

Quantitative structure-activity relationships: the Van't Hoff heats of transfer of resorcinol monoethers from water to n-octanol

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The partition coefficients of resorcinol and some resorcinol monoalkyl-ethers from water to n-octanol have been measured as a function of temperature. These data are used to evaluate ΔG , ΔH and ΔS for the solute transfer process. The data suggest direct calorimetric measurement of these parameters should be performed. Correlation with calorimetrically determined 'biological response' will permit a more detailed and precise commentary upon the quantitative structure-activity relationships for these compounds.

Inspection of the literature describing partition coefficient measurements for materials partitioned between water and n-octanol suggests that little attention is normally given to the precise temperature at which observations are made (Leo et al 1971; Fujita et al 1964). The reason for this lack of attention may, in part, be due to the lengthy experimentation and tedium of recording such data over a wide range of environmental and system conditions. Recently, a more rapid method has been described and its implications for pharmaceuticals briefly discussed (Davis et al 1976). For systems of pharmaceutical interest, particularly with respect to quantitative structure-activity relationships (QSAR) amongst drugs, the inherent uncertainty in 'biological response' may render unnecessary any greater degree of precision in the partition coefficients (Leo et al 1971).

The measurement of biological response, however, has often been a subjective element but should more accurate quantitative measurements of such responses be available, this might well justify more detailed analysis of the factors that contribute to the partition coefficient data as measured. For compounds that inhibit the growth of yeasts and bacteria such measurements are already available (Beezer et al 1977, 1979; Beezer & Chowdhry 1980a). In particular, the microcalorimetric method of Beezer et al (1977, 1979) has been shown to be more reproducible ($\pm 3\%$), more sensitive (0.5 I.U. ml⁻¹), and more rapid (ca 1 h) than the classical agar

diffusion method ($\pm 5-10\%$, 20 I.U. ml⁻¹, ca 16 h respectively) for the bioassay of polyenes.

Thus a main reason (Leo et al 1971) for not measuring partition coefficients as a function of temperature in n-octanol-water systems may no longer be valid. Moreover, such studies would make accessible the estimation of the enthalpy and entropy contributions involved in the partition of drugs between n-octanol and water and would permit calculation of the 'internal' and 'external' contributions to these parameters, i.e. to solvent-solute interactions (Hepler 1969; James 1974; Krishnan & Friedman 1976).

In an attempt to extend 'Hansch' analysis into these areas this paper reports the measurement of the partition coefficients of the monoethers of resorcinol as a function of temperature. These data are used to calculate the Gibbs free energy, Van't Hoff enthalpy and entropy of transfer for these compounds from water to n-octanol at 298 K. The antibacterial activity of these compounds has previously been correlated with their *calculated* partition coefficients (Lien et al 1968). The measurement of the antibacterial activity by microcalorimetry is described by Beezer et al (1981).

The thermodynamic parameters, ΔH , ΔS and ΔG for the transfer of a molecule from water to, in this case, n-octanol may permit discussion of the role in the partition of drugs, of such factors as solvation, relative magnitude of the energy of solvation, group transfer parameters and the effect of conformation upon solvation (Allawala & Riegelman 1954; Pimental 1960; Hegna 1977).

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MATERIALS AND METHODS

Materials

The methoxy and ethoxy derivatives of resorcinol were purchased from Eastman Kodak Co. Ltd. and were repeatedly distilled under vacuum before assay. The other resorcinol monoalkyl ethers were prepared by dissolving equimolar amounts of resorcinol and alkyl bromide in the appropriate alcohol followed by gentle boiling under reflux. The theoretical amount of a 25% aqueous potassium hydroxide solution was added drop by drop during 3 h followed by boiling under reflux for a further 2 h. Small quantities of diethers were formed in these reactions particularly with the higher members of the series for which prolonged reaction times were necessary. The reaction mixture was allowed to cool, poured onto ice, acidified and extracted with ether. Excess resorcinol was removed by repeated washing with water and the monoalkyl ethers extracted into potassium hydroxide solution. Acidification of the alkaline solution followed by ether extraction yielded the alkyl ethers which were distilled several times under reduced pressure.

