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Temperature dependence and thermodynamics of partitioning of clofazimine analogues in the *n*-octanol/water system

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Summary

The partition coefficient of eight N^2-substituted phenazines was measured at four temperatures (range 20–55°C) in the *n*-octanol/water system. The apparent molal partition coefficients, K'_m, were calculated from the distribution results. Plots of log K_m vs. T^{-1} were linear and the thermodynamic parameters for the solute transfer were evaluated from the Van't Hoff isochore. A relationship between log K_m and log P was derived.

Introduction

Distribution or partition studies can provide information on the solution thermodynamics of molecules of pharmaceutical interest and the thermodynamic data derived can be used for the prediction of absorption and membrane permeability. Although a large collection of data on partition coefficients exists (Hansch and Leo 1979), most work has concentrated on the free-energy based coefficient itself, which reveals little information about the underlying mechanisms involved. Such information can be obtained by a more complete thermodynamic description of the system, including the enthalpic and entropic contributions to the solute distribution. However, log P values show little variation with temperature and are often difficult to determine with the required degree of accuracy.

The distribution behaviour of solutes between organic and aqueous phases has been widely studied in medicinal chemistry (Collander, 1951; Leo et al., 1971). Various solvent pairs have been studied (Brodin et al., 1976; Dearden, 1976; Breslauer et al., 1978; Beezer et al., 1980, 1983; Rogers and Davis, 1980; Rogers and Wong, 1980; Dearden and Bresnen, 1981, 1982; James et al., 1981; Kinkel et al., 1981; Riebesehl and Tomlinson, 1981, 1984; Anderson et al., 1983; Tomlinson, 1983; Kojima and Davis, 1984; Riebesehl et al., 1984; Betageri and Rogers, 1987). Semipolar solvents have been found to yield better correlations with solute partitioning in biological membranes than non polar solvents (Leo et al., 1971; Diamond and Katz, 1974). The *n*-octanol/water system is frequently employed as a model system (Hansch and Fujita, 1964; Lien et al., 1968;

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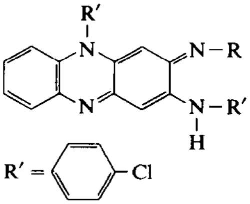
TABLE 1

The N^2-substituted phenazines



Compound	R
B628	H
B663 [a]	$CHMe_2$
B749	$(CH_2)_2NEt_2$
B826	$(CH_2)_3NEt_2$
B3779	$CH(Me)(CH_2)_3NEt_2$
B3785	$(CH_2)_3\underline{N(CH_2)_3C}H_2$
B3770	$(CH_2)_3\underline{N(CH_2)_4C}H_2$
B3640	$CH_2\underline{CH(CH_2)_2NHCH_2C}H_2$

[a] Clofazimine.

Hansch and Dunn, 1972; Roberts et al., 1977; Wallace et al., 1978).

The iminophenazine, clofazimine (British Pharmacopoeia, 1988) has been used for many years, either alone or in combination with a sulphone, dapsone, in the treatment of leprosy. The development of resistant strains of *M. Leprae* has led to the synthesis and testing of new analogues of clofazimine (O'Sullivan et al., 1988).

The N^2-substituted phenazines used in this study are shown in Table 1. These compounds are incompletely absorbed from the gastro-intestinal tract and accumulate in relatively large amounts in spleen, liver, lungs and fat, from which they are slowly released. In the present work the partition coefficients of the derivatives shown in Table 1 were determined as a function of temperature. The distribution constants (ln K_D) of these compounds have been recently reported (Fahelelbom et al., 1989) and the results analysed for enthalpy-entropy compensation effects.

The relationship between molal partition coefficients (log K_m) and those calculated based on a volume concentration scale (log P) is derived. It is found that the values of K_m and P differ by a factor involving the density ratio of the two phases.

