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PARAMETRIZATION OF LIPOPHILIC PROPERTIES OF SOME AROMATIC–ALIPHATIC ACIDS IN PAPER CHROMATOGRAPHY

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SUMMARY

The relationship between hydrophobicity and R_M has been studied for four series of acids, namely arylacetic, cinnamic, β-arylisobutyric, and α-methylcinnamic. The possibility of using pK values or polar constants to correct for dissociation of the acids investigated is discussed. The influence of substituent alkoxy groups on the relationship was investigated, and it was found that polar constants were important, even in systems in which dissociation was inhibited by addition of formic acid. In these circumstances, the polar constants probably compensate for modification of the hydrogen ion donor–acceptor properties of alkoxy derivatives. This phenomenon evidently plays a decisive role in those chromatographic systems markedly different from the reference partition system *n*-octanol–water.

INTRODUCTION

One of the important properties influencing interaction between biologically active substances and biomacromolecules is lipophilicity. Partition coefficients can be used in its quantitative measurement. To express quantitative structure–activity relationships, the logarithm of the partition coefficient has been introduced as an extrathermodynamic quantity, with linear dependence on changes in free energy during the partition process. As a model representing the aqueous and organic biophase system, Hansch and co-workers[1–3] had chosen the system *n*-octanol–water. Partition coefficients from this system are considered as a conventional measure of lipophilicity in quantitative structure–activity relationships. Hansch *et al.*[1] also defined the hydrophobicity, π, by eqn. 1; this parameter represents the contribution of a given substituent X to the total lipophilicity of a compound.

$$\pi = \log P_X - \log P_H \quad (1)$$

Studies published by Martin and co-workers[4–6] indicate that the relationship between the partition coefficient P and the value R_F in a chromatographic system can be expressed by eqn. 2, in which A_M and A_S represent the respective cross-sectional areas of the mobile and stationary phases. Taking the logarithm of eqn. 2, intro-

ducing the quantity R_M defined by Bate-Smith and Westall[7], and applying the Collander's relationship between the partition coefficients determined in a pair of closely related partition systems, we obtain eqns. 3 and 4, respectively.

$$P = A_M/A_S\,(1/R_F - 1) \tag{2}$$

$$\log P = a_1 \cdot R_M + b_1 \tag{3}$$

$$\pi = a_1 \cdot R_M - b_2 \tag{4}$$

This linear relationship has been documented for many series of compounds. The R_F values are usually determined by the reversed-phase method on silica-gel thin layers[9–15] impregnated with a suitable lipoid solvent, usually silicone oil or *n*-octanol[16,17]. Linear relationships between π and R_M have been also found with lipoid phases differing markedly in character[18] from *n*-octanol (*e.g.*, liquid paraffin[11,12,16,17,19], undecane[17], squalene[17]). These findings are partly explained by the observation that a substitution in the basic structure in such instances produced no marked modification of the hydrogen ion donor–acceptor properties in the entire series of compounds. It is known[20] that the hydrogen ion donor–acceptor activity significantly modifies the linearity of the relationship between the logarithms of partition coefficients of substances determined separately in a pair of partition systems whose organic phases markedly differ in lipophilicity. Leo[20] takes the solubility of water in the given solvent as a measure of lipophilicity.

As we had found[21] in several series of aromatic–aliphatic acids, linear relationships also occur between π and R_M values derived from paper chromatography, provided that dissociation of the acid is prevented, for example by the presence of formic acid in the stationary phase. In a chromatographic system in which acids do dissociate, a correction for dissociation[17] is usually made according to eqn. 5.

$$R_M{}^n = R_M + \log\,\{(K_a + [H^+])/[H^+]\} \tag{5}$$

where $R_M{}^n$ is the value of R_M calculated with respect to the non-dissociated portion of the acid, K_a is the dissociation constant, R_M is the value obtained from the experimental value R_F, and $[H^+]$ is the hydrogen ion concentration in the given system. In the arrangement of paper chromatography used in our study the value of $[H^+]$ is not known, and thus the corrected $R_M{}^n$ values cannot be calculated from eqn. 5. Thus to correct for dissociation we used the pK values or the polar constants σ, so that the relationship between π and R_M is expressed in general form by eqn. 6 or 6a. The applicability of this method of correction is discussed in the experimental part.

