An Investigation of Some Anomalous

Partition Coefficients

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G.M.Bresnen

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Abstract

A brief discussion of the development of structure-activity work leads to the contribution made by Hansch and fellow workers, with particular emphasis on the additive-constitutive nature of the substituent constant - π. However, it is pointed out that disagreement often occurs between the calculated and experimental values which questions the validity of the calculation methods. Two main reasons are suggested for the occurrence of such anomqlies : inaccuracies in the method of measurement of the partition coefficient which leads to inaccurate substituent constant values; and, interactions within the molecule which affect group contributions to the partition coeeficient and hence alter the value of the substituent constant.

In order to investigate these theories a group of substituted phenols was chosen. Chapter 2 describes the selection and preparation of these compounds.

Various methods of measuring partition coefficients are considered and an investigation of the most widely used methods is described in Chapter 4.

Methods of partition coefficient calculation are described in Chapter 5 and are used to calculate the partition coefficients of the study compounds. The results of the investigation in Chapter 4 are used to select a method for measuring the partition coefficients of the study compounds and the two sets of results are compared.

The importance of the choice of solvent phases is emphasised in Chapter 3 which gives the reasons for the choice of the octanol/ water partitioning system. This work highlights the necessity of ensuring both phases are mutually saturated prior to partitioning.

Ultraviolet and infrared spectroscopy, computer graphics and the thermodynamics of partitioning are used to illustrate the interactions within the molecule which affect its partitioning behaviour. Intra-molecular hydrogen bonding and steric effects give rise to partition anomqlies and it is shown that the 'ortho' effect must be considered since it is the proximity of substituent groups which leads to many anomqlies.

Finally, it is suggested that partition coefficient may not be the ideal parameter to use in correlations with biological activity. The rate of transfer is suggested as an alternative, but correlation of neither the forward nor the reverse rate constants with biological activity of a group of substituted hydroxyacetanilides improved upon the correlation with Log P.

SUMMARY

A brief discussion of the development of structure-activity work leads to the contribution made by Hansch and fellow workers, with particular emphasis on the additive-constitutive nature of the substituent constant - π. Use of this concept allows the calculation of partition coefficients, thus eliminating the need for experimental measurement. However, it is pointed out that disagreement often occurs between the calculated and experimental values which questions the validity of the calculation methods. Two main reasons are suggested for the occurrence of such anomalies : inaccuracies in the method of measurement of the partition coefficient which leads to inaccurate substituent constant values; and, interactions within the molecule which affect group contributions to the partition coefficient and hence alter the value of the substituent constant.

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Finally, it is suggested that partition coefficient may not be the ideal parameter to use in correlations with biological activity. The rate of transfer is suggested as an alternative, but correlation of neither the forward or the reverse rate constants with biological activity of a group of substituted hydroxyacetanilides improved upon the correlation with Log P.

No single conclusion was reached and a conclusion chapter has not been included. This decision is based upon the fact that a whole set of conclusions may be reached which are discussed in the last four chapters.

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CHAPTER ONE

INTRODUCTION

For many centuries man has utilised chemical agents in the treatment of specific diseases. About the year 1500, Carpensis employed mercury compounds in the treatment of syphilis, and Cinchona bark (quinine) was used to combat malaria in the middle of the 17th century. Of course, none of these early medicaments resulted from organised research but were the end-products of years of empirical testing. Then, in 1868, Crum-Brown and Fraser (86) discovered a relationship between the structure and properties of curariform neuromuscular relaxants and their biological effects. They found that quarternization of such drugs as morphine, codeine, thebaine and strychnine, having widely differing actions, resulted in the compounds acquiring curariform activity. From this observation they inferred that the physiological activity of a drug must be intimately related to its structure.

This discovery led to attempts to rationalise drug design which have continued to the present day. Indeed, today it is more important than ever that reliable methods for predicting drug structure and activity are available since costs of drug development continue to increase. The traditional method of synthesising hundreds of analogues and testing each one for potency and toxicity is no longer economically viable since the percentage reaching the

clinical testing stage is minute.

Many factors are involved in drug design, including the form in which the drug is presented to the body and the pharmacology of the drug itself. This latter feature is concerned with the action and mechanism of action due to an interaction between the drug molecule and the molecules constituting the biological object. Thus the chemical structure of the drug is important to its activity. The term chemical structure however does not simply mean the structural formula, but the complex of spatial arrangements of, and interrelations between, the atoms in the molecules which presents information on the possible conformations and energetic priorities in these conformations as well as the inherent charge distribution and mobility of these charges in the molecule. In the effort to interpret the structure-activity relationship, two main approaches have evolved:-

1. The group or moiety approach, which places emphasis on the significance of ceratin chemical groups in the drug molecule for the action. (9)
2. The integral approach, which considers the drug molecule as a whole, and is particularly concerned with overall physicochemical properties such as lipid/water solubility, polarity and charge distribution. (177)

In the second approach emphasis is also placed on group characteristics within the molecule as is evident from the use of substituent constants (measures of the contributions of specific chemical groups to particular physico-chemical parameters of a compound). (173)

These approaches form the basis of modern drug design but the original theories were postulated as far back as 1899 by Meyer and Overton (286,306-308), who both found that the narcotic action of many structurally diverse compounds could be related to their partition coefficients between a lipoidal phase, such as chloroform, and an aqueous phase. Within the limits imposed by the system, it was found that the higher the partition coefficient the greater the narcotic activity, until lipid solubility became so high that the substance was virtually insoluble in water and activity decreased. This relationship was taken to indicate that biological membranes have properties in common with the organic solvent used in the partition coefficient measurements. Thus partition coefficient is a model for certain of the rate constants or equilibria which affect the drug in the biological system.

Ferguson (128) attempted to explain this by assuming that the biological effect (B) of each of a series of non-specific drugs on a given organism was proportional to the thermodynamic activity (a) of a drug at the site of action;

$$B = ka$$

where k is a constant for the class of non-specific drugs for the organisms under test. He concluded that since these drugs could diffuse from the external medium into the cell to the site of action, each drug would be in equilibrium in all these phases; the thermodynamic activity of a drug would therefore have the same value, a, in all these regions. Ferguson also proposed a general relationship of biological

activity to the logarithm of the partition coefficient; this is known as Ferguson's Principle and is described fully by Albert.(5)

Thus, Ferguson implied that non-specific drugs present in the same proportional saturation, in a given medium, have the same biological effect on a given organism. Since the medium is usually aqueous, this can be interpreted as indicating that potency is proportional to solubility in water. Unfortunately, Ferguson's theory was rather misleading since it attributed biological response not to specific structural features of the drug, but merely to its presence in the nonaqueous biological (receptor) phase. In fact several types of weak interaction usually occur in concert between a drug and its receptor and these are a source of specificity since if only one type of interaction is varied within a structural series its importance can be recognised. Accordingly, the relative potency of analogues may depend on their lipophilicity but the total strength of the drug - receptor interaction may be due to electrostatic, charge transfer and hydrogen bonds as well.

Collander (76) suggested the existence of a linear relationship between the logarithms of the partition coefficients of substances between two pairs of solvents.

$$\text{Log } P_1 = a \text{ Log } P_2 + b$$

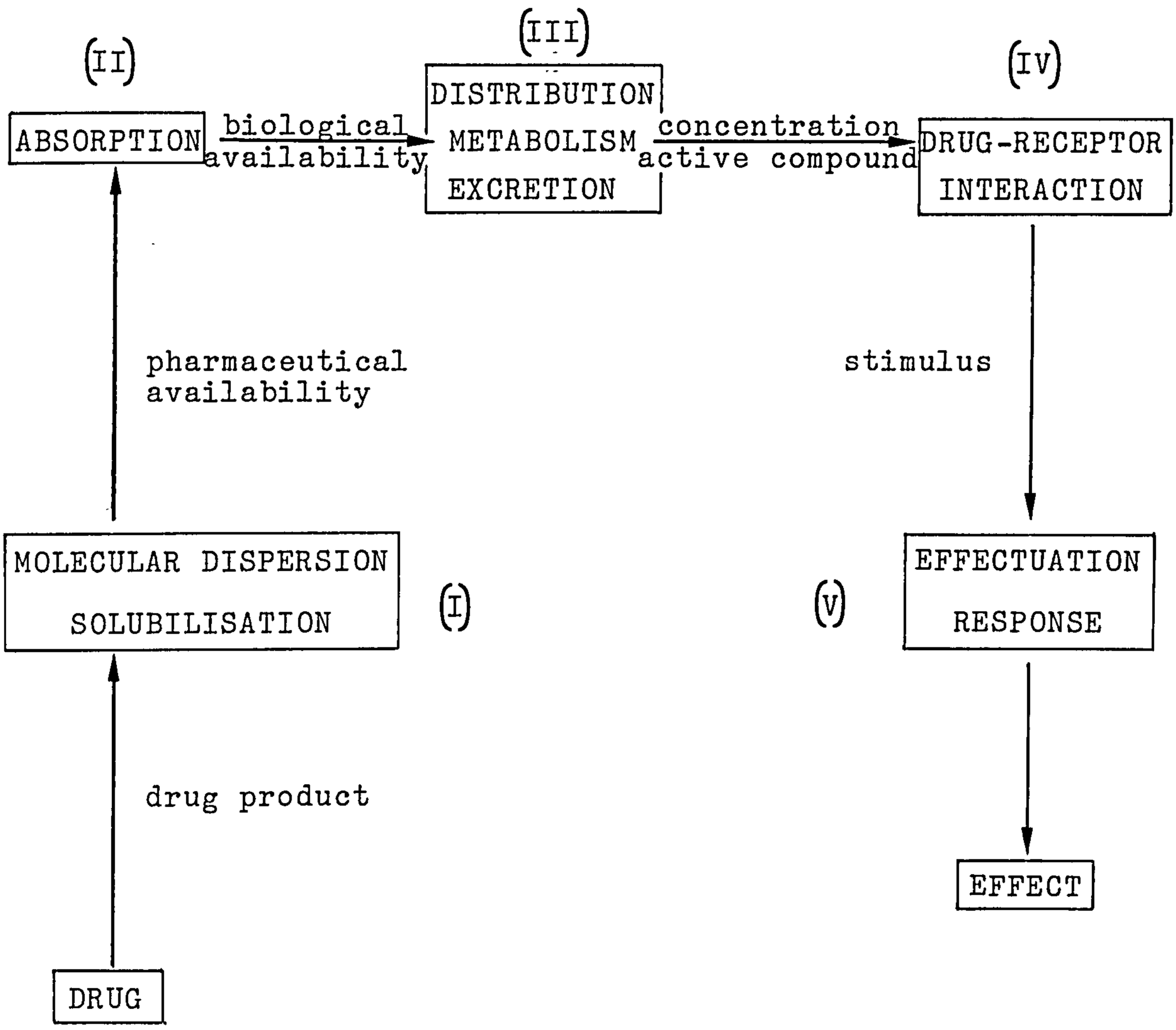
so that, if the drug was also thought of as partitioning between lipid and water, phases in the biological situation, information could be inferred about that process from the

situation between an organic solvent and water.

Whilst these avenues were being pursued, other groups were concentrating on the very specific interactions between a drug and its receptor, where very small structural changes could result in large differences in biological effect. As early as 1914, Dale (87) had recognised the ability of acetylcholine to reproduce responses to stimulation of parasympathetic nerves. This led to Dale et al (88) discovering that acetylcholine is the substance transmitting the nerve impulse between nerve and muscle. Barlow and Ing in 1948 (23) continued work into specific structure-activity relationships of quarternary ammonium compounds concerned in ganglionic and neuromuscular stimulation and blockade. They considered factors such as the electrostatic bonding between the charged nitrogen atom and proposed anionic receptor site, steric factors, and the influence through the secondary van der Waal's and London dispersion forces provided by larger aromatic groupings. This led to much valuable information about the nature of the acetyl-choline receptor, but the usefulness of the technique was limited since it required knowledge of the mechanism of the drug's response. However, more recently, the application of quantum mechanical techniques to the elucidation of receptor structure and conformation has been introduced with some success. The calculation of various parameters such as electron densities, bond angles and lengths for active drugs yields similar information about the receptor as the technique of Barlow and Ing.

Thus, for many years it has been observed that knowledge of physical properties of a drug can be used to predict its activity. However, it is not sufficient to concentrate simply on its behaviour at the final receptor site, even assuming this is known. It is also necessary to consider other factors as illustrated in Figure 1.

Figure 1



I. Drug is applied in its dosage form which makes the active compound available for absorption.

II. Absorption of drug resulting in biological activity.

III. Distribution, metabolic conversion and excretion of drug. These, together with phases I and II determine the concentration of drug in its active form reached in the target tissue.

IV. Induction of stimulus often based on interaction of the drug molecules with specific sites of action or receptors in the target tissue.

V. The stimulus induced, finally leading, via a sequence of biochemical processes to the response. The relationship between the stimulus and the response is independent of the properties of the drug molecule.

The complexity of these processes proved rather daunting and it was realised that a semi-empirical method of analysis was needed which did not require too extensive a knowledge of the biochemical processes involved.

However, although the importance of hydrophobicity to biological activity was understood, it was not until the 1960's that many of the problems were solved. Firstly, there was no agreement as to which organic solvent should be used as a model for biological partitioning. Secondly, little attention had been paid to the prediction of partition coefficients from structure. Thirdly, the applicability of statistical methods to the problem was not recognised. Fourthly, high-speed computers were not generally available.

Also in the 1930's, a different path was being followed by a group of physical-organic chemists who provided the theoretical and practical basis of the extrathermodynamic approach which is one of the methods in use in modern drug

design. These chemists, the most famous of whom was Hammett, studied the structure-activity relationships of the effect of sterically remote substituents on the equilibrium or rate constants of organic reactions. (165). Substituent effects in such reactions are electronic in nature; that is, they are due to changes in the electrostatic forces at the reaction centre. Electronic or polar effects are further subdivided into inductive-field and resonance effects.

The electronic or Hammett constant σ (sigma) is defined as follows:-

$$\rho\sigma = \log K_X - \log K_H$$

ρ = series constant

K_H = ionization constant for benzoic acid at 25^oC

K_X = ionization constant for a meta or para derivative under the same experimental conditions

This equation is known as the Hammett equation. Positive values of σ represent electron withdrawal by the substituent from the aromatic ring ($\sigma_{4\text{-}NO_2} = 0.78$); negative σ values indicate electron release to the ring ($\sigma_{4\text{-}OMe} = -0.27$).
K may be either a rate or equilibrium constant for a reaction at a centre normally insulated from resonance interaction with the aromatic ring.
ρ (rho) is a proportionality constant which is characteristic of the sensitivity of the reaction to electronic effects, defined as 1.0 when measurements are made in water at 25^oC.

In effect, the Hammett equation states that the electronic effect of substitiuents on the ionization of benzoic acids can be used as a model for the effect of substituents on other reaction centres attached to aromatic systems.

The Hammett equation does not hold in general for ortho substituents that exert a steric effect on the reaction centre but Charton (67) compiled a collection of σ values for ortho substituents and Fujita (146) developed a general approach for dealing with the electronic effects of ortho substituents.

Sigma constants are position dependent; that is, σ for a given substituent in the meta position (σ_m) is different from that in the para position (σ_p).

Properties correlated by sigma constants are described as additive and constitutive. Additive means that multiple substituents exert an influence equal to the sum of the individual constituents. Constitutive means that the effect of a substituent may differ depending on the molecule to which it is attached or its environment, hence the different sigma values for a substituent in the meta or para position.

A relationship such as the Hammett equation is reffered to as a Linear Free Energy Relationship (LFER) and is a specific example of an 'Extrathermodynamic Relationship'. These relationships are extrathermodynamic because although the relationships between the reactions are stated in thermodynamic terms (ΔG or logP values) there is no thermodynacic principle which says that these relationships should be true.

The modern application of this method to the study of chemical or biological properties of molecules uses statistical procedures, particularly regression analysis, to describe the observed relationship. Thus, two synonyms

for the method are regression and correlation analysis.

The Hammett relationship provides a quantitative equation which allows the comparison of the relative sensitivity of reactions to electronic or polar effects, or conversely, a method to examine the relative electronic effect of different substituents.

Although the original Hammett equation is valid only where polar effects are concerned, and breaks down when ortho substituents are involved, many adaptations of the basic equation have been formulated to diversify its applications. For example, many meta and para substituents with large resonance contributions such as the nitro- group, having a -M effect and amino with a +M effect (210) deviate markedly from the usual equation and these cases have been rationalised by the introduction of the σ^+ constant by Brown and Okamoto (40) and σ^- by Jaffe (212). In view of the complications arising for both ortho substituents and aliphatic systems. where both steric and polar effects may coexist, little progress was made until Taft (353) introduced the polar substituent constant σ^* and devised a procedure for separating polar, steric and resonance effects based on the analysis of the rate coefficients of basic and acidic hydrolysis of esters. This approach became known as Taft Analysis (355).

Taft followed a suggestion by Ingold and defined the steric effect of a substituent, E_s, as:-

$$E_s = \log \left[\frac{K_X}{K_H}\right]_A$$

where K refers to the rate constant for the acid hydrolysis (denoted by A) of esters of type:-

$$X—CH_2COOR$$

Unfortunately, variations of this structure cannot be used to obtain E_s values for many common substituents that are unstable under the conditions of acid hydrolysis (e.g. when $X\text{-}CH_2 = NO_2$,CN,halogen,OR,etc.). Charton (64) opened up a route to E_s values for such groups when he showed that E_s is related to the van der Waals radii of substituents. Kutter and Hansch (398) used this finding to formulate E_s values for many other substituents.

The various forms of E_s and Charton's steric parameter, υ, were developed with the idea of rationalising intramolecular proximity effects of substituents on a reaction centre. Since Charton showed the close relationship between E_s and van der Waals radii it was not surprising that E_s could be employed to rationalise intermolecular steric effects occurring between ligands reacting with macromolecules.

The steric influence of substituents in the interaction of organic compounds with macromolecules or drug receptors is much more complicated than the steric effects in simple homogenous organic reactions for which E_s was designed. Thus, more complex procedures are needed for such interactions.

This formed the basis of one aspect of modern investigation. Verloop et al (372) undertook a multiparameter approach to steric interactions of this type which could lead to more detailed analysis. They selected five dimensions for each

substituent and developed a computer programme using van der Waals radii, standard bond angles and lengths, and 'reasonable' conformations to define the space requirements of a substituent.

The five dimensions are labelled: L,B_1,B_2,B_3 and B_4 as shown in Figures 2 and 3.

The length parameter, L, is defined as the length of the substituent along the axis of the bond between the first atom of the substituent and the parent molecule. The four width parameters, B_1-B_4, are determined by the distance at their maximum point perpendicular to this attachment bond axis and each other. B_1 is the smallest and B_4 the largest width. In effect, these parameters define a box around the substituent. (273)

To make a statistically justified study of these parameters in a biochemical system, five steric parameters would be needed for substituents for each position substituted on the parent structure. Since a reaction is rarely dependent solely on steric factors, π,MR,σ_I and σ_R must also be considered at each position when developing a structure activity relationship. Thus the analysis of a single compound can be complex and much detailed work is necessary to apply this method to experimental data which limits its usefulness for QSAR studies.

Generally the Hammett and the Taft extrathermodynamic relationships may be studied by use of the statistical technique of regression analysis (least squares fit). Using this method the best fit of the data to the equation is

Figure 2.

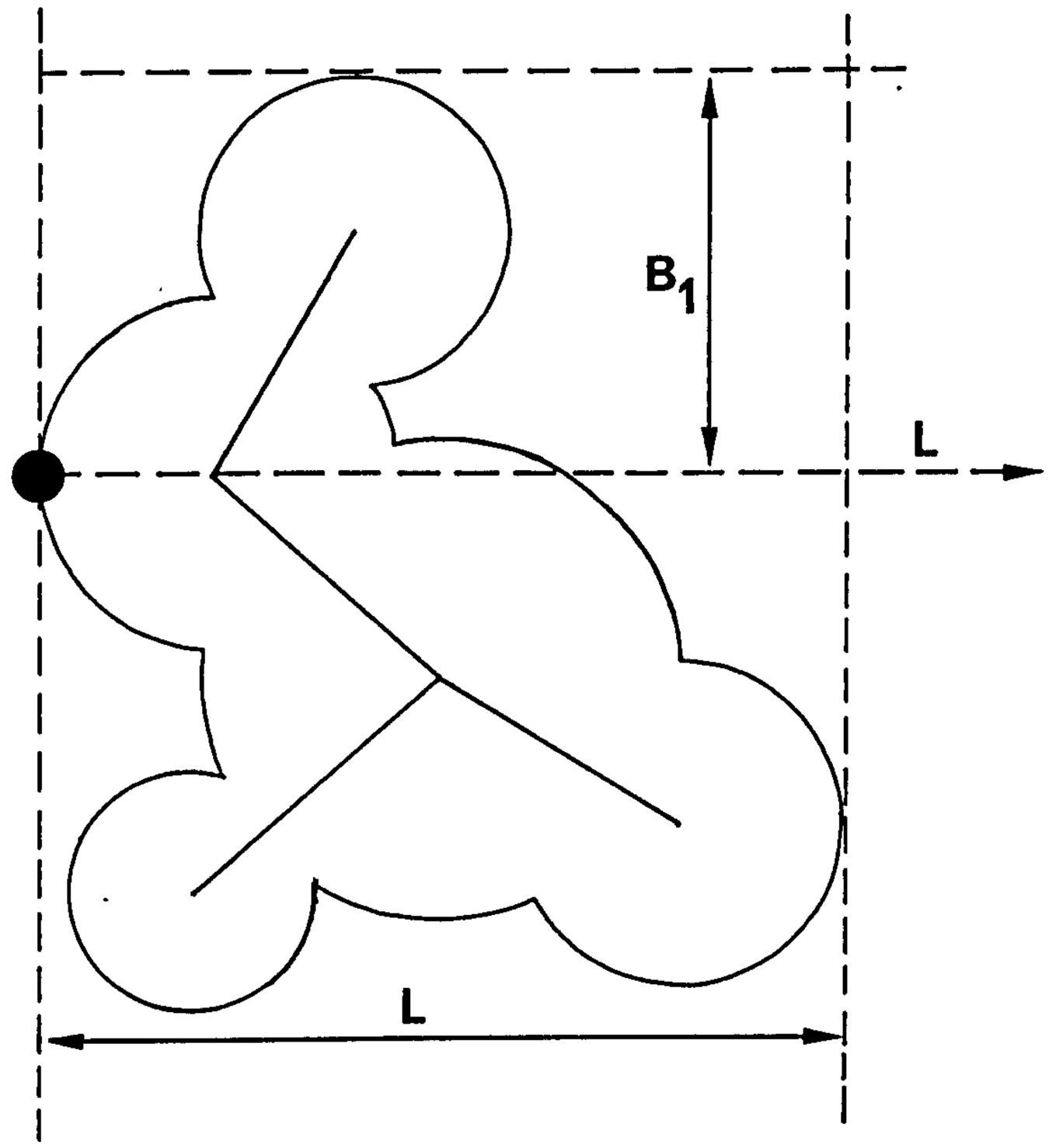
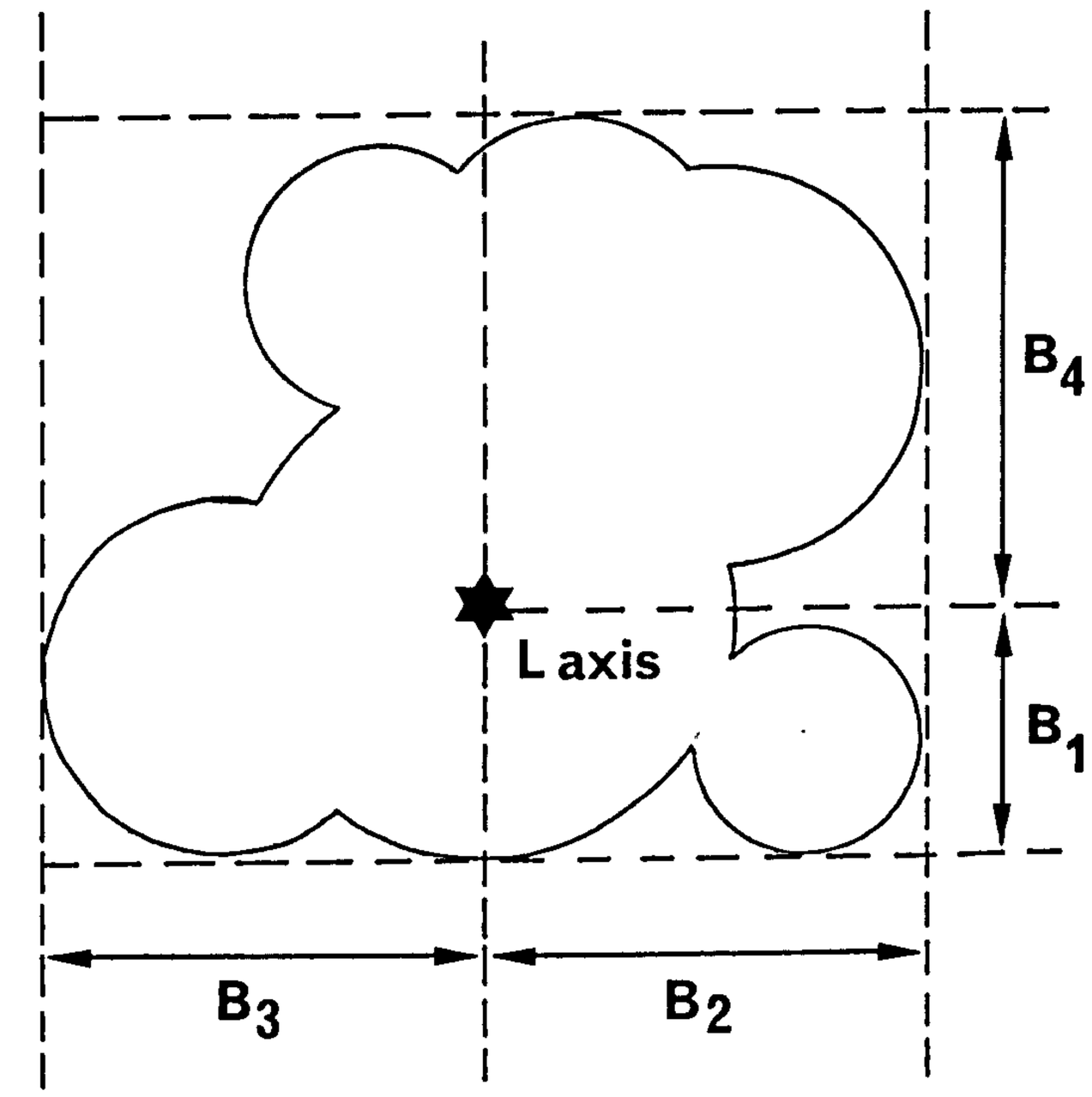


Figure 3.



calculated, and additionally, the statistical significance of the overall relationship, the significance of the contribution of each term in the equation, and the precision with which the calculated values approximate the observed are evaluated.

An important criterion of the usefulness of a regression equation is the R^2 statistic. R^2 equals the fraction of the variation in the dependent property (e.g. potency in drug work) which is accounted for by the stated relationship. For example, if the correlation between potency and log P has an R^2 of 0.95, 95% of the variation in potency is explained by variation in log P. The second criterion is the standard error of estimate, S, which is a measure of how closely the observed values are approximated by the theoretical relationship. The smaller is S, the closer the fit.

Early studies in QSAR attempted to correlate the effect of substituents on the biological properties of molecules with the substituent constants formulated by Hammett and Taft, but no real advance was made until two additional important steps were taken.

Hansch et al (169) searched for another physical property which would be more relevant and realised the significance of the partition coefficient as a measure of hydrophobicity. However, prior to this Hansch had already done much in the QSAR field. Hansch and Muir (167) had investigated the action of a variety of acids with very different structures, which had been found to cause elongation of cell sections

when these were placed in baths containing the various chemicals. They had found that an aromatic ring was essential for such activity and electro-negative substituents increased activity. With this in mind they formulated a model to describe how a drug reached its site of action.

They postulated that observed biological activity was a function of both the rate of transport of the drug to its active site and rate of attachment to the receptor. With respect to the transport function it was assumed that, in order to reach the site in question, the drug must pass through a series of membranes and aqueous compartments, its passage through these various compartments being described as a 'random walk' process.

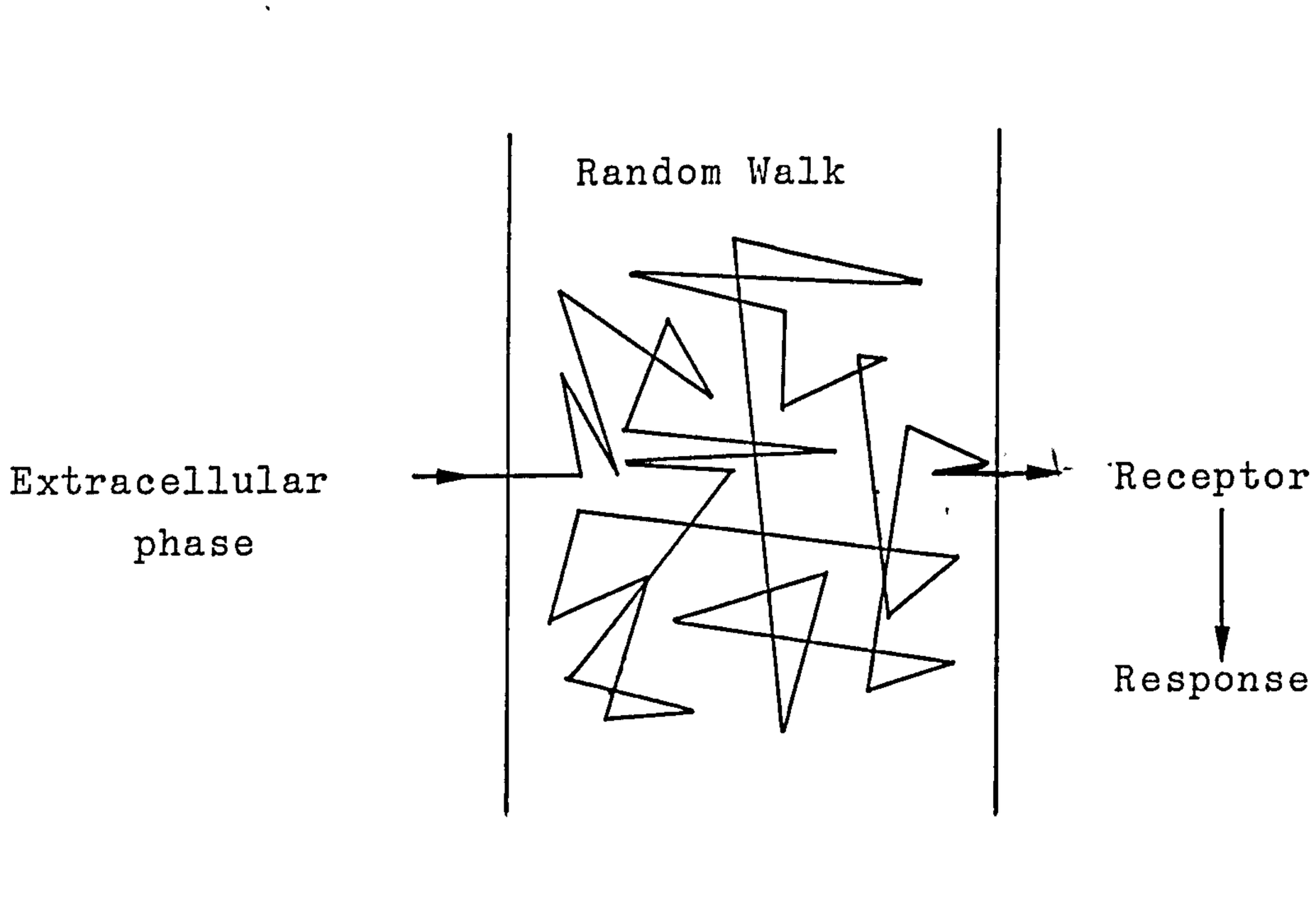


Figure 4. The Random Walk

It was also felt that the actual transfer from an aqueous phase to a largely lipid membrane could be thought of as a partitioning process which could be modelled using water and octanol as a model of the lipid. This was an extension of the Meyer-Overton theory, which had till then been applied only to very non-specific reactions. As stated earlier, Collander's work in 1951 suggested the existence of a linear relationship between the logarithms of the partition coefficients of a substance between two pairs of solvents:-

$$\log P_1 = a \log P_2 + b$$

From this work, Hansch drew support for the theory that partition coefficients from the octanol/water system should be linearly related to partitioning in the biological model. Thus transport rate could be thought of as depending on partition coefficient. i.e.

$$\text{Transport rate} = f(P)$$

Since the partition coefficient is a free energy-related quantity, a Hammett-type equation may be generated. It was also possible to relate the transport rate to a new substituent constant, π, which had been postulated by Hansch and was defined by Fujita et al (144) as:-

$$\pi_X = \log P_{YX} - \log P_{YH}$$

where π_X is the hydrophobic substituent constant for any substituent X, logP refers to the logarithm of the octanol/water partition coefficient and Y is any appropriate parent structure.

Since π is related to logP, it may be substituted for logP to give a comparative rather than a specific variable within a congeneric series. Thus:-

$$\log(\text{Transport rate}) = f'\log(\pi)$$

The rate of attachment to the receptor site was assumed to be a function of electron density and this was expressed in terms of the Hammett equation:-

$$\log(\text{rate of attachment}) = \rho\sigma$$

Thus a total equation can be formulated:-

$$\log(\text{biological activity}) = \log(\text{rate of transport}) + \log(\text{rate of attachment})$$

$$= f'\log(\pi) + \rho\sigma + K$$

where K is a constant.

Thus the significance of the partition coefficient was realised and the model used to describe the random walk process could be applied to other biological situations.

The Hansch group also demonstrated that log P is an additive-constitutive property (211). Hence, the change in log P brought about by substitution is characteristic of the substituent and is reasonably predictable. It had in fact been known for years (127) that each successive addition of a methylene group into a molecule increases log P by a fairly constant amount, but no one prior to Hansch (169) had emphasised the utility and generality of this observation for quantitative structure-activity studies.

The solvent system octanol/water was chosen for partition studies since octanol was presumed to be more like a biological membrane than an almost nonpolar solvent such as chloroform. Thus, log P refers to the partition coefficient of the neutral form of the compound between octanol and water, unless otherwise stated.

The concept and definition of π meant that it was not necessary to measure the partition coefficient of every molecule because a reasonable estimate of it could be calculated.

At this time it was known that a substituent produces three different major changes in the physical properties of a molecule: electronic, steric and hydrophobic. The effect of the substituents on the biological properties of a molecule was thus postulated by Hansch et al (169) to be the result of the change of some or all of these physical properties. It was further assumed that the electronic, steric and hydrophobic influences of substituents on potency (symbolised by 1/C) are independently additive. Thus, the linear form of the Hansch equation was proposed.

$$\log \frac{1}{C} = a \log P + b E_s + \rho\sigma + d$$

Multiple regression analysis can again be used to fit this equation, which can be seen to be very similar to the equation suggested by the Random Walk process.

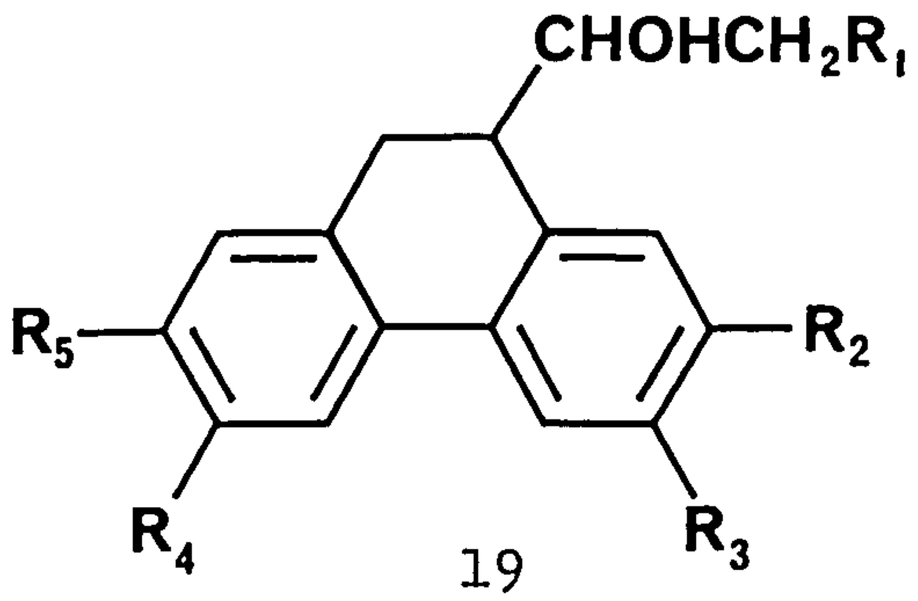
However, it was soon observed that many series do not follow a linear relationship between potency and log P, but rather, the relationship is linear and positive at relatively low

log P, then potency reaches a maximum, from which it gradually decreases with further increases in log P. This can be described by a parabolic relationship, giving the equation:-

$$\log \frac{1}{C} = a \log P - b \log P^2 + c E_s + \rho\sigma + d$$

This proposal has since been confirmed by many experimental findings, including work by Hansch and Clayton (183) in 1973. However, it was still felt that a linear relationship would be observed where only one partition step was involved, such as in the case of enzyme binding, or where drug action was occurring on the outside of a cell membrane, since the drug or substrate would probably have to pass through only one aqueous and one lipid-like phase.

A further approach to quantitative structure-activity relationships which was postulated at this time is that of Free and Wilson (139), known as the de-novo or Free-Wilson approach. This method is based on a statistical (multiple regression) statement of a typical unstated assumption of drug analogue design: that within a series of related compounds modified at more than one position, the effect of a particular substituent at a specific position is independent of the effect of the substituents at the other positions. For example, consider the hypothetical compound I :



(I)

If, in one pair of analogues for which R_1-R_4 are constant and R_5 is chloro or methyl, the methyl compound is one-tenth as potent as the chloro analogue, the Free-Wilson method assumes that every R_5 methyl analogue will be one-tenth as potent as the corresponding chloro analogue. A requirement for the application of the method is thus a series of compounds which have changes at more than one position. In addition, each type of substituent must occur more than once at each position in which it is found.

The result of the application of the Free-Wilson method is a table of the contribution to potency of each substituent at each position. It is not necessary to calculate physical properties for the compounds and the solution provides no information as to the relevance of model reactions to potency. This method is related to the extrathermodynamic method (83) particularly if the free energy relationships are linear or position specific. (300)

Since the development of these methods for investigating structure-activity relationships, research in this field has not remained static and many new methods have been evolved, particularly methods utilising statistical procedures and thus the capabilities of modern computers. Some of these methods are described below.

One of the most important of the modern methods is that of molecular connectivity suggested by Kier and Hall (162) This is a method for the quantitation of molecular structure that encodes information about size, branching, cyclization unsaturation and heteroatom content.(224). It was developed

in several stages, beginning with an alkane branching index proposed by Randic (319), a treatment of unsaturation (224), a development of extended bond quantitation (224) and finally, a rational way of quantifying heteroatom content (224). The treatment of heteroatoms, introduced by Kier and Hall (161) and called valence molecular connectivity, brought molecular connectivity into the role of a structure quantitation method useful in structure-activity analyses of drug molecules.