Materials and Methods

Chemicals

The eight N^2-substituted phenazines used here were synthesised in the laboratories of the Medical Research Council of Ireland, Trinity College, Dublin. The solvents *n*-octanol and methanol were both BDH-Analar grade. The Sorenson phosphate buffer (pH 5.15) was prepared using KH_2PO_4 and Na_2HPO_4 (both from BDH). Tris buffers were used in the pK_a determination and were prepared using trishydroxymethylaminomethylmethane (BDH-Analar). The pH range for these buffers was pH 7.7–8.9. Boric acid-borax (BDH-Analar) buffer solutions were required for the higher range pH 8.5–9.7.

Instrumentation and procedure

Ionisation constants. The pK_a values were determined using a spectrometric method (Albert and Serjeant, 1971). Absorbance readings were obtained at λ_{max} (482 nm) using an SP8-100 ultraviolet spectrophotometer (Pye-Unicam). A pH-meter 26 (Radiometer-Copenhagen) was used for the determination of the pH values at 20 °C.

Partition coefficients. The partition behaviour of the iminophenazines was studied using an aqueous phosphate buffer and *n*-octanol at four different temperatures (20, 37, 45, 55 °C). The partitioning process was carried out in a thermostatically controlled shaker water bath. A shaking time of 6 h was sufficient to produce equilibration in each case. The absorbance of the organic solvent layer was determined spectrophotometrically as described above for ionisation constants. The true partition coefficients were calculated by applying an ionisation correction factor to the apparent values.

Determination of partition coefficients

The apparent partition coefficients (P') were calculated from the distribution results by employing Eqn. 1

$$P' = [x/(x_o - x)](V_w/V_o) \qquad (1)$$

where x_o and x represent the weights of solute in the organic layer before and after distribution, respectively. V_w and V_o denote the volumes of the aqueous and octanol phases, respectively. Ion-corrected values were calculated from

$$P = P'(1-\alpha)^{-1} \tag{2}$$

where α is the degree of ionisation, and is given by

$$\alpha = (1 + 10^{\mathrm{pH} - \mathrm{p}K_a})^{-1}. \tag{3}$$

Thus

$$P = P'(1 + 10^{\mathrm{p}K_a - \mathrm{pH}}). \tag{4}$$

The apparent molal partition coefficients, K'_m, were calculated from the distribution results by employing Eqn. 5:

$$K'_m = [x/(x_o - x)](w_w/w_o) \tag{5}$$

where x_o and x are defined above while w_w and w_o are the weights of the aqueous and organic phases, respectively. The ion-corrected molal partition coefficients are calculated using equations similar to Eqns. 2–4 above.

Substituting Eqn. 1 into Eqn. 5, we obtain

$$K'_m = P'(V_o w_w / V_w w_o). \tag{6}$$

Since the weight of phase $i(w_i)$ is $\rho_i V_i$ (ρ_i = density of phase i) Eqn. 6 reduces to

$$K'_m = P'(\rho_w/\rho_o). \tag{7}$$

Hence

$$\log K'_m = \log P' + \log(\rho_w/\rho_o). \tag{8}$$

The ion-corrected values are related in a similar manner, i.e.

$$\log K_m = \log P + \log(\rho_w/\rho_o). \tag{9}$$

Since, under the experimental conditions, $\mathrm{p}K_a \gg \mathrm{pH}$

$$\log K_m \cong \log K'_m + \mathrm{p}K_a - \mathrm{pH}. \tag{10}$$

The standard change in free energy, $\Delta G_{w \to o}$, due to partitioning is given by

$$\Delta G_{w \to o} = -2.303RT \log K_m. \tag{11}$$

The temperature variation of the partition coefficient provides enthalpic and entropic contributions to the free energy of transfer (Davis et al., 1976; Beezer et al., 1980; Rogers and Wong, 1980; Kinkel et al., 1981; Anderson et al., 1983; Betageri and Rogers, 1987)

Since

$$\log K_m = -\Delta H_{w \to o}/RT + \Delta S_{w \to o}/R. \tag{12}$$

a linear plot of $\log K_m$ vs. T^{-1} enables calculation of $\Delta H_{w \to o}$ from the slope and $\Delta S_{w \to o}$ from the intercept. The latter may be conveniently calculated from

$$\Delta S_{w \to o} = (\Delta H_{w \to o} - \Delta G_{w \to o})/T. \tag{13}$$

The quantities $\Delta H_{w \to o}$ and $\Delta S_{w \to o}$ represent, respectively, the change in enthalpy and entropy when one mole of solute is transferred from the aqueous phase to the n-octanol phase at infinite dilution (Katz and Diamond, 1974).

