THE ANTIFUNGAL ACTIVITY OF SOME ALIPHATIC AND AROMATIC ACIDS

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The present paper deals with the relationship between biological activities of some aliphatic and aromatic acids and their physico-chemical parameters expressing the influence of hydrophobic factors. The test strain in the biotest of growth inhibition was the fungus *Fusarium moniliforme* CCMF-180 and *Penicillium expansum* CCMF-576. Significant relationship between antifungal activities of un-ionized form of aliphatic acids and their capacity factors ($\log k'_0$) extrapolated to pure water, partition coefficients determined in 1-octanol–water system ($\log P_{oct}$) and the first order of molecular connectivity indices ($^1\chi$) were calculated. The ionized form of aliphatic acids were antifungally active, too. For benzoic acids significant relationships between antifungal activities and capacity factors of anionic form ($\log k'_{ia}$) were calculated.

Aliphatic and aromatic acids are compounds generally known to have broad applicability. Certain short fatty acids affect growth of various plant organs and can induce seed dormancy[1-3]. Derivatives of benzoic acid are the parts of tree leaves and nuts[4] and have also been formed by tissue cultures[5,6]. Their utilization as preservatives of agricultural crops and foods against deterioration caused by microorganisms and fungi[7-9] is of particular importance.

Hydrophobicity is a major physico-chemical parameter in quantitative structure activity relationships (QSAR) studies[10]. The hydrophobicity can be expressed by the partition coefficients determined in 1-octanol–water system[11-13] ($\log P_{oct}$, Hansch's or Rekker's parameters). Chromatographic data (capacity factors $\log k'$) determined by reversed-phase high-performance liquid chromatography (RP-HPLC) can be also used[14]. Some authors[15-17] showed significant correlations between $\log P_{oct}$ or $\log k'$ and topological indices known as the molecular connectivity indices χ.

The aim of this paper was to study the relationships between hydrophobic parameters both aliphatic and aromatic acids and their antifungal activities.

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EXPERIMENTAL

Chemicals and Equipment

The compounds investigated were of analytical grade. The aliphatic, phenylacetic, indole-3-propionic acids were obtained from Lachema (Brno, Czechoslovakia), indole-3-acetic acid (IAA) from Loba (Wien, Austria) and benzoic acids from Fluka (Buchs, Switzerland).

The test strain was the fungus *Fusarium moniliforme* CCMF-180 and *Penicillium expansum* CCMF-576. The microorganisms were obtained from the microbiological laboratory of the Dermatological Clinic of Faculty of Medicine, Masaryk University, Brno, Czechoslovakia.

Experimental Conditions

The antifungal activity of the acids was examined using the inhibition zone method at the different concentrations of tested ones. The acids were dissolved in mixture ethanol–water (1 : 1) and micropipetted into the pits in solidified Czapek–Dox agar which had been inoculated by a spore

TABLE I

Biological activity and physico-chemical parameters of aliphatic acids. *F.m. Fusarium moniliforme* CCMF-180, *P.e. Penicillium expansum* CCMF-576, *A.s. Avena sativa* cv. SELMA

No.	Acid	$\log 1/C_m$			$\log S$	$\log P_{oct}$	$\log k'_0$	$\log P_i$	$^1\chi$	pK_A
		F.m.	*P.e.*	*A.s.*						
I	Formic	3·663	3·664	—	—	−0·54	0·112	−4·70	1·41	3·75
II	Acetic	2·802	2·795	—	—	−0·17	0·367	−4·20	1·73	4·75
III	Propionic	2·769	—	—	—	0·33	0·623	−3·70	2·27	4·87
IV	Butyric	3·080	2·996	—	—	0·79	0·878	−3·20	2·77	4·81
V	Pentanoic	3·117	2·983	1·947	−0·620	1·21	0·999	−2·70	3·27	4·72
VI	Hexanoic	3·210	3·130	2·097	−1·167	1·88	1·464	−2·20	3·77	4·88
VII	Heptanoic	—	—	2·319	−1·886	2·39[a]	—	−1·70	4·27	4·89
VIII	Octanoic	3·648	3·463	2·367	−2·658	2·93	1·974	−1·20	4·77	4·89
IX	Nonanoic	—	—	2·678	−3·125[a]	3·45[a]	—	−0·70	5·27	4·96
X	Decanoic	—	—	2·481	−3·842	3·99	2·410	−0·20	5·77	4·92
XI	2-Methyl-propionic	2·849	2·849		—	—	—	−3·40	2·64	4·84
XII	2-Methyl-butyric	2·484	2·924	—	—	—	—	—	3·13	4·77

[a] Calculated value.

