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REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY IN THE PARAMETRIZATION OF LIPOPHILICITY OF SOME SERIES OF ARYLALIPHATIC ACIDS

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SUMMARY

In the series of arylacetic acid and β-aryl-*n*-butyric acids, chromatography was carried out on a thin layer of silica gel impregnated with silicone oil, with 50% acetone as mobile phase. A separation mechanism in this system was evaluated using relationships between R_M values and the concentration of the lipophilic solvent in the silica-gel layer. It was found, that both partition and adsorption mechanisms participate, and that the adsorption effect increases with decreasing lipophilicity of the acids. The dichotomy of the mechanism manifests itself in the non-linear course of the relationships between R_M values and π parameters, or fragmental constants f derived from the partition system *n*-octanol—water. Such relationships can be expressed by a single quadratic dependence between lipophilic parameters and R_M values, or by two separate linear expressions with different slopes for different regions of substituent lipophilicities. The linear dependence between R_M and π at the lower range of lipophilicity is most probably made possible by significant linear dependence between π parameters and molecular surface areas of the substituents.

INTRODUCTION

Partition chromatography is one of the most important methods for the experimental evaluation of lipophilicity in quantitative structure—activity relationships. As evident from studies published by Martin and co-workers [1, 2], the relationship between R_M values and the logarithm of the partition coefficient can be expressed by eqn. 1. The quantity R_M is defined by Bate-Smith and Westall [3], P_S is the partition coefficient determined in a system identical with the chromatographic system, V_S and V_M are volumes of stationary and mobile phases, respectively. Provided the Collander [4] linear relationship (eqn. 2) is valid for the chromatographic system and for the reference system [5, 6] *n*-octanol—water, eqn. 1 can be rewritten as eqn. 3. An analogous linear relationship holds also when using π parameters instead of log P, or fragmental constants f [7]. These equations are valid on the a priori assumption that the partition mechanism prevails and that the adsorption effects are negligible.

$$R_M = \log P_s + \log (V_S/V_M) \quad (1)$$

$$\log P_s = a \cdot \log P + b \quad (2)$$

$$R_M = a \cdot \log P + c \quad (3)$$

The partition chromatography may be carried out by various methods differing in the character of the stationary phase. In paper chromatography [8, 9] a paper impregnated with a polar solvent is usually used. When reversed-phase thin-layer chromatography is performed an absorbent as support for lipoid stationary phase is used. Amongst others, silicone oil [10—13], *n*-octanol [14, 15] and liquid paraffin [15, 16] have been preferentially used as suitable solvents.

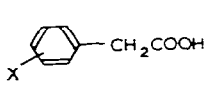
In many recent papers concerning chromatographic determination of lipophilicity, the partition mechanism has been assumed but not, however, always proved. Only recently Hulshoff and Perrin [17, 18] have described the possibility of verifying the partition mechanism in reversed-phase thin-layer chromatography. Deriving a condition for the validity of the partition mechanism, they started from eqn. 1 assuming that a ratio of volumes of both phases is linearly dependent on a volume concentration of lipophilic solvent in the stationary phase according to eqn. 4. Substituting into eqn. 1, eqn. 5 was obtained which holds for dissociable compounds, provided the dissociation is prevented by proper selection of the pH of the chromatographic system. Eqn. 5, on substituting for R_M and taking antilogarithm, yields eqn. 6, expressing the relationship between R_F values and C_{oil}. It is evident from eqn. 5 that a linear relationship with slope equal to 1 holds between R_M values and the log of the concentration of a lipophilic solvent in the stationary phase.

$$V_S/V_M = k \cdot C_{oil} \quad (4)$$

$$R_M = \log P_s + \log k + \log C_{oil} = \log C_{oil} + \text{constant} \quad (5)$$

$$1/R_F = P_s \cdot k \cdot C_{oil} + 1 \quad (6)$$

Hulshoff and Perrin [17] have found that in a system with Kieselguhr G impregnated with oleyl alcohol as stationary phase, the chromatography of phenothiazines was directed by a partition mechanism. However, verification of this mechanism for silica gel impregnated with a suitable lipoid solvent has not so far been made. We have been dealing with this problem in the series of arylacetic (I) and β-aryl-*n*-butyric (II) acids which were chromatographed on a silica-gel thin layer impregnated with silicone oil with 50% acetone as mobile phase.