All the monoethers were assayed for purity by g.l.c. on a Perkin-Elmer Gas Chromatograph F33 fitted with a 1 m column containing 3% SE30 on Chromasorb W 80-100 mesh (oven temperature 110 °C; injector/detector temperature 150 °C; carrier gas N₂ at 20 p.s.i.; mixture of hydrogen (17 p.s.i.) and air (25 p.s.i.) used for flame ionization detector). N.m.r. spectroscopy was used to confirm the structures of the compounds and to detect impurities if present. n-Octanol (specially pure grade, BDH Ltd.) was used for the determination of partition coefficients and for determining the mutual solubilities of octanol and water.

Methods

The amount of water present in n-octanol at saturation was determined at 288, 293, 303 and 308 K on a Rayleigh differential refractometer fitted with a thermostatically controlled cell holder, and compared with Karl Fischer titration results. Rayleigh differential refractometry was used for convenience; calibration curves were constructed at each of the temperatures by making up a range of concentrations of water in n-octanol up to the saturation concentration. The difference in refractive index between such a solution and pure octanol was read off from the instrument and plotted against the concentration of water. Measurements on each solution were repeated until two consecutive readings were obtained which differed by less than $\pm 0.5\%$ of

the observed values. All solutions were kept at the required temperature in a water bath maintained at ± 0.1 K of the required temperature. Saturated solutions were made up by shaking water in n-octanol vigorously for 1 min and then placing in the appropriate water bath. Over the next 24 h each solution was shaken twice for 30 s then replaced in the water bath and left for a further 24 h to complete separation.

The concentration of n-octanol in water is quoted (Leo & Hansch 1971) as 0.0045 M at 298 ± 0.1 K. The difference in refractive index between pure distilled water and that saturated with n-octanol is only just measurable on a Rayleigh refractometer.

The amount of water present in n-octanol at saturation was determined to be 5.075 ± 0.01 g per 100 g of octanol, a value that remained constant over the range 288-308 K. At 298 K this represents a 2.33 M solution in n-octanol; this compares favourably with the published value of 2.30 M determined by Karl Fischer titration (Leo & Hansch 1971).

Partition coefficients were determined for each monoether and for resorcinol at a range of concentrations at each of the five temperatures. A range of concentrations of each compound was made up in n-octanol (saturated with water), 10^{-3} - 10^{-1} M for resorcinol and the methyl and ethyl ethers, 10^{-2} - 10^{-1} M for the C₃-C₅ ethers. Measured volumes of such solutions were mixed with approximately equal volumes of distilled water (saturated with n-octanol) in 25 ml stoppered flasks and shaken at the required temperatures (± 0.1 K) for 24 h. The phases were then separated and subjected to high speed centrifugation for 20 min to complete the separation. Measured volumes of each phase were then diluted with the appropriate solvent for analysis.

Solutions of all compounds in each of the solvents (n-octanol saturated with water and water saturated with n-octanol) were used to construct calibration curves for spectrophotometric measurement of concentrations at $\lambda_{\text{max}} = 273$ nm on a Cary 1650 spectrophotometer. Change of temperature of the solutions had no effect upon the shape of these curves.

RESULTS AND DISCUSSION

The calibration plots of refractive index vs concentration of water in n-octanol were linear and coincident at all temperatures. The mean values of the partition coefficients as a function of temperature for each compound are shown in Table 1. Table 2 shows the observed values of K_D at 298 K compared

Table 1. Mean values for $\ln K_D$ as a function of temperature.^(a)

