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K_s VALUES OF SOME HOMOLOGOUS SERIES OF BARBITURATES AND THE RELATIONSHIP WITH THE LIPOPHILICITY AND METABOLIC CLEARANCE

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Abstract—The K_s values of several homologous series of barbiturates were determined. The relationship with the lipophilicity and the rate at which the barbiturates were eliminated by the isolated perfused rat liver, was investigated. The K_s values decreased with larger substituents, indicating a higher affinity for cytochrome P-450. The bromoallyl substituted and *N*-methylated barbiturates had lower K_s values than their allyl and non-methylated homologues. This corresponded generally very well with the rate of the metabolism, which was expressed as heptabarbital clearance ratio. The relationship between the K_s values and the lipophilicity in the various series could be described by a parabolic function. However, it appeared that the length of the side chain rather than the lipophilicity is responsible for the decline in affinity for cytochrome P-450 as was found for the derivatives with long substituents. It is concluded that although an affinity for cytochrome P-450 is a primary requirement for metabolism, other factors as the reactivity of the substituent are involved in the quantification of the metabolism.

For many drugs there seems to exist a relationship between the ability to produce a spectral change with liver microsomes and to undergo oxidative metabolic conversion [1]. This suggests that drug binding is an obligatory step in the mechanism of drug oxidation. The dissociation constant obtained by spectral difference measurement, is regarded as a measure of the affinity of the substrate for cytochrome P-450.

Although various studies have been undertaken to measure the K_s values of barbiturates [2–4], systematic studies involving large homologous series of barbiturates, are still lacking. We have, therefore, determined the K_s values of a large number of barbiturates and have correlated these values with the lipophilicity and the hepatic clearance of the compounds.

MATERIALS AND METHODS

Drugs. Barbiturates not commercially available were prepared by condensing the appropriate malonic esters with urea according to Fischer *et al.* [5]. *N*-Methylated barbiturates were prepared by methylation according to Martin *et al.* [6]. All barbiturates were recrystallized from ethanol–water. The purity was better than 99 per cent as determined by G.C. All other reagents were of analytical grade.

Preparation of microsomes. Livers of male Wistar rats were excised immediately after decapitation, weighed, transferred to 9 vol. ice-cold 0.25 M sucrose and homogenized in a Potter–Elvehjem homogenizer. Nuclei and cell debris were sedimentated by centrifuging at 600 *g* for 5 min. The supernatant was centrifuged at 18,000 *g* for 15 min and the microsomal fraction was obtained by centrifuging the 18,000 *g* supernatant for 1 hr at 105,000 *g*. The microsomal pellet was resuspended in 0.15 M KCl and centrifuged once more at 105,000 *g* for 30 min to remove haemoglobin and finally resuspended in 4 vol. 0.15 M KCl–0.05 M Tris–HCl (pH 7.4) to a concentration of about 1 mg microsomal protein per ml.

Recording of difference spectra. The barbiturates were dissolved in KCl–Tris–HCl buffer to concentrations ranging from 0.02 to 2 mM. Two cuvettes were filled with 1 ml microsomal suspension and 2 ml barbiturate solution was added to the suspension, while to the reference cuvet an equal volume of buffer was added. With varying substrate concentrations, spectra were recorded from 500–350 nm on a Carry 118 C double beam spectrophotometer with a scattered transmission accessory. The extent of spectral change was determined as the difference in absorption at 420 and 390 nm. K_s values were obtained from double reciprocal plots with the help of a linear regression computer program.

Partition coefficients. Octanol–water partition coefficients were measured by shaking 5 ml octanol [1] solution containing 20 mg barbiturate per 100 ml, vigorously for 0.5 hr with an equal volume of phosphate buffer (Sorensen 1/15 M). After equilibration and centrifuging the two layers were separated. The partition coefficient was calculated from the decrease of concentration in the octanol layer. Because of the large differences in partition of the members of the various series, measurements had to performed at different pH's. From these apparent partition coefficients (APC) the true partition coefficients (TPC) were calculated with the formula: $TPC = APC\ (1 + 10^{pH - pK_a})$. pK_a values were taken from Doornbos *et al.* [7], Krahl [8], Sitsen [9] and Kakemi *et al.* [10].