Results and Discussion

The $\mathrm{p}K_a$ values obtained at 37 °C and the corresponding values of α (Eqn. 3) at pH 5.15 are

TABLE 2

Ionisation constants ($\mathrm{p}K_a$) of the iminophenazines determined at 37°C

The degree of ionisation (α), expressed as $\log(1-\alpha)^{-1}$, at pH 5.15 is also tabulated

Compound	$\mathrm{p}K_a$	$\log(1-\alpha)^{-1}$
B628	8.480	3.330
B663	8.511	3.361
B749	8.850	3.700
B826	8.813	3.663
B3779	8.662	3.512
B3785	8.596	3.446
B3770	8.800	3.650
B3640	8.085	2.936

TABLE 3

n-Octanol/water log P values of iminophenazines at various temperatures

Compound	Temperature (K)			
	293.15	310.15	318.15	328.15
B628	3.135	3.307	3.397	3.480
B663	4.299	4.396	4.475	4.540
B749	4.274	4.853	5.010	5.244
B826	4.387	4.496	4.536	4.694
B3779	4.408	4.578	4.757	4.862
B3785	3.719	4.386	4.543	4.754
B3770	4.527	4.782	4.860	4.977
B3640	3.499	3.579	3.627	3.690

listed in Table 2. The lowest value of α in the series is that for B3640 ($\alpha = 0.999$) and so all derivatives studied are more than 99.9% ionised in the aqueous buffer solutions. The values of log P determined at each temperature using Eqn. 4 are presented in Table 3 while the log K_m values, determined using the correction factor (Table 4), are listed in Table 5.

The Van't Hoff plots of the data given in Table 5 are shown in Fig. 1. The values of $\Delta G_{w \to o}$, $\Delta H_{w \to o}$ and $\Delta S_{w \to o}$ calculated according to Eqns. 11 and 12 at the harmonic mean temperature (T_{hm}, Eqn. 14) are reported in Table 6, together with the correlation coefficient for the Van't Hoff plots.

$$T_{hm} = n / \sum_{i=1}^{n} T_i^{-1}. \qquad (14)$$

The good linearity observed (Fig. 1) indicates that the change of free energy is constant over the temperature range studied.

TABLE 4

Densities of n-octanol (ρ_o) and water (ρ_w) at various temperatures

Temperature (K)	ρ_o	ρ_w	$\log(\rho_w/\rho_o)$
293.15	0.8255	0.9970	0.0820
310.15	0.8213	0.9934	0.0826
318.15	0.8185	0.9903	0.0827
328.15	0.8149	0.9857	0.0826

TABLE 5

n-Octanol/water log K_m values of iminophenazines at various temperatures

Compound	Temperature (K)			
	293.15	310.15	318.15	328.15
B628	3.217	3.390	3.480	3.563
B663	4.381	4.479	4.558	4.623
B749	4.356	4.936	5.093	5.327
B826	4.469	4.579	4.619	4.777
B3779	4.490	4.661	4.840	4.945
B3785	3.801	4.469	4.626	4.837
B3770	4.609	4.865	4.943	5.060
B3640	3.581	3.662	3.710	3.773

The magnitudes of $\Delta G_{w \to o}$ are indicative of the spontaneity of the partition process. The values (Table 6) are all large and negative, being in the

