RELATIONSHIPS BETWEEN BIOLOGICAL ACTIVITY OF PHENOLS AND THEIR PHYSICO-CHEMICAL PARAMETERS

Miroslav POLSTER[a], Bohuslav RITTICH[b]* and Renata ŽALUDOVÁ[b]

[a] *Faculty of Medicine, Purkyně University, 662 44 Brno and*
[b] *Research Institute of Animal Nutrition, 691 23 Pohořelice*

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The present work deals with the relationship between biological activities of differently substituted phenols and their physico-chemical parameters expressing the influence of hydrophobic, electronic and steric factors. The testing was performed with the fungi *Trychophyton gypseum* and *Trychophyton gypseum* var. *Kaufman–Wolf* and the yeast *Candida albicans*. Significant relationship between biological activity and pK_A values was calculated. The interactions between individual factors as well as the influence of the position of substituents on quantitative structure–activity relationships are discussed.

Phenols are compounds generally known to have broad applicability. Of particular importance is their medical utilization as desinfectants or antimicrobial and antifungal agents. In dermatology, for instance, dermatophytoses (superficial skin infections caused by fungi) are successfully treated with the preparation "Nitrofungin", the effective component of which is 2-chloro-4-nitrophenol.

It has been recognized earlier[1–4] that the structure of phenols or their physico-chemical properties correlate well with their fungicidal effect. Most of the phenols seem to have the same mechanism of action which may be either accelerated or decelerated by various substituents of the phenyl ring.

One of the approaches relating biological activity to physical properties of the molecule of an active compound is that based on linear combination of the free energy terms representing physico-chemical parameters. It can be expressed as follows[5]:

$$\log 1/C_n = k_1\pi + k_2\sigma + k_3E_s + k_4\,, \qquad (I)$$

where C_n is the molar concentration of the substance producing an equivalent biological or biochemical effect, π, σ and E_s are constants referring to hydrophobic, electronic and steric effects, respectively.

Hydrophobic factor can be expressed by the partition coefficients determined in 1-octanol–water system (log P or Hansch's π parameters). Electronic factor can be expressed by Hammett's σ constants[6]. For ionized compounds with substituents in the *meta* and *para* positions, Hammett's constants can be replaced by pK_A values. These have also been successfully correlated with the biological activity in compounds with *ortho* substituents[4,7,8]. Most frequently, for the expression of the steric effect of substituents Taft's steric constants E_s have been used[9]. When using steric parameters problems remain with multiple substitution and with asymetric substituents. In such cases several authors[10] made use of the parameters reflecting the total volume of substituents — for example of molecular mass or molar refractivity.

* The author to whom correspondence should be addressed.

The objective of this work was to study the influence of hydrophobic, electronic, and steric parameters of phenols on their fungicidal activity.

EXPERIMENTAL

Chemicals and Equipment

The phenol derivatives used were commercial preparations of analytical grade (Lachema, Brno, Czechoslovakia, and Merck, Darmstadt, G.F.R.). The compounds used (*I*—*XXV*) are listed in Table I. Their identities were confirmed by means of ultraviolet and infrared spectrophoto-

TABLE I

Biological activity and physico-chemical parameters of phenols
Fungicidal activity: *A Candida albicans, B Trichophyton gypseum* var. *Kaufman–Wolf, C Trichophyton gypseum.*

