Biochemical Pharmacology, Vol. 27, pp. 783–788. Pergamon Press, 1978. Printed in Great Britain.

THE ROLE OF SUBSTRATE LIPOPHILICITY IN DETERMINING TYPE 1 MICROSOMAL P450 BINDING CHARACTERISTICS

K. A. S. AL-GAILANY*, J. B. HOUSTON† and J. W. BRIDGES
Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K.

(*Received* 20 *May* 1977; *accepted* 5 *October* 1977)

Abstract—The Type 1 cytochrome P450 binding of 53 aliphatic, alicyclic and aromatic compounds to microsomes from phenobarbitone or 3 methylcholanthrene pre-treated or normal hamsters has been studied. A good correlation between binding affinity and substrate lipophilicity was observed for each series of compounds. Sterically hindered molecules tended to partially deviate from this relationship. The pronounced slopes of plots of K_s against log P indicate that substrate lipophilicity is the predominant requirement for Type 1 binding of these compounds. Microsomes from phenobarbitone pre-treated animals showed very similar substrate binding characteristics to those of normal animals whilst 3-methylcholanthrene pre-treated animals showed a spectral shift but similar binding affinities.

In 1966, Remmer *et al.* [1] and Imai and Sato [2] reported that various xenobiotics produced characteristic absorbance changes in the difference spectrum of liver microsomes which they proposed reflected the interaction of the added compound with cytochrome P450. These spectral changes, which are obtained upon addition of various compounds to suspensions of liver microsomes in buffer systems, have been classified into three types termed Type 1, Type 2 and reverse Type 1 [3]. Type 1 spectral changes are characterised by an absorption peak at about 385 nm and a trough at about 420 nm, and are generally acknowledged to arise as a consequence of the binding of a substrate to the apoprotein of the cytochrome P450. The Type 2 spectral change which is indicated by an absorption peak in the region of 425–435 nm and a trough at about 390 nm is probably associated with ferrihaemochrome formation arising from the interaction of the haem iron with a basic nitrogen of the added compound. The reverse Type 1 spectral change is characterised by a peak at about 420 nm and a trough at about 390 nm, its cause remains uncertain.

The relative binding affinities of various compounds to cytochrome P450 is defined by the spectral dissociation constant 'K_s' which is the concentration of substrate giving rise to half maximal spectral change.

The Type 1 spectral change was initially correlated with metabolism, since many compounds which elicit this response in liver microsomes are known substrates of the mono-oxygenase system, and because similarities in spectral dissociation constant 'K_s' and Michaelis constant 'K_m' were observed for a number of substrates [4]. However, Anders *et al.* [5] showed no correlation between rates of *N*-demethylation and Type 1 binding spectra of several enantiomerically related drugs. Furthermore, Al-Gailany *et al.* [6], have reported that the K_m values for dealkylation of some *p*-nitrophenylalkylethers are generally one order of magnitude lower than the corresponding K_s values.

The work of Imai and Sato [7] using the spectral changes technique and of Al-Gailany *et al.* [8] and Cohen and Mannering [9] using the fluorescent probe technique have indicated that the 'active site' of cytochrome P450 is hydrophobic implying that there could be some relationship between a compound's binding affinity and its partition coefficient. Jansson *et al.* [10] using a series of barbituric acid derivatives found that all the compounds studied gave rise to a Type 1 spectral change when added to suspensions of rat liver microsomes but only a weak correlation was found between the partition coefficients and the binding affinities. They could find no statistically significant correlation between partition coefficient and rates of metabolism despite an indication that the more lipid-soluble barbiturates underwent a more rapid oxidation than the less lipid-soluble compounds.

In the present work, a wide variety of organic compounds including aliphatic carbamates, aromatic hydrocarbons, alicyclic hydrocarbons, fatty acids and methyl esters have been used to establish the relationship between Type 1 binding affinities and lipid solubility.

