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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. RELATIONSHIP BETWEEN R_M VALUES AND HANSCH'S π PARAMETERS FOR A SERIES OF PHENOLS

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SUMMARY

Experimental R_M values for a series of mono- and di-substituted phenols were determined using high-performance liquid chromatography. Statistically significant linear relationships were found between these R_M values and Hansch's π parameters or literature R_M values based on various chromatographic methods. The effect of the composition of the mobile phase on the linearity of the relationships is discussed. Physico-chemical parameters of the phenols are correlated with their fungicidal activities.

INTRODUCTION

A frequently used approach to the assessment of quantitative relationships between the biological activity and the chemical structure of drugs has been Hansch's analysis[1,2]. The mechanism of biological action can be described as a chain of consecutive processes in which the most important factor is the hydrophilic or lipophilic nature of the biophases in which the processes occur between the site of application and the site of action of a drug. Lipophilicity of drugs can be characterized by the logarithm of the partition coefficient, P, in the reference system *n*-octanol–water. This parameter[1,2] has frequently been used to interpret the relationship between structure and activity for a number of drugs with fungicidal[3], haemolytic[4], tranquillizing[5] and anticonvulsive[6] activities. Another parameter of hydrophobicity is the R_M value[7–11], derived from reversed-phase thin-layer chromatography (TLC) and paper chromatography (PC). There is a linear relationship between R_M and log P[10,11]. Partition coefficients in some phenols have been determined by means of high-performance

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liquid chromatography (HPLC) using a chemically bound non-polar stationary phase[12].

The aim of this study was to ascertain whether in a series of phenols the R_M values obtained by liquid chromatography correlate with fungicidal activity, and to check the linearity of the relationship between the partition coefficients obtained by different chromatographic techniques.

THEORETICAL

Hansch *et al.*[1,2] measured the partition coefficients in the *n*-octanol–water system. From these values they established the substitution constant, π, defined by the equation

$$\pi = \log P_X - \log P_H \tag{1}$$

where P_X is the partition coefficient of the compound with a substituent X and P_H is the partition coefficient of the unsubstituted compound. The relationship between the partition coefficient P and the R_M values obtained by partition chromatography can be expressed by Collander's equation[13]:

$$\log P = bR_M + a \tag{2}$$

where a and b are constants.

The value of R_M is defined by the equation[14]

$$R_M = \log (1/R_F - 1) \tag{3}$$

for thin-layer and paper chromatography, and by the equation

$$R_M = \log \left(\frac{V_x - V_0}{V_0} \right) \tag{4}$$

for liquid chromatography[15], where V_x is the elution volume of a separated compound and V_0 is the elution volume of an unretained compound.

For a reference system where $P_H = 1$, by inserting eqn. 2 into eqn. 1 the following expression is obtained:

$$\pi = bR_M + a \tag{5}$$

This relationship also holds for related partitioning systems with water as the polar phase and with the same type of interaction occurring between the solute and the solvent in the non-polar phase.

The effect of dissociation on the chromatographic behaviour of bases and acids has been reported by Golumbic *et al.*[16]. If association of molecules in the organic phase can be ignored, it is possible to express the effect of environmental pH by the equation

$$R_M = R_M' + \log \frac{K_A + [H^+]}{[H^+]} \tag{6}$$

where R_M is the corrected value, R_M the experimental value, K_A is the dissociation constant of the solute and $[H^+]$ is the hydrogen ion concentration of the mobile phase.

The relationship between biological activity and physico-chemical parameters can be expressed by the equation[17]

$$\log 1/C_x = a \log P + \varrho\sigma + bS + d \quad (7)$$

where a, b and d are constants, ϱ is the reaction parameter and C_x is the molar concentration of a substance producing an equivalent biological or biochemical effect. P, σ and S refer to the effects of hydrophobic, electronic and spherical factors, respectively. P can be expressed as the *n*-octanol–water partition coefficient according to Hansch *et al.*[1] and σ is Hammett's constant[18]. Steric effects can be ignored if an appropriate choice of derivatives is made. For ionized compounds with substituents in the *meta* and *para* positions, Hammett's σ parameters can be replaced by pK_A values[18]. These have also been successfully correlated with the biological activity in compounds with *ortho* substituents[19,20].