No	Substituent	log $1/C_n$			pK_A	log *P*
		A	*B*	*C*		
I	H	3·819	3·819	3·819	9·89	1·46
II	2-CH_3	3·879	4·356	4·356	10·20	1·95
III	3-CH_3	3·879	4·356	4·356	10·01	1·96
IV	4-CH_3	3·879	4·356	4·356	10·17	1·94
V	2-OH	3·887	3·887	3·887	9·48	0·88
VI	3-OH	4·364	4·364	—	9·81	0·80
VII	4-OH	3·887	3·887	3·887	9·96	0·59
VIII	3-NH_2	4·360	4·360	4·360	9·87	0·17
IX	4-NH_2	4·360	3·883	3·883	10·30	0·04
X	2-Cl	4·857	4·857	4·857	8·49	2·15
XI	3-NO_2	5·164	—	—	8·28	2·00
XII	4-NO_2	6·010	6·176	6·176	7·15	1·91
XIII	3,5-di-OH	3·946	3·946	3·946	9·35	0·16
XIV	2,6-di-CH_3-4-NO_2	5·983	6·080	6·080	7·16	—
XV	3,5-di-CH_3-4-NO_2	5·545	5·802	5·802	8·24	—
XVI	2-Cl-4-NO_2	8·252	8·427	8·348	3·74	—
XVII	2-OH-4-NO_2	5·551	6·074	6·074	6·70	—
XVIII	2-NH_2-4-NO_2	5·543	5·963	5·543	7·20	—
XIX	3-Cl-4-NO_2	6·524	6·649	6·524	5·68	—
XX	2-OH-3-NO_2	6·454	—	6·454	5·73	—
XXI	2-NO_2	4·008	4·008	4·008	7·23	1·77
XXII	2,4-di-NO_2	6·356	6·809	6·613	4·09	1·50
XXIII	2,5-di-NO_2	5·905	6·067	6·067	5·10	1·75
XXIV	2,6-di-NO_2	6·424	6·850	6·424	3·77	1·55
XXV	4-Cl-2-NO_2	4·687	4·687	4·687	6·99	—

metry. Analytical reagent grade solvents were redistilled before use. The test strain was the fungus *Trichophyton gypseum* and *Trichophyton gypseum* var. *Kaufman–Wolf* and the yeast *Candida albicans*. The microorganisms were obtained from the microbiological laboratory of the Dermatological Clinic of the Faculty of Medicine, Purkyně University, Brno, Czechoslovakia.

Conditions

The fungicidal activity of the phenols was examined using the inhibition zone method. Experimental conditions have been described in papers[11,12]. The molar concentration of each phenol that did not produce a visible inhibition zone was used as a basis for correlation of fungicidal activity with physico-chemical parameters. Since phenols are dissociable compounds and generally only undissociated molecules can produce a fungicidal effect[13], the molar concentration of phenols was expressed as the concentration of undissociated molecules using the equation[14]

$$\log 1/C_n = \log 1/C + \log \frac{K_A + [H^+]}{[H^+]}, \qquad (2)$$

where C_n is the molar concentration of the neutral form of a phenol, C is the molar concentration of the phenol, $[H^+]$ is the hydrogen ion concentration of external medium (in this case a Czapek–Dox agar, pH 5·6, was used). Values of the dissociation constants, pK_A, were taken from literature[15,16], as well as Hansch's π parameters[1,14], F constants from paper Swain and Lupton[17], molecular refractivities (MR) from Martin[18] and Taft's steric constants of *ortho*-substituted compounds E_s^0 from Exner[19]. The value pK_A of 2-nitro-4-chlorophenol was determined spectrophotometrically according to Perrin[20], Albert and Serjeant[21].

RESULTS AND DISCUSSION

Hydrophobic Effect

Partition coefficients ($\log P$) were used for correlations with fungicidal activities (Table I). Out of the set of the tested phenols, it was possible to distinguish 2 different types of regression equations with different slopes. For the phenols with electrone-acceptor substituents (2-chlorophenol, nitrophenols) the following slope values were calculated: $b = 1{\cdot}16 - 1{\cdot}92$. For phenols with electrone-donor substituents the calculated values were $b = 0{\cdot}05 - 0{\cdot}35$. As the correlations were found to be rather poor and the values of regression coefficients b were not statistically different from zero, it is not possible to draw conclusions on the type of hydrophobic bond with the acceptor.

Similarly, the equations with quadratic member $(\log P)^2$ were statistically non-significant.

From the values of correlations it follows that in this case the effect of hydrophobic factor is much less important than would be expected on the basis of earlier analysis performed by authors[2,22,23]. The statistically significant linear and quadratic relationships quoted in the literature were calculated mostly for phenols with alkyl or halogen substituents.

