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Thermodynamics and mechanism of partitioning of pyridylalkanamides in *n*-octanol/water and di-*n*-butyl ether/water

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Summary

The partition coefficient of 12 isomeric and homologous pyridylalkanamides was measured at 4 temperatures in *n*-octanol/water and di-*n*-butyl ether/water. The thermodynamic results were analyzed for enthalpy–entropy compensation effects by plots of ΔG vs ΔH and by isokinetic relationships. In the DBE/water system, a single mechanism prevails, and all solutes are postulated to display the same solvation characteristics irrespective of the presence or absence of an intramolecular N ... H–N bond. In the OCT/water system on the other hand, solutes able to form an intramolecular H-bond, and those which are not, have different mechanisms of partitioning. 4-Pyridylethanamide had a slightly deviant behavior in both systems presumably due to electronic delocalization.

Introduction

The partition coefficient (as expressed by log P) is one of the most important parameters in QSAR (quantitative structure–activity relationship) studies since interactions between xenobiotics and biological systems are frequently determined by lipophilicity. However, log P values as found in the literature only describe the overall process of distribution and contain limited thermodynamic information.

Measurements of log P values at various temperatures, by unraveling enthalpic and entropic contributions to free energy changes, appear as a promising means of better understanding partition and distribution phenomena. Nevertheless, this approach raises a number of difficulties, mainly because log P values show little variation with temperature, and because they are difficult to determine with the required degree of accuracy and precision. A number of studies have been published (Anderson et al., 1983; Beezer et al., 1980, 1983; Breslauer et al., 1978; Bresnen, 1984; Brodin et al., 1976; Dearden, 1976, 1985; Dearden and Bresnen, 1981, 1982; James et al., 1981; Kinkel et al., 1981; Riebesehl and Tomlinson, 1981, 1984; Riebesehl et al., 1984; Rogers and Davis, 1980;

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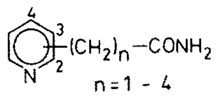

Fig. 1. Structure of investigated solutes.

Rogers and Wong, 1980; Tomlinson, 1983) particular attention being given to entropy–enthalpy compensation effects.

In the present study, we have examined the thermodynamics of partitioning of 12 homologous and isomeric pyridylalkanamides (Fig. 1). Several among these compounds are strong ligands of cytochrome P-450 (Repond, 1985; Repond et al., 1986). Two different solvent systems were chosen, namely *n*-octanol/water and di-*n*-butyl ether/water. *n*-Octanol, which dissolves ca. 4% water at saturation, is both a H-bond donor and acceptor, while di-*n*-butyl ether, a constitutional isomer of octanol with only H-bond acceptor ability, dissolves limited amounts of water. The thermodynamic results were analyzed for enthalpy–entropy compensations by two different approaches (plots of ΔH vs ΔG, and isokinetic relationships), and the results interpreted in terms of solvation and conformational behaviour.

Theoretical

The standard free energy change of drug transfer between two immiscible liquids can be expressed as (Anderson et al., 1983)

$$\Delta G = -RT \cdot \ln K_D \tag{1}$$

where K_D is the thermodynamic liquid–liquid distribution coefficient when concentrations are expressed in mole fractions. K_D is related to the familiar partition coefficient P as follows:

$$K_D = P(V_{org}/V_{aq}) \tag{2}$$

where V_{org} and V_{aq} are the molar volumes of the organic and aqueous phase, respectively. When the change in enthalpy ΔH is constant over the temperature range examined, or in other terms, when the change in heat capacity can be neglected, then ΔH can be obtained from the Van't Hoff isotherm:

$$\ln K_D = -(\Delta H/RT) + (\Delta S/R) \tag{3}$$

or

$$\log P = -(\Delta H/2.303RT) + (\Delta S/2.303R) - \log(V_{org}/V_{aq}) \tag{4}$$

It is a common observation that changes in enthalpy are linearly correlated with changes in entropy (Tomlinson, 1983). The existence of such proportionality is believed to indicate that a single mechanism predominates in the process investigated (Leffler and Grunwald, 1963). However, when both enthalpy and entropy are derived from the same equation (Van't Hoff plot), these correlations may be largely artefactual (Kinkel et al., 1981; Krug et al., 1976). To avoid the latter problem entropy–enthalpy compensations can be established by plotting ΔH vs ΔG at the harmonic mean temperature (T_{hm}), as suggested by several groups (Anderson et al., 1983; Krug et al., 1976; Tomlinson, 1983). The harmonic mean temperature is defined as:

$$T_{hm} = n/\sum_{1}^{n}(1/T_i) \tag{5}$$

An enthalpy–entropy compensation exists when the following relationship is verified:

$$\Delta G = \Delta H(1 - T/\beta) \tag{6}$$

where β is a compensation proportionality factor between ΔH and ΔS; this factor has the dimension of absolute temperature, and is also termed the isokinetic, the isoequilibrium or the compensation temperature. In isoenthalpic series (ΔH constant), β equals zero, whereas in isoentropic series (ΔS constant) β approaches infinity (Tomlinson, 1983).