$$\pi = a \cdot R_M + b \cdot \mathrm{p}K + c \tag{6}$$

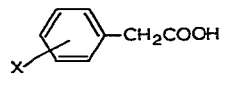
$$\pi = a \cdot R_M + b' \cdot \sigma + c' \tag{6a}$$

The present study attempts to elucidate how far the relationship between π and R_M is modified by the presence of alkoxy substituents in various series of acids, when the chromatographic system used differs considerably from the reference partition system. We were also interested in potential application of the above-mentioned method of correction for dissociation in additional series of aromatic–aliphatic acids.

EXPERIMENTAL

Chromatography

A 1% solution of each of the acids I–IV in ethanol–chloroform (1:1) was prepared, and 10 μl samples were applied to Whatman No. 4 paper, which was subsequently impregnated with a 40% ethanolic solution of formamide containing 5% of formic acid (system A) or 5% of ammonium formate (system B). The ethanol was evaporated off at 20°, and descending one-dimensional chromatography was carried out at a constant temperature of 20° with one of the following mobile phases: 1, benzene–cyclohexane (1:1); 2, benzene–cyclohexane (3:7), 3, benzene–cyclohexane (1:4), 4, benzene–cyclohexane (7:3). (The chromatographic systems are designated in the following text by the symbols A1–A4 and B1–B4.) The chromatogram was then dried at 80°. The acids I, II, and IV were visualized in UV light ($\lambda = 254$ nm), and the acids III were detected with a mixture of amylose, iodine, and potassium iodate[22]. Each chromatogram contained six compounds in each series; two acids serving as reference samples were repeated in each chromatogram. In the chromatograms evaluated the R_F values of the standards did not differ by more than 0.02.



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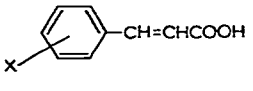

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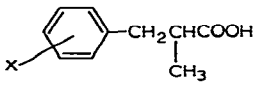

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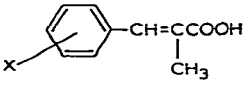

IV

Sample preparation

Derivatives of cinnamic (II) and α-methylcinnamic (IV) acids were prepared by the Wittig reaction[23,24]. Arylacetic acids (I) were obtained from the corresponding substituted benzyl chlorides by treatment with sodium cyanide in dimethyl sulphoxide and subsequent hydrolysis[25]. β-Arylisobutyric acids (III) were prepared by methylation of diethyl esters of corresponding substituted benzylmalonic acids and subsequent hydrolysis and decarboxylation[26].

Calculation of parameters

The parameters π used in the regression analysis were taken from ref. 27; for the acids II and IV, the values derived for benzoic acids, and for the acids I and III, the values derived for arylacetic acids were used. The parameters π of alkoxy groups were calculated from the value for the methoxy substituent and the following increments: $\Delta\pi = 0.5$ for CH_2, $\Delta\pi = -0.2$ for branching, and $\Delta\pi = -0.3$ for a double bond[2]. For unsubstituted derivatives a value of $\pi = 0.23$ was used, which corresponds to 0.5 log P of the hydrogen molecule[28]. This value is in agreement with the data published by Nys and Rekker[29,30], in connection with the concept of so-called "frag-

mental constants", f. Because a common thermodynamic basis exists for both methods of parametrization of lipophilicity[31], an interrelationship exists between π and f for a substituent X, which is expressed by eqn. 7:

$$f(X) - \pi(\mathrm{H}) = f(\mathrm{H}) \tag{7}$$

For the series of substituents presented in Table VI of ref. 30, the difference is 0.28 ($s = 0.02$), a value evidently corresponding to the fragmental constant of hydrogen. The lipophilicity of hydrogen was likewise taken into account in our calculations of π for disubstituted derivatives, in the sense that we subtracted the value of $\pi(\mathrm{H}) = 0.23$ from the sum of π of the two substituents.