The simple molecular connectivity index arises from the assignment of numerical adjacency values to each atom other than hydrogen (the molecular skeleton) in a molecule. These values, called δ values, are cardinal numbers enumerating the presence of non-hydrogenic-bonded neighbours irrespective of what the element is or what multiplicity of bonding is present. The assignment of δ values for 2,4-dimethylpentane is shown in Figure 5.

Figure 5. The Assignment of δ Values for 2,4-dimethylpentane

The molecule is dissected into bonds, each described by two δ values. A term for each bond (C_{ij}) is calculated according to:-

$$C_K = (\delta_i \delta_j)_K^{-\frac{1}{2}}$$

These terms are summed over the entire molecule to give the simple molecular connectivity index (chi) of the first order ($^1\chi$)

$$^1\chi = \Sigma(\delta_i\delta_j)_K^{-\frac{1}{2}} = C_K$$

This value may be useful in predicting size and shape of a molecule, but to include structural differences, Kier and Hall considered the count of the valence electrons participating in sigma, pi and lone-pair orbitals on each atom, exclusive of bonds to hydrogen. This gave a valence molecular connectivity index ($^1\chi^V$) which can give information on both volume and electronic character. Further extension of molecular connectivity index values have shown the method to be capable of describing structure-activity relationships and it has emerged as a valuable new method in drug design.

Discriminant analysis is a further technique employed in drug design which is used to calculate that combination of predictor variables which best distinguish members of pre-established groups. In drug design studies, for example, the weighting and statistical significance of various physical properties which might distinguish active from inactive analogues can be examined.

It is a useful extension to regression analysis because it allows the inclusion of inactive compounds in the analysis and nonquantitative data of other types can be analysed. It is closely related to regression analysis in that in discriminant analysis the function minimised is the sum of the squared deviations of observations from their respective

group means while the sum of squares from the opposite group is maximised; in regression analysis the function minimised is the sum of the squared deviations of observations from a line.

Cluster analysis is used to study the relationships between observations which have associated with them a number of properties. Primarily two types of questions are answered by a cluster analysis: "Which of these observations are similar to each other?" and "How many different groups does this data represent?". For example, cluster analysis was used to study a large set of substituents to establish which subsets of the total were similar in physical properties (184). If one wished to synthesize a series of compounds with the maximum variation in the minimum number of examples, then one member from each subset (cluster) would be chosen.

Two principle measures are used for the distance between multimembered clusters. The first, named nearest neighbour, considers the distance between clusters to be the distance between the two closest members of different clusters. The second, or centroid method, considers the distance between clusters to be the distance between the centres of the clusters. For either method one may calculate the distances in terms of the units of the original measurements, or first weight the data.

The clustering calculation may either start from one cluster per observation and progressively coalesce clusters to form fewer and fewer, or it may start with all of the observations as one large cluster and progressively form more and more clusters. The former method is agglomerative, the latter

divisive. If in successive steps the decisions (amalgamations or divisions) made in previous steps are not changed, then the method is hierarchical.

Pattern recognition is the name given to a collection of computer-based methods used for detecting previously unknown relationships (patterns) within large masses of multivariate data. It is done without making assumptions about the underlying statistics of the data. Although certain of the methods of calculation resemble regression, principle component, factor, discriminant or cluster analysis, in concept pattern recognition is much broader. Because of the lack of assumptions with respect to the distribution of the data, probability estimates are not made and statistical tests are not applied to the results of calculations.

Pattern recognition methods may be used in a manner similar to cluster analysis in which case the goal of the calculations is to examine the distance or relatedness between observations. This is unsupervised learning. In contrast, with supervised learning the value of the dependent variable is specified and attempts are made to discover which function of the other properties (features) best predicts the dependent variable. Hence supervised learning is analogous in objective to regression or discriminant analysis. The distances, the function to be maximised or minimised, may be calculated by the least squares or analogous procedure, or by the nearest neighbour method; variables may be in the original units, weighted, transformed or standardised.

An important part of pattern recognition is the development of methods for the display of multidimensional relationships in two dimensions. A number of methods have been described by Kowalski and Bender (242). The eigenvector or principle components analysis is an especially useful summary of data for plotting. The eigenvector calculation creates new variables which are a linear combination of the original ones. Usually the first two or three principle components contain most of the variance. Hence a plot of λ_1 vs λ_2 can give an indication of clustering and/or independence of observations in a data set.

To date, the biological structure-activity studies which have applied pattern recognition methods have not used the classic extrathermodynamic physical properties such as logP etc. but rather have used structural features such as the presence or absence of a certain group, number of a certain type of atom, or mass spectral fragmentation pattern (243,360) Thus it is not possible to compare pattern recognition to the classic statistical methods. However, since the methods have the potential of reducing a large amount of redundant and noisy information they will continue to be of use in QSAR.

These examples indicate the variety of different approaches which are being used in the study of structure-activity relationships, but despite the introduction of new methods the extrathermodynamic methods are still the most powerful. The strengths and weaknesses of this method can be summarised:

<u>Strengths</u>

1. It is based on the assumption of an analogy between the

interactions between small molecules and those between a small and a large molecule. For example, it is assumed that the interaction of compound A with a proton in water is parallel to the interaction of the same compound A with an electrophilic site in a cell. Similarly it is assumed that the octanol-water partition coefficient of compound A parallels the partitioning of A between a lipophilic biological substance and the surrounding aqueous environment. The importance of this is that data from simple model systems can be used to predict behaviour in complex biological systems, without full understanding of all the processes involved in the model.

2. Its predictions are quantitative with statistical confidance limits, and thus the results for predicted potency may be evaluated unambiguously. This is not possible with receptor mapping and rigid analogue techniques which only suggest that certain compounds should be active.

3. It is relatively easy and inexpensive to use.

4. The final advantage of the extrathermodynamic approach in contrast to the de novo method, is that the conclusions reached have application beyond the substituents included in the analysis and the particular structure class calculated. Thus similar physical properties are observed for series of varying structure but similar mode or site of action.

Limitations

1. There must be parameter values available for the substituents in the data set, or the compound may have to be omitted from the analysis. It is often possible to

measure the necessary parameter but this can be time consuming.

2. It is necessary to include a large number of compounds in the analysis to be confident that all possible predictors have been studied (365)

3. It is necessary to have a good understanding of statistics to obtain the most from the method.

4. The results depend on the parameters used, which may be the wrong ones. In contrast, the Free-Wilson parameters are perfect for the system investigated.

5. Models of small molecule interactions with which the drug actions are correlated are imperfect models for the biological system. In particular, steric interactions are very difficult to extrapolate from system to system. With both steric and electronic effects it is difficult to decide which is the key atom from which substituent effects should be measured. However, partitioning is easier to measure and the results seem less ambiguous. Thus the hydrophobic properties of drug molecules have been pursued in greater detail.

Hansch analysis, in particular the relationship of partition coefficient to activity, has therefore been extensively used in drug design and has proved itself to be an important and useful method. There have emerged different methods of measuring log P or related parameters. The basic Hansch shake flask method will be discussed in greater detail in Chapter 4, but here, other techniques which have been investigated will be described for comparison.

1. Evaluation of Octanol/Water Partition Coefficients by Using High-Performance Liquid Chromatography (299)

This technique extends the shake flask method of measuring partition coefficients. Normally this method is encumbered by the difficulties in measuring solute concentration in both phases accurately, but the use of HPLC allows more accurate analysis. Solutes are equilibrated between the two phases by the shake flask approach. An internal reference having an accurately known partition coefficient, which is similar to that of the compound under investigation, is added to the system. Samples of both phases are chromatographed by HPLC. The area ratios for the solute and internal reference in both phases are measured and used together with the known partition coefficient of the solute in question. The technique is rapid and has the advantages that small samples suffice, the substances need not be pure and the exact volumes of the phases need not be known.

The partition coefficient of the solute of interest, P_s, is calculated from the relationship:

$$P_s = P_r \frac{(As,o/Ar,o)}{(As,w/Ar,w)} .$$

where P_r is the 1-octanol/water partition coefficient of the internal reference, As,o and As,w are the peak areas of the solute from the 1-octanol and aqueous phases and Ar,o and Ar,w are the respective peak areas of the internal reference.

The accuracy of the method depends greatly on choosing the proper internal reference, which should meet the following

requirements:

a. Its partition coefficient should be known accurately.

b. It should have a high absorbance at the same wavelength where the elution of the substance under investigation is monitored to give an appropriate peak with photometric detectors even at low concentrations.

c. Its chromatographic retention should be similar to that of the compound investigated, yet it should be completely resolved from the solute of interest and its contaminants.

d. It should not contain ionogenic groups, so that its partition coefficient is independent of the pH of the aqueous phase.

e. It should not be subject to secondary chemical equilibria which would result in concentration-dependent partition coefficients.

f. It should be readily available in pure form, or at least should not contain interfering impurities.

The solute of interest and the internal reference do not however have to be pure for this method as chromatographic conditions can be adjusted to separate impurities from the two eluates of interest. Also the introduction of an internal reference and an analysis of both phases eliminates the need to know the exact volume of each phase, the extinction coefficients of the solutes and the size of the injection volume of the sample.

This method would also be of use for extending the calculation methods of log P values to complex molecules, since many of these compounds are not available in pure form or in large quantities.

2. Use of Reversed Phase HPLC to Measure Partition Coefficients and to Predict Biological Activity

The traditional method for determining octanol/water partition coefficients by shaking, followed by analysis of the equilibrium concentration of solute in each phase is tedious to perform and requires the compound to be in a high state of purity. It has been demonstrated that k' values of solutes in reversed phase HPLC can be correlated with their octanol/water partition coefficients as well as directly with their biological activity. An advantage of this approach (shared by the related reversed phase TLC method) is that solute purity is less critical than with the traditional method. In a typical reversed phase system using an octadecyl-silica column and a methanol/water eluent, the more hydrophobic a solute, the longer it will be retained.

Martin (274) deduced on theoretical grounds that for plate or paper chromatography:

$$\log P = \log K + Rm$$

where P is the partition coefficient, K is a constant for the system and Rm is the log of $(1/R_f) - 1$ where R_f is the retention in the system. In work with HPLC, a compound's retention is routinely expressed by the term k' which is defined as:

$$k' = (t_R - t_o)/t_o$$

where t_R is the elution time of a retained peak and t_o is the elution time of an unretained peak. The terms k' and

$(1-R_f)$ - 1 are analogous. Therefore, for partition between a stationary and mobile phase:

$$\log P = \log K + \log k'$$

Thus, for HPLC with a stationary lipid phase and an aqueous mobile phase, k' should be linearly related to a measured liquid-liquid partition coefficient.

The general method used for measuring partition coefficients by HPLC is that described by McCall (281). Corasil C-18[4] was used as the stationary chromatographic phase. This hydrolytically stable, reverse phase packing material is a pellicular silica gel to which octadecyl chains have been chemically bonded. A silyl ether terminates one end of the octadecyl chain. This terminus is more hydrophilic than the other which is alkyl and therefore more lipophilic. This combination of polar and lipid properties is less extreme than that of octanol.

Corasil C-18[4] has a low percentage of active silanol sites, which will interfere with the desired liquid-liquid partition process and so are blocked by treating the Corasil C-18[4] with hexamethyldisilazane (HMDS) and trimethylsilyl chloride (TMSCl) in hot pyridine. The preferred mobile phases were 1% triethylamine (TEA) in water and 15% CH_3CN in water. Because of the diverse lipophilicities of compounds, a single solvent system could not be used for all members of a series, so a spectrum of solvents was used, from water for the most hydrophilic analogues to 1% TEA in water or 1% TEA in 40% methanol-water for the more lipophilic analogues.

McCall found that retention (k') values are linearly related to octanol-water partition values, showing that log P values can be obtained directly from log k' values. Thus, HPLC on columns which are packed with vigorously silylated octadecyl supports and which are eluted with an aqueous solvent present a useful alternative to the often tedious octanol/water partition coefficient measurement. The HPLC technique is fast and reproducible, a typical assay time being 10 minutes. Because of such rapid analysis time, compounds which are unstable in solution can be assayed. Because solvent lipophilicity can be rapidly adjusted, compounds whose partition coefficients vary by several orders of magnitude can be quickly measured. Samples need not be pure since contaminants do not interfere with k' determinations. Finally, because both refractive index and UV HPLC detectors are available, any compound can be detected.

The disadvantage of the method described by McCall was that octadecylsilane is more like an alkane than an alcohol phase. Therefore, the differential effects of hydrogen bonding limit use of these procedures to close analogues.

Hulshoff and Perrin (205) compared reversed-phase HPLC and TLC procedures and also investigated oleyl alcohol supported on Porasil C' as an HPLC procedure to measure the lipophilicities of a series of benzodiazepines. Mirrlees et al (289) used 1-octanol supported on Hyflo supercel and compared some of the earlier work on reversed-phase TLC and HPLC. Henry et al (192) compared various HPLC techniques such as C-18 Corasil and 1-octanol and squalene on Corasil-11

and Porasil A. These procedures using 1-octanol physically adsorbed on a solid support, would be close to ideal (a physically bonded 'octanol'-like alcohol with the hydroxyl end free). However, attempts to obtain a stable baseline were unsuccessful.

However, Unger et al (370) found that a chemically bonded octadecylsilane support persilated and coated with 1-octanol produced good agreement with partition coefficients obtained by the shake flask method. Since 1-octanol is very lipophilic itself (logP=3.15 (189)), it should bond strongly to octadecylsilane and give a stable column with minimal free silinol sites. Providing the aqueous mobile phase is saturated with octanol, a system much like that of the octanol-aqueous shake flask system should result.

3. Measurement of Partition Coefficient by Reversed-Phase Thin-Layer Chromatography

In general the compounds are partitioned between a nonpolar stationary phase and a polar mobile phase. The nonpolar stationary phase can be obtained by impregnating a silica gel G layer with a suitable liquid such as a silicone. The mobile polar phase can be an aqueous buffer, alone or mixed with various proportions of acetone and saturated with the stationary phase liquid. The plates are developed by the ascending technique in a chromatography tank under conditions of vapour equilibrium. The plates are then dried and the compounds detected by a suitable method. Boyce and Millborrow (34) found that the N-n-alkyltritylamines they investigated obeyed the Martin (272) equation (p.30) that is, there was a linear relationship between Rm and

the number of methylene carbon atoms in the side-chain, and thus the biological activity could be related to Rm values. Biagi et al (31-3) investigated penicillins and found the Rm values correlated well with structural variations.

The choice of chromatographic method is a matter of convenience. No important differences have been found between paper and thin-layer chromatography except that the latter was quicker. Water is a constituent of the cell membrane and the protoplasm, so its use in one phase of the model system is obvious, but the lipoidal constitution of the cell membrane is unknown and the choice of hydrophobic phase must be somewhat arbitrary. From thermodynamic considerations, the nature of the non-aqueous phase should not affect the results qualitatively (74) provided there is no hydrogen bonding.

The scope of the method can be increased by varying the concentration of acetone in the mobile phase. This allows more lipophilic or hydrophilic compounds to be investigated and Soczewinski and Wachtmeister (346) have shown a linear relationship between Rm and solvent composition.

The advantages of this method over conventional partition measurements are:-

a. Quicker and less tedious because fewer manipulations are involved.

b. Up to 25 compounds can be run simultaneously on one plate so Rm values can be compared directly.

c. The detection of spots by simple nonspecific methods i.e.by iodine absorption or the use of a fluorescent

indicator, avoids the need for specific quantitative analytical methods.

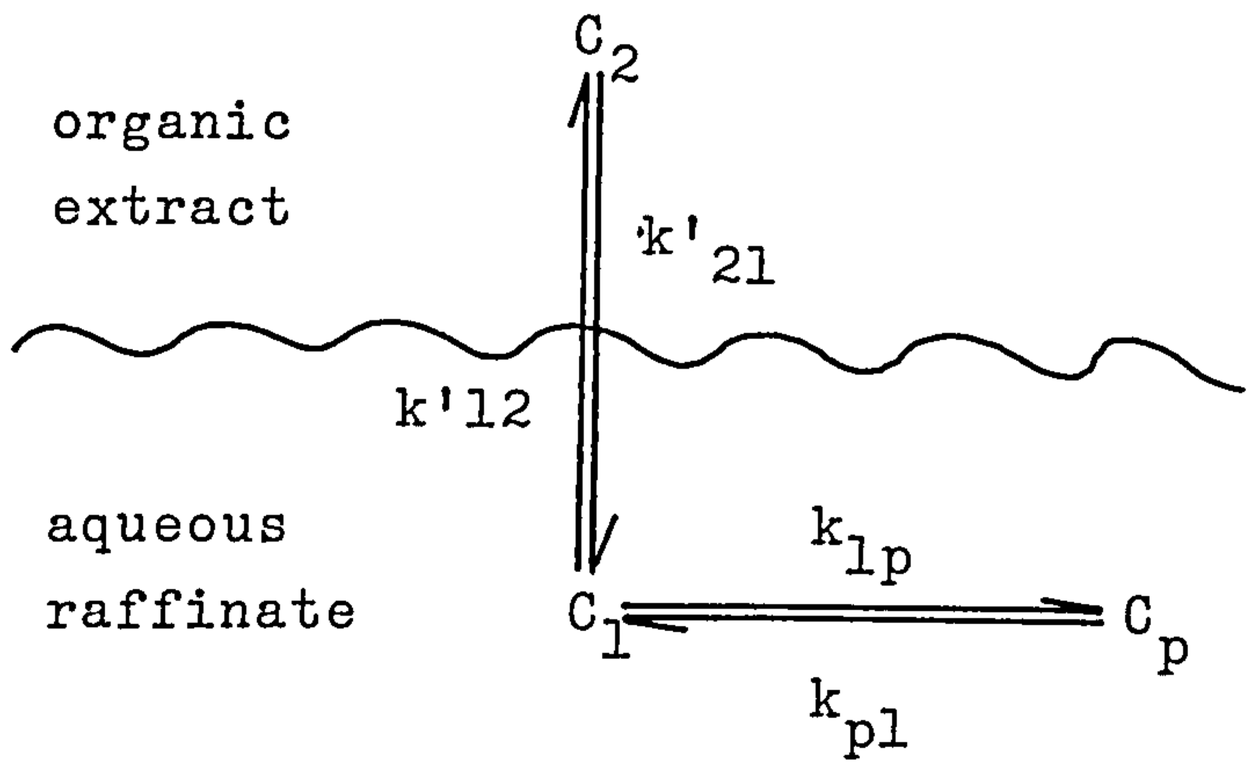
d. The material need not be pure if the salient spot can be identified.

e. Very little material is needed for detection.

4. Calculation of Partition Coefficient of an Unstable Compound Using Kinetic Methods

Many compounds are unstable in solution and are thus not suitable for standard methods of partition coefficent measurement. Thus a method has been described by Byron et al (59) for measuring partition coefficients of unstable compounds by kinetic methods. A stirred transfer cell containing equal volumes of light liquid paraffin and an aqueous phase at 37°C was used. Cyclohept-2-enone was chosen since it is a neutral molecule and therefore should have a pH-independent oil-water partition coefficient, K_D. Also, this cyclic α,β-unsaturated ketone undergoes hydrogen-ion-catalyzed hydration but is sufficiently stable at neutral pH to determine K_D. The system chosen represents first-order transfer between the aqueous (C_1) and organic (C_2) phases with simultaneous, reversible, first-order hydration. The transfer constants, k'_{12} and k'_{21}, were determined at 37°C in the absence of degradation where asymptotic values for C_1 agreed with the observed equilibrium values in nonkinetic partitioning studies. The first-order rate constants for hydration in 0.1N HCl were determined at 37°C in the absence of the organic phase. Partitioning with simultaneous hydration was then studied using 0.1N HCl and light liquid paraffin. Data were analysed by non-linear

regression based on the equation for C_1 as a function of time. The values for k'_{12} and k'_{21} from these experiments were comparable to the estimates obtained under stable conditions.



The fact that different methods of measuring log P have been investigated indicates that the original shake flask method is not ideal and improvements are possible. This becomes apparent if the Hansch data base (189) is studied for any compound in a given solvent system. Tables 1 and 2 give the partition coefficients for compounds studied in this thesis in the octanol/water system and the cyclohexane/water system. Simply by looking at the values for phenol it becomes apparent that there is a wide range. This can of course be due to a number of factors such as the pH of measurement, temperature etc., but in many cases it is not immediately apparent that such factors are responsible.

This was felt to be important since although QSAR methods have successfully been used to predict biological activity, examples being erythromycins (274) and thyroxine analogues (216), there are many groups of compounds whose activities are

Table 1. Published LogP Values in the Octanol/Water System

Compound	Log P	Conditions of Measurement	Ref
Phenol	1.48	No details	269
	-0.22	18°C±2°C pH6.8 Phosphate buffer	339
	2.20	Details not given	356
	1.46	Shake flask.Room temp. pH≃2	145
	1.48	Private communication Not known	359
	1.49	20°C Acidified with HCl pH≃2	232
	1.51	pH=5.6 Min.charge Max.P Phosphate buffer. Also P values at other pH's	369
	1.51	20°C Acidified with HCl pH≃2	236
	0.62	pH=7.4 Phosphate buffer Not ion-corrected	209
o-Clphenol	2.17	No details	269
	2.15	Shake flask.Room temp. pH≃2	145
	2.19	Private communication Not known	359
	2.12	20°C Acidified with HCl pH≃2	234
m-Clphenol	2.50	Shake flask.Room temp. pH≃2	145
	2.47	Private communication Not known	359
	2.52	Russian journal Not known	235
p-Clphenol	2.35	No details	269
	2.39	Shake flask.Room temp. pH≃2	145
	2.44	Private communication Not known	359
	2.40	Russian journal Not known	235
	2.53	pH=5.6 Min.charge Max P Phosphate buffer. Also P values at other ph's	369
o-NO_2 phenol	1.26	18°C±2°C pH6.8 Phosphate buffer	339
	1.79	Shake flask.Room temp. pH≃2	145
	1.73	Private communication Not known	359
	1.77	Tech.Note Not known	202
	1.79	20°C Acidified with HCl	238

Table 1 cont'd.

Compound	Log P	Conditions of Measurement	Ref
m-NO_2phenol	2.01	No details	269
	2.00	Shake flask. Room temp. pH 2	145
	2.00	Private communication Not known	359
	2.00	pH=5.6 Min.charge Max.P Phosphate buffer. Also P values at other pH's	369
	2.00	20°C Acidified with HCl	238
p-NO_2phenol	0.76	18°C±2°C pH6.8 Phosphate buffer	339
	1.91	Shake flask.Room temp. pH≃2	145
	1.91	Private communication Not known	359
	1.90	By modified or extended Brandstrom Technique	338
	1.38	pH=7.4 Phosphate buffer Not ion-corrected	209
	2.08	Not known	202
	1.95	20°C Acidified with HCl	238
2-NO_2resorcinol	1.36	pH=6.8 Ion-corrected 18°C	339
	1.56	pH=1.4 Using HCl	301
3-Me-2-NO_2phenol			
4-Me-2-NO_2phenol			
5-Me-2-NO_2phenol			
6-Me-2-NO_2phenol			
o-Mephenol	1.95	Unpublished analysis Not known	118
	1.97	Unpublished results Not known	142
	2.04	20°C Acidified with HCl pH≃2	231
m-Mephenol	1.95	No details	269
	1.96	Shake flask.Room temp. pH≃2	145
	2.01	Private communication Not known	359
p-Mephenol	1.95	No details	269
	1.94	Shake flask.Room temp. pH≃2	145
	1.92	Private communication Not known	359
	1.99	pH=5.6 Min.charge.Max P Phosphate buffer	369

Table 11 cont'd.

Compound	Log P	Conditions of Measurement	Ref
2,3-Me_2phenol			
2,4-Me_2phenol	2.30	20°C Acidified with HCl pH≃2	233
2,5-Me_2phenol	2.33	20°C Acidified with HCl pH≃2	233
2,6-Me_2phenol	2.36	Unpublished analysis Not known	251
3,4-Me_2phenol	2.23	20°C Acidified with HCl pH≃2	233
3,5-Me_2phenol	2.35	Unpublished analysis Not known	251
	2.31	20°C Acidified with HCl pH≃2	232
2,3,5-Me_3phenol			
2,3,6-Me_3phenol			
2,4,6-Me_3phenol			
2,3,5,6-Me_4phenol			
o-OHbenzaldehyde	1.70	Private communication Not known	361
	1.81	pH=5.4 Unpublished analysis	151
	1.65	pH=5.6 Min.charge.Max P Phosphate buffer	369
	1.62	Not known	202
m-OHbenzaldehyde	1.38	Not known	302
p-OHbenzaldehyde	1.35	pH=5.6 Min.charge.Max P Phosphate buffer	369
Benzoic acid	1.87	Shake flask.Room temp. pH≃2	145
	2.03	Not known	266
o-Mebenzoic acid			
m-Mebenzoic acid	2.37	Shake flask.Room temp. pH≃2	145
p-Mebenzoic acid	2.27	Shake flask.Room temp. pH≃2	145
2,6-Me_2benzoic acid			
3,5-Me_2benzoic acid			

Table 1 cont'd.

Compound	Log P	Conditions of Measurement	Ref
o-OHbenzoic acid	2.26	Shake flask.Room temp. pH≈2	174
	2.21	Unpublished analysis Not known	6
	2.24	20°C Acidified with HCl pH≈2	237
	2.25	pH=1.0 Using HCl	259
	0.95	pH=4.0	259
m-OHbenzoic acid	1.50	Shake flask.Room temp.	145
	1.48	20°C Acidified with HCl pH≈2	237
p-OHbenzoic acid	1.58	Shake flask.Room temp. pH≈2	145
	1.57	20°C Acidified with HCl pH≈2	237
2,6-OH_2benzoic acid	2.20	Unpublished analysis Not known	71
3,5-OH_2benzoic acid			
Acetanilide	1.14	Measured by HPLC	289
	1.16	Shake flask.Room temp. pH≈2	145
	1.17	Private communication Not known	323
	1.36	Private communication Not known	323
o-Meacetanilide			
m-Meacetanilide			
p-Meacetanilide	1.39	25°C Buffer pH=7.2	363
2,6-Me_2acetanilide			
3,5-Me_2acetanilide			

Table 2 Published LogP Values in the Cyclohexane/Water System

Compound	Log P	Conditions of Measurement	Ref
Phenol	-1.00	pH=9.2 0.05M Borate buffer Not ion corrected	58
	-0.72	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152
	-0.93	Calc.from mol.fraction part.coeff. pMF from expr. P=P-MF18(DO)/MWO DO=density org.solvent MWO=mol.wt.org.solvent	262
	-0.77	0.1%NaCl soln. by countercurrent dist^{n}	334
	-0.85	Unpublished analysis Not known	201
	-0.81	Unpublished analysis Not known	71
	-0.66	20^{o}C Acidified with HCl pH≃2	234
	-1.00	25^{o}C Shake 20min^{-1}/24hr 10^{-3}M	315
	-0.81	25^{o}C	206
	-0.74	Japanese journal Not known	208
	-0.74	Not known	1
o-Clphenol	0.08	pH=11.86 0.5M Phosphate buffer.Not ion corrected	58
	0.86	20^{o}C Acidified with HCl pH≃2	234
m-Clphenol	-0.70	pH=11.86 0.5M Phosphate buffer.Not ion corrected	58
	0.08	20^{o}C Acidified with HCl pH≃2	235
p-Clphenol	-0.70	pH=11.86 0.5M Phosphate buffer.Not ion corrected	58
	-0.26	Unpublished analysis Not known	71
	0.08	20^{o}C Acidified with HCl pH≃2	235
	-0.35	German journal. Not known	276
	-0.30	25^{o}C	
o-NO_2phenol	1.49	pH=8.38	220
	1.58	Not known	202
	1.45	20^{o}C Acidified with HCl	238
m-NO_2phenol	-1.57	pH=8.38	221
	-1.52	Not known	335
	-1.22	20^{o}C Acidified with HCl	238

Table 2 cont'd.

Compound	Log P	Conditions of Measurement	Ref
p-NO_2 phenol	-1.93	pH=8.38	220
	-1.79	Not known	335
	-2.01	Not known	202
	-1.70	20°C Acidified with HCl pH≃ 2	238
2-NO_2 resorcinol			
3-Me-2-NO_2 phenol			
4-Me-2-NO_2 phenol			
5-Me-2-NO_2 phenol			
6-Me-2-NO_2 phenol			
o-Mephenol	0.04	pH=9.2 0.05M Borate buffer Not ion corrected	58
	0.13	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152
	0.10	0.1%NaCl soln. By counter current distⁿ	334
	0.20	Shake method 0.05mg/ml 0.5MNaCl aq.phase	141
	0.00	25°C	206
	0.15	20°C Acidified with HCl	231
	0.01	Not known	1
m-Mephenol	-0.30	pH=9.2 0.05M Borate buffer Not ion corrected	58
	-0.15	2 min.shake with H_2O or 0.5M Phosphate buffer No temp.control	152
	-0.20	0.1%NaCl soln. Counter current distⁿ	334
	-0.10	Shake method 0.05mg/ml 0.5MNaCl aq.phase	141
	-0.34	25°C	206
	-0.30	Not known	1
p-Mephenol	-0.10	2 min.shake with H_2O or 0.5M Phosphate buffer No temp.control	152
	-0.19	0.1%NaCl soln. Counter current distⁿ	334
	-0.35	25°C	206
2,3-Me_2 phenol	0.51	0.1%NaCl soln. Counter current distⁿ	334
	0.14	Not known	1
2,4-Me_2 phenol	0.76	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152

Table 2 cont'd

Compound	Log P	Conditions of Measurement	Ref
2,4-Me_2phenol	0.55	0.1%NaCl soln. Counter current distⁿ	334
	0.34	20°C Acidified with HCl pH≃2	231
2,5-Me_2phenol	0.77	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152
	0.57	0.1%NaCl soln. Counter current distⁿ	334
	0.96	25°C	206
	0.56	20°C Acidified with HCl pH≃2	231
2,6-Me_2phenol	1.28	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152
	0.93	0.1%NaCl soln. Counter current distⁿ	334
	1.11	Not known	1
3,4-Me_2phenol	0.20	0.1%NaCl soln. Counter current distⁿ	334
	-0.80	pH=11.86 0.5M Phosphate buffer. Not ion corrected	217
	0.28	20°C Acidified with HCl pH≃2	231
3,5-Me_2phenol	0.54	2 min.shake with H_2O or 0.5M Phosphate buffer No temp. control	152
	0.27	0.1%NaCl soln. Counter current distⁿ	334
	-0.85	pH=11.86 0.5M Phosphate buffer. Not ion corrected	217
	0.21	25°C	206
	0.23	Not known	1
	0.38	20°C Acidified with HCl pH≃2	231
2,3,5-Me_3phenol	0.97	0.1%NaCl soln. Counter current distⁿ	334
2,3,6-Me_3phenol			
2,4,6-Me_3phenol	1.24	0.1%NaCl soln. Counter current distⁿ	334
2,3,5,6-Me_4phenol	1.77	pH=11.86 0.5M Phosphate buffer. Not ion corrected	217
o-OHbenzaldehyde	0.38	pH=9.2 0.05M Borate buffer Not ion corrected	72
	1.37	Not known	202

Table 2 cont'd

Compound	Log P	Conditions of Measurement	Ref
m-OHbenzaldehyde	-1.93	Unpublished analysis Not known	201
p-OHbenzaldehyde	-2.54	Unpublished analysis Not known	201
	-1.93	Not known	202
Benzoic acid			
o-Mebenzoic acid	0.655	6hr rocking method. 45° & 1cpm 25°C	321
m-Mebenzoic acid			
p-Mebenzoic acid			
2,6-Me_2benzoic acid			
3,5-Me_2benzoic acid			
o-OHbenzoic acid	-1.02	24°C Equil.over 3 days Occasional shaking. pH adjusted so no dissoc.	22
	-0.50	6hr rocking method. 45° & 1cpm 25°C	321
	-0.47	20°C Acidified with HCl pH≃2	237
m-OHbenzoic acid	-2.04	20°C Acidified with HCl pH≃2	237
p-OHbenzoic acid	-1.77	20°C Acidified with HCl pH≃2	237
2,6-OH_2benzoic acid			
3,5-OH_2benzoic acid			
Acetanilide			
o-Meacetanilide			
m-Meacetanilide			
p-Meacetanilide			
2,6-Me_2acetanilide			
3,5-Me_2acetanilide			

not adequately explained by QSAR. There can be many reasons for this, including 1) basing the prediction on a poorly designed series or an invalid or ambiguous regression equation 2) basing it on an extrapolation outside the range of the physical properties represented by the original substituents and 3) the conditions of the biological tests were different (275).

These reasons however, assume that the parameter values used for the series are correct. It was felt by this author that this was not necessarily the case and that it was possible that many anomalies encountered in quantitative structure-activity studies could arise from inaccurate measurement or calculation of physicochemical properties such as partition coefficient.

To investigate this theory we initially studied the information provided by the Hansch data base (Tables 1 & 2) A series of substituted phenols was chosen for the study, the criteria for selection being that the compounds were easy to obtain, either by purchase or synthesis, and the degree and type of substitution was such that factors affecting the expected partition coefficient could be identified without interference from other substituents.

Tables 1 and 2 , as has been said, illustrate the range of partition coefficient values available for each compound. Such a range is important since if all the values are included in an average estimate of log P an obvious large error will result. Therefore, certain values must be excluded from such an average. Those values to be included

must have been measured under similar conditions so knowledge of experimental conditions is necessary. To find this information, the original reference was consulted, but as the two tables show, such information was not always available. From the information that was available however, it is apparent that pH is very important and possibly temperature also.

From this study of the Hansch data base it became apparent that it would be necessary to select an adequate method of measuring partition coefficient and apply that method to all the compounds included in the study. This would have a number of advantages: 1) the conditions of measurement, technique used and operator would be the same for each compound so comparisons of log P would be valid, 2) compounds with previously unmeasured log P's could be included in the study, 3) the log P values measured by the chosen method could be compared with those given in the Hansch data base.

Thus Chapter 2 describes the selection and preparation of the compounds and Chapter 4 describes the selection of a suitable method. It was decided to pursue variations of the shake flask method for measuring partition coefficients since this technique was already widely used and appeared to give satisfactory results. However, attention was paid to the effect that length of shaking time, vigour of shaking, temperature and pH had on the partition coefficient. This also meant that different methods of shaking were utilised and thus investigated.

In addition to the method used for measuring log P, it is

also possible to vary the solvent system in which log P is measured. The octanol/water system is probably the system in most common use and seems to be fairly representative of the membrane systems encountered in biological systems, but the initial choice of octanol as the lipid phase seems to have been made fairly arbitrarily by Collander (76) so Chapter 3 includes a fairly detailed discussion of different phases available and the reasons for selecting octanol. However, it was felt that different organic phases could provide information about interactions occurring within molecules, between molecules and between solvent and molecule and so the apolar phase, cyclohexane, was also investigated.

Apolar solvents such as cyclohexane are not as simple to work with, and for correlation between polar and apolar solvent systems, it is necessary to introduce a term for hydrogen bonding (256).

i.e. $\log P_{octanol} = 0.5 \log P_{cyclohexane} + 2.43$

n	r	s
9	0.791	0.391

$\log P_{octanol} = 1.0 \log P_{cyclohexane} + 1.2 \log K_{HB} + 2.35$.

n	r	s
9	0.979	0.140

The linear relationship for the solutes (phenols) in the two systems is quite poor as shown in the first equation. However, when a term for hydrogen bonding is added ($\log K_{HB}$ is from Higuchi (198)) a good correlation is obtained. Also, the slope relating the log P values is unity, indicating

that the two processes are quite similar except for H-bonding. The intercept indicates that phenols of equivalent log P values are more easily partitioned into octanol than cyclohexane. The octanol/water reference system tends to indicate that hydrogen bonding is involved in the partitioning process. This simplification may well be desirable since for solutes moving from an aqueous to non-aqueous phase in living tissue, the free-energy change in hydrogen bonding, especially for a single hydrogen bond, must be small. However, for instances where hydrogen bonding is critical the use of cyclohexane can possibly give valuable information and was thus included as a reference system in this study.

Chapter 3 also includes the results of an investigation into the mutual solubility of octanol and water. Cyclohexane and water are virtually insoluble in each other so mixing is unlikely to produce any unexpected effects, but octanol and water display a fairly high degree of mutual solubility.

Hydophobic forces appear to be mainly the result of a desolvation process which is determined by the unique properties of water, such that, when an apolar organic compound is dissolved in water, a so-called 'flickering cluster' of water molecules envelops it. This is accompanied by a small evolution of heat and a rather large decrease in entropy. When such a molecular complex leaves the water phase and moves into a nonpolar solvent or a nonpolar phase of a protein, the cluster of water molecules is stripped from the organic compound. The resultant increase in entropy largely accounts for what is poorly termed hydrophobic bonding. Thus the main driving force for

leaving the aqueous phase and moving into the octanol (or lipoprotein) phase can be expressed as the desolvation of the small molecule. Since octanol is not a simple solvent under these conditions, in that it contains about 4% water and is also self-associated, a kind of desolvation of octanol is also possible in that a solute molecule may take control of a water molecule normally held by octanol. It is therefore important to ensure a standard amount of water contained in the octanol, i.e. the two phases must be in equilibrium so that these desolvation forces are constant for every experiment. Another important point is that since solubility varies with temperature, the desolvation forces will also vary with temperature. This is important for thermodynamic work since ΔH, ΔG, and ΔS will contain these variations. However, this should be constant, more or less, for all compounds.

Once the method of measuring log P was established, a set of partition coefficients for the compounds under investigation was determined and these were compared with published figures. In the event, two methods were eventually used and so further comparison was possible.

Partition coefficient is one of the most useful parameters of Hansch analysis because of the relevance of hydrophobic effects both to drug transport and to drug binding with some lipophilic site. However, the usefulness of log P is greatly increased by the fact that log P for any molecule more complex than a monosubstituted benzene can be estimated by adding known π values for substituents to some molecular fragment (the nucleus) for which log P has been

experimentally determined.

Thus, π is defined:- $\pi_X = \log P_{YX} - \log P_{YH}$

Y = appropriate parent structure

logP = octanol/water partition coefficient

So that in a molecule RXYZ the log P value may be estimated by adding $\log P_R$ for the nucleus, R, and π values π_X, π_Y and π_Z for substituents, assuming:-

$$\log P = \log P_R + \pi_X + \pi_Y + \pi_Z$$

This is known as the additive-constitutive nature of π and of course is of great value since it eliminates the necessity to measure every partition coefficient of every molecule, of particular value for unstable or insoluble compounds. However, if the calculated values of log P are to be used confidently in analyses, an accurate value of π must be available and this is the source of difficulty. Various assumptions made by early workers in this field have been found to be incorrect and are responsible in some degree for failure of additivity. The most basic error was the assumption that $\pi_H = 0$. Later work revealed that the hydrogen atom in fact has a π value of $\sim$0.2 which must be included in calculations. The proximity effect is another factor affecting additivity. It was found that the π value of certain groups was influenced by the type and proximity of their neighbours. Correction factors have been devised to account for deviations from additivity, examples being:

Proximity effect	(2C)	2 x 0.23	
	(1C)	3 x 0.27	(Rekker 325)
Double bond		-0.30	(Leo 189)

However, rather than devising correction factors, deviations from additivity can be used to give information about structure and interactions.

