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Review

QSAR Studies in Pesticide Research in Japan

Hajime IWAMURA and Toshio FUJITA

Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan

Use of QSAR (quantitative structure-activity relationship) analysis in pesticide research, as well as in other diverse fields involving biologically active compounds, provides us with a deeper insight into the molecular mechanisms of action and a guide to the development of novel compounds having potent activity. In the earlier stages of study in Japan, the methodology owed much of its progress to the efforts of our group in Kyoto University, and its utility has gradually come to the attention of practicing chemists. Much work of practical interest has been reported during the past ten years and it is the purpose of this article to review that in pesticide chemistry and related fields.

INSECTICIDES

1. *Phenyl N-Methylcarbamates*

The steps by which substituted phenyl *N*-methylcarbamates (ArOCONHMe) react with acetylcholinesterase (AChE) are shown in Eq. (1), where EOH denotes the enzyme.[1]

$$\mathrm{ArOCONHMe}+\mathrm{EOH} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{Reversible complex} \xrightarrow{k_2} \begin{matrix}\mathrm{EOCONHMe}\\ + \\ \mathrm{ArOH}\end{matrix} \qquad (1)$$

After establishing experimental conditions to determine a reliable set of kinetic parameters $K_d(=k_{-1}/k_1)$ and k_2, for the inhibitory reaction, Nishioka *et al.*[2] studied the molecular mechanism of enzyme inhibition using bovine erythrocyte AChE. Depending upon the position and nature of substituents, the value of K_d showed significant variations whereas that of k_2 did not. Thus formation of the reversible complex was considered to be the step which governs the variation in overall inhibitory activity.

Eq. (2) is the result of QSAR analysis for 53 *o*-, *m*- and *p*-derivatives (Fig. 1) where $\Delta \log 1/K_d$ is the value referring to that of the unsubstituted compound.* π is the hydrophobic parameter derived from the 1-octanol/water partition coefficient,[3] and the subscripts 2,3, and 4 indicate substituents at the *o*-, *m*- and *p*-positions, respectively. The slopes of the $\pi_{2,3}$ and π_4 terms suggest that the hydrophobic nature of the enzyme surface corresponding to the *o*- and *m*-positions is approximately equivalent, and higher than that of the surface corresponding to the *p*-position.

$$\begin{aligned}\Delta \log (1/K_d) = {} & \underset{(\pm 0.168)}{1.399\pi_{2,3}} + \underset{(\pm 0.159)}{0.306\pi_4} \\ & + \underset{(\pm 0.380)}{1.659\sigma^{o}_{\rho>0}} - \underset{(\pm 0.371)}{1.784\sigma^{o}_{\rho<0}} \\ & + \underset{(\pm 0.132)}{0.168E_s} + \underset{(\pm 0.536)}{0.770F} \\ & + \underset{(\pm 0.248)}{1.358HB} + \underset{(\pm 0.162)}{0.072} \\ & n=53,\ r=0.947,\ s=0.238 \end{aligned} \qquad (2)$$

σ^o is the electronic constant which supposedly contains no through-resonance effect.[4] The substituents are classified in terms of electronic effect into two groups: those in one group are more electron withdrawing and promote an attack by a nucleophile of the

* In this and the following equations, the figures in parentheses express the 95% confidence intervals.

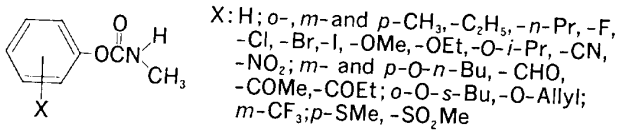


Fig. 1 Structure of phenyl *N*-methylcarbamates.

enzyme on the carbonyl carbon of the carbamyl group, and those in the other group are more electron-releasing and assist an electrophilic attack by an acidic group of the enzyme on the carbonyl oxygen atom. Substituents in the first group are those at the *o*-position, and those which are electron-withdrawing at the *m*- and *p*-positions, such as NO_2, CN and acyl. Their electronic effect is expressed by the $\sigma^o_{\rho>0}$ term. All other substituents belong to the second group, the electronic effect of which is represented by the $\sigma^o_{\rho<0}$ term. The significance of these two terms in Eq. (2) suggests different mechanisms for the two groups of substituents, leading to a common tetrahedral intermediate as shown in Fig. 2. Electron-donating *o*-substituents do not follow the negative ρ mechanism because the acid-catalytic site of the enzyme does not fit the carbonyl oxygen atom due to hindrance exerted by these *o*-substituents.

E_s is the Taft-Kutter-Hansch steric parameter,[5] the reference of which is shifted to that of H, and F is the Swain-Lupton-Hansch field effect constant,[6] both for *o*-substituents. The coefficient values of these terms, 0.17 and 0.77, are close enough to those for the alkaline hydrolysis of *o*-substituted phenyl acetates.[7] Thus, in support of the above discussion, the proximity effects of *o*-substituents are considered to be those on the tetrahedral intermediate formation.

HB, an indicator variable for the hydrogen bonding effect of substituents, is 1 for hydrogen bonding substituents such as *o*-OR, *m*-acyl, -CN, $-NO_2$ and $-NMe_2$, but otherwise is zero. The significance of the term in Eq. (2) indicates a specific hydrogen bond formation of these groups with a hydrogen donor on the enzyme. The hydrogen donor site is supposed to be located unsuitably for interaction with other hydrogen bonding groups such as *o*-NO_2, CN and *m*-OR. Fig. 3A shows the stereospecific situation schematically.

