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THE DEPENDENCE OF AMITRIPTYLINE PARTITION COEFFICIENTS ON LIPID PHASE

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SUMMARY

Equations for the determination of the absolute partition coefficient of weak bases in the ionized and the unionized form have been developed to study the partitioning behaviour of amitriptyline in a series of n-alcohols and a series of n-alkanes. The variation in the absolute partition coefficients of the unionized compound in the various alcohols is discussed and the meaning of the absolute partition coefficient of the ionized molecule is commented upon.

INTRODUCTION

Partition coefficients have been used effectively as physicochemical parameters in quantitative structure activity work (Hansch, 1973).

Usually, the absolute partition coefficients are used instead of the apparent values determined in buffered solutions so that prior to statistical analysis, the observed partition coefficients are transformed to the absolute values using appropriate equations.

More recently (Tsuji et al., 1977), equations have been derived relating the partition coefficient of the unionized species (P_u) of weak acids with that of the ionized molecule (P_i). This P_i term has often been neglected in structure–activity work. Although in systems where alkanes are used as partitioning systems this is a reasonable assumption, with systems consisting of alcohols, especially the lower homologues, it may be unjustified, at least in some of the cases. The P_i term, however, is itself an ambiguous parameter in that it depends very closely on the system used for its determination. For an ion to partition into an organic phase it must carry with it a counter-ion. Hence the P_i term will be subject to variations in the concentration and type of counter-ions present.

In the literature partition coefficients have often been determined for one single organic solvent. To investigate the possible relationships between the partition coeffi-

cients obtained using different members of a given homologous series of organic solvents, we have studied the partitioning of amitriptyline hydrochloride in a series of straight chain alcohols and a series of alkanes. Equations relating P_i and P_u for weak bases have also been developed to study the behaviour of the unionized and the ionized species in the partitioning systems.

THEORY

The dissociation of a conjugate acid BH^+ of a base B can be represented by Eqn. 1

$$BH^+ + H_2O \rightleftharpoons B + H_3O^+ \tag{1}$$

and the dissociation in water may be expressed as

$$K_a = \frac{[B][H_3O^+]}{[BH^+]} \tag{2}$$

where [ ] represents concentration. It is to be noted that the dissociation constant can also be expressed as the thermodynamic dissociation constant, in which case all the concentration terms are expressed as activities, and as the apparent dissociation constant where only the hydrogen ion concentration is expressed in terms of activity (Levy and Rowland, 1972). The same authors have also shown that with weak bases with no hydroxy groups the difference between the stoichiometric and thermodynamic pKa is insignificant.

During partitioning between an oil and an aqueous phase, the distribution of the various species can be illustrated as follows:

$$\begin{array}{ccc} [BH^+]_w & \overset{P_i}{\rightleftharpoons} & [BH^+]_o \\ \downarrow\uparrow \; K_a^w & & \downarrow\uparrow \; K_a^0 \\ [H_3O^+]_w + [B]_w & \overset{P_u}{\rightleftharpoons} & [H_3O^+]_o + [B_o] \end{array}$$

AQUEOUS PHASE | OIL PHASE

Scheme 1.

where the subscripts o and w represent the oil and water phase, respectively.

Usually, in deriving the equations for relating the dissociation constant with the partition coefficient only the aqueous K_a^w is taken into account. In the scheme adopted in our study (Scheme 1), K_a^o is also defined once K_a^w, P_i and P_u are known.

The apparent partition coefficient of the free base can be expressed as

$$P_{app} = \frac{[BH^+]_o + [B]_o}{[BH^+]_w + [B]_w} \tag{3}$$

and the true partition coefficients of the free base P_u and of the conjugate acid P_i are given by

$$P_u = \frac{[B]_o}{[B]_w} \tag{4}$$

$$P_i = \frac{[BH^+]_o}{[BH^+]_w} \tag{5}$$

Removal of the oil phase data by substitution of Eqns. 4 and 5 into Eqn. 3 leads to

$$P_{app} = \frac{P_i[BH^+]_w + P_u[B]_w}{[BH^+]_w + [B]_w} \tag{6}$$

From Eqn. 2

$$B_w = \frac{K_a[BH^+]_w}{[H_3O^+]} \tag{7}$$

Therefore, Eqn. 6 can be rearranged to

$$P_{app}\left(1 + \frac{Ka}{[H_3O^+]}\right) = P_i + P_u\left(\frac{Ka}{[H_3O^+]}\right) \tag{8}$$

A plot of

$$P_{app}\left(1 + \frac{Ka}{[H_3O^+]}\right) \text{ against } \frac{Ka}{[H_3O^+]}$$

should therefore give a straight line of slope P_u and intercept P_i.