Another way to explore the existence of linear entropy–enthalpy compensations is the statistical treatment proposed by Exner (Exner, 1973, 1974). This approach is based on the argument that in the case of linear enthalpy–entropy compensation a common point of intersection exists in the Arrhenius or Van't Hoff plot. Exner's method allows to directly determine the characteristic parameters of the isokinetic relationship, i.e. isokinetic temperature and isokinetic rate or equilibrium constant. Furthermore this test is not restricted to the temperature range of measurement.

Exner's analysis of isokinetic (isoequilibrium) relationships, as slightly modified by Linert (Linert et al., 1982, 1985), can be summarized as follows. For a given set of straight lines in the Arrhenius or Van't Hoff plot the common point of intersection is calculated at an arbitrary position along the abscissa. For this point one calculates the sum of squares of deviations of the measurement points from the corresponding straight line, including this point of intersection S_x (called the constrained lines). By varying the common point of intersection along the abscissa (the $1/T$ axis) the minimum of S_x, is determined and denoted by S_0. By means of an F-test this value (somewhat modified to get a nearly F-distributed variable) is compared with the sum of deviations of the measured points from the unconstrained lines S_{00}. The existence of an isokinetic relationship is usually accepted when the hypothesis, "there is a common point of intersection", cannot be rejected at a certain significance level, i.e. when F $(=((S_0 - S_{00})f_2)/S_\infty \cdot f_1))$ is smaller than the corresponding F-table value. The alternative hypothesis, "there is no common point of intersection", for which the statistical test is much stricter, can be rejected when the value $F' = 1/F$ is greater than the F-table value with f_2 and f_1 degrees of freedom. The equations of the statistical treatment as well as the related computer programs have already been published (Linert et al., 1982, 1985).

Materials and Methods

Materials

Pyridylalkanamides were synthesized as already described (Mayer and Testa, 1982). All other chemicals were from commercial sources. n-Octanol purissimum (OCT) (Fluka, Buchs, Switzerland) was used as purchased, while di-n-butyl ether pro analysis (DBE) (Fluka, Buchs, Switzerland) was passed over a basic silica activity I (Merck, Darmstadt, F.R.G.) column to remove peroxides (Dasler and Bauer, 1946).

Analytical

An HPLC Anacomp 220 system (Kontron, Zürich, Switzerland) consisting of a LC 414 T pump, an automatic injector RSI 660 and a Uvikon 730 SLC UV-detector (operating at 262 nm) was used. Columns of 15 cm length with LiChrosorb RP18, particle size 10 μm, or Hypersil ODS, particle size 5 μm, were used for the OCT/H_2O and DBE/H_2O system, respectively. The flow rate was 1 ml/min. The mobile phase for measuring partition coefficients in OCT/H_2O consisted of an aqueous solution containing 0.1 M NaCl, 0.15% (v/v) n-decylamine (El Tayar et al., 1985) and 15% MeOH. The pH was adjusted to pH = 8 with NaOH. This phase was saturated with n-octanol at the working temperature. A similar mobile phase was used for the DBE/H_2O experiments, the difference being that only 10% MeOH was added and that it contained 0.3% n-decylamine.

Partition coefficients

The aqueous phase contained NaCl 0.1 N and traces of NaOH to reach a pH of 8.0. The organic and aqueous phases were mutually saturated (4 h) and then separated after settling for 48 h. The pH was adjusted when necessary. The solubility of H_2O in OCT ranges from 3.75 to 4.25% (v/v) between 20 and 40 ° C (Beezer et al., 1980; Brodin et al., 1976; Kinkel et al., 1981). We have measured the solubility of H_2O in DBE by Karl Fischer titration (E 547 automatic titrator, Metrohm, Herisau, Switzerland). The results are presented in Table 1, and appear to compare well with literature values (Riddick and Bunger, 1970). The plot of log concentration vs $1/T$ is linear ($r = 0.996$).

The ratios of organic: aqueous phase were chosen so as to obtain 40–70% extraction from the starting phase, i.e. 2–5 ml water and 5–8 ml octanol in the OCT/H_2O system. In the DBE/H_2O system, however, and despite 200:1 volume ratios, only 5–25% of the solute was ex-

TABLE 1

Water content of di-n-butyl ether at various temperatures

	20 °C	25 °C	27 °C	34 °C	40 °C
Mean	0.119	0.128	0.134	0.148	0.168
S.D.	±0.005	±0.003	±0.004	±0.004	±0.007

The results are expressed in % (v/v) and are the means ± S.D. of 5 measurements.

tracted. The solvent systems were placed in 10 ml Sovirel tubes (OCT/H_2O) or 300 ml Erlenmeyer flasks (DBE/H_2O).