suspension (7-day-old culture). The diameter of inhibition zones was recorded after 48 h cultivation at the temperature 25°C in the dark. Four replications were carried out for each acid concentration. The molecular concentration of acid that produced an inhibition zone with diameter 15 mm was used as a basic correlation of biological activity with hydrophobic parameters. Since acids are dissociable compounds the molar concentration was expressed as the concentration of undissociated molecules (Eq. (*1*)) and dissociated ones (Eq. (*2*)):

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

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

where C_m is the molar concentration of the neutral form of an acid, C is the molar concentration of an acid, C_{ia} is the molar concentration of anionic form, $[H^+]$ is the concentration of the solvated proton of external medium (in the case a Czapek–Dox agar, pH 5·6 was used).

The biological activities and some physico-chemical parameters used are listed in Tables I and II.

The capacity factors $\log k'$ of aliphatic acids were taken from literature[18], as well as capacity factors of the anionic form of aromatic acids[19,20] — $\log k'_{ia}$. The capacity factor of the anionic form of phenylacetic acid was determined at the same chromatographic conditions as the other acids (Table II) given in paper[20]. From literature were also taken negative logarithms of disso-

TABLE II

Biological activity and physico-chemical parameters of aromatic acids. *P.e.Penicillium expansum* CCMF-576, *F.m. Fusarium moniliforme* CCMF-180

No.	Acid	$\log 1/C_{ia}$		$\log k'_{ia}$		pK_A	$\log P_{oct}$
		P.e.	*F.m.*	*I*[a]	*II*[b]		
XIII	Benzoic (B)	2·603	2·602	−0·538	−1·222	4·19[a]	1·87
XIV	2-Hydroxy B	2·554	2·476	−0·456	−1·620	2·97[a]	2·26
XV	3-Hydroxy B	2·278	2·234	−0·750	−2·720	4·06[a]	1·32
XVI	4-Hydroxy B	2·120	2·049	−0·770	−1·765	4·48	1·31
XVII	2-Methoxy B	2·562	2·550	—	−1·326	3·91	1·59
XVIII	3-Methoxy B	2·464	2·407	−0·523	−1·525	4·27	2·02
XIX	4-Methoxy B	2·471	2·430	—	−1·698	4·36	1·96
XX	Phenylacetic	2·475	2·381	−0·538	−1·765	4·28	1·96
XXI	Indole-3-acetic	2·526	2·425	−0·553	−1·824	4·65[a]	1·41
XXII	Indole-3-propionic	2·436	—	−0·444	—	4·81[a]	—

[a] Values were taken from reference[19]; [b] values were taken from reference[20].

ciation constants[19,21] — pK_A, partition coefficients determined in 1-octanol–water system[11] — log P_{oct} (log P_{oct} values of heptanoic and nonanoic acids were calculated from regression equation log P_{oct} vs C_n), solubilities in water[22] — log S (log S value of nonanoic acid was calculated from regression equation log S vs C_n), air–water partition coefficients[23] — log k_{aw}. The molecular connectivity indices were calculated according to Kier and Hall[24]. The partition coefficients of ionized form of aliphatic acids determined in 1-octanol–water system (log P_i) were taken from the paper published by Hansch and Lien[25].

The inhibition activity of aliphatic acids in seed germination of oat (*Avena sativa* cv. SELMA) were taken from the literature[26]. The coefficients of regression equation $Y = a + bX$ were tested on the hypothesis that $a, b = 0$ according to a procedure presented by Eckschlager et al.[27].

RESULTS AND DISCUSSION

Aliphatic Acids

As the tested aliphatic acids were of different polarity the log k' values were determined using various compositions of methanol–water mixture as the mobile phase[18]. Therefore capacity factors extrapolated to pure water (log k'_0) were used as a basis for correlation with antifungal activity of aliphatic acids. To verify the possibility to use log k'_0 values as hydrophobic parameters they were correlated with partition coefficients determined in 1-octanol–water system and/or the first order of molecular connectivity indices $^1\chi$ (Eqs (*3*) and (*4*)).

$$\log P_{oct} = -0{\cdot}858 + 1{\cdot}960 \log k'_0 \quad n = 8,\ r = 0{\cdot}998,\ s = 0{\cdot}099,\ t_b = 41{\cdot}595,\ P < 0{\cdot}01, \tag{3}$$

$$\log k'_0 = -0{\cdot}592 + 0{\cdot}526\,{}^1\chi \quad n = 8,\ r = 0{\cdot}997,\ s = 0{\cdot}062,\ t_b = 33{\cdot}715,\ P < 0{\cdot}01. \tag{4}$$

The relationships between hydrophobic parameters, $^1\chi$ values and antifungal activities of un-ionized form of aliphatic acids (tested on *Fusarium moniliforme* CCMF-180 and *Penicillium expansum* CCMF-576) were studied. The linear regression equations calculated were statistically highly significant (Table III). Formic acid was not included into the set because the value of antifungal activity (log $1/C_m$) was outside the regression curve. Due to low value of pK_A formic acid is fully ionized and anionic form is less antifungally active than un-ionized one (see below).