The mechanism of the inhibition reaction against AChE prepared from the fly head was found to be quite similar to that against bovine erythrocyte AChE (Eq. (3)).[8] One of the slight differences to be noted is that the effect of hydrogen bonding substituents is represented by two indicator variable terms, HB_1 for *o*-OR, -CN, $-NO_2$, *m*-CN, $-NO_2$ and -acyl, and HB_2 for *m*-OR, with a larger coefficient for the HB_1 term. A likely explanation is that the hydrogen donor group on fly head AChE is more suitably located for interaction with the HB_1 substituents than that with *m*-OR as shown in Fig. 3B.

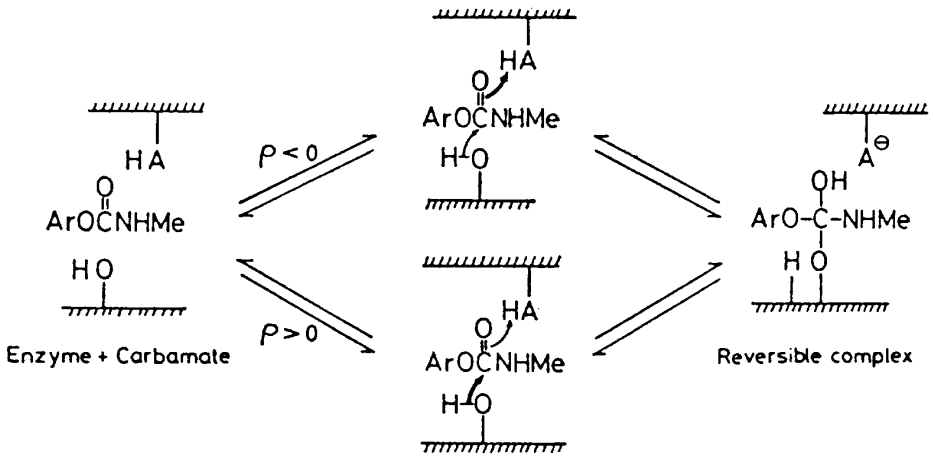


Fig. 2 Electronic mechanism of the reaction of phenyl *N*-methylcarbamates leading to the tetrahedral intermediate with AChE (reproduced with permission from Academic Press, Inc.).[2]

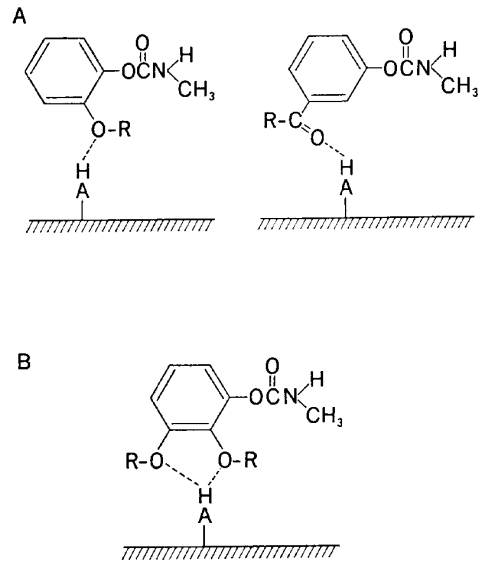


Fig. 3 Hydrogen bonding formation of phenyl *N*-methylcarbamates with A: bovine erythrocyte[2] and B: flyhead AChE[8] (reproduced with permission from Academic Press, Inc.).

$$\log(1/K_d) = \underset{(\pm 0.212)}{1.554\pi_2} + \underset{(\pm 0.143)}{1.134\pi_3} + \underset{(\pm 0.134)}{0.238\pi_4} + \underset{(\pm 0.279)}{1.147\sigma^{o}_{\rho>0}} - \underset{(\pm 0.328)}{1.592\sigma^{o}_{\rho<0}} + \underset{(\pm 0.168)}{1.188HB_1} + \underset{(\pm 0.231)}{0.435HB_2} + \underset{(\pm 0.131)}{4.027}$$

$$n=54,\ r=0.961,\ s=0.210 \qquad (3)$$

Carbamates are metabolically detoxicated by a variety of biochemical reactions in the body of the housefly. Among these, the oxidative metabolism generally associated with the mixed-function oxidases has been shown to be of prime importance.[9] Under conditions where oxidative metabolism was suppressed with piperonyl butoxide, the insecticidal LD_{50} values were determined. QSAR analysis gave Eq. (4) which resembles Eq. (3) for AChE inhibition but the slope of each term is generally lower, suggesting that a detoxication mechanism(s) other than oxidation may be involved in the whole body activity.[10,11] The insecticidal-activity values of compounds with strongly electron-withdrawing substituents such as NO_2 and CN are not included in Eq. (4). They were considerably lower than those to be expected from their inhibitory activity against AChE, suggesting the possibility of spontaneous hydrolysis during the test period.

$$\log(1/LD_{50}) = \underset{(\pm 0.079)}{0.375\pi_{2,3}} - \underset{(\pm 0.079)}{0.082\pi_4} + \underset{(\pm 0.390)}{0.995\sigma^{o}_{\rho>0}} - \underset{(\pm 0.244)}{0.668\sigma^{o}_{\rho<0}} + \underset{(\pm 0.162)}{0.852HB_1} + \underset{(\pm 0.114)}{0.328HB_2} + \underset{(\pm 0.074)}{8.419}$$

$$n=37,\ r=0.955,\ s=0.104 \qquad (4)$$

The above QSAR results were considered helpful in designing new compounds having higher activity. Polysubstituted derivatives having a hydrophobic OR group at the *o*-position and a hydrophobic alkyl group at the *m*-position were designed and, of these, 2-*i*-propoxy-5-*n*- and -*s*-butyl derivatives showed high potency as expected.

