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## PARTITIONING OF ALIPHATIC ALCOHOLS IN SEPHADEX G-15 AT 25°C WITH WATER AS SOLVENT

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### SUMMARY

The partitioning of 51 alcohols, including linear, branched, cyclic and linear-cyclic types, was measured chromatographically at 25°C. There was a clear tendency for the distribution coefficients,  $K_d$ , to increase with increasing carbon chain length,  $n_c$ , and within each series the  $\ln K_d$  values were best correlated, not with  $n_c$ , but with  $(n_c)^2$ . The reasons for the increasing affinity and the quadratic relationship are discussed. It is concluded that a hydrophobic interaction (HI) is a major determinant of affinity. The considerably higher  $K_d$  values of the cycloparaffins compared with the corresponding alcohols are in accord with this concept; the values of the linear alcohols should thus be due to the HI-suppressing effect of the polar hydroxyl group (short-range effect). It is, however, unlikely that HI can account for the quadratic relationship, which, it is concluded, implies that there is another additional (energetic) interaction. The latter, it is suggested, may be due to van der Waals-London dispersion forces which increase with increasing bulk of the non-polar moiety of the molecule.

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### INTRODUCTION

The covalently cross-linked but otherwise unsubstituted dextran (Sephadex®) gels were first introduced for their exclusion properties<sup>1,2</sup>. This property is exhibited by even the most highly cross-linked members G-15 and G-10, although the molecular sieving ranges over only about 1000 daltons or less. Not unexpectedly, however, sorptive properties also become increasingly significant as the degree of cross-linking and the matrix concentration increases<sup>3</sup>, and the partitioning of a variety of solutes in Sephadex G-15 has been described previously<sup>4</sup>. It was concluded that there were two basic patterns of behaviour. Weakly polar and non-polar saturated compounds exhibited an affinity for the gel, and within an homologous series such as the 1-alkanols, the standard free energy of transfer,  $\Delta G^0$ , into the gel decreases with increasing molecular size. Affinity is not limited to weakly polar or non-polar saturated compounds but is also exhibited by polar unsaturated compounds such as urea and thiourea and also by phenols and other aromatics. Towards polar saturated com-

pounds such as oligosaccharides, polyhydric alcohols or poly(ethylene oxide)s, however, the gel behaves as a molecular sieve with  $\Delta G^\circ$  increasing linearly with increasing molecular size.

The addition of a hydroxyl group to a hydrocarbon reduces the affinity; each subsequent hydroxyl addition has a qualitatively similar effect until, in the limit of perhydroxylation, the polar polyhydric alcohols exhibit characteristic molecular-sieving behaviour. The thermodynamic changes associated with alcohol affinity are consistent with a major contribution from a hydrophobic interaction (HI). The presence of the hydroxyl group apparently suppresses HI and this is a short range effect, as evidenced, for example, by the partial molar heat capacities at infinite dilution of monofunctional solutes<sup>5</sup>.

This paper extends the scope of an earlier study on the partitioning of monofunctional aliphatic alcohols<sup>4</sup>, and it is shown that the suggested linear free energy relationship (LFER) for the transfer into the gel of 1-alkanols up to a chain length of eight carbon atoms is not valid if longer homologues are also included.

#### MATERIALS AND METHODS

The data given below were all obtained in water-swollen Sephadex® G-15 (Batch No. 2014) at  $25 \pm 0.1^\circ\text{C}$ . The  $K_d$  values were determined by liquid chromatography as described previously<sup>6</sup>. The authenticity of most of the alcohols was checked by mass fragmentography [Finnigan gas chromatographic-mass spectrometric (GC-MS) data system using electron impact (JT 70 eV)] and NMR Spectroscopy (Jeol FX90Q instrument). The most poorly soluble alcohols were washed repeatedly with water purified in a Q4 system (Millipore, Bedford, MA, U.S.A.) which was also used as eluent, to remove water-soluble impurities which may in some cases<sup>7</sup> be present in significantly high concentrations in an aqueous saturated solution of the solute. In some cases the identities of the most poorly soluble alcohols in the effluent were checked by mass fragmentography (MS 9000; LKB Instruments, Stockholm, Sweden) after gas chromatography (Carbowax 20M) of a dichloromethane extract.

