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STRUCTURE–ACTIVITY RELATIONSHIPS IN THE EFFECTS OF 1-ALKYLIMIDAZOLES ON MICROSOMAL OXIDATION *IN VITRO* AND *IN VIVO*

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Abstract—The inhibitory activity of a series of 13 1-alkylimidazoles toward microsomal epoxidation of aldrin in enzyme preparations from rat liver and armyworm (*Prodenia eridania*) gut is optimal in compounds with a chain length of 8–10 carbon atoms. The capacity of the imidazoles to bind to cytochrome P-450 (type II) appears to be closely related to their inhibitory activity. The activity of the compounds *in vivo* in synergizing the toxicity of carbaryl to houseflies and potentiating pentobarbital sleeping time in mice closely parallels the data *in vitro*. Regression analyses clearly establish that both activity patterns *in vitro* and *in vivo* can be satisfactorily described by linear equations in terms of the hydrophobic bonding constant (π and π^2) indicating a close correlation between biological activity and lipophilic character.

THE REMARKABLE potency of many 1- and 4(5)-arylimidazoles as inhibitors of drug oxidation,[1–5] potentiators of barbiturate sleeping time in mammals,[3,5,6] and insecticide synergists to houseflies[6] has recently been established. In view of the high activity of the imidazoles, their relatively simple structure, and the considerable amount of background information on their interaction with hemoproteins,[7–9] it would appear that they might represent a useful class of model compounds for studying ligand interaction with cytochrome P-450. Structure–activity correlations could be of considerable value in more clearly defining the topography of the active site of the microsomal oxidases and could provide useful information on the physicochemical parameters important in the binding and interaction of foreign compounds with cytochrome P-450.

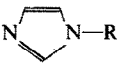
With this objective, several homologous series of substituted imidazoles have been synthesized and regression analyses made to determine the effect of substituents on their biological activity *in vitro* and *in vivo*.

This report is concerned with structure–activity relationships in a homologous series of 1-alkylimidazoles.

MATERIALS AND METHODS

Chemicals. The 1-alkylimidazoles (II–XIII) were obtained in good yield by the reaction of imidazole (I) with the appropriate alkylhalide.[10] The preparation of 1-hexylimidazole is typical. A mixture of imidazole (6·81 g, 0·1 mole) and *n*-hexylbromide (19·8 g, 0·12 mole) was heated under reflux for 1 hr. The resulting mixture was cooled, water was added, and the crude 1-hexylimidazole was liberated from solution by addition of solid KOH. The dark, oily product was extracted into chloroform and the extract washed with water and dried over anhydrous Na_2SO_4. The chloroform was removed, and the resulting oil distilled under vacuum to yield 10·3 g (68%) 1-hexylimidazole, b.p. 104–6° (1 mm Hg). Physical properties and elemental analyses (Schwarzkoff Microanalytical Laboratory, Woodside, New York) of the 1-alkylimidazoles are shown in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF 1-ALKYLIMIDAZOLES

General structure: 

Compound	Substituent (R)	Boiling point (°)	Elemental analysis			
			Calc.		Found	
			C	H	C	H
I	H					
II	CH_3	199–200	58·74	7·32	58·17	7·42
III	C_2H_5	207–209	62·50	8·33	62·12	8·50
IV	C_3H_7	220–223	65·45	9·09	65·26	9·26
V	C_4H_9	234–237	67·74	9·68	67·46	9·80
VI	C_5H_{11}	245–248	69·57	10·14	68·58	10·36
VII	C_6H_{13}	104–106 (1 mm)	71·05	10·53	70·79	10·54
VIII	C_7H_{15}	118–121 (2 mm)	72·29	10·84	71·84	10·69
IX	C_8H_{17}	124–126 (1 mm)	73·33	11·11	73·59	11·15
X	C_9H_{19}	134–136 (1 mm)	74·23	11·34	73·43	11·39
XI	$C_{10}H_{21}$	142–144 (0·8 mm)	75·00	11·54	76·07	11·95
XII	$C_{12}H_{25}$	161–163 (1 mm)	76·33	11·96	76·71	12·30
XIII	$C_{14}H_{29}$	177–179 (0·8 mm)	77·34	12·22	76·69	12·12

Analytical grade samples of aldrin (1,2,3,4,10,10-hexachloro-1,4,4a-5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) and its 6,7-epoxide, dieldrin, were kindly provided by the Shell Development Co., Modesto, California.