The electron effects of aromatic substituents were assessed with the aid of Hammett's polar constants σ, taken from ref. 32. Values for polar constants of substituents which were not found in the tables were obtained from experimental pK values, and a relationship between the pK values and polar constants of suitably substituted cinnamic acids[33]. The correction for dissociation according to eqn. 6 or 6a is applicable, provided that the relationship between the correction term in eqn. 5 can be approximated by a linear dependence expressed by eqn. 8. Insertion of the latter into eqn. 10, which is an extended form of eqn. 9, gives eqn. 11. This equation can be readily rearranged to eqn. 6, or, if Hammett's eqn. 12 is applied, to eqn. 6a.

$$\log \{(K_a + [\mathrm{H}^+])/[\mathrm{H}^+]\} = k_1 \cdot \mathrm{p}K + k_2 \tag{8}$$

$$\pi = a_1 \cdot R_M{}^n + b_1 \tag{9}$$

$$\pi = a_1 \cdot R_M + a_1 \cdot \log \{(K_a + [\mathrm{H}^+])/[\mathrm{H}^+]\} + b_1 \tag{10}$$

$$\pi = a_1 \cdot R_M + a_1 \cdot k_1 \cdot \mathrm{p}K + (a_1 \cdot k_2 + b_1) \tag{11}$$

$$\mathrm{p}K = \varrho\sigma + \text{constant} \tag{12}$$

The linear relationship (eqn. 8) is satisfied if the R_F values are obtained at pH values an order of magnitude higher than the range of pK values of the acids investigated, regardless of the extent of these values. If the pH of the chromatographic system lies within the range of pK values of the investigated acids, then the validity of the linear relationship 8 diminishes with the expanding range of pK values. In our studies of several series of aromatic–aliphatic acids, we found a maximum range of pK values in the series of substituted cinnamic acids, namely, from 5.67 (pK of 4-nitrocinnamic acid) to 6.67 (pK of 4-benzyloxycinnamic acid). For this range linearity holds, as is evident from eqns. 13–15, derived for the cinnamic acid series for pH values 6, 7, and 9. The pK values figuring in the eqns. 13–15 were determined in 80% methylcellosolve at 25°. Eqn. 16 expresses the linear relationship using the polar constants σ.

	n	s	r	F	
$\log [(10^{-6} + 10^{-\mathrm{p}K})/10^{-6}] = -0.394\,\mathrm{p}K + 2.683$	14	0.024	0.978	268.8	(13)
$\log [(10^{-7} + 10^{-\mathrm{p}K})/10^{-7}] = -0.849\,\mathrm{p}K + 6.141$	14	0.012	0.999	$4.5 \cdot 10^3$	(14)
$\log [(10^{-9} + 10^{-\mathrm{p}K})/10^{-9}] = -0.998\,\mathrm{p}K + 8.989$	14	0.000	0.999	$2.9 \cdot 10^7$	(15)
$\log [(10^{-7} + 10^{-\mathrm{p}K})/10^{-7}] = 0.708\,\sigma + 7.743$	14	0.012	0.999	$4.8 \cdot 10^3$	(16)

The coefficients in the regression equations were calculated from experimental data 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 minimal significance level $\alpha = 0.005$, with the exception of eqn. 23, in which the significance level of the parameter σ is 0.05.

RESULTS AND DISCUSSION

In a system in which dissociation is inhibited by the addition of formic acid, alkoxy substituents exert statistically significant effects on the influence of the σ constants on the relationship between π and R_M .In the series of arylacetic acids (Table I) the influence of the σ constants is evident from eqn. 17, obtained by regression analysis of data obtained from all the acids 1–16, the series of acids having been classified by the presence or absence of an alkoxy group in the molecule. For the acids 1–7, containing no alkoxy group, eqn. 18 was obtained, statistical significance of which cannot be increased by insertion of σ constants. For the alkoxy derivatives 8–16 eqn. 19 was derived, from which a considerable effect of the σ constants on the relationship between π and R_M is evident. Analogous differences between both groups of derivatives were also found in the series of cinnamic (Table II) and β-arylisobutyric (Table III) acids, as is evident from eqns. 20 and 22 for acids not con-