MATERIALS AND METHODS

Chemicals. Phenanthrene was used as purchased (Koch Light and Co. Ltd.), naphthalene (m.p. 80°) and biphenyl (m.p. 70°) were twice recrystallised from absolute alcohol. 2-Naphthol was thrice recrystallised from aqueous alcohol, m.p. 93–95°. The following compounds were twice re-distilled at their boiling points: benzene, 78–79°; cyclohexylbenzene, 235–236°; diphenylmethane, 262–263°; dicyclohexyl, 235–237°; ethylbenzene, 133–135°; cyclohexane, 81°; toluene, 110° and dekalin (mixture of *cis*- and *trans*-hexahy-

* Present address: Department of Chemistry, University of Basrah, Iraq.
† Present address: Department of Pharmacy, University of Manchester, Manchester, U.K.

dronaphthalene), 188–190° *n*-Butyl carbamate (Kodak Ltd., Kirby) and benzyl carbamate (Aldrich Chemical Co., Milwaukee, Wisconsin) were used without further purification. Tert-Pentyl (2,2'-dimethylpropyl, m.p. 80°), *n*-pentyl (m.p. 53–54°), terthexyl (3,3'-dimethylbutyl, m.p. 55–56°), *n*-hexyl (m.p. 59°), *n*-heptyl (m.p. 62°) *n*-octyl (m.p. 67°), *n*-decyl (m.p. 69°) carbamates were synthesised by the Chemistry Division of the Chemical Defence Establishment. The purity of the carbamates used was checked by infra-red spectroscopy and g.l.c. and was found in all cases to be >99 per cent. All other compounds were at least of reagent grade.

Tissue preparation. Hepatic microsomes were obtained from either male Wistar albino rats (140–180 g) or male Syrian hamsters (12–16 weeks). The animals were killed by cervical dislocation, the livers were immediately removed and immersed in ice-cold 1.15% potassium chloride solution followed by homogenisation in 0.25 M sucrose solution using a motor-driven Potter–Elvehjem type Teflon–glass homogeniser. Washed microsomal fractions were then obtained by differential centrifugation. The final microsomal suspension was diluted with 0.1 M sodium phosphate buffer, pH 7.4 to a final concentration of 2 mg/ml. The protein content of the microsomal suspension was determined by the method of Lowry *et al.* [11] and cytochrome P450 content using the method of Ullrich [12].

Microsomes from phenobarbitone and 3-methylcholanthrene pre-treated animals were prepared following injections of either 100 mg/kg body wt sodium phenobarbitone intraperitoneally once daily for 3 days, or 50 mg/kg body wt of 3-methylcholanthrene in Saladin groundnut oil, once daily for 2 days.

Determination of apparent partition coefficient. The apparent partition coefficient of each carbamate was determined in duplicate at 37° using n-octanol and 0.1 M phosphate buffer. Both the octanol and buffer were saturated with the relevant aqueous or organic phase before use. Equal volumes (25 ml) of both phases were used and 6 hr agitation was allowed to achieve equilibrium [13]. The initial concentration of carbamate was 20 mM dissolved either in the aqueous or the octanol phase. The amount of carbamate in both phases at equilibrium was assayed by g.l.c. and good agreement was found between duplicates.

The partition coefficients of the other compounds were obtained from the tables of Leo *et al.* [13].

Apparent spectral changes. The difference spectra produced by the compounds studied were determined as previously described using either a Pye-Unicam SP 1800 or Perkin Elmer 356 spectrophotometer in the split beam mode [4]. The spectral dissociation constant (K_s) was determined using a double reciprocal Lineweaver–Burk plot of the absorption differences of the spectral changes against the concentration of the drug. The absorbance (ΔA) was measured between 420 nm trough and 385–390 peak.

Statistical evaluation of results. Linear regression analysis based on the method of least squares was performed on a Hewlett–Packard 9810A programmable calculator. Where appropriate, data have been expressed as mean ± S.D. Tests for statistical significance were performed using either an 'F'-test or an appropriate '*t*' test.

RESULTS

Spectral interactions. The addition of low concentrations of benzene (0.02–0.50 mM) to liver did not elicit a detectable spectral change, but as the concentration of the compound increased, a classical Type 1 spectrum was obtained giving an absorption peak at

Table 1. Type 1 interaction of various aromatic and alicyclic compounds to cytochrome P450* of hamster hepatic microsomes