Phenol	Temperature (K)				
	288·16	293·16	298·16	303·16	308·16
Resorcinol	2·226 ± 0·008	2·152 ± 0·035	1·970 ± 0·023	1·900 ± 0·034	1·831 ± 0·021
<i>m</i> -Methoxy	3·920 ± 0·015	3·851 ± 0·022	3·802 ± 0·027	3·748 ± 0·020	3·704 ± 0·030
<i>m</i> -Ethoxy	4·855 ± 0·012 ^(b)	4·809 ± 0·013	4·748 ± 0·012	4·703 ± 0·014	4·660 ± 0·010
<i>m</i> -Propoxy	6·237 ± 0·014 ^(b)	6·205 ± 0·020	6·145 ± 0·004	6·121 ± 0·005	6·096 ± 0·016
<i>m</i> -Butoxy	7·250 ± 0·014 ^(c)	7·213 ± 0·019	7·168 ± 0·007	7·145 ± 0·019	7·122 ± 0·006
<i>m</i> -Pentoxy	8·621 ± 0·008	8·599 ± 0·009	8·554 ± 0·011	8·527 ± 0·007	8·502 ± 0·012

^(a) The standard deviations of the means of 5 observations are also shown.

^(b) T = 290·16 K.

^(c) T = 290·86 K.

Table 2. Observed and theoretical^(a) values for partition coefficients.

Compound	K_D (Molal) at 298 K	K_D (M) ^(a) at 298 K	$\log K_D$ at 298 K (expt)	$\log K_D$ at 298 K (theor)
Resorcinol	7·17	5·92	0·77	0·80
<i>m</i> -Methoxy	44·78	36·96	1·57	1·58
<i>m</i> -Ethoxy	115·5	95·3	1·98	2·08
<i>m</i> -Propoxy	466·3	384·8	2·59	2·58
<i>m</i> -Butoxy	1295·7	1069	3·03	3·08
<i>m</i> -Pentoxy	5186	4280	3·63	3·58

^(a) Calculated by Lien et al (1968).

with the theoretical values calculated by Lien et al (1968).

The graphs of \ln (partition coefficient) vs $1/T$ are all linear (the correlation coefficient and slope of the line obtained are shown in Table 3). Table 4 describes the value of ΔH derived from the slope of this plot together with the values of ΔG and ΔS calculated at 298 K from standard relationships (Hepler 1969).

Table 3. Values of the slope and correlation coefficient derived from plots of $\ln K_D$ vs $1/T$.

Phenol	slope	corr. coeff.
Resorcinol	1800 ± 146	0·990
<i>m</i> -Methoxy	952 ± 37	0·998
<i>m</i> -Ethoxy	869 ± 45	0·996
<i>m</i> -Propoxy	711 ± 84	0·980
<i>m</i> -Butoxy	629 ± 65	0·979
<i>m</i> -Pentoxy	613 ± 48	0·992

Fig. 1 shows the plots of ΔG , ΔS and ΔH derived from the experimental data as a function of side chain length. Not unexpectedly, resorcinol, the parent compound, with two hydroxy groups and no methylene groups does not fall into the pattern revealed by the alkyl monoethers. It thus has a large enthalpy change associated with the water-octanol transfer. The enthalpy change declines with increasing side chain substitution. This accords with the

Table 4. Values of the derived thermodynamic parameters, ΔG , ΔH , ΔS and $\Delta(\Delta G)$ for the transfer of phenols from water to *n*-octanol at 298 K.

Phenol	$-\Delta H$ (kJ mol ⁻¹)	$-\Delta G$ (kJ mol ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)	$\Delta(\Delta G)$ (kJ mol ⁻¹)
Resorcinol	15·0 ± 1·2	4·88	-33·9	
<i>m</i> -Methoxy	7·91 ± 0·3	9·42	5·0	2·35
<i>m</i> -Ethoxy	7·20 ± 0·4	11·77	15·4	3·46
<i>m</i> -Propoxy	5·92 ± 0·7	15·23	31·2	2·55
<i>m</i> -Butoxy	5·22 ± 0·5	17·78	42·3	3·41
<i>m</i> -Pentoxy	5·12 ± 0·4	21·19	54·0	

decline in H-bond strength noted for similar systems (Pimental 1960). However after *m*-butoxy phenol the enthalpies of transfer appear to become insensitive to increasing substitution. There is only a limited compensation between ΔH and ΔS in the data reported here; we note the discussions of Lumry & Rajender (1970) and Exner (1975) and do not attach any significance to this result. The compensation does, however, hold for the series *m*-methoxy to *m*-butoxy (resorcinol itself is excluded from the series) and the apparent compensation temperature is 78 K. This value is much lower than the values of 250–320 K observed for small molecule systems in water (Lumry & Rajender 1970).