TABLE I

CHROMATOGRAPHIC PROPERTIES OF ARYLACETIC ACIDS

No.	*X*	*System A1*		*System B4*		σ	π	$\pi_{calc.}$	
		R_F	R_M	R_F	R_M			A^*	B^{**}
1	H	0.08	1.06	0.05	1.28	0	0.23	0.24	0.15
2	3-Cl	0.16	0.72	0.07	1.12	0.37	0.68	0.62	0.58
3	4-Cl	0.16	0.72	0.09	1.00	0.23	0.70	0.62	0.63
4	4-*tert*.-C_4H_9	0.65	−0.27	0.53	−0.05	−0.20	1.68	1.74	1.53
5	4-iso-C_4H_9	0.70	−0.37	0.62	−0.21	−0.11	1.90	1.87	1.77
6	4-iso-C_3H_7	0.47	0.05	0.42	0.14	−0.15	1.40	1.38	1.35
7	4-C_2H_5	0.30	0.37	0.24	0.50	−0.15	0.90	1.02	0.94
8	4-CH_3O	0.06	1.19	0.06	1.19	−0.27	0.01	−0.03	0.07
9	4-*n*-$C_6H_{13}O$	0.88	−0.87	0.87	−0.83	−0.23***	2.51	2.43	2.40
10	4-iso-C_4H_9O	0.52	−0.03	0.53	−0.05	−0.37***	1.31	1.36	1.42
11	4-iso-C_3H_7O	0.30	0.37	0.25	0.48	−0.45	0.81	0.84	0.76
12	3-Cl, 4-*n*-$C_6H_{13}O$	0.93	−1.12	0.93	−1.12	0.14***	2.96	2.93	2.79
13	3-Cl, 4-iso-C_4H_9O	0.67	−0.31	0.68	−0.33	0.01***	1.76	1.90	1.90
14	3-Cl, 4-iso-C_3H_7O	0.38	0.21	0.38	0.21	−0.08	1.26	1.23	1.32
15	3-Cl, 4-*n*-C_3H_7O	0.45	0.08	0.43	0.12	0.01***	1.46	1.44	1.48
16	3-Cl, 4-CH_3O	0.10	0.95	0.11	0.91	0.10	0.46	0.45	0.64

* Values of π for alkoxy derivatives were calculated from eqn. 19, for other derivatives from eqn. 18.