In applying the equation for log P to polysubstituted benzenes, a correction for the dependence of π values on electronic interactions between substituents can usually be made by suitable choice of the fragment R and relevant π values so that an accurate estimate of log P can be made.

However, many drug molecules are frequently heterocyclic compounds and often are so flexible that they can exist in more than one conformation in aqueous solution. In certain conformations, or in rigid but sterically crowded molecules, the groups chosen as X,Y and Z may interact with each other or with the nucleus, R, through space by intramolecular hydrogen bonding. An experimental measurement of log P for such molecules can then be compared with a calculated value to examine the relevance of the additivity equation and to give insight into intramolecular and other effects.

Log P and π have been applied to Hansch analysis in two ways:

1. Transport alone is rate determining

$$\log BR = a \log P - b(LogP)^2 + ... + k$$

In this equation log P refers to the complete molecule and an optimal value is predicted from the 'random walk' theory when drug transport is rate determining. If this is applied as a model equation to complex molecules where additivity of π constants does not apply, log P must be measured, or large deviations will occur.

2. Transport is Not rate determining

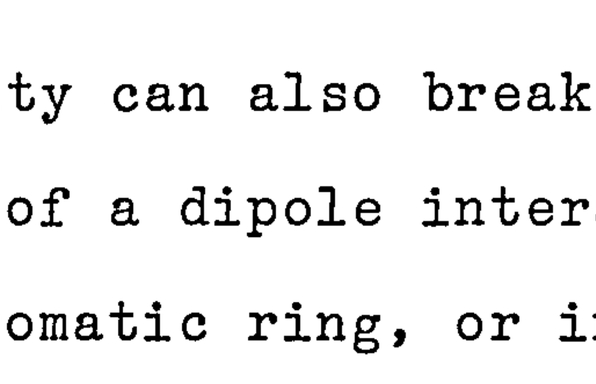
$$\log BR = C_{\pi} + ... + k$$

where this applies, π refers to that part of the molecule desolvated on binding to the receptor. This equation is often applicable to equilibrium situations, such as those found in vitro. Sometimes, different dependencies on π are found for substituents in different parts of the molecule. For example, the hydrolysis rates of substituted phenyl β-D-glucosides by emulsin have been shown by Hansch (172) to depend on π for the para substituents but not for the meta substituents.

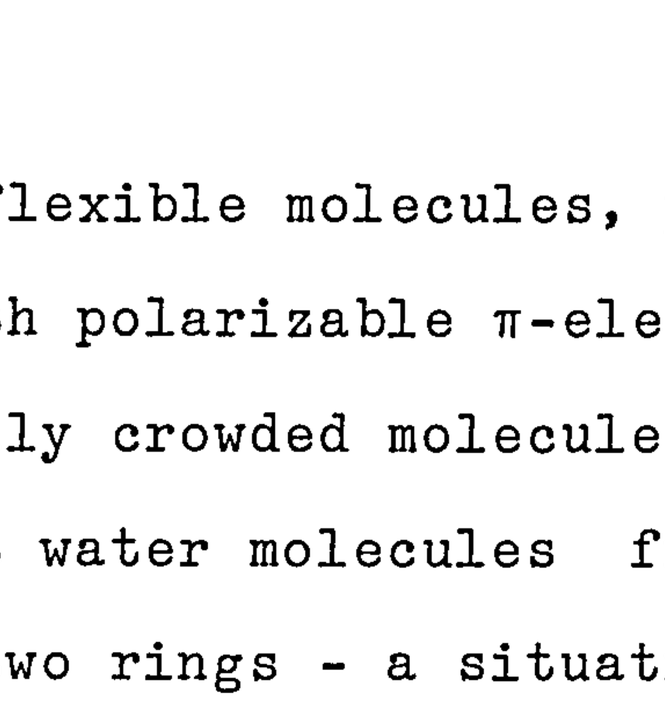
One problem that frequently occurs is that when π is taken from one series and applied to another, an electrical component may be needed to compensate for the remoteness of the model, especially when the series has strongly polar or hydrogen-bonding functions. (I)

Additivity can also break down in flexible molecules, possibly because of a dipole interacting with polarizable π-electrons of an aromatic ring, or in sterically crowded molecules where the overall shape may prevent water molecules from forming a solvate iceberg between two rings - a situation which can be considered as an intramolecular hydrophobic bond.

(I)



(II)



In compound II, a combination of intramolecular hydrophobic effects, and possibly interaction of the side chain dipole with one or both aromatic rings, leads to a wide discrepancy between experimentally determined and calculated log P values.

Sterically crowded molecules can also present additivity problems. In compounds with two aromatic rings there may be interaction between the rings which can be considered as an intramolecular hydrophobic effect. This can lead to a raising of log P. In addition, substitution on such rings can be involved in the interaction and may not produce the expected increase or decrease in log P. Therefore, whenever intramolecular interactions are possible either because of steric crowding or conformational flexibility, it is necessary to measure log P.

However, the need to measure log P values for such molecules reduces the advantages of the additivity function of π and therefore ways have been sought to account for these factors in log P calculations. Hansch has devised a fragment method for calculating log P and Rekker has developed his hydrophobic fragmental constant system. Both these methods are discussed in Chapter 5.

Those factors which affect additivity of π are an inherent part of the structure of the molecule and may be expressed as spatial (or steric) and electronic features. A drug molucule is usually considered in terms of the contributions of its component atoms or functional groups, and of the relationships of these to one another in the total molecule. Electronic features determine the potential bonding

propensities of a molecule or its components with other molecules (or with itself) including receptor structures, whilst spatial or steric features have an influence on the ability of the molecule to exercise its bonding propensities. Electronic properties of component atoms may, of course, influence configurational or spatial relationships within molecules.

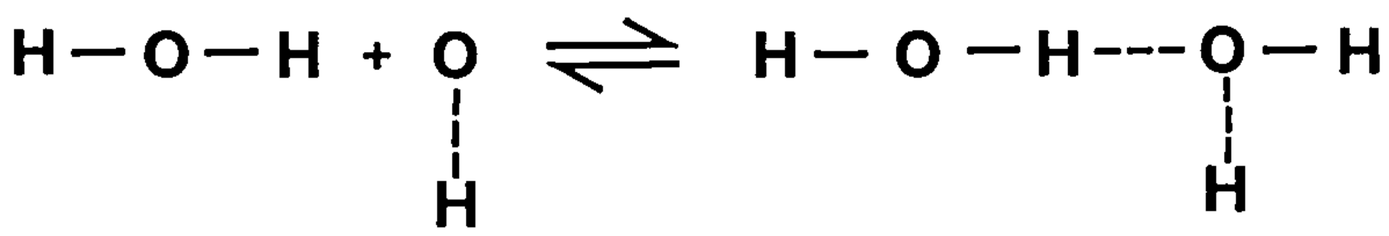
Electronic characteristics include the formation, disruption or modification of covalent, ionic, chelate, hydrogen, van der Waals, hydrophobic and charge (electron) transfer complex bonding. Covalent bonds are irreversible and therefore associated only with drugs which are potentially more toxic and thus have a separate role in structure-activity studies.

Ionic bond interactions are probably the most widely implicated drug-receptor bonding phenomenon, since the majority of drugs are ionized to some extent at physiological pH ranges. Four types of interaction may occur; ion-ion, ion-dipole, ion-induced dipole and dipole-dipole. There is a strong interaction between two oppositely charged ions. The energy of interaction between ions can be influenced by ion-dipole interactions in the solvent and substituents can also have an effect by modifying the distance between the charges. Substituents which tend to delocalise a charge will decrease the strength of interaction of the ion with another ion because with a diffuse charge the average distance between the charges will be larger. Substituents might also sterically prevent close approach of the two charges. Because of the angular relationship between the

centres of charge and the dipole moment, equivalent substituents at different positions will produce different net effects on dipole moment. Hence, separate structure-activity relationships may be found for analogues substituted at different positions. This aspect of substitution is investigated in this thesis.

Chelation, that is intramolecular hydrogen bonding which occurs with certain molecules such as β-blockers, that contain both electrophilic and nucleophilic functions in a relationship capable of interacting to form rings, as a form of bonding interaction is not an important feature in drug design.

The hydrogen bond however, has been found to be important in drug interactions. A hydrogen bond may form between a hydrogen atom and an electronegative atom such as F,O or N. The attraction of the electrons by the electronegative atom means that the hydrogen atom gains a partial positive charge which can interact with the partially negative atom of another molecule or an atom involved in a different covalent bond in the same molecule. For example:-



The hydrogen bond is considered unique to hydrogen because hydrogen is the only atom which can carry a positive charge while covalently bonded in a molecule and which is also small enough to allow a close approach of a second electro-

negative atom. However, lithium also has this ability to a slight extent.

The effect of hydrogen bonding on the partition coefficient of a molecule is investigated in this thesis. The effect of intermolecular hydrogen bonding between solvent and solute is investigated by studying the two solvent systems, octanol/water and cyclohexane/water.

Van der Waals intermolecular forces are weak, short-range forces and are not considered to be of any great importance in affecting log P in a system such as octanol/water, but they may be of importance in the cyclohexane/water system.

Hydrophobic bonds occur if two solute molecules at low concentration interact with each other rather than with the solvent. Thus a measure of hydrophobicity is the relative solubility of the liquid phase of the substance in water. Hydrophobicity is important since the overall hydrophobicity of a molecule is considered to be the determinant of the rate or extent of passage of the substance through membranes. This is the basis of the value of log P which is a measure of hydrophobicity.

Charge-transfer bonding occurs when a good electron donor comes in contact with a molecule which is a good electron acceptor and the donor transfers some of its charge to the acceptor. These bonds do not appear to be of general import in drug interaction and have not been investigated in this work.

Steric factors are also important in determining the potency

and specificity of many kinds of biologically active molecules. Spatial factors may be looked on in terms of size and shape and are governed by component atom radii, weights and bond distances and angles. Size may be expressed in dimensions of weight, volume or area of the molecule or of certain of its parts. The shape assumed by the molecule as a whole is related to the stereochemical arrangement of the parts and the shapes of the parts. Conformational differences in shape of a molecule result from the freedom of rotation of single bonds which permits substituents to assume different positions relative to one another. Thus positional isomers were included in the study since steric and electronic factors inherent in each isomer can be responsible for anomalies in partition coefficients and hence in structure-activity relationships.

Thus, having ascertained the existence of anomalies which cannot be accounted for by log P measurement techniques, it was necessary to investigate the actual source of such anomalies. To do this two techniques were investigated. Computer graphics was utilised to confirm molecular shape and substituent position relative to bond length, angles and area. This gave some idea of the importance of steric and electronic factors within each molecule. The results of this work are recorded in Chapter 8. The thermodynamics of partitioning was also investigated and this is recorded in Chapter 9. Spectroscopic data were utilised in conjunction with the thermodynamic and partition data to aid interpretation of the results. These have been recorded in Chapter 7.

Finally, Chapter 10 investigates the rate of partitioning and its relationship to biological activity. Quantitative structure activity relationships are based on the biological response to a given dose of drug, measured at a predetermined time after administration. Thus, the rate at which a drug is partitioned through membranes will influence the amount of drug present at the target site at any one time and hence the degree of response. The octanol/water partition coefficient of a drug has been found to correlate with biological activity although, as has been explained, anomalies occur which are difficult to explain. Since partition coefficient may be expressed as the ratio of the forward and reverse rate constants, an anomalous partition coefficient may be due to abnormal partitioning rates, either forward or reverse. Therefore, it is possible that the rate of partitioning into or from octanol will correlate better with biological activity than the partition coefficient. This thoery was investigated with a series of substituted hydroxyacetanilides.

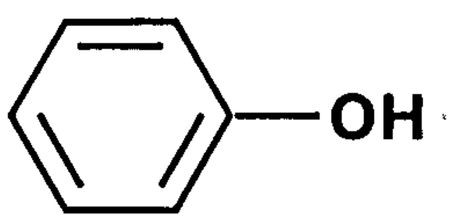
The purpose of this introduction has not been to provide a comprehensive review of all structure-activity work performed to date, even if this were possible in view of the advances made in recent years. Rather, an attempt has been made to illustrate the avenues pursued and the need for the work undertaken in this thesis.

CHAPTER TWO

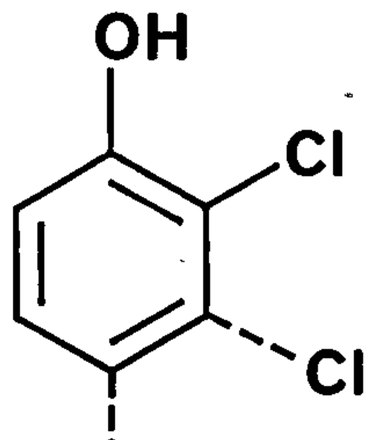
SELECTION AND PREPARATION OF COMPOUNDS

The prime aim of this investigation was to study intramolecular steric effects and intramolecular hydrogen bonding. Different series of compounds were selected that would demonstrate these effects. The meta- and para- isomers within each series were included as controls.

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Phenol		
C_6H_6O	QR	94.11



Chlorophenol		
C_6H_5ClO	1.QR BG	128.56
	2.QR CG	
	3.QR DG	



1.ortho

(1-chloro-2-hydroxybenzene)

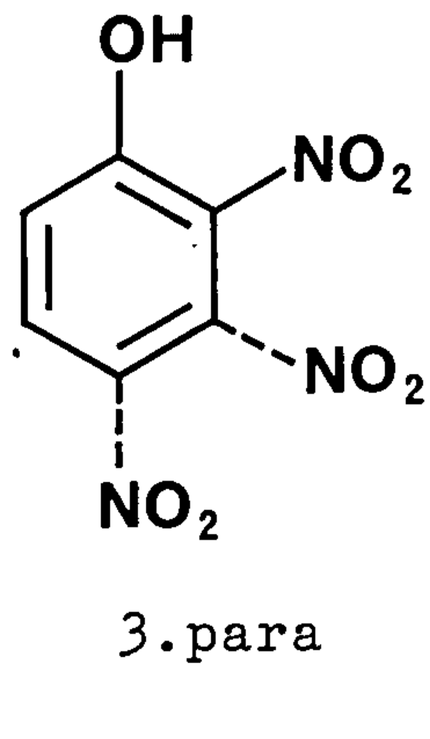
2.meta

(1-chloro-3-hydroxybenzene)

3.para

(1-chloro-4-hydroxybenzene)

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Nitrophenol		
$C_6H_5NO_3$	1.WNR BQ	139.11
	2.WNR CQ	
	3.WNR DQ	



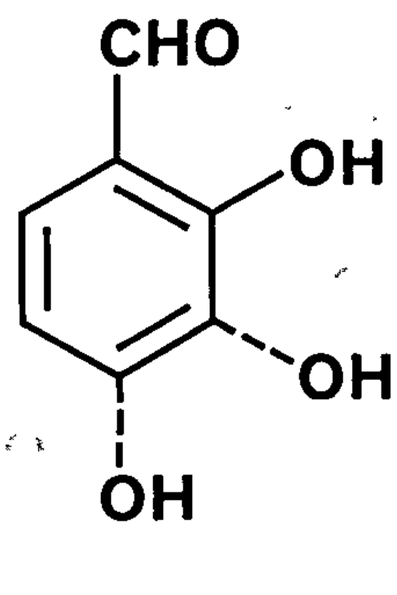
1.ortho

2.meta

3.para

2-Nitroresorcinol		
$C_6H_5NO_4$	WNR BQ FQ	155.11

Hydroxybenzaldehyde		
$C_7H_6O_2$	1.VR BQ	122.12
	2.VR CQ	
	3.VR DQ	



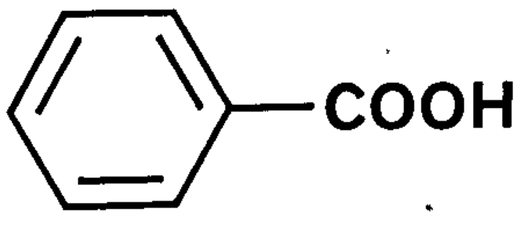
1.ortho

(Salicylaldehyde)

2.meta

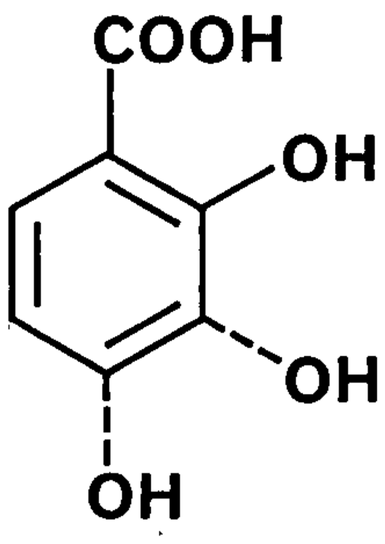
3.para

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Benzoic Acid		
$C_7H_6O_2$	QVR	122.12



Hydroxybenzoic Acid

$C_7H_6O_3$	1.QVR BQ 2.QVR CQ 3.QVR DQ	138.12



1.ortho

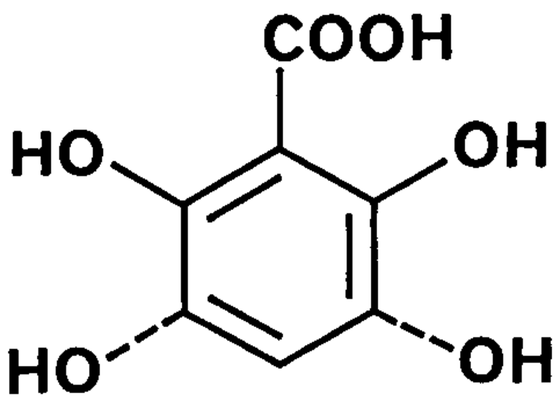
(Salicylic Acid)

2.meta

3.para

Dihydroxybenzoic Acid

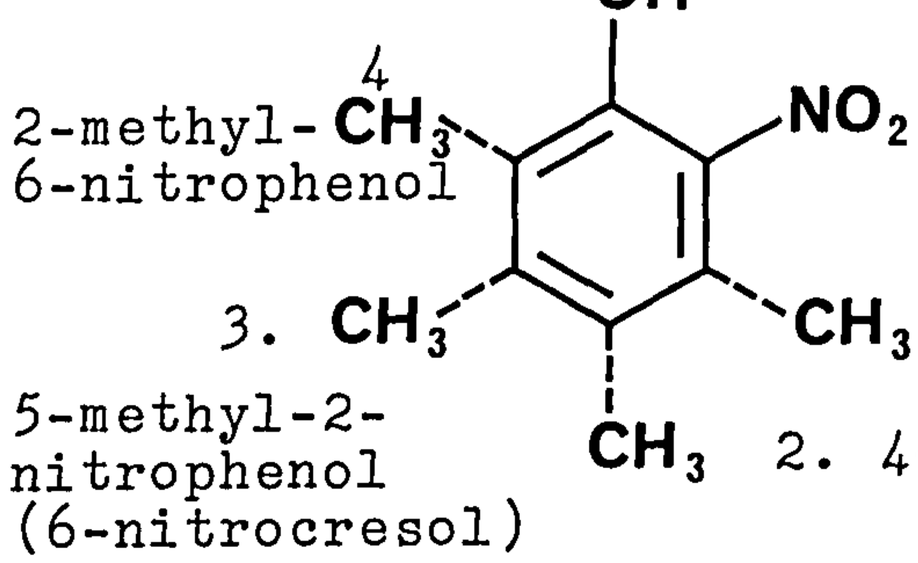
$C_7H_6O_4$	1.QVR BQ FQ 2.QVR CQ EQ	154.12



1. 1.2,6-dihydroxybenzoic Acid

2. 2.3,5-dihydroxybenzoic Acid

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Methyl-o-Nitrophenol		
$C_7H_7NO_3$	1.WNR BQ F1	153.13
	2.WNR BQ E1	
	3.WNR BQ D1	
	4.WNR BQ C1	



2-methyl-6-nitrophenol

3. 5-methyl-2-nitrophenol (6-nitrocresol)

1. 3-methyl-2-nitrophenol

2. 4-methyl-2-nitrophenol

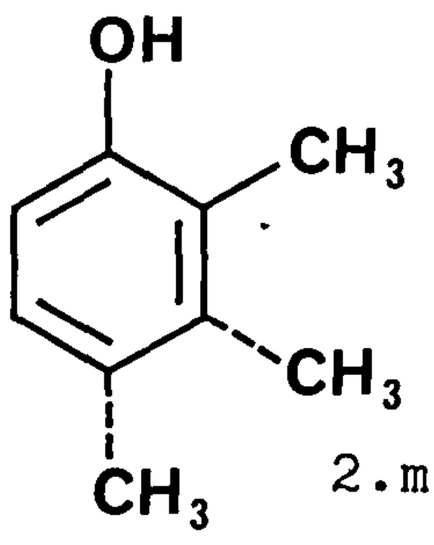
Methylphenol

C_7H_8O

1.QR B1 108.13

2.QR C1

3.QR D1

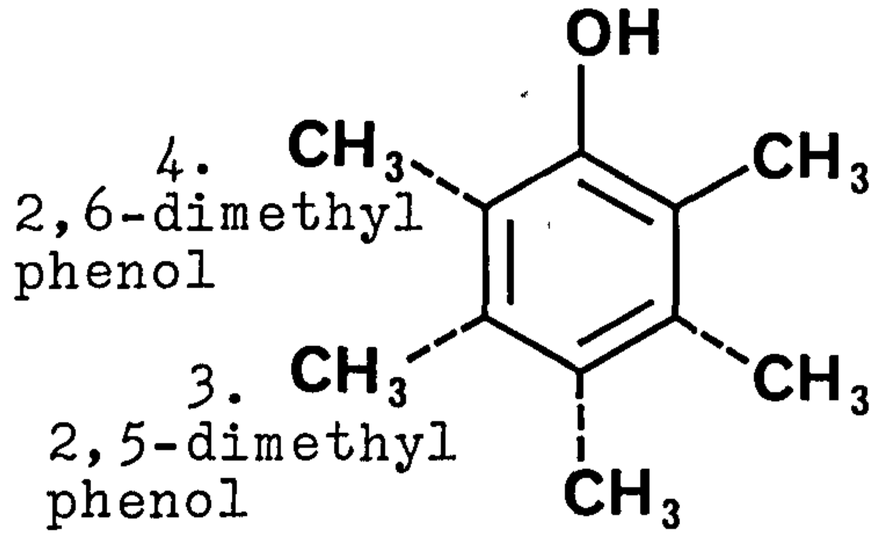


1.ortho (o-cresol)

2.meta (m-cresol)

3.para (p-cresol)

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Dimethylphenol		
$C_8H_{10}O$	1.QR B1 C1	122.16
	2.QR B1 D1	
	3.QR B1 E1	
	4.QR B1 F1	
	5.QR C1 D1	
	6.QR C1 E1	

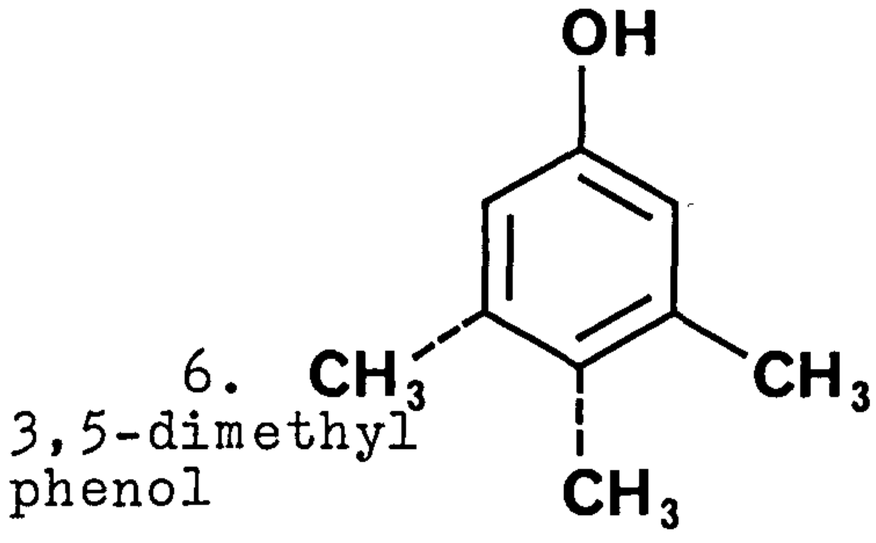


4. 2,6-dimethyl phenol

3. 2,5-dimethyl phenol

1. 2,3-dimethylphenol

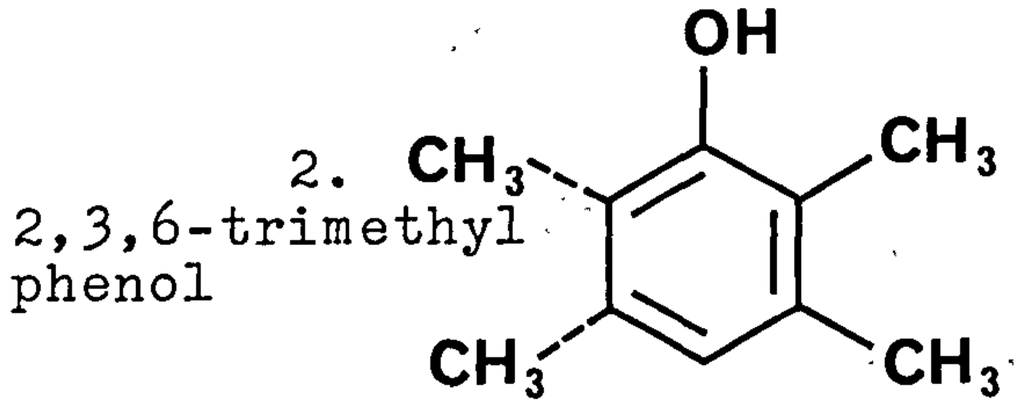
2. 2,4-dimethylphenol



6. 3,5-dimethyl phenol

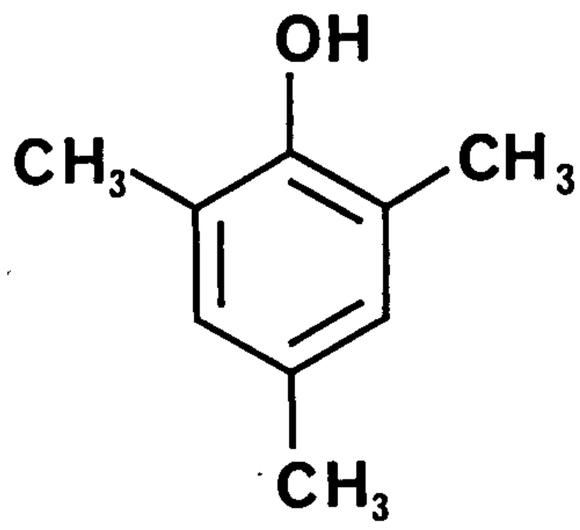
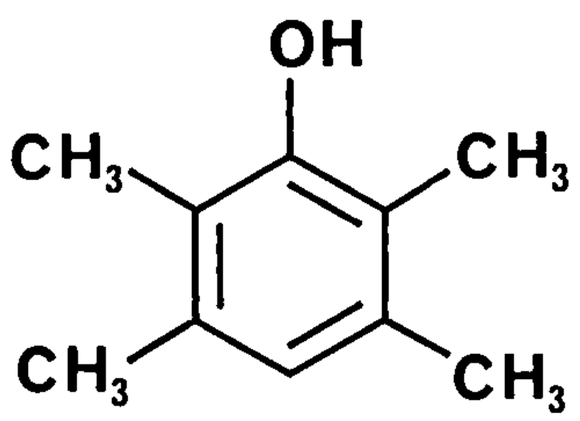
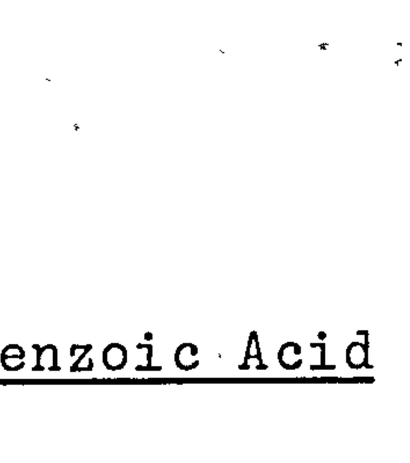
5. 3,4-dimethylphenol

Trimethylphenol		
$C_9H_{12}O$	1.QR B1 C1 E1	136.19
	2.QR B1 C1 F1	
	3.QR B1 D1 F1	

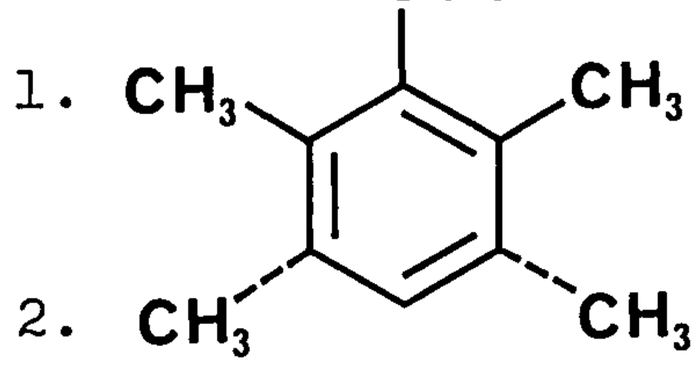


2. 2,3,6-trimethyl phenol

1. 2,3,5-trimethylphenol

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Trimethylphenol		
	3. 2,4,6-trimethylphenol	
Tetramethylphenol		
$C_{10}H_{14}O$	QR B1 C1 E1 F1	150.22
	2,3,5,6-tetramethylphenol	
Methylbenzoic Acid		
$C_8H_8O_2$	1.QVR B1 2.QVR C1 3.QVR D1	136.14
	1.ortho (o-toluic acid) 2.meta (m-toluic acid) 3.para (p-toluic acid)	

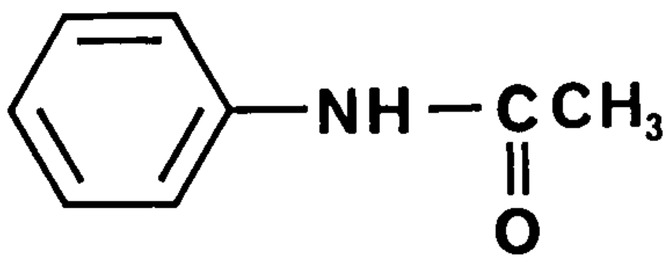
COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Dimethylbenzoic Acid		
$C_9H_{10}O_2$	1.QVR B1 F1	150.17
	2.QVR C1 E1	



1. 2,6-dimethylbenzoic acid (2,6-xylic acid)
2. 3,5-dimethylbenzoic acid (3,5-xylic acid)

Acetanilide

C_8H_9O MV1R 135.16

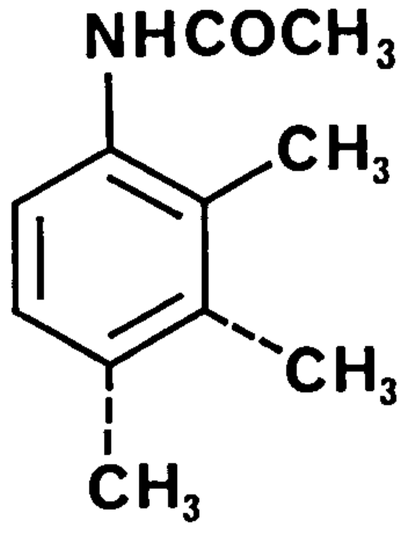


Methylacetanilide

$C_9H_{11}NO$ 1.1VMR B1 149.19

2.1VMR C1

3.1VMR D1

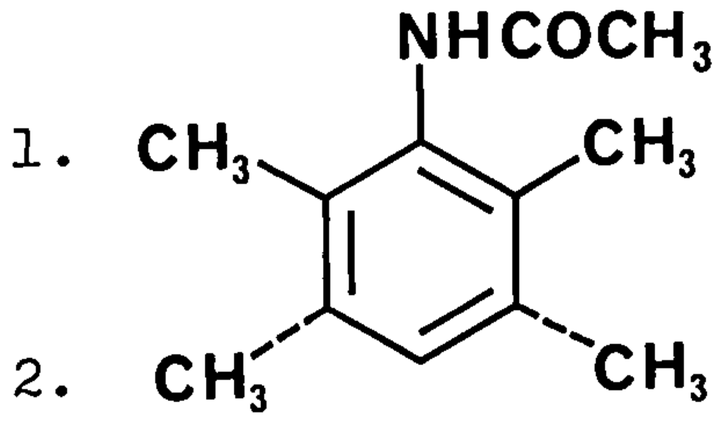


1.ortho (o-acetotoluide or N-acetyl-o-toluidine)

2.meta (m-acetotoluide or N-acetyl-m-toluidine)

3.para (p-acetotoluide or N-acetyl-p-toluidine)

COMPOUNDS	WISWESSER LINE NOTATION	MOLECULAR WEIGHT
Dimethylacetanilide		
$C_{10}H_{13}NO$	1.1VMR B1 F1	163.21
	2.1VMR C1 E1	



1. 1. 2,6-dimethylacetanilide

2. 2. 3,5-dimethylacetanilide

A further factor which influenced the choice of these compounds was that they were all, except for the acetanilides, available commercially from the Aldrich Chemical Company Ltd.

The acetanilides were synthesised in the laboratory as follows: ortho-, meta-, and para-methylacetanilide, 2,6-dimethylacetanilide and 3,5-dimethylacetanilide were all prepared by N-acetylation of the corresponding aniline.

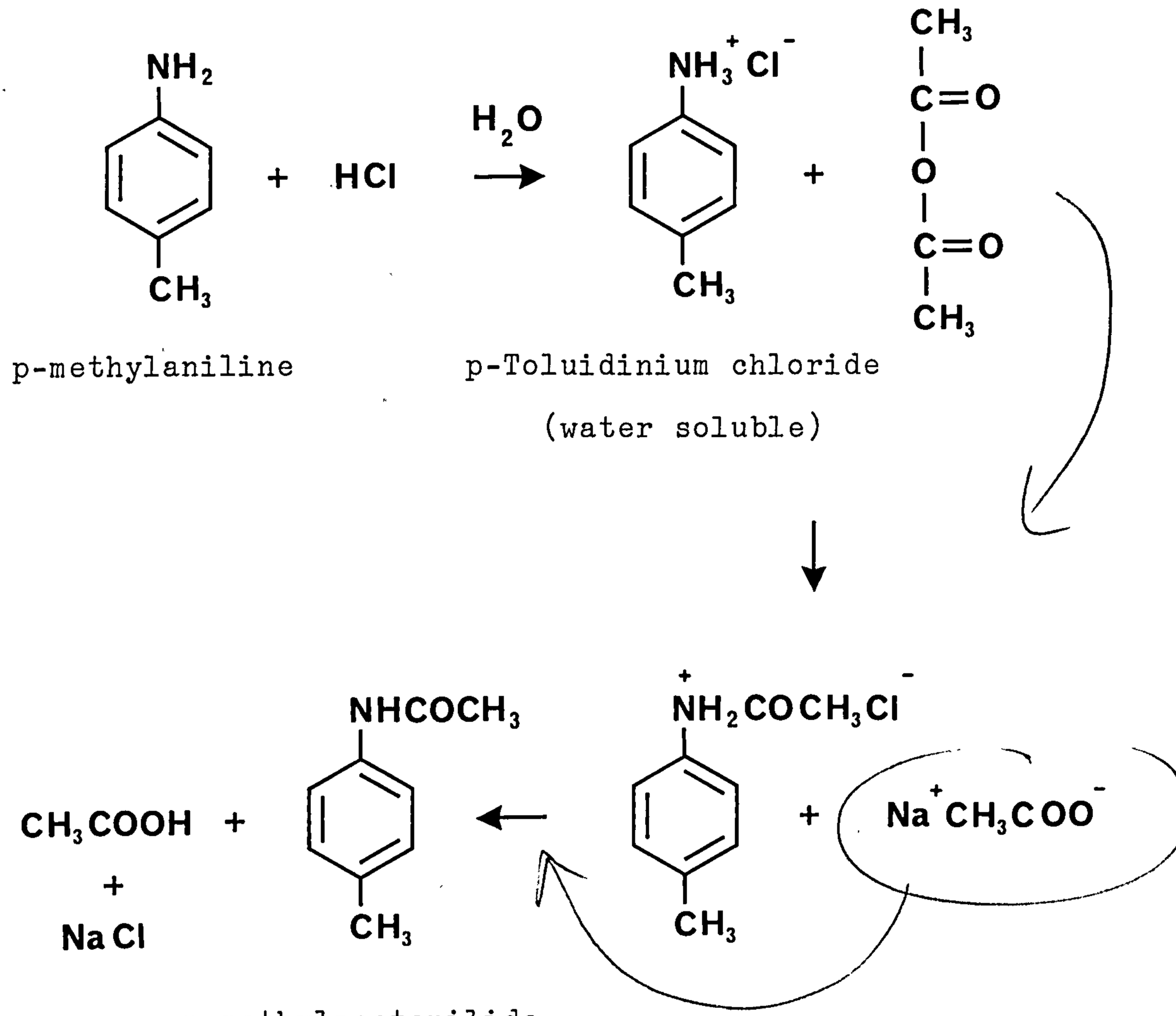
Two methods of preparation were tried.

Method 1

The amine was dissolved with an equivalent amount of hydrochloric acid in water. Then acetic anhydride was added, followed by enough sodium acetate to react with the hydrochloride salt and liberate the free amide.

