)	0.295	0.38	4.279

* π parameters taken from refs. 24 and 25.

** Chromatography was performed on silica gel containing 7.5% of silicone oil.

*** Activities taken from ref. 12.

§ Insoluble under the conditions of the test.

§§ These values, calculated from R_M values using eqn. 26, were used in correlation analysis of the inhibition of serum albumin denaturation (eqn. 27).

eleven derivatives with lipophilicity not exceeding $\Sigma\pi = 2.2$ (acid IVn). For these compounds, the linear dependence on the lipophilicity was calculated, characterized either by the π parameters (eqn. 27) or by R_M values (eqn. 28).

	n	*r*	*s*	*F*	
$\log(1/C^I) = 0.487\,\pi + 3.123$	11	0.975	0.076	172	(27)
$\log(1/C^I) = 1.734\,R_M + 3.657$	11	0.981	0.066	235	(28)

CONCLUSION

For the four series of acids investigated, the results of regression analysis of various biological activities showed that the use of R_M values, obtained by reversed-phase TLC, for the characterization of lipophilicity was not markedly influenced by deviations from linearity of the π *versus* R_M correlations. Of nine examples of relationships between biological activity and π parameters studied, the application of R_M values for the characterization of lipophilicity led to a significant change in the activity–lipophilicity relationships solely in eqns. 4, 5 and 18. In these instances a linear relationship between the activity and π parameters is substituted by the fallacious parabolic dependence on lipophilicity expressed by R_M values. These pitfalls in the use of R_M values can probably be expected only in instances when the statistical

significance of the above linear relationship is characterized by a correlation coefficient approaching 0.99. Such exceptional instances also require a highly accurate determination of the biological activity concerned.

In several examples of compounds with combinations of substituents in which the additivity of π parameters could be expected to fail (acids Io–Is, IIt, IIIn–IIIp, IVm and IVn), it was possible to characterize their lipophilicity by quantities obtained from partition chromatography. For this purpose either the R_M values were used directly, or π parameters calculated from the relationship between π and R_M values were used.

REFERENCES

1 M. Kuchař, V. Rejholec, M. Jelínková, V. Rábek and O. Němeček, *J. Chromatogr.*, 162 (1979) 197.
2 G. L. Biagi, A. M. Barbaro, M. C. Guerra, G. Cantelli-Forti and M. E. Fracasse, *J. Med. Chem.*, 17 (1974) 28.
3 G. L. Biagi, O. Gandolfi, M. C. Guerra, A. M. Barbaro and G. Cantelli-Forti, *J. Med. Chem.*, 18 (1975) 868.
4 I. D. Turnball, G. L. Biagi, A. J. Merola and D. E. Cornwall, *Biochem. Pharmacol.*, 20 (1971) 1383.
5 G. L. Biagi, A. M. Barbaro, M. C. Guerra and M. F. Gamba, *J. Chromatogr.*, 44 (1969) 195.
6 A. Hulshoff and J. H. Perrin, *J. Chromatogr.*, 120 (1976) 65.
7 A. Hulshoff and J. H. Perrin, *J. Med. Chem.*, 20 (1977) 430.
8 M. Kuchař, B. Brůnová, V. Rejholec, Z. Roubal and O. Němeček, *Collect. Czech. Chem. Commun.* 41 (1976) 633.
9 M. Kuchař, B. Brůnová, Z. Roubal and O. Němeček, *Česk. Farm.*, 25 (1976) 105.
10 M. Kuchař, V. Rejholec, Z. Roubal and O. Němeček, *Collect. Czech. Chem. Commun.*, 44 (1979) 183.
11 V. Čepelák, Z. Roubal and M. Kuchař, *Folia Haematol. (Leipzig)*, 103 (1976) 343.
12 M. Kuchař, B. Brůnová, V. Rejholec, Z. Roubal and O. Němeček, in J. F. Davidson, M. M. Samama and V. Čepelák (Editor), *Progress in Chemical Fibrinolysis and Thrombolysis*, Vol. IV, Churchill Livingstone, Edinburgh, 1979, p. 48.
13 M. Kuchař, B. Brůnová, V. Rejholec and J. Grimová, *Eur. J. Med. Chem.*, 13 (1978) 363.
14 M. Asano, H. Motomatsu and O. Tamemasa, *J. Pharm. Soc. Jap.*, 70 (1950) 622.
15 M. Kuchař, B. Brůnová and V. Rejholec, *Česk. Farm.*, 26 (1977) 239.
16 M. Kuchař, B. Brůnová, Z. Roubal, J. Schlanger and O. Němeček, *Collect. Czech. Chem. Commun.*, in press.
17 M. Kuchař, B. Brůnová, V. Rejholec, Z. Roubal, J. Grimová and O. Němeček, *Collect. Czech. Chem. Commun.*, 40 (1975) 3546.
18 Y. Mizushima, *Arch. Int. Pharmacodyn. Ther.*, 149 (1964) 1.
19 K. N. von Kaulla, *J. Med. Chem.*, 8 (1965) 164.
20 D. A. Kalbhen, P. Gelderblom and R. Domenjoz, *Pharmacology*, 3 (1970) 353.
21 M. Kuchař, J. Grimová, Z. Roubal, O. Němeček and B. Kakáč, *Česk. Farm.*, 22 (1973) 388.
22 J. Hillebrecht, *Arzneim.-Forsch.*, 9 (1959) 625.
23 M. Kuchař, B. Brůnová, V. Rejholec, J. Grimová and O. Němeček, *Collect. Czech. Chem. Commun.*, 42 (1977) 1723.
24 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1864) 5175.
25 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
26 G. G. Nys and R. F. Rekker, *Eur. J. Med. Chem.*, 9 (1974) 361.
27 A. Leo, P. Y. C. Jow, C. Silipo and C. Hansch, *J. Med. Chem.*, 18 (1975) 865.