Structure-Activity Correlations in Fungitoxicity of Imides and Their Imide-$SCCl_3$ Compounds

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The fungitoxicity of imides and their *N*-$SCCl_3$ compounds to spore germination of *Stemphylium sarcinaeforme*, and the toxicity of the imide *N*-$SCCl_3$ compounds against *Erysiphe graminis* on oats have been correlated with the partition coefficient. Parabolic equations, derived from the method of least squares, account for more than 91% of the variance in the data. Similar equations have been derived for the fungitoxicity of the imide *N*-$SCCl_3$ compounds against *Neurospora crassa*, and a linear equation highly correlates the activity against *Alternaria tenuis*.

Since the first report of Kittleson (1952), a wide variety of compounds containing the trichloromethylthio group (S-CCl_3) have been shown to have different degrees of antifungal activity (Lukens *et al.*, 1965; Lukens, 1966; Lukens and Horsfall, 1967; Richmond *et al.*, 1964). It has been suggested by Horsfall (1956) and by Horsfall and Rich (1957) that the toxiphore of captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) is the imide and that the —$SCCl_3$ group serves as a "shape charge" to drive the compound across the lipoid membrane. It has also been proposed by Lukens and Sisler (1958) that the actual toxiphore of captan is the S-CCl_3 group, which breaks down to thiophosgene ($SCCl_2$) and this reacts with some nucleophiles of the cell, or binds through the free bond of sulfur to certain vital groups in the cell. Owens and Novotny (1959) on the other hand have suggested that the oxidation of cellular thiols is the most important reaction in captan toxicity.

Since 1959 Richmond and Somers (1966, 1968) have shown that the reaction of captan with soluble cell thiols is, in fact, a detoxication process and that neither oxidation of soluble thiols nor reaction of thiophosgene can explain the toxicity of captan. It has been postulated that the inhibition of cell division may be due to the reaction of captan with the sulfhydryl groups of the nuclear protein (Richmond *et al.*, 1967).

The purpose of this paper is to show that the fungitoxicity of some imides and their $SCCl_3$ derivatives can be correlated with the partition coefficient measured in oleyl alcohol/water, and an alternative mechanism of action can be advanced from the consideration of the molecular structure, the lipo-hydrophilic character, and some other biological data.

METHOD

The fungitoxicity data and the partition coefficients, taken from the careful study of Lukens and Horsfall (1967), that of Richmond *et al.* (1964), and the work of Richmond and Somers (1962) are assembled in Table I. The method of least squares is used to derive Equations 1–9, using a Honeywell-800 computer.

RESULTS

Equations 1 and 2 are derived from the fungitoxicity to spore germination of *Stemphylium sarcinaeforme;* Equations 3 and 4 are from the toxicity on *Erysiphe graminis*. The number of data points is represented by *n*. Equation 1 shows that a linear equation of log $1/ED_{50}$ and log *P* gives fairly good correlation (where *P* is the oleyl alcohol/water partition coefficient and *r* is the correlation coefficient). About 94% (r^2 = 0.94) of the variance in the data can be explained by this simple one-parameter equation. Addition of a $(\log P)^2$ term (Hansch *et al.*, 1968, Lien *et al.*, 1968) gives Equation 2. The correlation is excellent (r = 0.997, r^2 = 0.994), and the standard deviation *s* is appreciably smaller than that of Equation 1 (0.16 *vs.* 0.44). An *F*-test indicates that the addition of the $(\log P)^2$ term is significant at 99.5% level ($F_{1,4}$ = 34.1; $F_{1,4\,\alpha.995}$ = 31.3). The log P_o of 2.77 represents the optimum lipo-hydrophilic character for the fungitoxicity in the spore germination test against *S. sarcinaeforme*. The relatively broad 95% confidence interval (2.32–4.02) for the log P_o is due to the fact that only one compound with log $P > 2.77$ was studied. One can readily visualize this parabolic dependence of the activity on log *P* in Figure 1.

