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Role of Hydrophobic Interactions in Enzyme Inhibition by Drugs

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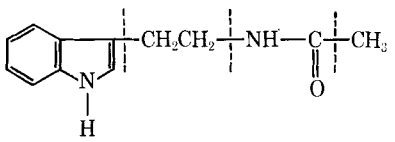
Abstract □ The role of hydrophobic interactions in inhibiting the relatively specific enzymatic reactions of five enzyme systems by series of congeneric drugs has been illustrated by the use of substituent constants and regression analysis. The inhibition of lipoxygenase by alcohols, the inhibition of D-amino acid oxidase by maleimides, and the inhibition of hydroxyindole-*o*-methyltransferase by *N*-acyltryptamines are found to be linearly dependent on the lipohydrophilic character of the inhibitors (log *P* or π). The inhibition of carbonic anhydrase by sulfonamides is found to be linearly dependent on the log *P* and Hammett's σ constant. For monoamine oxidase inhibition by substituted β-carbolines, a parabolic equation of log *P* gives the most significant correlation. The ideal lipohydrophilic character (log P_0) for maximum inhibition is found to be 2.74.

Keyphrases □ Enzyme inhibitory activity—hydrophobic interactions, drugs □ Hydrophobic interactions, drugs—enzyme inhibitory activity □ Physicochemical constants, enzyme inhibition—correlation

In recent years much effort has been focused on elucidation of weak intermolecular forces in biological systems (1), especially on the importance of hydrophobic interactions (2–6). Various experimental methods have been used to estimate the hydrophobic bonding tendency of drug molecules, such as partitioning and chromatographic methods (3, 7). Organic solvents capable of forming hydrogen bonds (*e.g.*, alcohols and esters) appear to give better correlations than hydrocarbons (8). The purpose of this paper is to correlate quantitatively enzyme inhibitory activity with the tendency of hydrophobic interactions of series of drugs, as measured by the partition coefficient of 1-octanol–water. It is hoped that this work may shed some light on the intermolecular forces involved in enzyme inhibition and provide some clues in designing new enzyme inhibitors.

METHOD

The biological data given in Table I are taken from the literature (4, 9–12). The Hammett's sigma constants (σ) are from the compilation of Jaffé (13) unless otherwise stated. The log *P* values are either experimentally determined or calculated from the π constants (14, 15) of Hansch. For example, the log *P* of *N*-acetyltryptamine is calculated as follows:



$$\Sigma\pi = 2.14 + 1.00 - 1.71 + 0.50 = 1.93 = \log P$$

The steric constants, E_s, are taken from Leffler and Grunwald (16). The equations listed in Table II are derived *via* the method of least squares using an IBM 360/65 computer. The inhibition constant K_I, $k' = (k_1/K_I)$, or the concentration of an inhibitor giving 50% inhibition of the enzyme (I_{50}) is converted to the molar basis, and pK_I, log k', or log $1/I_{50}$ is used as a measure of the inhibitory activity.

RESULTS

The equations correlating enzyme inhibition with the physicochemical constants are summarized in Table II, where *n* is the number of data points used in the analysis, *r* is the correlation coefficient, and *s* is the standard deviation.

In the inhibition of lipoxygenase by monohydric alcohols, the relative inhibitory activity is mainly determined by the lipohydrophilic character (log *P*). More than 98% ($r^2 = 0.983$) of the variance in the data can be accounted for by the simple linear equation (Eq. 1*a*). Equation 1*b*, derived by Mitsuda *et al.* (4), gives a slightly lower correlation coefficient, presumably due to the slightly different log *P* values used.

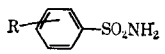
For the carbonic anhydrase inhibition by sulfonamides, by comparing Eq. 2*a* with Eq. 2*b* one can see that the electronic term σ is slightly more important than the log *P* term. The positive coefficient associated with σ indicates that electron-withdrawing groups will increase the inhibitory activity. By using both terms simultaneously, a much better correlation is obtained (Eq. 2*c*). The log *P* term in Eq. 2*c* is significant at 97.5-percentile level, as indicated by an *F*-test ($F_{1,16} = 7.2$; $F_{1,15\ 0.975} = 6.2$).

The π-constant alone gives almost perfect correlation for inhibition of D-amino acid oxidase by *N*-alkylmaleimides (Eqs. 3*b* and 3*c*). By using π and σ terms together, high correlation is obtained for the *N*-aryl as well as *N*-alkyl derivatives. The σ term in Eq. 3*a* is highly significant ($F_{1,5} = 111$; $F_{1,5\ 0.995} = 63.6$).