Biochemicals were purchased from CalBiochem, Los Angeles, California, and all other chemicals and solvents employed were of analytical reagent grade.

Enzyme preparations. Livers from male Sprague–Dawley rats, purchased from Blue Spruce Farms, Altamont, New York, were homogenized in ice-cold 1·15% KCl (1:4, w/v) and microsomes were sedimented from the post-mitochondrial supernatant (20,000 g_{max} for 20 min) by centrifugation at 100,000 g_{max} for 1 hr in an International Equipment Co. (IEC) B-60 preparative ultracentrifuge equipped with an IEC A-321 angle-head rotor. For enzyme assay the microsomal pellet was resuspended in 1·15% KCl to a concentration of approx. 0·6 to 1·0 mg/ml.

The insect preparation routinely employed consisted of a crude homogenate of cleaned midguts from sixth instar southern armyworm larvae (*Prodenia eridania*) (2 guts/ml) in cold 1·15% KCl and had a protein concn of approx. 1 mg/ml. Microsomal

suspensions of armyworm midguts were employed for the binding studies and were prepared by a procedure similar to that followed for rat liver.

Protein concn were determined by a modified biuret method[11] using bovine serum albumin as a standard.

Assay of aldrin epoxidation. Incubations were carried out aerobically in 25-ml Erlenmeyer flasks shaken in a water bath at 30°. The standard 5-ml incubation mixture consisted of 0·5 ml of the appropriate enzyme suspension and the following components (final concentration): Tris-HCl buffer (5×10^{-2} M), pH 7·4 (rat) or 7·8 (armyworm); G-6-P ($2{\cdot}4 \times 10^{-3}$ M); KCl ($1{\cdot}2 \times 10^{-2}$ M); NADP ($5{\cdot}2 \times 10^{-5}$ M); G-6-P dehydrogenase (1·6 units) and 100 μg aldrin in 25 μl ethanol. The 15-min reaction was initiated by addition of enzyme, and terminated with 4 ml acetone. Extraction and gas chromatographic assay of aldrin and dieldrin were as previously described.[12] The 1-alkylimidazoles were added to the incubations in 10 μl dilute ethanol and I_{50} values were determined from the means of duplicate incubations with at least four different inhibitor concentrations.

Binding studies. Optical difference spectra were recorded with a Norelco Unicam SP-800 spectrophotometer equipped with a scale expansion device and accessory recorder. Microsomal suspensions employed for this purpose contained 1–2 mg protein/ml in 67 mM phosphate buffer at either pH 7·4 (rat liver) or pH 7·8 (armyworm gut). Spectral dissociation constants (K_s) were determined from the abscissal intercepts of double reciprocal plots of ΔO.D.$_{430-390\,nm}$ vs imidazole concn. The means of duplicate determinations with five or six different imidazole concn were employed.

Sleeping time. Female mice (18–25 g) of the Swiss Webster strain purchased from Marlands Farms, Hewitt, New Jersey, were employed. Each mouse was weighed and injected i.p. with sodium pentobarbital (65 mg/kg) in approx. 0·5 ml water. The test animals were considered to be asleep when the righting reflex was lost, usually within 4–5 min, and at this time the animals were placed on their backs. The period which elapsed until the mouse regained control of its righting reflex was recorded as the sleeping time. SKF 525-A (β-diethylaminoethyldiphenyl propyl acetate), imidazole (I) and the lower 1-alkylimidazoles (II–VI) were dissolved in water (3 mg/ml), and solutions of the same concentration of the higher analogs (VII–XIII) were prepared by addition to the water of a few drops of conc. HCl. After dilution, appropriate aliquots of these solutions (97·5 mg/kg and/or 9·75 mg/kg) were injected i.p. 30 min prior to pentobarbital administration and sleeping time was recorded as described.