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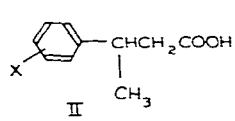

II

EXPERIMENTAL

Chromatography

For the preparation of the stationary phase, 25 g of silica gel G F_{254} were shaken for 90 sec with a mixture of x% (v/v) of silicone oil, 6 ml of acetone and diluted with dioxane to 50 ml. The glass plates (10 cm × 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 solution were evaporated off within 16 h at 20°.

Solutions of 1% of acids I and II in methanol were prepared, and 5-μl samples were applied to the plate 3 cm from the lower edge. After evaporating off the methanol at 20°, ascending one-dimensional chromatography was carried out using 50% acetone containing a buffer (pH 3.4) as mobile phase. The chromatographic chamber had been equilibrated for 16 h with the mobile phase. The temperature was kept at 20°. When 15 cm migration was attained, the plates were removed and, after evaporating off the remaining mobile phase, acids I and II were visualized in UV light (λ = 254 nm). For evaluation of R_M—log C_{oil} relationships, each chromatogram containing five compounds was repeated three times; the mean R_F values were taken for calculation of R_M. For evaluation of π—R_M dependencies, each chromatogram contained six compounds; two acids serving as reference samples were repeated on each chromatogram. In the chromatograms evaluated, the R_F values of the standards did not differ by more than 0.02.

Sample preparation

Arylacetic acids (I) were prepared from the corresponding substituted benzyl chlorides and sodium cyanide in dimethylsulphoxide with subsequent hydrolysis [19]. Benzyloxyphenylacetic acid (Io) and its *m*-chloro- (Ip) and *m*-methoxy- (In) derivatives were prepared [20] by the reaction of benzyl chloride with esters of *p*-hydroxyphenylacetic acid, or its *m*-chloro- and *m*-methoxy-analogues in the presence of sodium methoxide with subsequent hydrolysis.

β-Aryl-*n*-butyric acids (II) were prepared by the method of Asano et al. [21], which has been described in detail elsewhere [22, 23].

Calculations

In the regression analysis, the π parameters derived [24] for arylacetic acids were used. The π parameters for alkoxy groups and for higher alkyls were calculated from the value for the methoxy group, or the methyl, and from the following increments [25]: $\Delta\pi$ = 0.5 for CH_2, $\Delta\pi$ = —0.2 for branching. For calculation of $\Sigma\pi$ for disubstituted derivatives, a difference between the lipophilicity [7] of the remaining aromatic parts —C_6H_4— and —C_6H_3= was taken into consideration, so that the value 0.23 corresponding [26] to 0.5 log P of hydrogen was substracted from the sum of both substituents [9]. The values of the fragmental constants f were taken from ref. 7.

For calculation of the molecular surface areas of substituents, the spheric areas of single atoms were used, according to Bondi [27], because of the absence of generally accepted pear surface areas [28]. The values of Van der

TABLE I

VAN DER WAALS' RADII AND SURFACE AREAS OF ATOMS

Atom	Radius* (A)	Surface area ($10^2 A^2$)
C	1.7	0.363
H	1.1	0.152
O	1.4	0.246
Cl (aromatic)	1.8	0.407
Br (aromatic)	1.9	0.453

*Values are taken from ref. 28.

TABLE II

CORRECTION VALUES OF VAN DER WAALS' SURFACE AREA FOR SPHERE OVERLAPPING DUE TO COVALENT BONDING

Bond	Bond length (A)	Correction value ($10^2 A^2$)
C—C	1.5	0.203
C⋯C (aromatic)	1.4	0.214
C—H	1.1	0.132
C—O	1.4	0.162
C—benzene*	1.5	0.101
H—benzene*	1.1	0.091
O—benzene*	1.4	0.091
Cl—benzene*	1.8	0.091
Br—benzene*	1.9	0.091

*Correction value includes only overlapping of the atom bound to the aromatic nucleus.