The concentration of the solute in the loading solution was always less than saturated. It was checked in one case (nonan-1-ol) that the  $K_d$  value was independent of concentration. The linear flow-rate in the column ( $40 \times 0.8$  cm) never exceeded  $3 \text{ cm h}^{-1}$  and the effluent concentrations were monitored with a differential refractometer (Optilab 901; B. Philip, Stockholm, Sweden).

The dynamic distribution coefficient,  $K_d$ , is defined<sup>8</sup> as

$$K_d = \frac{V_e - V_o}{V_i} \quad (1)$$

where  $V_e$  and  $V_o$  are the peak elution volumes of a solute and a void-volume indicator (Dextran 500), respectively;  $V_i$ , the internal water volume of the swollen gel beads, can be obtained from the peak elution volume,  $V_w$ , of water. For convenience, the internal volume reference used was deuterated water<sup>9</sup>, which, however, gives an overestimate of  $V_w$  due to isotope exchange with the hydroxyl hydrogens on the gel matrix. Since the oxygens of water should not exchange with oxygens on the gel

matrix<sup>10</sup>, the elution volume of [<sup>18</sup>O]water should represent the true value of  $V_w$ . Using deuterated water as the reference, the distribution coefficient,  $K_d$ , of a solute is therefore

$$K_d = K_d^D \cdot \frac{V_e - V_o}{V_D - V_o} \quad (2)$$

where  $V_D$  denotes the peak elution volume of deuterated water, and  $K_d^D$ , the distribution coefficient of deuterated water is:

$$K_d^D = \frac{V_D - V_o}{V_w - V_o} \quad (3)$$

For Sephadex G-15,  $K_d^D$  has the value 1.075<sup>11</sup>.

## RESULTS AND DISCUSSION

$K_d$  values at 25°C for linear and branched non-cyclic alcohols are given in Table I and those of cyclic and linear-cyclic alcohols and cycloparaffins are given in Table II. Within each homologous series the affinity increases with the number of carbon atoms. Within each linear isomeric group the 1-alkanol has the highest affinity, the 2-alkanol has approximately the same affinity as the 1-alkanol with one carbon atom less and the affinity is further reduced the farther the hydroxyl is located from the terminal position. Branching also reduces the affinity. Thus, 4-methylpentan-1-ol has a lower  $K_d$  than its isomer 1-hexanol, and the  $K_d$  value of a tertiary 2-methyl-2-alkanol is similar or slightly lower than that of a 2-alkanol with one carbon atom less.

It is evident that addition of a hydroxyl group to a cycloparaffin reduces  $K_d$  and it is reasonable to assume a similar change with the linear paraffins. Further, comparing the affinity of 3-cyclohexylpropan-1-ol with that of cyclohexane shows that the  $K_d$ -increasing effect of the three linear methylene groups is completely cancelled by the  $K_d$ -reducing effect of the hydroxyl group. The affinity of each of the cycloalkanols is higher than that of the corresponding secondary linear isomers.

Comparing cycloheptanol with its isomer cyclohexylmethanol (or comparing corresponding C<sub>6</sub> isomers) it is evident that when a hydroxyl group is transferred from a secondary position on a ring to a primary position on the methyl group the affinity is increased, and the cycloalkylmethanols have roughly the same  $K_d$  values as their isomeric linear 1-alkanols.

The tertiary adamantan-1-ol has a lower affinity than its secondary isomer adamantan-2-ol, which is consistent with the behaviour of the non-cyclic alcohols.