Reaction scheme see over:

Preparation of Methylacetanilide: Method 1



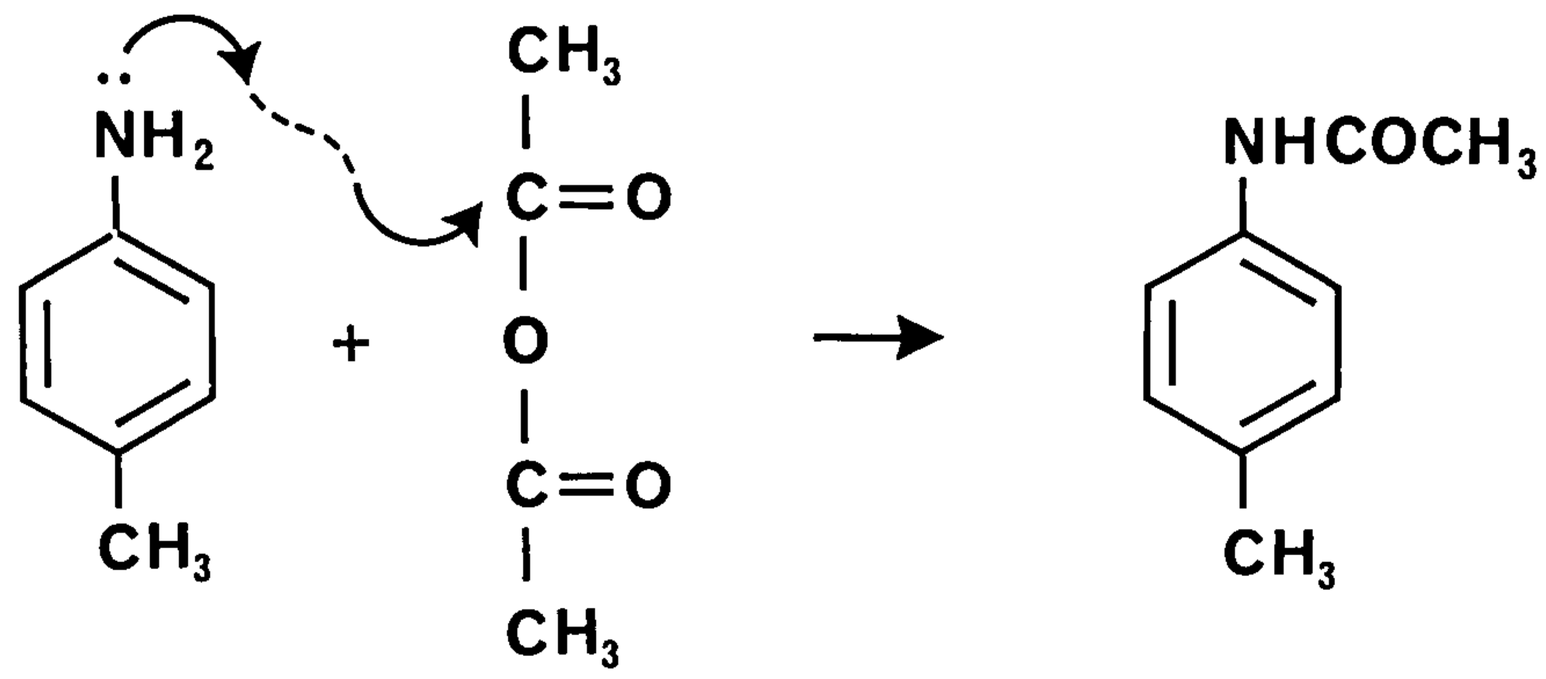
p-methylacetanilide

Method 2

One mole of the amine e.g. p-methylaniline was boiled with 400ml of glacial acetic acid in a one litre round bottomed 3-necked flask fitted with a reflux condenser, stirrer and thermometer, for two hours. The mixture was then cooled in ice and the acetanilide e.g. p-methylacetanilide, crystallized out. This was then recrystallised from ethanol.

Reaction scheme see over:

Preparation of Methylacetanilide Method 2



p-methylaniline p-methylacetanilide

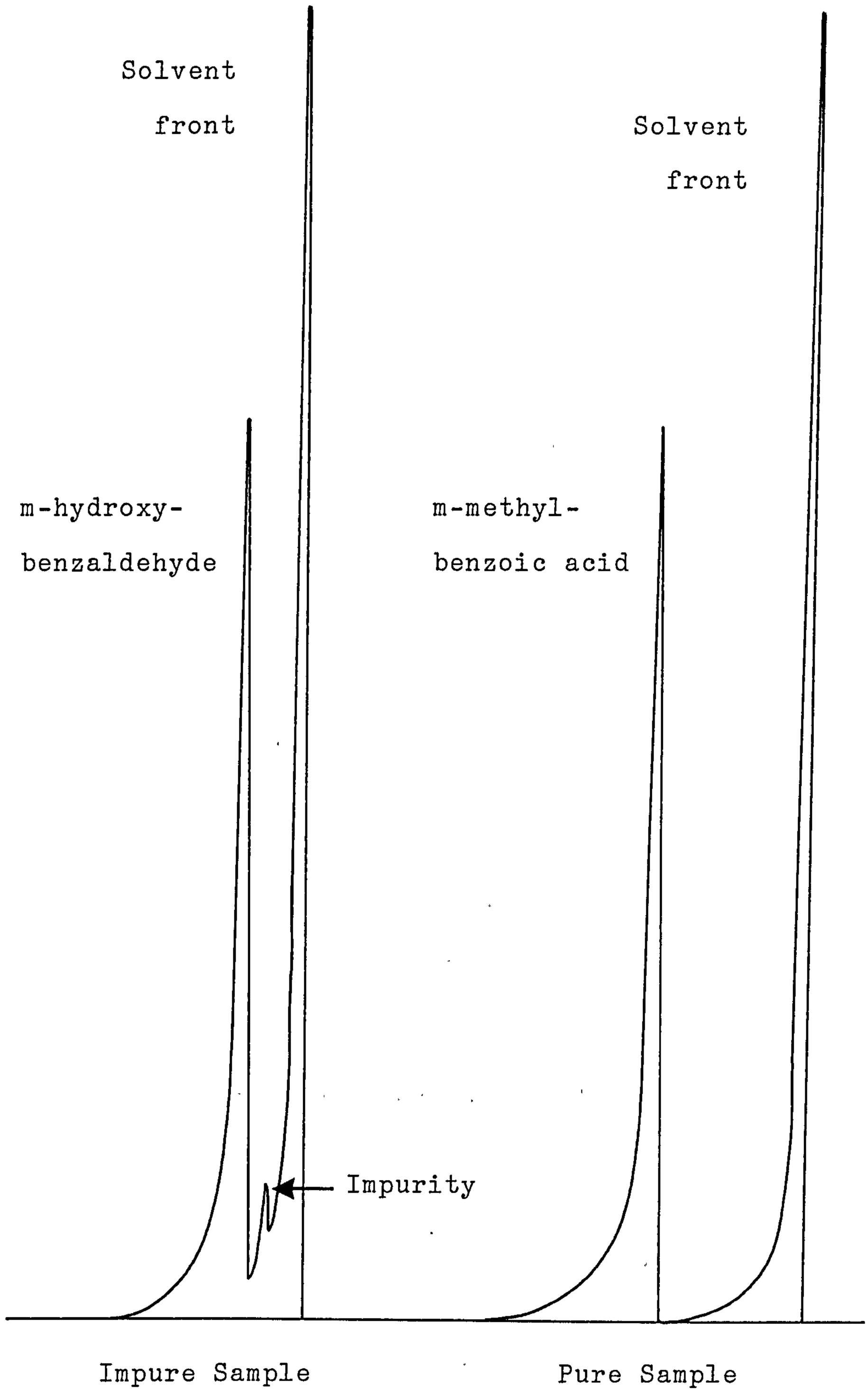
Method 2 was finally chosen to prepare all five acetanilides since this method produced the best yields.

	YIELD
o-methylacetanilide	35%
m-methylacetanilide	29%
p-methylacetanilide	25%
2,6-dimethylacetanilide	37%
3,5-dimethylacetanilide	45%

The prepared acetanilides contained coloured contaminants which were removed by adsorption onto charcoal. A small amount of decolourising charcoal (1 - 2 %) was added to a solution of the acetanilide which was then boiled for about 5 minutes and filtered.

The purity of all the compounds was checked by gas-liquid chromatography. For this, each compound was dissolved in methanol (10mg/ml) and a 1µl sample was injected onto the

Figure 6 Example of GLC Chromatograph to Show Impurity in Compound.



column.

Column packing : 3% SP2100 Supelco 80/100

Supelcoport 1-1987

Column temperature : 100°C (adjusted if necessary)

Detector : Flame ionisation

Detector temperature : 250°C

Chromatograph : Pye Unicam Series 104

Recorder : Perkin Elmer

The column temperature was adjusted for each compound to obtain satisfactory separation of the sample peak from the solvent front.

Any compounds showing impurities were either recrystallised from an ethanol/water mixture if solid, or redistilled by vacuum distillation if liquid, until GLC showed no detectable impurity. (Fig. 6.)

Recrystallisation

The solid to be recrystallised was placed in a 100ml round bottom flask with slightly less than the estimated quantity of solvent. Anti-bumping granules were added and the mixture was refluxed . If after 4-5 minutes the solid had not dissolved, more solvent was added slowly and refluxing continued.

Once the solid had dissolved the solution was filtered through a fluted filter into a suitable size flask, both funnel and flask having been previously warmed to prevent crystallisation in the stem of the funnel. This filtration removed dust and other insoluble particles. Gravity filtration was necessary rather than suction filtration at this stage because of:

1) the danger of crystallisation occurring in the stem of the funnel. (Hence warming of the funnel)
2) the difficulty of ensuring that a Buchner or Hirsch funnel is quite clean. Hot solvent may clean it and thus contaminate the solution being clarified.

After filtration the solution was covered to prevent dust entry and was allowed to cool slowly whilst remaining undisturbed. The crystal crop was then filtered off using a Buchner funnel. The mother liquor was removed by washing with small quantities of pure solvent. Air was drawn through the crystals for 4-5 minutes and they were then dried in an oven at a temperature at least 30°C below their melting point.

Vacuum Distillation

The liquid to be redistilled was placed in a round bottom flask fitted with a thermometer, fractionation column and condenser. A vacuum of 40 mmHg was applied to the system and the flask was heated to the boiling point of the liquid to obtain a slow uniform rate of distillation. The first and last cuts of distillate were rejected and intermediate fractions collected.

Pure solvents were also obtained by distillation.

Melting Points

The melting points of all the commercial compounds were checked and if necessary the compounds were recrystallised to constant melting point.

Melting points were determined using an electrically heated block. (Gallenkamp melting point apparatus). An approximate value of the melting point was determined by gently heating the block and noting the temperature at which the substance melted. An accurate determination was then made by inserting the capillary tube containing the compound into the block when the temperature was 10-20°C below the melting point and maintaining a uniform rise of 3°C per minute until the sample melted. The temperature was noted when the substance commenced to melt and when the last trace of solid disappeared. A narrow range gave an indication of a pure compound. Depression of the melting point or a wide range over which the substance melted indicated the presence of a contaminant and the need for recrystallisation.

Melting points can be corrected for the length of the thermometer stem exposed to the atmosphere. The correction to be applied is 1°C for every 100°C of exposed stem. The figures given are uncorrected.

Table 3. MELTING POINTS °C

COMPOUND	EXPERIMENTAL	LITERATURE VALUE
1. Phenol	39.5-41.5	39.5-41.5
2. o-Chlorophenol		9.0 (bpt 174.9^{760})
3. m-Chlorophenol	33-35	33 (35-36)
4. p-Chlorophenol	42-44	43.2-43.7
5. o-Nitrophenol	44-46	45.3-45.7
6. m-Nitrophenol	96-98	97
7. p-Nitrophenol	113-115	114.9-115.6
8. 2-Nitroresorcinol	81-82	
9. o-Hydroxybenzaldehyde	196-197bpt^{760}	-7 (bpt 197^{760})
10.m-Hydroxybenzaldehyde	104-105.	108 (101-103)
11.p-Hydroxybenzaldehyde	115-117	117
12.Benzoic Acid	122-123	122
13.o-Hydroxybenzoic Acid	157-159	159
14.m-Hydroxybenzoic Acid	201.5-204	201.3 (199-200)
15.p-Hydroxybenzoic Acid	213.5-215	213 (214.5-215.5)
16.2,6-Dihydroxybenzoic Acid	155-157	167d (150-170)
17.3,5-Dihydroxybenzoic Acid	232-235	238-240,232-233
18.3-Methyl-2-Nitrophenol	39-40	35-39*
19.4-Methyl-2-Nitrophenol	32.5-33.5	32-35*
20.5-Methyl-2-Nitrophenol	55-56	56
21.2-Methyl-6-Nitrophenol	67.5-68.5	68
22.o-Methylphenol	31-32	30.94
23.m-Methylphenol		11.5 (bpt 202.2^{760})
24.p-Methylphenol	34-35.5	34.8
25.2,3-Dimethylphenol	73-74	73-75
26.2,4-Dimethylphenol	25.4-26	26
27.2,5-Dimethylphenol	71-73	74.5

Table 3 cont'd

COMPOUND	EXPERIMENTAL	LITERATURE VALUE
28.2,6-Dimethylphenol	45-46	49
29.3,4-Dimethylphenol	64-66	65
30.3,5-Dimethylphenol	63-64	68
31.2,3,5-Trimethylphenol	91-93	92-95*
32.2,3,6-Trimethylphenol	62-63	62-64*
33.2,4,6-Trimethylphenol	70-71.5	69 (72)
34.2,3,5,6-Tetramethylphenol	108-109	108-110*
35.o-Methylbenzoic Acid	106-108	107-108
36.m-Methylbenzoic Acid	110-112	111-113
37.p-Methylbenzoic Acid	180-182	182
38.2,6-Dimethylbenzoic Acid	115-116	116
39.3,5-Dimethylbenzoic Acid	170-172	170-171
40.Acetanilide	113-114	114
41.o-Methylacetanilide	109-111	110
42.m-Methylacetanilide	65-67	65.5
43.p-Methylacetanilide	147.5-149.5	148.5 (153)
44.2,6-Dimethylacetanilide	178-180	177.5** (176.5***)
45.3,5-Dimethylacetanilide	139-142	141.5-142.5****

All values obtained from C.R.C Handbook of Chemistry and Physics 57th Edition 1976-1977.
Melting points of questionable accuracy given in brackets.
Boiling points given for liquids.. Pressure at which it was obtained appears as a superscript.

d = decomposed
* = Aldrich Chemical Company Limited. 1977-1978 Catalogue
** = P.B.D.De la Mare and M.Hassan. J.Chem.Soc.1958.1519-24
*** = S.V.Zhuravlev and E.V.Nikolaev. Zhur.Obshchei.Khim. 30.1155-7 (1960)
**** = A.van Loon,P.E.Verkade and B.M.Webster. Rec.Trav.Chim. (1960) 79.977-1001

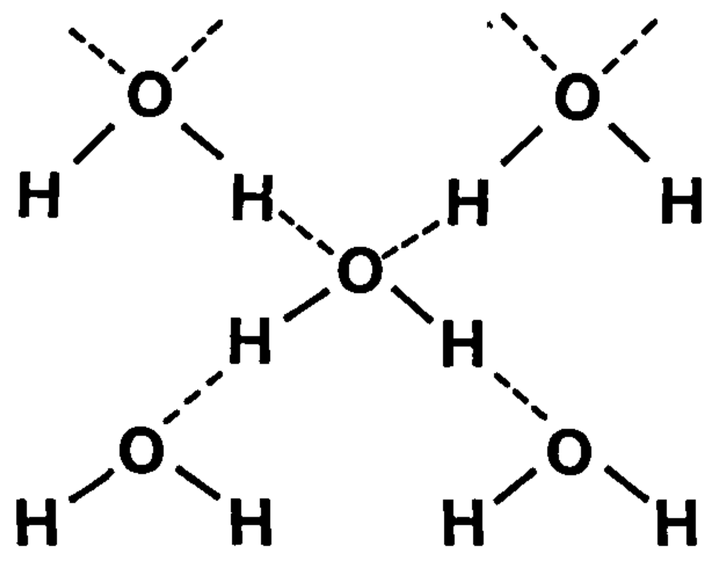
CHAPTER THREE

CHOICE OF SOLVENT FOR PARTITIONING WORK

Apart from its intrinsic chemical ability to act at the active site, a biologically active species can exert its influence only if it can reach the active site by a transport process that involves passage through both hydrophilic and lipophilic barriers in the biological system. Once at the active site the drug must interact with either an essentially aqueous system, an essentially nonaqueous one or both. Thus the nature of the liquid phases encountered by the drug are important as is the relationship of the receptor to the liquid phases since this may be disturbed by the drug. For example structured water molecules may be released which may cause a conformational change of the receptor.

In a biological system, two types of solvent are encountered, water and nonpolar.

The aqueous phase is essentially a dilute solution of salts in water. Water however is a unique solvent. In ice, the water molecules are arranged in a tetrahedral array so that each oxygen atom is surrounded by four hydrogen atoms; the two to which it is covalently bonded and two to which it is hydrogen bonded.



In liquid water the interactions present in the solid phase have been either eliminated or their goemetry changed and the hydrogen bonds are either broken or bent and stretched (230,99). This structure is not fixed in time, but is continually broken and reformed with different molecules.

The strength of solute-solute interactions in water is considerably influenced by the strong intermolecular forces between water molecules, plus the ability of water to interact with solutes by virtue of its large dipole moment, its polarizability and its ability to form hydrogen bonds.

The structure of liquid water changes in the presence of solutes. Water molecules tend to orient their dipoles to neutralise the charge of dissolved ions, whereas around apolar molecules they tend to form local 'icebergs'. In the former case the orientation of the molecules is different from that in the pure solvent, whilst in the latter it is similar. Thus ions and polar molecules are said to be water-structure breakers and apolar molecules are structure formers.

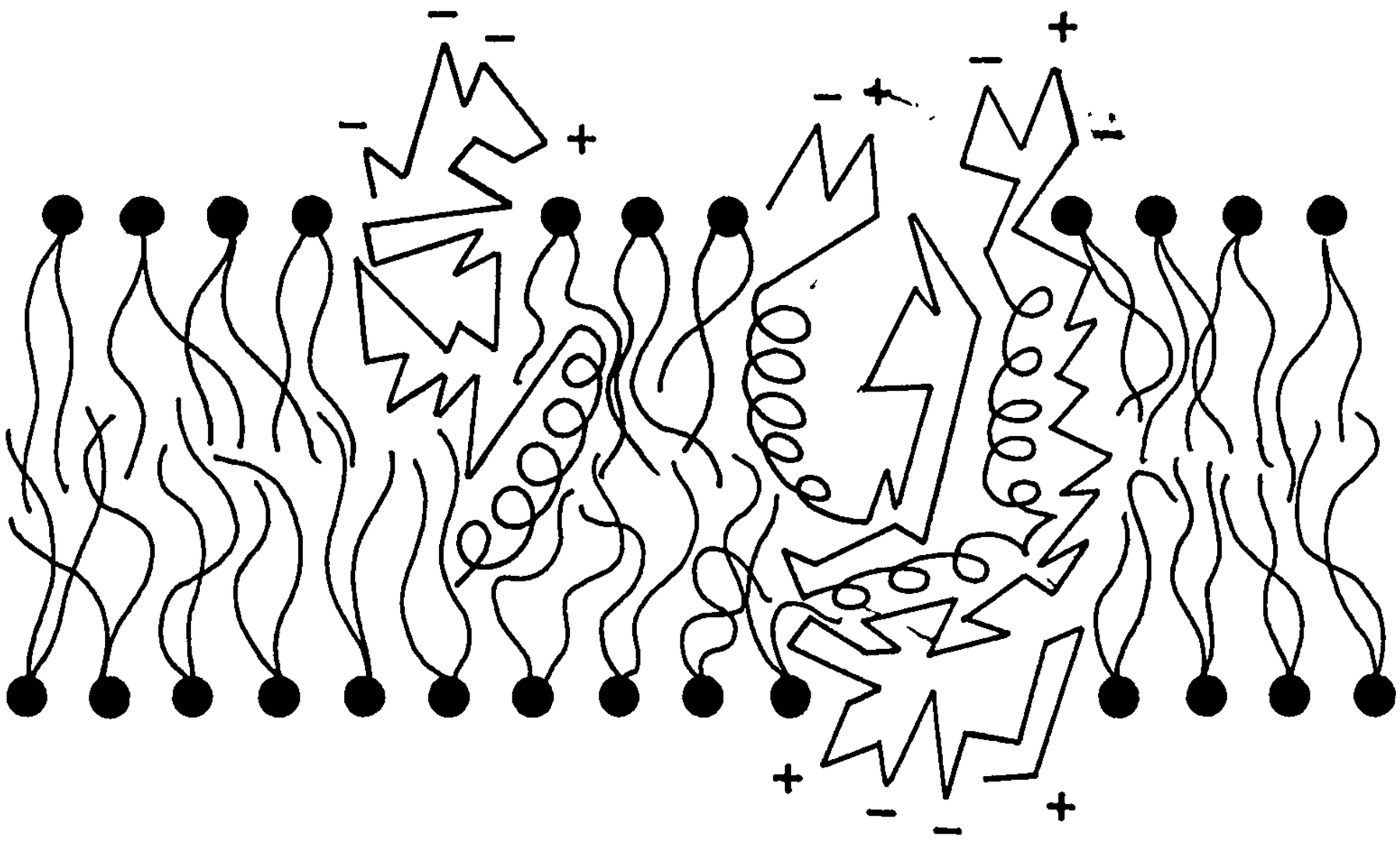
The membrane is probably the most important non-aqueous biological phase. It forms a lipophilic barrier to the entrance and exit of water soluble substances in the cell and it may also form a surface to which enzymes and other proteins may be attached to produce a localization and structural organization of function; thirdly, because of its relative impermeability to ions the membrane may separate solutions of different electrochemical potential.

Membranes are composed of lipids, the main ones being phosphatidylcholine, phosphatidyl ethanolamine or a glyco-

lipid. These are all amphiphiles: the amino or sugar group forms the polar, water-soluble 'head' and the R group the hydrophobic tail, usually a chain of 16 to 24 carbon atoms.

The lipids form two layers. The polar groups of one set of lipids is on one surface in contact with water, the hydrocarbon chains of these molecules are in the next layer, then there is an'empty'region in which the ends of the hydrocarbon chains are found, next are the hydrocarbon chains of the lipids of the other side of the membrane, and finally there are the polar groups of the second layer of molecules in contact with the second aqueous phase.

STRUCTURE OF MEMBRANE (331)



The stability of the membrane arises from the stabilisation of the ionic charges by ion-dipole interactions with water as well as from the association of the nonpolar groups with each other. The hydrocarbon chains move freely and thus this region is similar to liquid hydrocarbon. The lipid layer is interrupted more or less frequently by islands of

protein which, with the individual lipid molecules are free to move within the plane of the membrane.

An important aspect of drug design therefore is the modification of the hydrophobic character of compounds possessing a desired intrinsic chemical activity so as to optimise their transport to, and binding at, the active site. This means that partitioning is an important measure of activity and in order to obtain meaningful experimental partitioning data which can be related to activity, a synthetic reference biological system must be developed. Thus various solvent-water systems have been utilised such that the solvent represents the lipophilic phase and water the hydrophilic phase. However, problems arise in relating one system to another so ideally a standard system should be agreed upon.

As can be seen from the description of the biological systems, they are far too complex to be closely modelled, even with respect only to hydrophobic characteristics, by a simple two-phase system. Also, in biological systems the lipophilic phases are not pure hydrocarbons but are associated with significant amounts of water held by the polar and/or ionic portions of the lipid molecules. These polar areas and their associated water molecules have a profound effect on the lipophilicity of these phases.

Partition coefficients are often thought of as coming from reference systems comprising one pure solvent phase and one pure water phase, but in fact, partition coefficients refer to systems that are composed of two binary phases, a water-saturated solvent phase and a solvent-saturated water phase. The effect of such saturation on the structure and properties

of a given phase will be discussed later.

Another point to note is that to be useful, a partition coefficient must characterise the transfer of only a simple molecular form in which it exists in these phases. To determine P, the experimentally determined amounts of partitioned solute in each phase must be corrected to take into account the various molecular species with which it is in equilibrium. This can often present difficulties so ideally the reference system should minimise these uncertainties for as wide a range of solutes as possible.

A large number of solvent systems have been examined. These are illustrated in Table 4 where they have been placed in order of increasing lipophilicity, which in this case has been defined by the solvent's inability to accommodate water molecules.

Within these systems, the least lipophilic solvents in the left hand column demonstrate a linear relationship between log P values measured in any two systems.

$$\log P_2 = a \log P_1 + b$$

This is not surprising if it is considered that in transferring from the polar aqueous phase to the lipoid phase, a solute molecule would exchange 'hydration' forces for those forces provided by an alkyl chain, plus a hydroxyl group as found in the alcohol solvent systems. However, this relationship becomes more complex as lipophilicity increases in the solvent, and the solute must be considered as having lipophilic and hydrophilic segments.

Table 4. Lipophilicity of Solvent Systems

	LIPOID PHASE	MOLES $H_2O(x10^3)l^{-1}$
INCREASING LIPOPHILICITY ↓	1.n-Butyl alcohol	9440
	2.Cyclohexanol	6510
	3.2-Butanone	5460
	4.2-Pentanol	5320
	5.Primary Pentanols	5000
	6.Cyclohexanone	4490
	7.Octanol	2300
	8.Ethyl Acetate	1620
	9.Methyl isobutyl ketone	950
	10.Oleyl alcohol	712

	LIPOID PHASE	MOLES $H_2O(x10^3)l^{-1}$
INCREASING LIPOPHILICITY ↓	11.Ether	690
	12.Isopentyl acetate	456
	13.Nitrobenzene	180
	14.Oils	72.5
	15.$CHCl_3$	68.4
	16.Benzene	26.0
	17.Toluene	25.6
	18.Xylene	18.8
	19.CCl_4	10.0
	20.Heptane	3.3
	21.Cyclohexane	2.5

Thus, each system will present different problems and will alter the partitioning value of a solute. Further changes may exist if a pure solvent is used, or one saturated with water. These factors are included in the following discussion.

Movement, or diffusion of a molecule through a liquid matrix of others identical to it is simply related to the viscosity of the liquid. Diffusion of a different solute through a liquid matrix of identical but different solvent molecules is also related to the viscosity of the solvent, but there is also a component related to a concentration gradient and thus to the entropy and enthalpy of dilution. In addition to this, the solvent usually controls the motions of the isolated solute molecules in ways that relate viscosity to the intermolecular forces and to the molar ratio of free volume to intrinsic volume. The free volume of a liquid is considered to be the sum of a large number of empty 'holes' into which diffusing molecules may move in the transport process. The molecule has to overcome resistance as it moves from one hole to another. This resistance is unique to each solvent and may change if the phases are mutually saturated. In fact, the resistance to molecular transport has been shown to be changed by saturation by only 3% in all phases except for n-butanol and ethylacetate (345)

Another property of a solvent which may be altered by the addition of water is the dielectric constant. This gives a measure of the resistance to molecular 'flipping' in response to alterations of an applied electric field. This involves the size, polarizability and permanent dipole (if any) of the molecules and any tendency to self-associate to

form larger aggregates. The addition of significant amounts of highly polar water to organic solvents may result in either an increase or decrease in the dielectric constant and so molecular characteristics of liquid structure may be inferred.

3.1 Solvent Characteristics

1. Cyclohexane is a nonpolar solvent and has no capacity for hydrogen bond formation. Therefore it has negligible tendency for self-association into molecular aggregates, there is no liquid structure, and water is extremely insoluble in it. In a water-saturated solution, the dissolved water has no significant effect on density, viscosity or dielectric constant.

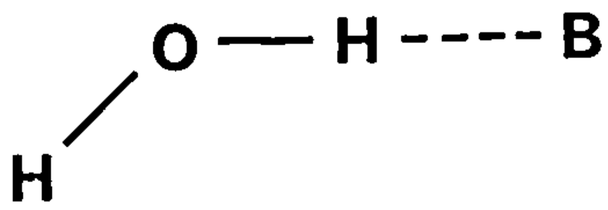
Isopiestic studies (214) indicate that the very small amount of water that does dissolve in cyclohexane is not associated and exists only in monomeric form.

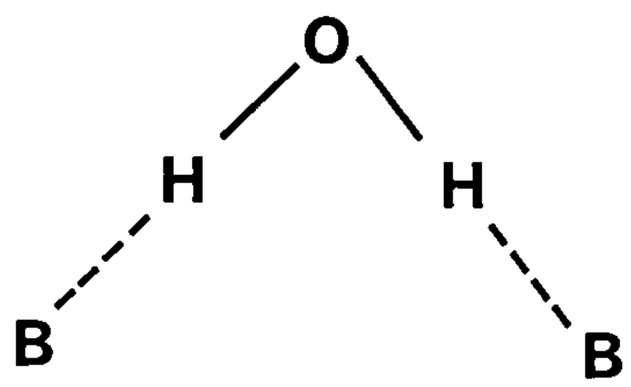
As a partitioning liquid, this binary phase has a great affinity for nonpolar compounds and their values of P will be extremely high (the reverse is true for very polar and ionic compounds), a fact which makes the determination of P very difficult because of the low solute solubility in one phase or the other. This problem can usually be solved by selecting a suitable volume ratio of the phases, but with one almost totally nonpolar phase this can be impossible.

This very pure nonpolar characteristic also leads to other experimental difficulties which may be impossible to overcome when measuring P values for polar solutes. It has been found (348, 70) that when a polar solute 'S' is extracted from water, there may be a many fold increase in the solubility of water in the nonpolar solvent due to the formation of hydrated solute complexes: $S(H_2O)$, $S(H_2O)_2$ etc. which are in equilibrium with each other; the nonpolar character

of the phase may be substantially changed by the process of partition itself. This will be especially so with hydroxylic compounds, amines and other hydrogen bonding substances. This problem is in addition to the solute association (such as carboxylic acid dimerisation) that often occurs.

It has been shown(290) in a study of the association of water with Lewis bases (substances that can donate electron pairs) in inert nonpolar solvents that for all N- and O-containing Lewis bases a 1:2 water-base complex formed when base is in excess, and a 1:1 water-base complex formed when water is in excess. It was also shown that hydrophobic cations are not hydrated at all in nonpolar solvents, but water is hydrogen bonded to halide ions in the same way as for uncharged Lewis bases.



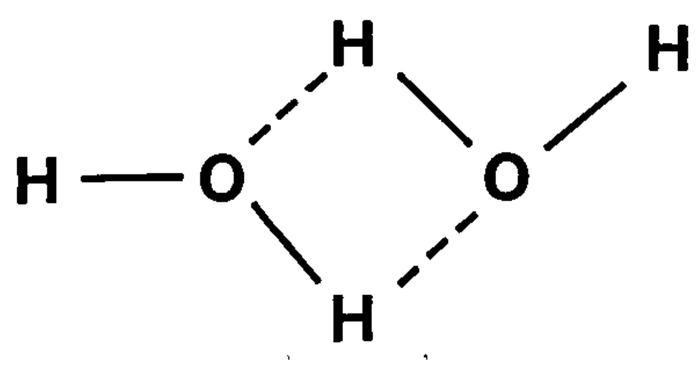


NMR studies (383) have shown that when polyfunctional solutes are being partitioned, allowance must be made for intramolecular binding as well as intermolecular binding.

The structural studies of associated polar solutes in non-polar solvents have so far not considered the effect of the water that is drawn into the solvent by the solute when it is partitioned.

The water concentration, and all equilibria between solute hydrates, solute polymers, hydrated solute polymers and intramolecularly bound molecules are dependent on solute concentration; hence, to avoid the necessity for complicated corrections it is advisable for the solute concentration in the nonpolar solvent not to exceed 10^{-3}M , but this may cause analytical difficulties in the aqueous phase. However, biological correlations with log P values determined in alkane-water systems are not as good as those with log P values from the more polar n-octanol-water system (132,58)

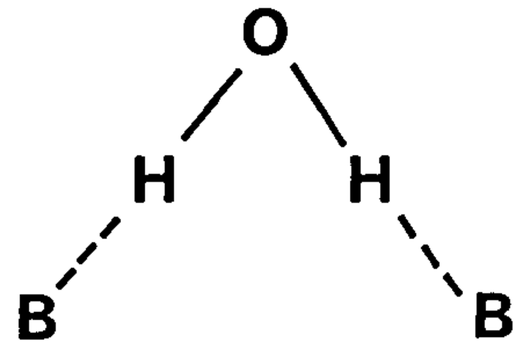
2. Carbon tetrachloride is a nonpolar solvent with characteristics similar to cyclohexane, although water is four times more soluble in carbon tetrachloride than in cyclohexane, and a small amount (about 3.6%) of the dissolved water is present as a dimer, probably with the structure:-



(270)

3. Benzene has many properties comparable to those of cyclohexane, being nonassociated and nonpolar with great affinity for lipophilic hydrocarbon-like substances. However, it is polarisable and its π - electron system can act as an electron pair donor for hydrogen bond formation. This means generally that polar compounds are more soluble in it and more specifically water is 15 times more soluble in benzene than in cyclohexane and thus the binary phase, water-

saturated benzene, is more polar than cyclohexane but its dielectric constant is barely larger than that of pure benzene and its density and viscosity are the same as those of pure benzene. The water dissolved in benzene, up to saturation is not self-associated in any polymeric form and exists primarily in a hydrogen bonded solvated form such as:



where B represents benzene, the base donor of the electron pair. (290,201)

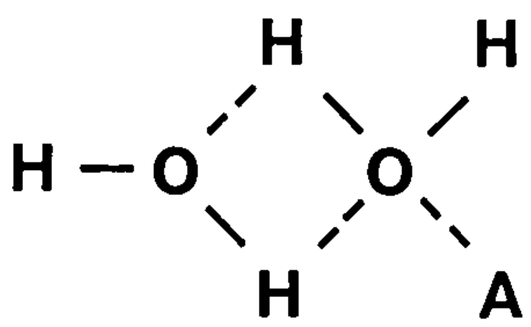
All structural and partitioning problems associated with cyclohexane also hold for benzene, and there are extra complications introduced by solutes such as alcohols competing with water for hydrogen bonding with the benzene.

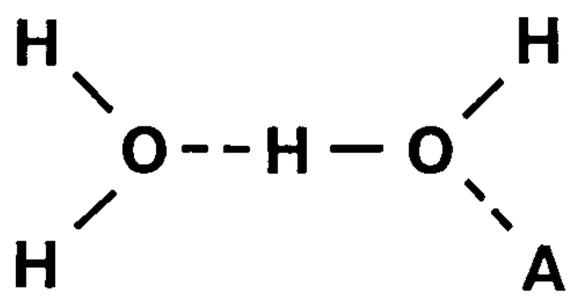
Analytical problems are also encountered due to the extreme solubility of lipophilic solutes in benzene and insolubility in water (and the reverse for hydrophilic solutes) and also the fact that benzene has very strong UV absorption so this method of analysis is inapplicable, even in the benzene-saturated water phase, at wavelengths shorter than 270nm.

4. Chloroform is a slightly polar, nonassociated liquid which has a great affinity for lipophilic materials. Its lone proton is an effective electron pair acceptor in hydrogen bond formation, so that the solubility of water is almost twice that in benzene. This greater solubility

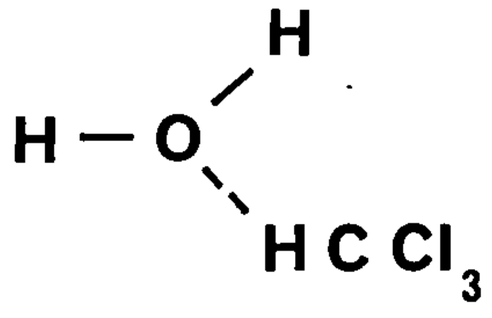
leads to a small but significant decrease in the dielectric constant of chloroform, yet it is still too small to have a significant effect on the density or viscosity. However, the amount of dissolved water is large enough to introduce a structural complexity not observed in benzene or cyclohexane.

NMR studies (278) revealed that a small amount of water dimer is hydrogen bonded to chloroform, probably as a mixture of: (343)





but the majority is monomeric and hydrogen bonded to chloroform:

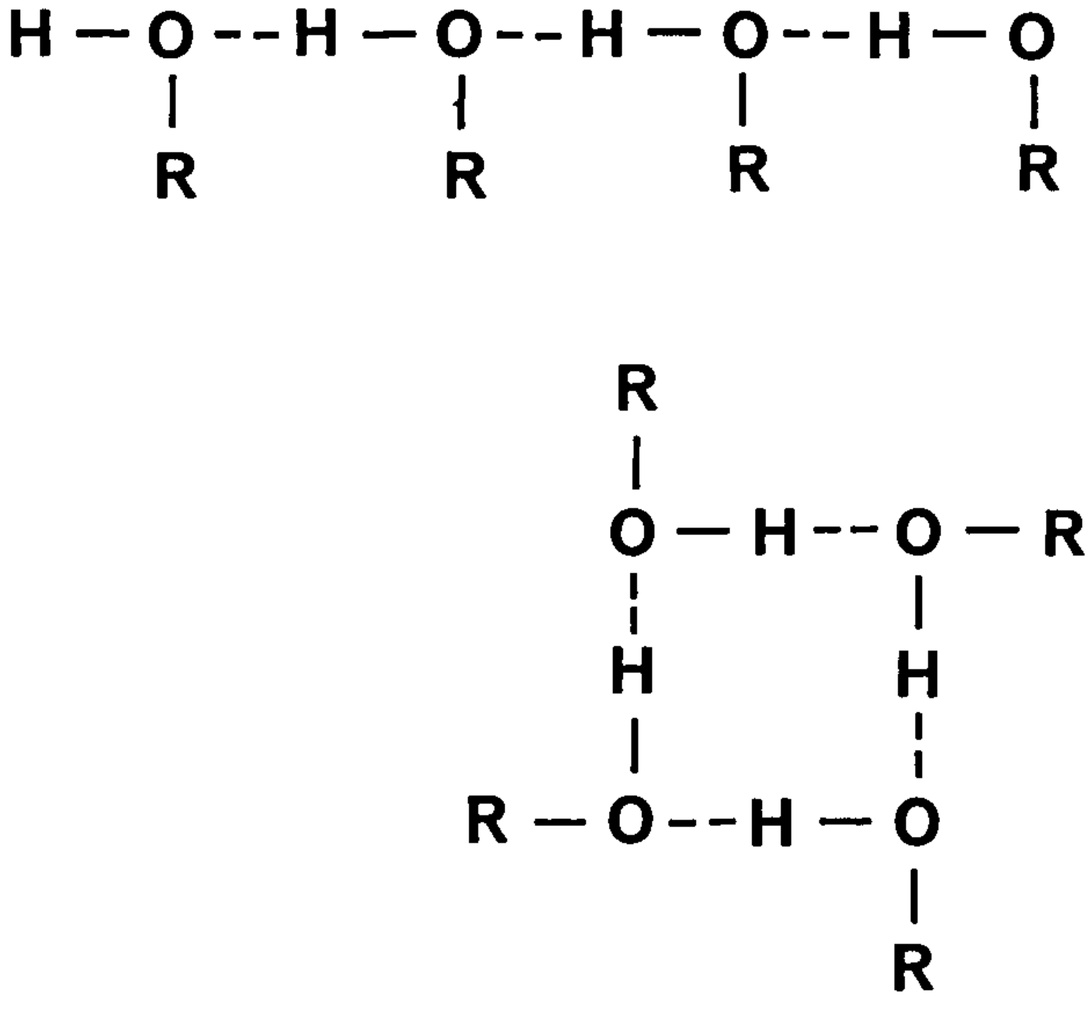


where A represents chloroform, the electron pair acceptor.

Chloroform does not have UV absorption problems, but has all other partition problems associated with benzene, although in a less extreme form, with the added complication of water association.

5. Alcohols have a highly polar hydroxyl group and thus a great capacity for hydrogen bonding. The pure alcohols are associated liquids with multiple equilibria existing

between monomers and linear and cyclic multimers. For non-sterically hindered alcohols IR spectral evidence indicates the predominance of linear and cyclic tetramers, in equilibrium with monomers.