The partition experiments were carried out in a water bath (WTR 1, Ismatec, Zürich, Switzerland) at 20.0, 27.0, 34.0 and 40.0 °C (±0.1 °C) under gentle shaking for 2 h (OCT/H_2O) or 4 h (DBE/H_2O). The aqueous phases at time zero contained the amide under study at a concentration of 10^{-3} M, preliminary experiments had shown log *P* values to be independent of concentration below and above 10^{-3} M.

After settling of the phases at the temperature of study, an aliquot of the aqueous phase was removed avoiding contamination by the organic phase. An internal standard (another pyridylalkanamide) was added, and the concentration of solute in the aqueous phase was measured by HPLC as described above. This method has the advantage of being faster, more efficient and more precise than direct UV measurements of concentration.

No amide hydrolysis was detectable; when both phases were analyzed, 100% (±3%) of solute were recovered. When calculating the partition coefficients in the DBE/H_2O system, a correction was introduced to account for the changes in the volumes of the phases with temperature. In the OCT/H_2O system, temperature effects on volumes could be neglected.

The investigated compounds have pK_a values ranging from 4.34 to 5.92 at 20 °C (Mayer and Testa, 1982), meaning that the protonated forms were present in negligible proportion ($< 0.5\%$) and that no correction for ionization was necessary.

TABLE 2

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

Solute	293.15 K	300.15 K	307.15 K	313.15 K
2[a]–1[b]	−0.638 ± 0.055[c]	−0.635 ± 0.037	−0.590 ± 0.034	−0.552 ± 0.058
2–2	−0.231 ± 0.018	−0.171 ± 0.052	−0.126 ± 0.063	−0.108 ± 0.037
2–3	0.020 ± 0.021	0.061 ± 0.009	0.083 ± 0.013	0.113 ± 0.013
2–4	0.364 ± 0.003	0.399 ± 0.021	0.419 ± 0.020	0.452 ± 0.007
3–1	−0.640 ± 0.049	−0.592 ± 0.032	−0.562 ± 0.034	−0.537 ± 0.033
3–2	−0.286 ± 0.032	−0.254 ± 0.028	−0.233 ± 0.029	−0.214 ± 0.035
3–3	0.051 ± 0.010	0.072 ± 0.028	0.086 ± 0.031	0.095 ± 0.043
3–4	0.385 ± 0.052	0.414 ± 0.037	0.440 ± 0.036	0.460 ± 0.034
4–1	−0.626 ± 0.023	−0.591 ± 0.041	−0.574 ± 0.042	−0.564 ± 0.050
4–2	−0.352 ± 0.071	−0.317 ± 0.068	−0.301 ± 0.086	−0.285 ± 0.062
4–3	0.039 ± 0.024	0.056 ± 0.042	0.065 ± 0.038	0.094 ± 0.028
4–4	0.407 ± 0.028	0.433 ± 0.026	0.472 ± 0.011	0.471 ± 0.009

[a] Position of side-chain attachment to the pyridine ring.

[b] Number of methylenes in side-chain.

[c] Mean ± S.D. of 3–7 experiments.

TABLE 3

Di-n-butyl ether / water log *P values of pyridylalkanamides at various temperatures*

Solute	293.15 K	300.15 K	307.15 K	313.15 K
2[a]–1[b]	−3.052 ± 0.040[c]	−2.931 ± 0.089	−2.871 ± 0.066	−2.809 ± 0.036
2–2	−2.976 ± 0.031	−2.876 ± 0.300	−2.751 ± 0.173	−2.667 ± 0.150
2–3	−2.806 ± 0.118	−2.647 ± 0.076	−2.527 ± 0.032	−2.422 ± 0.064
2–4	−2.599 ± 0.100	−2.485 ± 0.015	−2.405 ± 0.029	−2.257 ± 0.007
3–1	−3.717 ± 0.211	−3.716 ± 0.123	−3.788 ± 0.199	−3.762 ± 0.157
3–2	−3.213 ± 0.116	−3.030 ± 0.094	−3.005 ± 0.182	−2.949 ± 0.244
3–3	−2.987 ± 0.005	−2.906 ± 0.028	−2.719 ± 0.016	−2.701 ± 0.037
3–4	−2.527 ± 0.047	−2.434 ± 0.023	−2.269 ± 0.044	−2.207 ± 0.040
4–1	−3.649 ± 0.093	−3.609 ± 0.095	−3.555 ± 0.154	−3.467 ± 0.035
4–2	−3.240 ± 0.199	−3.194 ± 0.016	−3.135 ± 0.116	−3.138 ± 0.072
4–3	−2.978 ± 0.012	−2.907 ± 0.067	−2.815 ± 0.017	−2.793 ± 0.038
4–4	−2.593 ± 0.012	−2.496 ± 0.010	−2.403 ± 0.037	−2.328 ± 0.018

[a,b] See Table 2.

