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The effect of ionization on the partitioning of clofazimine in the 2,2,4-trimethylpentane–water system

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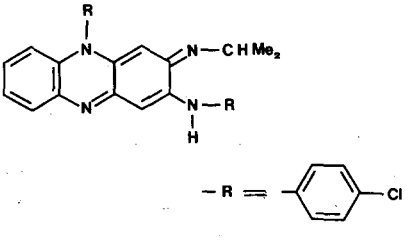
Summary

The pH dependence of the 2,2,4-trimethylpentane–water partition coefficient of clofazimine is studied at 37 °C. A model is presented which includes contributions from the ionized (P_i) and unionized (P_u) species to the observed apparent partition coefficient (P'). The value of P_i is much smaller than that of P_u with the ratio P_i/P_u, designated Q, equal to 1.012×10^{-4}. However, at low pH, the former term becomes increasingly predominant and it is desirable to assess its contribution quantitatively to the apparent partition coefficient.

Introduction

A high percentage of biologically active compounds are ionized at the physiological pH. The degree of ionization has important implications with regard to drug absorption, transport and receptor interactions. Drug absorption entails a random passage of the solute from a hydrophilic phase to a lipid phase (membrane) and from a lipid phase to an aqueous phase (bloodstream pH = 7.4). The driving force for the transport of a weak electrolyte may be associated with the difference between the pH values of the intermediate and final compartments. In many cases, molecules interact with receptor sites in the ionized form and it is very important therefore to assess the dependence of lipophilicity on pH to analyze in quantitative terms the relationship between physicochemical properties and biological activity (La Rotonda et al., 1985).

Clofazimine (**I**), a phenazine derivative developed as a potential antituberculosis agent in the Laboratories of the Medical Research Council of Ireland, has been clinically used as an antileprotic agent for a number of years and is included in the



[I]

Scheme 1.

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British Pharmacopoeia 1988. A range of analogues have been synthesised and tested for antimicrobial activities (O'Sullivan et al., 1988). Recent studies have centered on the influence of lipophilic and steric parameters on the transport of phenazines to the spleen of mice following oral administration (Canavan et al., 1986) and the thermodynamics of the oil/water distribution of 8 selected derivatives (Fahelelbom et al., 1989). Since these studies were carried out at pH 5.15, the phenazine would be predominantly in the ionized form and it would be desirable to quantify the contribution of this species to the apparent partition coefficient (P'). As P' is pH-dependent (Gupta et al., 1968; Gupta and Cadwallader, 1968; Leo et al., 1971; Leo and Hansch, 1971), the purpose of this study was to investigate the effect of ionization on the partition behaviour.

Materials and Methods

Materials

A sample of clofazimine was generously supplied by the Medical Research Council of Ireland, Trinity College. The solvent 2,2,4-trimethylpentane was BDH-Analar grade. The buffer systems used were the Sorensen phosphate buffer (KH_2PO_4/ $Na_2HPO_4 \cdot 2H_2O$) for the range 5.1–6.0 and the McIlvain citrate–phosphate buffer (0.1 M $C_6H_8O_7 \cdot H_2O$/0.2 M $Na_2HPO_4 \cdot 2H_2O$) for the pH range 2.2–4.6.

Before partitioning, the organic solvent and buffer solution were mutually saturated.

Methods

The pK_a value was determined using a spectrometric method (Albert and Serjeant, 1971).

The partition coefficients were measured using the shake-flask method (Leo et al., 1975; Hansch and Leo, 1979) at 37 °C. 10 ml of organic phase containing the drug and 40 ml of buffer were equilibrated over a period of 6 h. The concentration of solute in each layer was determined by UV spectrophotometry (Pye Unicam SP8–100 spectrophotometer) at $\lambda_{max} = 283$ nm.

The pH measurements were taken using a Radiometer 26 pH meter. The apparent partition coefficient is calculated from the distribution results by employing Eqn. 1.

$$P' = (C_o/C_w) \tag{1}$$

where C_o and C_w are, respectively, the solute concentrations in the organic and aqueous layers.

Results and Discussion

Ion partitioning

The apparent partition coefficients (P') of clofazimine (RNH) are determined using 2,2,4-trimethylpentane as the lipophilic phase in the pH range 2.2–6.0 at 37 °C. The solute is a weak base, the dissociation of which may be represented by

$$RNH + H^+ \rightleftharpoons RNH_2^+ \tag{2}$$

with

$$K_a = [RNH][H^+]/[RNH_2^+] \tag{3}$$

The value of pK_a determined using the spectrometric method was 8.511.