Krug et al (1976a,b) have shown from statistical analysis of Van't Hoff data that linear enthalpy-entropy compensation may arise from experimental error factors alone. They point out, however, that enthalpy-Gibbs energy plots are not subject to the

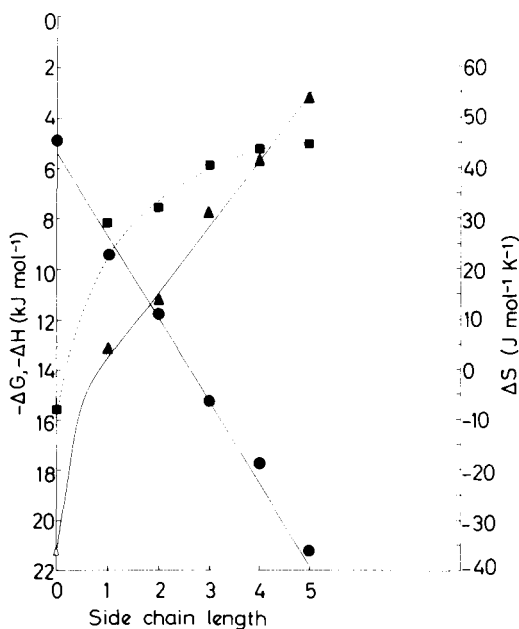


FIG. 1. Plots of ΔG , ΔH , and ΔS versus side-chain length. ΔG , \circ , ΔH , \square , ΔS , \triangle .

same constraint. The approximately linear plot for the data described in Table 4 (excluding resorcinol) indicates, according to Krug et al (1976a,b), that chemical compensation is occurring, i.e. there is a linear dependence between $\Delta H/\Delta S$ and between $\Delta H/\Delta G$ that arises not from experimental error but rather from systematic chemical effects. Thus resorcinol is noted again as the parent member of the alkyl monoether series under investigation and the results once more suggest enhanced H-bonding of resorcinol to octanol compared with the other members of the series.

Whilst the data shown do exhibit reasonable linear correlation coefficients for plots of $\ln K_D$ vs $1/T$, the graphs for butoxy and pentoxy phenols exhibit a slight convex curvature. This suggests that ΔH for these systems at least is not independent of temperature as is assumed by use of the Van't Hoff isotherm. That ΔH be independent of T is a requirement of 'Hansch' analysis since calculation from a group contribution equation is often used to calculate the partition coefficients for higher members of a series. Moreover, to employ K_D values measured 'at room temperature' with 'biological response' recorded at 310 K could be dangerous, as has been emphasized previously by, for example, Kaufman (1975). The actual variation of $\log K_D$ with T was found to be

of the order of $0.01 \log \text{unit K}^{-1}$ for resorcinol, falling to $0.003 \log \text{unit K}^{-1}$ for m-pentoxy phenol.

Indeed, inspection of the K_D data in Table 3 and the ΔG data in Table 4 reveals that for the observations reported here there is an alternation in $\Delta(\Delta G)$ on advancing through the series (see Table 4). This is in contrast to the 'assumed' increment in $\log K_D$ of 0.5 calculated by Hansch (Lien et al 1968). Moreover the values of ΔH reported in Table 4 and shown in Fig. 1 appear to be becoming asymptotic with chain length at a value of ca -5 kJ mol^{-1} from $n = 5$ onward. Thus for the higher alkyl monoethers the $\Delta(\Delta H)$ appears to be tending to zero. If this is true it would mean that the transfer process becomes increasingly entropically driven as n increases.

Both ΔH and ΔS become increasingly positive in magnitude as the chain length increases. A possible explanation of this is that the effects of these solute molecules on solvent octanol are much more drastic than they are upon water. This seems reasonable since, as the K_D values readily indicate, solubility increases markedly in n-octanol as chain length increases. Thus these solute molecules appear to have a dramatic effect on the structure of the bulk octanol leading to marked breakdown of solvent-solvent hydrogen bonding.

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