From Eqn. 6

$$P_{app} = \left(\frac{[BH^+]_w}{[BH^+]_w + [B]_w}\right) P_i + \left(\frac{[B]_w}{[BH^+]_w + [B]_w}\right) P_u \tag{9}$$

If x is the fraction ionized then this may be rewritten as

$$P_{app} = xP_i + (1 - x)\,P_u \tag{10}$$

Hence, knowing P_i and P_u, Eqn. 10 can be used to calculate P_{app} at any pH value and any experimentally measured P_{app} can be checked.

The values of P_u can also be compared with that obtained using Eqn. 11 (Leo et al., 1971)

$$(P/P_{app}) - 1 = \text{antilog}(pK_a - pH) \tag{11}$$

Note, however, that in the derivation of Eqn. 11, $[BH^+]$ in the oil phase is taken as being negligible, an assumption which is not always valid.

MATERIALS AND METHODS

Experimental materials

All the partitioning solvents and buffering materials used were purchased from BDH Ltd., in the purest form available. Amitriptyline hydrochloride was obtained from Merck, Sharpe and Dohme Ltd.

Apparent partition coefficients

These were determined at 37°C by presaturating the buffers (Sorensen's citrate adjusted to 0.5 M ionic strength with potassium chloride) with each of the organic solvents, separating the phases and using the aqueous phase to make the amitriptyline hydrochloride solutions (1×10^{-3} M). Twenty-five ml of the aqueous solutions and 2 ml of the buffer saturated organic solvents were used for each determination and a duplicate run for each point. The aqueous phase was assayed for residual drug by UV at 240 nm and the concentration in the organic phase calculated by mass balance after assay of the original solutions.

pKa determination

The non-logarithmic titration method described by Levy and Rowland (1971) was used to determine the pKa of amitriptyline at 37°C. Fifty ml of aqueous amitriptyline hydrochloride solution containing 3×10^{-4} M of drug were titrated with 1 N NaOH. The aqueous solubility of the free base was determined in 0.01 N NaOH as described by Green (1967) except that equilibration was carried out at 37°C and absorbance readings at 450 nm were read in thermostated cells.

Dissociation constant

The pKa of amitriptyline has previously been reported by Green (1967) to be 9.4 at 24°C. However, since the partitioning experiments were carried out at 37°C and previous experiments (Gescher and Li Wan Po, 1978) have suggested significant hydrophobic behaviour for amitriptyline base, an accurate value for the dissociation constant at the higher temperature was essential since a small difference in pKa will be translated into a very large difference in the partition coefficient of the unionized species. The non-logarithmic titration method described by Benet and Goyan (1965) and Levy and Rowland (1971) was adopted because of the more precise pKa values obtainable with this method when dealing with highly water-insoluble compounds. A pKa value of 9.31 (37°C) was obtained for amitriptyline conjugate acid.

RESULTS AND DISCUSSION

Partitioning in the n-alkanols

The apparent partition coefficients for amitriptyline hydrochloride over the range of alcohols and pH studied are listed in Table 1 and as predicted by Eqn. 8, plots of P_{app} $(1 + (Ka/[H_3O]^+))$ against $(Ka/[H_3O]^+)$ produced straight lines (Eqns. 12–18).

TABLE 1

THE APPARENT PARTITION COEFFICIENT OF AMITRIPTYLINE HYDROCHLORIDE AS A FUNCTION OF pH AND PARTITIONING ALCOHOL SYSTEM AND THE P_i AND P_u VALUES CALCULATED FROM THE APPARENT PARTITION COEFFICIENTS

P_u is the partition coefficient of the free base calculated from a plot of $(Ka/[H_3O]^+)$ vs $P_{app}(1 + (Ka/[H_3O]^+))$. P_i is the partition coefficient of the conjugate acid using the same plot.