The degree of ionization, α, is given by

$$\alpha = [RNH_2^+]/\{[RNH] + [RNH_2^+]\} \tag{4}$$

which, in combination with Eqn. 3 yields

$$\alpha = (1 + 10^{pH - pK_a})^{-1} \tag{5}$$

In the distribution of the solute between two phases where ion partitioning is assumed to be negligible, the corrected partition coefficient, P, is the ratio of the concentration in the organic phase to that of all forms in the aqueous phase. Thus

$$P = [RNH]_o/\{[RNH]_w + [RNH_2^+]_w\} \tag{6}$$

where the subscripts o and w refer, respectively, to concentration terms in the organic and aqueous phases. The ion-corrected partition coefficient, P, can be calculated from the apparent value by using the equation

$$P = P'(1 - \alpha)^{-1} \tag{7}$$

which, in combination with Eqn. 5 yields

$$P = P'(1 + 10^{pK_a - pH}) \quad (8)$$

Since to a first approximation the ionized form of the molecule does not partition into the organic phase, the true partition coefficient (P) may be calculated from the observed value using Eqn. 8. If the pH of the aqueous phase is far from the pK_a value of the molecule, the above equation simplifies to

$$\log P' \cong \log P - pK_a + pH. \quad (9)$$

However, at a pH greater than pK_a

$$\log P' \cong \log P. \quad (10)$$

Eqns. 7 and 8 presuppose that only the unionized species partitions from the aqueous phase into the organic phase. For differences between pH and pK_a larger than 4 log units, the assumption that ion partitioning is negligible is no longer valid (Kubinyi, 1979; Martin, 1980; Scherrer, 1984).

The above model may be extended to treat the partitioning of ionized (P_i) and unionized species (P_u) individually. For different types of ionizable compounds P_u is in the range 10^3–10^5 P_i (Leo et al., 1971; Tsuji et al., 1977; Kubinyi, 1979). However, if the fraction of unionized species, $1 - \alpha$, is within the range or smaller than P_i/P_u, the ionized species will significantly contribute to the apparent partition coefficient. The contributions of P_i and P_u to P' can be expressed by

$$P' = \{[RNH]_o + [RNH_2^+]_o\} / \{[RNH]_w + [RNH_2^+]_w\} = (1 - \alpha)P_u + \alpha P_i. \quad (11)$$

The model is presented in Fig. 1.

Combining Eqns. 5 and 11 yields

$$P'(1 + 10^{pH - pK_a}) = P_u(10^{pH - pK_a}) + P_i \quad (12)$$

The parameters P_u and P_i can thus be determined by plotting $P'(1 + 10^{pH - pK_a})$ against $10^{pH - pK_a}$.

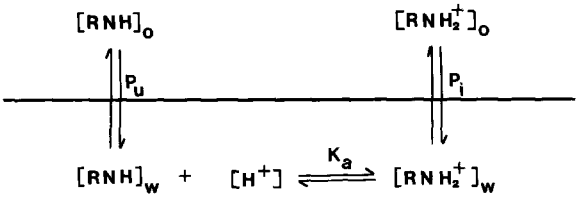

Fig. 1. Partitioning and dissociation equilibria of clofazimine (RNH) between water and organic solvent (2,2,4-trimethylpentane).

The above relationships can be conveniently expressed in terms of K_a and $[H^+]$ i.e.

$$P' = \{K_a/(K_a + [H^+])\}P_u + \{[H^+]/(K_a + [H^+])\}P_i \quad (13)$$

which, on rearranging, yields

$$P' = (P_u - P_i)\{K_a/(K_a + [H^+])\} + P_i \quad (14)$$

The effect of ionization on the apparent partition coefficient can be expressed as a ratio (Q) of the ionized and non-ionized partition coefficients (Loftsson, 1985).

$$Q = P_i/P_u. \quad (15)$$

Results

The absorbance (A) of the calibration solutions can be expressed in terms of the concentration, c (mg/10 ml), by the equation

$$A = ac + b \quad (16)$$

where the parameters a and b are determined by least squares fitting procedures. The values of the coefficients are listed in Table 1 along with the correlation coefficients. The calibration equations are used to estimate the concentration of clofazimine in both the organic and aqueous phases after equilibration. The fraction of ionized clofazimine, $1 - \alpha$, and the apparent partition coefficient, P', are given in Table 2 at each pH value. The value of P' at pH 5.15 was determined by analyzing the

TABLE 1

The parameters a and b (Eqn. 16) derived from the calibration data

System	a	$10^3 b$	n	r
Organic phase	9.3820	3.46	7	0.9995
pH 2.21	2.1828	−6.30	8	0.997
pH 2.28	1.4852	−0.04	6	0.9995
pH 3.41	1.3610	−4.08	6	0.997
pH 4.42	1.0869	−2.08	6	0.998
pH 5.15	–	–	–	–
pH 5.99	0.4283	−8.88	7	0.974

The number of points (n) and the correlation coefficient (r) are also listed.