Waals' radii and calculated surface areas of atoms are listed in Table I together with the bond lengths and correction values for sphere overlapping due to covalent bonding (Table II). For the atoms directly bound to the aromatic nucleus, the correction corresponds only to overlapping of the bound atom. For 3,4-disubstituted derivatives, the area of one hydrogen atom was subtracted from the sum of the surface areas.

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 statistically evaluated by the Student's t-test at the minimal significance level $\alpha = 0.005$; the exceptions in eqns. 12 and 22 are noted in the text.

RESULTS AND DISCUSSION

To determine the relationships between R_M values and concentration of silicone oil in the silica gel, the support was impregnated with 2.5, 3.5, 5.0, 6.2 and 7.5% of silicone oil. The influence of dissociation on chromatographic behavior of acids I and II was suppressed by suitably arranging the pH of the mobile phase. At pH 3.4, β-(*m*-bromophenyl)-*n*-butyric acid, the most acidic compound in both series (pK = 6.85 in 50% acetone), exists almost exclusively in unionized form.

The experimental results for five arylacetic and five β-aryl-*n*-butyric acids are summarized in Table III and Fig. 1. The linear dependences were evaluated by regression analysis; the results are summarized in Table IV. The variability of the slopes from 0.45 to 0.82 clearly demonstrates that the mechanism of chromatographic separation of the acids is not uniform. Provided the value of the slope can be taken as a measure of the contribution of the partition mechanism to the chromatographic process, the results show that the share of this mechanism increases with increasing lipophilicity of the substituents in both series of acids. The ratio of partition and adsorption mechanisms is also apparently influenced by other physico-chemical characteristics of these acids. The slopes for arylacetic acids (I) are generally higher than those for β-aryl-*n*-butyric acids (II), although the total lipophilicity, expressed as log P, is lower for arylacetic acids.

In both series of acids, we have studied an effect of a non-uniform mechanism of chromatographic separation upon relationships between R_M and other lipophilic quantities. The lipophilicity of the substituents was expressed either by π parameters, or by fragmental constants f. The chromatogra-

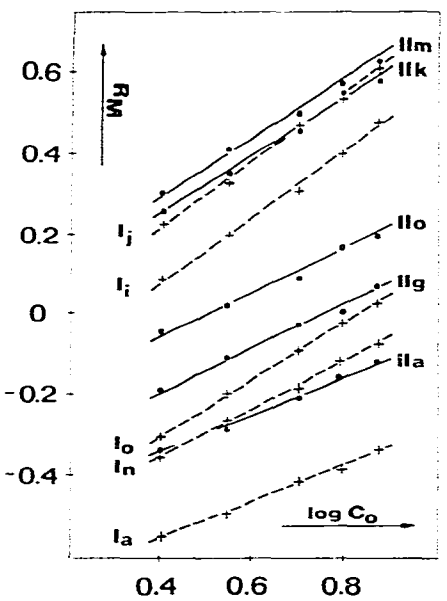

Fig. 1. Effect of the concentration of silicone oil (C_o) in the impregnating mixture on R_M values of arylacetic acids (I, - - -) and β-aryl-*n*-butyric acids (II, - - -).