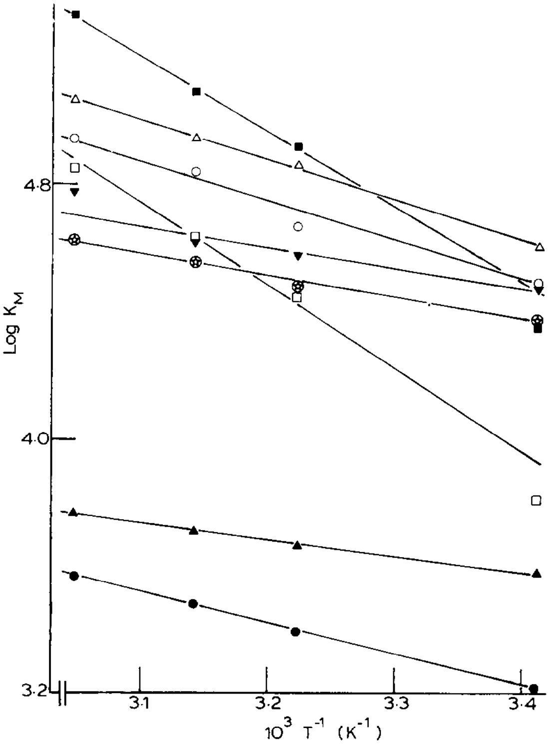


Fig. 1. Van't Hoff plots for the partitioning of N^2-substituted phenazines in the n-octanol/phosphate buffer system at pH 5.15. Solutes: ●, B628; ⊕, b663; ■, B749; ▼, B826; △, B3779; □, B3785; , ○, B3770; ▲, B3640.

TABLE 6

Thermodynamic parameters for partitioning of iminophenazines in n-octanol/water at the harmonic mean temperature (T_{hm} = 312.15 K)

Solute	r [a]	$-\Delta G_{w\rightarrow o}$ (kJ · mol^{-1})	$\Delta H^{\circ}_{w\rightarrow o}$ (kJ · mol^{-1})	$\Delta S_{w\rightarrow o}$ (J · K^{-1} · mol^{-1})
B628	0.999	20.409	18.365	124.22
B663	0.992	26.964	12.836	127.50
B749	0.898	28.755	53.431	263.29
B826	0.957	27.568	15.132	136.79
B3779	0.985	28.311	24.489	169.15
B3785	0.990	26.542	54.913	260.95
B3770	0.998	29.119	23.644	169.03
B3640	0.994	22.009	9.975	102.46

[a] Correlation coefficient of Van't Hoff isotherm.

range -20 to -30 kJ · mol^{-1}. The data indicate a high degree of lipophilicity of the solutes and a favourable energy of transfer from the aqueous to the oil phase.

For all solutes under study, with the exception of B749 and B3785, the increase in log K_m with temperature is in the range 0.005–0.013 per °C. Leo et al. (1971) reported that the temperature dependency for the *n*-octanol/water system is of the order of 0.01. The enthalpy of transfer ($\Delta H_{w\rightarrow o}$) for all derivatives studied is positive, being particularly high for B749 and B3785. In these cases, the entropy of transfer ($\Delta S_{w\rightarrow o}$) is also higher than those of the other six solutes in the series.

It is well known that the distribution constant of an organic solute increases by a factor of about four for each methylene group added to the parent molecule (Davis et al., 1974). Among the solutes under study, the pairs B749/B826 and B3785/3770 may illustrate the effect of a methylene group on the free energy of transfer. The latter pair yields a value of -2.58 kJ · mol^{-1} for $(\Delta G)_{CH_2}$. Repond et al. (1987) obtained a value of -1.96 kJ · mol^{-1} in their work on pyridylalkanamides. These observations are corroborated by the ¶ values in Table 7, calculated from the equation

$$\log P(R = X) - \log P(R = H) = ¶(X) \qquad (15)$$

The value of ¶ for B3770 exceeds that for B3785 by 0.43 which agrees quite well with the methylene group contribution for the *n*-octanol/water system (0.52) quoted by Davis et al. (1974).