	Compound	$K_s \times 10^{-4}$ M†	A_{max}‡ A × 10^{-2}/2 mg protein)	Log P
1	Benzene	75.00	4.30	2.13
2	Toluene	11.00 ± 1.00	4.56 ± 0.75	2.63
3	Ethylbenzene	4.60 ± 0.40‡	3.03 ± 0.50	3.13
4	Butylbenzene	1.40 ± 0.15‡	4.76 ± 0.90	4.13
5	*n*-Hexylbenzene	0.62 ± 0.10‡	3.18 ± 0.80	5.13
6	*iso*-Propylbenzene	2.90 ± 0.25	5.55 ± 0.50	3.43
7	*tert*-Butylbenzene	1.60 ± 0.10	5.71 ± 0.42	3.73
8	Cyclohexane	9.00 ± 1.50	4.70 ± 0.65	2.73
9	Methylcyclohexane	6.00 ± 0.75‡	4.00 ± 0.40	3.23
10	Ethylcyclohexane	2.80 ± 0.35‡	3.60 ± 0.50	3.73
11	Butylcyclohexane	0.70 ± 0.15‡	1.80 ± 0.30	4.73
12	Naphthalene	3.40 ± 0.20	3.36 ± 0.43	3.37
13	Tetralin	1.90 ± 0.11	4.50 ± 1.00	3.97
14	Dekalin	1.10 ± 0.05	3.46 ± 0.35	4.57
15	α-Naphthol	5.25	—	3.00
16	β-Naphthol	4.0	—	2.84
17	Biphenyl	5.00 ± 0.55	7.20 ± 1.10	4.04
18	Phenanthrene	3.07 ± 0.25	5.20 ± 0.50	4.46
19	Diphenylmethane	2.50 ± 0.15	3.00 ± 0.15	4.54
20	Cyclohexylbenzene	2.20 ± 0.20	2.40 ± 0.20	4.64
21	Dicyclohexyl	0.88 ± 0.14	0.87 ± 0.09	5.24
22	Fluorene	2.40 ± 0.10	3.80 ± 0.30	4.30
23	Perhydrofluorene	0.79 ± 0.05	2.20 ± 0.40	5.50

* Values are mean S.D. of three experiments, except for benzene, α-Naphthol and β-Naphthol where values represent the mean of two determinations.

† Students '*t*' test was applied comparing the results of each compound with that of the preceding one, ‡$P < 0.001$.

‡ The absorbance A was measured between 420 nm trough and 385–390 nm peak. Log P is the log of the partition coefficient between octanol and phosphate buffer, pH 7.4.

about 388 nm and a trough at about 418 nm. The K_s value of the interaction of benzene with hamster hepatic microsomes was found to be higher than its saturated analogue cyclohexane (Table 1). Similarly, the K_s value of dekalin was less than that of naphthalene, although there was no significant difference in the A_{max} values. In line with this trend, tetralin, the partially saturated molecule, showed intermediary binding affinity between naphthalene and dekalin. This trend was also observed in the series: biphenyl, cyclohexylbenzene and dicyclohexane (Table 1).

The effect of increasing number of carbon atoms attached to the aromatic compound, benzene, was also studied. Toluene was found to interact strongly with cytochrome P450 giving a binding affinity approximately six times higher than that of benzene. Ethyl, butyl and hexyl benzenes were also found to interact in a Type 1 manner. The K_s value determined for the alkylbenzenes was found to decrease with increasing number of carbon atoms in the straight chain aliphatic alkyl group but no systematic differences in A_{max} values were found for these compounds (Table 1). The interaction of the branched-chain compound *tert*-butylbenzene was lower than that of the other branched-chain compound studied, iso-propylbenzene.

A decrease in K_s value was also observed as the number of carbon atoms in the aliphatic side chain of cyclohexane was increased. Methyl and ethyl cyclohexane produced a Type 1 spectral change with no significant difference in A_{max} values, while *n*-butylcyclohexane produced a relatively smaller A_{max} value (Table 1).

Type 1 spectral interactions were also found with other hydrocarbons:— diphenylmethane, fluorene, phenanthrene, perhydrofluorene, α-naphthol and β-naphthol.

The binding spectra of a series of C_3—C_{14} aliphatic carboxylic acids were examined. Propionic acid and butyric acid at concentrations as high as 5.0 mM failed to produce a detectable spectral interaction, but acids containing more than five carbon atoms were found to elicit a distinct Type 1 difference spectra. A significant increase in the binding affinity of the acids with increasing number of carbon atoms was noticed but no similar trend was observed in the A_{max} values (Table 2). Myristic acid, unlike the other acids studied, gave no definite peak in the range 390–400 nm, but showed a trough at about 422 nm. At concentrations below 0.5 mM myristic acid did not affect the stability of P450, but some destruction of P450, monitored by the appearance of a P420 peak was clearly noticed at high concentration. At 25 mM a 25 per cent decrease in P450 was observed. Rats and hamsters gave very similar K_s values for all the fatty acids.