Synergistic activity. Synergistic activity of the 1-alkylimidazoles was determined by the extent to which they enhanced the toxicity of the insecticide carbaryl (1-naphthyl *N*-methylcarbamate) to female houseflies (*Musca domestica* L.) of the standard insecticide-susceptible World Health Organization strain. Appropriate μl aliquots of acetone solutions to carbaryl plus the test compound (1:5, w/w ratio) were topically applied to the dorsal thoraces of groups of 20 female flies under carbon dioxide anaesthesia. Treated flies were provided with sucrose, and mortality was assessed 24 hr post-treatment. The LD_{50} values were determined from the mean mortalities observed in duplicate treatments made on each of two different days with at least four concentrations of carbaryl.

Regression analysis. Structure–activity correlations were examined by regression analysis[13] using the IBM 360/65 computer at Cornell University. The program employed provides estimates of the parameters of single equation models by the

TABLE 2. BIOLOGICAL ACTIVITY OF 1-ALKYLIMIDAZOLES

Compound	I_{50}M aldrin epoxidation		Spectral dissociation constant (K_s)		Synergistic ratio in houseflies*	Mean sleeping time in mice (min)†
	Rat liver	Armyworm gut	Rat liver	Armyworm gut		
I	$3{\cdot}6 \times 10^{-3}$	$2{\cdot}9 \times 10^{-3}$	$2{\cdot}10 \times 10^{-4}$		~1·00	68·8 ± 6·8 (5)‡
II	$2{\cdot}9 \times 10^{-3}$	$1{\cdot}25 \times 10^{-3}$	$1{\cdot}99 \times 10^{-4}$		>12·2	43·1 ± 5·5 (10)
III	$1{\cdot}6 \times 10^{-4}$	$5{\cdot}3 \times 10^{-4}$	$2{\cdot}10 \times 10^{-5}$	$1{\cdot}02 \times 10^{-5}$	>17·4	50·1 ± 6·2 (10)
IV	$3{\cdot}0 \times 10^{-5}$	$1{\cdot}4 \times 10^{-5}$	$1{\cdot}14 \times 10^{-5}$		>14·5	125·7 ± 13·1 (10)
V	$4{\cdot}1 \times 10^{-5}$	$2{\cdot}6 \times 10^{-6}$	$9{\cdot}4 \times 10^{-6}$		>14·7	169·7 ± 12·4 (10)
VI	$3{\cdot}1 \times 10^{-5}$	$3{\cdot}9 \times 10^{-7}$	$4{\cdot}10 \times 10^{-6}$		>21·3	243·0 ± 14·7 (10)
VII	$1{\cdot}3 \times 10^{-5}$	$1{\cdot}3 \times 10^{-7}$	$3{\cdot}39 \times 10^{-6}$	$3{\cdot}05 \times 10^{-6}$	>24·4	355·6 ± 20·5 (28)
VIII	$5{\cdot}6 \times 10^{-6}$	$1{\cdot}15 \times 10^{-7}$	$2{\cdot}78 \times 10^{-6}$		>40·0	373·1 ± 21·8 (15)
IX	$2{\cdot}35 \times 10^{-6}$	$9{\cdot}6 \times 10^{-8}$	$3{\cdot}2 \times 10^{-6}$	$6{\cdot}9 \times 10^{-7}$	>66·7	397·0 ± 22·1 (13)
X	$8{\cdot}0 \times 10^{-7}$	$8{\cdot}0 \times 10^{-8}$	$3{\cdot}9 \times 10^{-6}$		>98·0	202·2 ± 13·5 (10)
XI	$4{\cdot}0 \times 10^{-7}$	$1{\cdot}02 \times 10^{-7}$	$3{\cdot}5 \times 10^{-6}$		>126·6	172·1 ± 18·5 (10)
XII	$2{\cdot}25 \times 10^{-6}$	$1{\cdot}78 \times 10^{-7}$	$4{\cdot}5 \times 10^{-6}$	$8{\cdot}4 \times 10^{-7}$	>116·3	209·0 ± 20·5 (9)
XIII	$6{\cdot}8 \times 10^{-6}$	$8{\cdot}4 \times 10^{-7}$	$5{\cdot}7 \times 10^{-6}$		>84·7	162·2 ± 15·6 (9)