** Values were calculated from eqn. 25.

*** Constants σ were calculated from the linear dependence between pK and σ constants in the series of cinnamic acids.

TABLE II

CHROMATOGRAPHIC PROPERTIES OF CINNAMIC ACIDS

No.	*X*	*System A1*		*System B1*		σ	π	$\pi_{calc.}$	
		R_F	R_M	R_F	R_M			A^*	B^{**}
17	H	0.11	0.91	0.04	1.38	0	0.23	0.35	0.09
18	3-Cl	0.20	0.60	—	—	0.37	0.83	0.73	—
19	4-Cl	0.20	0.60	0,12	0.87	0.23	0.87	0.73	0.79
20	3-Br	0.31	0.35	0.12	0.87	0.39	0.99	1.03	0.89
21	4-*tert.*-C_4H_9	0.59	−0.16	0.64	−0.25	−0.20	1.70	1.65	1.81
22	4-iso-C_4H_9	0.67	−0.31	0.74	−0.45	−0.11	1.90	1.83	2.05
23	4-iso-C_3H_7	0.56	−0.10	0.40	0.18	−0.15	1.40	1.58	1.36
24	4-*n*-$C_6H_{13}O$	0.82	−0.66	0.87	−0.83	−0.23***	2.58	2.36	2.45
25	4-cyclo-$C_6H_{11}O$	0.76	−0.50	0.73	−0.43	−0.23***	2.18	2.14	2.00
26	4-iso-C_4H_9O	0.60	−0.18	—	—	−0.37***	1.38	1.49	—
27	4-iso-C_3H_7O	0.41	0.16	0.20	0.60	−0.45	0.88	0.91	0.72
28	4-CH_2=$CHCH_2O$	0.22	0.55	0.26	0.45	−0.29***	0.78	0.64	0.97
29	4-CH_3O	—	—	0.06	1.19	−0.27	0.08	—	0.15
30	3-Cl, 4-*n*-$C_6H_{13}O$	0.85	−0.75	0.95	−1.28	0.14***	3.18	3.06	3.17
31	3-Cl, 4-iso-C_4H_9O	0.63	−0.23	—	—	0.01***	1.98	2.15	—
32	3-Cl, 4-iso-C_3H_7O	0.53	−0.05	0.50	0	−0.08	1.48	1.77	1.60
33	3-Cl, 4-CH_2=$CHCH_2O$	0.27	0.43	0.35	0.27	0.08***	1.38	1.37	1.39
34	3-Cl, 4-C_2H_5O	0.20	0.60	0.31	0.35	0.13***	1.18	1.22	1.32
35	3-Cl, 4-CH_3O	0.06	1.06	—	—	0.10	0.68	0.56	—
36	3-CH_3O, 4-*n*-$C_6H_{13}O$	0.58	−0.14	0.68	−0.33	−0.09***	2.49	1.88	1.96
37	3-CH_3O, 4-i-C_3H_7O	0.19	0.63	0.14	0.79	−0.33	0.79	0.46	0.57

* Values of π for alkoxy derivatives were calculated from eqn. 21, for other derivatives from eqn. 20.

** Values were calculated from eqn. 26.

*** See third footnote to Table I.

TABLE III

CHROMATOGRAPHIC PROPERTIES OF β-ARYLISOBUTYRIC ACIDS

No.	*X*	*System A3*		*System B2*		σ	π	$\pi_{calc.}$	
		R_F	R_M	R_F	R_M			A^*	B^{**}
38	H	0.24	0.50	0.25	0.48	0	0.23	0.33	0.30
39	3-Cl	0.39	0.19	0.29	0.39	0.37	0.68	0.68	0.66
40	4-Cl	0.38	0.21	0.38	0.21	0.23	0.70	0.66	0.77
41	4-Br	0.44	0.10	0.40	0.18	0.23	0.90	0.78	0.81
42	3-NO_2	—	—	0.06	1.19	0.71	−0.01	—	0.01
43	4-NO_2	—	—	0.04	1.38	0.78	−0.04	—	−0.18
44	4-*tert.*-C_4H_9	0.85	−0.75	0.85	−0.75	−0.20	1.68	1.74	1.57
45	4-iso-C_4H_9	0.87	−0.83	0.89	−0.91	−0.11	1.90	1.83	1.81
46	4-iso-C_3H_7	0.77	−0.53	0.80	−0.60	−0.15	1.40	1.49	1.43
47	4-iso-C_3H_7O	0.54	−0.07	0.60	−0.18	−0.45	0.81	0.83	0.74
48	3-CH_3O	0.17	0.69	0.18	0.66	0.12	0.04	0.15	0.18
49	4-CH_3O	0.15	0.75	0.19	0.63	−0.27	0.01	−0.03	−0.06
50	3-Cl, 4-iso-C_4H_9O	0.85	−0.75	0.89	−0.91	0.01***	1.76	1.73	1.90
51	3-Cl, 4-iso-C_3H_7O	0.71	−0.39	0.70	−0.37	−0.08	1.26	1.30	1.22
52	3-Cl, 4-CH_3O	0.24	0.50	0.31	0.35	0.10	0.46	0.36	0.52
53	3-CH_3O, 4-*n*-$C_6H_{13}O$	0.81	−0.63	0.81	−0.63	−0.09***	2.32	1.56	1.51
54	3-CH_3O, 4-iso-C_3H_7O	0.25	0.48	0.31	0.35	−0.33	0.62	0.25	0.22

* Values of π for alkoxy derivatives were calculated from eqn. 23, for other derivatives from eqn. 22.