Hepatic clearance of the compounds. In order to determine the hepatic metabolism of the barbiturates, independent of distribution processes and other routes of elimination as is the case in intact animals, the isolated perfused rat liver was selected.

The perfusions were mainly performed according

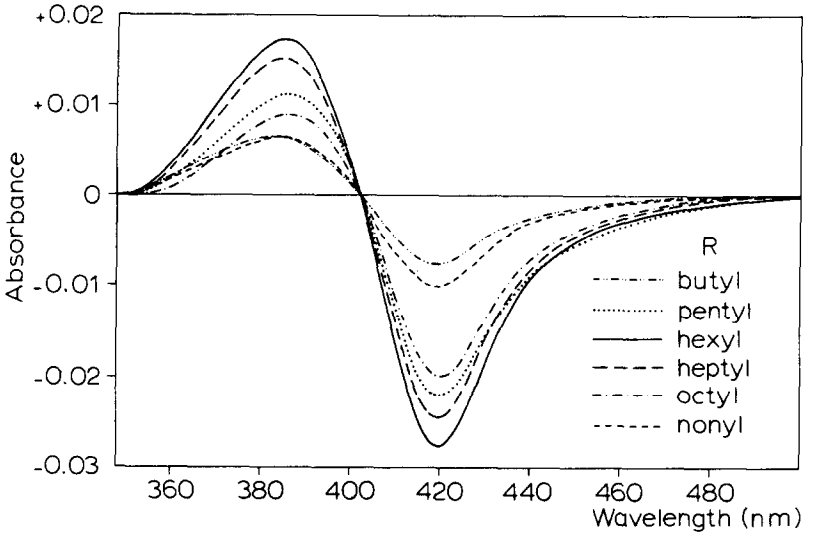


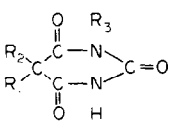
Fig. 1. Type I spectral change caused by 5-ethyl-5-alkyl substituted barbiturates. Final concentration 0.22 mM.

to the methods of Miller *et al.* [11]. In order to correct for variations in metabolic activity of the livers used, the metabolic clearance of heptabarbital was choosen as internal standard of each perfusion, since this barbiturate has been reported to be eliminated mainly by metabolic conversion [12]. The metabolic clearance of the barbiturates has been expressed as the heptabarbital clearance ratio (i.e. clearance compound/clearance heptabarbital in the same perfusion). Detailed information on the perfusion system and analysis of the barbiturates is given by Yih [13].

RESULTS

In the concentration ranges used, all barbiturates showed a type I spectral change with an absorption

Table 1. K_s values, partition coefficients and metabolic clearances of a number of barbiturates