Fig. 1 shows the relationship between the affinity and the number of carbon atoms,  $n_c$ , in the solute for 1-, 2- and 4-alkanols, 2-methyl-2-alkanols, cycloalkanols and two cycloalkanes. For comparison, the  $\ln K_d$  values of poly(ethylene oxide)s (PEOs) and perhydroxylated alcohols (POLs) are also included as they exhibit so-called molecular-sieving behaviour. The curvature of the plot for the monofunctional solutes implies a non-linear relationship between the affinity and the carbon chain length, and therefore in Fig. 2 the  $\ln K_d$  values are instead shown as a function of

TABLE I  
DISTRIBUTION COEFFICIENTS OF NON-CYCLIC ALCOHOLS AT 25°C

<i>Alcohol</i>	$K_d^*$	$SR^{**}$
Methanol	0.8714 (11)	0.0017
Ethanol	0.8724 (6)	0.0012
Propan-1-ol	0.9589 (9)	0.0021
Propan-2-ol	0.8554 (10)	0.0036
Butan-1-ol	1.0941 (7)	0.0021
Butan-2-ol	0.9710 (10)	0.0017
2-Methylpropan-1-ol	1.0274 (4)	0.0269
2-Methylpropan-2-ol	0.8423 (6)	0.0023
Pentan-1-ol	1.2780 (15)	0.0027
Pentan-2-ol	1.0944 (14)	0.0013
Pentan-3-ol	1.0700 (10)	0.0031
2,2-Dimethylpropan-1-ol	1.1694 (7)	0.0023
Hexan-1-ol	1.5271 (19)	0.0032
Hexan-2-ol	1.2750 (8)	0.0045
Hexan-3-ol	1.2210 (8)	0.0016
4-Methylpentan-1-ol	1.4636 (10)	0.0019
2-Methylpentan-2-ol	1.0691 (6)	0.0006
Heptan-1-ol	1.9052 (17)	0.0028
Heptan-2-ol	1.5256 (11)	0.0052
Heptan-3-ol	1.4398 (9)	0.0014
Heptan-4-ol	1.3996 (6)	0.0004
2-Methylhexan-2-ol	1.2245 (6)	0.0014
Octan-1-ol	2.4136 (9)	0.0067
Octan-2-ol	1.8919 (11)	0.0035
Octan-4-ol	1.6578 (12)	0.0042
2-Methylheptan-2-ol	1.4706 (6)	0.0014
Nonan-1-ol	3.2339 (8)	0.0275
Nonan-2-ol	2.4547 (8)	0.0152
Nonan-4-ol	2.0738 (9)	0.0035
Nonan-5-ol	2.0204 (9)	0.0078
Decan-1-ol	4.4491 (5)	0.0447
Decan-2-ol	3.3245 (7)	0.0205
Decan-3-ol	3.0081 (8)	0.0249
Decan-4-ol	2.7760 (13)	0.0200
Undecan-1-ol	6.279 (3)	0.148

\* The number in parentheses is the number of measurements.

\*\* SR is the standard error of the mean when  $n > 3$  and the range when  $n < 4$ .

$(n_c)^2$  which gives a very good fit for the 1-alkanols. Also included in Fig. 2 are cyclic alkanols, 2- and 4-alkanols, 2-methyl-2-alkanols and two cycloalkanes, and it seems likely that these monofunctional series also exhibit the same behaviour. Linear regression analysis was done for the 1-alkanols alone, since the numbers of solutes belonging to the other series were too few to permit a reliable statistical analysis. The  $\ln K_d$  values of the 1-alkanols are thus best represented by a second-degree equation:

TABLE II

DISTRIBUTION COEFFICIENTS OF CYCLIC AND LINEAR-CYCLIC ALCOHOLS AND CYCLOALKANES AT 25°C

Compound	$K_d^*$	SR**
Cyclopentanol	1.2127 (11)	0.0011
Cyclohexanol	1.4000 (30)	0.0010
Cyclopentylmethanol	1.5522 (22)	0.0016
Cycloheptanol	1.7386 (27)	0.0022
Cyclohexylmethanol	1.8666 (17)	0.0014
2-Methylcyclohexanol	1.5550 (6)	0.0022
3-Methylcyclohexanol	1.6017 (7)	0.0027
4-Methylcyclohexanol	1.6073 (14)	0.0016
Cyclooctanol	2.1591 (29)	0.0026
1-Cyclohexylethanol	1.9214 (10)	0.0030
4-Ethylcyclohexanol	2.0345 (6)	0.0033
Cyclooctylmethanol	3.2414 (6)	0.0044
3-Cyclohexylpropan-1-ol	3.0740 (7)	0.0203
Cyclodecanol	3.4886 (12)	0.0111
Adamantan-1-ol	2.2276 (7)	0.0031
Adamantan-2-ol	2.9828 (7)	0.0085
Cyclopentane***	2.60 (4)	0.05
Cyclohexane***	3.12 (2)	0.06

\* The number in parentheses is the number of measurements.