[c] Mean ± S.D. of 6–20 experiments.

Results

Log P and thermodynamic parameters

The log *P* values of the pyridylalkanamides determined at different temperatures in the OCT/water and DBE/water system are presented in Tables 2 and 3, respectively. The Van't Hoff plots of these data (Figs. 2, 3) show that in both systems log *P* decreases linearly with increasing $1/T$, excepting 3-pyridylethanamide which in DBE/water displays temperature-independent partitioning.

The values of ΔG, ΔH and ΔS calculated according to Eqns. 1–4 for 303.21 K (the harmonic mean temperature, Eqn. 5), are reported in Tables 4 and 5 together with correlation coefficient for the van't Hoff plots.

Comparison of log *P values in the two solvent systems*

Collander's equation (Collander, 1951) links partition coefficients measured in different solvent systems.

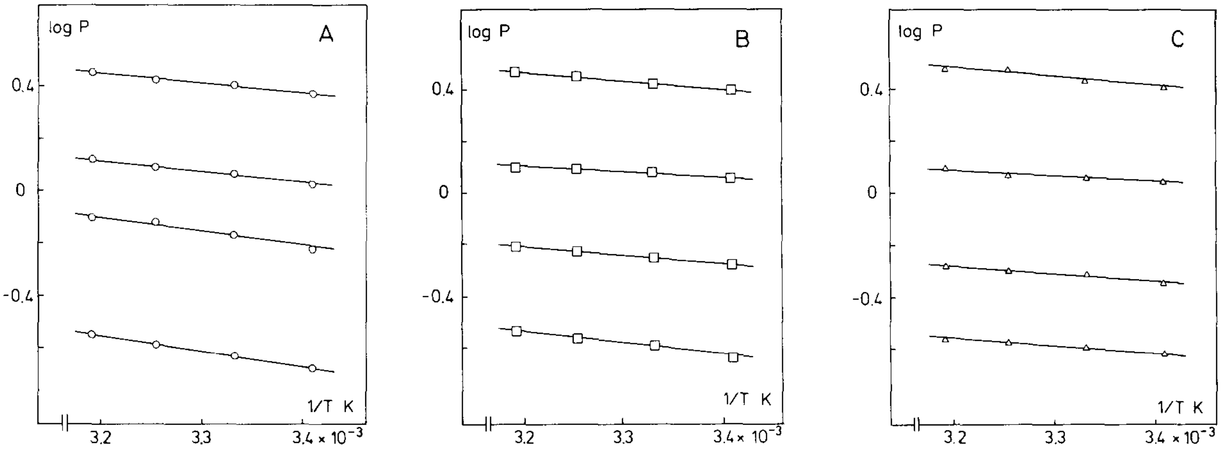

Fig. 2. Van't Hoff plots for the partitioning of 2-, 3- and 4-pyridylalkanamides (A, B and C, respectively) in the *n*-octanol/water system.

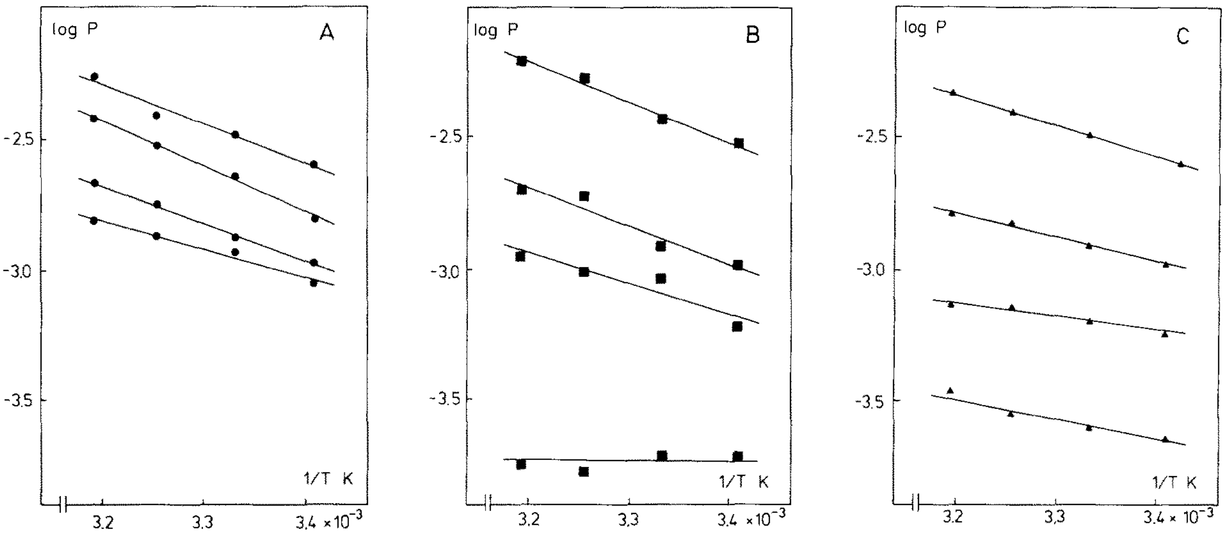

Fig. 3. Same as Fig. 2, but in the di-*n*-butyl ether/water system.

$$\log P_1 = a \cdot \log P_2 + b \qquad (7)$$

However, good correlations are obtained only when the two organic phases are not too dissimilar and involve comparable solute–solvent interactions (Leo et al., 1971; Leo and Hansch, 1971).