Compound	R_1	R_2	R_3	Log TPC oct-water	K_s (mM)	Heptabarbital clearance ratio
Propyl	ethyl	propyl	H	0.87	0.235 ± 0.044	0.004 ± 0.001 (2)*
Butyl	ethyl	butyl	H	1.70	0.089 ± 0.009	0.068 ± 0.005 (5)
Pentyl	ethyl	pentyl	H	2.23	0.032 ± 0.004	0.289 ± 0.056 (5)
i-Pentyl	ethyl	3-methylbutyl	H	2.11	0.038 ± 0.017	0.161 ± 0.023 (4)
s-Pentyl	ethyl	1-methylbutyl	H	2.13	0.045 ± 0.012	0.310 ± 0.062 (4)
Dm-Butyl	ethyl	2,3-dimethylbutyl	H	2.39	0.025 ± 0.002	0.425 ± 0.017 (4)
Hexyl	ethyl	hexyl	H	3.08	0.019 ± 0.003	0.809 ± 0.065 (5)
Heptyl	ethyl	heptyl	H	3.64	0.020 ± 0.004	1.025 ± 0.122 (6)
Octyl	ethyl	octyl	H	3.85	0.024 ± 0.002	1.110 ± 0.272 (4)
Nonyl	ethyl	nonyl	H	4.13	0.056 ± 0.011	1.611 ± 0.225 (4)
Aprobarbital	allyl	isopropyl	H	1.61	0.219 ± 0.047	0.015 ± 0.005 (3)*
Talbutal	allyl	1-methylpropyl	H	1.76	0.098 ± 0.008	0.066 ± 0.006 (3)
Nealbarbital	allyl	neopentyl	H	2.53	0.061 ± 0.007	0.049 ± 0.007 (3)
Secobarbital	allyl	1-methylbutyl	H	2.59	0.028 ± 0.002	0.303 ± 0.064 (4)
Brallobarbital	bromoallyl	allyl	H	2.05	0.136 ± 0.030	0.037 ± 0.005 (5)†
Noctal®	bromoallyl	isopropyl	H	2.21	0.105 ± 0.015	0.047 ± 0.004 (3)†
Pernocton®	bromoallyl	1-methylpropyl	H	2.58	0.031 ± 0.005	0.314 ± 0.030 (3)
Rectidon®	bromoallyl	1-methylbutyl	H	3.13	0.013 ± 0.001	0.965 ± 0.175 (4)
m-Brallobarbital	bromoallyl	allyl	CH_3	2.34	0.103 ± 0.013	0.270 ± 0.060 (3)†
Eunarcon®	bromoallyl	isopropyl	CH_3	2.78	0.034 ± 0.001	0.524 ± 0.042 (3)
m-Pernocton	bromoallyl	1-methylpropyl	CH_3	3.20	0.012 ± 0.001	0.594 ± 0.036 (3)
Norhexobarbital	methyl	1-cyclohexen-1-yl	H	1.16	0.099 ± 0.003	0.092 ± 0.008 (3)*
Cyclopal®	allyl	2-cylopenten-1-yl	H	1.64	0.080 ± 0.010	0.134 ± 0.010 (3)
Cyclobarbital	ethyl	1-cyclohexen-1-yl	H	2.02	0.041 ± 0.003	0.299 ± 0.028 (3)
Heptabarbital	ethyl	1-cyclohepten-1-yl	H	2.45	0.022 ± 0.002	1
Reposal®	ethyl	bicyclo-3,2,1-oct-2-en-2-yl	H	2.78	0.026 ± 0.004	0.757 ± 0.063 (3)
Hexobarbital	methyl	1-cyclohexen-1-yl	CH_3	2.04	0.036 ± 0.003	0.815 ± 0.092 (3)
m-Cyclopal	allyl	2-cyclopenten-1-yl	CH_3	1.95	0.025 ± 0.001	1.252 ± 0.054 (4)
m-Cyclobarbital	ethyl	1-cyclohexen-1-yl	CH_3	2.32	0.024 ± 0.002	1.554 ± 0.208 (4)
m-Phenobarbital	ethyl	phenyl	CH_3	2.03	0.023 ± 0.003	0.076 ± 0.009 (3)
m-Heptabarbital	ethyl	1-cycloheptten-1-yl	CH_3	2.95	0.015 ± 0.002	2.570 ± 0.301 (3)
m-Reposal	ethyl	bicyclo-3,2,1-oct-2-en-2-yl	CH_3	3.26	0.017 ± 0.001	2.428 ± 0.414 (3)

* Mean ± S.D.; number of experiments in parentheses.
† Slowly metabolized in perfusion [16].