\*\* SR is the standard error of the mean when  $n > 3$  and the range when  $n < 4$ .

\*\*\* Data from ref. 4.

$$\ln K_d = 0.01668 (n_c)^2 - 0.1791 \quad (4)$$

$n = 11, r^2 = 0.9997, \text{S.D.}_{\text{slope}} = 0.00010, \text{S.D.}_{\text{intercept}} = 0.0061$

For comparison the linear regression equation for the  $\ln K_d$  values of the 1-alkanols plotted against  $n_c$  is:

$$\ln K_d = 0.2001 n_c - 0.6124 \quad (5)$$

$n = 11, r^2 = 0.9475, \text{S.D.}_{\text{slope}} = 0.0157, \text{S.D.}_{\text{intercept}} = 0.1065$

The regression analysis for  $\ln K_d$  against  $n_c$  gives a "fair" correlation coefficient due to the fact that the correlation coefficient for an assumed linear relationship between  $n_c$  and  $(n_c)^2$  is relatively high ( $r^2 = 0.9487$  for  $n_c = 1-11$ ) and is even higher when the range is smaller. It is therefore difficult to discriminate between a first- or second-degree regression equation if the range is too small, and it may be that some of the free energy relationships reported in the literature as linear may in fact be erroneous and actually curved if longer homologues are included.

We have not been able to find a similar dependence of  $\Delta G^0$  (or  $\ln K_d$ ) in the literature, which is, however, not surprising, since the curvilinear relationship does not become evident in this gel until the range for the 1-alkanols is extended to  $C_9$  or  $C_{10}$ , and their accurate detection presents some difficulty due to their very poor

