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Physico-chemical properties of barbituric acid derivatives. III: Partition coefficients of cycloalkane-1′,5-spirobarbituric acids at 25°C

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Summary

The partition coefficients between 0.001 M HCl and octan-1-ol at 25°C for eight cycloalkane-1′,5-barbituric acids and one cycloalkane-1′,5-spiro-2-thiobarbituric acid derivative are reported. Both phases were analyzed for the barbituric acid concentration. The mean deviation for duplicate measurements of the partition coefficients was ±0.76% (largest deviation ±0.006 log units). The alicyclic methylene group contribution to partitioning was found to be dependent on the alicycle size for rings containing up to five methylene groups, but independent for larger rings. The log *P* values support a solvent exclusion hypothesis which accounts for previously reported steric acid-strengthening in barbituric, mono- and dicarboxylic acids. The lack of biological (CNS) activity for some derivatives is discussed.

Introduction

This study continues a program aimed at (i) providing carefully measured physico-chemical data for the barbiturates and (ii) the use of that data in predictive relationships relating structure to physico-chemical properties and to biological activity. Studies of the physico-chemical properties of 5,5-disubstituted barbituric acid derivatives (**1**) in water have led to a steric acid-strengthening hypothesis for the first ionization constant for these compounds (McKeown, 1976; Prankerd, 1977; McKeown, 1980; McKeown and Prankerd, 1981) for which 5,5-dimethylbarbituric acid ($pK_1 = 8.51$) and 5,5-diethylbarbituric acid ($pK_1 = 7.98$) provide the most convincing examples. Acid strength was thought to be controlled by the interactions of the C5 substituents with the hydration shell around the barbituric acid in both its un-ionized (initial) and ionized (final) states (McKeown, 1980; McKeown et al., 1986). The hypothesis suggested that un-ionized 5,5-dimethylbarbituric acid was more highly solvated in aqueous solution than the 5,5-diethyl derivative.

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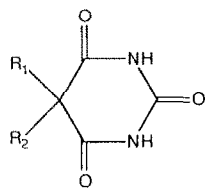

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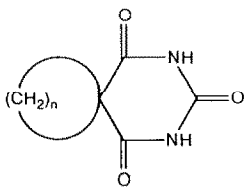

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Further support for the steric-solvent exclusion hypothesis came from a study of the partition coefficients of the 5,5-disubstituted barbituric acid (**1**) and cycloalkane-1′,5-spirobarbituric acid (**2**) derivatives between octan-1-ol and 0.1 M HCl (Prankerd, 1977). The partition coefficient for cyclopropane-1′,5-spirobarbituric acid (**2**; $n = 2$, X = O) (log $P = -0.45$) was about the same as that of its acyclic analogue, 5,5-dimethylbarbituric acid (**1**; $R_1 = R_2 = Me$) (log $P = -0.49$). However, the partition coefficient for cyclopentane-1′,5-spirobarbituric acid (**2**; $n = 4$, X = O) (log $P = 0.14$) was significantly less than that of its acyclic analogue, 5,5-diethylbarbituric acid (**1**; $R_1 = R_2 = Et$) (log $P = 0.66$). There were no data available for 5-ethyl-5-methylbarbituric acid to compare with its alicyclic analogue, cyclobutane-1′,5-spirobarbituric acid. These data implied that the triketopyrimidine ring was more highly hydrated when it had a cycloalkane-1′,5-spiro substituent than with 5,5-dialkyl substituents.

In the preliminary study (Prankerd, 1977), the differences between measured partition coefficients for three members of the series of cycloalkane-1′,5-spirobarbituric acid derivatives (**2**; $n = 2$–4, X = O) were not constant. This indicated that the previously accepted single value for the alicyclic methylene group contribution to partitioning (AMGC = 0.41) (Tute, 1971) might not always be correct. In the present work, the series of cycloalkane-1′,5-spirobarbituric acid derivatives has been extended to include compounds with $n = 2$–7, 10 and 11 methylene groups in the spiro ring. The partition coefficients for the compounds previously reported were redetermined, as that study (Prankerd, 1977) used a non-specific method for analysis of barbituric acid concentrations. The log P values for the series of cycloalkane-1′,5-spirobarbituric acid derivatives in this study are used to re-examine the AMGC for partitioning of alicycles between aqueous and octan-1-ol phases.

Experimental

Materials

Instrumental techniques and materials were as described in the preceding article (Part II; Prankerd and McKeown, 1992).