TABLE 7

Values of ¶ obtained by comparing log P values of substituted derivatives with that of the parent compound (B628) at the harmonic mean temperature

Compound	¶
B663	1.096
B749	1.511
B826	1.198
B3779	1.322
B3785	1.026
B3770	1.457
B3640	0.267

The incremental thermodynamic parameters for partitioning of the compounds under investigation are given in Table 8. These have been calculated as the difference between the thermodynamic constants for the substituted and unsubstituted phenazine as listed in Table 6. These values provide a measure of the partial molal free energy,

TABLE 8

Incremental thermodynamic functions for partitioning of iminophenazines in n-octanol/water at the harmonic mean temperature (T_{hm})

Solute	$\delta\Delta G_{w\rightarrow o}$ (kJ·mol^{-1})	$\delta\Delta H_{w\rightarrow o}$ (kJ·mol^{-1})	$\delta\Delta S_{w\rightarrow o}$ (J·K^{-1}·mol^{-1})
B663	−6.555	−5.529	3.28
B749	−8.346	35.066	139.07
B826	−7.159	−3.233	12.57
B3779	−7.902	6.124	44.93
B3785	−6.133	36.548	136.73
B3770	−8.710	5.279	44.81
B3640	−1.600	−8.390	−21.76

enthalpy and entropy of the N^2-functional groups on the transfer of one mole of each compound from the aqueous phase to the *n*-octanol phase. The value of $\delta\Delta G$ is negative in all cases, indicating that substitution at the N^2-position favours transfer to the *n*-octanol phase. The values of $\delta\Delta H_{w\to o}$ and $\delta\Delta S_{w\to o}$ for B749 and B3785 are both large and positive. The most negative $\delta\Delta H_{w\to o}$ value is that for B3640, indicating that the 4-methylpiperidinyl group enhances transfer to the *n*-octanol phase. However, the strengthened interactions with *n*-octanol are somewhat offset by a negative $\delta\Delta S_{w\to o}$ value, indicating that the transfer process is entropically unfavourable. For all other compounds $\delta\Delta S_{w\to o}$ is positive.

The *n*-octanol/water solvent pair is much studied with regard to the distribution process (Hansch and Leo, 1979). It is a suitable medium for describing solute hydrophobicity, but the complexity of the system has led to its criticism as a standard state (Rytting et al., 1970). Lippold and Adel (1972) have suggested that water-centred aggregates exist in *n*-octanol with a 4 : 1 alcohol : water ratio and that a solute transferring to this phase may replace one octanol molecule in the complex (Smith et al., 1975). Moreover, linear aliphatic alcohols have been shown to exist in both monomeric and polymeric forms (Anderson et al, 1978). Octan-1-ol dissolves 4% water at saturation and is both a hydrogen-bond donor and acceptor.

Since the iminophenazines are highly lipophilic and since pH $\ll$ p*K*a, the presence of the compounds in the predominantly ionised form introduces the possiblity of the extraction of the ionised form as ion-pairs into the organic phase.

Linear relationships obtained from enthalpy-entropy compensation plots suggest a single mechanism of transfer for a series of solutes or solvents (Leffler and Grunwald, 1963). When both enthalpy and entropy are derived from the Van't Hoff plot such correlations may be artefactual (Kinkel et al., 1981; Tomlinson, 1983). To avoid this problem entropy-enthalpy compensations can be established by plotting the thermodynamic parameters at the harmonic mean temperature (Eqn. 14). However, in the *n*-octanol/0.15 M NaCl solution system, no linear relationship was observed (Anderson et al., 1983). Likewise, a poor correlation was observed between $\Delta G_{w\to o}$ and $\Delta H_{w\to o}$ for β-blockers in the *n*-octanol/buffer system (Betageri and Rogers, 1987). The system under study does not exhibit true linear compensation behaviour. Direct calorimetric determinations are only suitable in examining compensation for some solvent pairs as in the case of micellisation the micellar number may be temperature dependent.

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