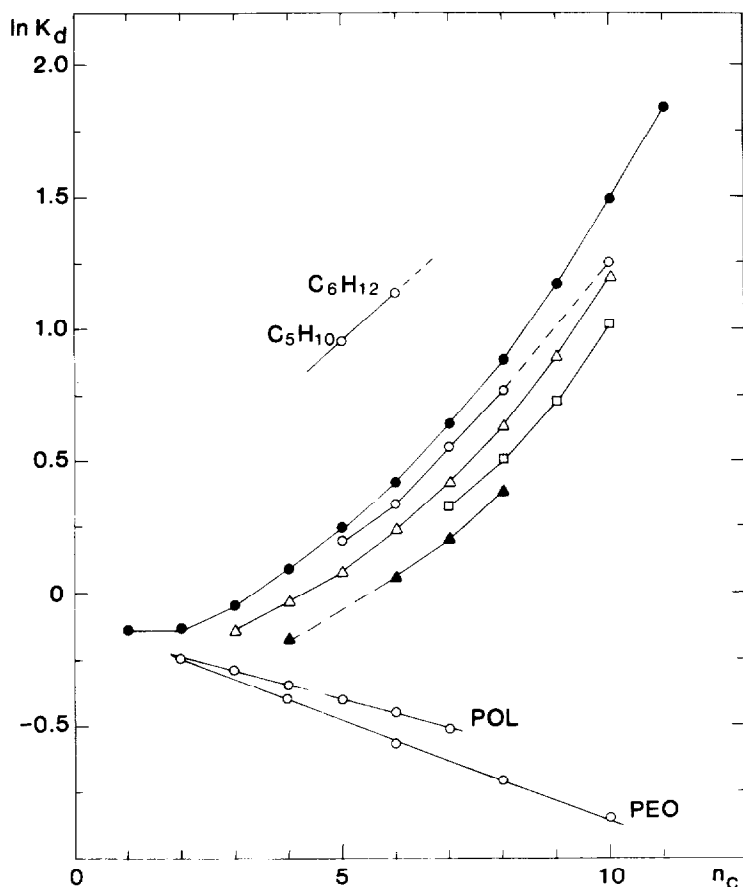


Fig. 1. The relationship between  $\ln K_d$  and number of carbon atoms in the solute,  $n_c$ . POL denotes perhydroxylated linear alcohols and PEO denotes poly(ethylene oxide). Further notation is 1-alkanols (●), cycloalkanols (○), 2-alkanols (△), 4-alkanols (□) and 2-methyl-2-alkanols (▲).

aqueous solubility. Due to this, the loading solution was nearly saturated with the test solute and under these conditions, there is a danger of association phenomena occurring<sup>12,13</sup>. This question was therefore tested with nonan-1-ol which was employed at three different concentrations, *i.e.*, saturated, half-saturated and quarter-saturated. There was no obvious trend within the  $K_d$  values, and further, the differences between them were of the same magnitude as those within the total number of measurements.

The transfer of monofunctional alcohols from water to the interior of the gel is associated with positive standard enthalpies,  $\Delta H^0$ , and entropies,  $\Delta S^0$ , of transfer<sup>14</sup>, and within a homologous series such as the 1-alkanols both  $\Delta H^0$  and  $\Delta S^0$  increase with increasing carbon chain length. These values together with the decrease in  $\Delta G^0$  with increasing carbon number are consistent with the hypothesis that a hydrophobic interaction (HI) is involved in this partitioning behaviour<sup>3,11,14-17</sup>.

The non-linearity of  $\ln K_d$  and hence  $\Delta G^0$  might of course reflect a similar

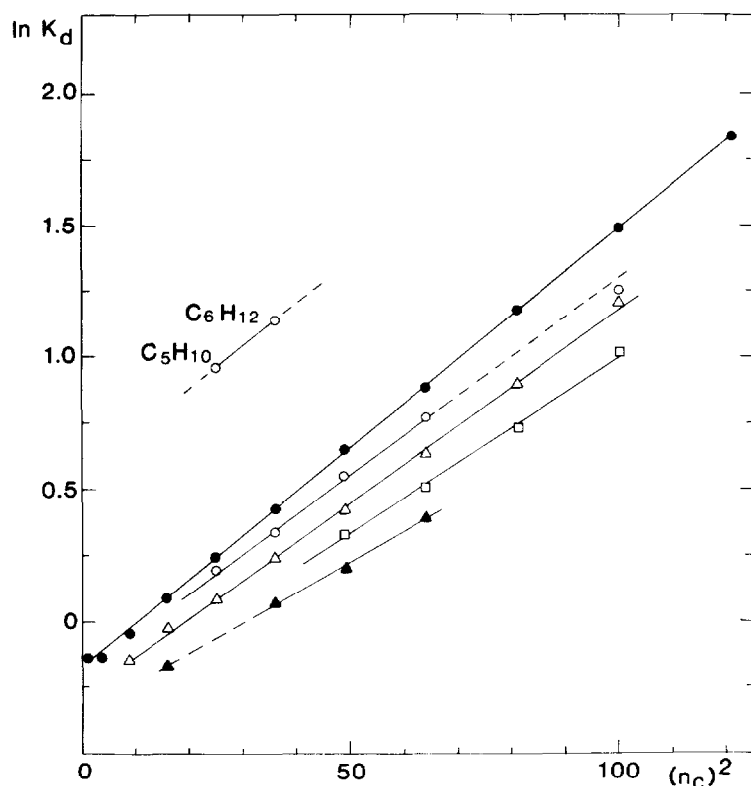


Fig. 2. The relationship between  $\ln K_d$  of two cycloalkanes and monofunctional solutes and the square of the number of carbon atoms,  $(n_c)^2$ . The notation is the same as in Fig. 1.

behaviour of either  $\Delta H^0$  or  $T\Delta S^0$ , since  $\Delta G^0 = \Delta H^0 + T\Delta S^0$ , but although the thermodynamic data cited above<sup>14</sup> must be regarded as only approximate because of the relatively large errors involved in the determination of  $\Delta H^0$  ( $\pm 4\%$  for 1-decanol) and  $\Delta S^0$ , they provided no hint of a curvilinear dependence of  $\Delta H^0$  (or  $T\Delta S^0$ ) on  $n_c$ .