EXPERIMENTAL

Chemicals and equipment

The phenol derivatives used were commercial preparations of analytical-reagent grade (Lachema, Brno, Czechoslovakia, and Merck, Darmstadt, G.F.R.). The compounds used are listed in Table II (I–XXVI). Their identities were confirmed by means of ultraviolet and infrared spectrophotometry. Analytical-reagent grade solvents were redistilled before use. The test strain was the fungus *Trichophyton gypseum* var. Kaufman-Wolf.

Chromatography was performed on a Varian LC 8500 chromatograph using a MicroPak CH-10 column (250 × 2 mm I.D., Merck), packed with silica gel (particle size 10 μm) with C_{18} chemically-bound non-polar stationary phase. An ultraviolet detector ($\lambda_{max.} = 254$ nm) and Hamilton 705 chromatographic syringes (50 μl) were also used.

Conditions

Phenols were separated on a MicroPak CH-10 column using various compositions of methanol–water and dioxan–water mixtures as the mobile phase at a flow-rate of 60 ml/h. Samples were dissolved in the mobile phase at a concentration of about 1 mg/ml and applied to the column in 5–10-μl volumes. The antifungal activity of the phenols was examined on a strain of *Trychophyton gypseum* var. Kaufman-Wolf using the inhibition zone method[21,22]. The molar concentration of each phenol that did not produce a visible inhibition zone by this method was used as a basis for correlation of biological activity with physico-chemical parameters. Values of the dissociation constants, pK_A, were taken from the literature[23,24], as also were Hansch's π parameters[25,26].

RESULTS AND DISCUSSION

Elution volumes of the phenols studied were measured and R_M values were calculated using a methanol–water system of varying composition as the mobile phase. The aim was to determine a concentration range within which the relationship between R_M values and methanol concentration in the mobile phase was constant, that is, to find out which values could be included in correlations by eqn. 5. For the phenols, constants for the regression equation and regression coefficients were calculated for methanol concentrations in the mobile phase in the range 20–70% (see Table I). As indicated by the regression coefficients, within this concentration range the relationship between R_M and methanol concentration was linear, which is in accordance with the results of other workers[12]. The best partitioning was achieved in the mobile phase methanol–water (1:4). The results are presented in Table II.

TABLE I

RELATIONSHIP BETWEEN R_M VALUES AND METHANOL CONCENTRATION IN THE MOBILE PHASE

$R_M = bc + a$.

a and b = constants; c = methanol concentration (%, v/v); n = number of compounds in the set; s = standard deviation; r = regression coefficient. Concentration of methanol in the mobile phase = 20–70% (v/v).

Compound	*a*	*b*	*n*	*s*	*r*
Phenol	0.769	−0.946	4	0.082	0.954
p-Cresol	0.997	−1.193	4	0.071	0.976
p-Chlorophenol	1.322	−1.656	4	0.080	0.985
p-Bromophenol	1.644	−2.130	4	0.197	0.952
p-Phenylphenol	2.090	−2.634	4	0.150	0.995

Experimental R_M values calculated by eqn. 4 were correlated with Hansch's π parameters. For the methanol–water (1:4) mobile phase eqn. 8 was derived; included in the calculation were phenols having $pK_A \geqslant 7.00$, for which the effect of correction by eqn. 6 could be ignored.

Mobile phase: methanol–water (1:4)

Compound	*Equation*	*n*	*s*	*r*	*Equation No.*
I–XIV	$\pi = 3.333\ R_M - 2.597$	14	0.153	0.874	(8)
I–XII	$\pi = 2.359\ R_M - 1.735$	12	0.279	0.936	(9)
I–XII, XV–XVII	$\pi = 2.234\ R_M - 1.578$	15	0.275	0.921	(10)
I–XII, XV–XVII	$\pi = 2.294\ R_M - 1.710$	15	0.303	0.904	(11)

On reverse calculation of π values according to eqn. 8, large differences were found between tabulated and calculated values for *m*- and *p*-aminophenols. After eliminating these compounds from the set tested, the relationship was defined by eqn. 9. Eqn. 10 determines the relationship between the parameters and R_M values for phenols, including those with $pK_A < 7.00$, with no correction for dissociation, and eqn. 11 expresses the same relationship after correcting the R_M values with eqn. 6.