Methyl, ethyl and propyl carbamates did not give detectable binding spectra with hamster or rat liver microsomes, or with phenobarbitone-pretreated rat liver microsomes, but a classical Type 1 interaction was found with C_4—C_{10} carbamates. A decrease in the K_s value was observed with increasing number of carbon atoms in the straight chain compounds. The lowest K_s value was produced by *n*-decyl carbamate, but like the fatty acids, there was no significant change in K_s values between rat and hamster hepatic microsomes. The branched-chain compounds, *tert*-pentyl and *tert*-hexyl carbamates did interact with microsomal suspensions but their K_s values were higher than those of the corresponding straight chain compounds (Table 2).

The interaction characteristics of aliphatic methyl esters with microsomal P450 was similar to those observed with the fatty acids. Methyl-*n*-butyrate produced no detectable difference spectra even at concentrations as high as 10 mM, whereas a classical Type 1 spectrum was found with the methyl esters of hexanoic acid, octanoic acid, decanoic acid and lauric acid (Table 2).

The relationship between binding affinities and partition coefficients. For all the compounds studied, a good correlation was observed between the logarithm of the octanol/water partition coefficient (log P) and the logarithm of K_s.

The results from regression analysis on the aromatic compounds are shown in Table 3. Equation 1 describes the relationship between the straight chain alkylbenzenes and log P. The statistical significance of this relationship was not altered by the inclusion of the two branched-chain alkyl benzenes in the analysis (Equation 2). The log K_s–log P relationship, for the alkyl cyclohexanes (Equation 3) was not statistically different from Equation 2. Regression analysis using the data from all the mono-cyclic compounds gave Equation 4 which is also shown in Fig. 1.

Similar relationships were obtained for the naphthalene analogues (compounds 12–16) and the biphenyl analogues (compounds 17–23) as quantified by Equations 5 and 6.

Table 3 presents the regression analysis in the three aliphatic series of compounds. Equation 7 describes the relationship for the straight chain carbamates (compounds 27–32) and Equation 8 for both the straight chain and branched chain carbamates (compounds 27–35). As was found with the alkyl benzenes, inclusion of the branched chain molecules did not lessen the statistical significance of the relationship.

Equations 9 and 10 describe the relationship for the fatty acids (compounds 38–43) and their methyl esters (compounds 47–50) respectively.

The effect of induction on spectral interaction. Pretreatment of hamsters with phenobarbitone increased the amplitude of the spectral changes produced by Type 1 compounds. Biphenyl, cyclohexylbenzene, cyclohexane, ethylcyclohexane and butylcyclohexane elicit a Type 1 spectral interaction giving similar K_s values for control and phenobarbitone microsomes (Table 4). However, dicyclohexane behaved atypically, giving a K_s value of about 40 μM with phenobarbitone microsomes compared to 88 μM with normal microsomes. A statistically significant increase in A_{max} for control and phenobarbitone microsomes was observed with all but one of the compounds studied.

The effect of pretreatment of hamsters with 3-methylcholanthrene on the Type 1 binding site was studied using biphenyl, cyclohexylbenzene, dicyclohexane, cyclohexane, ethylcyclohexane and butylcyclohexane. All these compounds produced a Type 1 difference spectrum with 3-methylcholanthrene microsomes giving a typical peak at about 385 nm, but showing a 5 nm shift towards shorter wavelength in the trough

Table 2. Binding characteristics of various aliphatic compounds to cytochrome P450* of hamster hepatic microsomes