The ionized forms of the tested acids were antifungally active, too. This phenomenon agrees with well-known experience that salts of formic and propionic acids have been used as preservatives[7,8]. By our experience, the salts of these acids are less active than undissociated ones. In distinction to free acids they are less corrosive and more convenient from the point of view of occupational hygiene[8]. The relationships between antifungal activity of onized form (log $1/C_{ia}$) and log P_i were statistical-

ly highly significant – see Eqs (*9*) and (*13*). The slopes is mentioned equations do not agree with these ones given by Hansch and Lien[25]. It may be explained by the fact that in this paper antifungal activities were examined at pH 5·6 in contrary to pH 6·5 employed in the paper[25].

To the finding that both acid forms are biologically active can be added that the undissociated form is the transport one and the ionized form is the active agent for many compounds.

Aliphatic acids with long chain (C_{12} and C_{14}) were not active. For explanation of this phenomenon data published in the literature[26] were used. Oat (*Avena sativa* cv. SELMA) showed reduced germination in the presence of fatty acids with chain lengths of C_5 to C_9. Highly significant linear regression equation for relationship between biological activity of aliphatic acids (C_5-C_9) and log P_{oct} was calculated (Table III, Eq. *14*). The decanoic acid was less effective than nonanoic one. The data are presented graphically in Fig. 1. The graph in Fig. 1 identifies "aquatic biological activity space" as the region below the water solubility. There were

TABLE III

Relationship between biological activity of aliphatic acids and their physico-chemical parameters

Acid No.	Equation $\log 1/C_m =$	n	r	s	t_b	Eq. No.
Fusarium moniliforme CCMF-180						
II–VI, VIII	$2{\cdot}783 + 0{\cdot}277 \log P_{oct}$	6	0·967	0·082	8·481[a]	*5*
	$2{\cdot}550 + 0{\cdot}527 \log k'_0$	6	0·963	0·087	7·957[a]	*6*
	$2{\cdot}227 + 0{\cdot}283\,^1\chi$	6	0·965	0·084	8·200[a]	*7*
II–VI, VIII, XI, XII	$2{\cdot}164 + 0{\cdot}288\,^1\chi$	8	0·912	0·121	5·895[a]	*8*
I–VI, VIII	$3{\cdot}241 + 0{\cdot}311 \log P_i$	7	0·989	0·057	16·061[a,b]	*9*
Penicillium expansum CCMF-576						
II, IV–VI, VIII	$2{\cdot}798 + 0{\cdot}208 \log P_{oct}$	5	0·973	0·057	8·413	*10*
	$2{\cdot}621 + 0{\cdot}398 \log k'_0$	5	0·977	0·053	9·126[a]	*11*
	$2{\cdot}385 + 0{\cdot}211\,^1\chi$	5	0·961	0·069	6·953[a]	*12*
I, II, IV–VI, VIII	$2{\cdot}984 + 0{\cdot}249 \log P_i$	6	0·931	0·126	5·725[a,b]	*13*
Avena sativa cv. SELMA						
VI–X	$1{\cdot}543 + 0{\cdot}312 \log P_{oct}$	5	0·977	0·060	9·145[a]	*14*
	$0{\cdot}802 + 0{\cdot}346\,^1\chi$	5	0·981	0·054	10·052[a]	*15*

[a] $P < 0{\cdot}01$; [b] in Eqs (*9*) and (*13*), values the C_{ia} were used instead of C_m ones.

founded the parabolic dependences between biological activities and log P_{oct} values in some cases[25]. They have been caused by the compounds transport in the biological system. In the region above the water solubility the biological activity could not be measured under equilibrium conditions. Nonlinear dependences between biological activities and log P_{oct} have been effected by other influences than by the compounds transport. The point of maximum activity varied according to the organisms tested and depended on the resistance of organism to the active agent[28]. This biological activity–structure relationship is consistent with the concept of

TABLE IV

Relationship between biological activity of aromatic acids and their capacity factor of anionic form (log k'_{ia})