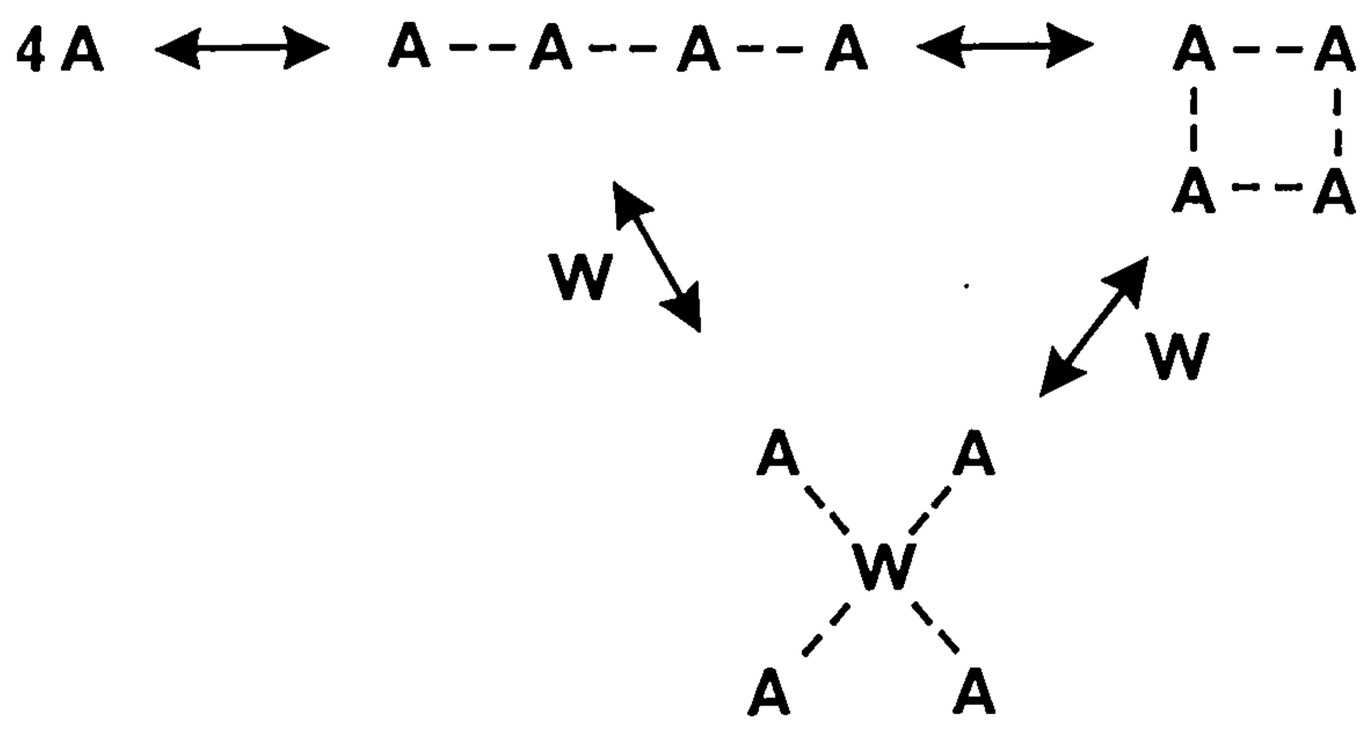
Increasing the temperature seems to decrease the relative proportions of cyclic to linear tetramers and decrease the proportion of tetramers to monomers. Highly substituted alcohols, especially those substituted on the α - carbon atom, have severe steric hindrance, which tends to reduce the concentration of monomers and encourage the formation of cyclic polymers (282)

When water is added to alkanols, the simpler ones undergo more profound intermolecular interactions, particularly if water-saturated solutions are considered as is the case with partition measurements. Two catogories, C_4 - C_5 and C_6 - C_{12} are indicated by water solubility. C_{13} alkanols and above are solids at room temperature and C_3 alkanols and below

are miscible with water in all proportions. Once the molar ratio of water to alcohol exceeds about 0.40, the structure and properies of the binary phase are so altered that much more water is associated, probably in a three dimensional hydrogen bonded network.

At room temperature when water is added to the C_1 - C_5 n-alkanols there is a steady increase in viscosity and dielectric constant with increasing water concentration, but for C_6 - C_{10} alkanols there is an initial decrease in these properties. In the case of 1 - octanol a minimum is reached at saturation.

The following equilibria are thought to exist:



A = Alcohol monomer

W = Water

Thus water-alcohol solutions are very complex, but it appears that water-saturated n-octanol binary phase has some advantages for partitioning. (345) All the alcohol and water molecules are involved in the same type of A_4W complex with a small amount of water probably involved in cross-

linking some of the complexes. Because of the large quantity of dissolved water, it is unlikely that the water concentration or the structural characteristics of this binary phase will be significantly changed as is the case of the nonpolar solvents. This means that low but convenient analytical concentrations of partitioned solute may be used, regardless of the polar functional groups it may possess. The balance of polarity and nonpolarity is such as to minimise solute self-association while retaining the predominantly lipophilic character required for a biolipid system.

The complicated association equilibria that exist for alcohol and hydroxylic solutes partitioned into nonpolar or slightly polar solvents are virtually eliminated in water-saturated n-octanol, with each such partitioned molecule probably replacing one octanol in an A_4W complex to maintain the equivalent of a monomeric dispersion of the partitioned solute. The A_4W complex also allows, in very high concentration (~6.2M), a highly lipophilic solute to 'dissolve' in the matrix of highly lipophilic carbon chains of neighbouring A_4W complexes.

Octanol is the alcohol of choice since it is easy to work with, it is not too viscous and it does not form stable emulsions with the ease of other alcohols

n-Heptanol and n-Nonanol might be considered as comparable alternatives to n-octanol for partitioning. Water-saturated n-heptanol has the advantage of being somewhat less viscous but the disadvantage of having a little too much water.

Water-saturated n-nonanol has the advantage of having a lower water concentration but the disadvantage of being more viscous.

Water saturated n-butanol has a water-to-alcohol ratio of about 1:1 and thus its structure is very complex. It is not suitable for use in a reference partitioning system because it offers little sensitivity for discriminating between the relative hydrophobicities of solutes; the water-saturated alcohol is too similar in solvent properties to the alcohol-saturated water phase.

6. Esters are polar Lewis bases whose oxygen atoms have a high potential for hydrogen bonding. Thus they tend to dissolve more water than the slightly polar hydrogen bonding solvents like chloroform or benzene. The binary ester phase will tend to discourage the formation of self-association complexes of the partitioned solute, but the solute may carry additional water into the solvent phase. It also seems likely that hydroxylic solutes will participate in different varieties of association complexes with water, ester and alcohol, depending on the degree of steric hindrance, hydrophobicity and concentration, making the determination of true partition coefficients very difficult.

Ethyl acetate has been fairly widely used but it is lacking in hydrophobic character and slowly hydrolyses, which reaction may be catalysed by some partitioned solutes. The hydrolysis products could drastically alter the apparent partition coefficient.

Olive oil is derived from a natural source and is theoretically a good reference biolipid phase, but it does not dissolve much water and thus problems of self-association of partitioned solute, structural problems, and problems of varying degrees of hydration of polar partitioned solutes by additional water brought in by the partitioning process associated with nonpolar or slightly polar solvents will occur. Olive oil is also difficult to work with because it is very viscous and it tends to form stable emulsions. Additional problems make it a very unsatisfactory reference phase, it is for example difficult to obtain consistent quality, particularly regarding its UV absorption characteristics.

Solvent-Saturated Water

Tables 5,6 and 7, show the principle properties of some solvent-saturated water phases. With the exception of n-butanol and ethylacetate, the solvents have very little effect. Ethylacetate and n-butanol affect the physical and structural characteristics of the hydrogen bonded water molecule network. An increase in viscosity probably results from the water structure-forming influence of the nonpolar hydrocarbon portion of the molecules. (195)

The other solvents in the table have solubilities in the range from 6×10^{-4} to 6×10^{-2} M. These concentrations have little effect on structure and properties, but may affect partitioning. Hydrophobic interaction between solute and solvent in the water phase may result in a higher solute solubility in solvent-saturated water than in pure water,

DENSITY OF PURE AND SATURATED SOLVENTS AT 15-35°C

Table 5.

Solvent	State	ρ g/ml 15°	25°	35°
Cyclohexane	Pure	0.784	0.774	0.765
	Water saturated	0.784	0.774	0.765
Benzene	Pure	0.884	0.874	0.863
	Water saturated	0.884	0.874	0.864
Chloroform	Pure	1.493	1.475	1.453
	Water saturated	1.498	1.479	1.459
n-Butanol	Pure	0.814	0.807	0.799
	Water saturated	0.849	0.844	0.836
n-Octanol	Pure	0.828	0.822	0.815
	Water saturated	0.834	0.829	0.824
Oleyl Alcohol	Pure	0.854	0.847	0.841
	Water saturated	0.855	0.850	0.840
Ethyl Acetate	Pure	0.906	0.894	0.884
	Water saturated	0.911	0.900	0.899
Olive Oil	Pure	0.916	0.910	0.904
	Water saturated	0.916	0.910	0.904
Water	Pure	0.999	0.997	0.994
	Cyclohexane saturated	0.999	0.997	0.994
	Benzene saturated	0.998	0.997	0.994
	Chloroform saturated	0.999	0.997	0.994
	n-Butanol saturated	0.989	0.987	0.983
	n-Octanol saturated	0.998	0.997	0.994
	Oleyl Alcohol saturated	0.999	0.997	0.994
	Ethyl Acetate saturated	0.999	0.997	0.993
	Olive Oil saturated	0.999	0.997	0.994

VISCOSITY OF PURE AND SATURATED SOLVENTS AT 15-35°C

Table 6.

Solvent	State	η centipoises 15°	25°	35°
Cyclohexane	Pure	1.074	0.896	0.761
	Water saturated	1.074	0.896	0.761
Benzene	Pure	0.700	0.602	0.524
	Water saturated	0.699	0.601	0.524
Chloroform	Pure	0.595	0.538	0.489
	Water saturated	0.596	0.539	0.490
n-Butanol	Pure	3.320	2.520	1.930
	Water saturated	3.860	2.800	2.060
n-Octanol	Pure	11.000	7.610	5.430
	Water saturated	10.600	7.260	5.170
Oleyl Alcohol	Pure	45.200	28.400	18.700
	Water saturated	44.100	27.900	18.400
Ethyl Acetate	Pure	0.482	0.430	0.387
	Water saturated	0.520	0.460	0.410
Olive Oil	Pure	101.300	63.100	41.700
	Water saturated	100.500	62.600	41.400
Water	Pure	1.140	0.894	0.720
	Cyclohexane saturated	1.140	0.894	0.720
	Benzene saturated	1.140	0.896	0.721
	Chloroform saturated	1.180	0.919	0.736
	n-Butanol saturated	1.570	1.160	0.895
	n-Octanol saturated	1.130	0.887	0.714
	Oleyl Alcohol saturated	1.140	0.893	0.718
	Ethyl Acetate saturated	1.360	1.040	0.811
	Olive Oil saturated	1.150	0.895	0.720

SATURATION CONCENTRATIONS OF WATER-IN-SOLVENT AND SOLVENT-IN-WATER AT 25°C

Table 7.

SOLVENT	STATE	MOLES WATER / MOLES SOLVENT	WATER CONC M
Cyclohexane	Water saturated	0.000266	0.00245
Benzene	Water saturated	0.00314	0.035
Chloroform	Water saturated	0.00542	0.067
n-Butanol	Water saturated	1.05	9.53
n-Octanol	Water saturated	0.28	1.72
Oleyl Alcohol	Water saturated	0.228	0.712
Ethyl Acetate	Water saturated	0.162	1.6
Olive Oil	Water saturated	0.07	0.0725

		MOLES SOLVENT / MOLES WATER	SOLVENT CONC M
Water	Cyclohexane saturated	1.18×10^{-5}	0.000651
	Benzene saturated	4.13×10^{-4}	0.0228
	Chloroform saturated	1.16×10^{-3}	0.0637
	n-Butanol saturated	1.92×10^{-2}	0.977
	n-Octanol saturated	7.29×10^{-5}	0.00404
	Oleyl Alcohol saturated	6.70×10^{-8}	0.0000037
	Ethyl Acetate saturated	1.64×10^{-2}	0.842
	Olive Oil saturated	2.60×10^{-8}	0.0000014

which may cause the observed log P to be lower than expected, particularly for nonpolar solutes whose water solubility is less than that of the solvent. However, this problem can be minimised by keeping solute concentration low.

Thus a wide variety of solvents are available for partitioning studies, with a great range of properties. From these it is necessary to select one which fulfils the criteria required for accurate work. These criteria include:

1. Availability as a pure solvent - or ease of purification in the laboratory. This excludes the natural products such as olive oil.

2. Low absorption in the UV range of interest - this usually requires low absorption at wavelengths above 230nm since many compounds of interest absorb at wavelengths between 230nm and 280nm. Benzene and olive oil are unsuitable in this respect since benzene, and even benzene saturated water absorbs at wavelengths lower than 270nm and olive oil has variable UV absorption due to its variable quality.

3. Stability during use. This may be in terms of physical stability so that deterioration in the quality of the solvent does not occur during use giving products which may affect the partition coefficient. This can happen with ethyl acetate which slowly hydrolyses. The solvent must also be inert with respect to the solute, for instance it must not cause oxidation or hydrolysis.

4. Low viscosity. A viscous liquid is difficult to work with, prolonging drainage times from measuring vessels such as pipettes and because of this reducing the accuracy of measurement due to incomplete drainage. The viscosity

problem would eliminate the higher alcohols (C_9 - C_{12}).

5. Affinity for a wide range of solutes. This can have an effect in two ways, if one phase has great affinity for a solute the volume ratio will have to be great for partitioning to be measurable and if the phase which is to be analysed has a very low concentration of solute or is of very small volume, analysis will be difficult. The ability to dissolve a wide range of solutes also means one solvent can be used to gain a large amount of directly comparable data. This means that the nonpolar solvents such as cyclohexane or benzene are not ideal partitioning phases.

Thus the alcohols n-heptanol, n-octanol and n-nonanol appear to be most suitable for partitioning experiments as a reference biolipid phase. Of these solvents, n-octanol has proved to be the most popular. Its advantages seem to stem from having most of the n-octanol and water tied up in a tetrahedral hydrogen bonded complex that retains a high degree of hydrophobicity because of the four 8-carbon non-polar chains surrounding the polar centre. It discourages self-association of partitioned polar solutes by having a reasonable degree of polarity and by providing an opportunity for a solute molecule to exchange with an alcohol molecule in the tetrahedral complex. Because it contains a relatively high concentration of water, its structure, polarity and partitioning properties will not be altered appreciably by additional water introduced by polar solutes as is the case for nonpolar and slightly polar solvents. No analytical or storage problems are associated with water-saturated n-octanol.

The popularity of octanol also means that there are large amounts of data already available for many solutes and this can be compared with data revealed in this thesis.

Thus octanol was chosen as the main system for partitioning work. However, this thesis is concerned with anomalies in partition coefficients and it was felt that valuable information could be obtained by comparing the partition coefficients and the thermodynamics of partitioning of the compounds selected for study in both a polar and nonpolar system. Cyclohexane was chosen because it has been the most widely used nonpolar solvent so many experimental values are already known for it; furthermore, being completely nonpolar it should allow distinct differences to be observed and explained. Cyclohexane is also relatively easy to work with and does not present any particular analytical problems.

The two phases having been selected, it was decided to investigate the conditions necessary to ensure mutual saturation which was felt to be one possible reason for anomalies observed in partition coefficients. This was only considered for the octanol-water system since octanol can contain a relatively high concentration of water. It was not considered as important in the cyclohexane-water system because water is virtually insoluble in cyclohexane. However, for this system the effect on the mutual solubility of raising the temperature was investigated.

The Karl Fischer apparatus was used to determine the concentration of water in the solvent and gas-liquid chromatography to determine the concentration of solvent in water.

3.2 Determination of Water Uptake Using Karl Fischer Reagent

For the determination of small amounts of water, Karl Fischer (1935) proposed a reagent prepared by the action of sulphur dioxide upon iodine dissolved in pyridine and methyl alcohol. The main reaction in methanol appears to proceed in two distinct steps.

1. $3C_5H_5N + I_2 + SO_2 + H_2O \longrightarrow 2C_5H_5NH^+I^- + C_5H_5N^+—SO_2—O^-$

2. $C_5H_5N^+—SO_2—O^- + CH_3OH \longrightarrow C_5H_5NH—OSO_2OCH_3$

The first step is the oxidation of the sulphur dioxide by the iodine and takes place only in the presence of an oxygenated molecule. This leads to the intermediate compound 'pyridine-sulphur trioxide'. The second step, the formation of the methyl ester (pyridinium methyl sulphate) prevents the pyridine complex from reacting with another molecule of water, or other active hydrogen compound. Hence one molecule of iodine is equivalent to one molecule of water.

The method used is that of titration, the end-point being determined electrometrically, although the reagent may serve as its own indicator since it has a deep reddish-brown colour when freshly prepared and a pale straw colour when spent.

The procedure involving the dead-stop end-point is usually used. If a small e.m.f is applied across two platinum electrodes immersed in the reaction mixture, a current will flow as long as free iodine is present to remove hydrogen

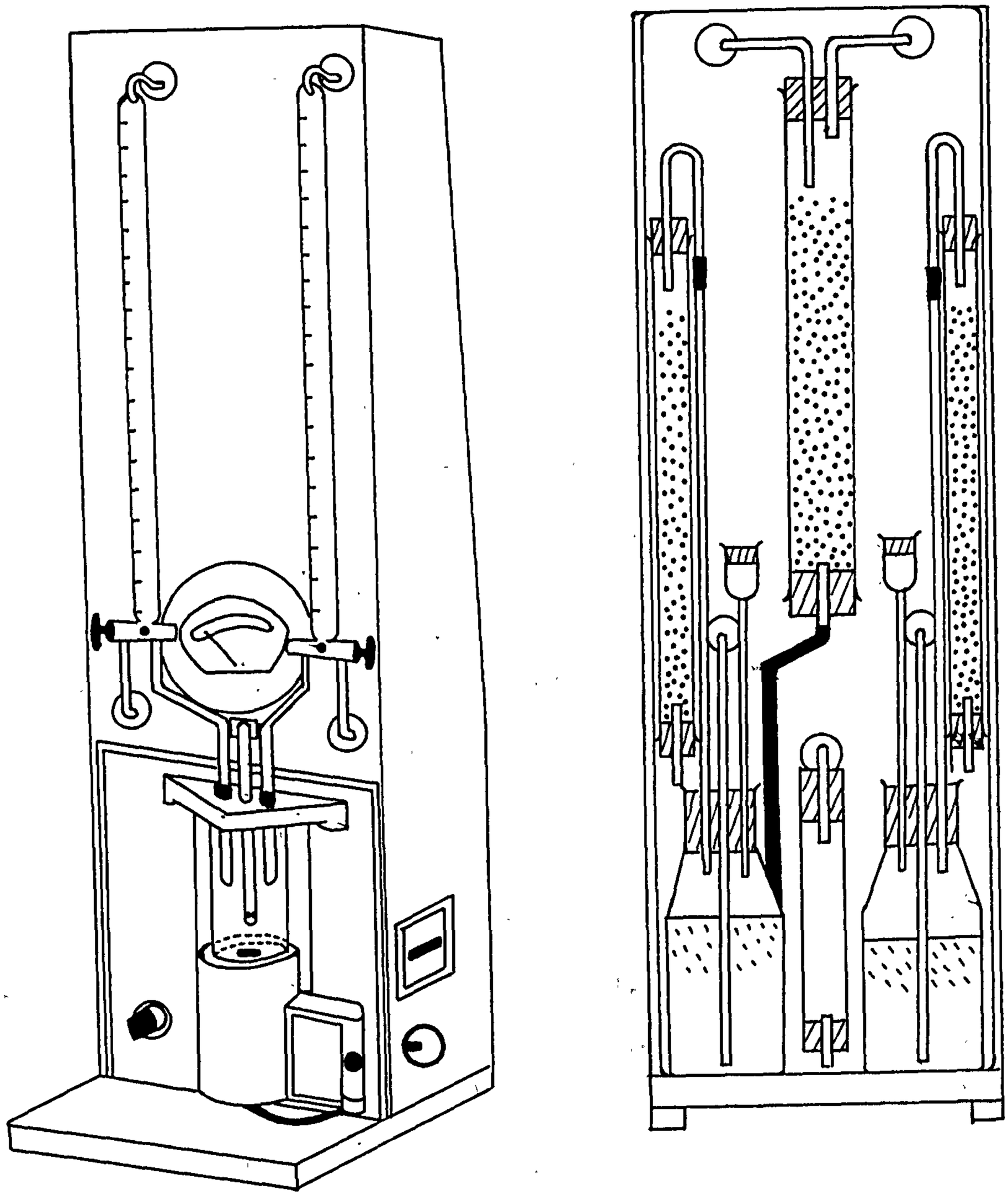
and depolarise the cathode. When the last trace of iodine has reacted, the current will decrease to zero. Conversely the sample may be titrated directly with Karl Fischer reagent when the current suddenly increases at the first appearance of unused iodine in the solution.

Liquids used in the titration must comply with certain requirements.

1. They do not react with the reagent or a component of the reagent, nor with the hydrogen iodide formed in the reaction, to yield water.
2. They are miscible with the reagent and preferably do not cause precipitation of the pyridine complexes formed during titration.
3. They will conduct an electric current.

The apparatus used for the titration is shown in Figure 17. It consists of two 25ml burettes mounted on the face of the metal cabinet, which are fitted with three-way stopcocks which permit suction of the reagents into the burettes and either delivery into the titration vessel or drainage back into the reservoir. Each reservoir can hold one litre of reagent and each is fitted with a special funnel to facilitate rapid filling. Both the reservoirs and the burettes are protected by large guard tubes filled with desiccant. The titration vessel has a ground top which fits into a rubber gasket and is held in position by a spring loaded stirrer housing, thus ensuring an air tight joint. The stirrer has a Teflon-coated iron core and is operated magnetically, stirring speed being controlled by a rheostat, located to the left of the stirrer unit. The

Figure 7. Karl Fischer Apparatus



electrode unit consists of a pair of bright platinum electrodes in a partitioned glass tube: they are connected to a micro-ammeter, which acts as the end-point indicator. The scale of the micro-ammeter is marked 'Excess Fischer' and 'Excess Water'; warning of the approach of the end-point is given by momentary kicks of the needle as each drop of reagent is added.

The Karl Fischer reagent undergoes auto-decomposition with time and thus has to be standardised each time it is used. To do this, sufficient Karl Fischer reagent is run into the titration vessel to cover the electrodes and titrated with standard water solution: a solution of methanol containing 5-6mg/ml of water. This is repeated until results agree to within 0.1ml. The number of mg of water equivalent to 1ml Karl Fischer reagent can then be calculated.

The sample is then introduced into the titration vessel and titrated with Karl Fischer reagent.

Experimental Details

Two experiments were performed, one to determine the conditions necessary to ensure complete saturation of the phases and the other to determine how increased temperature affected the solubilities.

Experiment 1.

To determine the conditions necessary for complete saturation six 100ml conical flasks were taken and into each was placed 50ml distilled water and 50mL octanol. These were then placed in a water bath at a temperature of 25°C. The contents of two flasks were left undisturbed for the duration

of the experiment, another two flasks were agitated at intermittent intervals by shaking and the contents of the final two flasks were stirred continuously by means of a magnetic stirrer and follower - stirring was strong enough to produce a vortex.

Samples of 0.5ml octanol were then removed from each flask at specified time intervals and the water content of each was determined by the Karl Fischer method described above.

Experiment 2.

A series of conical flasks was prepared as in Experiment 1 and using the conclusions drawn from this experiment, two flasks were placed in each of a series of water baths maintained at temperatures of 25°C, 30°C, 35°C, 40°C, 45°C and 50°C and stirred continuously for five hours. At the end of this time the two phases were carefully separated. The water content of the octanol phase was measured using the Karl Fischer method on 0.5ml samples and the octanol content of the aqueous phase was measured as described below.

3.3 G.L.C Determination of the Octanol Content of Water

The octanol content of the aqueous phase was measured by means of gas-liquid chromatography. A Pye Unicam Series 104 Chromatograph with Perkin Elmer recorder was used with a column of one metre length and 4mm internal diameter packed with 15% Carbowax 20M on a Chromosorb W 100-120 mesh support. The packed column was placed on the machine and conditioned by raising the oven (and thus column) temperature by approximately 50°C every 30 minutes until the final column

temperature of 170°C was reached. The detector temperature was 320°C. A flame-ionization detector was used, with a nitrogen flow rate of 40mm^3/min. through the column.

A calibration graph was prepared using known concentrations of octanol and an external standard. Hexanol was chosen as the standard because it could be clearly identified as a separate peak from that of octanol. To prepare the calibration graph, 1µl of hexanol was placed in 0.2ml butanol and different standards were prepared using quantities of octanol from 1µl to 5µl. A 1µl sample of each standard was then injected onto the column and the peak heights of octanol and hexanol measured. A graph was then plotted of the ratio of peak heights of octanol and hexanol against the volume of octanol.

The samples were prepared by taking 10ml of aqueous phase and shaking it vigorously with 2ml of butanol to extract octanol. Butanol was used for extraction because of the high solubility of octanol in butanol and because when separated by GLC the butanol peak did not interfere with either the octanol peak or the external standard hexanol peak. An extraction had to be performed since direct injection of an aqueous sample onto the column did not produce a measureable octanol peak. Two extractions were performed which were sufficient to extract 99.9% octanol from the aqueous phase. This was calculated from the fact that although the partition coefficient of octanol between butanol and water was not known, the partition coefficient of octanol between butyl acetate and water was known to be

997.2 (logP = 2.99) and that of octanol between octanol and water was 1412.5 (logP = 3.15) therefore it could be assumed that the partition coefficient of octanol between butanol and water was approximately 1000 (logP = 3.0). This assumption enabled the efficiency with which butanol could extract octanol from water to be determined from the equation:

$$w_n = w\left(\frac{kV_1}{kV_1 + V_2}\right)^n$$

where w grams of a solute are extracted repeatedly from V_1 ml of one solvent with successive portions of V_2 ml of a second solvent which is immiscible with the first. The weight of solute remaining in the original solvent after extracting with the other solvent, n times, is given by w_n.

$$k = \frac{\text{concentration of solute in original solvent}}{\text{concentration of solute in extracting solvent}}$$

This equation assumes complete immiscibility of the two phases, but when a solvent such as butanol is used to extract organic compounds from water, this is not true since butanol is partially miscible with water (7.9g per 100ml of water at 20°C). However, the equation provides approximate values which are satisfactory for practical purposes.

Extraction was achieved by shaking the two solvents together. Vigorous shaking was possible since emulsification did not occur. However, to ensure complete separation of the two phases, the sample was centrifuged and a 0.2ml aliquot of butanol removed. To this was added 1µl of hexanol as an external standard. A 1µl sample was then injected onto the column and the peaks recorded. By measuring the peak

heights and reading the ratio from the calibration graph, the equivalent octanol content could be calculated.

3.4 Results

1. Rate of Water Uptake by Octanol

Time	Water Concentration M		
	Unstirred	Intermittent Stirring	Continuous Stirring
Initial	0.250	0.25	0.25
1 hr	0.265	0.31	1.24
2 hr	0.274	0.63	1.74
3 hr	0.286	0.70	2.03
5 hr	0.401	1.02	2.36
24 hr	0.464	1.24	2.36
48 hr	0.910	1.55	2.35
100 hr	0.834	1.73	2.36

2. Rate of Octanol Uptake by Water

Time	Octanol Concentration M		
	Unstirred	Intermittent Stirring	Continuous Stirring
Initial	0.00000	0.00000	0.00000
1 hr	0.00025	0.00060	0.00152
2 hr	0.00050	0.00113	0.00410
3 hr	0.00068	0.00221	0.00605
5 hr	0.00100	0.00247	0.00660
24 hr	0.00123	0.00285	0.00662
48 hr	0.00147	0.00300	0.00658
100hr	0.00162	0.00375	0.00660

3. Water Content of Cyclohexane

The amount of water present in cyclohexane at saturation was determined to be 0.003M. This value remained constant over the range 293 - 323 K.

Figure 8. Rate of Water Uptake by Octanol

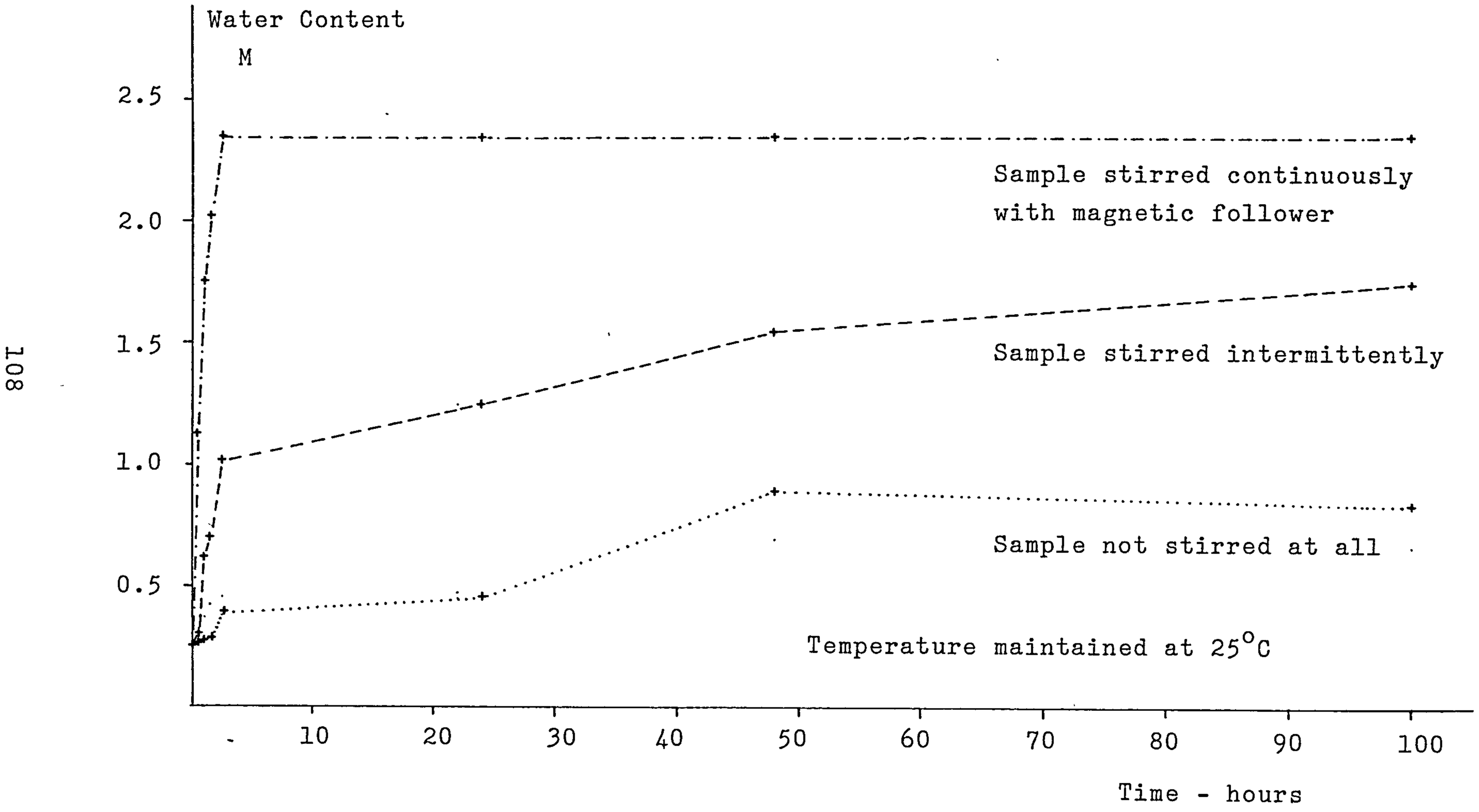
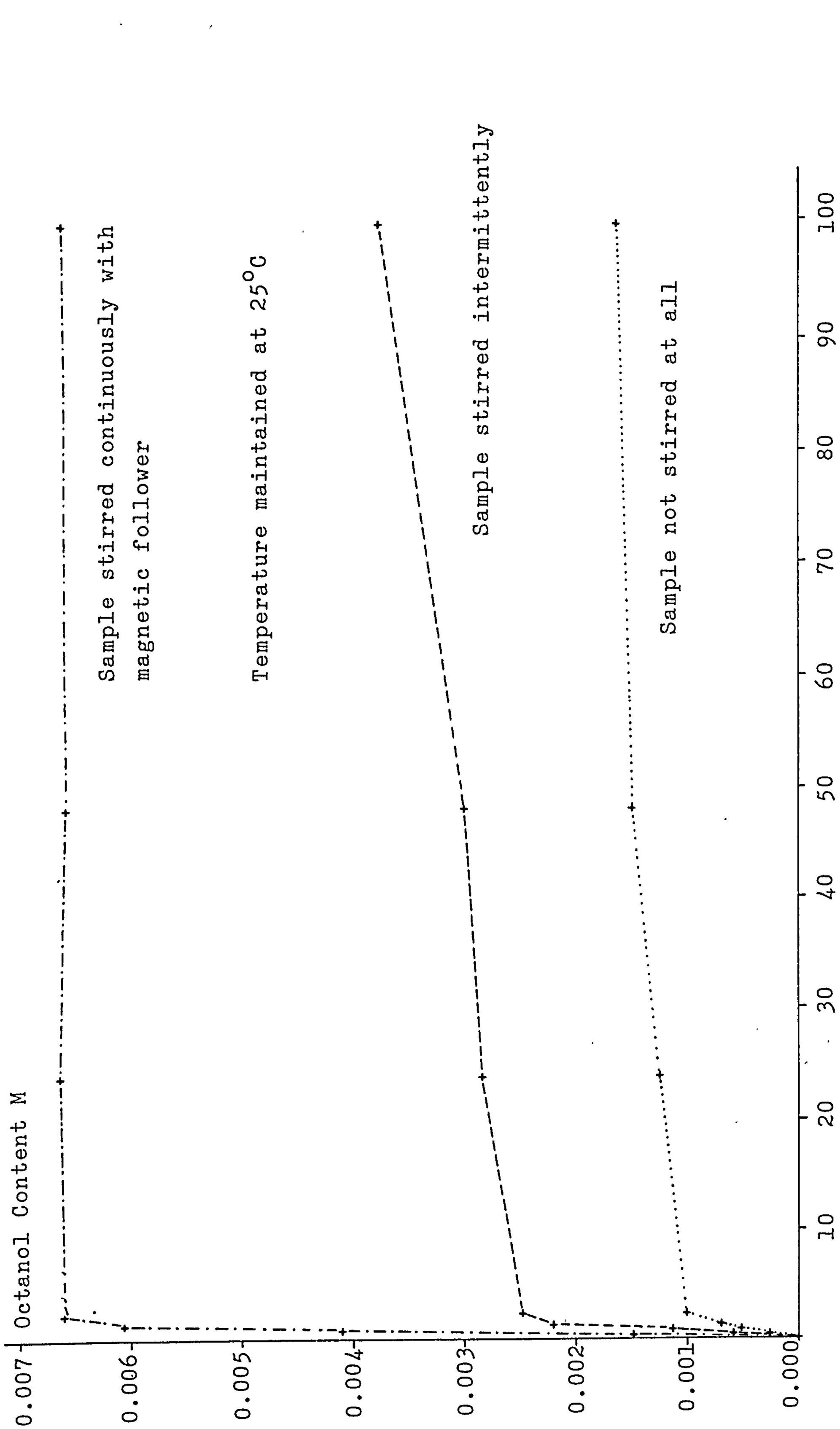


Figure 9. Rate of Octanol Uptake by Water



4. Change in Equilibrium Solubility with Temperature

Temperature	Conc^{n} of Water in Oct.		Conc^{n} of Oct. in Water	
^{o}C	mg/ml	M	mg/ml	M
5	28.6	1.59	1.16	0.0089
25	42.5	2.36	0.86	0.0066
30	43.7	2.43	0.82	0.0063
35	44.8	2.49	0.78	0.0060
40	44.6	2.48	0.73	0.0056
45	44.3	2.46	0.70	0.0054
50	38.4	2.13	0.65	0.0050
55	36.4	2.02	0.67	0.0052
70	26.4	1.47	1.12	0.0086

5. Thermodynamics

Calculation of ΔH, ΔG and ΔS for the solubility of water in octanol and octanol in water.

ΔH may be calculated from the slope of a plot of logP(mole fraction) against $1/T^{o}A$. The slope of the graph is equal to $-\Delta H/2.303R$. ΔG is equal to $-2.303RT\log P$ and ΔS is equal to $(+\Delta H-\Delta G)/T^{o}A$. In order to calculate the mole fraction partition coefficient it is necessary to know the molar volume of water and octanol at each temperature. This may be calculated from a density vs mass graph.