Plots of the log P_{oct} versus log P_{DBE} values are linear (Fig. 4) with fair correlation coefficients ($r = 0.958$, 0.991 and 0.990 for 2-, 3- and 4-pyridylalkanamides, respectively). According to Zaslavsky et al. (1981), the slope in Collander's equation expresses the ratio of free energy for the transfer of a CH_2 group from water to the organic phase. Fig. 4 shows that the 2-pyridylalkanamides

TABLE 4

Thermodynamic parameters of pyridylalkanamides in n-octanol/water at the harmonic mean temperature (T_{hm} = 303.21 K)

Solute	r [c]	ΔG ($kJ \cdot mol^{-1}$)	ΔH ($kJ \cdot mol^{-1}$)	ΔS ($J \cdot mol^{-1} \cdot K^{-1}$)
2 [a]–1 [b]	0.999	3.57	11.56	26.35
2–2	0.986	0.96	9.90	29.49
2–3	0.996	−0.40	7.95	27.53
2–4	0.996	−2.37	7.46	32.40
3–1	0.993	3.38	8.96	18.40
3–2	0.996	1.43	6.20	15.72
3–3	0.989	−0.44	3.81	14.00
3–4	0.994	−2.47	6.76	30.44
4–1	0.970	3.42	5.41	6.59
4–2	0.984	1.82	5.74	12.91
4–3	0.975	−0.37	4.56	12.26
4–4	0.943	−2.59	6.06	28.53

[a,b] See Table 2.

[c] Correlation coefficient of Van't Hoff isotherm (Eqn. 4).

TABLE 5

Thermodynamic parameters of pyridylalkanamides in di-n-butyl ether/water at the harmonic mean temperature (T_{hm} = 303.21 K)

Solute	r [c]	ΔG ($kJ \cdot mol^{-1}$)	ΔH ($kJ \cdot mol^{-1}$)	ΔS ($J \cdot mol^{-1} \cdot K^{-1}$)
2 [a]–1 [b]	0.988	16.93	20.84	12.89
2–2	0.999	16.35	27.50	36.76
2–3	0.998	15.09	33.41	60.41
2–4	0.987	14.15	28.73	48.10
3–1	–	21.58	0	−71.17
3–2	0.930	17.70	22.23	14.95
3–3	0.968	16.42	27.61	36.90
3–4	0.911	13.69	29.79	53.10
4–1	0.970	20.76	13.97	−22.38
4–2	0.936	18.46	9.46	−29.62
4–3	0.986	16.69	16.70	0.04
4–4	0.999	14.26	23.34	29.97

[a,b] See Table 2.

[c] Correlation coefficient of Van't Hoff isotherm (Eqn. 4).

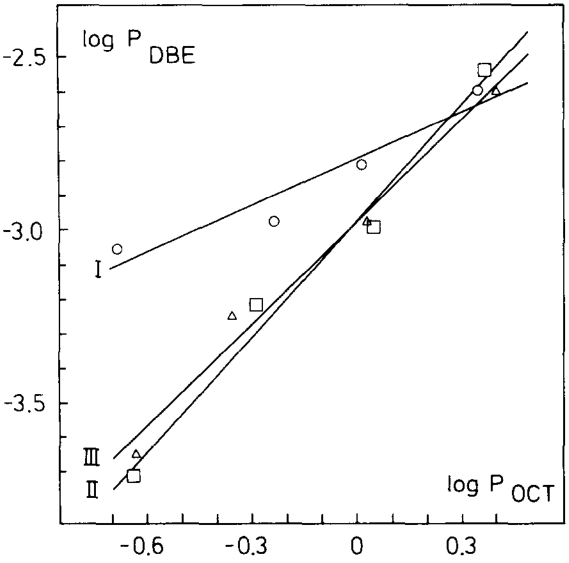

Fig. 4. Comparison of log P values obtained at 20 °C in the two solvent systems. The 2-, 3- and 4-pyridylalkanamides are represented by ○, □ and △, respectively, and the calculated lines are designated by I, II, III.

clearly differ from the two other series. For the 3- and 4-pyridylalkanamides the slope is close to one (1.11 and 0.97, respectively) indicating that the contribution of the methylene group to the free energy of transfer is the same for the two solvent systems. The slope for the 2-pyridylalkanamides, however, is only about half of that of the other series, suggesting different partition mechanisms.