** Values were calculated from eqn. 27.

*** See third footnote to Table I.

taining alkoxy groups, and eqns. 21 and 23 for alkoxy derivatives. In the series of α-methylcinnamic acids (Table IV), eqn. 24 was calculated for the derivatives 55–66 regardless of the character of substituents. In view of the small number of alkoxy derivatives in this instance partial equations are statistically irrelevant.

TABLE IV

CHROMATOGRAPHIC PROPERTIES OF α-METHYLCINNAMIC ACIDS

No.	*X*	*System A2*		*System B4*		σ	π	π_{calc}	
		R_F	R_M	R_F	R_M			A^*	B^{**}
55	H	0.27	0.43	0.28	0.41	0	0.23	0.13	0.14
56	3-Cl	0.43	0.12	0.50	0	0.37	0.83	0.89	0.86
57	3-Br	0.47	0.05	0.58	−0.14	0.39	0.99	0.99	1.05
58	4-Br	0.45	0.09	0.55	−0.09	0.23	0.98	0.86	0.88
59	4-NO_2	—	—	0.09	1.00	0.78	0.02	—	0.09
60	4-*tert*.C_4H_9	0.85	−0.75	0.89	−0.91	−0.20	1.70	1.69	1.61
61	4-iso-C_4H_9	0.87	−0.83	0.93	−1.12	−0.11	1.90	1.83	1.92
62	4-iso-C_3H_7	0.79	−0.58	0.84	−0.72	−0.15	1.40	1.50	1.41
63	4-iso-C_3H_7O	0.65	−0.27	0.73	−0.43	−0.45	0.88	0.95	0.88
64	4-CH_2=$CHCH_2O$	0.52	−0.03	0.62	−0.21	−0.29***	0.78	0.74	0.71
65	3-CH_3O	0.26	0.45	0.37	0.23	0.14	0.14	0.35	0.45
66	4-CH_3O	0.21	0.58	0.33	0.31	−0.27	0.08	−0.02	0.10
67	3-CH_3O, 4-*n*-$C_6H_{13}O$	0.87	−0.83	0.93	−1.12	−0.09***	2.49	1.78	1.93
68	3-CH_3O, 4-iso-C_3H_7O	0.36	0.25	0.51	−0.02	−0.33	0.79	0.40	0.46

* Values of π were calculated from eqn. 24.
** Values of π were calculated from eqn. 28.
*** See third footnote to Table I.

Arylacetic acids

	n	s	r	F	
$\pi = -1.216\,R_M + 0.410\,\sigma + 1.489$	16	0.082	0.995	703.7	(17)
$\pi = -1.131\,R_M + 1.435$	7	0.098	0.992	184.2	(18)
$\pi = -1.186\,R_M + 0.553\,\sigma + 1.525$	9	0.074	0.998	636.5	(19)

Cinnamic acids

$\pi = -1.210\,R_M + 1.458$	7	0.131	0.978	110.5	(20)
$\pi = -1.347\,R_M + 1.562\,\sigma + 1.829$	11	0.170	0.981	103.8	(21)

β-Arylisobutyric acids

$\pi = -1.126\,R_M + 0.891$	7	0.093	0.990	251.3	(22)
$\pi = -1.119\,R_M + 0.294\,\sigma + 0.887$	6	0.096	0.994	129.9	(23)

α-Methylcinnamic acids

$\pi = -1.259\,R_M + 0.526\,\sigma + 0.850$	11	0.114	0.983	116.3	(24)