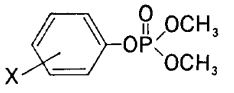
2. *O,O,-Dimethyl O-Phenylphosphates*

The formation of a reversible complex of *O,O*-dimethyl *O*-phenylphosphates (Fig. 4) with housefly-head AChE was similarly analyzed to give Eq. (5).[12]

$$\log(1/K_d) = \underset{(\pm 0.161)}{0.176\pi_{2,3}} + \underset{(\pm 0.304)}{2.253\sigma^{\#}} + \underset{(\pm 0.215)}{2.892}$$

$$n=19,\ r=0.970,\ s=0.153 \qquad (5)$$

$\pi_{2,3}$ is the π value for *o*- and *m*-substituents. The π term for *p*-substituents was not significant in determining activity. The $\sigma^{\#}$ (sigma mixed) term[13] is a composite of σ^{o} for *o*- and *m*-substituents and σ^{-} for *p*-substituents.[14] Thus, the electron-withdrawing effect of *o*- and *m*-substituents includes practically no through-resonance factor but it is supposed that this is contained in that of *p*-substituents.



X: *o*-,*m*-and *p*-CN, -NO_2, -CHO; *m*-and *p*-$COCH_3$, -Br; *m*-I, -Cl

Fig. 4 Structure of *O,O*-dimethyl *O*-phenylphosphates.

At any rate, the electronic effect represented by the $\sigma^{\sharp}$ term with the positive ρ value indicates the prime importance of a nucleophilic attack by the serine-OH on the P=O moiety of the drugs. The inhibition reaction, only obeying the $\rho>0$ mechanism, differs from that of carbamates with fly-head AChE.

3. *BHC Analogs*

A number of γ-BHC analogs, in which some of the chlorine atoms are replaced by other substituents while maintaining the γ-configuration, were synthesized. Their insecticidal activity against mosquitos seemed highly dependent on the positions of the "heteroatom" substituents. Thus, they were stereochemically classified into two groups, *meso*- and *dl*-analogs, according to their heterosubstituent positions as shown in Fig. 5. Analyses of these groups were performed separately to give Eqs. (6) and (7).[15] The Δr_w and ΔV_w are the van der Waals radius of the atom of substituents directly bound to the ring and the van der Waals molar volume of substituents, respectively, those of the chlorine substituent being taken as the point of reference.

meso-Analogs:

$$\log(1/LD_{50}) = \underset{(\pm 0.67)}{1.66\Delta r_w} + \underset{(\pm 0.19)}{3.53}$$
$$n=9,\ r=0.911,\ s=0.205 \qquad (6)$$

dl-Analogs:

$$\log(1/LD_{50}) = \underset{(\pm 0.01)}{-0.02\Delta V_w^2} - \underset{(\pm 0.03)}{0.10\Delta V_w} + \underset{(\pm 3.01)}{3.09}$$
$$n=16,\ r=0.920,\ s=0.350 \qquad (7)$$

The variation in activity is governed mainly by the steric effect of substituents within the compounds examined in both series. However, the modes of interaction seem to be different, as revealed by the different steric parameters incorporated. The significance of the Δr_w in Eq. (6) may be that the *meso*-substituents interact in such a manner that they come into contact with the receptor surface or walls, whereas that of the ΔV_w in Eq. (7) may indicate the importance of fitting into a cavity or pocket on the receptor surface in the region where the *dl*-substituents direct. The ΔV_w value for Cl (0.0) is nearest to the optimum steric condition ($\Delta V_w \simeq -2.0$) calculated from Eq. (7). Thus, *dl*-Cl substituents in γ-BHC are thought to be those which best fit the receptor cavity among the *dl*-substituents.

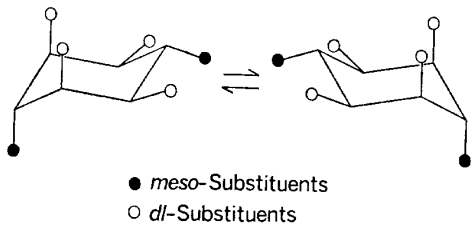


Fig. 5 Structure and conformation of substituted cyclohexanes of γ-BHC type.

Among BHC isomers, γ-BHC is most toxic not only to insects but also to mammals, whereas δ-BHC is more effective than other isomers in its toxicity to higher plants and its inhibition of the growth of microorganisms. From observation of the symptoms of poisoning in insects, γ-BHC is better characterized as an excitant, whereas δ-BHC is a typical depressant.[16] Thus, the excitatory and depressant effects of BHC isomers seem to obey different modes of action.

In this respect, Uchida *et al.*[17] analyzed the inhibition of BHC isomers against beef brain Na^+-K^+-ATPase, yeast growth and cockroach nerve conduction to yield Eqs. (8)–(10). Some compounds having similar activity, such as 1-alkanols and a few other aromatic and aliphatic compounds, were also included in the analysis. In Eq. (10), *MIC* is the minimum molar concentration which gives complete inhibition. The results show that variation of these activities is governed only by the hydrophobicity of the molecule.