Determination of partition coefficients

Partition coefficients were determined for nos 24–29 and 33 between octan-1-ol and 0.001 M HCl as described for the previous paper (Prankerd and McKeown, 1992). For cycloundecane-1′,5-spirobarbituric acid (no. 30), about 16.5 mg was weighed accurately into duplicate 120 ml water-jacketed glass cells, then the aqueous and octan-1-ol phases were pipetted into each cell and stirred until equilibrium was established. A standard solution of cycloundecane-1′,5-spirobarbituric acid (1.00×10^{-4} M) was also prepared in methanol. After equilibration, in all cases, the octan-1-ol phase was removed with a Pasteur pipette and the phases were analysed for the barbituric acid derivative by one of the following methods:

(i) An aliquot of the aqueous phase was added to a volume of 5,5-diethylbarbituric acid (internal standard), diluted with 0.001 M HCl, and then the concentration of the barbituric acid was determined by HPLC. The octanol phase was analysed similarly, except that it was diluted with methanol. For cycloundecane-1′,5-spirobarbituric acid, the internal standard was cyclododecane-1′,5-spirobarbituric acid (no. 34). Standards prepared for each phase were also chromatographed and the method of peak height ratios used to determine the concentrations of the barbituric acid in each phase.

(ii) The aqueous and octan-1-ol phases were diluted with 0.001 M HCl and either methanol or ethanol, respectively, and then the absorbance at 286.5 nm compared with standards.

Method (i) was used for the cycloalkane-1′,5-spirobarbituric acids, while method (ii) was used for cyclohexane-1′,5-spiro-2-thiobarbituric acid. UV absorbance measurements and subsequent calculations were performed as described in the preceding work (Prankerd and McKeown, 1992). Results are reported in Table 4. No. 34 was too insoluble in water for reliable data to be obtained by actual partitioning experiments. The reported log P value for no. 34 was obtained by extrapolation of a plot of the log (HPLC capacity factor, k') for a large number of barbituric acid derivatives vs their measured log P values. Details will be reported in a subsequent paper in this series.

Results and Discussion

The errors listed in Table 1 are the deviations of the mean values from duplicated partition coefficient measurements. For the spectrophotometric analyses, correlation coefficients for Beer's law plots were $\geqslant 0.9990$ and all plots passed through the origin.

Validation of the experimental methods has been described in Part II (Prankerd and McKeown, 1992). For most of the derivatives, recoveries (Table 1) were outside the range regarded as acceptable (97.5–102.5%). The mean recovery for duplicated measurements on all compounds was $101.9 \pm 9\%$. Preliminary UV and HPLC work with the spiro derivatives suggested that they were unstable, even under the weakly acidic conditions used for partitioning. Consequently, high apparent recoveries might be expected as the standard aqueous solution of the barbituric acid (used to prepare the partitioning systems and for subsequent calibration plots) hydrolysed at the same time as did the derivative in the aqueous phase of the partitioning system. However, in the octan-1-ol phase, the derivative was protected from hydrolysis (Davis et al., 1976; El-Sayed and Repta, 1983). Conversely, where a standard solution was prepared in methanol (stored in a refrigerator) and the partitioning system set up with a weighed quantity of the solid acid, recoveries might be expected to be low, as was observed (Table 1, no. 30). Decomposition was confirmed in several cases by the appearance of extra peaks in chromatograms after partitioning. All compounds gave single peaks on chromatography of freshly prepared solutions in methanol or 0.001 M HCl. Although the recoveries were outside the desired range for these compounds, this is not expected to have serious effects on the partition coefficients (P), as these are the *ratios* of the equilibrium phase concentrations at any time (Davis et al., 1976), and are thus independent of recovery. This will be true so long as the rate of transfer of the derivative from the octan-1-ol phase is faster than the rate of loss of the derivative from the aqueous phase. No studies were performed to

TABLE 1

Partition coefficients between octan-1-ol and 0.001 M HCl at 25°C for cycloalkane-1′,5-spirobarbituric acids (2)

No.	n	X	Partition coefficient (P)	Log P	Recovery (%)	Analytical method
24 [a]	2	O	0.280 ± 0.002	−0.533	102.5 ± 1.4	(i)
25 [a]	3	O	0.542 ± 0.008	−0.266	98.5 ± 3.8	(i)
26 [a]	4	O	1.75 ± 0.02	0.242	96.3 ± 0.7	(i)
27 [b]	5	O	8.18 ± 0.04	0.913	106.0 ± 2.0	(i)
28 [b]	6	O	23.1 ± 0.1	1.363	108.5 ± 0.5	(i)
29 [b]	7	O	61.1 ± 0.5	1.786	109.3 ± 0.3	(i)
30 [b]	10	O	1407 ± 3	3.148	92 ± 5	(i)
34 [b]	11	O	3240 ± 650	3.51 ± 0.08	–	[c]
33 [b]	5	S	59.6 ± 0.8	1.775	108.3 ± 0.7	(ii)

[a] McKeown and Prankerd (1981).
[b] Present work.
[c] From HPLC capacity factors (k').

determine the rates or mechanism for the decomposition of these derivatives.