Comparison of thermodynamic parameters

The good linearity obtained for the Van't Hoff plots (Figs. 2, 3) indicates that the change of free energy is constant over the range of temperatures investigated. This shows that the simple Van't Hoff model, which neglects the heat capacity contribution, can be applied to our series. The same observation was made for other solutes (resorcinol monoethers (Beezer et al., 1980), substituted phenols (Kojima and Davis, 1984; Rogers and Wong, 1980), substituted benzenes (Kinkel et al., 1981)).

The free energy of partitioning ΔG_{TRm} (calculated at the harmonic mean temperature) decreases linearly with increasing number of CH_2 groups (nC) in the side-chains (Fig. 5). The linear

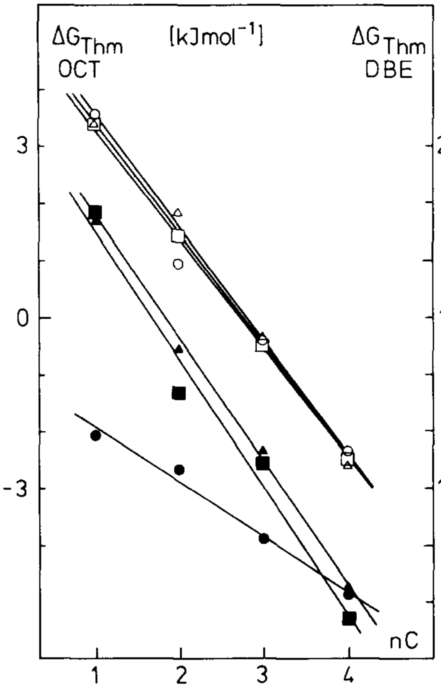

Fig. 5. Plot of free energy at the harmonic mean temperature (ΔG_{Thm}) versus number of CH_2 groups in side-chain (nC) for 2-, 3- and 4-pyridylalkanamides (○●, □■ and △▲, respectively) Open symbols OCT/water system; closed symbols DBE/water system.

regression equations are: *(a) for the OCT/water system*

$$(2\text{-})\quad \Delta G_{\mathrm{Thm}} = -1.92(\pm 0.33)nC + 5.24(\pm 0.92)$$

$$n = 4;\ r = 0.993 \qquad (8)$$

$$(3\text{-})\quad \Delta G_{\mathrm{Thm}} = -1.94(\pm 0.04)nC + 5.43(\pm 0.12)$$

$$n = 4;\ r = 0.999 \qquad (9)$$

$$(4\text{-})\quad \Delta G_{\mathrm{Thm}} = -2.02(\pm 0.22)nC + 5.62(\pm 0.59)$$

$$n = 4;\ r = 0.997 \qquad (10)$$

where (2-), (3-) and (4-) indicate the side-chain attachment and nC the number of $-CH_2-$ groups; 95% confidence intervals are given in parentheses. Clearly all three regio-isomeric series behave similarly in the OCT/water solvent system. The regression including all compounds gives a mean value for ΔG_{CH_2} equal to -1.96 ± 0.06 kJ · mol^{-1}.

(b) for the DBE/water system

$$\text{(2-)} \quad \Delta G_{Thm} = -0.960(\pm 0.190)nC + 18.0(\pm 0.5)$$

$$n = 4;\ r = 0.991 \qquad (11)$$

$$\text{(3-)} \quad \Delta G_{Thm} = -2.27(\pm 0.49)nC + 22.8(\pm 1.3)$$

$$n = 4;\ r = 0.989 \qquad (12)$$

$$\text{(4-)} \quad \Delta G_{Thm} = -2.13(\pm 0.17)nC + 22.9(\pm 0.5)$$

$$n = 4;\ r = 0.998 \qquad (13)$$

Eqns. 11–13 reveal that the contribution of the methylene group to the free energy of transfer in the 2-series is about half that of the two other regio-isomeric series. The ΔG_{CH_2} mean value of 3- and 4-pyridylalkanamides in the DBE/water system (-2.2 ± 0.15 kJ · mol^{-1}) is close to that determined for the OCT/water system. Furthermore, 2-pyridylpentanamide presents a similar behavior compared to its two regio-isomers.

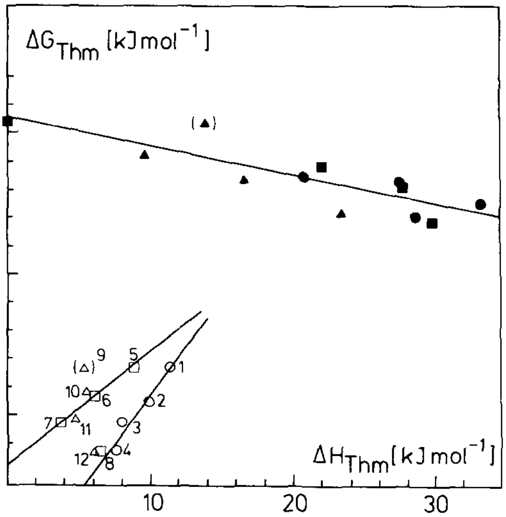

Fig. 6. Search for enthalpy–entropy compensation in the OCT/water (open symbols) and DBE/water (closed symbols) systems; 2-, 3- and 4-pyridylalkanamides: ○●, □■ and △▲, respectively. The 3 regression lines are described by Eqns. 14–16. 4-Pyridylethanamide is shown in parentheses.