TABLE III

R_M VALUES OF ACIDS I AND II FOR 2.5—7.5% SILICONE OIL IN THE IMPREGNATING MIXTURE

Compound		Silicone oil (%, v/v)									
No.	X	2.5		3.5		5.0		6.2		7.5	
		R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
Arylacetic acids											
Ia	4-CH_3O	0.78	−0.55	0.755	−0.49	0.72	−0.41	0.705	−0.38	0.68	−0.33
In	3-CH_3O,4-$C_6H_5CH_2O$	0.685	−0.34	0.645	−0.26	0.60	−0.18	0.565	−0.11	0.54	−0.07
Io	4-$C_6H_5CH_2O$	0.665	−0.30	0.61	−0.19	0.547	−0.09	0.51	−0.02	0.48	0.03
Ii	4-*n*-$C_6H_{13}O$	0.447	0.09	0.385	0.20	0.327	0.31	0.295	0.40	0.247	0.48
Ij	3-Cl,4-*n*-$C_6H_{13}O$	0.37	0.23	0.32	0.33	0.25	0.47	0.225	0.54	0.193	0.62
β-Aryl-n-butyric acids											
IIa	H	0.685	−0.34	0.65	−0.27	0.62	−0.21	0.59	−0.16	0.57	−0.12
IIg	3-Br	0.61	−0.19	0.565	−0.11	0.52	−0.03	0.50	0	0.46	0.07
IIo	4-$C_6H_5CH_2O$	0.53	−0.05	0.49	0.02	0.45	0.09	0.405	0.17	0.38	0.21
IIk	4-*i*-C_5H_{11}	0.355	0.26	0.31	0.35	0.257	0.46	0.22	0.55	0.215	0.56
IIm	4-*n*-$C_6H_{13}O$	0.335	0.30	0.28	0.41	0.24	0.50	0.213	0.57	0.193	0.62

TABLE IV

CALCULATED VALUES OF SLOPES AND INTERCEPTS OF THE GENERAL EQUATION $R_M = a \cdot \log C_{oil} + b$

Experimental values from Table III were employed; $n = 5$.

Compound	π_X	Log P	a	b	r	s	F	Eqn. No.
Arylacetic acids								
Ia	0.01	1.46**	0.457	−0.734	0.998	0.006	732	(7)
In	1.09*	2.54	0.572	−0.570	0.998	0.007	954	(8)
Io	1.33*	2.78	0.692	−0.572	0.999	0.005	2986	(9)
Ii	2.51	3.96	0.808	−0.238	0.998	0.012	642	(10)
Ij	2.96	4.41	0.821	−0.105	0.999	0.010	1081	(11)
β-Aryl-n-butyric acids								
IIa	0	2.15**	0.455	−0.521	0.999	0.005	1301	(12)
IIg	0.91	3.06	0.522	−0.397	0.995	0.011	310	(13)
IIo	1.31***	3.46	0.551	−0.277	0.994	0.013	253	(14)
IIk	2.25	4.40	0.699	−0.023	0.996	0.014	389	(15)
IIm	2.51	4.66	0.666	0.040	0.999	0.006	1535	(16)

*Values are calculated from eqn. 20.
**Log P values of phenylacetic and β-phenyl-n-butyric acids are taken from ref. 25.
***Value is calculated from eqn. 30.

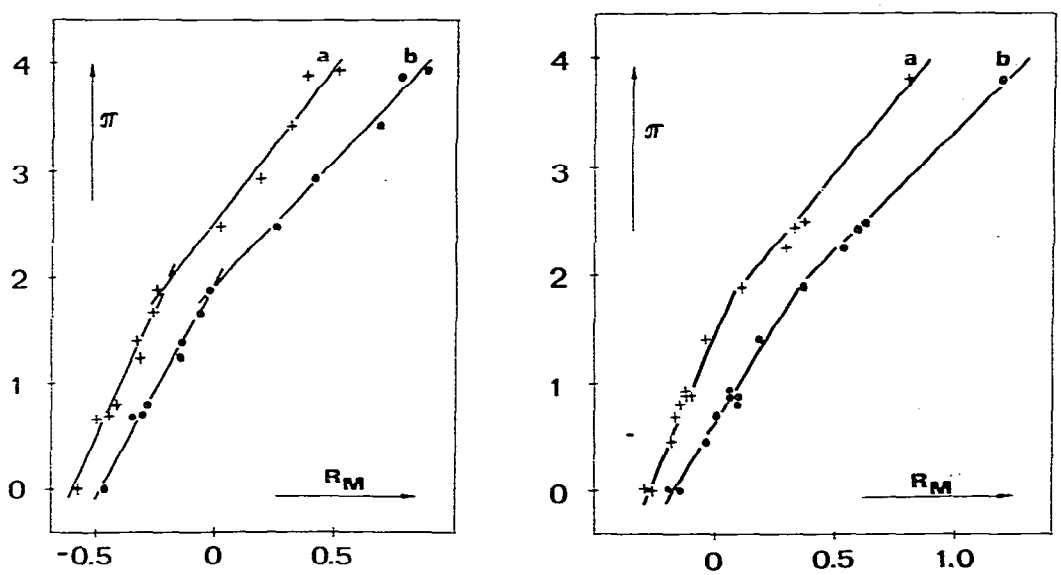

Fig. 2. Arylacetic acids: relationships between π and R_M values. (a) 2.1% Concentration of silicone oil. (b) 4.2% Concentration of silicone oil.