The partition coefficients reported in Table 1 may be regarded as being at concentrations for which solute-solute interactions are negligible (Prankerd and McKeown, 1992). Log P values for nos 24–26 (**2**; $n = 2$–4, X = O) are similar to those previously recorded (Prankerd, 1977). The data from the present work are preferred, as a specific method of phase analysis (HPLC) was used, whereas the previous work used Kjeldahl nitrogen determination. This non-specific method of analysis would not distinguish between the barbiturates and their immediate decomposition products (monoureides of cycloalkane-1,1-dicarboxylates).

Steric hindrance to solvation development

Comparison of the log P values for the following derivatives makes clear the importance of steric hindrance to hydration in aqueous solutions of barbiturates (Table 2). Except for no. 24, the spiro derivatives are more hydrophilic than their open chain analogues by about −0.38 log unit. This corresponds approximately to a 2.4-fold decrease in partition coefficient on 'tying back' the C5 substituents into the cycloalkane ring. The reduced log P values are thought to result from minimization of the ability of the C5 substituents to sterically hinder solvation development in the un-ionized barbituric acid nucleus, especially at the 4- and 6-carbonyl groups. This has the effect of making the spiro derivative more hydrophilic than the corresponding 5,5-dialkyl derivative. Symmetry or asymmetry of 5,5-dialkyl substitution seems to be unimportant from comparisons of log P values for no. 28 and the isomeric 5,5-di-*n*-propyl- or 5-*n*-butyl-5-ethylbarbituric acids (Table 2).

The small increase in hydrophilicity in going from (**1**; $R_1 = R_2 = Me$) to no. 24 (**2**; $n = 2$, X = O) (Table 2, Δ log $P = -0.09$) probably can be rationalized on the basis that the C5 methyl groups, due to their small size, produce slightly more steric hindrance to hydration than the cyclopropane spiro ring of no. 24. The small steric effects of C5 methyl groups are supported by the very rapid base-catalyzed hydrolysis rates observed for (**1**; $R_1 = R_2 = Me$) (McKeown, 1976). The increase in hydrophilicity that is observed on comparing (**1**; $R_1 = R_2 = Me$) with no. 24 (**2**, $n = 2$, X = O) probably results from the following factors: (i) the C5 methyl groups are able to rotate freely and thus occupy a larger volume (and hence are slightly more efficient at reducing hydration of the nucleus) than the rigidly held methylene groups of the immobilized cyclopropane spiro ring; and (ii) the partial double bond character of the cyclopropane ring with an increased electron density may allow more water molecules to be involved in solute-solvent interactions (increased solvation).

The above observations are consistent with the steric solvent exclusion acid strengthening hypothesis (McKeown, 1980), especially as they only relate to the solute as the un-ionized molecule. Previous studies of steric effects on acid strength had involved acid-weakening with bulky substituents and this was attributed to steric solvent exclusion in the anion (final state) and consequent destabilization (Hammond and Hogle,

TABLE 2

Comparison of log P values for cycloalkane-1',5-spirobarbituric acid derivatives and their corresponding 5,5-dialkyl analogues

No.	5,5-Dialkyl analogue	Log P_{cyclic}	Log $P_{dialkyl}$	Δ log P = (log P_{cyclic} − log $P_{dialkyl}$)
24	(**1**; $R_1 = R_2 = Me$)	−0.533	−0.44 [a]	−0.09
25	(**1**; $R_1 = Me$, $R_2 = Et$)	−0.266	0.083 [a]	−0.35
26	(**1**; $R_1 = R_2 = Et$)	0.242	0.666 [a]	−0.42
28	(**1**; $R_1 = R_2 = n$-Pr)	1.363	1.75 [b]	−0.39
28	(**1**; $R_1 = Et$, $R_2 = n$-Bu)	1.363	1.73 [b]	−0.37

[a] Prankerd and McKeown (1992)

[b] Wong and McKeown (1988).