TABLE II

Relationship between biological activity of phenols and pK_A values.
n Number of compounds in the set, r correlation coefficient, s standard deviation, t_b Student's characteristic for the coefficient b of the regression equation $Y = bX + a$ (the coefficient was tested on the hypothesis $b = 0$).

Tested material	Compounds	$\log 1/C_n$	n	r	s	t_b	Eq. No
Candida albicans	*XXI–XXV*	$-0{\cdot}440\ pK_A + 8{\cdot}170$	5	0·827	0·554	2·550	(*3*)
	I–XX	$-0{\cdot}627\ pK_A + 10{\cdot}260$	20	0·969	0·309	16·647[a]	(*4*)
	I–XX	$-0{\cdot}618\ pK_A + 10{\cdot}281$	20	0·978	0·257	19·703[a]	(*5*)
Trychophyton gypseum	*XXI–XXV*	$-0{\cdot}831\ pK_A + 10{\cdot}142$	5	0·995	0·160	16·675[a]	(*6*)
var. *Kaufman-Wolf*	*I–X, XII–XIX*	$-0{\cdot}662\ pK_A + 10{\cdot}707$	18	0·967	0·336	15·224[a]	(*7*)
	I–X, XII–XIX	$-0{\cdot}646\ pK_A + 10{\cdot}664$	18	0·977	0·271	18·430[a]	(*8*)
Trychophyton gypseum	*XXI–XXV*	$-0{\cdot}736\ pK_A + 9{\cdot}506$	5	0·979	0·283	8·368[a]	(*9*)
	I–V, VII–X XII–XX	$-0{\cdot}629\ pK_A + 10{\cdot}369$	18	0·962	0·360	14·093[a]	(*10*)
	I–V, VII–X XII–XX	$-0{\cdot}627\ pK_A + 10{\cdot}449$	18	0·975	0·286	17·672[a]	(*11*)

[a] Statistically highly significant difference ($P < 0{\cdot}01$).

Electronic Effect

The effect of the electronic factor on the fungicidal activity of phenols was expressed by means of pK_A values. In equations (*3*), (*6*), and (*9*) the relationships are calculated for phenols with NO_2-substitutents in position 2, and in equations (*4*), (*7*), and (*10*) for the other phenols. The regression equations have a different slope for each group (Table II). Collecting both groups into one set resulted in a decrease of the value of correlation coefficient – Eqs (*12*), (*15*), (*18*) (Table III). The other phenols with the substituents in position 2 showed no influence on the value of regression line slope.

The equilibrium and rate constants in molecules with several equivalent functional groups are dependent even on the number of these groups. It is necessary to introduce a correction for this influence – the so-called statistical factor[24] (the equilibrium or rate constant is divided by the number of functional groups). The values of pK_A were not divided by the number of functional groups because pK_A values were used in the analysis as a counterpart of Hammett's σ constants. In this case the effect of dissociation should not be confused with pK_A. The relationships for corrected values of fungicidal activities and pK_A values were given in equations (*5*), (*8*), and (*11*). They reveal that the statistical factor did not substantially increase the value of correlation coefficient, which, perhaps, was due to lower precision of measuring fungicidal activities.

As for phenols with the NO_2-groups in position 2 regression equations were calculated having a different slope than for other phenols, it was tested whether this phenomenon was caused by proximity effect[25]. To express it for phenols with ortho substituent, F constants according to Swain–Lupton[17] were used. The relationship between $\log 1/C_n$, pK_A and F for the whole set was given in Eqs (*13*), (*16*), (*19*).

The increase of values of correlation coefficients agrees with the assumption that the deviation in the behaviour of 2-nitrophenols may be caused by proximity effect. Further evaluation of this phenomenon is presented in the following chapter.