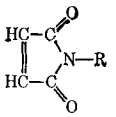
For the inhibition of hydroxyindole-*o*-methyltransferase by *N*-acyltryptamines, log *P* alone gives fairly good correlation (Eq. 4*a*). By deleting three molecules with deviation greater than 2*s*, a better

Table I—Enzymological Data and the Physicochemical Constants Used in Deriving the Equations in Table II

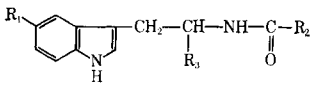
pK_I Calcd.[a]	pK_I Found[b]	R—OH log P[c]	R—
−0.30	−0.18	−0.66	CH_3—
0.18	0.18	−0.16	C_2H_5—
0.46	0.37	0.14	*iso*-C_3H_7—
0.65	0.68	0.34	*n*-C_3H_7—
0.72	0.49	0.41	*tert*-C_4H_9—
0.91	0.86	0.61	*s*-C_4H_9—
0.94	1.13	0.64	*iso*-C_4H_9—
1.13	1.15	0.84	*n*-C_4H_9—
1.39	1.34	1.11	*iso*-C_5H_{11}—
1.61	1.61	1.34	*n*-C_5H_{11}—
2.08	2.10	1.84	*n*-C_6H_{13}—
2.56	2.60	2.34	*n*-C_7H_{15}—



pK_I (log $1/K_I$) Calcd.[d]	pK_I (log $1/K_I$) Found[e]	log P[f]	σ[g]	R—
4.72	4.96	−0.28	−0.59	*p*-CH_3NH—
4.53	4.60	−0.78	−0.66	*p*-NH_2—
5.15	5.30	0.27	−0.27	*p*-CH_3O—
5.38	5.50	0.83	−0.17	*p*-CH_3—
5.47	5.22	0.82	−0.07	*m*-CH_3—
5.39	5.13	0.31	0.00	H—
5.78	5.96	1.01	0.23	*p*-Cl—
5.86	5.96	1.33	0.23	*p*-Br—
5.92	5.92	1.07	0.37	*m*-Cl—
6.07	5.89	−0.06	0.87	*p*-$CH_3C(=O)$—
5.89	6.19	−0.01	0.65	*p*-CN—
6.05	6.12	0.42	0.71	*m*-NO_2—
6.15	6.26	0.55	0.78	*p*-NO_2—
6.22	6.52	1.77	0.50	3,4-Cl_2—
6.44	6.60	1.12	0.94	3-NO_2—4—Cl—
6.80	6.66	1.62	1.20	3-CF_3—4—Cl—
5.45	4.92	0.99	−0.14	2-CH_3—
5.73	5.62	0.90	0.20	2-Cl—
5.82	5.46	0.08	0.55	2-NO_2—



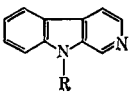
log k' (log k_1/K_I) pH 7.0 Calcd.[h]	log k' (log k_1/K_I) pH 7.0 Found[i]	log k' (log k_1/K_I) pH 7.5 Calcd.[j]	log k' (log k_1/K_I) pH 7.5 Found[i]	π	σ	R—
1.44	1.43	1.89	1.88	1.00	−0.15	C_2H_5—
1.84	1.86	2.27	2.27	2.00	−0.15	*n*-C_4H_9—
2.04	2.04	2.46	2.46	2.50	−0.15	*n*-C_5H_{11}—
2.24	2.23	2.65	2.64	3.00	−0.15[k]	*n*-C_6H_{13}—
2.44	2.45	2.84	2.87	3.50	−0.15[k]	*n*-C_7H_{15}—
2.64	2.61	3.03	3.01	4.00	−0.15[k]	*n*-C_8H_{17}—
2.64	2.51[l]	—	—[m]	2.13	0.01	C_6H_5—
2.72	2.85[l]	—	—[m]	2.32	0.01[k]	4-$(CH_3)_2N$—3,5—$(NO_2)_2$—Ph—



log $1/I_{50}$ Calcd.[n]	log $1/I_{50}$ Found[o]	log P	R_1—	R_2—	R_3—
2.65	2.85	1.93	H—	CH_3—	H—
3.93	3.74	4.06	H—	$C_6H_5CH_2$—	H—
3.63	3.43	3.56	H—	C_6H_5—	H—
4.01	4.21	4.19	F—	$C_6H_5CH_2$—	H—
4.50	4.30	5.00	Br	$C_6H_5CH_2$—	H—
4.02	3.96	4.21	H—	*p*-F—$C_6H_4CH_2$—	H—
4.35	4.17	4.76	H—	*p*-Cl—$C_6H_4CH_2$—	H—
3.68	3.60	3.64	H—	1-Cyclohexenyl—	H—
3.64	3.80	3.57	H—	*o*-F—C_6H_4—	H—
3.71	3.70	3.69	H—	*m*-F—C_6H_4—	H—
3.72	3.47	3.71	H—	*p*-F—C_6H_4—	H—
3.99	4.26	4.15	H—	*o*-Cl—C_6H_4—	H—
4.09	4.00	4.32	H—	*m*-Cl—C_6H_4—	H—
4.05	4.18	4.26	H—	*p*-Cl—C_6H_4—	H—
4.41	4.26	4.85	H—	2,4—$Cl_2C_6H_3$—	H—