Na^+-K^+-ATPase inhibition:

$$pI_{50} = \underset{(\pm 0.08)}{0.77\log P} + \underset{(\pm 0.23)}{0.53}$$
$$n=14,\ r=0.988,\ s=0.237 \qquad (8)$$

Yeast growth inhibition:

$$pI_{50} = \underset{(\pm 0.04)}{0.92\log P} + \underset{(\pm 0.11)}{0.53}$$
$$n=12,\ r=0.998,\ s=0.101 \qquad (9)$$

Inhibition of nerve conduction:

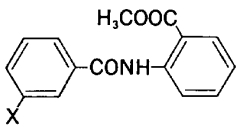
$$\log(1/MIC) = \underset{(\pm 0.08)}{0.91}\log P + \underset{(\pm 0.19)}{0.19}$$
$$n=12,\ r=0.993,\ s=0.162 \qquad (10)$$

The coefficient values of the log P term in Eqs. (8) and (9) are significantly different. Thus the growth inhibition of yeast by BHC isomers is not necessarily related to the concomitant Na^+-K^+-ATPase inhibition observed by Lyr[18] and Batterton *et al.*,[19] since yeast Na^+-K^+-ATPase inhibition can be assumed to have a similar dependence on log P to that demonstrated in Eq. (8) for beef brain Na^+-K^+-ATPase.[17] The fact that stereochemistry of the BHC isomers does not play any role in Eqs. (8)–(10), as well as the fact that the activities of structurally unrelated compounds such as alcohols and related compounds are at the same time explained by these single parameter equations, leads to the conclusion that neither Na^+-K^+-ATPase inhibition nor nerve blocking is related to the insecticidal activity of γ-BHC. The neuroexcitatory effect to induce excessive afterdischarges was shown to be the direct cause of the insecticidal activity of γ-BHC.[20]

FUNGICIDES

1. *N-Benzoylanthranilic Acid Esters*

Because benzanilides exhibit fungicidal activity,[21] and anthranilic acid esters have fungicidal and bactericidal activities,[22] Kirino *et al.* attempted to derive compounds with preventive activity against powdery mildew of cucumber from those having a hybrid structure, methyl *N*-(substituted benzoyl)anthranilates.[23] Among various derivatives, *m*-substituted compounds were shown to exhibit appreciable fungicidal activity. Eq. (11) was obtained for 14 *m*-substituted compounds listed in Fig. 6, where L and B_4 are the STERIMOL parameters developed by Verloop *et al.*[24] L is the length of the substituents along the axis which connects them to the rest of the molecule and B_4 is the maxium width perpendicular to the L-axis as shown schematically in Fig. 7. The wider the substituents are in the B_4 direction, the lower the activity. The activity is also related parabolically to the L value. The steric fit with the target site is thus supposed to be critical for activity. Compounds such as *m*-F, -Cl and -CN with smaller B_4 values and lengths as near as possible to the optimum, exhibited high potency as expected.



X: H, CH_3, C_2H_5, F, Cl, Br, I, CF_3, CN, $CH(CH_3)_2$, OCH_3, OC_2H_5, O-*n*-Pr, O-*i*-Pr

Fig. 6 Structure of *N*-(*m*-substituted benzoyl)-anthranilates.

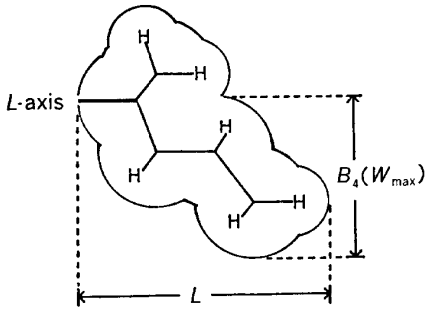
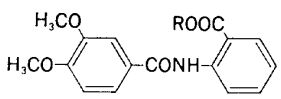


Fig. 7 Schematic representation of the STERIMOL $B_4(W_{max})$ and L-parameters.

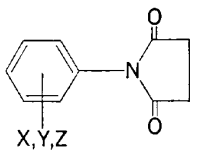
$$pI_{50} = \underset{(\pm 0.104)}{-0.114L^2} + \underset{(\pm 0.839)}{1.082L} - \underset{(\pm 0.291)}{0.715B_4} - \underset{(\pm 1.640)}{3.349}$$
$$n=14,\ r=0.942,\ s=0.229 \qquad (11)$$

Tests of activity against powdery mildew of other plants have revealed that the *m*-OMe derivative is highly active against that of barley as well. The extension of the study also showed that the activity of the 3,4-$(OMe)_2$ derivative is the highest among the poly-methoxy compounds, and the transformation of the alcoholic structure of the ester moiety further alters the activity.[25] The 3,4-$(OMe)_2$ benzoyl compounds thus prepared are shown in Fig. 8 and the result of the QSAR analysis is Eq. (12). The hydrophobic parameter used here is the R_M value determined from reversed phase thin layer chromatography[26] and correlates well with the π value of the ester substituents.[25] The significance of the R_M^2 term means that there is parabolic dependence of activity on hydrophobicity, with the

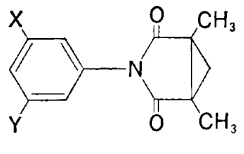


R: CH_3, C_2H_5, *n*-Pr, *i*-Pr, *n*-Bu, *i*-Bu, *s*-Bu, *n*-Pent, *i*-Pent, *s*-Pent, 3-Pent, Neopent, 3-CH_3-2-Bu, *n*-Hexyl, 3-Hexyl, 4-CH_3-2-Pent, 2-Propenyl, 2-Propynyl, 2-Butenyl, 2-CH_3-2-Propenyl, 3-Butenyl

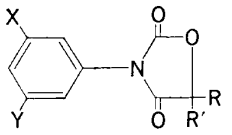
Fig. 8 Structure of *N*-(3,4-dimethoxybenzoyl)-anthranilates.