The search for an enthalpy–entropy compensation by plotting ΔG_{Thm} versus ΔH_{Thm} is shown in Fig. 6. Clearly such a compensation effect exists in the DBE/water system. Excluding 4-pyridylethanamide from the regression due to its slightly deviant behavior (see later and Discussion), the following equation is obtained:

$$\Delta G_{Thm} = -0.202(\pm 0.038)\Delta H_{Thm} + 20.9(\pm 0.9)$$

$$n = 11;\ r = 0.873 \qquad (14)$$

The situation in the OCT/water system appears more complex. Here, *two* lines are seen. The first connects all 2-pyridylalkanamides as well as 3- and 4-pyridylpentanamide according to:

$$\Delta G_{Thm} = 1.16(\pm 0.14)\Delta H_{Thm} - 10.2(\pm 1.2)$$

$$n = 6;\ r = 0.974 \qquad (15)$$

The second line connects the remaining compounds, excluding, however, again 4-pyridylethanamide:

$$\Delta G_{Thm} = 0.778(\pm 0.136)\Delta H_{Thm} - 3.39(\pm 0.83)$$

$$n = 5;\ r = 0.958 \qquad (16)$$

These two lines are statistically different having significantly different slopes and intercepts.

Analysis of isokinetic relationships

Exner's analysis has been performed using all individually measured log P values. In the DBE/water system, a common intersection point exits for all compounds, except 4-pyridylethanamide. This can be substantiated for both statistical hypotheses ($F = 0.419$; $f_1 = 10$, $f_2 = 237$).

Analysis of the OCT/water partition data reveals the presence of two groups of compounds with distinct isokinetic temperatures. The first group is composed of the 2-pyridylalkanamides plus 3- and 4-pyridylpentanamide ($F = 0.204$; $f_1 = 5$, $f_2 = 82$). The remaining compounds (excluding the outlier 4-pyridylethanamide) constitute the second group ($F = 0.048$; $f_1 = 4$, $f_2 = 78$). These analyses were in agreement with the $\Delta G/\Delta H$ plots which showed a single line for the DBE/water data and two lines for the OCT/water system. In all analyses, the 4-pyridylethanamide had to be excluded since it does not follow the entropy (OCT/water) or enthalpy behavior (DBE/water) of its homologs.

The position of the isokinetic temperatures in the OCT/water system ($1/T_{\mathrm{iso}}$ approaches zero) indicates an isoentropic partitioning behavior for both groups. For the DBE/water system, the isokinetic temperature of 247.5 K ($1/T_{\mathrm{iso}} = 4.04 \times 10^{-3}$) shows that the entropic effects must be of considerable importance. The fact that T_{iso} is found below the mean experimental temperature implying that the selectivity is increased with temperature in contrast to the usual chemical behaviour (Exner, 1974).

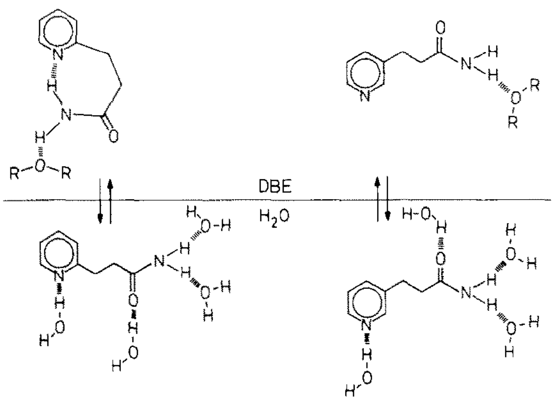

Fig. 7. Hypothetical mechanism for the transfer of pyridylalkanamides between DBE and water. Only polar (H-bond) solute–solvent interactions are shown, but not nonpolar (van der Waals) interactions. 4-Pyridylalkanamides are not represented but are considered to behave like their 3-regio-isomers.

Discussion

A number of findings are reported in the present study:

(a) Adding a CH_2 group in the side-chain of pyridylalkanamides increases their free energy of transfer by ca. 2 kJ · mol^{-1} in both the OCT/water and DBE/water system. Exceptions are the 2-pyridylalkanamides in DBE/water (ca. 1 kJ · mol^{-1}).