These results can be summarized as follows. In the investigated series of acids the relationships between π and R_M are modified by the polar constants σ. As formic acid is present in the stationary phase it is probable that the effect of the σ constants is not due to acid dissociation, as is also indicated by eqns. 18, 20 and 22. Consequently, the σ constants modify the relationship between π and R_M solely in alkoxy derivatives. A plausible explanation is that the presence of an alkoxy group modifies the hydrogen ion donor–acceptor properties of these derivatives because of its hydrogen ion acceptor character. As found by Leo[20], the linearity of relationship between the logarithms of partition coefficients determined in systems with markedly different lipophilicity of the organic phase is substantially diminished in a series of compounds containing both hydrogen ion donors and hydrogen ion acceptors. In a series of phenols, Leo found that the poor correlation of logarithms of partition coefficients determined in different systems could be markedly improved by introduction of logarithms of association constants[34], which reflect the extent of hydrogen-bond formation. These association constants exhibit linear correlations with the σ constants. The presence of terms with σ constants in eqns. 17, 19, 21, 23 and 24 indicates modifications in the degree of hydrogen-bond formation consequent upon the introduction of alkoxy groups.

The dialkoxy derivatives 37, 38, 54, 55, 68 and 69 gave results deviating significantly from those obtained with the monoalkoxy derivatives. They exhibited decreased lipophilicity against the tabulated parameters π (*cf.* Tables II, III and IV). This difference can be explained by further changes in the extent of hydrogen-bond formation. However, an interaction between the two alkoxy substituents themselves cannot be ruled out.

The series of acids I–IV were also investigated in analogous systems, but in the presence of ammonium formate in the stationary phase, which does not inhibit acid dissociation. By regression analysis of the experimental data (Tables I–IV), eqns. 25–28 were calculated for arylacetic, cinnamic, β-arylisobutyric and α-methylcinnamic acids, respectively. The equations include data for all derivatives investigated. The σ constants in these equations evidently compensate both the effects of dissociation and the differing extent of hydrogen-bond formation. In these systems the differences between alkoxy and other derivatives are apparent as well. Classification of the acids by the presence or absence of an alkoxy group led to equations expressing the relationship between π and R_M. From the results (Table V) an increase in the

TABLE V

SLOPES AND INTERCEPTS IN EQUATIONS: $\pi = a \cdot R_M + b$

Series of acids	*Substitution**	*a*	*b*
Arylacetic	*Y*	−1.229	1.473
	Z	−0.980	1 599
Cinnamic	*Y*	−1.286	1.572
	Z	−0.826	1.535
β-Arylisobutyric	*Y*	−1.141	0.737
	Z	−0.858	0.976
α-Methylcinnamic	*Y*	−1.167	0.441
	Z	−0.922	0.825

* Alkoxy derivatives are denoted by *Y*, others by *Z*.

absolute value of the slope of the relationship foı alkoxy derivatives in comparison with the other derivatives is apparent. The slope is a relative measure of changes in free energy of substance partition on the phase interface with respect to the system *n*-octanol–water. The observed differences among the slopes indicate different changes in free energy of the interphase partition between alkoxy and other derivatives, respectively.

	n	*s*	*r*	*F*	
$\pi = -1.137\,R_M + 0.669\,\sigma + 1.610$	16	0.119	0.990	339.3	(25)
$\pi = -1.128\,R_M + 0.562\,\sigma + 1.646$	15	0.157	0.984	283.3	(26)
$\pi = -1.145\,R_M + 0.705\,\sigma + 0.850$	15	0.117	0.987	230.2	(27)
$\pi = -1.205\,R_M + 0.614\,\sigma + 0.638$	12	0.124	0.985	135.6	(28)

CONCLUSION

Introduction of alkoxy groups as substituents into series of otherwise structurally related acids complicates the relationships between the parameters π and the R_M values. These complications result above all from modifications of the hydrogen ion donor–acceptor properties of the derivatives concerned. This phenomenon plays a decisive role in those chromatographic systems which differ markedly from the reference system *n*-octanol–water. In the chromatographic system under study, the deviations of alkoxy derivatives from π–R_M linearity are probably caused not only by different lipophilicity of lipoid phases but also by different hydrogen ion donor activity of formamide and water. It has to be borne in mind, particularly in connection with their application in quantitative structure–activity relationships, that the R_M values thus obtained do not represent an actual measure of lipophilicity of the alkoxy derivatives concerned.

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