	Compound	Type of binding	$K_s \times 10^{-4}$ M	A_{max} (A $\times 10^{-2}$/2 mg protein)	Log P
24	Methyl carbamate	N.D.†	—	—	—
25	Ethyl carbamate	N.D.	—	—	—
26	Propyl carbamate	N.D.	—	—	—
27	*n*-Butyl carbamate.	I	19.00	3.45	0.85
28	*n*-Pentyl carbamate	I	9.30	3.33	1.35
29	*n*-Hexyl carbamate	I	5.00	3.45	1.85
30	*n*-Heptyl carbamate	I	1.70	4.76	2.36
31	*n*-Octyl carbamate	I	0.71	5.26	2.84
32	*n*-Decyl carbamate	I	0.35	6.45	3.85
33	*t*-Pentyl carbamate	I	9.50	3.12	0.94
34	*t*-Hexyl carbamate	I	7.40	3.23	1.45
35	Benzyl carbamate	I	19.00	3.85	1.23
36	Propionic acid	N.D.	—	—	—
37	Butyric acid	N.D.	—	—	—
38	Hexanoic acid	I	85.00 ± 10.0	1.9 ± 0.30	1.88
39	Heptanoic acid	I	21.00 ± 3.0‡	1.3 ± 0.20	2.41
40	Octanoic acid	I	3.45 ± 0.50‡	1.7 ± 0.15	2.94
41	Nonanoic acid	I	1.61 ± 0.15†	1.1 ± 0.10	3.47
42	Decanoic acid	I	1.28 ± 0.15*	1.6 ± 0.10	4.00
43	Undecanoic acid	I	0.78 ± 0.08†	2.0 ± 0.40	4.53
44	Lauric acid	I	0.79 ± 0.07	1.3 ± 0.15	—
45	Myristic acid	I	0.67 ± 0.05	1.3 ± 0.10	—
46	Methyl-*n*-butyrate	N.D.	—	—	—
47	Methyl-*n*-hexanoate	I	12.50 ± 1.00	8.80 ± 0.70	2.20
48	Methyl-*n*-octanoate	I	5.50 ± 0.50‡	8.60 ± 0.50	3.20
49	Methyl-*n*-decanoate	I	2.10 ± 0.30‡	7.20 ± 1.0	4.20
50	Methyl-*n*-laurate	I	1.00 ± 0.10†	—	5.20

* Values are mean ± S.D. of three experiments. †N.D. No detectable binding spectra. Students '*t*' test was applied comparing the results of each compound with that of the preceding one ‡$P < 0.001$. †$P < 0.01$. *$P < 0.05$.

compared to normal or phenobarbitone microsomes. Similar K_s values were obtained in normal and in 3-methylcholanthrene microsomes and an increase in A_{max} was only apparent with one compound.

The effect of 3-methylcholanthrene pretreatment on Type 1 binding was further tested with compounds containing carboxylic acid methyl ester functional groups. Methyl hexanoate, methyl octanoate and octanoic acid all produced a Type 1 spectral interaction with both normal and 3-methylcholanthrene microsomes, showing an absorption peak at about 385–390 nm with both preparation, but like the hydrocarbons, a shift in the trough towards shorter wavelength when the interaction with 3-methylcholanthrene microsomes was compared to controls.

All the compounds studied in the phenobarbitone and 3-methylcholanthrene microsomes showed a similar trend to that of untreated microsomes; namely a decrease in their K_s values with increasing partition coefficient.

DISCUSSION

The structural requirements for Type 1 binding were studied using a wide variety of hydrocarbons and oxygen-containing compounds. Lineweaver–Burk type plots for the difference spectral interaction of most of the compounds studied showed that a single line could be adequately fitted to the experimental data. Deviations from linear plots at very low concentrations (<0.05 mM) were observed for several compounds, possibly due to the difficulties accompanying the determinations of very small changes in optical density. However, Lineweaver–Burk type plots of the Type 1 interactions for biphenyl and methyl-*n*-hexanoate displayed a distinct bimodal shape from which two K_s values could be derived, implying the presence of more than one P450 Type 1 binding site. These observations are in agreement with those of Burke who found a biphasic interaction between biphenyl and hamster hepatic microsomes [14]. Bimodal kin-

Table 3. Results of regression analysis between log K_s and log P for the aliphatic and cyclic compounds studied