The fact that the fungicidal activity of phenols is associated with the presence of a hydroxyl group in the molecule was confirmed by the determination of fungicidal activity of ethers: methoxy-4-nitro-benzene and ethoxy-4-nitrobenzene. These two compounds had a lower fungicidal activity against the fungus *Trychophyton gypseum* var. *Kaufman–Wolf* ($\log 1/C_n = 4{\cdot}507$ and $\log 1/C_n = 4{\cdot}545$, respectively) as compared to 4-nitrophenol (Table I). The activity found in these two ethers can be perhaps explained by the presence of NO_2-group which may be subjected – owing to metabolic pathways of a fungus cell – to the following reactions[26]:

$$—NO_2 \rightarrow —NH_2OH \rightarrow -NH_2 .$$

The fungicidal activity of aniline derivatives has not been fully elucidated as yet, but, in general, these compounds are known as toxic to organisms[27]. Contrary to this

TABLE III

Relationship between biological activity of phenols and steric parameters. *F* Denotes Snedecor's characteristic

Tested material	Compounds	$\log 1/C_n$	*n*	*r*	*s*	*F*	Eq. No
Candida albicans	*I—XXV*	$-0{\cdot}476\ pK_A + 8{\cdot}870$	25	0·883	0·556	39·128[a]	(*12*)
		$-0{\cdot}639\ pK_A + 0{\cdot}354\ F + 9{\cdot}963$	25	0·952	0·373	66·995[a]	(*13*)
		$-0{\cdot}611\ pK_A + 1{\cdot}355\ E_s^o + 10{\cdot}151$	25	0·968	0·306	103·533[a]	(*14*)
Trychophyton gypseum var. *Kaufman–Wolf*	*I—X, XII—XIX, XXI—XXV*	$-0{\cdot}499\ pK_A + 9{\cdot}183$	23	0·885	0·594	36·163[a]	(*15*)
		$-0{\cdot}663\ pK_A + 0{\cdot}344\ F + 10{\cdot}313$	23	0·943	0·435	50·887[a]	(*16*)
		$-0{\cdot}643\ pK_A + 1{\cdot}338\ E_s^o + 10{\cdot}549$	23	0·959	0·372	71·911[a]	(*17*)
Trychophyton gypseum	*I—V, VII—X, XII—XXV*	$-0{\cdot}480\ pK_A + 8{\cdot}993$	23	0·871	0·612	31·352[a]	(*18*)
		$-0{\cdot}644\ pK_A + 0{\cdot}360\ F + 10{\cdot}102$	23	0·940	0·434	48·301[a]	(*19*)
		$-0{\cdot}621\ pK_A + 1{\cdot}410\ E_s^o + 10{\cdot}332$	23	0·962	0·348	78·663[a]	(*20*)

[a] Statistically highly significant difference ($P < 0{\cdot}01$).

the ester 2-chloro-4-nitrophenyl acetate, which hydrolyzes easily, showed the same biological activity as did 2-chloro-4-nitrophenol.

Steric Effect

Fungicidal activities were also correlated with molecular masses, molar refractivities and Taft's constants[19] E_s^o. The relationships calculated were statistically non significant.

Because for the phenols with the NO_2-groups in position 2, regression equations with a different slope than for other phenols were calculated, it was tested whether this phenomenon was caused by a steric effect. Using multiple linear regression the relationship between biological activities, pK_A values and steric parameters expressing the influence of the substituent in position 2 was studied.

In the regression equations, where the steric effect was expressed by molar refractivity, correlation coefficients varied in the range of $r = 0{\cdot}899 - 0{\cdot}911$. The relationships with Taft's constants E_s^o are given in Eqs (*14*), (*17*), and (*20*). It is necessary to take into regard that similar values of correlation coefficients were calculated for equations with F parameter. This can be explained by the fact that E_s^o values may be completely accounted for in terms of electrical effects parameters[28].

The paper paid attention to phenols with substituents in position 2. Compounds with the above mentioned type of substitution are not frequently compared with other derivatives in the same correlation. From this point of view, the results obtained here present a contribution to the investigation into the relationship between the biological activity of compounds and their structure.

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