* LD_{50} Carbaryl alone (>100 μg/fly)/LD_{50} carbaryl in combination with imidazole. Insectide imidazole ratio 1:5.
† Mice treated (i.p.) with 9·75 mg/kg of imidazole 30 min before i.p. injection of 65 mg/kg of sodium pentobarbital. Control sleeping time was 56·8 ± 8·4 min.
‡ Number of animals employed.

method of least squares. The π values employed in the regression analyses were those reported by Fujita *et al.*[14] for the substituted phenoxyacetic acids; values for the higher homologs were calculated on the principal of the additive character of this parameter.

RESULTS

The data in Table 2 and Figs. 1 and 2 clearly show that, on ascending the homologous series, the changes in each type of biological activity follow a remarkably similar pattern, activity typically increasing with increasing chain length, passing through a maximum, and decreasing again in the higher homologs.

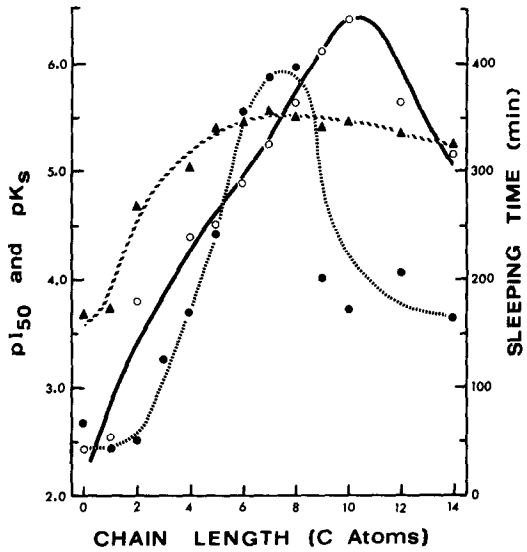


FIG. 1. Activity of 1-alkylimidazoles in mammals *in vitro* and *in vivo*. Solid line, open circles—pI_{50} aldrin epoxidase activity in rat liver microsomes; striped line, solid triangles—pK_s rat liver microsomes; broken line, solid circles—pentobarbital sleeping time in mice.

The ability of the compounds to inhibit aldrin epoxidation in rat liver microsomes increases by almost four orders of magnitude on going from the unsubstituted imidazole (I) to the 1-decyl derivative (XI) and with an I_{50} of $4{\cdot}0 \times 10^{-7}$ M this was the most active member of the series; further increase in chain length was associated with a decrease in inhibitory activity. A similar pattern was observed with respect to the inhibition of epoxidation in the armyworm gut preparation (Fig. 2) although in this case the most active compound was 1-nonylimidazole (X) and activity only changed approx. two-fold from the hexyl (VII) to the dodecyl (XII) homologs. In general, all members of the series above butyl (V) were 8- to 100-fold more potent inhibitors of the armyworm gut enzyme than of that from rat liver.