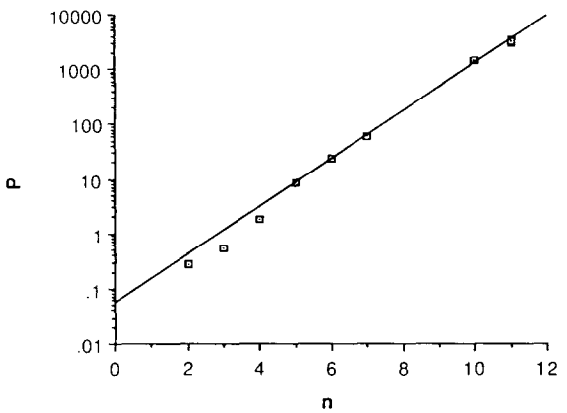

Fig. 1. Semilogarithmic plot of partition coefficient (P) as a function of number of ring methylenes (n) for the cycloalkane-1',5-spirobarbituric acids. The regression line is for nos 27–30 and 34 (see text). Except for $n = 11$, experimental errors are within the plot symbols.

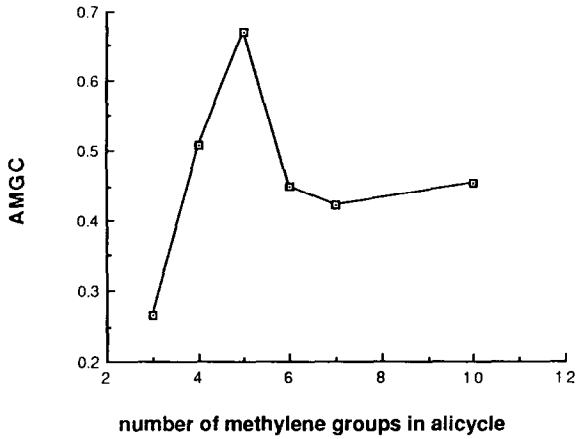

Fig. 2. Plot of alicyclic methylene group contribution (AMGC) as a function of ring size (number of methylene groups in the alicycle) for cycloalkane-1',5-spirobarbituric acids.

1955), although this mechanism has been disputed more recently (McKeown et al., 1986). The present study, as well as earlier investigations (McKeown, 1980; McKeown and Prankerd, 1981; McKeown et al., 1986), support the important concept that steric and solvation effects in the un-ionized molecule (initial state), as well as in the anion (final state) have an influence on ionization equilibria.

Alicyclic methylene group contributions (AMGCs)

The partition coefficients in Table 1 are plotted as a function of ring size in Fig. 2. The AMGC was initially assigned a value for $\pi = 0.41$ (Tute, 1971). A similar value may also be calculated from the difference between log P for cyclohexane (log $P = 3.44$) and cyclopentane (log $P = 3.00$) (Hansch and Leo, 1979). Data obtained in the present work was used to calculate the AMGC in two ways:

(i) The log P values for nos 27–30 and 34 were fitted to a linear equation in which the independent variable was the number of methylene groups (n) and the dependent variable was log P (Fig. 1). The slope of the line is the AMGC or $\pi(CH_2)$ and the intercept is the partition coefficient for the unsubstituted nucleus or 'head group'. This gave the following regression data:

$$\log P = 0.437n - 1.267 \quad r = 0.9997 \quad F = 5092$$

S.E. (π) = 0.006; S.E. (intercept) = 0.0499;

$t(\pi) = 71.3$; t (intercept) = -25.4

The correlation coefficient (r), Fisher F-ratio (F) and the Student t-values (t) indicate that the

TABLE 3

Alicyclic methylene group contributions, $\pi(CH_2)$, from log P differences

Nos	Δn	Log P_1	$-\log P_2 =$	Δ log P	$\pi(CH_2) = \Delta \log P/\Delta n$
25–24	1	−0.266	−0.533	0.267	0.267
26–25	1	0.242	−0.266	0.508	0.508
27–26	1	0.913	0.242	0.671	0.671
28–27	1	1.363	0.913	0.450	0.450
29–28	1	1.786	1.363	0.423	0.423
30–29	3	3.148	1.786	1.362	0.454

regression equation is significant at the 99% confidence interval or better. The intercept in (i) above (−1.267) is a little less than the log P value commonly assigned to the barbituric acid nucleus (log $P = -1.35$) although it is within 2 S.D. units of that value. However, there are only three degrees of freedom for the regression and the agreement might be fortuitous.