Fig. 3. β-Aryl-n-butyric acids: relationships between π and R_M values. (a) 3.5% Concentration of silicone oil. (b) 7.5% Concentration of silicone oil.

TABLE V

CHROMATOGRAPHIC PROPERTIES OF ARYLACETIC ACIDS

No.	X	π		f		A_W	2.1% Silicone oil		4.2% Silicone oil	
		Tab.	Calc.*	Tab.	Calc.**	($10^2 A^2$)	R_F	R_M	R_F	R_M
Ia	4-CH_3O	0.01	0.11	1.97	2.08	0.416	0.793	-0.585	0.747	-0.47
Ib	3-Cl	0.68	0.58	2.65	2.61	0.316	0.76	-0.50	0.693	-0.35
Ic	4-Cl	0.70	0.73	2.65	2.79	0.316	0.74	-0.45	0.673	-0.315
Id	4-*i*-C_3H_7O	0.81	0.83	2.96	2.90	0.816	0.726	-0.42	0.66	-0.29
Ie	3-Cl,4-*i*-C_3H_7O	1.26	1.34	3.65	3.47	1.071	0.66	-0.29	0.585	-0.15
If	4-*i*-C_3H_7	1.40	1.38	3.42	3.51	0.722	0.68	-0.33	0.58	-0.14
Ig	4-*t*-C_4H_9	1.68	1.61	4.01	3.77	0.922	0.653	-0.275	0.543	-0.08
Ih	4-*i*-C_4H_9	1.90	1.72	3.95	3.90	0.922	0.643	-0.255	0.525	-0.04
Ii	4-*n*-$C_6H_{13}O$	2.51	2.61	4.62	4.87	1.417	0.496	0.01	0.36	0.25
Ij	3-Cl,4-*n*-$C_6H_{13}O$	2.96	3.02	5.31	5.31	1.672	0.40	0.18	0.28	0.41
Ik	3-Cl,4-*n*-$C_7H_{15}O$	3.46	3.61	5.84	5.93	1.872	0.33	0.31	0.173	0.68
Il	4-2'-Ethylhexyl	3.90	3.76	6.07	6.08	1.722	0.30	0.37	0.147	0.76
Im	3-Cl,4-*n*-$C_8H_{17}O$	3.96	3.94	6.37	6.26	2.072	0.24	0.50	0.12	0.87
In	3-CH_3O,4-$C_6H_5CH_2O$***	—	1.09	—	3.19	1.545	0.705	-0.38	0.625	-0.22
Io	4-$C_6H_5CH_2O$***	—	1.33	—	3.45	1.190	0.693	-0.36	0.585	-0.15
Ip	3-Cl,4-$C_6H_5CH_2O$***	—	1.81	—	3.99	1.445	0.64	-0.25	0.505	-0.01

*Values are calculated from eqn. 20.
**Values are calculated from eqn. 22.
***Not included in the regression analysis.