Each of the 1-alkylimidazoles exhibited a type II optical difference spectrum in oxidized rat liver microsomes, and spectral dissociation constants (K_s) obtained from double reciprocal plots of ΔO.D.$_{430-390}$ vs imidazole concn decreased almost 100-fold in going from imidazole to the 1-pentyl (VI) derivative (Table 2, Fig. 1). Further increase in chain length caused only slight changes in K_s, the lowest values ($\sim 3{\cdot}0 \times 10^{-6}$ M) occurring between C_6 and C_8. Although these data do not exactly

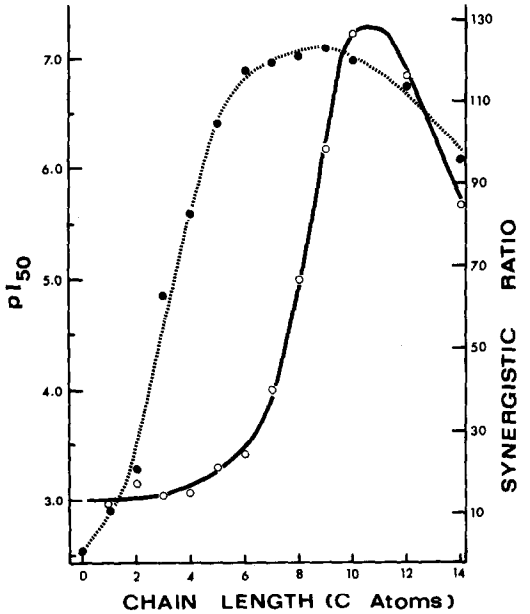


FIG. 2. Activity of 1-alkylimidazoles in insects *in vitro* and *in vivo*. Broken line, solid circles—pI_{50} aldrin epoxidase activity in armyworm gut preparation; solid line, open circles—synergistic ratio with carbaryl to houseflies.

correlate with the corresponding I_{50} values, the patterns of activity are sufficiently similar to suggest that the binding of the imidazoles to cytochrome P-450 is closely associated with their inhibitory activity. Spectral dissociation constants (K_s) in armyworm gut microsomes appear to be up to 5-fold lower than those observed in rat liver microsomes (Table 2).

The activity of the 1-alkylimidazoles *in vivo* in potentiating pentobarbital sleeping time in mice and synergizing the toxicity of carbaryl to houseflies appears to closely reflect their effects on the microsomal enzymes *in vitro*.

Although at a dosage level of 9·75 mg/kg the first three members of the series (I–III) showed little or no potentiation of barbiturate sleeping time, significant activity was observed at a dosage of 97·5 mg/kg (I = 126·5 ± 13·4 min; II = 111·0 ± 7·0 min; III = 213·4 ± 14·1 min).

Each of the other members of the series showed considerable activity at the lower dosage level (Table 2) and a maximum was exhibited by 1-octylimidazole (X) which caused an almost seven-fold increase in sleeping time (397·0 ± 22·1 min) relative to the controls. This is approx. 2·2-fold greater than that caused by SKF 525-A tested under similar conditions at the same dosage (180·1 ± 9·0 min), although when measured on a molar basis, the activity of IX (mol. wt 180) is almost the same as that of SKF 525-A (mol. wt 353).

The degree to which the various 1-alkylimidazoles synergize the toxicity of carbaryl to houseflies (Table 2) is not so great as that observed with many other types of insecticide synergists, and only two compounds (IX, X) approach the effectiveness of the well known material piperonyl butoxide (synergistic ratio of >175 under similar test conditions). The pattern of synergistic activity, however, is qualitatively quite similar to those observed with the other types of activity studied.

Regression analysis on the IBM 360/65 computer was employed to investigate correlations between the biological activity of the series and the hydrophobic bonding character as measured by π.[14] As shown by the regression equations in Table 3, only poor correlation coefficients were obtained in terms of π alone. In each case, however, the correlation coefficient was considerably improved by addition to the equation of a π^2 term. Since each type of biological activity measured clearly passes through an optimum as the series is ascended (Figs. 1 and 2), the importance of the squared term was expected, and the close agreement between observed values and those calculated using the appropriate equation in terms of π and π^2 (Table 4) indicates that compounds with π values in excess of the optimal value (π_0) conform to a normal distribution. In view of the fact that in the test systems *in vitro* over 90 per cent of the data variance can be accounted for in terms of π, the lipophilic character of the compounds clearly plays the major role in determining biological activity. Even *in vivo* where the factors determining activity are more complex, the importance of π is still clearly shown by the high correlation coefficients of the regression equations.