pH	Butanol	Pentanol	Hexanol	Heptanol	Octanol	Decanol	Dodecanol
2.1	73.5	46.3	48.7	30.7	24.5	18.5	11.80
3.0	54.5	45.8	40.4	26.7	21.7	12.2	5.36
4.0		48.8	43.7	34.4	29.1	14.7	7.20
5.80	55.5	80.5	62.4	60.9	54.3	42.9	40.9
5.97	64.5	100.3	84.0	74.6	69.2	61.1	47.0
6.07	69.6	110.4					
6.19			120.1	104.8	113.8	74.9	72.9
6.37	90.2	158.7	164.2	145.7	142.8	114.1	110.2
6.58	100.4	283.7	260.2	273.5	224.5	183.7	158.8
6.80	138.2	416.4	422.2	410.7	394.0	335.0	296.2
P_u ($\times 10^{-5}$)	0.2897	1.246	1.357	1.302	1.207	1.043	0.9152
P_i	53.43	32.98	16.57	13.05	13.30	2.98	4.89
γ (linear regression coefficient)	0.996	0.992	0.998	0.996	0.997	0.995	0.996

Solvent

Butanol $y = 0.290 \times 10^5 X + 50.74 \quad \gamma = 0.996$ (12)

Pentanol $y = 1.246 \times 10^5 X + 32.98 \quad \gamma = 0.992$ (13)

Hexanol $y = 1.357 \times 10^5 X + 16.57 \quad \gamma = 0.998$ (14)

Heptanol $y = 1.302 \times 10^5 X + 13.05 \quad \gamma = 0.996$ (15)

Octanol $y = 1.207 \times 10^5 X + 13.30 \quad \gamma = 0.997$ (16)

Decanol $y = 1.043 \times 10^5 X + 2.96 \quad \gamma = 0.995$ (17)

Dodecanol $y = 0.915 \times 10^5 X + 4.87 \quad \gamma = 0.996$ (18)

where $y = P_{app}(1 + (Ka/[H_3O]^+))$

$x = (Ka/[H_3O]^+)$

γ = correlation coefficient

solvent = partitioning solvent

Although the slopes of the equations give a good estimate of P_u, the intercepts only provide rough guides to the value of P_i owing to the very large differences in P_i and P_u in all the systems studied. While a linear relationship was observed between P_u and the alkyl chain length between hexanol and dodecanol

$$(P_u = 7600\,C + 1.819 \times 10^5 \text{ and } \gamma = 0.998). \quad (19)$$

This equation is clearly not applicable to the whole range of alcohols and water since, in the hypothetical case where partitioning between water and water is studied, one would expect a partition coefficient of unity whereas extrapolation of Eqn. 19 predicts a value of 1.82×10^5. Marked deviations were apparent with pentanol and butanol (Fig. 1). The change in P_u probably reflects the large differences in the solubility of water in the solvents and of the solvents in water. The very low solubility of amitriptyline free base in water relative to the organic solvents in fact indicates that the deviation shown by the lower alcohols is to be expected since in going from water to the alcohols an increase in solubility and hence in partition coefficient must be observed initially, the maximum value being attained when the solute is at ideality in the organic phase. The water content at saturation has been reported (Leo et al., 1971) as being 2.3 M for octanol, 5 M for pentanol and 9.4 M for butanol.

This disproportionate solubility in the lower alcohols could, therefore, account for the particularly marked deviation observed (Table 1 and Fig. 2). The high water content of the lower alcohols in the partitioning systems could mean that the partition and dissociation of a cation will depend upon the nature of the lipid phase. The breakdown of the linear relationship between carbon numbers and P_u with the lower alcohols is most likely due to the fact that the polarity and the solution properties of these alcohols in the

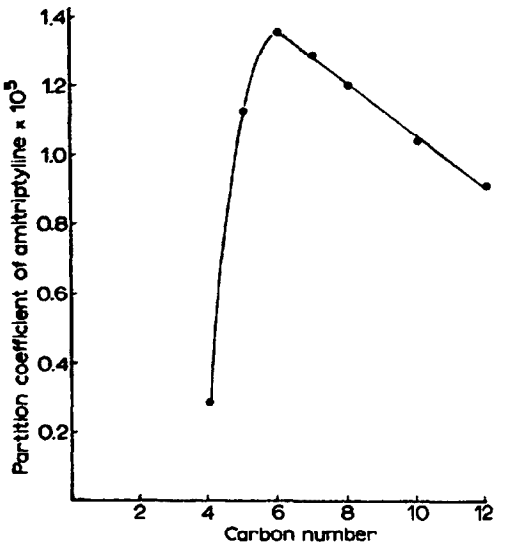

Fig. 1. The effect of carbon chain length of n-alkanols on the partition coefficient of amitriptyline free base.