Table I—(*Continued*)

log $1/I_{50}$ Calcd.[n]	log $1/I_{50}$ Found[o]	log P	R_1—	R_2—	R_3—
4.51	4.70	5.02	H—	3,4—Cl_2—C_6H_3—	H—
3.75	3.70	3.76	H—	3,4,5—$(CH_3O)_3$—C_6H_2—	H—
3.71	3.77	3.69	F—	C_6H_5—	H—
4.20	4.15	4.50	Br—	C_6H_5—	H—
4.59	4.66	5.15	F—	3,4—Cl_2—C_6H_3—	H—
5.08	5.30	5.96	Br—	3,4—Cl_2—C_6H_3—	H—
4.11	3.57[p]	4.36	H—	$C_6H_5CH_2$—	CH_3—
4.05	3.42[p]	4.26	H—	3,4,5—$(CH_3O)_3$—$C_6H_2CH_2$—	H—
4.54	3.96[p]	5.08	H—	3,5—Cl_2—C_6H_3—	H—



log $1/I_{50}$ Calcd.[q]	log $1/I_{50}$ Found[r]	E_s	log P	σ	R—
4.38	4.54	1.24	2.08	0.00	H—
4.65	5.00	0.00	2.58	−0.17	CH_3—
4.59	4.32	−0.07	3.08	−0.15	C_2H_5—
4.19	3.82	−0.36	3.58	−0.13	n-C_3H_7—
3.45	3.85	−0.39	4.08	−0.16	n-C_4H_9—
2.84	2.74	−0.35	4.38	−0.23	iso-C_5H_{11}—
3.71	3.40	−0.19	1.55	0.06	—CH_2OCH_3
3.68	3.82	−0.47	1.53	0.52	—$COCH_3$
2.10	3.32[s]	−0.39	2.42	−0.13	—$CH_2CH_2CH_2OH$
2.18	3.96[s]	−0.36	1.92	−0.07	—CH_2CH_2OH
2.59	4.47[s]	−0.07	1.42	0.06	—CH_2OH

[a] Calculated from Eq. 1*a* of Table II. [b] From Fig. 2 of *Reference 4*. [c] From *Reference 19*. [d] Calculated from Eq. 2*c* of Table II. [e] From *Reference 9*. [f] From *References 14* and *15*. [g] From *Reference 13*. [h] Calculated from Eq. 3*a* of Table II. [i] From *Reference 10*. [j] Calculated from Eq. 3*c* of Table II. [k] Estimated values. [l] These points are not included in Eq. 3*b* of Table II. [m] Not measured. [n] Calculated from Eq. 4*b* of Table II. [o] From *Reference 11*. [p] These points are not included in Eq. 4*b* of Table II. [q] From *Reference 12*. [r] Calculated from Eq. 5*g* of Table II. [s] These points are not included in Eq. 5*g* of Table II.