TABLE 3. REGRESSION EQUATIONS FOR BIOLOGICAL ACTIVITIES OF 1-ALKYLIMIDAZOLES*

	n	r	S.D.
In vitro			
$pI_{50}RL = 0{\cdot}455\pi + 3{\cdot}240$	13	0·829	0·712
$pI_{50}RL = -0{\cdot}125\pi^2 + 1{\cdot}337\pi + 2{\cdot}274$	13	0·964	0·356
$pI_{50}AW = 0{\cdot}589\pi + 3{\cdot}734$	13	0·775	1·119
$pI_{50}AW = -0{\cdot}215\pi^2 + 2{\cdot}100\pi + 2{\cdot}078$	13	0·983	0·335
$pK_sRL = 0{\cdot}209\pi + 4{\cdot}353$	13	0·721	0·468
$pK_sRL = -0{\cdot}086\pi^2 + 0{\cdot}812\pi + 3{\cdot}692$	13	0·961	0·197
In vivo			
Log SR $= 0{\cdot}223\pi + 0{\cdot}073$	13	0·866	0·299
Log SR $= -0{\cdot}038\pi^2 + 0{\cdot}489\pi + 0{\cdot}439$	13	0·922	0·243
Log PST $= 0{\cdot}829\pi + 1{\cdot}938$	13	0·585	0·268
Log PST $= -0{\cdot}043\pi^2 + 0{\cdot}383\pi + 1{\cdot}610$	13	0·871	0·170

* Abbreviations: $pI_{50}RL$ and $pI_{50}AW = -\log I_{50}M$ aldrin epoxidation in preparations from rat liver and armyworm gut, respectively; $pK_s = -\log$ spectral dissociation constant (K_sM); SR = synergistic ratio with carbaryl to houseflies; PST = potentiation of pentobarbital sleeping time in mice; n = number of data points; r = correlation coefficient; S.D. = standard deviation from regression.

DISCUSSION

In recent years, significant advances have been made in correlating biological activity with chemical structure, through the use of regression analysis to investigate the effect of substituent groups in terms of physicochemical parameters describing hydrophobic, electronic and steric character.[13,15] In addition to indicating structural parameters which are of importance in determining biological activity and allowing predictions to be made concerning the activity of untested molecules, the results of the regression analyses often provide information on the mechanism of the interaction at the active site and on the nature of the active site itself.

In agreement with previously reported data on a large number of 1-aryl- and 4(5)-arylimidazoles,[1–5] many of the 1-alkylimidazoles employed in this investigation are

TABLE 4. BIOLOGICAL ACTIVITY OF 1-ALKYLIMIDAZOLES—OBSERVED VS CALCULATED DATA*

	pI_{50}†				pK_s‡		Log SR§		Log ST‖	
	Rat liver		Armyworm gut		Rat liver		Houseflies		Mice	
Compound	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
I	2·27	2·44	2·08	2·54	3·69	3·68	0·44	0·00	1·61	1·84
II	2·93	2·54	3·11	2·90	4·09	3·70	0·68	1·09	1·80	1·63
III	3·52	3·80	4·03	3·28	4·44	4·68	0·91	1·24	1·96	1·70
IV	4·05	4·52	4·83	4·85	4·75	4·94	1·11	1·16	2·10	2·10
V	4·51	4·39	5·52	5·59	5·01	5·03	1·29	1·17	2·22	2·23
VI	4·90	4·51	6·09	6·41	5·22	5·39	1·45	1·33	2·32	2·39
VII	5·23	4·89	6·54	6·89	5·39	5·47	1·60	1·39	2·39	2·55
VIII	5·48	5·25	6·88	6·94	5·51	5·56	1·72	1·60	2·44	2·57
IX	5·67	5·63	7·09	7·02	5·59	5·49	1·82	1·82	2·46	2·60
X	5·78	6·10	7·20	7·10	5·62	5·41	1·90	1·99	2·47	2·31
XI	5·84	6·40	7·20	6·99	5·60	5·46	1·96	2·10	2·45	2·24
XII	5·73	5·65	6·82	6·75	5·42	5·35	2·02	2·07	2·34	2·32
XIII	5·36	5·17	5·98	6·08	5·06	5·24	1·99	1·93	2·14	2·21