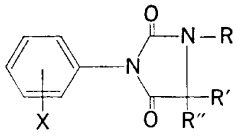
N-Phenylsuccinimides



N-Phenyl-1,2-dimethyl-cyclopropanedicarboximides



3-Phenyloxazolidine-2,4-diones



3-Phenylimidazolidine-2,4-diones

Fig. 9 Structures of *N*-phenyl cyclic imides.

optimum condition being $R_M=0$. The negative sign of the Taft's σ^* term indicates that activity decreases with increasing electron-withdrawing property of the ester substituents.

$$pI_{50} = -5.642R_M^2 - 1.287X - 0.902\sigma^* + 5.410 \quad (12)$$
$$(\pm 1.840)\quad (\pm 0.230)\quad (\pm 0.492)\quad (\pm 0.143)$$
$$n=21,\ r=0.963,\ s=0.207$$

The electronic effect of ester substituents suggests the existence of an inactivation process(es) in which the esters are hydrolyzed to anthranilic acid and/or cyclized to quinazolones, with the elimination of the alcohol moiety. X is an indicator variable for compounds having α-branching in the ester moiety, and the negative coefficient was attributed to an unsuitable fit of the branching structure with the critical site of fungicidal action. The *n*-propyl derivative having $R_M \simeq 0$ and a slight electron-releasing property showed the highest activity and has been selected for further practical tests.

2. *N-Phenyl Cyclic Imides*

Information obtained from QSAR study of one class of compounds may be transposed to another having structural similarities. Takayama *et al.*[27] showed that antifungal activities against *Botrytis cinerea* in terms of pI_{50} values, of *N*-phenyl succinimides and *N*-phenyl-1,2-dimethylcyclopropanedicarboximides (Fig. 9) with the same aromatic substituents, correspond very nicely with each other. Since the slope and intercept of the plots of pI_{50} values are almost 1 and 0, respectively (Fig. 10), the substituent effects are considered identical in the two series. The structure-activity correlation for the *N*-phenyl succinimides is shown as Eq. (13), where the $\sum\pi_{3,5}$ and $\sum E_s^{2,6}$ are the sums of π and E_s values of

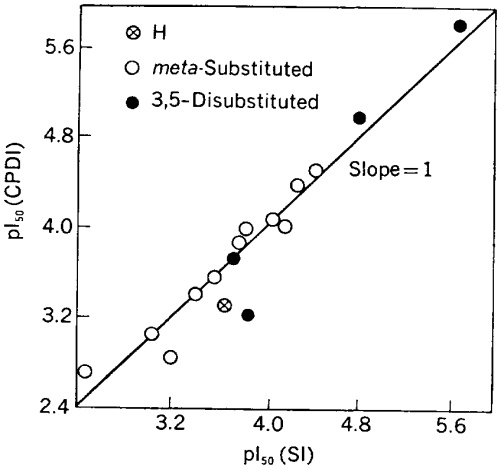


Fig. 10 Plots of pI_{50} value for *N*-phenyl-1,2-dimethylcyclopropanedicarboximides (CPDI) against that for *N*-phenylsuccinimides (SI) (reproduced with permission from Takayama[27] and Academic Press, Inc.).

substituents at designated positions and $\sum\sigma^o$ is the sum of the σ^o values of all ring substituents.

$$pI_{50} = 0.723\sum\pi_{3,5} + 1.464\sum\sigma^o + 0.894\sum E_s^{2,6} + 0.671E_s^m + 0.345E_s^p - 0.543HB + 3.690 \quad (13)$$
$$(\pm 0.201)\quad (\pm 0.266)\quad (\pm 0.195)\quad (\pm 0.235)\quad (\pm 0.207)\quad (\pm 0.183)\quad (\pm 0.233)$$
$$n=61,\ r=0.952,\ s=0.293$$

The E_s^m is that of either 3- or 5-substituents having bulkier dimensions in terms of E_s, and

E_s^p is that of *p*-substituents. *HB* is the indicator variable for hydrogen-bonding substituents such as OR, COR, COOMe, NO_2 and CN. The electron-withdrawing effect favors this activity, but the hydrogen-bonding factor reduces it. The hydrophobic effect is position-specific, and only the effects of the *m*-substituents are significant and they additively enhance the activity. The steric effect is also position-specific and additive, and the bulkier the *m*-substituent, the lower the activity. Accordingly, the high antifungal activity exerted by *N*-(3,5-dihalophenyl)succinimides[27] was nicely rationalized by the hydrophobic and electron-withdrawing properties of halogen atoms, and also because the steric bulk of only one of the two halogen substituents is unfavorable to activity.

Substantially the same effects are thought to be at work in the interaction of the *N*-phenyl dimethylcyclopropanedicarboximides[28] with the critical site, and it is also expected to be so with the other *N*-phenylimide types of fungicides, such as 3-phenyloxazolidine-2,4-diones[29] and 3-phenylimidazolidine-2,4-diones[30] (Fig. 9).