Table II—Equations Correlating Enzyme Inhibition with Physicochemical Constants

Enzyme	Inhibitors	Equation	n	r	s	Eq. No.	log P_0, (95% c-l.)
Lipoxygenase	ROH	$pK_I = 0.954 \log P + 0.329$	12	0.992	0.110	1*a*	
		$pK_I = 0.944 \log P + 0.830$	12	0.984	—	1*b*[a]	
Carbonic anhydrase	Sulfonamides	$pK_I = 0.553 \log P + 5.378$	19	0.609	0.492	2*a*	
		$pK_I = 1.026 \sigma + 5.438$	19	0.886	0.288	2*b*	
		$pK_I = 0.259 \log P + 0.886 \sigma + 5.314$	19	0.923	0.247	2*c*	
D-Amino acid oxidase	Maleimides (at pH 7.0)	$\log k' = 0.339 \pi + 4.705 \sigma + 1.745$	8	0.988	0.085	3*a*	
	(at pH 7.5)	$\log k' = 0.395 \pi + 1.051$	6	0.999	0.018	3*b*	
		$\log k' = 0.382 \pi + 1.503$	6	0.999	0.019	3*c*	
Hydroxyindole-*o*-methyltransferase	*N*-Acyltryptamines	$\log 1/I_{50} = 0.561 \log P + 1.590$	24	0.870	0.255	4*a*	
		$\log 1/I_{50} = 0.601 \log P + 1.491$	21	0.948	0.170	4*b*	
Monoamine oxidase	β-Carbolines	$\log 1/I_{50} = 0.675 E_s + 4.017$	11	0.509	0.578	5*a*	
		$\log 1/I_{50} = -0.216 \log P + 4.494$	11	0.355	0.628	5*b*	
		$\log 1/I_{50} = -0.373 (\log P)^2 + 1.907 \log P + 1.864$	11	0.604	0.568	5*c*	2.56 (∞)
		$\log 1/I_{50} = 0.454 E_s - 0.304 (\log P)^2 + 1.564 \log P + 2.283$	11	0.648	0.556	5*d*	2.57 (∞)
		$\log 1/I_{50} = 0.635 E_s + 3.983$	8	0.507	0.649	5*e*	
		$\log 1/I_{50} = -0.215 \log P + 4.550$	8	0.341	0.707	5*f*	
		$\log 1/I_{50} = -0.679 (\log P)^2 + 3.719 \log P - 0.422$	8	0.900	0.360	5*g*	2.74 (2.32–2.98)
		$\log 1/I_{50} = 0.140 E_s - 0.645 (\log P)^2 + 3.544 \log P - 0.227$	8	0.905	0.390	5*h*	2.75 (1.99–3.13)

[a] From *Reference 4*.

correlation is obtained (r = 0.948 for Eq. 4*b*). The authors also explored the role of the electronic parameter. For the 15 molecules with X-Ar as R_2 (Table I), neither the σ of X nor the σ of R_1 gives significant improvement in correlation. Of the three poorly predicted molecules, two may be due to steric hindrance, one with the methyl group as R_3, and the other with the 3,4,5-trimethoxybenzyl group as R_2. The fact that the observed activities of these three compounds are lower than the predicted values supports this argument. The third molecule has the 3,5-dichlorophenyl group as R_2. It is difficult to explain why this is poorly predicted since the one with the 3,4-dichlorophenyl group as R_2 is slightly more active than predicted.

For the inhibition of monoamine oxidase by 9-substituted β-carbolines, Taft's steric constant, E_s, gives somewhat better correlation than log P (Eq. 5*a versus* 5*b*). However, the correlation coefficients are too low to be considered significant. Even when a $(\log P)^2$ term is included, the correlation coefficient is still below 0.70 (Eqs. 5*c* and 5*d*). When three compounds with an alcoholic OH group are excluded, a parabolic equation of log P gives fairly good correlation (Eq. 5*g*). The $(\log P)^2$ term in Eq. 5*g* is significant at the 99-percentile level ($F_{1,5}$ = 18.2; $F_{1,5\ 0.99}$ = 16.3). For the eight compounds without an OH group, neither the linear equation of E_s nor that of log P gives a good correlation (Eqs. 5*e* and 5*f*); the addition of the E_s term to the parabolic equation does not improve the correlation significantly (Eq. 5*h versus* 5*g*, $F_{1,4}$ = 0.21). It is felt that an active function like an OH group may have its own *intrinsic activity* not possessed by the other inert substituents. For example, the H of the OH group might form a hydrogen bond with an atom having unshared electron pair(s). The fact that the activities of the compounds with the OH group are appreciably higher than what are predicted from Eq. 5*g* is in accordance with this explanation. The optimum lipohydrophilic character ($\log P_0$) for the maximum inhibition is derived by setting $(d \log 1/I_{50})/(d \log P) = 0$ (17–19). This is the apex of the parabolic curve. Once this $\log P_0$ is obtained, it may serve as a useful guidepost in designing new inhibitors.

The importance of the hydrophobic interactions for the enzyme inhibition is clearly shown by the good correlations obtained by using log P or π with or without a σ term.

DISCUSSION

From the correlations obtained, it is clear that in the five enzyme systems examined the lipohydrophilic character of the inhibitors plays a very important role in inhibition. The rather nonspecific hydrophobic interactions may be involved in two different ways: (*a*) adsorption and desorption on the macromolecule, since all proteins including enzymes contain 20–45% of amino acids with nonpolar side chains (20), and (*b*) inducing proper fit at the active site or the allosteric site (21) by the association of the nonpolar groups in the presence of water molecules. At present, not enough data are available to differentiate which of these two is more important.

It will be interesting to apply the method used in this study to other systems where drugs exert their activity by enzyme inhibition, such as choline esterase inhibitors and histidine decarboxylase inhibitors.

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