TABLE 2

The values of $(1-\alpha)$ and P' for clofazimine ($pK_a = 8.511$) at each pH value

pH	$10^j(1-\alpha)$	$10^k P'$	j	k
2.21	5.000	8.7344	7	2
2.82	2.037	2.0407	6	1
3.41	7.925	1.6391	6	0
4.42	8.109	8.2283	5	0
5.15	4.354	1.7073	4	−1
5.99	3.004	7.9634	3	−1

concentration in the 2,2,4-trimethylpentane phase before and after partitioning.

The plot of log P' against pH is given in Fig. 2.

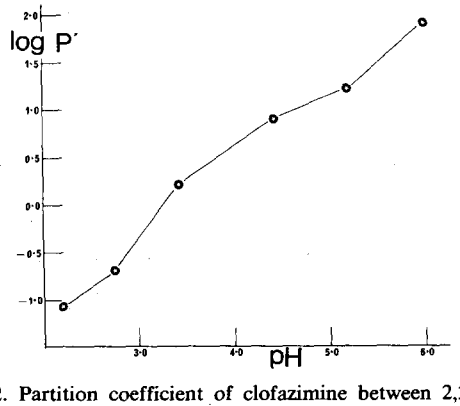

Fig. 2. Partition coefficient of clofazimine between 2,2,4-trimethylpentane and the aqueous phase as a function of pH at 37 °C.

Discussion

The steady increase in the value of a with decreasing pH (Table 1) reflects the concomitant increase in α. The observed increase in a is due to the fact that the optical density of the ionized species (RNH_2^+) is greater than that of the molecular species (RNH).

Applying Eqn. 7 to the data given in Table 2 yields a series of values of P which decrease with increasing pH. The values of log P decrease from 5.242 at pH 2.21 to 4.423 at pH 5.99. This indicates that the model must be modified to include an extra term which expresses the contribution of ion-partitioning to the value of P'. This contribution is accounted for by using Eqn. 11 as the value of $[RNH_2^+]_o$ is non-negligible.

Using regression techniques, the data in Table 2 are analyzed to yield the parameters P_u and P_i in Eqn. 14. The least-squares line is

$$P' = 2.58249 \times 10^4 \{ K_a/(K_a + [H^+]) \} + 2.613;$$

$$r = 0.996.$$

Hence $P_u = 2.58275 \times 10^4$ and $P_i = 2.613$.

The contribution of the ion partitioning to the measured partition coefficient can be expressed in terms of Q (Eqn. 15).Thus

$$Q = 1.012 \times 10^{-4}$$

The quantity pQ, defined as log Q^{-1}, is equal to 3.995. For $1 - \alpha < Q$ Eqs. 11, 12 and 14 reduce to

$$P' \cong P_i. \tag{17}$$

Since $\alpha = [H^+]/(K_a + [H^+])$, this condition is equivalent to

$$pH < pK_a - pQ. \tag{18}$$

Therefore at a pH value less than 4.52 the values of P' should approach the limiting value of P_i. The contributions from the ionized and unionized species are equivalent when $pH = pK_a - pQ$, i.e. when $(1-\alpha)P_u = \alpha P_i$. When $pH = pK_a$, $\alpha = 0.5$ and $2P' = P_u + P_i$ (Eqn. 11). The value of log P' = 4.11 at this pH value.

The data presented in Table 2 and Fig. 2 concord quite well with the model presented in Fig. 1 for the individual contributions to the apparent partition coefficient, P'. The value of the correlation coefficient, r, obtained by fitting the results to Eqn. 14 is 0.996. The value of $pQ \cong 4.0$, indicating that the contribution of the molecular species to the measured partition coefficient is a factor of 10^4 greater than that of the ionized species.