TABLE VI

CHROMATOGRAPHIC PROPERTIES OF β-ARYL-*n*-BUTYRIC ACIDS

No.	X	π		A_W	3.5% Silicone oil		7.5% Silicone oil	
		Tab.	Calc.*	($10^2 A^2$)	R_F	R_M	R_F	R_M
IIa	H	0	0.12	0.061	0.65	−0.27	0.58	−0.14
IIb	4-CH_3O	0.01	−0.06	0.416	0.657	−0.28	0.61	−0.19
IIc	4-CH_3	0.45	0.48	0.321	0.60	−0.18	0.525	−0.04
IId	4-Cl	0.70	0.66	0.316	0.59	−0.16	0.495	0.01
IIe	4-*i*-C_3H_7O	0.81	0.93	0.816	0.58	−0.14	0.45	0.09
IIf	4-Br	0.90	0.86	0.363	0.57	−0.12	0.46	0.07
IIg	3-Br	0.91	0.86	0.363	0.565	−0.11	0.46	0.07
IIh	4-C_2H_5	0.90	0.93	0.522	0.56	−0.10	0.45	0.09
IIi	4-*i*-C_3H_7	1.40	1.26	0.722	0.52	−0.03	0.39	0.19
IIj	4-*i*-C_4H_9	1.90	1.82	0.922	0.435	0.11	0.30	0.37
IIk	4-*i*-C_5H_{11}	2.25	2.32	1.122	0.335	0.30	0.225	0.54
IIl	4-*n*-C_5H_{11}	2.45	2.48	1.122	0.32	0.33	0.20	0.60
IIm	4-*n*-$C_6H_{13}O$	2.51	2.54	1.417	0.30	0.37	0.193	0.62
IIn	4-2′-Ethylhexyl	3.90	3.88	1.722	0.136	0.80	0.06	1.19
IIo	4-$C_6H_5CH_2O$**	—	1.31	1.190	0.505	−0.01	0.38	0.21

*Values are calculated from eqn. 30.
**Not included in the regression analysis.

phy in both series of acids was performed with two concentrations of silicone oil in the silica gel. The experimental results are summarized in Table V (for the acids I) and in Table VI (for the acids II) and on the corresponding graphs (Figs. 2 and 3). A non-linearity of the dependence between R_M and π is clearly visible from the plots.

Eqn. 17 gives the π—R_M linear relationship for the 2.1% concentration of silicone oil, and eqn. 19 for the 4.2% concentration of silicone oil in the series of arylacetic acids. Equations 18 and 20 show the parabolic dependences between π and R_M. The quadratic term R_M^2 becomes significant on the significance level $\alpha = 0.005$ for the higher concentration of the silicone oil; for the lower concentration, the significance of this term declines to $\alpha = 0.025$ (the confidence intervals are introduced in brackets). Application of the fragmental constants *f* yielded similar results. Eqns. 21 and 22, corresponding to eqns. 19 and 20 are given for the sake of comparison. Since the introduction of the fragmental constants instead of π did not improve the correlation, only the π values were used in further analysis.

The relationships between lipophilic parameters and R_M values can be substituted in the given range of lipophilicity, by two separate linear dependences for lower and higher lipophilicity. For the first group of acids (Ia—Ih), eqns. 23 and 25 were derived, and for the second group (Ig—Im), eqns. 24 and 26. A comparison of these equations reveals significant differences in the slopes in the sense of decreasing slopes with increasing lipophilicity. This experimental finding shows that a change of Gibbs energy accompanying the separation in the chromatographic system used tends to be lower in a region of higher lipophilicity.

	n	r	s	F	
$\pi = 3.543\ (\pm 0.570)\ R_M + 2.422(\pm 0.215)$	13	0.988	0.206	472	(17)
$\pi = 3.426(\pm 0.487)\ R_M - 1.394(\pm 1.361)\ R_M^2 + 2.603(\pm 0.293)$	13	0.994	0.163	381	(18)
(4.2% of silicone oil)					
$\pi = 2.836(\pm 0.410)\ R_M + 1.693(\pm 0.184)$	13	0.991	0.186	582	(19)
$\pi = 3.251(\pm 0.412)\ R_M - 0.985(\pm 0.771)\ R_M^2 + 1.855(\pm 0.169)$	13	0.997	0.111	830	(20)
$f = 3.088(\pm 0.520)\ R_M + 3.843(\pm 0.233)$	13	0.988	0.236	430	(21)
$f = 3.600(\pm 0.552)\ R_M - 1.212(\pm 1.034)\ R_M^2 + 4.042(\pm 0.226)$	13	0.996	0.149	549	(22)
(2.1% of silicone oil)					
$\pi = 5.232(\pm 1.778)\ R_M + 3.105(\pm 0.723)$	8	0.982	0.127	161	(23)
$\pi = 2.994(\pm 0.921)\ R_M + 2.551(\pm 0.281)$	7	0.990	0.143	241	(24)
(4.2% of silicone oil)					
$\pi = 4.080(\pm 0.915)\ R_M + 1.991(\pm 0.246)$	8	0.992	0.084	371	(25)
$\pi = 2.405(\pm 0.496)\ R_M + 1.929(\pm 0.267)$	7	0.995	0.097	538	(26)