TABLE II

CHROMATOGRAPHIC SEPARATION OF PHENOLS

Compound	*No.*	R_M	
		*I**	*II***
Phenol	I	0.653	0.000
m-Cresol	II	0.845	0.333
p-Cresol	III	0.813	0.348
Resorcinol	IV	0.477	−0.296
Hydroquinone	V	0.477	−0.269
Phloroglucinol	VI	0.301	−0.637
o-Chlorphenol	VII	0.875	0.454
p-Chlorphenol	VIII	1.060	0.558
p-Bromphenol	IX	1.393	0.692
o-Nitrophenol	X	0.954	0.417
m-Nitrophenol	XI	0.954	0.348
p-Nitrophenol	XII	1.000	0.284
m-Aminophenol	XIII	0.477	−0.301
p-Aminophenol	XIV	0.602	−0.367
2,6-Dinitrophenol	XV	0.544	−0.813
2,5-Dinitrophenol	XVI	0.740	−0.269
2,4-Dinitrophenol	XVII	0.602	—
p-Iodophenol	XVIII	—	0.845
p-Cyanophenol	XIX	—	0.208
o-Aminophenol	XX	0.699	0.333
o-Cresol	XXI	0.845	0.317
Pyrocatechol	XXII	0.398	−0.269
Guiacol	XXIII	0.778	0.208
p-Phenylphenol	XXIV	1.591	1.158
Pyrogallol	XXV	0.352	−0.269
2-Chloro-4-nitrophenol	XXVI	0.602	−0.367

* I = MicroPak CH-10 column, methanol–water (1:4) mobile phase, flow-rate 60 ml/h.
** II = MicroPak CH-10 column, dioxan–water (1:4) mobile phase, flow-rate 60 ml/h.

The same procedure was employed to compute the relationship between π and R_M values in the mobile phase dioxan–water (1:4). In this system the chromatographic behaviour of *m*- and *p*-aminophenols was less anomalous than in the system methanol–water (eqns. 12 and 13). Phenols with R_M values calculated according to eqn. 4 were represented by eqn. 14 and phenols with R_M values corrected according to eqn. 6 were defined by equation 15.

Mobile phase: dioxane–water (1:4)

Compound	*Equation*	*n*	*s*	*r*	*Equation No.*
I–XIV, XVIII–XIX	$\pi = 2.139\ R_M - 0.294$	16	0.239	0.969	(12)
I–XII, XVIII–XIX	$\pi = 1.889\ R_M - 0.168$	14	0.127	0.987	(13)
I–XVI, XVIII–XIX	$\pi = 1.587\ R_M - 0.079$	18	0.490	0.843	(14)
I–XVI, XVIII–XIX	$\pi = 1.832\ R_M - 0.141$	18	0.273	0.954	(15)

Eqns. 11 and 15 indicate a statistically significant ($P < 0.01$) linear relationship between π and R_M values obtained by reversed-phase HPLC.

TABLE III

RELATIONSHIP BETWEEN EXPERIMENTAL AND LITERATURE R_M VALUES

R_M (exp.) = bR_M (lit.) + a.

a and b = constants; n = number of compounds in the set; s = standard deviation; r = regression coefficient.

Mobile phase	*Chromatographic method**	*a*	*b*	*n*	*s*	*r*	*Reference*
Dioxan–water (1:4)	HPLC	0.585	1.096	7	0.031	0.942	12
	TLC	0.506	0.851	5	0.030	0.994	27
	PC	0.760	0.650	9	0.070	0.982	28
Methanol–water (1:4)	TLC	0.730	0.568	7	0.028	0.993	27
	PC	1.279	0.618	9	0.103	0.957	28

* HPLC: μBondapak C_{18}; methanol–water mobile phase. TLC: cellulose–5% ethyl oleate; ethanol–water (1:4) mobile phase. PC: Whatman No. 4–5% ethyl oleate, ethanol–water (1:4) mobile phase.

Eqn. 16 shows the R_M values measured with the methanol–water (1:4) mobile phase ($R_{M\text{-I}}$) and those with the dioxan–water (1:4) system ($R_{M\text{-II}}$) for phenols with $pK_A \geqslant 7.00$. Eqn. 17 holds for the test set of phenols without aminophenols. Eqn. 18 expresses the relationship between $R_{M\text{-I}}$ and $R_{M\text{-II}}$ values for the complete set of phenols, where the R_M values for nitrophenols with $pK_A < 7.00$ were corrected according to eqn. 6.

Compound	*Equation*	*n*	*s*	*r*	*Equation No.*
I–XIV, XX–XXV	$R_{M\text{-I}} = 0.724R_{M\text{-II}} + 0.666$	20	0.111	0.947	(16)
I–XII, XXI–XXV	$R_{M\text{-I}} = 0.773R_{M\text{-II}} + 0.655$	17	0.093	0.969	(17)
I–XII, XV–XVI	$R_{M\text{-I}} = 0.641R_{M\text{-II}} + 0.709$	20	0.188	0.874	(18)

TABLE IV

RELATIONSHIP BETWEEN EXPERIMENTAL R_M VALUES AND DATA REPORTED BY MARCINKIEWICZ *et al.*[28]

R_M (exp.) = bR_M (lit.) + a.