Temperature	Molar Volume of Octanol	Molar Volume of Water
5^{o}C	155.4 ml	18.020 ml
25^{o}C	157.8 ml	18.073 ml
30^{o}C	158.4 ml	18.100 ml
35^{o}C	159.1 ml	18.128 ml
40^{o}C	159.7 ml	18.161 ml
45^{o}C	160.4 ml	18.197 ml
50^{o}C	161.0 ml	18.238 ml
55^{o}C	161.6 ml	18.281 ml
70^{o}C	163.4 ml	18.429 ml

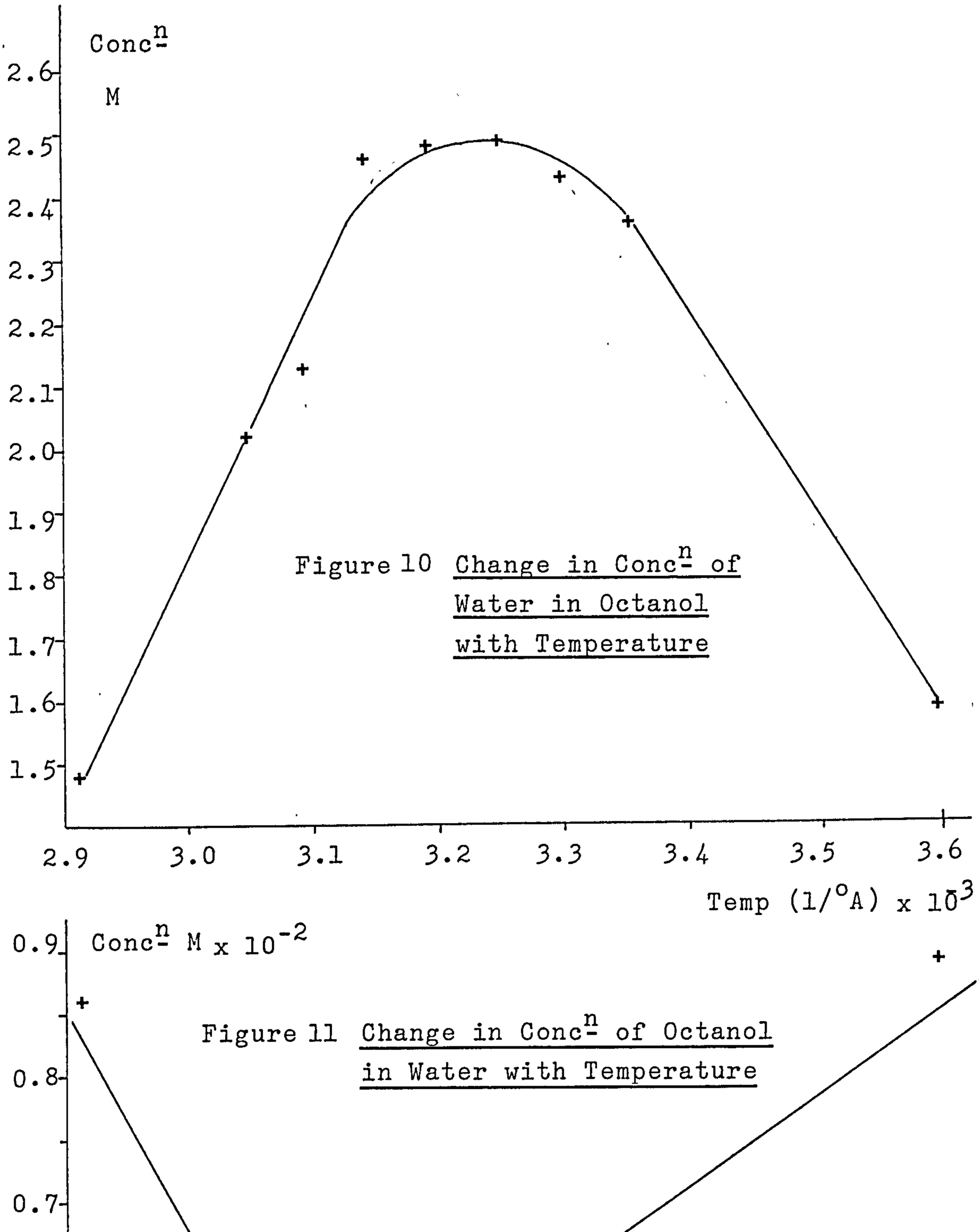


Figure 10 Change in Conc$^{n}_{-}$ of Water in Octanol with Temperature

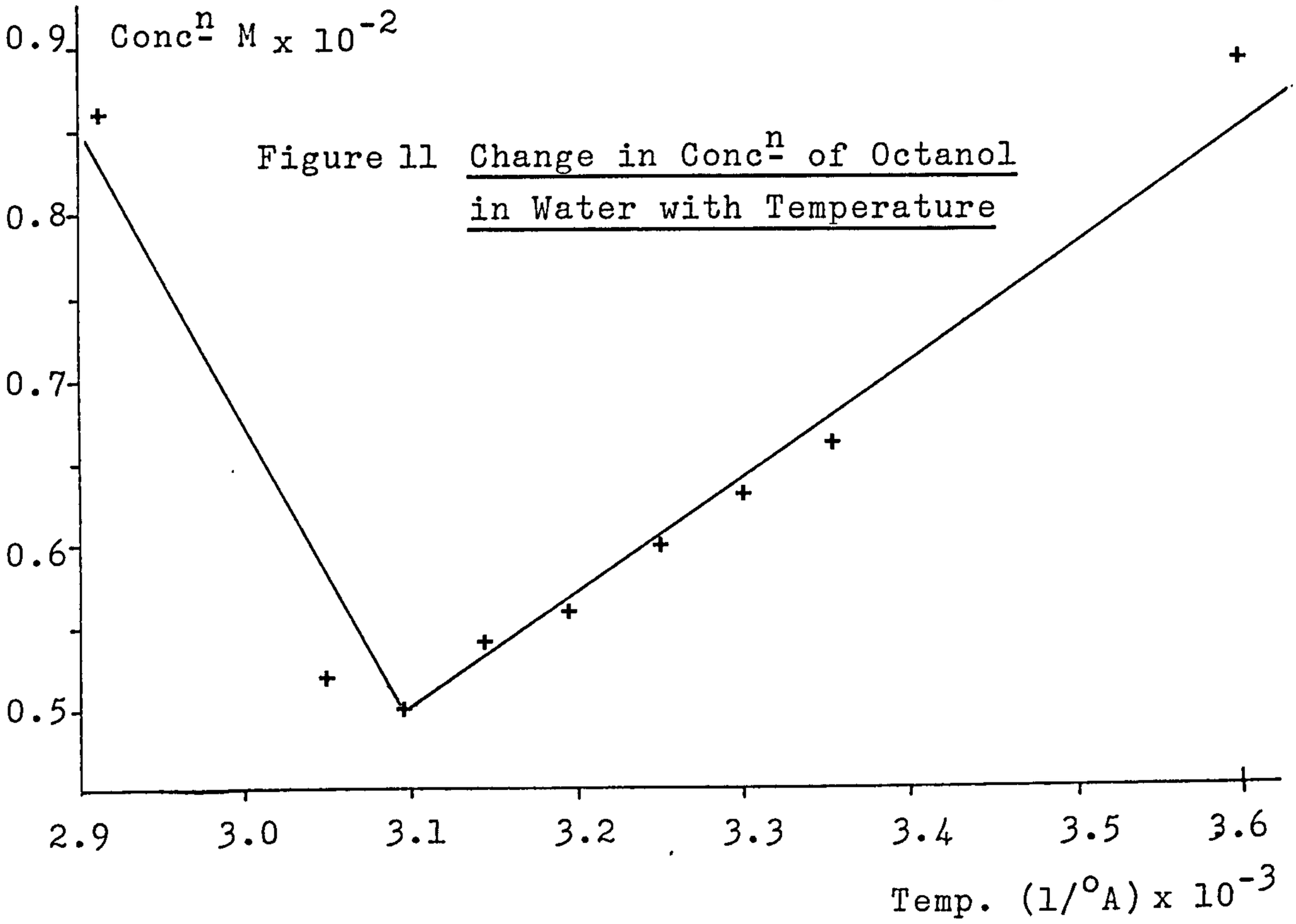


Figure 11 Change in Conc$^{n}_{-}$ of Octanol in Water with Temperature

Sample calculation: Mole fraction of octanol in octanol.

Temperature: 25°C

2.36M H_2O per litre octanol = 42.65ml H_2O per litre.

Vol.octanol per litre = 1000 - 42.65 = 957.35ml

= 6.067 M

$$\text{Mole fraction of octanol in octanol} = \frac{6.067}{6.067+2.36}$$

= 0.72

Mole fraction of water in octanol = 0.28

Temperature	Mole Fraction Oct. in Oct.	Mole Fraction Water in Octanol
5°C	0.799	0.201
25°C	0.720	0.280
30°C	0.713	0.287
35°C	0.707	0.293
40°C	0.707	0.293
45°C	0.707	0.293
50°C	0.737	0.263
55°C	0.747	0.253
70°C	0.802	0.198

Temperature	Mole Fraction Water in Water	Mole Fraction Octanol in Water
5°C	0.999640	0.000161
25°C	0.999881	0.000119
30°C	0.999886	0.000114
35°C	0.999891	0.000109
40°C	0.999898	0.000102
45°C	0.999902	0.000098
50°C	0.999909	0.000091
55°C	0.999905	0.000095
70°C	0.999841	0.000159

Partition Coefficients

Temperature	Water in Octanol		Octanol in Water	
	P	Log P	P	Log P
5°C	0.2011	-0.6966	2.015×10^{-4}	-3.696
25°C	0.2800	-0.5528	1.653×10^{-4}	-3.782
30°C	0.2870	-0.5421	1.599×10^{-4}	-3.796
35°C	0.2930	-0.5331	1.542×10^{-4}	-3.812
40°C	0.2930	-0.5331	1.443×10^{-4}	-3.841
45°C	0.2933	-0.5327	1.386×10^{-4}	-3.858
50°C	0.2632	-0.5796	1.235×10^{-4}	-3.908
55°C	0.2532	-0.5965	1.272×10^{-4}	-3.896
70°C	0.1980	-0.7033	1.983×10^{-4}	-3.703

Van't Hoff plots are shown for the dissolution of water in octanol (Figure 12) and for the dissolution of octanol in water (Figure 13). The thermodynamic parameters, ΔH, ΔG and ΔS were calculated from these plots. It can be seen that the plot for the dissolution of water in octanol is not linear, therefore, a tangent was constructed at 25°C and the slope of the tangent was used for the calculation of ΔH.

	Octanol in Water	Water in Octanol
ΔH	-7.66 kJ mol^{-1}	+7.45 kJ mol^{-1}
ΔG	+21.58 kJ mol^{-1}	+3.15 kJ mol^{-1}
ΔS	-98.1 j mol^{-1} $^{o}K^{-1}$	+35.57 j mol^{-1} $^{o}K^{-1}$

ΔG and ΔS were calculated at 25°C.

Figure 12 Van't Hoff Plot for the Dissolution of Water in Octanol

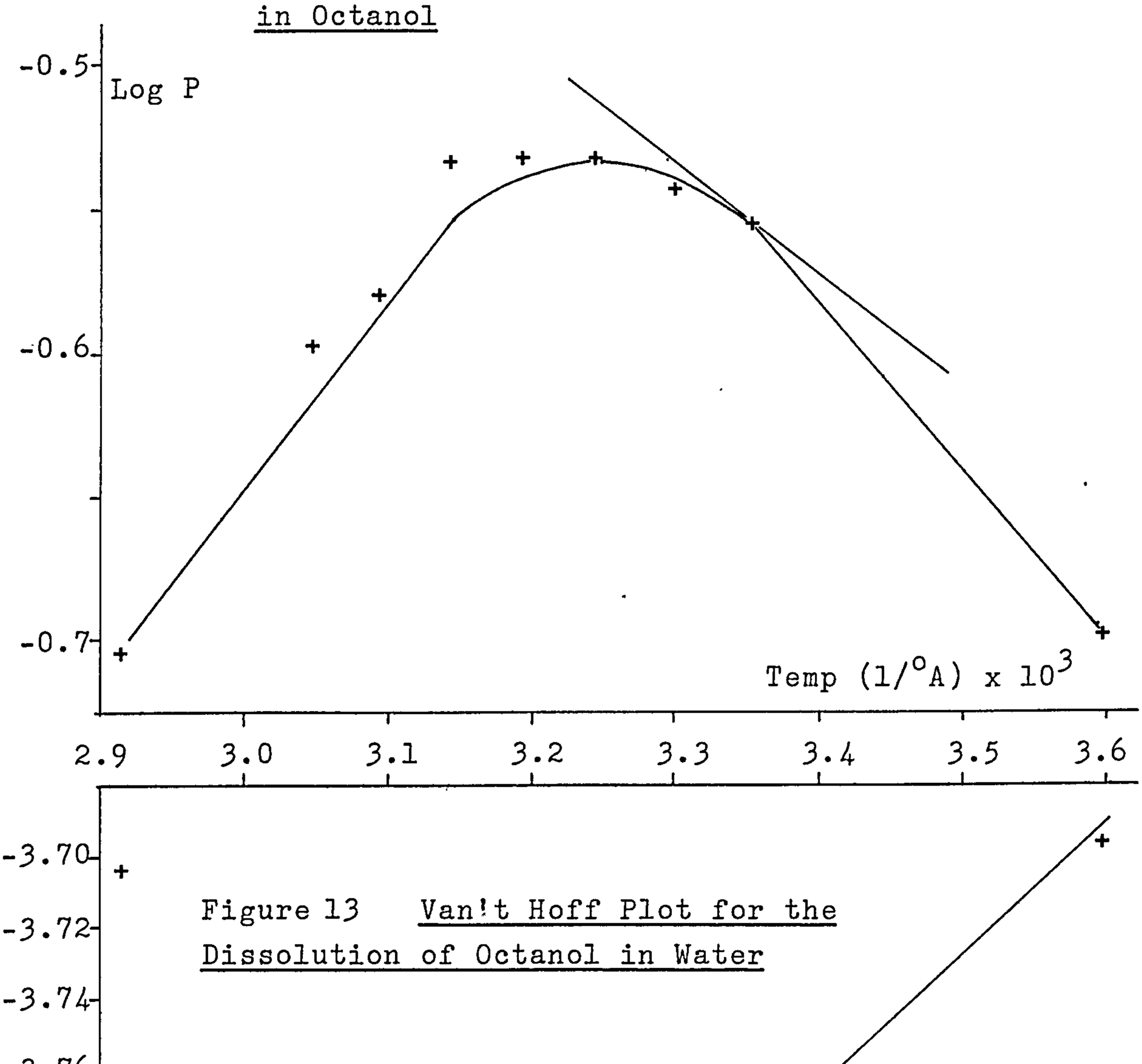
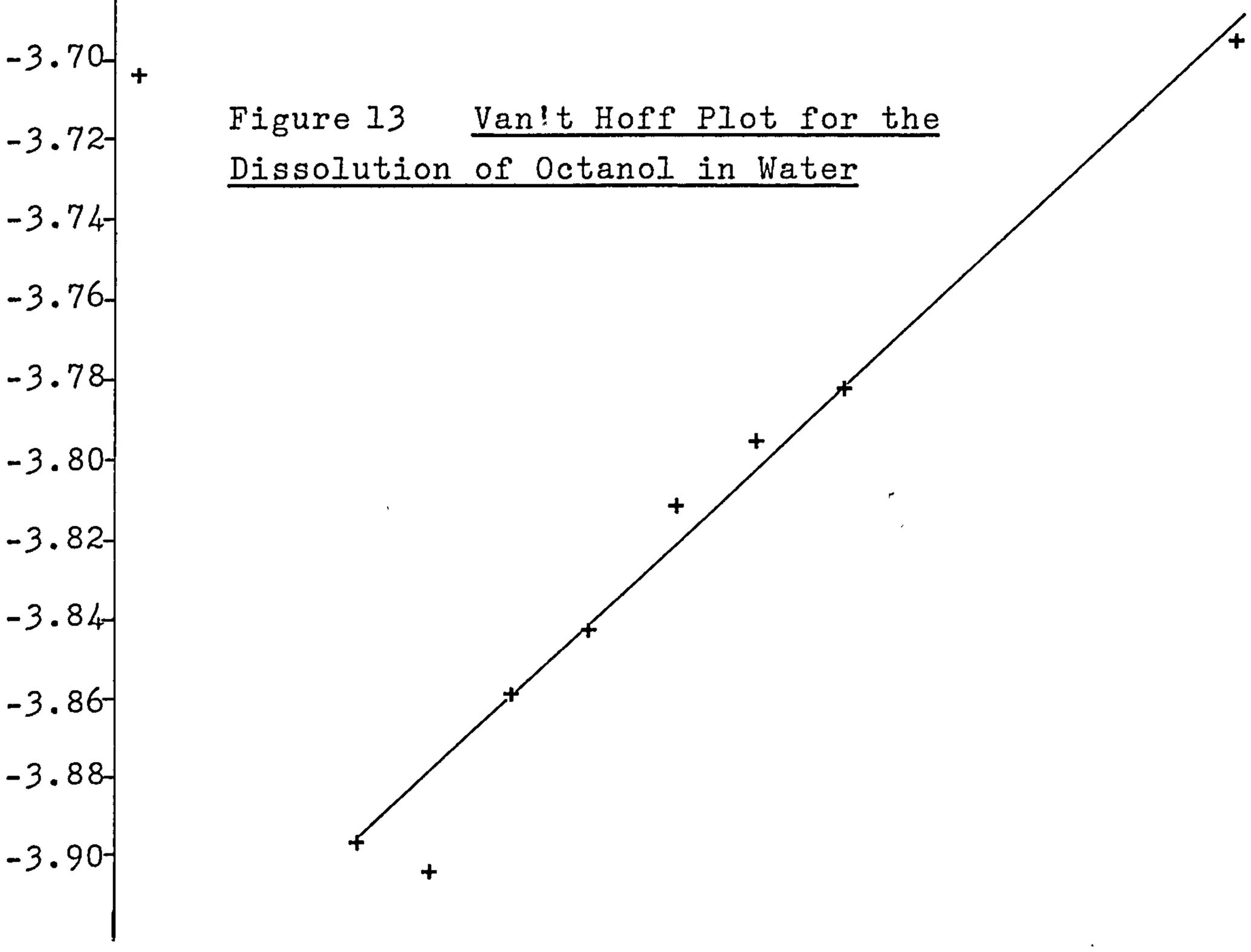


Figure 13 Van't Hoff Plot for the Dissolution of Octanol in Water



3.5 Discussion

1. Rate of Water Uptake by Octanol

This experiment illustrated the importance of thorough mixing of the two phases prior to use in partitioning experiments. The final saturation concentration of water in octanol was found to be 2.36M which could not be achieved within a reasonable time (less than 100 hours) either by simply storing the two phases together or by intermittent stirring. To attain mutual saturation it was necessary to mix the two phases thoroughly for a period of 5 hours. In practice, the two phases were mixed together a few days prior to use and then left to stand with intermittent mixing until needed.

The need for thorough mixing to ensure mutual saturation is clear, but there is also a need for a precise definition of the meaning of the term 'mutual saturation'. Many authors state, 'we mutually saturated the two phases', but do not specify how this was achieved or if checks were made to confirm saturation concentration. This experiment indicates that simply storing the two phases together does not guarantee saturation and the probability of error is high. Care is essential with saturation to eliminate errors in partition coefficient measurement, particularly for compounds with low log P values. The amount of water in the organic phase can affect the solubility of hydrophilic compounds and thus can affect the partition coefficient. In addition, the introduction to this chapter discusses the effect of water in alcohols. The viscosity and dielectric constant of octanol decrease with the addition of water, reaching a minimum at saturation. This alteration in physical properties

of octanol may also influence the partition coefficient.

2. Rate of Octanol Uptake by Water

Saturation of the aqueous phase with octanol is possibly not as important since the amount of octanol present is very small (0.0066M) and this has little, if any, effect on the physical properties and structure of water. However, failure to saturate could affect the partition coefficient of very lipophilic compounds.

Figures 8 and 9 show that saturation of each phase occurs within a similar period of time, under similar conditions, so that preparation of both phases a day or more before use is recommended.

3. Thermodynamics of Water-Octanol Dissolution

The results of experiments 1 and 2 were utilised for the third experiment which aimed to show the variation of saturation concentration with temperature and from this calculate the thermodynamics of the dissolution of water in octanol and vice versa.

It was found that the concentration of each phase in the other did vary with temperature such that:

a. The concentration of water in octanol increased with increasing temperature, reaching a maximum at about 40°C, at which temperature the concentration of water in octanol began to decrease.

b. The concentration of octanol in water decreased with increasing temeprature, reaching a minimum at about 50°C, at which point the concentration of octanol in water began to increase.

The findings for the dissolution of octanol in water are in agreement with work performed by James (399) and also with similar work on Hexan-1-ol and Pentan-1-ol (400,401).

Thermodynamic data reveals that for the dissolution of octanol in water, ΔH is negative (-7.66kJ), which indicates a favourable enthalpy change by the evolution of heat. However, octanol is considered to be insoluble in water, as indicated by the very low saturation concentration (0.0066M). This reluctance to mix with water is a result of a large ΔS for the process (-98.1 $Jmol^{-1}$). The large energy of reordering the hydrocarbon solute and the water solvent molecules keeps them in separate phases when placed together. These results are in agreement with work performed by Kauzmann (219) on the thermodynamic changes in hydrocarbon transfer, notably the transfer of liquid propane and liquid butane to water.

	$\underline{T}$	$\underline{\Delta S_u}$	$\underline{\Delta H}$	$\underline{\Delta G_u}$
Liquid propane → C_3H_8 in H_2O	298	-23	-1800	+5050
Liquid butane → C_4H_{10} in H_2O	298	-23	-1000	+5850

ΔS_u and ΔG_u refer to the unitary entropy and free energy in cal/mol.

A variety of work (219,137) supports the conclusion that the entropic component of ΔG plays a large role in the position of equilibrium (partition coefficient) taken by nonpolar compounds in nonpolar water-solvent sysytems.

A mixture of octanol or any lower aliphatic alcohol (219), with water, shows a positive deviation from Raoult's law, indicating an increase in unitary free energy ($\Delta G > 0$:

$\Delta G = +21.58 kJmol^{-1}$) for the transfer of octanol from octanol to water phase, despite the fact that heat is evolved ($\Delta H<0$) on the addition of octanol to water. Therefore, $\Delta S < 0$ when an alcohol (octanol) molecule is transferred to water.

The solubility of octanol in water decreases with increasing temperature. Hence ΔH for the transfer process must, according to the principle of Le Chatelier, be <0. ΔG is greater than zero, therefore, ΔS for the mixing must be negative.

The origin of the large negative unitary entropy change and the small negative enthalpy change involved in partitioning between aqueous and nonaqueous phases was first clearly appreciated by Frank and Evans (137). They concluded that when organic compounds are placed in water, the water molecules arrange themselves around the apolar parts in what was termed 'iceberg' structures. This terminology was not meant to imply a rigid structure or one as extensive as in pure ice, but rather a structure which was denser rather than lighter than water. Therefore, these structures were later referred to as 'flickering clusters' to indicate their lack of stability.

The Frank-Evans point of view was that the stripping of the 'form-fitting sweater' (402) of water molecules from the apolar part of the solute results in a large entropy change in the randomization of the water molecules. Aranow and Witten (8) suggested an alternative view point. They reasoned that in the aqueous phase the apolar chain of a

solute molecule is rigidly held in a favoured rotational configuration by the structured layer of water molecules surrounding it. In the organic solvent its rotational oscillations are relatively unrestricted. Partitioning data (255) however, favour the Frank-Evans hypothesis.

The solubility of water in octanol follows a more usual pattern for partially miscible liquids. The solubility of water in octanol increases with increasing temperature, hence, ΔH for the transfer process must, according to the principle of Le Chatelier, be greater than zero, which is found to be the case. This indicates an unfavourable enthalpy change by the absorption of heat. This is characteristic of non-ideal solutions and is due to cohesion among the solute molecules and among the solvent molecules. Non-ideal solutions also show positive deviations from Raoult's law, indicating an increase in unitary free energy ($\Delta G > 0$). This is expected since ΔH is greater than zero. Similarly, $\Delta S > 0$ when the water molecule is transferred to octanol. This indicates an increase in disorder as the water molecules enter the alcohol. The positive value for ΔS explains the greater solubility of water in octanol than octanol in water, although since ΔH is positive, the solubility of water in octanol is limited.

CHAPTER FOUR

EXPERIMENTAL DETERMINATION OF PARTITION COEFFICIENTS

4.1 Experimental Technique

Various methods are available for determining the partition coefficient of a compound between aqueous and lipophilic phases. These methods will be discussed in greater detail further on in this chapter, but which ever method is chosen certain experimental techniques must be used to ensure reliable and accurate results. These techniques are discussed in the following section and are those observed in preparing the results obtained in this thesis.

A partition coefficient represents the distribution of a substance between an organic and an aqueous phase. The ratio is defined as:-

$$P = \frac{(C)\text{organic}}{(C)\text{aqueous}}$$

Since P is a quantity with no dimensions, any units of concentration are appropriate, although the use of mole fractions allows direct comparison between various groups of compounds. Care must be taken if mole fractions are used since different values are obtained than if the amount per unit volume is used. P is not independent of concentration and ideally, infinite dilutions should be used in the calculations. However, for neutral compounds which have little tendency to associate, 10^{-2} to 10^{-3}M is sufficiently dilute (316). For acids and other molecules which tend to associate, measurements should be made at several concentrations and log P plotted against concentration to

obtain the value at infinite dilution. For very lipophilic compounds it may be necessary to have a concentration as low as 10^{-5}M in the aqueous phase so that work is done below the critical micelle concentration (316). As indicated in Chapter 3, mutual solubility of the two solvent phases must also be considered.

Partition coefficients of ionizable molecules must be corrected for ionization and expressed as the partition coefficient of either the neutral or the ionized species. Alternatively, the molecule may be partitioned at a pH where only one species is present. For this purpose 0.1N HCl or 0.1N NaOH may be used.

Partitioning

The general procedure is to dissolve the carefully weighed compound completely in the phase in which it is more soluble. A calculated amount of the second phase is added and the phases are mixed. The mixture may then be centrifuged, a sample of one phase withdrawn and analysed.

Compound Preparation

Care must be taken to ensure that the compounds to be measured are quite pure. This was done in the present work by gas chromatography and where necessary the compounds were recrystallised if solid or redistilled if liquid. Melting points and boiling points were checked, although they alone are not sufficient standards of purity.

Weighing

Accurate weighing is important. For all the work in this thesis, a Beckman LM500 microbalance was used. This was

recalibrated before use and checked repeatedly during use. If possible a large enough sample should be used to reduce the error to less than 1%. Care must be taken to ensure no loss when transferring the sample to the partitioning vessel. In this case the aluminium weighing pan was carefully washed with solvent into the partitioning flask.

Partition Vessels

It is convenient to partition and centrifuge in one bottle, when centrifugation is necessary. This was not possible in the shake flask method used in this work since 250ml conical flasks were used as the partitioning vessels. Great care was taken to ensure the flasks were clean and they were carefully rinsed with distilled water after washing to remove any traces of detergent since this could affect the ultraviolet absorption of the sample. Flasks of a uniform size were chosen since differences in size and shape were found to affect the results. (This will be explained in greater detail later on). Aluminium foil caps were used to close the flasks; glass stoppers would have been preferable but were not available.

For the thermodynamics work a jacketted beaker was used (see Figure 17). This was also carefully cleaned and rinsed and was fitted with a ground glass lid. Uniformity of size was no problem since a single vessel was used repeatedly.

Solvents

Three solvents were used in the partitioning work, water, n-octanol and cyclohexane. Other solvents were used in spectroscopic work and for the gas chromatographic work.

In every case the best grade possible was obtained or a laboratory grade solvent was redistilled. Freshly distilled water was used throughout and the cyclohexane was spectroscopic grade from B.D.H. which had a minimum U.V transmittance of 80% at 230nm and 98% at 250nm. Octanol is usually the solvent which presents most purity problems since even the best grade contains impurities which may affect analysis. Usually, purification of octanol is recommended, but work was done on various methods of purification of octanol (115) and none was found noticeably to improve the U.V. absorption spectrum of octanol. Therefore octanol was obtained from Koch-Light, this having been found to give consistently the least spectroscopically contaminated samples. It was found not to absorb above 220nm and was therefore used as supplied.

The choice of solvents for partitioning work is discussed in more detail in Chapter 3 where the importance of mutual saturation is also described.

When using the more viscous organic solvents it is also important to allow longer time to drain the pipette when samples are pipetted.

Dissolving Compounds for Partitioning

It is often difficult to dissolve certain solids in either phase. Particle size reduction may help, as may gentle heating, although care must be taken on cooling to ensure a true solution is obtained and not a supersaturated one. Dissolving the compound initially in a small amount of alcohol also aids solubility and this was the method used

throughout the experimental work. The compounds were dissolved in 2ml ethanol, with the aid of slight heating if necessary, and then made up to volume with distilled water. Checks were made to ensure that this did not appreciably alter the partition coefficient.

Mixing

The phases can be mixed by a number of methods. Studies by Craig (82) indicate that when approximately equal volumes of solvent are used equilibrium is rapidly attained. With high ratios (e.g. 2ml octanol and 200ml water) longer shaking is necessary. Vigorous shaking must be avoided since emulsification may result, particularly with octanol and water, and in fact it was found to be better if a magnetic stirrer was used since emulsions produced by shaking were difficult to crack.

To minimise errors in calculation, the phases should be adusted in volume so that roughly equal weights of compound are in each phase at equilibrium. Sometimes it is difficult to adhere to this ideal situation if the molecules have a particularly high or low partition coefficient.

Care must be taken to ensure that after partitioning, each phase is still under-saturated. This can be checked by determining partition coefficients at several different solute concentrations. A constant value indicates no special interaction of substrate is occurring in either phase and that neither phase is saturated.

Centrifugation

Most, if not all, the papers and books which describe

partitioning methods stress the importance of centrifuging the partitioned phases, particularly if the mixing process has produced an emulsion. During the work for this thesis, the conclusion was reached that it was essential not to cause emulsification since it was impossible to crack an emulsion once formed. For this reason the method used for partitioning was adapted slightly and either gentle shaking in an orbital incubator or a magnetic stirrer were used to mix the phases. However, it was felt important to centrifuge the phases before analysis since even slight contamination could seriously affect the observed absorbance, especially with extreme P values. In the thermodynamics work the phases were separated by filtration which was found to be very efficient.

Sampling

Ideally samples of each phase should be analysed and the partition coefficient calculated from two separate values. This means a material balance can be made to ensure against unforseen losses, e.g. by adsorption. However, this is often inconvenient and time consuming since an analytical procedure must be worked out for each phase. Therefore, generally only one phase is analysed, but a number of separate partitionings are run. The amount of solute found in one phase is then subtracted from the total sample to obtain the amount in the second phase. In this work, at least five runs were used to determine log P. The standard curves were run in duplicate, using separate weighings of solute. Compounds with a log P outside the range ±3.0 are difficult to run, but if a compound has a strong U.V.absorption

it is possible to obtain log P values from -5 to +5.

Usually it is the aqueous phase which is sampled. One method which is recommended (316) is to thrust a pipette with a finger over the top through the octanol phase into the aqueous phase. A small amount of air should then be blown through to force out any octanol which may have entered the tip. However, this method was found to be unsatisfactory since it was very difficult to ensure no octanol remained in the sample and even the slightest amount would contaminate the U.V. cell walls and cause inaccuracies in absorbance readings. Therefore the two phases were separated so that it was not necessary to put the pipette through an octanol layer.

In the thermodynamics experiments, a continuous flow sampling method was used and the two phases were separated by filtration.

Standards

A carefully weighed sample was dissolved, usually in the aqueous phase, and diluted in a volumetric flask to produce a standard solution. With insoluble solutes, a few millilitres of ethanol were used to aid solution. This does not change the absorbance providing the concentration of ethanol does not exceed 5%. Ethanol was also used in the blank.

Spectrophotometry

This is the most convenient method of analysis and since all the compounds investigated had fairly high molar absorptivities it was the method used throughout the experimental work for this thesis. The absorption spectrum

of each compound can be taken from 350 - 400nm down to 220 - 240nm where octanol begins to absorb. Having a complete spectrum can be useful as a comparison before and after partitioning to check purity and stability. The peak of strongest absorption is used for analysis unless it is below 220nm. The blank consists of water saturated with octanol.

Two spectrophotometers were employed in the course of the experimental work. A Perkin Elmer 550 U.V. spectrophotometer with Perkin Elmer 551 recorder was used for the basic partitioning work and a Cecil CE595 Double Beam Digital U.V spectrophotometer with Cecil CE500 control record module Series Two was used for the thermodynamics work.

Calculation of Partition Coefficient

1. The calibration graph is drawn from the results obtained with the standard solutions.
2. The concentration per ml. is multiplied by the volume of the sample phase to find the concentration in one phase.
3. The weight in the sample phase is subtracted from the original sample weight to give the weight in the octanol phase.
4. The ratio is calculated:

$$P = \frac{\text{mg. in octanol/volume of octanol}}{\text{mg. in water / volume of water}}$$

Temperature Dependence

It has been stated (255) that partition coefficient is not very sensitive to temperature changes if the phases employed are quite immiscible. However, this was not felt

to be totally accurate and in fact was shown in the thermodynamics work not to be the case. Therefore, all partition coefficients were measured at controlled temperatures.

Solvent Volumes

Most organic compounds are more soluble in octanol than water so normally the volume of octanol used was less than the volume of aqueous phase. In the cyclohexane system this is not so and the volume of cyclohexane often exceeded that of water. To ensure accuracy the ratio of the phases was adjusted so that each phase held approximately the same weight of solute at equilibrium. The reason for this can be demonstrated by the following example.

For a compound with P = 200, if equal volumes of 100ml and a 20mg sample are used, then:-

$$P = \frac{20}{0.1mg}$$

An error of ±0.5mg in the aqueous phase means that P can be between 133 - 400.

By adjusting the solvent volumes to 200ml H_2O and 5ml octanol

$$P = \frac{17.5}{3.5} \times \frac{200}{5} = 200$$

An error of ±0.5mg in the aqueous phase yields P = 197-203

Thus the ratio of phases was chosen individually for each compound.

Acids and Bases

The term partition coefficient usually refers to the unionised form of a molecule. No bases were investigated, but acids were studied at pH1.0 acidified with 0.1N HCl.

4.2 Methods of Measuring Partition Coefficients

1. Ref: The Hydrophobic Fragmental Constant. R.Rekker (325)
The solute is weighed accurately and dissolved in the most appropriate solvent phase. The second phase is added in an amount adapted to both the partition coefficient and the analytical facilities available. Mechanical shaking is usually recommended but some workers prefer a manual shaking procedure. (316). A Griffin flask shaker is very suitable for this purpose, as four shaking bottles can be treated at a time. Different shaking times have been reported in the literature, about ½hour seems most appropriate. Shaking should be followed by centrifugation at 2000rpm for 1-2hours. This centrifugation is the more important the higher the partition values. It is not strictly necessary to establish the total amount of solute partitioned, but it does facilitate a check of the analytical results. It is advisable to carry out at least four partition experiments for any one solute.

2. Ref: Strategy of Drug Design. A Molecular Guide to (316) Biological Activity. W.P.Purcell,G.E.Bass,J.M.Clayton
The general procedure is to dissolve a carefully weighed sample of compound completely in the phase in which it is most soluble. The calculated amount of the second phase is added and the bottles shaken, for approximately 2 minutes, by hand. The bottles are then placed in a centrifuge and turned at 2000rpm for 1-2hours. An aliquot of one phase is withdrawn and analysed.

3. Ref: Physico-chemical Parameters and Analgesic Activity in a Series of Substituted Acetanilides.

E.Tomlinson (363)

Pyrex glass shaking tubes, consisting of hollow tubes approximately 20cm in length, closed at both ends with a central, stoppered port for sampling, were used. These have a maximum volume of 100ml. (321)

Measurements were carried out at 25°C ±0.2°C. Optimum shaking speed was found to be 180cpm., shaking being along the horizontal axis of the tubes. Solvent volume had no effect on the coefficient, but drug concentration had, so concentrations of 0.01% w/v and 0.006% w/v were used. A four hour shake period was found to be satisfactory for complete partition. The tubes were removed from the shaker and the phases separated by decanting. The aqueous phase was poured carefully into a 15ml polythene centrifuge tube which was placed in a centrifuge and spun at 6000rpm at 25°C for 30 minutes to remove any trace of octanol. The drug concentration in this phase was then found spectrophotometrically.

4. Ref: Partition Coefficients and their Uses.

A.Leo,C.Hansch and D.Elkins (255)

Simple repeated inversion of a tube with the two phases establishes equilibrium in 1-2 minutes. About 100 inversions in roughly 5 minutes produce consistent results. Very vigorous shaking should be avoided since this tends to produce troublesome emulsions. The clarity of the two phases is not a dependable criterion of the absence of an emulsion and therefore a centrifugation step is recommended for

precise determinations. For convenience, partitioning can be carried out in 250ml centrifuge bottles fitted with glass stoppers. In this way centrifugation can be accomplished without transfer of material. Avoiding cork or rubber stoppers eliminates the possibility that impurities might be introduced by these materials or that some substances might be extracted by these stoppers. It is desirable to work at low concentrations in each phase (0.01M or less).

5. Ref: Quantitative Drug Design. Y.C.Martin (274)

Pipette into a tightly closeable vessel (ground glass or inert plastic stoppers) the aqueous and octanol phases which contain the appropriate amount of the compound. Since it is more viscous, octanol drains more slowly than water. The vessels are then stoppered tightly, and shaken gently for several minutes. Thirty minutes of continuous slow shaking on a mechanical shaker may be necessary for equilibration of compounds of high log P. The tubes are then centrifuged at moderate speeds (2000rpm) for twenty to thirty minutes. This centrifugation process is critical to the accurate determination of log P values. After centrifugation the tubes should be carefully handled and inspected for evidence of incomplete separation of the layers. A second centrifugation may be necessary. If the phases seem to resist separation (because of traces of detergent) it may be helpful to remove most of the octanol phase to a second vessel, to discard the material in the centre of the original tube, and to recentrifuge the aqueous and octanol phases separately. In the final analytical determination of concentrations, the phases must not be

contaminated with each other.

The five methods of partition coefficient measurement described above were investigated to determine the most suitable method for use in the determination of the partition coefficients of the compounds to be examined.

In each method the importance of centrifugation was stressed so initially this was investigated in depth. The compound chosen to demonstrate the different methods was 2,6-dimethyl phenol. The literature value for the log P for 2,6-dimethyl phenol is given as 2.36 (189); therefore, any experimental value obtained should be close to this figure.

Method 1. Two Minute Shake by Hand

This method was investigated first since it was the simplest and the most widely quoted. The system used consisted of a stoppered test-tube containing 50ml aqueous solution and 5ml octanol. As previously stated, each phase was mutually saturated before each experiment and only those solutions were used throughout. The two phases were mixed by gentle inversion 60 times in 2 minutes. An initial concentration of 10^{-3}M in the aqueous phase was used.

Calibration curves were calculated for each compound. The same procedure was used each time. Five solutions of different concentration were made by dissolving the solute in 2ml of ethanol if necessary, and making up to 100ml in a grade A volumetric flask with aqueous phase. The U.V. spectrum of the solution was recorded and the wavelength of maximum absorbance noted. This wavelength was used to produce the calibration curve. Each calibration curve was

duplicated for accuracy. The concentrations used in each case were such that they followed Beer's Law and a straight line calibration was produced.

The first partitioning demonstrated one problem. The phase volumes used were 50ml aq. : 5ml octanol; these were placed in a stoppered test tube and inverted gently 60 times in 2 minutes. At the end of this time the mixture was centrifuged at 2000rpm for 2 hours. This produced a partition coefficient of 75.00 (log P = 1.88), which was much too low. However, it was noted that octanol was contaminating the faces of the U.V. cell and this caused an increased absorbance which resulted in a low partition coefficient. The reason for the contamination was found to be that since the octanol phase was above the aqueous phase, to sample the aqueous phase a pipette had to be passed through the octanol layer. It was very difficult to eliminate all traces of octanol from the end of the pipette, either by wiping it carefully or by expelling air through it as it passed through the octanol. Therefore it was felt necessary to separate the two phases before centrifuging so that there was no risk of octanol contamination.

The experiment was then performed a number of times with different solute concentrations and centrifuge times and speeds.

1. 50ml H_2O : 5ml octanol. 10^{-2}M.

a.2000rpm	½hr	P=178 (log P = 2.25)
b.2000rpm	1hr	P=145 (log P = 2.16)
c.4000rpm	½hr	P=178 (log P = 2.25)
d.4000rpm	1hr	P=191 (log P = 2.28)
e.6000rpm	½hr	P=174 (log P = 2.24)

2. 50ml H_2O : 5ml octanol. 25mg/50ml. 4 x 10^{-3}M.

a.2000rpm	½hr	P=158 (log P = 2.20)
b.2000rpm	1hr	P=186 (log P = 2.27)
c.4000rpm	½hr	P=166 (log P = 2.22)
d.4000rpm	1hr	P=191 (log P = 2.28)
e.6000rpm	½hr	P=166 (log P = 2.22)

3. 100ml H_2O : 2ml octanol. 10^{-4}M.

At this concentration the absorbances were too small for accurate measurement and it was decided that 10^{-3}M was a more suitable concentration to use.

These experiments demonstrated three points:

1. The importance of gentle shaking. All the partition coefficients appeared to be low when compared with the literature value and although it was later found that the lower value was repeated in all experiments for this compound, it was noticed that this experiment was susceptible to the formation of emulsions which were difficult to crack.

2. The importance of the correct ratio of phases. It is necessary for accurate work for each phase to contain an equal weight of solute at equlibrium. For all succeeding work, if possible, the phase ratio was calculated, either from a previously recorded value of P or a calculated value.

$$\text{e.g.} \quad P = 230 = \frac{\text{x.mg.octanol}}{\text{x.mg.water}} \times \frac{\text{Vol.}H_2O}{\text{Vol.oct}}$$

Therefore: If Vol.H_2O = 100ml

$$\text{then: } 230 = \frac{100}{\text{y.ml.oct}}$$

Therefore: y.ml.octanol = 0.43

Therefore a ratio of 100mlaq.phase : 0.5ml octanol phase is used.