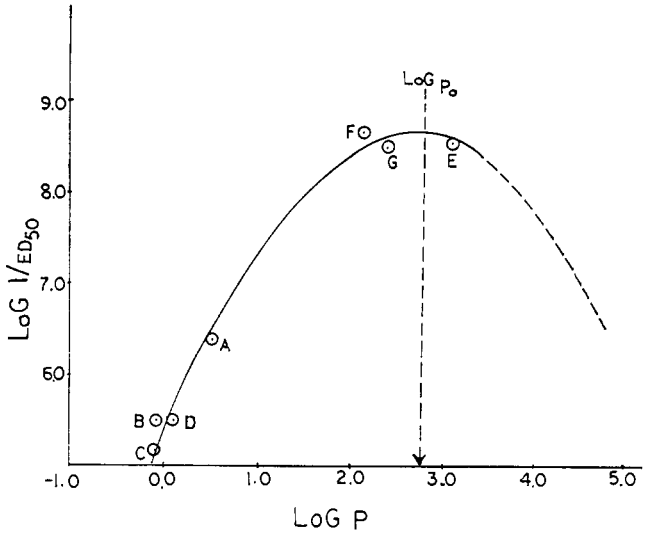

Figure 1. Parabolic dependence of fungitoxicity of imides and their $SCCl_3$ compounds on log *P* (test organism *S. sarcinaeforme*)

A. Phthalimide
B. Tetrahydrophthalimide
C. 3,6-Endomethylenetetrahydrophthalimide
D. Hexahydrophthalimide
E. *N*-$SCCl_3$ derivative of phthalimide (folpet, phaltan)
F. *N*-$SCCl_3$ derivative of cyclohexene-1,2-dicarboximide (captan).
G. *N*-$SCCl_3$ derivative of cyclohexane-1,2-dicarboximide

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Table I. Correlations of Fungitoxicity of Imides and Their N-$SCCl_3$ Compounds with Partition Coefficients

Imides	log P (oleyl alc/water)	log $1/ED_{50}$[a] (moles/cm²) (*vs. S. sarcinaeforme*) Obsd	Calcd[e]	log BR[b] (*vs. E. graminis*) Obsd	Calcd[f]	log $1/ED_{50}$[c] (moles/l.) (*vs. N. crassa*) Obsd	Calcd[g]	log RP[d] (*vs. A. tenuis*) Obsd	Calcd[h]
Phthalimide	0.55[i]	6.38	6.53						
Tetrahydrophthalimide	−0.01[i]	5.46	5.31						
3,6-Endomethylenetetrahydrophthalimide	−0.08[i]	5.19	5.14						
Hexahydrophthalimide	0.16[i]	5.58	5.71						
N-$SCCl_3$ Derivative of									
Phthalimide (Folpet, Phaltan)	3.13[j]	8.58	8.63	3.03	3.06	6.07	6.09	−0.03	−0.07
Cyclohexene-1,2-dicarboximide (Captan)	2.15[j]	8.70	8.52	3.52[k]	5.06[k]	5.90	5.55	0.00	−0.11
Cyclohexane-1,2-dicarboximide	2.40[j]	8.58	8.63	5.15	4.75	5.70	5.69	−0.12	−0.06
4-Methylhexahydrophthalimide	2.85[j]			3.65	3.85	5.80	5.93	−0.47[l]	−0.04[l]
4,5-Dimethyltetrahydrophthalimide	2.93[j]			3.67	3.64	6.00	5.98	−0.09	−0.04
3,6-Endohexahydrophthalimide	1.65[j]			4.94	5.24	5.01	5.28	−0.33	−0.28
4,5-Epoxyhexahydrophthalimide	0.85[j]			4.47	4.38	4.87	4.84	−0.77	−0.78