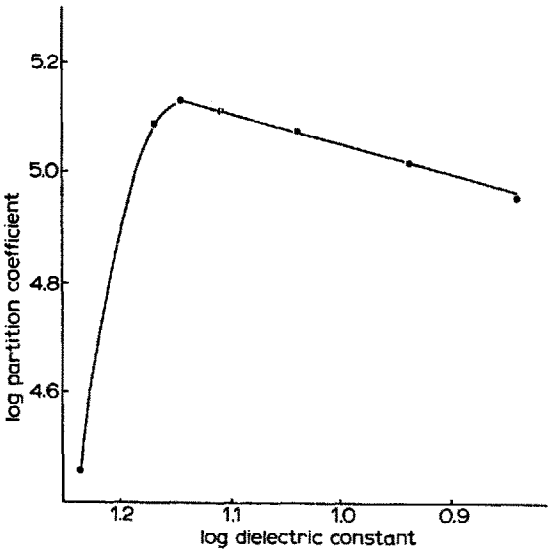

Fig. 2. The relationship between dielectric constant of the organic phase and the partition coefficient of amitriptyline free base.

biphasic systems is not solely dictated by the carbon chain length. In a study of the partitioning of carboxylic acids between water and alcohol, Pearson and Levine (1952) observed a linear relationship between partition coefficient and carbon number all the way from pentanol to decanol for formic acid. Deviation from linearity was however apparent with the somewhat more hydrophobic acetic acid. The importance of mutual solubilities in predicting partitioning has been indicated by Treybal (1963).

In the literature, the organic phase used for partitioning studies has been very varied. This has therefore led to the well known attempts (Collander, 1959; Leo et al., 1971) to find extrathermodynamic relationships between the partition coefficient values obtained using different solvent systems. Attempts to correlate our butanol and pentanol data with that of octanol using the solvent regression equations developed by Leo et al. (1971) failed. With pentanol, for example, a calculated log P of 4.41 compared to an experimental value of 5.12 was obtained. It is likely that there would be a better correlation between the higher alcohols. These have unfortunately not been reported.

Some of the difficulties associated with the exact analysis of pH data for similar compounds (phenothiazines) have been discussed by Murthy and Zografi (1970). The values obtained for the apparent partition coefficient depended upon the buffer concentration and the concentration of KCl used to adjust the ionic strength. In addition the relative ratio of the ionic species of the buffer also changes with pH so that the amount of available counter-ions for ion pair partitioning would also be altered. Even when all these variables are kept constant by fixing the pH and the ionic strength, the observed solvent effect on the apparent partition coefficients cannot be fully explained by their influence on the solute. Account must be taken of their solvating properties on the counterions. Solvation depends on the properties of both the solute and the solvent (Parker, 1962). Their polarity and hydrogen bonding ability with each other are of particular importance

(Schill, 1974). The low pH data (pH 2.2, 3.0, 4.0, 5.8) in Table 1 illustrate further anomalies when dealing with compounds having widely different P_i and P_u. At pH 5.0, for example, although in the aqueous phase (octanol/buffer system) the ratio of the ionized to the unionized amitriptyline can be calculated, using the Henderson–Hasselbach equation, to be 20,417, the corresponding ratio in the organic phase as given by Eqn. 20 (Leo et al., 1971):

$$\log\left(\frac{[BH]^+_o}{[B]_o}\right) = \log P_i - \log P_u - (pH - pKa) \qquad (20)$$

is only 2.2, thus showing that with compounds having large P_u/P_i ratios, one must be particularly careful in the choice of pH when determining P_i. The counter-ions will of course determine the P_i observed. From Eqn. 9,

$$P_{app} = P_i \text{ (fraction ionized)} + P_u \text{ (fraction unionized)} = P_i(X) + P_u(1 - X)$$