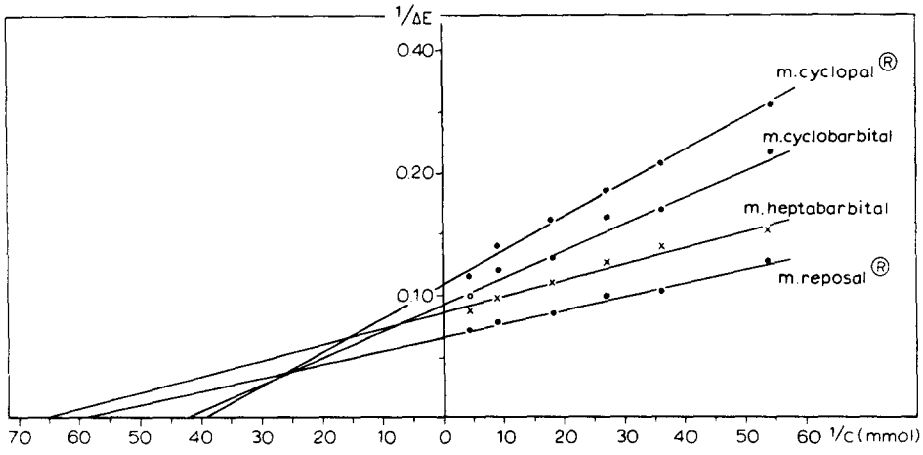


Fig. 2. Reciprocal plots of changes in absorbance at 420 nm relative to 390 nm caused by consecutive additions of *N*-methylated ring substituted barbiturates to microsomal suspensions.

minimum at 420 nm and a peak at 390 nm. Figure 1 shows the spectral change of a number of 5-ethyl-5-alkyl substituted barbiturates added to a final non-saturating concentration of 0.22 mM. There is an increase in spectral change from the propyl to the heptyl derivative, while a further lengthening of the alkyl side chain results in a smaller spectral change.

In Table 1 the dissociation constants of the 5-ethyl-5-alkyl series, based on the induced spectral change, are listed. Our values for the butyl and *s*-pentyl derivative are consistent with those reported by Jansson *et al.* [14]. The value of the *i*-pentyl derivative is in agreement with that of Topham [4] and Sitar [15]. Table 1 presents the values of a series of allyl and 2-bromoallyl substituted barbiturates together with some *N*-methylated homologues. In both groups there is an increase in affinity for cytochrome P-450 with increasing alkyl side chain. The K_s values of the bromoallyl substituted barbiturates are lower than those of their allyl substituted homologues.

Figure 2 shows the reciprocal plots of a number of *N*-methylated barbiturates with a cyclic substituent at the 5-position. There is apparently an optimum in affinity for cytochrome P-450 for the cycloheptenyl substituted derivative. In Table 1 the K_s values are listed for the cyclic substituted barbiturates. As in the previous series the *N*-methylated barbiturates have lower K_s values than their non-methylated homologues.

Attempts have been made to correlate the K_s values of the various barbiturates with their octanol–water partition coefficients. In Fig. 3 a curve is shown, which presents the relationship between log P and the affinity for cytochrome P-450 of a number of 5-ethyl-5-alkyl substituted barbiturates according to a parabolic function. The correlation coefficient and