[a] From Lukens and Horsfall, 1967.
[b] From Richmond *et al.*, 1964, BR (biological response = % reduction in infection/μmoles/cm²).
[c] From Richmond and Somers, 1962.
[d] From Richmond and Somers, 1962, RP = relative potency, captan is the reference compound.
[e] Calculated from Equation 2.
[f] Calculated from Equation 4.
[g] Calculated from Equation 5.
[h] Calculated from Equation 9.
[i] From Lukens and Horsfall, 1967.
[j] From Richmond *et al.*, 1964.
[k] This point was not used in deriving Equation 4.
[l] This point was not used in deriving Equation 9.

	n	r	s	log P_o	
Test organism *S. sarcinaeforme:*					
$\log 1/ED_{50} = 1.182 \log P + 5.523$	7	0.969	0.444	...	(1)
$\log 1/ED_{50} = -0.437 (\log P)^2 + 2.420 \log P + 5.334$	7	0.997	0.161	2.77 (2.32–4.02)	(2)
Test organism *E. graminis* on oats:					
$\log BR = -0.559 (\log P)^2 + 1.814 \log P + 3,354$	7	0.722	0.673	1.52	(3)
$\log BR = -1.117 (\log P)^2 + 3.867 \log P + 1.902$	6	0.955	0.317	1.73 (0.69–1.96)	(4)
Test organism *N. crassa:*					
$\log 1/ED_{50} = 0.548 \log P + 4.371$	7	0.921	0.207	...	(5)
$\log 1/ED_{50} = -0.044 (\log P)^2 + 0.726 \log P + 4.221$	7	0.922	0.229	8.24 (∞)	(6)
Test organism *A. tenuis:*					
$\log RP = 0.239 \log P - 0.804$	7	0.687	0.225	...	(7)
$\log RP = -0.220 (\log P)^2 + 1.127 \log P - 1.557$	7	0.809	0.203	2.56 (∞)	(8)
$\log RP = -0.205 (\log P)^2 + 1.126 \log P - 1.585$	6	0.975	0.084	2.75 (2.30–30.7)	(9)

In deriving Equation 3 all of the seven data points are included, among which captan is about 1.5 × s less active than the predicted value. The correlation is poor. After deleting captan we have Equation 4. The correlation is much better (r = 0.955, r^2 = 0.91). For the toxicity of the captan analogs against *Neurospora crassa*, Equations 5 and 6 are obtained. The standard deviation of Equation 6 is larger than that of Equation 5, and the correlation coefficients for these two equations are practically the same. Although the $(\log P)^2$ term in Equation 6 is not statistically significant, it suggests that the log P_o for the maximum activity would be appreciably higher than three. For the toxicity against *Alternaria tenuis*, Equations 7 and 8 are obtained for all the seven data points. By deleting 4-methylhexahydrophthalimide, which has a deviation greater than 2 × s, Equation 9 is obtained. In Equation 9 the $(\log P)^2$ term is significant at 95% level ($F_{1,3}$ = 10.6, $F_{1,3\,\alpha 0.95}$ = 10.1). Interestingly enough, the log P_o for Equation 4 is appreciably lower than those of Equations 2, 6, 8, and 9. The difference of one log unit in log P_o corresponds to a ratio of 10 of the P_o values.

DISCUSSION

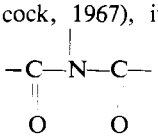
Since the antifungal activity of imides and their —$SCCl_3$ compounds can be correlated with the log P values of the *intact* molecules measured in oleyl alcohol/water, it seems to indicate that the intact molecules are responsible for the fungitoxicity, and the mechanism of action is probably the same for both series of compounds. Although it has been shown that captan breaks down to thiophosgene ($SCCl_2$), and this reacts with —SH, —NH_2, —OH or other nucleophiles (Lukens and Sisler, 1958), it may not necessarily be the mechanism of fungitoxic action. Owens and Blaak (1960) have provided circumstantial evidence that captan can react directly with cell thiols without the intermediate of thiophosgene. It has been reported that the fungitoxicity to yeast is not reversed by adding thiols to cells poisoned by captan (Lukens, 1966). This nonreversal of toxicity by thiols discounts the oxidation of thiols to disulfides by the molecules as an ultimate reaction of toxicity, although it does not necessarily distinguish toxicity by the intact molecules from that by decomposition products.