Equation number	Slope of regression line (S.D.)	Intercept of regression line (S.D.)	Correlation coefficient (statistical significance)	Compounds used in regression analysis
1	−0.64 (0.12)	2.90 (0.42)	−0.976 ($P < 0.005$)	1–5
2	−0.65 (0.11)	2.86 (0.39)	−0.968 ($P < 0.001$)	1–7
3	−0.56 (0.04)	2.25 (0.14)	−0.997 ($P < 0.005$)	8–11
4	−0.63 (0.07)	2.78 (0.25)	−0.973 ($P < 0.001$)	1–11
5	−0.37 (0.05)	1.75 (0.19)	−0.970 ($P < 0.01$)	12–16
6	−0.54 (0.06)	2.84 (0.28)	−0.970 ($P < 0.001$)	17–23
7	−0.62 (0.05)	1.77 (0.13)	−0.986 ($P < 0.001$)	27–32
8	−0.61 (0.06)	1.77 (0.12)	−0.971 ($P < 0.001$)	27–35
9	−0.76 (0.12)	3.11 (0.40)	−0.952 ($P < 0.005$)	38–43
10	−0.37 (0.01)	1.92 (0.04)	−0.999 ($P < 0.005$)	47–50

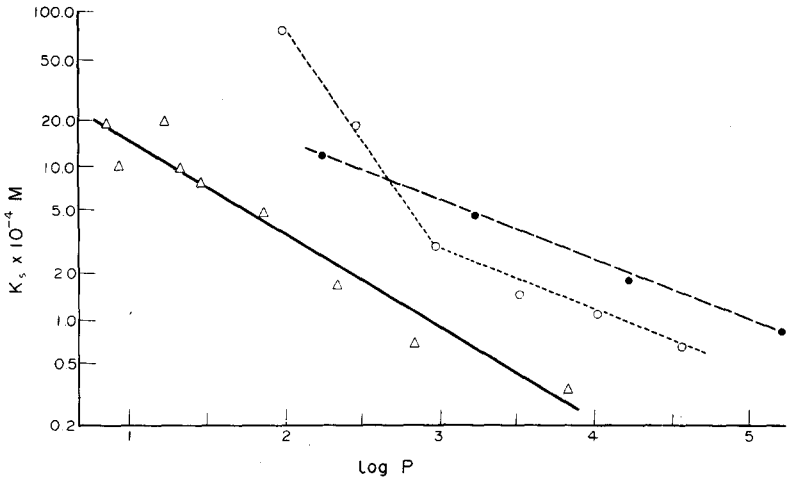

Fig. 1. The relationship between log K_s and log P for the aliphatic compounds. The solid line drawn is Equation 8 and the dashed line Equation 10. The dotted line is drawn by eye. Fatty acids (○) and their esters (●), carbamate (△).

etics were presented as evidence for the presence of high- and low-affinity P450 interactions with cyanide, a similar observation has been made for the interaction of some imidazoles with rat hepatic microsomes [15–17].

The binding affinity increases as the number of side-chain carbon atoms attached to benzene is extended. The decrease in K_s value with increasing number of carbon atoms is found also with aliphatic carboxylic acids (C_6—C_{12}), methyl esters (C_6—C_{12}), alkylcyclohexanes and aliphatic carbamates (C_4—C_{10}). It is well documented that an increase in octanol/water partition coefficient parallels an increase in chain length for a homologous series [18]. For each of the four series of homologues studied in the present investigation, statistically significant correlations between binding affinity and lipophilicity were obtained. Similar correlations were also obtained for the series of naphthalene analogues (compounds 12–16) and biphenyl analogues (compounds 17–23).

The slopes of the regression lines for the K_s-lipophilicity relationships vary from 0.37 to 0.76. It is of interest that similar slopes have been reported for relationship between the binding of various compounds to plasma proteins and partition coefficient [19–23].

Canady *et al.* have postulated that a hydrophobic interaction is the most important single factor in the binding of substrates to hepatic cytochrome P450, based on the correlation between the free energy of complex formation and the increment of carbon atoms added on the series of benzene, ethylbenzene, toluene, xylene, indene and naphthalene [24]. Our results support this hypothesis by demonstrating a clear relationship between partition coefficient and binding affinities for a much greater range of compounds. Also, Ichikawa and Yamano [25] have reported that the transition concentrations used for the conversion of cytochrome P450 to P420 by anilines and phenols closely paralleled the lipophilicity of the compound.