(b) Analyses of enthalpy–entropy compensation by ΔG versus ΔH plots and by isokinetic relationships yield identical conclusions, namely, (i) 4-pyridylethanamide has a slightly deviant behaviour in both systems. This behavior will be discussed later; (ii) all remaining 11 solutes partition between DBE and water by a single mechanism, the entropy differences in the series being larger than the enthalpy differences; and (iii) in the OCT/water system two mechanisms of transfer are indicated, one for the 2-pyridylalkanamides plus 3- and 4-pyridylpentanamide, the other for the 5 remaining solutes. In both subseries, however, enthalpy differences are larger than entropy differences.

The following mechanistic interpretation is offered to account for these results. If we first consider the DBE/water system, a single mechanism prevails for all compounds (excepting 4-pyridylethanamide). In the aqueous phase, 4 molecules of water can form H-bonds with a molecule of any pyridylalkanamide, whereas in DBE only one solvent molecule is believed to be able to bind by a H-bond, irrespective of the presence or absence of an intramolecular H-bond (Fig. 7). The bulkiness of DBE presumably prevents two molecules from binding to the NH_2 group. In this solvent of low polarity, intramolecular H-bonds are favored. If this is the case for 2-pyridylalkanamides as shown in Fig. 7, then the solvent-accessible area of each CH_2 group in the side-chain must be markedly decreased. This effect is believed to account for the smaller ΔG_{CH_2} values displayed by 2-pyridylalkanamides as compared to their 3- and 4-regio-isomers. The fact that entropy variations are of importance in the DBE/water system (see above) indicates that non-polar solute–solvent interactions play a predominant role in DBE, a fact compatible with the above proposals.

In the OCT/water system, 2-pyridylalkanamides plus 3- and 4-pyridylpentanamide behave differently than the other solutes. Interestingly, these compounds are the only ones in our series sterically able to form intramolecular N...HN bonds as revealed by molecular models. However, such intramolecular H-bonded conformations have a low probability of existence in water-saturated octanol. Nevertheless, the equilibrium must exist, and it must be temperature-dependent since different solute–solvent interactions occur (see Fig. 8). We therefore conclude that in the OCT/water system thermodynamically different partition behavior must be displayed by those pyridylalkanamides able to form an intramolecular H-bond and those which cannot.

As mentioned earlier, 4-pyridylethanamide was excluded from the analyses of ΔG versus ΔH plots and isokinetic relationship since its slightly deviant behaviour makes it a statistical outlier. We postulate that electronic delocalization (as shown in Fig. 9) causes this compound to be distinctly more polar than its isomers and homologs. In particular, the methylene protons should be more acidic than in the two isomers. As a result of such increased polarity, the compound can be expected to have distinct solvation characteristics.

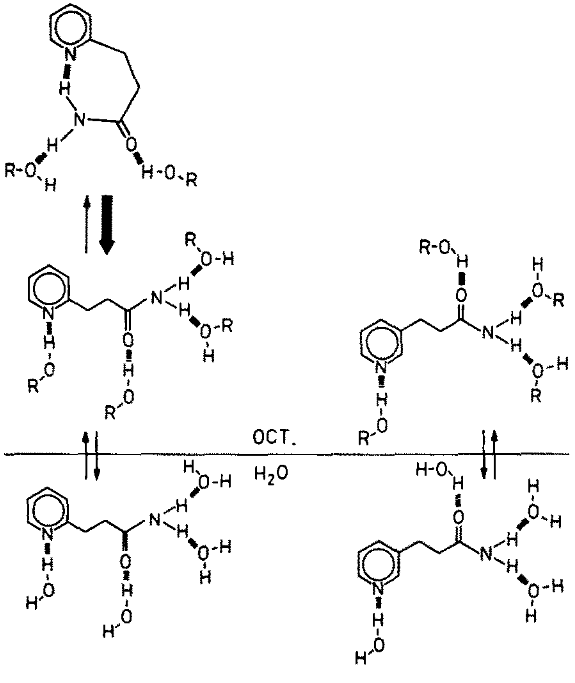

Fig. 8. Hypothetical mechanisms for the transfer of pyridylalkanamides between *n*-octanol and water. Only polar (H-bond) solute–solvent interactions are depicted but not non-polar (van der Waals) interactions. 2-Pyridylalkanamides (and 3- and 4-pyridylpentanamide (not shown)) are hypothesized to display a temperature-dependent conformational equilibrium in octanol whereas the remaining 3- and 4-pyridylalkanamides do not. R = *n*-octyl or H.

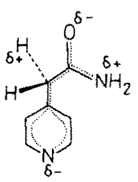

Fig. 9. Postulated electronic delocalization in 4-pyridylethanamide.

In conclusion, the present study establishes that solvation and intramolecular interactions markedly influence partitioning and account for differences between regio-isomeric solutes. The conformational differences between 2-pyridylalkanamides and their isomers may help to explain marked differences in the binding characteristics of these compounds to cytochrome P-450 (Repond et al., 1986).

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