In addition to the complications arising from dissociation of acids and bases, the values of P' can be significantly affected by the formation of ion pairs (Lee et al., 1978; Scherrer, 1984). Murthy and Zografi (1970) indicated the importance of this effect in polar organic solvents. They concluded that an increase in polarizability and lipophilicity of the counterion leads to a corresponding increase in P'. However, since 2,2,4-trimethylpentane is a highly non-polar solvent, the contribution of ion pairs is assumed negligible. This is reflected in the large and positive value of pQ, indicating that the concentration of $[RNH_2^+]_o$ is very low. However, it is desirable to account for this term at lower pH values (i.e. at values lower than pH 4.5).

The high log P values for clofazimine indicate a high degree of lipophilicity. The presence of the compound in the predominantly ionized form in the aqueous phase, $[RNH_2^+]_w$, introduces the possibility of the extraction of the ionized form into the non-aqueous phase.

References

Albert, A. and Serjeant, E.P., *Ionization Constants of Acids and Bases,* Methuen, New York, 1971, pp. 69–82.

Canavan, E.B., Esmonde, A.G., Feely, J.F.P., Quigley, J.M. and Timoney, R.F., The influence of lipophilic and steric properties on the transport of N^2-substituted phenazines to spleen of mice following oral administration. *Eur. J. Med. Chem.,* 21 (1986) 199–203.

Fahelelbom, K.M.S., Quigley, J.M., Timoney, R.F. and Corrigan, O.I., Thermodynamics of the distribution and interfacial transfer kinetics of antimicrobial iminophenazines in a two-phase system of *n*-octanol/aqueous buffer. *Proc. R. Ir. Acad.,* in press.

Gupta, V.D. and Cadwallader, D.E., Determination of first pK_a' value and partition coefficients of bromothymol blue., *J. Pharm. Sci.,* 57 (1968) 2140–2142.

Gupta, V.D., Cadwallader, D.E., Herman, H.B. and Honigberg, I.L., Effect of pH and dye concentration on the extraction of a thiamine dye salt by an organic solvent. *J. Pharm. Sci.,* 57 (1968) 1199–1202.

Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology,* Wiley, New York, 1979.

Kubinyi, H., Lipophilicity and drug activity. *Prog. Drug Res.,* 23 (1979) 97–198.

La Rotonda, M.I., Amato, G., Barbato, F., Caliendo, G., Silipo, G. and Vittoria, A., pH-dependence of hydrophobic parameters in sets of ionizable drugs. In Seydel, J.K. (Ed.), *QSAR and Strategies in the Design of Bioactive Compounds,* Weinheim, F.R.G., 1985 pp. 290–293.

Lee, H.K., Chien, Y.W., Lin, T.K. and Lambert, H.J., Functional group contribution of bile salt molecules to partitioning of a quaternary ammonium N,N-dimethyl derivative of propranolol. *J. Pharm. Sci.,* 67 (1978) 847–849.

Leo, A. and Hansch, C., Linear free-energy relationships between partitioning solvent systems. *J. Org. Chem.,* 36 (1971) 1539–1544.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. *Chem. Rev.,* 71 (1971) 525–616.

Leo, A., Jow, P.Y.C., Silipo, C. and Hansch, C., Calculation of hydrophobic constant (log P) from ¶ and *f* constants. *J. Med. Chem.,* 18 (1975) 865–868.

Loftsson, T., The effect of ionization on partition coefficients and topical delivery. *Acta Pharm. Suec.,* 22 (1985) 209–214.

Martin, Y.C., The quantitative relationships between pK_a, ionization, and drug potency: utility of model-based equations. In Yalkowsky, S.H., Sinkula, A.A. and Valvani, S.C. (Eds.), *Physical Chemical Properties of Drugs,* Dekker, New York, 1980, pp. 49–110.

Murthy, K.S. and Zografi, G., Oil-water partitioning of chlorpromazine and other phenothiazine derivatives using dodecane and *n*-octanol. *J. Pharm. Sci.,* 59 (1970) 1281–1285.

O'Sullivan, J.F., Conalty, M.L. and Morrison, N.E., Clofazimine analogues active against a clofazimine-resistant organism. *J. Med. Chem.,* 31 (1988) 567–571.

Scherrer, R.A., The treatment of ionizable compounds in quantitative structure-activity Studies with special consideration to ion partitioning. In Magee, P.S., Kohn, G.K. and Menn, J.J. (Eds.), *Pesticide Synthesis through Rational Approaches, ACS Symp. Ser. 255,* American Chemical Society, Washington, DC, 1984 pp. 255–246.

Tsuji, A., Kubo, O., Miyamoto, E. and Yamana, T., Physicochemical properties of β-lactam antibiotics: oil–water distribution. *J. Pharm. Sci.,* 66 (1977) 1675–1679.