The antibacterial activity of many series of compounds has been correlated with the log P measured in octanol/water (Lien *et al.*, 1968), where the log P_o values have been found to be around four for Gram-negative bacteria and about six for Gram-positive cells. The reason for the difference in log P_o values was attributed to the difference in the lipid content of the cell wall. The lower log P_o value found for the fungitoxicity against oat powder mildew (*E. graminis*) may also be due to the high lipid content of powdery mildew spores (Yarwood, 1950). The compounds with log P values close to log P_o would have the greatest opportunity to penetrate through lipoid as well as aqueous phases to reach the site of action in the period of the biological test, while the highly lipophilic compounds tend to be bound to lipids or other macromolecules by hydrophobic interactions.

Why the activity of captan is very well predicted from Equations 2, 5, and 9 but very poorly predicted from Equation 4 is difficult to explain, since the structural changes are gradual in all these cases. One possible explanation is that the host plant might metabolize captan faster than the other congeners, resulting in a decreased toxicity to the fungus.

Since it is known that the log P_1 value measured in one solvent system can be related to the log P_2 value obtained from a second system by the simple equation (Collander, 1951): $\log P_1 = a \log P_2 + b$, where a and b are constants. Similar correlations would be expected when the partition coefficient is measured in another system (e.g., octanol/water), although the numerical values of the slope, the intercept, and the log P_0 will be different.

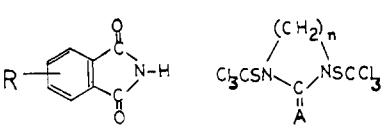
A recent paper by Siegel and Sisler (1968) on folpet suggests that the binding of sulfur-containing fragments of folpet to ethanol-insoluble entities of yeast cells is primarily responsible for the fungitoxicity. Electron microscopy studies (Richmond *et al.*, 1967) show that ED_{50} doses of captan give convoluted form of the nuclear membrane to dormant conidia of *Neurospora crassa*, and caused almost complete loss of intracellular fine structure after captan-treated spores have been incubated. Richmond and his coworkers (1967) have hypothesized that this effect may be due to the reaction of captan with the sulfhydryl groups of the nuclear proteins leading to an inhibition of cell division. No change in the cytoplasmic membrane is observed from the electron microscopic study. However, in the light of the recent theories of drug action (Bloom, 1967; Lien and Kumler, 1968; Shulman and Laycock, 1967), it seems that the highly polar imido group



of the title compound may anchor by dipole-dipole interaction and/or hydrogen bond on the protein portion of a lipoprotein membrane—e.g., the membrane of the nucleus, the endoplasmic reticulum, or the mitochondria. The alkylene or the phenyl group and the highly lipophilic —$SCCl_3$ group may then undergo hydrophobic interactions with the lipoid portion of the membrane and cause conformational change, which would then change the permeability of the membrane or even cause disruption of the lipoprotein membrane. The reason why no change in cytoplasmic membrane is observed may be due to the support of the relatively rigid cell wall, or may be that the change is too subtle to be photographed.

The difference in the pK_a values (8.9–9.6) of the imides appears to be of insignificance, since in a medium of pH 6–7, the degree of dissociation of the imido N—H will be less than 1%, therefore no correction for the ionization is necessary.

From Equation 2 one can predict that phthalimides with substituents on the benzene ring to make log P close to 2.77 in oleyl alcohol/water would be as active as folpet or captan in the same test. The following compounds would be of interest to investigate the fungitoxicity:



where R = SCF_3, C_4H_9, C_5H_{11}, C_4F_9, $SCCl_3$, and

n = 2–4, A = O, S.

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