The experimental results of chromatography of β-aryl-*n*-butyric acids using silica gel containing 3.5 and 7.5% of silicone oil (Table VI) were processed in a similar manner. The regression equations (eqns. 27—34) are summarized in Table VII. Also in this case the relationships between π and R_M were expressed both by the linear (eqns. 27 and 29) and quadratic dependences (eqns. 28 and 30). Replacement of the parabolic expression by two linear relationships for different regions of the lipophilicity, yielded eqns. 31 and 32 for a 3.5% concentration of the silicone oil, and eqns. 33 and 34 for the 7.5% concentration. The equations show that analogous rules hold as in the series of arylacetic acids.

The relationships between R_M values and the concentration of silicone oil demonstrate the presence of adsorption effects in the chromatographic separation of both series of acids. Notwithstanding, the linear dependences between R_M and π values were observed even in the groups of acids I and II with lower lipophilicity. It is probable that an adsorption of the molecules on a solid support is affected by the surface areas of the molecules. Therefore, we have undertaken the calculation of molar surface areas (A_W) of the substituents with the aim of searching for the relationship between A_W and π. Such relationships are given by eqns. 35 and 36 for the substituents of acids Ia—Im and IIa—IIn, respectively.

Taking these linear correlations into account, it is understandable that the

TABLE VII

RELATIONSHIPS BETWEEN π AND R_M VALUES OF β-ARYl-*n*-BUTYRIC ACIDS

General equation: $\pi = a \cdot R_M - b \cdot R_M^2 + c$.

Eqn. No.	Silicone oil (%)	Series	a	b	c	n	r	s	F
27	3.5	IIa—IIn	3.542(±0.531)	1.138(±1.129)*	1.232(±0.159)	14	0.989	0.172	525
28	3.5	IIa—IIn	4.030(±0.678)		1.316(±0.155)	14	0.994	0.131	460
29	7.5	IIa—IIn	2.914(±0.333)		0.641(±0.147)	14	0.993	0.132	898
30	7.5	IIa—IIn	3.481(±0.491)	0.695(±0.489)	0.622(±0.094)	14	0.998	0.082	1168
31	3.5	IIa—IIj	5.073(±0.900)		1.447(±0.151)	10	0.992	0.080	465
32	3.5	IIj—IIn	2.989(±1.449)		1.461(±0.412)	5	0.994	0.099	236
33	7.5	IIa—IIj	3.583(±0.661)		0.612(±0.176)	10	0.995	0.083	430
34	7.5	IIj—IIn	2.462(±0.378)		0.967(±0.272)	5	0.999	0.031	2358

*Confidence interval of the quadratic term on the significance level $\alpha = 0.01$.

linear relationships between π and R_M in both regions of lipophilicity are not affected by adsorption effects.

	n	r	s	F	
$\pi = 2.036\, A_W - 0.280$	13	0.959	0.401	138	(35)
$\pi = 2.180\, A_W - 0.225$	14	0.947	0.361	105	(36)

CONCLUSIONS

Summarizing the results obtained it is possible to observe that a non-uniform mechanism of the chromatographic separation of the acids being investigated manifests itself in significant departures from linearity in the relationships between π and R_M values. The role of a purely partition mechanism in this chromatographic system is influenced by the lipophilicity of the compounds, and most probably by certain other physicochemical characteristics. A non-linear relationship between lipophilic π parameters and R_M values can be replaced either by a quadratic dependence or by two lines with different slopes in different regions of the lipophilicity. In the broader region of lipophilicity the R_M values thus obtained do not represent the conventionally used measure of lipophilicity, that is lipophilic parameters derived from the octanol—water system. Therefore, the determination of the separation mechanism plays an important role in the chromatographic evaluation of lipophilicity. The use of the above-mentioned R_M values in correlating some biological activities of acids I and II is in progress in our laboratory.

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