The net standard free energy of transfer,  $\Delta G^0$ , into the gel can be regarded as the sum of at least three different terms<sup>14,18</sup>,

$$\Delta G^0 = \Delta G^0_{se} + \Delta G^0_{hi} + \Delta G^0_{int} \quad (6)$$

where the subscripts denote steric exclusion (se), hydrophobic interactions (hi) and energetic interactions (int) (van der Waals-London dispersion forces) respectively, although Ben-Naim<sup>19</sup> has warned that different interactions are not always additive and this may be an erroneous assumption. If any one of the three  $\Delta G^0$  terms were a non-linear function of  $n_c$  this could explain the non-linear dependence of  $\ln K_d$ . The term affinity is defined as the sum of the interactive terms (hi + int) in eqn. 6.

#### Steric exclusion

$\Delta G^0_{se}$  has been shown to be linearly related to  $n_c$  for a number of polar ho-

mologous series, such as the oligosaccharides<sup>20</sup>, and further, in Fig. 1 the poly(ethylene oxide)s and perhydroxylated alcohols also exhibit such a positive LFER. It is therefore unlikely that the contribution from this term can account for the observed non-linearity.

### *Hydrophobic interactions*

It would be preferable to study HI with non-polar hydrocarbons, but their low aqueous solubilities unfortunately limit their use as hydrophobic probes. However, the more soluble monofunctional aliphatic compounds may serve as satisfactory non-polar models<sup>21</sup>. For example, the hydroxyl group of the monofunctional alcohols has only a short-range influence<sup>5</sup> and the partitioning differences between two consecutive higher members of a series depend essentially on the additional methylene group<sup>22</sup>. As far as HI is concerned, the evidence suggests LFERs for processes usually regarded as models for this interaction. One of the simpler macroscopic models for the extent of HI is the aqueous solubilities of a series of solutes. Kinoshita *et al.*<sup>23</sup> have reported a LFER for the solubilities of 1-alkanols (C<sub>4</sub>–C<sub>10</sub>), and such a LFER has also been observed for linear paraffins (C<sub>4</sub>–C<sub>10</sub>)<sup>7</sup>. There is, of course, always the possibility that a curvilinear relationship will be missed by measurements over too short a range. However, Smith and Tanford<sup>24</sup> have shown that the partitioning of undissociated fatty acids between aqueous solution and liquid *n*-heptane exhibits a LFER up, to at least, 22 carbon atoms.

### *Energetic interactions*

Van der Waals–London dispersion forces depend on the inverse of the sixth power of the interatomic distance. If the solute is assumed to be contained in an interstice in the wet gel<sup>14,25</sup>, the distance between the solute and the dextran chains will decrease with increasing bulk of the solute. If the interstice is sufficiently small, the bulkier homologues may have to be very close to the matrix surface, thus increasing the energy of interaction, and this contribution to the affinity would almost certainly not be related linearly to  $n_c$ . Ultimately, if the solute were too big, it would be excluded partially or totally from the gel, resulting in a decrease in affinity, and possibly even a reversal of the sign of the slope of the plot of  $\ln K_d$  versus  $n_c$ .

It must be borne in mind that the experimentally observed partition coefficient is the net result of opposing effects, *i.e.*, steric exclusion and affinity, where the former seems to be operative over the whole range of carbon chain lengths studied as indicated by the POL and PEO series in Fig. 1. As previously pointed out<sup>4</sup>, the affinity term ( $\Delta G_{hi}^0 + \Delta G_{int}^0$ ) should thus be even larger than estimated from the experimental  $K_d$  values.

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