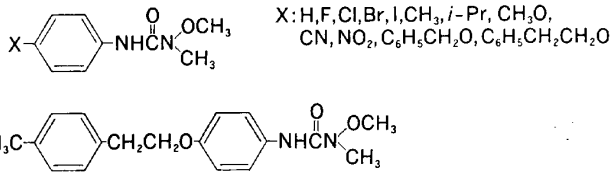
HERBICIDES

1. *N'-Phenyl N,N-Disubstituted Ureas*

Equation (14) is a classical QSAR equation derived by Hansch *et al.*[31] for the inhibitory activity of *N'*-phenyl *N,N*-dimethylureas against the Hill reaction.

$$pI_{50} = 0.544\sigma + 1.290\pi + 4.182$$
$$n=12,\ r=0.944,\ s=0.372 \qquad (14)$$

From this equation, electron-withdrawing as well as hydrophobic substituents are expected to enhance the activity, although in practice an electron-withdrawing substituent often tends to lower the hydrophobicity and *vice versa*. Takemoto *et al.*[32] introduced substituents with high hydrophobicity into the *p*-position of the benzene ring of the congeneric *N'*-phenyl *N,N*-dimethylureas and slightly modified *N'*-phenyl *N-methoxy-N*-methylureas, leading to the development of the highly herbicidal *N'*-4-(4-methylphenethyloxy)phenyl *N*-methoxy-*N*-methylurea (Fig. 11). The inhibitory activity against the Hill reaction of these



N'-(4-Methylphenethyloxy)phenyl-*N*-methyl-*N*-methoxyurea

Fig. 11 Structure of *N'*-phenyl *N,N*-disubstituted ureas.

compounds was then examined using radish chloroplasts. QASR analysis was performed with experimentally determined π values to give Eq. (15), which was different from Eq. (14) where estimated π value was used.

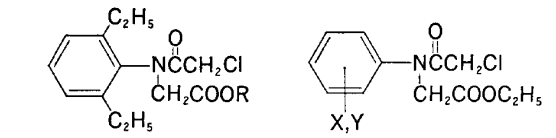
$$pI_{50} = -0.552\sigma + 1.044\pi + 4.436$$
$$(\pm 0.385)\ (\pm 0.204)\ (\pm 0.198)$$
$$n=12,\ r=0.975,\ s=0.189 \qquad (15)$$

2. *N-Phenylglycinates and Related Compounds*

Correlation Eqs. (16) and (17) have been developed for the herbicidal activities of *N*-chloroacetyl-*N*-(2,6-diethylphenyl)glycinates (Fig. 12) against the rice plant and barnyard grass, respectively.[33]

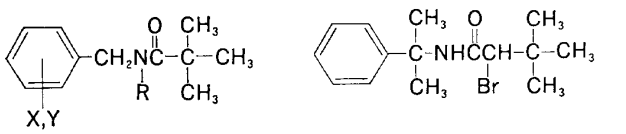
The rice plant:

$$pI_{50} = -0.278\,(\log P)^2 + 1.474\log P$$
$$(\pm 0.163)\qquad (\pm 1.131)$$
$$-1.949\sigma^* + 4.000$$
$$(\pm 0.572)\ (\pm 1.902)$$
$$n=22,\ r=0.922,\ s=0.359 \qquad (16)$$



N-Chloroacetyl-*N*-(2,6-diethylphenyl) glycinates

Ethyl *N*-chloroacetyl-*N*-phenylglycinates



N-Benzylacetamides

N-(1-Methyl-1-phenylethyl)-2-bromo-3,3-dimethylbutanamide

Fig. 12 Structures of herbicidal glycinates and related compounds.

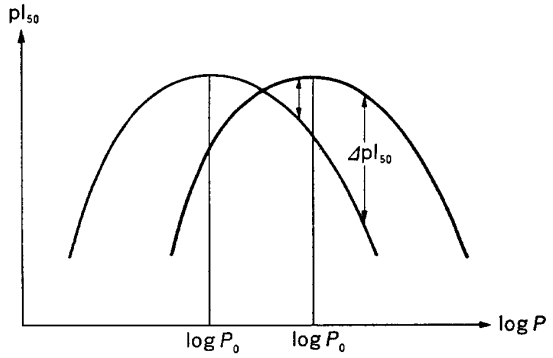


Fig. 13 Schematic representation of the parabolic relationship of pI_{50} to $\log P$.

Barnyard grass:

$$pI_{50} = -0.290(\log P)^2 + 1.884\log P - 0.996\sigma^* + 2.885$$
$$(\pm 0.170)\quad (\pm 1.185)\quad (\pm 0.600)\quad (\pm 1.993)$$
$$n=22,\ r=0.850,\ s=0.376 \qquad (17)$$

The activities vary parabolically with the change of log P value. The optimum log P value is 2.6 for the rice plant and 3.3 for barnyard grass. This situation is schematically shown by Fig. 13, illustrating that the selectivity in terms of ΔpI_{50} increases with increasing log P value. Thus, there is a range with sufficient herbicidal activity against barnyard grass within which compounds with the lowest possible toxicity against the rice plant can be designed. Fujinami *et al.*[33] suggested compounds having an ester moiety of C_3–C_5 as possible candidates.

Similar analysis of compounds having a fixed ethyl ester moiety and variated substituents on the benzene ring (Fig. 12) gave Eqs. (18) and (19).

The rice plant:

$$pI_{50} = -0.328\log P - 0.950E_s^o - 0.618\Sigma\sigma + 4.625$$
$$(\pm 0.182)\quad (\pm 0.142)\quad (\pm 0.458)\quad (\pm 0.193)$$
$$n=28,\ r=0.959,\ s=0.261 \qquad (18)$$

Barnyard grass:

$$pI_{50} = -0.767E_s^o - 0.218E_s^m + 3.990$$
$$(\pm 0.158)\quad (\pm 0.196)\quad (\pm 0.241)$$
$$n=28,\ r=0.905,\ s=0.295 \qquad (19)$$

The negative coefficient of the Taft's steric parameters of *o*- and *m*-substituents, E_s^o and E_s^m, indicates an enhancement of activity with an increase of steric bulk. Especially, the variation in activity against barnyard grass is mainly governed by steric factors. Because steric bulkiness is considered to prevent the proper fit with the target site, these results were taken to mean that it inhibits the interaction with hydrolytic enzymes which participate in the detoxication process(es) in the plant body. The negative slope of the σ^* term in Eqs. (16) and (17) coincides with this view, i.e. a molecule with a more electron-withdrawing substituent becomes more susceptible to hydrolytic attack.