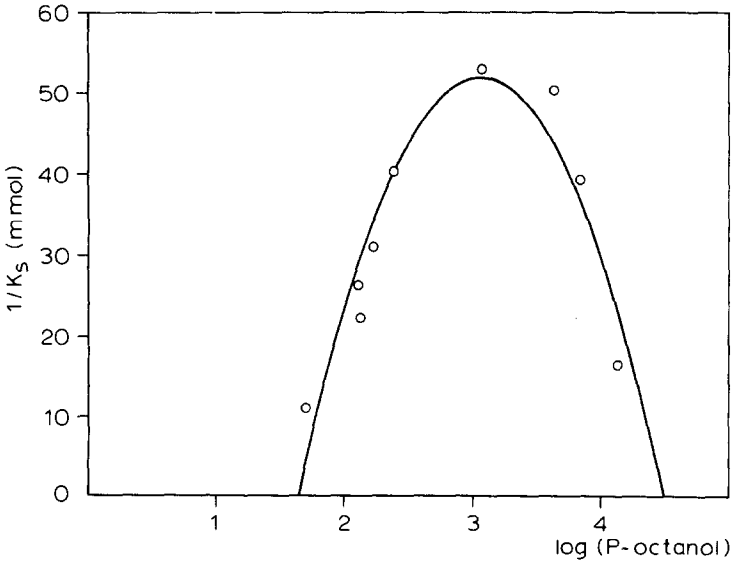


Fig. 3. Relationship between log P(octanol–water) and the affinity to cytochrome P-450 of a number of 5-ethyl-5-alkyl substituted barbiturates.

Table 2. K_s values and partition coefficients of some *N*-methylated 5-ethyl-5-alkyl substituted barbiturates

Compound	K_s (mM)	Log TPC oct-water
hexyl*	0.008 ± 0.001	3.26
heptyl	0.009 ± 0.002	4.16
octyl	0.011 ± 0.002	4.25
nonyl	0.020 ± 0.003	4.34

* Refers to alkyl substituent.

the residual standard deviation were 0.935 and 0.105 respectively. This is consistent with the reports of Hansch *et al.* [16] on the parabolic dependence of pharmacological activity upon the lipophilic character of the compounds.

In the 5-ethyl-5-alkyl series, the lipophilicity of the compounds was increased by the introduction of larger substituents. It was interesting to investigate whether the high lipophilicity was responsible for the higher K_s values of the octyl and the nonyl derivative. The lipophilicity of the last four members of the series was increased, therefore, in another way, namely by *N*-methylation. If the high lipophilicity is responsible for the decline in affinity for cytochrome P-450, these *N*-methylated derivatives should have higher K_s values than their non-methylated homologues and should be situated on the descending part of the parabola, presented in Fig. 3. The results, presented in Table 2, indicate that the length of the substituent, rather than the lipophilicity is responsible for the descending part of the curve.

DISCUSSION

The introduction of larger substituents either aliphatic or cyclic at the 5-position of the barbituric acid nucleus results in a decrease in K_s value. Further, the 2-bromoallyl substituted and *N*-methylated barbiturates have lower K_s values than the corresponding allyl and non-methylated derivatives. In the various homologous series this corresponds very well with the metabolic clearances of compounds with no more than 6 or 7 carbon atoms in the side chain. Generally, compounds with a low K_s value are metabolized faster than those with higher K_s values. Studying derivatives with no more than 6 or 7 carbon atoms in the side chain may lead to the conclusion that the K_s values are only dependent on the lipophilicity of the compounds [4]. Our results, however, indicate that with larger substituents the influence of a steric effect becomes visible resulting in a lower affinity for cytochrome P-450, despite the higher lipophilicity of the compounds. This decrease in affinity for cytochrome P-450 may be responsible for the parabolic relationship found, when plotting the metabolic clearances against the partition coefficients of the compounds [13].

Jansson *et al.* [14] did not find a good correlation between the lipophilicity and the rate of metabolism of barbiturates. One of the reasons may be that the barbiturates studied, were treated as belonging to one homologous series. They may indeed structurally. However, when looking at their routes of metabolism, they belong to different series [13].

Besides an affinity for the metabolic system, the rate of metabolism is also dependent on factors as the reactivity of the C—H bonds to be hydroxylated. For instance, nealbarbital and methylphenobarbital are slowly metabolized, despite the rather high affinity for cytochrome P-450. When going from the *n*-hexyl to the *n*-nonyl compound the number of reactive C—H bonds increases, explaining their greater clearances, despite a decrease in affinity for cytochrome P-450.

In conclusion we can say that a certain affinity for cytochrome P-450 is a primary requirement for metabolism. However, other factors as the reactivity of the C—H bonds to be hydroxylated and the number of those bonds, obviously play a role in the quantification of the rate of metabolism of the barbiturates.

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