Figure 14. The Effect of Centrifuge Speed on Partition Coefficient - 2,6-dimethylphenol

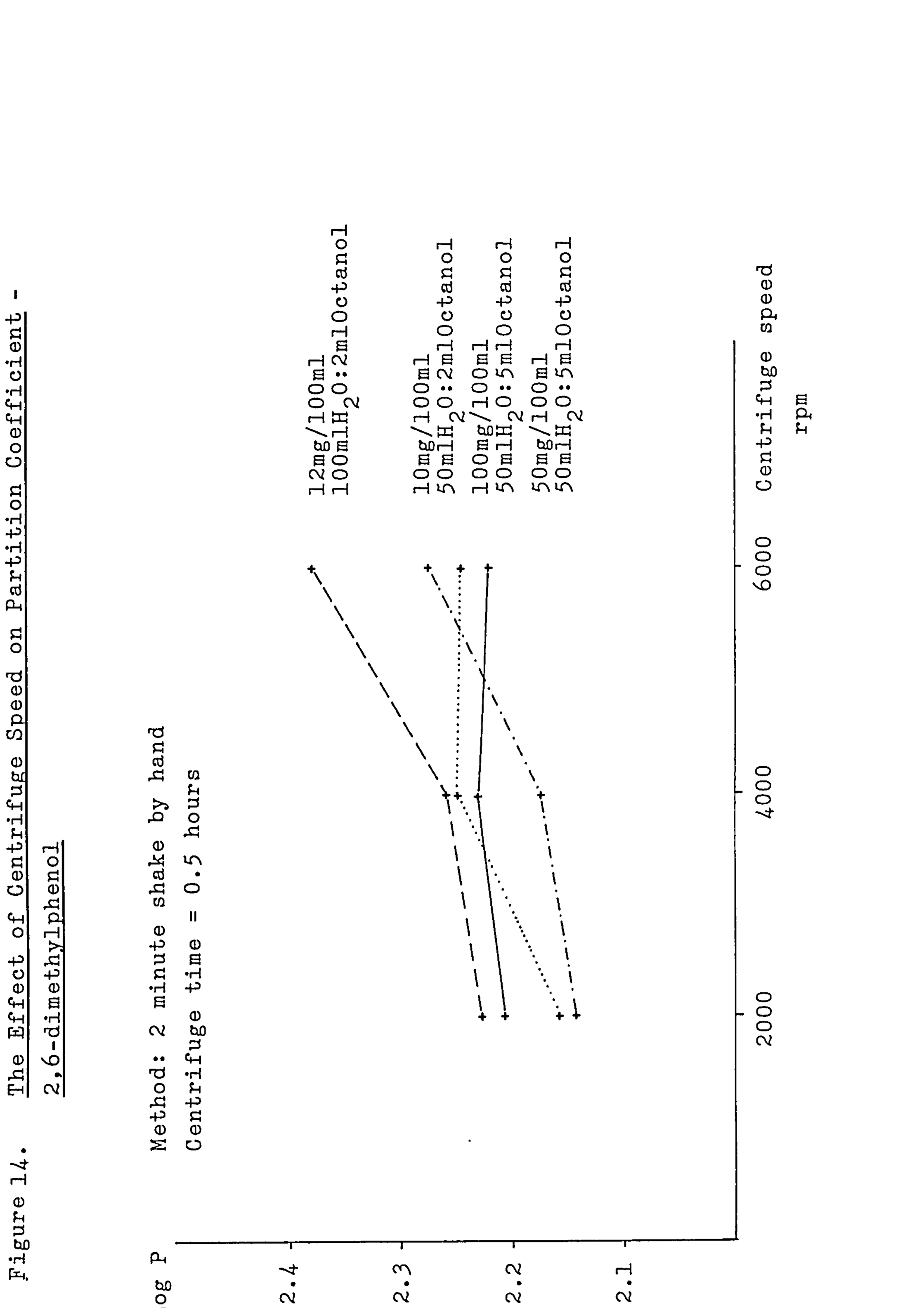
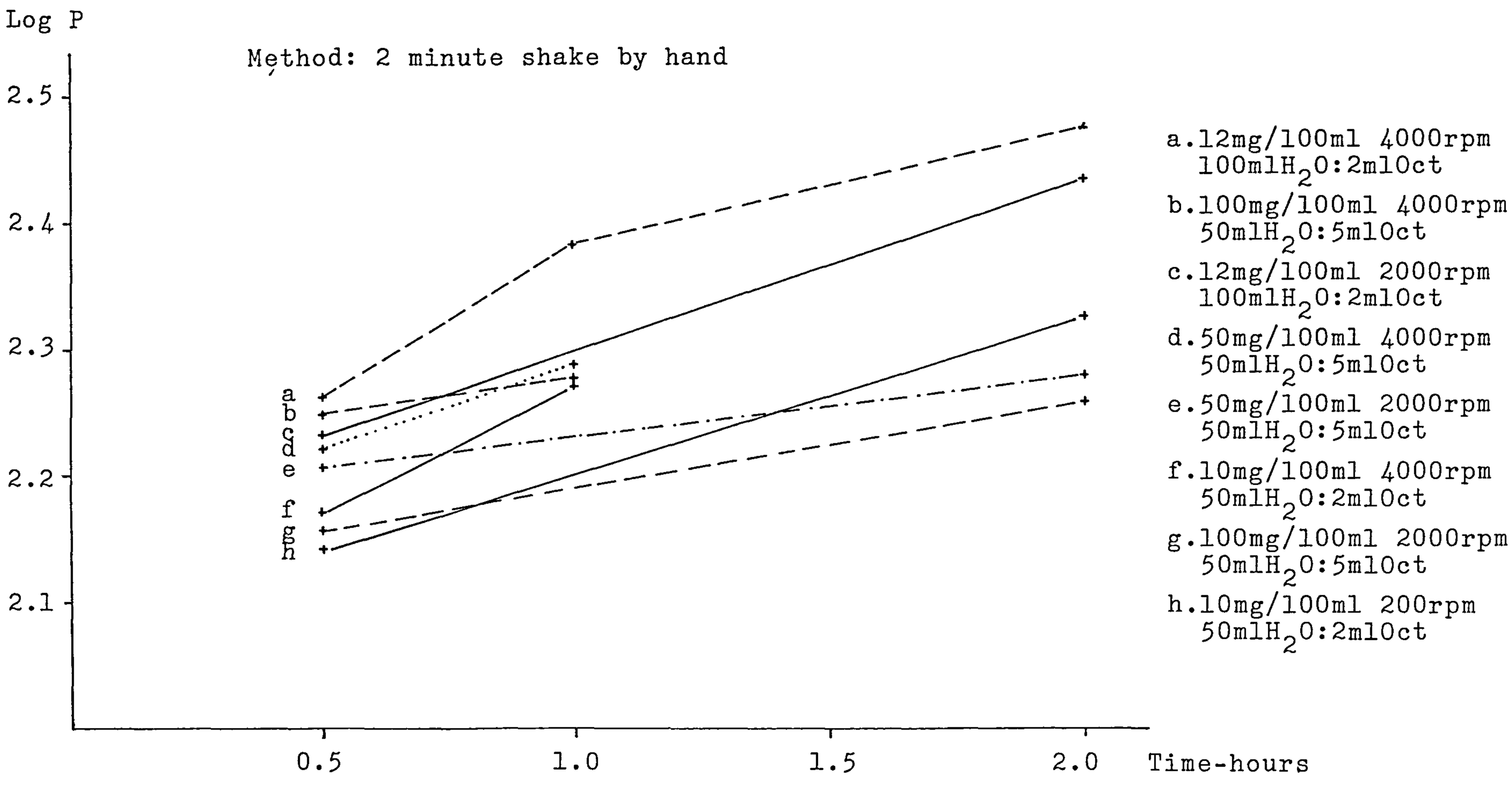


Figure 15. The Effect of Centrifuge Time and Speed on Partition Coefficient - 2,6-dimethylphenol



3. It can be noted from the figures above that different centrifuge times and speeds produce a variation in the value of log P. This is shown in Figures 14 and 15, and is investigated in the next set of experiments.

Method 2. Pyrex Tubes in Water Bath Shaker

Partition coefficients are known to vary with temperature and it was felt to be important that all values used for interpretation in this thesis were measured at a constant temperature (fixed at 25°C for convenience). For this method, 100ml stoppered pyrex tubes (see Figure 16) were shaken horizontally in a Gallenkamp water bath, which allowed temperature control. For efficient mixing, an air space had to be left in the tube and so a total working volume of 75ml was employed. To avoid emulsification, a shaking speed of 60rpm was used. The water bath held four tubes so each experiment was performed four times.

1. 75ml aq.phase : 1.5ml octanol. 10^{-3}M. ½hr mixing.

a.2000rpm	½hr	P=191 (log P = 2.28)
b.4000rpm	½hr	P=229 (log P = 2.36)
c.6000rpm	½hr	P=295 (log P = 2.47)
d.2000rpm	1hr	P=251 (log P = 2.40)
e.4000rpm	1hr	P=339 (log P = 2.53)
f.6000rpm	1hr	P=447 (log P = 2.65)
g.2000rpm	2hr	P=324 (log P = 2.51)
h.4000rpm	2hr	P=389 (log P = 2.59)
i.No centrifuging		P=141 (log P = 2.15)

As in the previous experiment, an increase in centrifugation time and speed produced an increase in the partition coefficient. A large value of P meant there was a lower concentration of solute in the aqueous phase. Removal of octanol contamination from the U.V. cell i.e. emulsification could account for an apparent decrease in absorbance of the

aqueous phase, but in this experiment P considerably exceeded the literature value, and although it was by no means certain that the literature value was correct, the fact that no constant value had emerged seemed to indicate some loss of solute.

Plastic centrifuge tubes had been used; hence, to eliminate the possibility of adsorption onto the plastic, P was compared after centrifugation in both glass and plastic tubes. Glass tubes shatter at speeds above 2000rpm so only the duration of centrifugation was investigated.

2. 90ml aq.phase : 1ml octanol. ½hr mixing. 10^{-3}M.

Glass tubes

a.2000rpm	½hr	P=72 (log P = 1.86)
b.2000rpm	1hr	P=90 (log P = 1.95)
c.2000rpm	2hr	P=94 (log P = 1.97)
d.2000rpm	3hr	P= 101 (log P = 2.00)

Plastic tubes

a.2000rpm	½hr	P=70 (log P = 1.84)
b.4000rpm	½hr	P=85 (log P = 1.93)
c.6000rpm	½hr	P=106 (log P = 2.03)
d.2000rpm	1hr	P=86 (log P = 1.94)
e.4000rpm	1hr	P=113 (log P = 2.05)
f.6000rpm	1hr	P=282 (log P = 2.45)
g.2000rpm	2hr	P=93 (log P = 1.97)
h.4000rpm	2hr	P=125 (log P = 2.10)
i.6000rpm	2hr	P=626 (log P = 2.80)

The phase volumes were altered for this experiment which explains the low results - inefficient mixing due to excessive volume in the tube - but it can still be seen that there is little difference in the values of P obtained using either glass or plastic tubes but a marked increase with increased centrifugation speed and time.

Since the centrifuge was not temperature controlled, one

possible cause was heat generated during centrifuging causing solute degradation. Thus the absorbance of a sample of 2,6-dimethylphenol solution was recorded before and after centrifuging at 2000rpm for 1hour.

Abs.before cent.	Abs. after cent.
1.24	1.04
0.77	0.66

Thus a decrease in absorbance occurs after centrifuging. The absorbance of the original sample after standing at room temperature for 1 hour had not changed showing that degradation did not occur at 25°C.

Therefore the effect of heat on a solution of 2,6-Me_2phenol was investigated.

Abs. of initial soln.	Abs.@ 66°C	
	1hr	2hr
1.175	0.840	0.255
1.380	0.680	0.290

Therefore the error introduced in the previous experiments would appear to be due to degradation during centrifugation due to heat production. This illustrates the care necessary in using heat to aid dissolution of compounds for testing.

A temperature controlled centrifuge was then used. The temperature was maintained at 12°C, but a small reduction in absorbance still occurred.

Initial Abs.	2000rpm.2hr.12°C	2000rpm.2hr.No°C control
0.29	0.275	0.200

Therefore, controlling the temperature reduces the problem

but does not prevent it. A second cause for absorbance reduction could be oxidation. The solute is a phenol and therefore susceptible to oxidation. To prevent this, nitrogen was bubbled through a solution prior to centrifugation.

Initial Abs.	2000rpm.2hr.12°C	2000rpm.2hr.No°C control
0.340	0.335	0.340

This prevented absorbance fall and so for all following experiments nitrogen is bubbled through the solution before partitioning.

This illustrates the necessity to record the U.V. spectrum of the compound before and after partitioning to ensure no change has occurred to the compound. It has already been mentioned that phenols are fairly susceptible to oxidation, but nevertheless, it is clear that the solute must be protected.

The two experiments were then repeated to demonstrate the effect of speed and duration of centrifugation.

3. 50ml aq.phase : 1ml octanol. 10^{-3}M.

	2min. shake by hand			Shaker bath ½hr.25°C		
rpm	½hr	1hr	2hr	½hr	1hr	2hr
2000	1.96	1.99	1.99	2.13	2.16	2.17
4000	2.02	2.04	2.00	2.16	2.17	2.15
6000	1.95	1.96	1.96	2.16	2.18	2.17

Thus it was shown that centrifuging at 2000rpm for 1hr was adequate to ensure complete separation of the phases.

However, neither of these two methods seemed suitable since reliable results were difficult to obtain. This was felt to be due to the short time allowed for partitioning in

Figure 16. Horizontal Shaking Tube

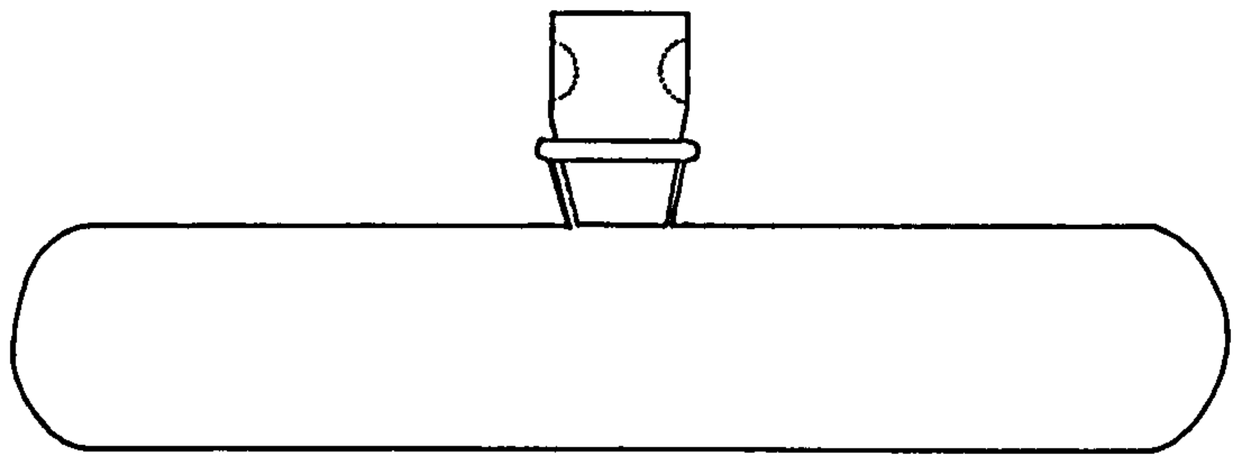
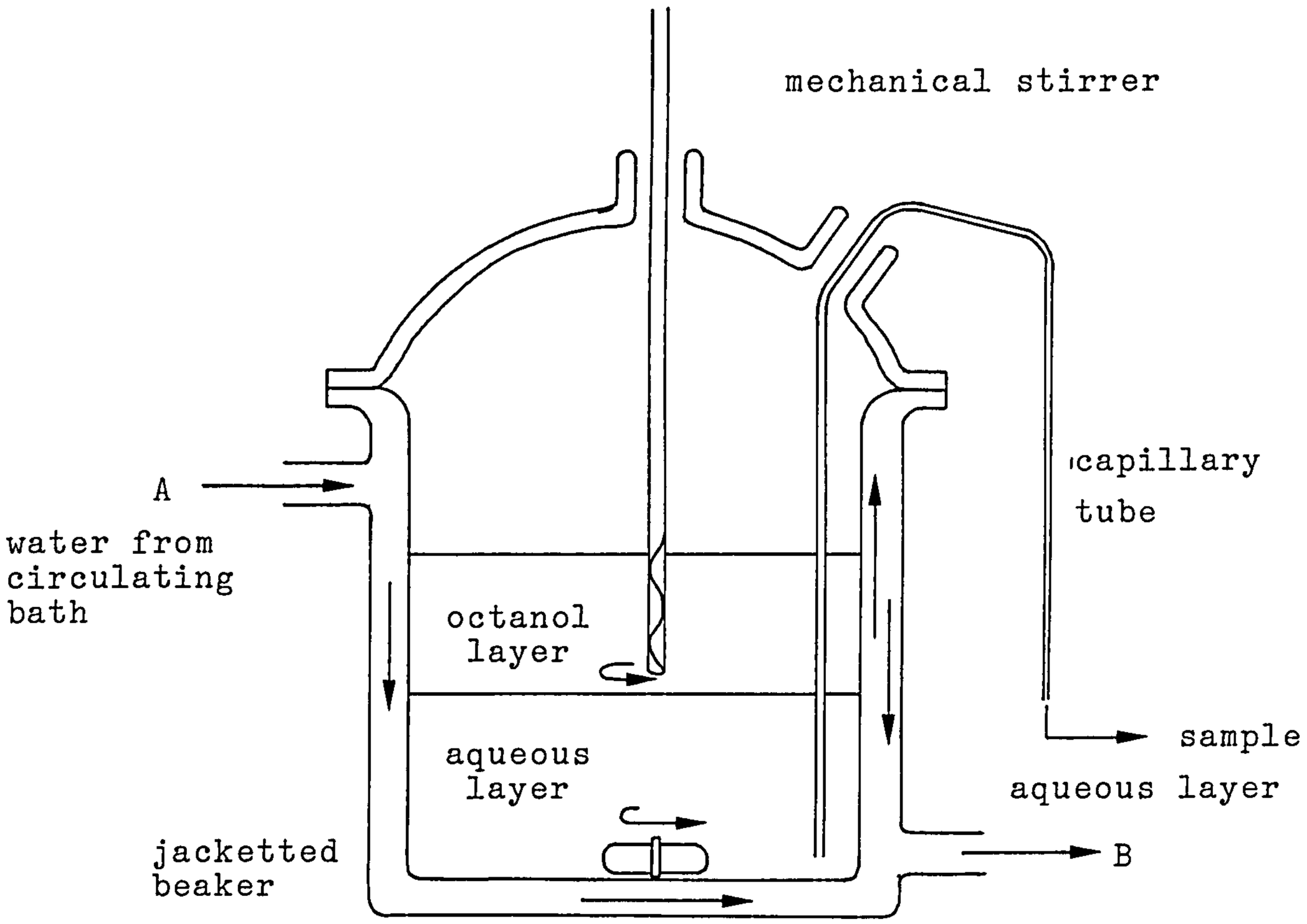


Figure 17. Jacketted Beaker



Method 1 and the restricted volume in Method 2 which prevented adequate mixing. Therefore methods allowing more flexibility on these points were considered.

Method 3. Gallenkamp Flask Shaker

The use of conical flasks as the partitioning vessel allows greater volumes to be partitioned and the mixing volume is greater and thus mixing is more efficient. Therefore a Gallenkamp flask shaker with 250ml conical flasks was chosen as the third method for investigation.

Unfortunately, this method did not prove successful since the shaking action was very vigorous and it proved impossible to prevent emulsification. As has been stated previously, once formed the emulsion was very stable and was not easily cracked by centrifugation.

Therefore the methods extracted from the literature had proved unsatisfactory but they did serve to illustrate the criteria necessary for an acceptable method.

1. Gentle but efficient mixing. A mechanical shaker would be most efficient since hand shaking is laborious, time consuming and inconsistent. However, a mechanical shaker must be capable of being controlled since emulsification is to be avoided at all costs.

2. Sufficient volume to allow adaptation of phase volumes and efficient mixing. Conical flasks seem to be suitable for this.

3. Temperature control so that all partitioning is performed at a known temperature. This is also most important for thermodynamics work, as described later in this thesis (Chapter 9).

Method 4. Orbital Incubator - Shake Flask

A Gallenkamp orbital incubator was chosen as the mechanical shaker for this experiment. It was capable of holding up to 28 250ml conical flasks and mixed by means of a swirling action, the speed of which could be controlled so that emulsification was not a problem. The incubator was adapted by the addition of a cooling system so that a temperature of 25°C ±0.1°C could be maintained.

The solutions were prepared as before and in each case, 100ml aqueous phase was partitioned with an appropriate volume of octanol. Each partitioning was performed at least five times and an average value of P calculated. The flasks were tightly sealed, usually with an aluminium cap. A shaking speed of 80rpm was maintained and experiments were performed to judge the most suitable partitioning time. At the end of this period the phases were separated and the aqueous phase was centrifuged at 2000rpm for 1 hour. The absorbance of the aqueous phase was then recorded and from this the partition coefficient calculated.

The ultraviolet spectrum of each compound was recorded before and after partitioning to confirm no degradation had occurred (see Figure 18).

1. The Effect of Different Partitioning Times

Solute: p-hydroxybenzaldehyde

100ml aq. phase : 3ml octanol. 10^{-3}M. 25°C. 2000rpm 1hr.

Time	P	Log P
½hr	17.5	1.24
1hr	20.1	1.31
2hr	22.3	1.35
3hr	22.4	1.35
4hr	22.4	1.35

Figure 18. Example of the Ultraviolet Spectrum of 4-Me-2-NO_2phenol Before and After Partitioning Between Water and Octanol

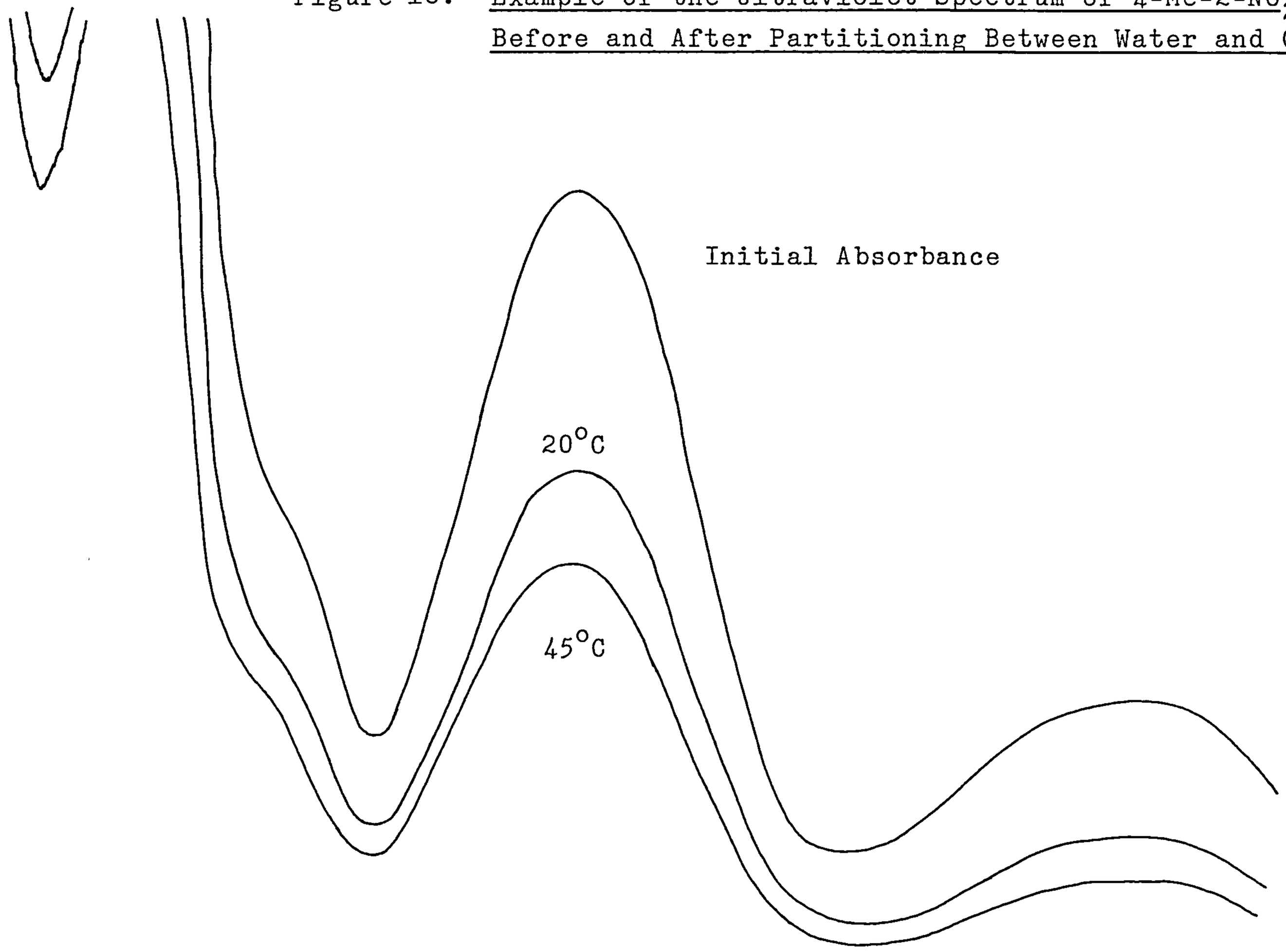
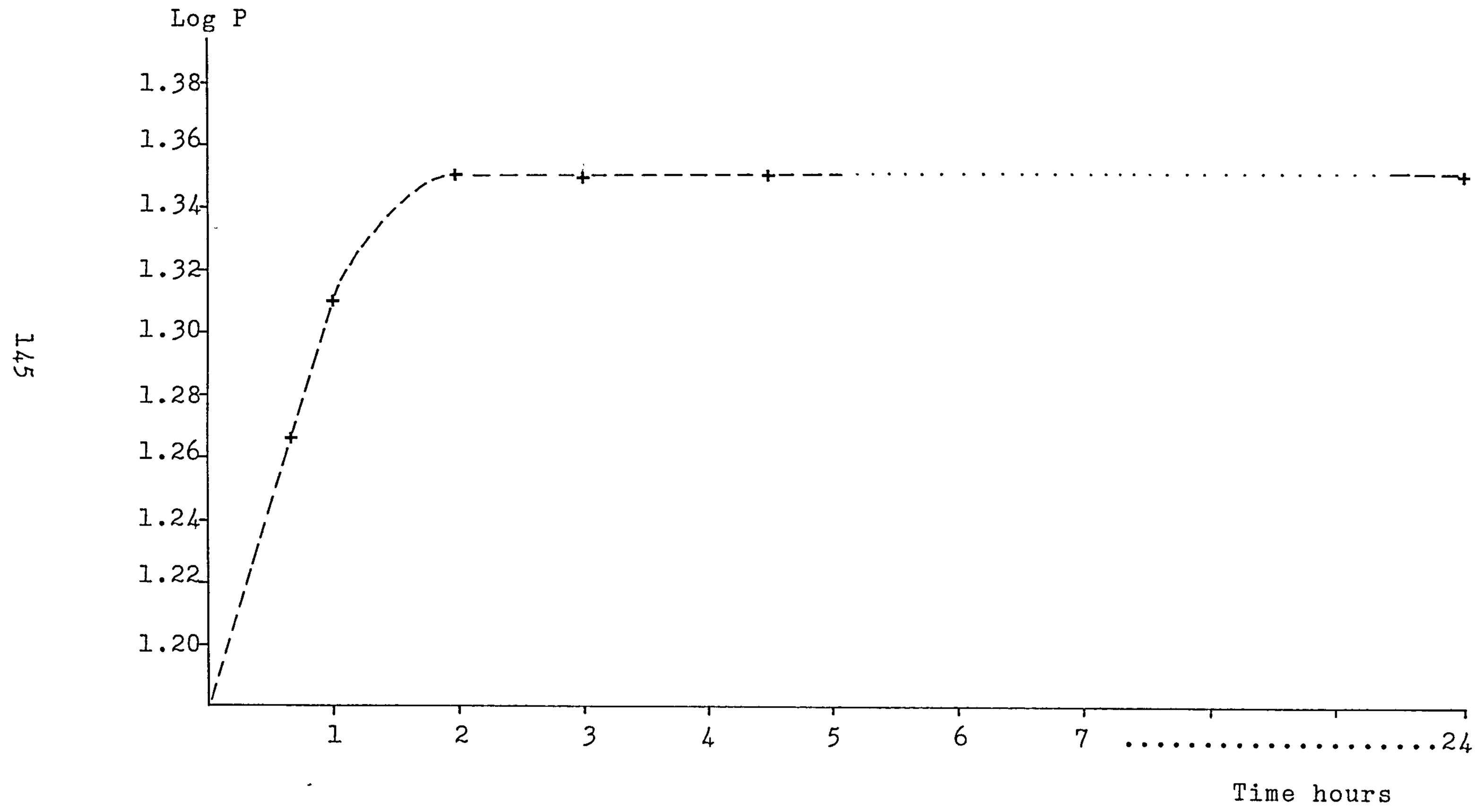


Figure 19. Variation of Log P with Time in Incubator

p-hydroxybenzaldehyde



From the results of this experiment it was decided that 2 hours was a suitable equilibration time for the compounds being studied. This aspect of partitioning is further discussed in the next method, but this decision was confirmed by further experimentation.

During this experiment however it was found that within a set of samples at ½ hour and 1 hour, there was a considerable variation in the partition coefficients which were calculated. This variation was not present after 2 hours equilibration, when in fact the values were all fairly close to the average. This discrepancy was found to be due to the shape of the conical flask. Two different shapes were used, one of which had a diameter of 8.59cm at the aqueous layer surface and the other a diameter of 7.32cm. The contents of the flasks with the smaller diameter were found to reach equlibrium at a lower rate than did the contents of the larger diameter flasks.

2. <u>The Effect of Flask Size</u>

Solute: p-methylacetanilide

100ml aq.phase : 5ml octanol

Small Flasks			Large Flasks		
Time	P	Log P	Time	P	Log P
½hr	16.1	1.21	½hr	29.7	1.47
1hr	27.7	1.44	1hr	30.1	1.48
2hr	29.6	1.47	2hr	30.1	1.48
3hr	31.1	1.49	3hr	29.9	1.48
24hr	31.1	1.49	24hr	30.2	1.48

Thus it is important to check all aspects of a method to eliminate possible sources of error. For all following experiments the large diameter flasks with an equlibration

time of 2 hours were used.

This method was chosen for use throughout this work since the results were reproducible, the system could be temperature controlled and a number of samples could be processed at one time.

Method 5. Filter Probe

This method was a modification of the system developed by Cantwell and Mohammed (62) and used by Kinkel et al (226) It consisted of a thermostatted mixing chamber of 200ml volume (see Figure 17). Thermostatic control was achieved by connecting ports A and B to a circulating water bath. The contents could either be stirred vigorously by means of a magnetic stirring bar and motor, or less vigorously by means of a stirring rod used in conjunction with the magnetic stirring bar. This latter method enabled each phase to be stirred independently so that if required, little phase interface disruption occurred. The vessel was sealed with a ground glass lid which had two ports to allow entrance of a stirring rod, thermometer or sampling tube or probe.

Two sampling modifications were used with this system. The filter probe technique is described in Chapter 9 and was the method used for the thermodynamics work, capillary sampling was used for the rate of partitioning experiment as described in Chapter 10. Both systems eliminated the need for centrifuging the sample prior to U.V. analysis. Vigorous mixing was employed in the filter probe method but the phases were separated by filtration and for the

capillary sampling method individual phase stirring was employed so that phase contamination was not a problem provided the capillary tube was carefully positioned. The capillary tube was made of Teflon and remained in position throughout the experiment, allowing single samples to be obtained or continuous sampling. Since sample extraction was achieved by capillary action a mechanical pumping system was unecessary. With both sampling systems the sample was returned to the partitioning vessel after analysis.

Continuous sampling and analysis by using a flow through cell in a recording UV.spectrophotometer (Cecil CE595 with Cecil CE500 recorder) allowed an investigation of the time required to reach equilibrium for the study compounds. The results are given in Table 8 and show that it is wrong to assume that all compounds reach equilibrium in similar times. Equilibration times vary from one compound to another and from one system to another. This supports work reported by Rogers and Wong (330) and questions the accuracy of merely shaking the two phases for a few minutes. However, the results support the choice of 2 hours as a suitable equilibration period. Two solvent systems were investigated and in general it appears that equilibration takes longer in the cyclohexane/water system than in the octanol/water system. The range of equilibration time in the octanol/water system is fairly narrow, being between 10 and 30 minutes, but in the cyclohexane/water system the range is much wider, between 8 and 120 minutes. The method involved maximum agitation and so inefficient mixing should not be responsible for the lengthy equilibration period.

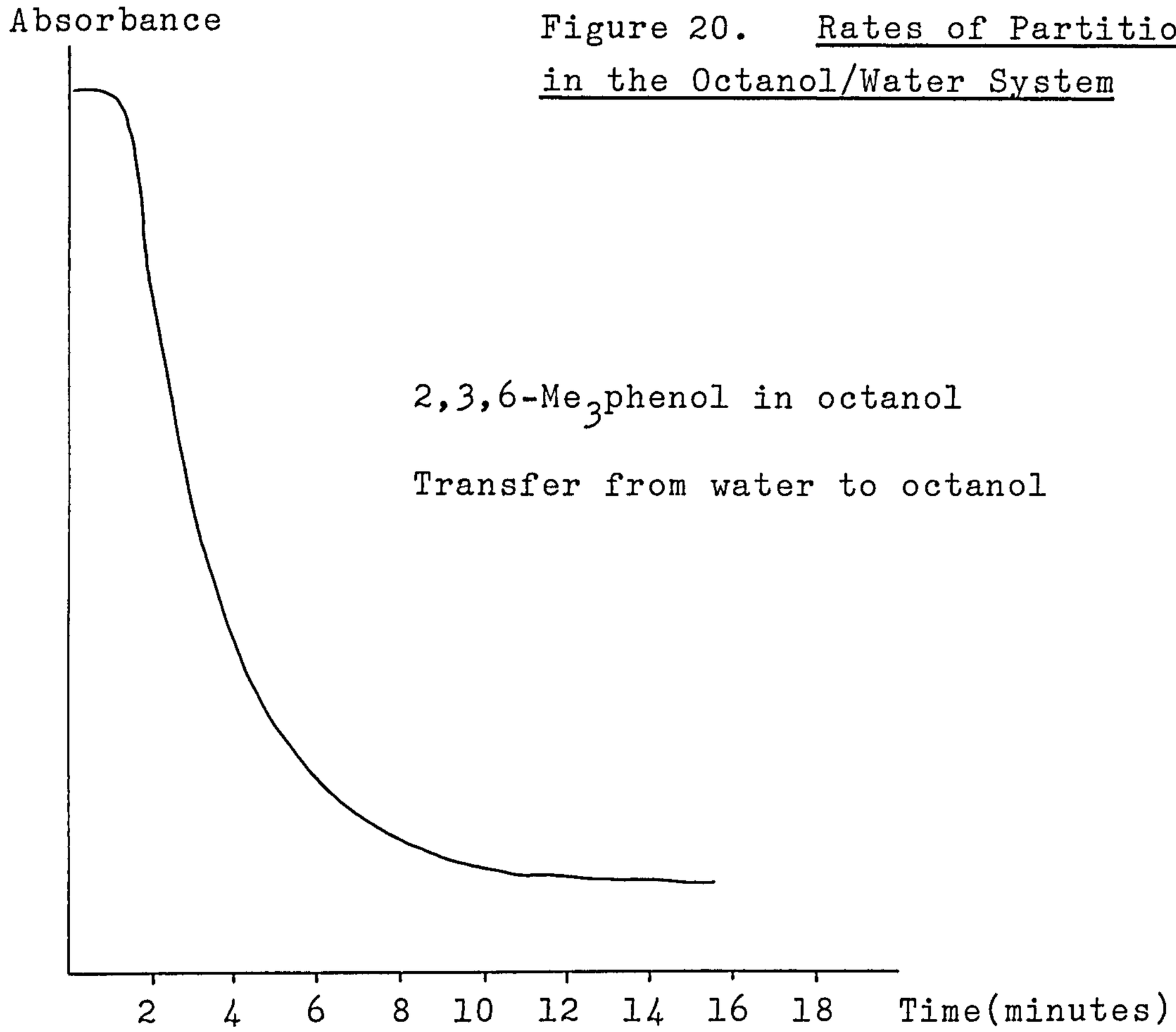
Table 8. Time Required to Reach Equilibrium in Two-Phase Partitioning Systems

Compound	Octanol/Water System	Cyclohexane/Water System
Phenol	12 mins	20 mins
o-Clphenol	20 mins	28 mins
m-Clphenol	15 mins	26 mins
p-Clphenol	15 mins	30 mins
o-NO phenol	22 mins	16 mins
m-NO phenol	20 mins	65 mins
p-NO phenol	16 mins	120 mins
2-NO resorcinol	10 mins	30 mins
o-OHbenzaldehyde	16 mins	30 mins
m-OHbenzaldehyde	16 mins	80 mins
p-OHbenzaldehyde	14 mins	120 mins
Benzoic acid	12 mins	14 mins
o-OHbenzoic acid	22 mins	20 mins
m-OHbenzoic acid	20 mins	-----
p-OHbenzoic acid	15 mins	-----
2,6-OH benzoic acid	30 mins	30 mins
3,5-OH benzoic acid	28 mins	-----
3-Me-2-NO phenol	10 mins	30 mins
4-Me-2-NO phenol	14 mins	60 mins
5-Me-2-NO phenol	30 mins	20 mins
6-Me-2-NO phenol	12 mins	10 mins
o-Mephenol	18 mins	10 mins
m-Mephenol	14 mins	12 mins
p-Mephenol	12 mins	16 mins
2,3-Me phenol	18 mins	16 mins
2,4-Me phenol	14 mins	16 mins
2,5-Me phenol	30 mins	16 mins
2,6-Me phenol	16 mins	10 mins
3,4-Me phenol	12 mins	8 mins
3,5-Me phenol	16 mins	16 mins
2,3,5-Me phenol	14 mins	16 mins
2,3,6-Me phenol	18 mins	14 mins
2,4,6-Me phenol	16 mins	34 mins
2,3,5,6-Me phenol	28 mins	25 mins
o-Mebenzoic acid	16 mins	30 mins
m-Mebenzoic acid	14 mins	20 mins
p-Mebenzoic acid	12 mins	28 mins
2,6-Me benzoic acid	16 mins	22 mins
3,5-Me benzoic acid	30 mins	22 mins
Acetanilide	30 mins	50 mins
o-Meacetanilide	25 mins	22 mins
m-Meacetanilide	22 mins	18 mins
p-Meacetanilide	26 mins	24 mins
2,6-Me acetanilide	20 mins	20 mins
3,5-Me acetanilide	22 mins	10 mins

In the octanol/water system the solute is initially dissolved in the aqueous phase.