Acid No.	Equation log $1/C_{ia}$ =	n	r	s	t_b	Eq. No.
Penicillium expansum CCMF-576						
XIII–XVI, XVIII	3·190 + 1·295 log k'_{ia}	5	0·919	0·080	4·653[a,b]	*16*
XIII–XVI, XVIII, XX, XXI	3·202 + 1·306 log k'_{ia}	7	0·917	0·069	5·630[a,b]	*17*
XIII–XV, XVII–IXX	2·836 + 0·206 log k'_{ia}	6	0·950	0·036	6·815[a,c]	*18*
XIII–XV, XVII–XXI	2·836 + 0·201 log k'_{ia}	8	0·923	0·038	6·328[a,c]	*19*
Fusarium moniliforme CCMF-180						
XIII–XV, XVII–IXX	2·829 + 0·225 log k'_{ia}	6	0·940	0·044	6·172[a,c]	*20*
XIII–XV, XVII–XXI	2·826 + 0·226 log k'_{ia}	8	0·929	0·041	6·619[a,c]	*21*

[a] $P < 0{\cdot}01$; [b] values of log k'_{ia} were taken from reference[19]; [c] values of log k'_{ia} were taken from reference[20].

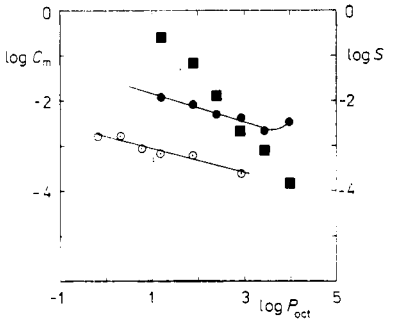

FIG. 1

Relationships between 1-octanol–water partition coefficient (log P_{oct}) and biological activity (log C_m) for *Fusarium moniliforme* CCMF-180 (⊙) and for *Avena sativa* cv. SELMA (●). ■ water solubility data

a toxicity "cut off" at the higher homologs published by Ferguson[28]. The decrease in biological activity observed for the higher homologs may result from a decrease in the chemical activity of bulkier compounds occupying larger molecular volumes[29,30].

Statistically less significant regression equation for relationship between fungicidal activity and air–water partition coefficients ($r = 0{\cdot}887$) was calculated for *Fusarium moniliforme* CCMF-180.

Aromatic Acids

To compare the mode of biological effect of various types of acids the antifungal activity of some aromatic acids was studied. The unsignificant linear relationship between antifungal activities of the neutral form of tested acids (compounds *XIII* to *XXI*) and log P_{oct} values were calculated ($r = 0{\cdot}609$ for *Penicillium expansum* CCMF-576 and $r = 0{\cdot}635$ for *Fusarium moniliforme* CCMF-180, resp.). On the contrary, the high significant relationship between activities and capacity factors of anionic form (log k'_{ia}) were calculated (Table IV).

This phenomenon agrees with well-known experience that salts of benzoic acid derivatives are antifungally active[31].

It is interesting that phenylacetic, indole-3-acetic and indole-3-propionic acids were also fungicidally active. Nevertheless, the value of biological activity of indole-3--propionic acid did not lay on regression curve log $1/C_{ia}$ vs log $k'_{ia}(1)$ ($r = 0{\cdot}833$ for *Penicillium expansum* CCMF-576; acids: *XIII*–*XVI*, *XVIII*, *XX*–*XXII*, $n = 8$).

From the above mentioned compounds indole-3-acetic acid (IAA – auxin) is ranked among the most effective plant growth regulators[32]. At higher concentrations (10^{-2} mol l^{-1}) this aid is responsible for correlative inhibition among plant organs. The authors cannot explain the different mechanism of IAA biological activity which depends evidently on its concentration. According to Bates and Goldsmith[33] all the weak acids, including IAA, at various concentrations have different mechanisms of effect on the plasma membrane potential in coleoptile cells of oat (*Avena sativa* L.).

SYMBOLS

C	molar concentration
C_{ia}	molar concentration of the anionic form of an acid
C_m	molar concentration of the neutral form of an acid
C_n	number of methylene groups in the alkyl chain
H^+	concentration of solvated proton
k'	capacity factor
k'_{ia}	capacity factor of the anionic form of an acid
k'_0	capacity factor extrapolated to pure water

k_{aw}	air–water partition coefficient
n	number of compounds in the set
P_i	partition coefficient of the anionic form determined in 1-octanol–water system
pK_A	dissociation constant
P_{oct}	partition coefficient determined in 1-octanol–water system
r	correlation coefficient
s	standard deviation
S	solubility in water
t_a, t_b	Student's characteristics for the coefficients a, b of the regression equation $Y = a + bX$
χ	molecular connectivity index

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