Compound	*Literature R_M values*[28]	*Measured R_M values*		*Calculated R_M values*	
		*I**	*II***	*I**	*II***
Phenol	−1.063	0.653	0.000	0.622	0.070
o-Cresol	−0.547	0.845	0.317	0.941	0.405
m-Cresol	−0.767	0.845	0.333	0.805	0.262
p-Cresol	−0.767	0.813	0.348	0.805	0.262
p-Bromophenol	−0.070	1.393	0.692	1.236	0.715
p-Iodophenol	0.204	—	0.845	—	0.893
o-Chlorophenol	−0.417	0.874	0.454	1.021	0.489
p-Chlorophenol	−0.417	1.060	0.558	1.020	0.489
p-Phenylphenol	0.556	1.591	1.158	1.623	1.121

* I = MicroPak CH-10 column, methanol–water (1:4) mobile phase, flow-rate 60 ml/h.

** II = MicroPak CH-10 column, dioxan–water (1:4) mobile phase, flow-rate 60 ml/h.

TABLE V

FUNGICIDAL ACTIVITY OF PHENOLS

Compound	*log 1/C**			
	Observed	*Calculated***		
		I	*II*	*III*
Phenol	3.819	4.207	3.935	4.036
o-Cresol	4.356	—	4.043	4.165
m-Cresol	4.356	4.610	4.177	4.321
p-Cresol	4.356	4.438	4.009	4.338
Phloroglucinol	3.946	—	3.717	3.121
m-Nitrophenol	4.487	5.714	5.578	5.589
p-Nitrophenol	6.396	6.412	6.451	6.334
o-Chlorophenol	4.857	5.706	5.297	5.671
p-Chlorophenol	5.694	5.452	5.111	5.157
2,4-Dinitrophenol	8.197	8.000	8.536	—
2,5-Dinitrophenol	7.353	7.560	7.486	7.222
2,6-Dinitrophenol	7.817	7.373	8.672	7.960
2-Chloro-4-nitrophenol	9.821	—	8.782	8.962

* C = fungicidal activity determined experimentally against *Trychophyton gypseum* var. Kaufman-Wolf.

** I, II and III = fungicidal activities as calculated by eqns. 19, 21 and 23, respectively.

The R_M values obtained in these experiments were correlated with those obtained by various chromatographic methods as reported in the literature (see Tables III and IV). The results in Table III show that the linear relationship between these R_M values was statistically significant ($P < 0.01$).

Both Hansch's π parameters and R_M values were used to correlate the fungicidal effect of mono- and di-substituted phenols (tested on *Trychophyton gypseum* var. Kaufman-Wolf) with their physico-chemical properties (Table V). For the π parameters, this relationship was expressed by eqn. 19. Eqns. 20 and 21 were derived for uncorrected R_M values and for R_M values corrected by eqn. 6, respectively, using methanol–water (1:4) as the mobile phase. Eqns. 22 and 23 were formulated for both the uncorrected and corrected R_M values measured with the mobile phase dioxan–water (1:4).

Equation	*n*	*s*	*r*	*Equation No.*
$\log 1/C = 0.858\ \pi - 0.648\ pK_A + 10.616$	10	0.574	0.952	(19)
Mobile phase: methanol–water (1:4)				
$\log 1/C = 1.267\ R_M - 0.743\ pK_A + 10.530$	13	0.661	0.950	(20)
$\log 1/C = 1.698\ R_M - 0.703\ pK_A + 0.779$	13	0.510	0.957	(21)
Mobile phase: dioxan–water (1:4)				
$\log 1/C = 0.921\ R_M - 0.758\ pK_A + 11.552$	12	0.904	0.900	(22)
$\log 1/C = 1.117\ R_M - 0.723\ pK_A + 11.186$	12	0.867	0.909	(23)

Correlation coefficients expressed by eqns. 19–23 indicate that for phenols hydrophobic R_M data have the same value as Hansch's π parameters when corre-

lating fungicidal activity with physico-chemical parameters. Moreover, HPLC is a method that requires less time and labour than the spectrophotometric determination of partition coefficients in a pair of solvents.

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