Although the branched-chain compounds show higher K_s values than their straight-chain analogues, their K_s binding could be predicted if their partition coefficients are taken into consideration. This was true both for the alkylbenzene series and the aliphatic carbamate. series. Also the binding affinities of the cyclohexane homologues could be predicted from their log P values using the alkylbenzene regression

Table 4. Comparison of K_s and A_{max} values of compounds reacting with untreated, phenobarbitone and 3-methylcholanthrene hamster microsomes*

Compound	Normal microsomes		Phenobarbitone microsomes		3-Methylcholanthrene microsomes	
	$K_s \times 10^{-4}$ M	A_{max} A $\times 10^{-2}$/2 mg protein	$K_s \times 10^{-4}$ M	A_{max} A $\times 10^{-2}$/2 mg protein	$K_s \times 10^{-4}$ M	A_{max} A $\times 10^{-2}$/2 mg protein
Biphenyl	5.00 ± 0.55	7.20 ± 1.10	3.50 ± 0.50	14.80 ± 0.30†	3.30 ± 0.45	7.50 ± 0.80
Cyclohexylbenzene	2.20 ± 0.20	2.40 ± 0.20	1.50 ± 0.30	9.30 ± 0.40†	2.00 ± 0.20	5.55 ± 0.50†
Dicyclohexyl	0.88 ± 0.14	0.87 ± 0.09	0.40 ± 0.05†	5.20 ± 0.65†	0.40 ± 0.06	2.00 ± 0.40
Cyclohexane	9.00 ± 1.50	4.70 ± 1.60	10.30 ± 0.65	15.80 ± 0.30†	16.00 ± 3.00	3.57 ± 0.53
Ethylcyclohexane	2.80 ± 0.35	3.60 ± 0.50	2.30 ± 0.40	5.50 ± 0.60	3.50 ± 0.40	3.70 ± 0.50
Butylcyclohexane	0.70 ± 0.15	1.80 ± 0.30	0.82 ± 0.20	4.16 ± 0.35†	1.60 ± 0.35	1.85 ± 0.30
Octanoic acid	3.45 ± 0.50	1.70 ± 0.15	—	—	5.20 ± 0.65	1.30 ± 0.30
Methyl-*n*-octanoate	5.50 ± 0.50	0.86 ± 0.05	—	—	4.40 ± 0.55	1.11 ± 0.25

* Values are mean S.D. of three determinations.

† Statistically significantly different ($P < 0.05$) from normal microsomes.

analysis. However, the binding affinities of the naphthalene and biphenyl analogues could not have been predicted accurately from this data on the alkylbenzenes and cyclohexanes (Equation 4). The fact that plots of log K_s against log P are not entirely superimposable implies that steric factors as well as hydrophobicity may influence P450 Type 1 binding. However this is not reflected in the molecular volume of biphenyl and naphthalene analogues which are no greater than the molecular volume range covered by the compounds used in obtaining Equation 4 [26].

The regression analysis on the data obtained from the aliphatic series of homologues in the main showed similar behaviour to that of the aromatic compounds. However, the chain length relationship for the fatty acids (Fig. 1) showed a distinct biphasic character with a breakpoint at C_6. The slope of the second phase being very similar to that obtained for the methyl fatty acid esters. It is noteworthy that Brown *et al.* studying the binding of fatty acids valerate, hexanoate, heptanoate and octanoate to bovine serum albumin using the ultra-filtration technique [27], also found a good correlation for C_5 to C_7 with log P, but that octanoate shows an unexpectedly high affinity. Although the mechanism of the break in the log K_s vs log P presented by the interaction of hexanoic acid with P450 is unknown, it may indicate that the P450 binding site for fatty acids containing six or less carbon atoms is different from that for heptanoic acid and higher analogues. This investigation could not be extended to acids containing more than eleven carbon atoms, because of the uncertainty of partition coefficient found. Leo *et al.* [13] have reported a value of 4.2 for log P (octanol/aqueous solution) of lauric acid (expected value 5) suggesting that when aliphatic chains become long enough they may tend to coil up in solution with the formation of molecular oil droplets, a phenomenon which has not been observed in the *n*-heptane/aqueous solution partition coefficient [8]. However, myristic acid which contains two extra methylene groups compared with decanoic acid showed statistically similar K_s values to that of decanoic acid. Furthermore, high chain length aliphatic carbamates (C_{12}—C_{14}) showed abnormal spectral changes, a phenomenon which was also found with the polycyclic hydrocarbons e.g. benzo(a)pyrene. Thus, the correlation between log K_s and log P may be considered to hold only for a certain range of partition coefficient for a particular series of compounds.