* Calculated from equations in Table 3 giving highest r values.
† $-\log I_{50}$M aldrin epoxidation.
‡ $-\log K_s$M.
§ Degree of synergism of carbaryl to houseflies.
‖ Pentobarbital sleeping time (min) in mice.

potent inhibitors of microsomal epoxidation in preparations from rat liver and armyworm gut (Table 2). The ideal hydrophobic value (π_0) for inhibitory activity can be calculated from the regression equations by taking the partial derivative $\delta pI_{50}/\delta\pi$ and setting it equal to zero. These values were 5·33 and 4·89 for the rat liver and armyworm gut systems, respectively, equivalent to alkyl chains of 9–10 carbon atoms. The ability of the 1-alkylimidazoles to bind to cytochrome P-450 also appears optimal at π values approximately equal to that of the 1-nonyl derivative ($\pi_0 = 4{\cdot}89$), indicating a close relationship between binding and inhibitory activity.

The importance of lipophilic character in the ability of compounds to interact with the microsomal drug-metabolizing enzymes was first recognized by Gaudette and Brodie,[16] and subsequent studies[17,18] have clearly established that the ability of many foreign compounds to undergo metabolic transformation depends on their ability to bind to the microsomal complex; good correlations have been observed between substrate binding (K_m) and the hydrophobic parameter log P, P being the octanol/water partition coefficient.[17,18]

Type II optical difference spectra of the type exhibited by the imidazoles[3,5,6] and many other nitrogen-containing compounds are considered to result from direct interaction between the non-bonded electrons of the nitrogen atom and the fifth or sixth ligand on the heme moiety of cytochrome P-450.[19] It is clear from the results of this investigation that the ability of compounds to interact in this way depends to a large extent on their lipophilic character. The fact that binding capacity exhibits an optimum value strongly suggests that, in order to successfully undergo ligand interaction with P-450, the imidazole must also bind to a hydrophobic patch close to the cytochrome which can accommodate only about nine methylene groups. The decreased binding ability of compounds containing larger alkyl substituents may result from steric effects or binding to other hydrophobic sites in a manner which changes the spatial orientation of the imidazole nitrogen and prevents its interaction with cytochrome P-450. It is probable that the hydrophobic sites with which the imidazoles interact are closely associated with the sites responsible for substrate binding.

Although activity in the whole animal depends on many factors other than those associated with target site interaction, the regression equations (Table 3) indicate a close correlation between activity and lipophilicity *in vivo*, and the activity patterns closely parallel those observed in the test systems *in vitro*. From the appropriate regression equation in Table 3, the π_0 value for the potentiation of pentobarbital sleeping time is 4·49, corresponding to an alkyl chain length of between 8 and 9 carbon atoms. The activity of the series as carbaryl synergists to houseflies shows a somewhat higher π_0 value (6·47) which may reflect the additional requirement of penetration through the lipophilic insect cuticle.

The results of this investigation show that the interactions *in vitro* of selected series of microsomal enzyme inhibitors, as well as the effects *in vivo* of these interactions, can be described in physico-chemical terms. It is hoped that studies currently in progress will further define the nature and mechanism of inhibitor interactions at cytochrome P-450 and will provide information leading to the design of new and more active materials of this type.

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