Aiming at the development of new herbicides, this information on the steric effect was transposed to another class of compounds, *N*-benzylacetamides[34] (Fig. 12), which are known to be herbicidal[35] and possess the amide moiety in common with the glycinates. A systematic transformation of the structure, in which substituents around the hydrolytic site were made bulkier to protect against the detoxication mechanism, resulted in the finding of a highly preferable herbicide for the paddy field with high selectivity in favor of the rice plant, *N*-(1-methyl-1-phenylethyl)-2-bromo-3,3-dimethylbutanamide (Fig. 12).

3. *N-Phenyl Tetrahydrophthalimides*

The structural requirements for herbicidal activity against sawa millet in this series of compounds (Fig. 14) were investigated by Ohta *et al.*[36] As shown by Eq. (20), the steric dimensions expressed by the STERIMOL length parameter L_p and maximum width B_4^p of *p*-substituents appear to play a decisive role in determining activity.

$$pI_{50} = -0.597\sigma + 1.768L_p - 0.312L_p^2 - 0.946B_4^p + 4.067$$
$$(\pm 0.348)\quad (\pm 0.316)\quad (\pm 0.084)\quad (\pm 0.212)\quad (\pm 0.217)$$
$$n=28,\ r=0.930,\ s=0.273 \qquad (20)$$

The lack of significance of the hydrophobic parameter term seems to mean that translocation to the target site is not critical among the compounds examined. Together with

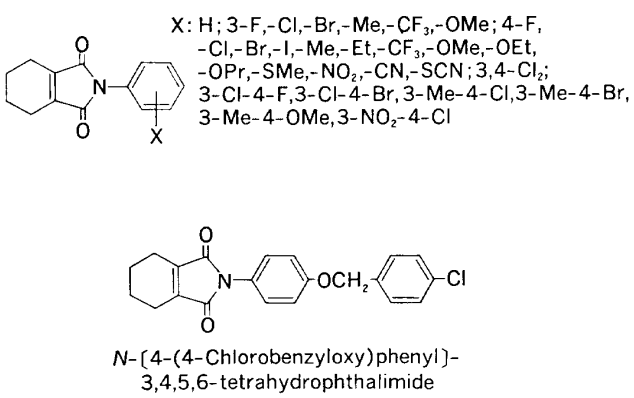


Fig. 14 Structure of *N*-phenyl-tetrahydrophthalimides and related compounds.

this, the significance of the specific directional steric effects suggests that the substituent effects are primarily related to the interaction with the critical site of herbicidal action. In this respect, hydrogen bond formation at the carbonyl oxygen atom with an acidic group at the site of action is a possibility, since the negative sign of the σ term shows that the electron-withdrawing effect of the substituents directed to the imide moiety is unfavorable to activity. These results are thus considerably different from those on the series of herbicides described above, where the activity was mainly governed by the events on the way to the target site.

The activity of the p-$OCH_2C_6H_4$-p-Cl derivative (Fig. 14) in the series was conspicuous and positively and significantly deviated from the value calculated by Eq. (20), while that of p-CH_2Ph derivative is in accord with the predictive value. There are additional factor(s) inherent in the p-$OCH_2C_6H_4$-p-Cl structure at work.

PLANT GROWTH REGULATORS

1. Cytokinin Agonists and Antagonists

The cytokinin activity of structurally very different N^6-substituted adenines and N,N'-diphenylureas (Fig. 15) is well known. To reveal the structural correspondence between the two classes of compounds with different structures but the same biological activity, Iwamura *et al.*[37] utilized the QSAR approach to obtain Eqs. (21) and (22).

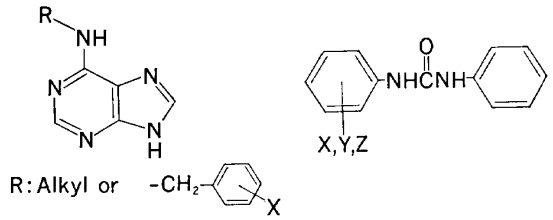


Fig. 15 Structures of N^6-substituted adenines and N,N'-diphenylureas.

N^6-Substituted adenines:

$$\log 1/E_{50} = \underset{(\pm 0.98)}{2.03\sigma^*} - \underset{(\pm 0.15)}{0.32W_{max}^2} + \underset{(\pm 1.71)}{3.35W_{max}} - \underset{(\pm 0.26)}{0.65W_{o,m}} - \underset{(\pm 4.74)}{8.50}$$
$$n=22,\ r=0.85,\ s=0.26 \qquad (21)$$

N,N'-Diphenylureas:

$$\log 1/C = \underset{(\pm 0.33)}{0.90\sigma} - \underset{(\pm 0.25)}{0.85L_o} - \underset{(\pm 0.22)}{0.27L_p} + \underset{(\pm 0.58)}{1.04\pi_m} + \underset{(\pm 0.32)}{2.00}$$
$$n=39,\ r=0.91,\ s=0.38 \qquad (22)$$

The E_{50} is the concentration at which 50% of maximum activity in the tobacco callus test is obtained and C is the minimum concentration at which activity is observed. The W_{max} term is the maximum width of the N^6-substituents which corresponds to the B_4 of the STERIMOL parameter (Fig. 7) and the $W_{o,m}$ is that for o- and m-substituents of N^6-benzyl derivatives (Fig. 15). The L_o and L_p terms are the length of o- and p-substituents of diphenylureas and the π_m is the hydrophobic parameter of the m-substituent.