At pH values lower than 6.80 (for calculating P_i) the fraction unionized can be taken as negligible so that within a given alcohol/buffer system the P_iX term can be regarded as a constant. A plot of P_{app} versus $(1 - X)$ should therefore give a straight line of slope P_u. Eqns. 21–26 confirm the validity of the assumption made.

$$P_{app} = P_uX + P_i(1 - X)$$

Solvent	P_uX	$P_i(1 - X)$	γ	Eqn.
Butanol	26,214	53.41	0.996	(21)
Pentanol	125,033	32.77	0.993	(22)
Hexanol	135,896	16.47	0.999	(23)
Heptanol	130,414	12.94	0.996	(24)
Octanol	120,800	13.20	0.997	(25)
Decanol	104,370	2.89	0.995	(26)

The P_u values obtained can be seen to be almost identical to those derived from plots of Eqn. 8. The intercept which is equal to $P_i(1 - X)$ approximates to P since $(1 - X)$ approximates to unity at pH lower than pH 6.8. Again because P_i is about 4 orders of magnitude smaller than P_u, the P_i estimate must be looked upon as guide rather than an absolute value.

Various attempts (for example, Mottolla and Freiser, 1966, 1967; Buchowski, 1962) have been made to relate observed partition coefficient with solvent properties like dielectric constant, mutual solubility and Hildebrand's solubility parameter. In studying the partitioning of 8-quinolinol between a series of solvents and aqueous buffer, Mottolla and Freiser found an approximately parabolic relationship between the logarithm of the partition coefficient and the logarithm of the dielectric constant (ϵ) of the solvent. A plot of our results (Fig. 2) also shows this approximate relationship with the data for hexanol

TABLE II

THE APPARENT PARTITION COEFFICIENT OF AMITRIPTYLINE HYDROCHLORIDE AS A FUNCTION OF pH AND PARTITIONING n-ALKANE SYSTEM AND THE CALCULATED TRUE PARTITION COEFFICIENT

pH	Heptane	Octane	Nonane	Decane	Dodecane	Hexadecane
5.80	9.6	9.3	10.5	13.3	11.9	8.9
5.97	13.3	21.2	26.5	19.6	17.2	17.8
6.19	20.6	39.5	35.3	39.6	43.7	32.6
6.37	34.1	49.0	56.2	53.6	43.4	50.7
6.58	68.4	78.0	77.4	82.5	71.0	73.2
P_u ($\times 10^{-5}$)	0.38	0.42	0.41	0.44	0.37	0.41
Mean $P_u = 0.4065 \times 10^5 \pm 0.028$						
P_i	−5.2	1.1	3.8	1.3	4.0	−0.7
γ	0.990	0.988	0.983	0.994	0.962	0.992

to dodecanol showing a linear relationship ($\log K = 0.561 \log \epsilon + 4.508$ $\gamma = 0.996$). A linear relationship was observed between the carbon number and the logarithm of the dielectric constant of pure alcohols ($\log \epsilon = 0.049C + 1.41$ $\gamma = 0.996$). The curve (Fig. 2) parallels that shown in Fig. 1. Although the solubility parameter theory rests upon the London theory of dispersion forces, there have been several attempts to use the solubility parameters for polar substances but their general use has been cautioned by Hildebrand et al. (1970).

No significant differences were observed in the P_u values obtained using the various alkanes. The homologues used gave a mean P_u of 4.065×10^4. These results are in agreement with those reported by Murthy and Zografi who showed that with several phenothiazines, the same partition coefficients were obtained with different alkanes. Seiler (1974) also indicated that when log P cyclohexane was correlated with log P obtained with hexane, octane and hexadecane, none of the slopes of the straight lines obtained was significantly different from 1.00, thus showing that the partition coefficients obtained in any of the alkanes studied should be close to each other. The intercepts ranged between −5.2 and 4.0 and it is probably reasonable to say that these point to a P_i of 0 and the values reflect the experimental errors involved.

CONCLUSION

This study has shown that although there was a linear relationship between the absolute partition coefficient of amitriptyline base and the carbon number between dodecanol and hexanol, this relationship breaks down with pentanol and butanol. The partition coefficient of the same species in the series of straight chain hydrocarbons show that there was no marked difference between the P_u observed. A method for the determination of the partition coefficient of the ionized and the free base forms of amitriptyline by measuring the apparent partition coefficients at various pH values has been described. A study of the influence of the lipophilic phase on these parameters has been undertaken.

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