(ii) The AMGC was calculated as the difference in log P (Δ log P) for successive pairs of derivatives divided by the number of additional methylene groups (Δn) in the larger alicyclic ring (Table 3). These data show that the AMGC is erratic until Δ log P is taken between derivative pairs with a minimum of five methylene groups in the ring (Fig. 2).

The mean value for the AMGC from the last three entries of Table 3 (0.442) is in excellent agreement with the value from (i) above (0.437) and with that from the difference between log P for cyclohexane and for cyclopentane (0.44). The AMGC from the difference between log P values for nos 30 and 34 is 0.36 ± 0.1 log unit. This is less than the differences between other cycloalkane-1′,5-spirobarbituric acids with $n > 5$ (Table 3), although it is within the experimental and statistical errors. The difference may result from the method for estimation of log P for no. 34 (correlation of HPLC capacity factors with log P values), or may be a real difference. Superficially, it would be expected that the AMGC for large rings ($n \geqslant 11$) should not decrease, but should gradually increase to approach the value for the aliphatic methylene group contribution (MCG). This supposition arises from previous literature data on large alicycles (Sicher, 1962) which demonstrate that rings for which $n \geqslant 11$ tend to exhibit less non-classical ring strain and behave more like open chain aliphatic hydrocarbons.

Although ring strain may be a factor in determining the AMGC, it is also likely that another factor is the accessibility of water molecules to the alicyclic methylene groups. Aliphatic chains are exposed to water molecules on all sides, but in alicycles, the inner part of the ring is less accessible. In large rings ($n \geqslant 11$) it is possible that the alicycle is not circular, but in the form of an elongated oval in which accessibility of water to the inner part of the ring is still restricted. This could account for the present observation of an AMGC (0.44) which remains about 0.1 log unit less than the MGC (0.54) (Prankerd and McKeown, 1992), even for large rings.

The present experimental log P values confirm the methylene group contribution obtained from literature log P values for alicycles containing five or more methylene groups. However, the range of AMGC values in Table 3 indicate that it is most unsafe to estimate log P values for small ($n = 2$–4) alicycles from the AMGC value obtained from common or larger ($n > 4$) alicycles. This is also demonstrated by the plot in Fig. 1, which clearly shows that the compounds with small alicycles do not conform to the partitioning behavior of those with larger alicycles.

Pharmacological activity

Some of the smaller spiro ring compounds (**2**; $n = 2$, 4 and 5, X = O) have been unsuccessfully examined for pharmacological activity following i.v. administration of solutions of their sodium salts (Swanson et al., 1950). Although it was claimed that solutions of the drugs were freshly prepared, it is likely that rapid hydrolysis of derivatives without substituents adjacent to the nucleus had occurred before administration of solutions to the test animals. This would explain an apparent lack of CNS activity. This possibility is supported by data showing that the spirobarbiturates with $n = 3$–5 have hydrolysis rates which are 3–4 orders of magnitude greater than for 5,5-dialkylbarbiturates under corresponding conditions (Mokrosz and Paluchowska, 1986). The lower partition coefficients reported in the present study (compared to acyclic analogues) support the likelihood of greater accessibility of nucleophiles (such as OH^- or H_2O) to the hydrolytically susceptible nucleus, thus increasing reaction rate constants. Such hydrolytic reactions may be either specifically base-catalyzed or else due to spontaneous water attack (McKeown, 1976).

The partition coefficients in this study suggest that some of the derivatives should in fact have CNS depressant pharmacological activity typical of hypnotically active barbituric acid derivatives,

provided that they are delivered intact to the site of action. In particular, nos 29 and 33 have log P values which approach the optimal value (log $P = 2$) for transport to the CNS in the Hansch parabolic relationship between log P and $-\log C$ (a measure of biological activity). Nos 24 and 25 probably do not have hypnotic activity, as their partition coefficients are very low and they may be too hydrophilic to partition to the site of action in sufficient concentration to elicit a response. Nos 30 and 34 are probably not active as they are practically insoluble in water and their log P values are significantly higher than the optimal log P value (log $P = 2$). Pharmacological testing of these derivatives may be difficult, given the instability of i.v. solutions of the sodium salts, although oral dosing would be feasible. Intravenous dosing of the free acids may be possible with submicron emulsion dosage forms (Prankerd et al., 1988; Prankerd and Stella, 1990). This approach has been previously shown to give good results with barbiturates (Jeppsson, 1972a,b).

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