The results are briefly that the steric dimensions and electron-withdrawing effects directed toward the NH function are important for activity through both series of compounds, and the situation is drawn schematically in Fig. 16. The stippled lines represent the steric interaction sites or spatial walls of the receptor deduced from the steric parameters incorporated into the correlations, and the smooth lines facing the NH group are the electron donating sites which presumably interact electrostatically with the acidic NH.

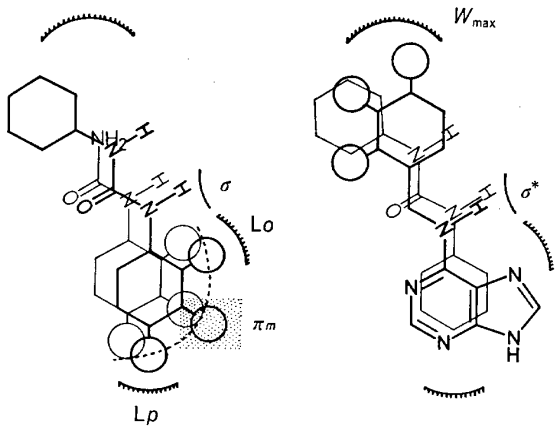


Fig. 16 Schematic cytokinin-receptor complex where a hexagon represents the benzene ring. Stippled and smooth lines are the receptor walls or interaction sites and the dotted square is the hydrophobic region deduced from the physicochemical parameters incorporated into Eqs. (21) and (22) (reproduced with permission from Pergamon Press Ltd.).[37]

The dotted square is the hydrophobic region where, as suggested by the π_m term in Eq. (22), the *m*-substituent of the diphenylureas comes when they fit with the receptor. Accordingly, the figure displays the structural correspondence and/or similarity between the two classes of cytokinins, as well as the rough size of the receptor cavity. For the latter, the optimum steric condition in terms of the W_{max} value is calculated to be ca. 5 (Å) from the quadratic Eq. (21) for N^6-substituted adenines.

The results of a QSAR study on a class of anticytokinins, developed by Iwamura *et al.*,[38] 4-substituted 2-methylthiopyrido[2,3-*d*]pyrimidines (Fig. 17), are shown graphically in Fig. 18, indicating a parabolic relation of the activity to the W_{max} value of N^4-substituents which correspond to the N^6-substituent of adenines. The optimum W_{max} value is ca. 4.6 (Å) and coincides well not only with that for the N^6-adenylate cytokinins shown above but also with the value of ca. 4.7[39] calculated for another class of anticytokinins, 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines (Fig. 17).[40] The fact that the optimum steric condition in terms of the W_{max} is the same in all three of these series of compounds, which have different structures but compete for the same receptor, may provide us with an insight

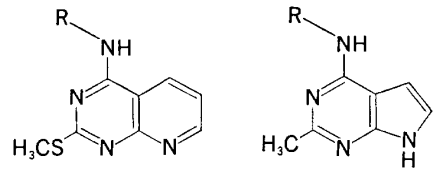


Fig. 17 Structures of 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines (left) and 4-substituted-2-methylpyrrolo[2,3-*d*]-pyrimidines (right).

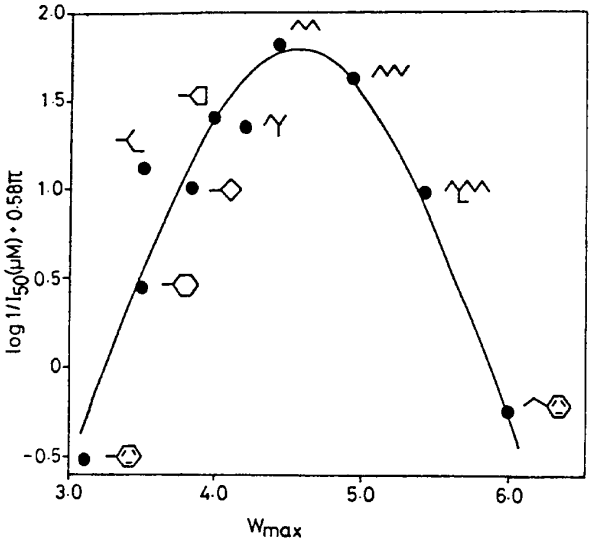


Fig. 18 Relation of anticytokinin activity of 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines to W_{max} (reproduced with permission from Pergamon Press Ltd.).[38]

into the size of the receptor cavity into which a compound has to fit to be active.

FINAL REMARKS

The QSAR approach offers several advantages in the study of the *chemical* basis of drug action as well as providing a more efficient method, compared to classical trial and error synthesis, to obtain drugs with high activity or high selectivity within a congeneric series. Although it sometimes leads to the finding that the compound of optimum activity has already been made, it prevents wasted effort, and the results will still be helpful in the selection of new group(s) of compounds to be investigated, as well as in understanding the precise mechanism of action. Accumulation of these QSAR analyses for compounds having the same type of activity but different structures is useful to obtain a thorough concept of

the mode of action that can sometimes result in generation of a novel lead structure. The examples we showed here are concerned with these aspects of QSAR study and our efforts are continuously directed toward the perfection of the methodology itself and exploration of action mechanisms, and ultimately toward the creation of new useful compounds. Endeavours and results from workers in other countries are an encouragement to us.

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