It is clear from the results presented that changes in A_{max} values were either insignificant (Tables 1, 2) or not systematic and there was no correlation between A_{max} values with either partition coefficient or K_s values. The correlation between log K_s and log P was found also to hold with phenobarbitone microsomes (Table 4), in which A_{max} values are generally much larger than in microsomes derived from control or 3-methylcholanthrene pretreated animals. This latter observation would tend to imply that the environment of the binding sites in P450 and P448 for this series of compounds is not fundamentally different.

The above data indicate that the ability of a compound to form a Type 1 spectrum with microsomal P450 is probably, in the majority of instances, determined primarily by its lipophilicity. It seems likely that the interaction point is a hydrophobic portion of the apoprotein of cytochrome P450 but the number of potential binding sites capable of producing a Type 1 spectrum remains uncertain.

REFERENCES

1. H. Remmer, J. B. Schenkman, R. W. Estabrook, H. Sasame, J. R. Gillette, S. Narasimhulu, D. Y. Cooper and O. Rosenthal, *Molec Pharmac.* **2,** 187 (1966).
2. Y. Imai and R. Sato, *Biochem. biophys. Res. Commun.* **22,** 620 (1966).
3. J. B. Schenkman, *Biochemistry* **9,** 2081 (1970).
4. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3,** 113 (1967).
5. M. W. Anders, M. J. Cooper and A. E. Takemori, *Drug Metab. Disp.* **1,** 642 (1973).
6. K. A. S. Al-Gailany, J. W. Bridges and K. J. Netter, *Biochem. Pharmac.* **24,** 867 (1975).
7. Y. Imai and R. Sato, *J. Biochem.* **62,** 239 (1967).
8. K. A. S. Al-Gailany, A. G. E. Wilson and J. W. Bridges, *Biochem. Soc. Trans.* **2,** 113 (1974).
9. G. M. Cohen and G. J. Mannering, *Molec. Pharmac.* **9,** 383 (1973).
10. I. Jansson, S. Orrenius, L. Ernster and J. B. Schenkman, *Archs Biochem. Biophys.* **151,** 391 (1972).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
12. V. Ullrich, *Hoppe-Seyler's Z. Physiol. Chem.* **350,** 357 (1969).
13. A. Leo, C. Hansch and D. Elkins, *Chem. Rev.* **71,** 525 (1971).
14. M. D. Burke and J. W. Bridges, *Xenobiotica* **5,** 357 (1975).
15. J. F. Gaylor, N. J. Moir, H. E. Seifried and C. R. E. Jeffcoate, *J. biol. Chem.* **245,** 5511 (1970).
16. T. Shimakata, M. Katsuyoshi and R. Sato, *Biochem. biophys. Res. Commun.* **44,** 533 (1971).
17. M. Dickins and J. W. Bridges (unpublished results).
18. C. Hansch, in *Drug Design* Vol. 1, (Ed. E. J. Ariens), *Med. Chem. Monographs* Vol. 11. Academic Press, London (1971).
19. A. E. Bird and A. C. Marshall, *Biochem. Pharmac.* **16,** 2275 (1967).
20. C. Hansch, K. Kiehs and G. L. Lawrence, *J. Am. Chem. Soc.* **87,** 5770 (1965).
21. C. Hansch and K. N. Von Kaulla, *Biochem. Pharmac.* **19,** 2193 (1970).
22. F. Helmer, K. Kiehs and C. Hansch, *Biochemistry* **7,** 2858 (1968).
23. J. M. Vanderbilt, C. Hansch and C. Church, *J. Med. Chem.* **15,** 787 (1972).
24. W. J. Canady, D. A. Robinson and H. D. Colby, *Biochem. Pharmac.* **23,** 3075 (1974).
25. Y. Ichikawa and T. Yamano, *Biochim. Biophys. Acta* **147,** 518 (1967).
26. I. Moriguchi, Y. Kanada and K. Komatsu, *Chem. Pharm. Bull.* **24,** 1799 (1976).
27. N. Brown and J. W. Bridges (unpublished results) (1975).
28. R. Smith and C. Tanford, *Proc. natn. Acad. Sci. U.S.A.* **70,** 289 (1973).