In the cyclohexane/water system the solute is initially dissolved in the cyclohexane.

Figure 20. Rates of Partitioning in the Octanol/Water System



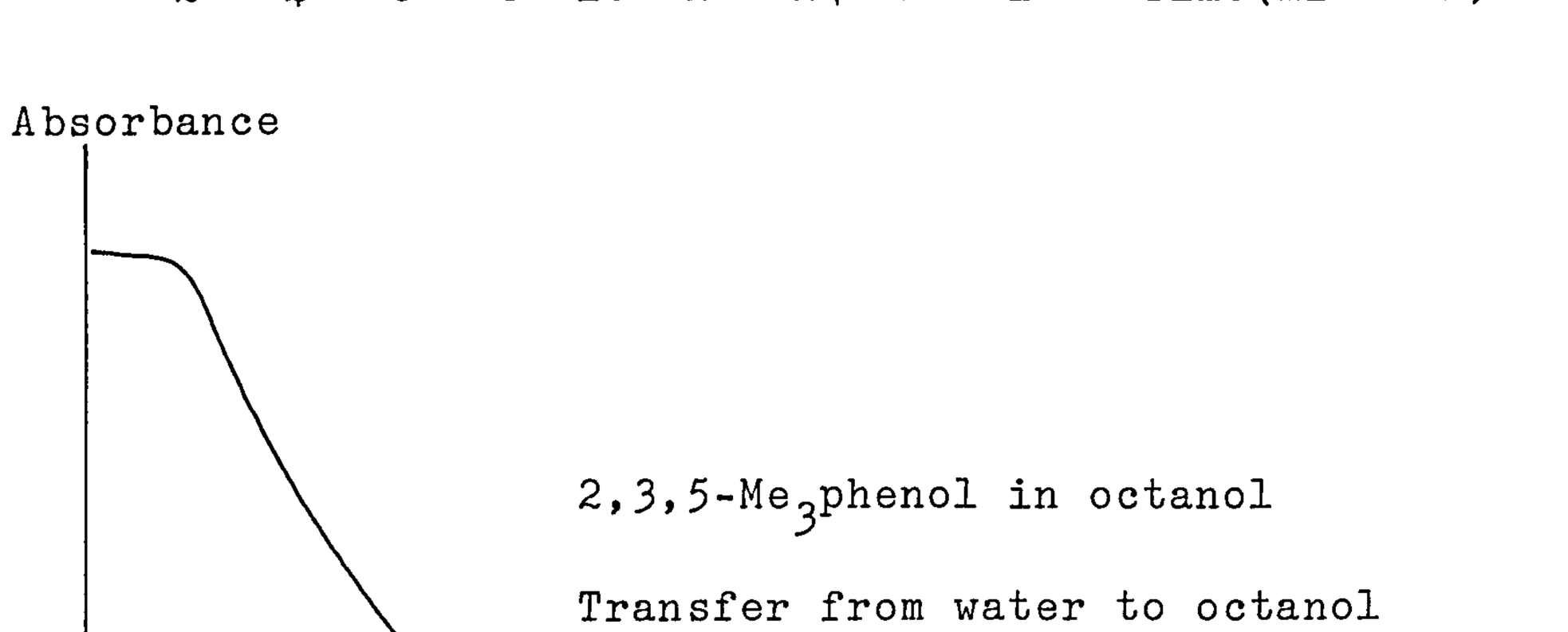
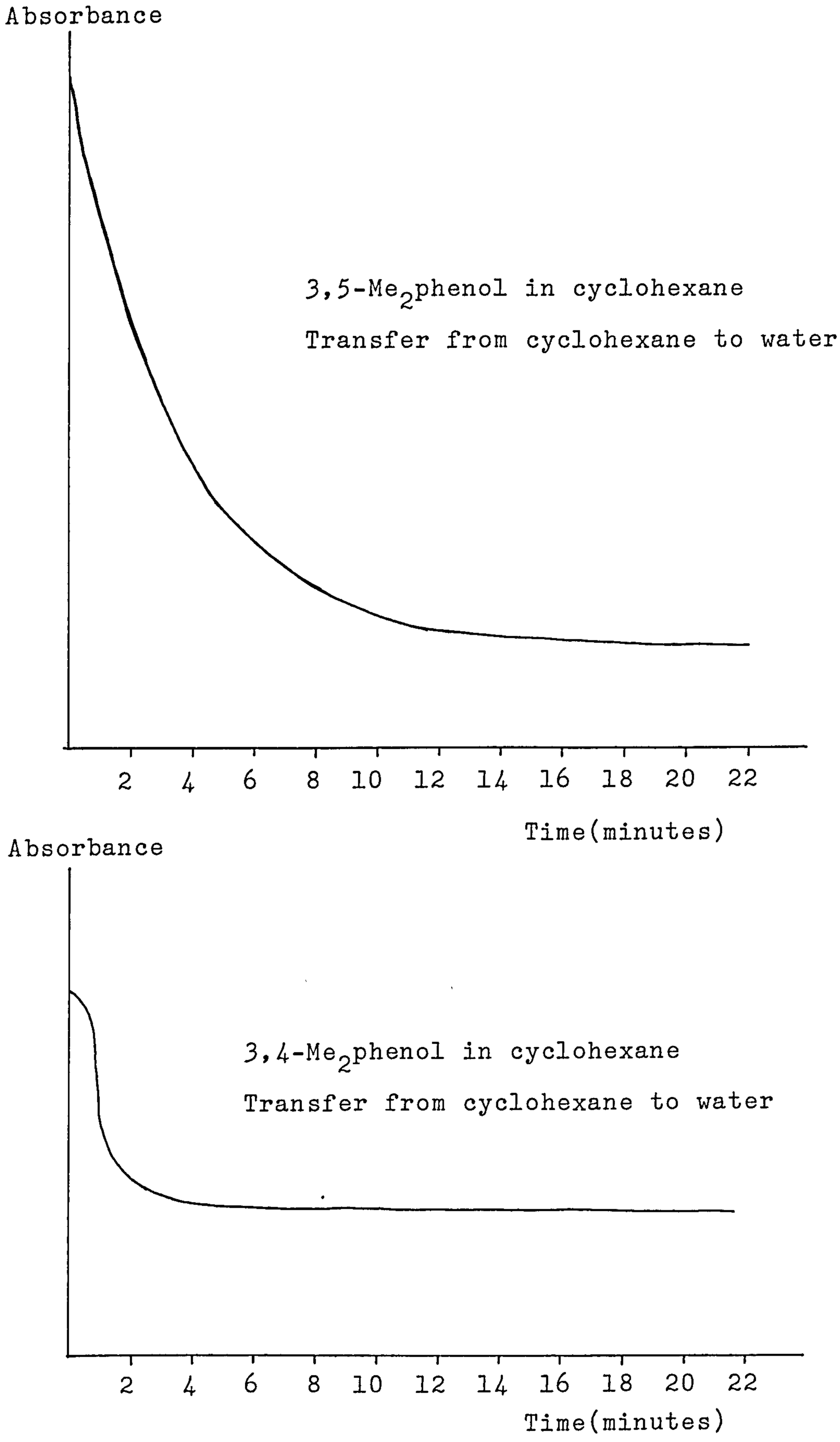


Figure 21. Rates of Partitioning in the Cyclohexane/Water System



4.3 Discussion of the Methods of Measuring Partition Coefficients

Comparison of the different methods used for determining partition coefficients illustrates the difficulties encountered when trying to compare results obtained by a number of groups of workers. Even within this study, where all experiments were conducted by one operator, the different methods were difficult to correlate. This presents one reason for anomalous results and indicates the necessity for stating experimental details.

Method 1 is the shaking method recommended by Hansch (255). This is probably the most universally used method, although the present work has shown its limitations. It has the advantage of being simple and requiring a minimum of laboratory equipment. Many workers have reported it to be reliable and a large proportion of the published data has been obtained using this method. The results of the present investigation indicate that it should not be the method of choice for a number of reasons:

1. Although simple, it is time consuming. The recommended partitioning time is two minutes, but only two samples can be handled simultaneously by one operator, so determinations on a large number of compounds would be tedious.
2. There is no facility for temperature control, so that the temperature of partitioning varies with room temperature. The latter may be fairly consistent in an air-conditioned, controlled environment, but many laboratories experience seasonal variation in ambient

temperature and the thermodynamics work of Chapter 9 shows the effect that even a 5°C temperature change can have on partition coefficient.

3. There is a possible volume restriction. The method quoted uses 50ml aqueous phase and 5ml octanol; this ratio severely restricts the range of partition coefficients which can be measured and although larger vessels can be used, shaking becomes more difficult.

4. Emulsification can be a problem, particularly in the octanol/water system. Too vigorous shaking can produce an emulsion which is difficult to crack.

5. Centrifugation is essential to ensure complete separation of the phases before analysis. This increases experiment time.

6. The recommended shaking time is 2 minutes. Table 8 and Figures 20 & 21, illustrate:-

a)that different compounds take different lengths of time to reach equilibrium, and,

b)that the compounds investigated in this work took longer than 2 minutes to reach equilibrium even when vigorous mechanical shaking was employed.

Thus it is possible that incorrect values of P are obtained simply because equilibrium has not been attained.

However, this method was used to demonstrate a number of areas where errors can occur.

1. <u>Sampling the aqueous phase</u>

Purcell (316) recommends sampling the aqueous phase by passing a pipette, with a finger over the top, through the octanol phase into the aqueous phase, then blowing gently

through the pipette to force out any octanol in the tip. This was found to be unsatisfactory since traces of octanol remained which contaminated the spectrophotometric cell and caused increased U.V. absorption. To overcome this it was necessary to separate the two phases before sampling.

2. Concentration of solute

The concentration of solute must be sufficiently low for association not to occur, but at the same time high enough for accurate analysis. For the compounds used in this investigation 10^{-3}M was found to be suitable. More sensitive analytical techniques allow lower concentrations to be used. The ratio of aqueous phase to organic phase is important here since for greatest accuracy the weight of solute in each phase should be the same at equilibrium. This varies with each solute depending on its partition coefficient so phase volume adjustment is necessary for each solute.

3. Centrifugation

Centrifugation of the analysed phase is stressed in most methods as being vitally important to separate any traces of contaminating phase and crack an emulsion if formed. Various times and speeds are quoted, but the emphasis is the same - centrifugation is essential for accurate results. However, this investigation found that although it was important to ensure complete separation of the two phases, it was better to remove most of one phase i.e.the octanol phase, before centrifuging since sampling the aqueous phase through a layer of octanol produced contamination of the sample which negated the effect of centrifugation.

It was also found to be essential not to produce an emulsion particularly in the octanol/water system, since once formed it was stable and extremely difficult to crack, even using centrifugation.

4. Stability of solute

This was exemplified by 2,6-dimethylphenol.

Initial results indicated that both the time and speed of centrifugation affected the chemical stability of 2,6-dimethylphenol. Increased time and temperature (the latter caused by generation of heat during centrifuging), caused a decrease in absorbance leading to an apparently increased partition coefficient. This effect was eliminated by purging the sample with nitrogen prior to partitioning, indicating that the problem was caused by oxidation of the phenol. This is particularly likely to happen in the aqueous phase and was of considerable importance in this investigation since the majority of solutes were phenols. Therefore, in later experiments the aqueous phase was purged with nitrogen.

This again indicates the importance of stating all experimental conditions since a change in conditions may produce previously unknown errors. The method of dissolving the solute must also be considered since heat or the addition of a solvent may be utilised and it is important that no aid to dissolution affects the solute.

5. pH of aqueous phase

The effect of different pH's on the partition coefficient can be seen from the literature:

Log P phenol at pH7.4 in phosphate buffer = 0.62, with

out correction for ionisation.

Log P phenol at pH2.0 and 20^{o}C = 1.49, acidified with HCl.

Care has to be taken when using buffers since the change in partition coefficient may be due to the buffer (261).

Method 2 is an improvement on Method 1 because temperature control is possible, shaking speed and time can be controlled and a number of measurements can be made at one time. The disadvantage of this method was the shape and volume of the partitioning vessels. Efficient mixing was difficult and equilibrium was difficult to ascertain. The limited volume imposed restrictions on the phase ratios and hence the range of compounds which could be investigated.

The four hour shake period used by Tomlinson supports the suggestion that mixing was inefficient and hence longer times were needed, and since the total volume in the vessel had to be restricted to 75ml to ensure adequate mixing, the range of log P which could be measured was confined to values of $1<\log P<2$, which is far too narrow.

However this method was used to demonstrate some of the problems which could be encountered with centrifugation and degradation or adsorption of solute, for which reasons it is always advisable to record the ultraviolet absorption spectrum of the solute before and after partitioning to ensure that no structural changes changes have occurred.

Method 3 is quoted by Rekker (325) and Martin (274) but shaking was found to be too violent making emulsification difficult to avoid. Also, again, temperature control was

impossible. It is interesting to note that Rekker and Martin suggest half-hour shaking periods, raising the question of how reliable they considered the two-minute hand-shake method of Hansch et al (255).

Emulsification proved such a problem with this method that it was impossible to obtain reliable results.

Method 4 proved to be the method of choice for initial partition coefficient measurements. Temperature and agitation control were possible and the shaker could accommodate a number of flasks so that a larger number of samples could be partitioned at one time. The range of solutes which could be tested was also extended since different flask sizes could be used which allowed greater volume flexibility. However, another source of error was revealed because it was shown that partitioning time could vary with the inter-facial area, a larger area producing shorter partitioning times. Thus, ideally, a single vessel should be used, or, as with this experiment, a set of identical vessels.

Mixing was carefully controlled so that agitation and hence the risk of emulsification, was minimised. This was found to require an equilibration time of two hours, again raising doubts as to the value of a two minute shake.

Method 5 was initially investigated for use in the thermodynamics work (Ch 9). It was found to be the most reliable method tried. Accurate temperature control could be achieved, mixing was efficient without emulsification, a standard vessel was used so uniformity was guaranteed, the vessel capacity permitted a fairly wide range of solutes to

be tested, and if necessary a larger vessel could be used since handling was not a problem. Sampling was no problem if the flow-through system was used and an accurate record of equlibration time could be kept by linking a chart recorder to the U.V. spectrophotometer analyser. Separation of the two phases by filtration proved particularly successful, eliminating the need for centrifugation. Temperature control of the U.V. cell was also possible.

Slight problems were encountered when cyclohexane was the circulating solvent, since it could interact with certain tubing materials, but these were overcome by the correct choice of pump and tubing materials. The system was found to be reliable and its only drawback was that only one experiment could be performed at a time.

4.4 Conclusions

These experiments revealed certain characteristics essential for a reliable partitioning method:-

1. Shape of vessel must allow efficient mixing.
2. Size of vessel must be sufficient for a reasonable range of log P values to be measured. Ideally the method should be adaptable for different vessel sizes.
3. Mixing should be efficient but controlled. Emulsification must be avoided at all costs. Centrifugation cannot be relied upon to crack an emulsion.
4. Sampling must be controlled so that contamination with the second phase does not occur. Centrifuging is important for producing a 'pure' sample but its effect can be nullified by poor sampling technique. Filtration of the two phases was found to produce the best sample and eliminated the

need for centrifugation.

5. Temperature control is essential.

Methods 4 and 5 (Shake flask and Filter probe) were satisfactory in these respects and were therefore chosen for the measurement of the partition coefficients of the study compounds.

In addition to choosing a reliable method for measuring partition coefficients, the conditions of measurement must also be controlled:

1. A consistent temperature must be maintained throughout the partitioning period (see point 5 above).
2. The pH must be adjusted to achieve minimum ionisation.
3. The phase volume ratio must be such as to achieve a concentration of solute in the analysed phase at equilibrium which may be easily measured by the analysis method of choice.
4. The method chosen for analysis must be appropriate for the solute.
5. The initial concentration of solute must be low enough to eliminate self-association, but high enough for detection after equilibration.
6. Aids to dissolution must not affect the solute.
7. Solute oxidation etc., must be avoided, for example by nitrogen purging prior to partitioning or protection from light etc.
8. Care must be taken to ensure mutual saturation of solvents.
9. It is important for all these conditions to be recorded so that results may be accurately interpreted by other workers.

CHAPTER FIVE

CALCULATION OF PARTITION COEFFICIENTS

Experience gained by workers such as Hansch, Leo, Fujita etc. in the use of hydrophobic parameters in the study of quantitative structure-activity relationships by regression analysis has indicated that measured log P's should be used whenever possible. However, when the number of compounds is great and the structural variation is limited, simple economics make it desirable to measure the log P's for only the key structures and to calculate the remainder where any group interactions not in the measured solutes can be assumed to be negligible. While regression analysis requires the most accurate log P values possible, there is an ever increasing need for reliable estimates of many chemicals for which experimental values may be difficult to obtain. An example of this would be the thousands of compounds being studied as potential hazards to the environment through bioaccumulation.

Therefore, the additive-constitutive nature of the partition coefficient is potentially one of its most valuable contributions to structure-activity investigations.

5.1 The Hansch Equation: The Hydrophobic Substituent Constant, π.

The Hammett equation makes it possible to calculate a rate or an equilibrium constant of a meta- or para-substituted derivative of C_6H_5-R with R as the reacting centre.

$$\log k_s/k_o = \rho\sigma \qquad 5.i.$$

where k_o represents the rate constant of an unsubstituted structure and k_s that of a substituted derivative. This left-hand term can be replaced by $\log K_s/K_o$ where K represents an equilibrium constant.

σ denotes a constant typical of the substituent, reflecting its ability to attract or repel electrons and ρ is a constant characteristic of the type of reaction.

Hammett standardised this equation to the dissociation of benzoic acid in water at 25°C, ρ being taken to be 1.000.

Thus: $\log K_s/K_o = \sigma$ 5.ii.

where K_s and K_o indicate the dissociation constants of a substituted benzoic acid and benzoic acid itself in water at 25°C.

By analogy with the Hammett equation, in 1962, Hansch proposed to describe lipophilicity as follows

$$\log P(SX)/P(SH) = \rho\pi(X) \qquad 5.iii.$$

where P(SH) and P(SX) represent the partition coefficients of SH and SX respectively; $\pi(X)$ represents the hydrophobic substituent constant, i.e. the contribution of substituent X to the lipophilicity of structure SH when X replaces an H atom in SH; and the constant ρ reflects the characteristics of the solvent pair used in determining the partition coefficient.

Standardisation here means choosing one of the several solvent systems as the reference, so that ρ = 1.000 and Eqn.5.iii. can become:-

$$\log P(SX) - \log P(SH) = \pi(X) \qquad 5.iv.$$

e.g. $\pi_{Cl} = {}^{\log P}C_6H_5Cl - {}^{\log P}C_6H_6$ (255,145)

$$\pi_{Cl} = 2.84 - 2.13 = 0.71$$

A positive value for π means that, relative to H, the substituent favours the octanol phase. A negative π value indicates its hydrophilic character relative to H.

Eqn.5.iv. can, where appropriate, be transformed into eqn.5.v.

$$\log P(S'X_1X_2....X_n) = \log P(S'H_n) + \sum_1^n \pi(X_n) \qquad 5.v.$$

with the practical implication that for a structure for which a partition value has not been or perhaps cannot be determined (because of unduly high or low logP values, whether or not combined with poor analytical detectability), any given moiety with known lipophilicity can serve as a basic fragment from which the total lipophilicity can be construed by simple addition of the proper π values.

Like the Hammett constant, σ , π is a free-energy related constant; it is a measure of the free energy changes involved in moving a substituent from one phase to another. It has been termed a "group contribution". (94-97)

Octanol-water was proposed by Hansch and Dunn (402) as the solvent system of choice (ρ = 1.000). Its relatively poor capacity to dissolve water (water-saturation concentration = 1.7M), combined with the presence of a hydroxyl group, which can act as a hydrogen-bond donor as well as as acceptor, is considered to constitute a reasonable model of the average macromolecular system in which drug interaction takes place.

Equation 5.iii. was designed primarily by analogy with the Hammett equation and is thus applicable to aromatic structures, but it may also serve in describing the lipophilicity of aliphatic structures, provided a separate set of π values is available.

Table 9. Hydrophobic Substituent Constants

Function	π Value		Correction for:	
	Aliphatic	Aromatic		
F	-0.17	0.14	Branching :-	
Cl	0.39	0.71	1.in C chain	-0.20
Br	0.60	0.86	2.of functional	
I	1.00	1.12	group	-0.20
OH	-1.16	-0.67	3.ring closure	-0.09
OCH_3	-0.47	-0.02	Double bond	-0.30
SCH_3	0.45	0.61	Folding	-0.60
NH_2	-1.19	-1.23	Intramolecular	
CN^2	-0.84	-0.57	H-bonding	0.65
COOH	-0.67	-0.28	Ring joining	-0.20
$COOCH_3$	-0.27	-0.01		
$COCH_3$	-0.71	-0.55		
$N(CH_3)_2$	-0.85	-0.28		
NO_2	-0.85	-0.28		
CH_3^2	0.50	0.56		

Table 9 shows the πvalues of the most important functional groups and the correction terms required by the π system in calculating lipophilicity. Most of the aliphatic π values tabulated were obtained from log P measurements for only one structure so that some caution is necessary in their practical application. (143,144,145,174)

The π value given for OH holds for primary, secondary and tertiary alcohols, provided that the appropriate branching corrections are made.

e.g. $\log P(\text{2-butanol}) = 4\pi(CH_3) + \pi(OH) + 1\text{br.c.}$

$= 2.00 + (-1.16) + (-0.20) = 0.64$

Observed = 0.61

$\log P(\text{tert-butanol}) = 4\pi(CH_3) + \pi(OH) + 2\text{br.c.}$

$= 2.00 + (-1.16) + (-0.40) = 0.44$

Observed = 0.37

Two branching corrections (br.c.) are needed in the last example since there are two alkyl branches in tert-butanol.

The branching correction for a functional group can also be accommodated in the π value of this group, as has been reported by Tute (367) who proposed π values of -1.16, -1.39 and -1.43 for primary, secondary and tertiary groups respectively. With these π values, the respective log P values obtained for the above two alcohols are 0.61 and 0.41.

The most important correction in the π system is that for folding. A study on the lipophilicity of a number of structures of the type: $C_6H_5CH_2CH_2CH_2$-X where X denotes OH,F,Cl,Br,I,COOH,$COOCH_3$,$COCH_3$,NH_2,CN,OCH_3 or $CONH_2$ revealed that log P in this series is about 0.60 ± 0.10 lower than calculations would indicate.

e.g. $\log P(C_6H_5CH_2CH_2CH_2OH) = 2.13 + 1.50 + (-1.16)$

$= 2.47$

Observed = 1.88

Hansch et al. claim that this decrease is due to folding, assuming that the dipole induced at the end of the side chain ($C^{\delta+}$-$X^{\delta-}$) interacts with the π-electrons of the phenyl ring with the X atom or group projecting away from the ring/side-chain complex. The net result will be a more compact structure, producing a greater than expected water solubility.

The correction for ring joining, -0.20, is necessary since the phenyl group was found not to give full additivity when attached to another aromatic ring.

e.g. log P(biphenyl) = 2 x 2.13 + (-0.20)

= 4.06

Observed = 4.09 (71)

In order to obtain information on the π pattern of functional groups attached to a phenyl ring, Fujita et al (145) determined the partition coefficients of 203 mono- and di-substituted benzene derivatives in the octanol-water system. A collection of π values for 67 functional groups could be derived from these partition values. The compounds were considered to belong to eight different systems: phenylacetic acid, phenoxyacetic acid, benzoic acid, benzyl alcohol, phenol, aniline, nitrobenzene and benzene.

Table 10 shows the π values for selected functional groups.

In general, for substituents on an aromatic ring, π values are reasonably constant whether the parent is benzene, benzoic acid or phenylacetic acid (274) i.e.parent molecules in which there is not a strong hydrophilic group attached directly to the ring. However, Table10 shows that large differences may occur with a few functional groups, so that for practical purposes the ultimate choice of the system to be used for the calculation of unknown lipophilicities depends on the mutual arrangements of the functional groups present on the benzene ring.

Table 10 shows that inert groups such as alkyl (CH_3) have a

Table 10. Examples of π_x From Various Aromatic Solute Systems

Function		Systems							
		$C_6H_5CH_2COOH$		$C_6H_5CH_2OH$			$C_6H_5NO_2$		
			$C_6H_5OCH_2COOH$		C_6H_5OH				C_6H_6
				C_6H_5COOH			$C_6H_5NH_2$		
CH_3	o-		0.84						
	m-	0.54	0.51	0.52	0.50	0.56	0.50	0.57	0.56
	p-	0.45	0.60	0.42	0.48	0.48	0.49	0.52	
OH	o-	-0.54							
	m-	-0.52	-0.49	-0.38	-0.61	-0.66	-0.73	0.15	-0.67
	p-		-0.61	-0.30	-0.85	-0.87	-1.07	0.11	
COOH	o-								
	m-	-0.27	-0.15	-0.19		0.04		-0.02	-0.28
	p-					0.12		0.03	
NH_2	o-								
	m-				-1.15	-1.29		-0.48	-1.23
	p-					-1.63		-0.46	
NO_2	o-		-0.04						
	m-	-0.01	0.11	-0.05	0.11	0.54	0.47	-0.36	-0.28
	p-	-0.04	0.24	0.02	0.16	0.50	0.49	-0.39	
Cl	o-		0.59			0.69			
	m-	0.68	0.76	0.83	0.84	1.04	0.98	0.61	0.71
	p-	0.70	0.70	0.87	0.86	0.93	0.80	0.54	
Br	o-		0.75			0.89			
	m-	0.91	0.94	0.99		1.17	1.13	0.79	0.86
	p-	0.90	1.02	0.98		1.13	1.12		

Table 11. π Constants - Octanol/Water System

Function	Aromatic Substituent Constant
CHO	-0.65
COOH	-0.32
CH_3	0.56
CH_2OH	-1.03
CH_2NH_2	-1.04
C_6H_5	1.96
Cl	0.71
H	0.00
NO_2	-0.28
NH_2	-1.23
OH	-0.67
$COCH_3$	-0.55

relatively constant π value, regardless of the electronic character of a second substituent attached in the para-position. The halogens are somewhat more sensitive, their values being changed considerably by strong electron-withdrawing and -releasing substituents. Most sensitive are those substituents carrying a lone pair of electrons on the atom attached directly to the ring. (e.g.OH,OCH_3,NH_2). Strong electron-attracting groups such as NO_2 or CN raise π values, whereas electron-releasing groups lower π values.

When two substituents are placed on an aromatic ring, the mutual electronic effect of one upon the other changes the π value of each. (145) Often, however, one substituent is dominant (i.e.the constant substituent is influenced electronically by the variable substance), and π values applicable to a new aromatic system can be estimated. Proximity effects of nearby substituents can also affect π values. The field effect appears to be more important than the resonance effect in changing π values. For example, o-, m- and p-nitroanilines have log P values of 1.79, 1.37 and 1.39 respectively. (189) Through resonance apparently has little effect on log P.

The additivity of π substituent constants can be seen in the following example:

$$\log P_{1,3,5\text{-tri-}NO_2\text{benzene}} = \log P_{C_6H_6} + 3\pi_{NO_2}$$

$$= 2.13 - 0.84 = 1.29$$

Observed logP = 1.18

Crowding on the aromatic ring and possibly electron release (favouring hydrogen bonding with, hence lowering log P) by

CH_3 lowers the partition coefficient. When strong electron interaction occurs between substituents (strong electron-donating and strong electron-attracting substituents on the same ring) simple additivity fails.

In the early work with partition coefficients of hydrocarbons, it was assumed that log P of H was essentially zero. By definition, π_H is zero. If this were so, then log P values of small molecules could be combined to get values for larger molecules. (181) More recent work has shown log P for H to be approximately 0.23. (189)

It has been stated that values of π_x vary for different solute systems and therefore a second set of π values - named π- by Fujita et al (145) is useful for substituents on phenols and anilines. Table 12 shows a selection of π and π- values as stated by Norrington et al. (302)

Table 12. πand π- Values For Substituents on an Aromatic Ring

Group	ortho		meta		para	
	π	π-	π	π-	π	π-
H	0.00	0.00	0.00	0.00	0.00	0.00
CH_3	0.84*	0.49	0.52	0.50	0.60	0.48
C_2H_5	1.39		0.99	0.94		
C_6H_5			1.92	1.77	1.74	1.74
OH	-0.41	-0.58	-0.50	-0.66	-0.61	-0.87
OCH_3	-0.33	-0.13	0.12	0.12	-0.03	-0.12
$OCOCH_3$	-0.58	-1.02	-0.60	-0.23	-0.58	-1.06
NH_2	-1.40	-0.84	-1.29	-1.29	-1.30	-1.42
$NHCOCH_3$	-0.14	-0.74	-0.78	-0.73	-0.56	-1.21
NO_2			0.11	0.54	-0.28	0.45
CHO	-0.43	0.24		-0.08	-0.47	-0.06
Cl	0.76	0.69	0.77	1.04	0.73	0.93

* this value for CH_3 has been shown to be in error. Hansch (189) gives a value of 0.67 which is still high but has been shown by Dearden and Wootton (113) to be a unique effect due to steric enhancement of resonance of the $-OCH_2-$ group. of parent compound phenoxyacetic acid

In this case the hydrophobic effect of a group is the net result of its intrinsic hydrophobicity plus its effect on the hydrophobicity of the parent molecule and vice versa. Hence, the largest difference (+0.73 log units) between normal π values and π- values as shown in Table 12 is for the p-NO_2 substituent. This group can interact by resonance directly with the -OH or -NH_2 group to decrease the ability of the substituents to interact with water. p-NO_2aniline substitution increases the log P of aniline, but decreases that of benzene. This situation is interesting since this statement seems to contradict a previous statement which said that resonance effects do not seem to be as important as field effects since the log P's of both m- and p-nitroaniline are similar. This is an example of anomalies which exist in partition coefficient measurement and calculation which will be investigated in this thesis.

Phenoxyacetic acid and benzyl alcohol appear to be intermediate between the two types of parent molecules.

The decision as to which set of aromatic π values to use should be based on a consideration of which parent molecule most resembles the one of interest in terms of possible interactions with water.

Further values of π and π- are listed by Fujita (145) and Leo (255).

Thus the π system was the first and most widely used system for calculating partition coefficients. However, since the definition of π constants for substituents is entirely

analogous to the definition of Hammett σ (electronic) constants, their use in linear free-energy relationships depends upon the same assumption; that is, π is also 'extrathermodynamic'.

Therefore,if the assumptions inherent in adding substituent free energies are accepted, log P values can be calculated by substituting groups for hydrogen (π system) or by summing the appropriate atructural elements (fragment system).

In the early work with π calculations (176) erroneous values for a few aliphatic hydrocarbons led to the conclusion that the intrinsic hydrophobicity of the hydrogen atom in the octanol-water system was close to zero, so a good approximation of log P could be obtained by summing π constants (174) Davis (94) however, showed that the hydrophobicity of a methyl group ($-CH_3$) was appreciably greater than that of a methylene ($-CH_2-$) group. This led to Nys and Rekker (303) developing a set of fragment values which could be used additively according to the equation :-

$$\log P = \sum_{1}^{n} a_n f_n$$

where a is the number of occurences for fragment f of the structural type n.

The π and fragment systems are closely related but each has its own advantages in application and in certain circumstances it is easier to use one than the other. For instance, when calculating log P of a complex structure it is easier to begin with the measured log P of a similar parent and use π constants where the structural differences

are substituents on an aromatic ring. If however, no log P value is available for a suitable parent, the fragment method of building up a log P value is preferable.

Two fragmental approaches to calculating log P have been evolved, that of Nys and Rekker, and that of Hansch and Leo. The hydrophobic fragmental constant of Nys and Rekker was the first to be developed, but Hansch and Leo felt that there was still scope for a more precise procedure suitable for computer programming and so developed their method. Both methods will now be described.

5.2 Hansch and Leo Fragment Method

This method can be considered constructionist or synthetic and is based on a few carefully measured partition coefficients for simple structures. The first value obtained was that for the ordinary hydrogen atom - ordinary in the sense that it was not attached to an electronegative atom such as oxygen, nitrogen or the carbon in carbonyl. The partition coefficient of the H_2 molecule was carefully measured, giving $f_H = 0.23$. This was followed by the value of a carbon atom in an alkyl chain, which was obtained from an average of the log P of a methyl group in methane and ethane.

$$f_{CH_3} = \log P\ CH_4 - f_H = 1.09 - 0.23 = 0.86$$

$$f_{CH_3} = \tfrac{1}{2}\log P\ CH_3\text{-}CH_3 = \tfrac{1}{2}(1.81) = 0.905$$

$$\text{Thus: } f_C = fCH_3 - 3fH = 0.88 - 3(0.23) = 0.20$$

This method therefore uses constant fragment values for the fundamental structural elements and looks for other factors (F) that affect the partitioning equilibrium in the more

complex solutes where summation of fragments alone leads to spurious values.

This means that although fewer fragment constants are used, the calculation is complicated by extra structural factors, but Hansch and Leo considered that this system recognised solvation forces operating in both the lipid and the aqueous phases and thus was likely to clarify the mechanism of the processes being studied.

Thus a set of definitions and symbols may be presented.

5.2.1 Fundamental Fragments

IC	Isolating carbon atoms: those having either 4 single bonds (at least two of which are to nonhetero atoms) or those which are multiply bonded to other carbon atoms.
NIC	Nonisolating carbon atoms: those multiply bonded to hetero-atoms e.g.Carbons in carbonyls, nitriles, imines etc.

A single atom fundamental fragment can only be:
1. An isolating carbon atom (IC)
2. A hydrogen or hetero-atom all of whose bonds are to IC's.

e.g. a. —C— in CH_4 and C= in $H_2C{=}CH_2$ but not in $(CH_3)_2C{=}NH$

b. —H in H—C≡C but not in R—C(=O)—H

c. —F in CH_3—F but not in R—SO_2—F

d. —O— in CH_3—O—CH_3 but not in CH_3—C(=O)—O—CH_3

A multiple atom fundamental fragment can be formed by joining directly any of these types:

a. an NIC
b. a hydrogen
c. a hetero-atom

A fundamental fragment is complete only when all its remaining bonds lead to isolating carbons.

Two atom fragments include:

a. —OH b. —C(=O)— c. NH

Multiple-atom fragments include:

a. —C(=O)—NH_2 b. —NH_2 c. —NH—NH—

H-polar fragment: one that can participate in hydrogen bonding, either as donor or acceptor.

S-polar fragment (for σ-polar): those with strong electron-withdrawing power, but little or no tendency to hydrogen bond. i.e.the halogens.

Derived Constants

These are for commonly used fragments such as —CH_3 and —C_6H_5. Prior calculation of such fragment constants make manual calculation of log P values easier.

As well as the basic fragment constants, there is also a set of factors. These values are based on interactions within the molecule and include:

A.Chains

Flexibility: A larger increase in log P occurs in going from C_1 to C_2 than from C_3 to C_4. This is possibly due to chain flexibility in the larger molecules which can reduce the degree of order in the solvation shell surrounding the molecule. The flexibility factor for rings is less than that for chains so the bond factor (F_b) which is -0.12 for chains is -0.09 for rings.

Unsaturation: The polar character of the bonds

between carbon atoms increases in the order: single<double <triple and, since polarity increases the accomodation by water relative to octanol, unsaturation should lead to a lower log P. However, only localised polarity favours accommodation by water so conjugation would be expected to reduce the log P-lowering effect of unsaturation.

Thus in log P calculations, the structure is first considered as saturated and then the appropriate $F_{=}$ and $F_{\equiv}$ constant is added.

Branching: Branched alkanes are more water-soluble than are their straight-chain isomers. The increased water-solubility and decreased partition coefficient are usually ascribed to the smaller cavity needed to accommodate the branched solute. i.e.Less hydrophobic surface.

B.Interactions

Halogen with Halogen: All polar groups are favoured by the solvating forces in water relative to those in octanol. The size of the polar group may outweigh its polar effect, causing a positive rather than negative fragment constant, but for calculation it is more important to distinguish between polar groups that can hydrogen bond and those that cannot. This puts the halogens in a separate group from nitrogen- and oxygen- containing fragments while sulphur is a little ambiguous.

When a halogen is attached to a hydrocarbon, only a localised dipole leads to better accommodation by the aqueous phase i.e.to a decrease in log P.

In alkane structures, multiple halogenation on the same or adjacent carbon atoms results in a higher log P than simple additivity predicts. This is probably accounted for by the bulk of the halogen atoms themselves shielding the localised dipole from complementary dipoles in the aqueous phase.

Each of these situations has a factor value assigned to it.

Halogen with H-Polar: The effect of one or more halogen or other electron-withdrawing fragments in a position to influence an H-polar fragment is not easily predictable, but 'enhanced' fragment constants (f^X) are used in this scheme for aromatic-attached, H-bonding fragments whenever a second substituent with high electronegativity is present.

A strongly electronegative group (S-polar) attached to the same aliphatic carbon as an H-polar also raises the log P over the simple additive value. These effects are difficult to predict and few values are available.

H-Polar Fragments: For uncharged H-polar groups, any interaction effect is usually only important when only one or two carbon atoms intervene. The proximity effect between charged H-polar fragments or between H^+polar and normal H-polar fragments is difficult to assess, but is felt to be proportional to the amount of intrinsic hydrophilic character of the two fragments. Thus two H-polar fragments separated by four or more carbon atoms are isolated and given their full value. When separated by two carbon atoms in a chain, the value of each is reduced by approximately 25% and by about 40% if only one carbon atom separates them. Since the sum of fragment values for H-polar groups is

always a negative number, its reduction results in an increase in log P.

Pi Electrons with Polar Fragments-Aromatic Systems: The hydrophobicity of polar groups is increased when pi electrons from resonating systems become available. This effect is greatest in the case of H-polar fragments, and is most commonly seen when polar fragments are attached to benzene rings. This 'f-enhancing' effect of pi electrons is the basis for defining 'isolating' carbon atoms and using them as markers to separate polar fragments.

Hansch and Leo decided to use separate fragment sets for aliphatic and aromatic attachments, thus making an aromatic carbon atom 'isolating'. This system can be extended to cover heterocyclic compounds and other more intricate systems, but these will not be covered since they are not relevant to this thesis.

Thus a system was developed for calculating log P values from fragment constants. Hansch and Leo felt that to be useful this system must be a compromise between two extremes. If the fragments are taken to be the smallest possible structural units, the separate atoms with associated bond types, connection and proximity factors are problems. However, to reduce interaction factors to zero the fragments must incorporate most of them so that they become parent molecules upon which small modifications are made, which is essentially the π-calculation system.

Table 13. Leo et al's Modified Fragmental Constants

Fragment	f
H	0.23
CH_3	0.89
CH_2	0.66
CH	0.43
C	0.20
b(single bond in chain)	-0.12
b(single bond in rings)	-0.09
cbr(chain branching)	-0.13
gbr(group branching)	-0.22

	Aliphatic	Aromatic
F	-0.38	0.37
Cl	0.06	0.94
Br	0.20	1.09
I	0.60	1.35
OH	-1.64	-0.40
COOH	-1.09	-0.03
COO	-1.49	-0.56
O	-1.81	-0.57
S	-0.79	0.03
NH_2	-1.54	-1.00
NH	-2.11	-1.03
N	-2.16	-1.17
NO_2	-1.26	-0.02
CN	-1.28	-0.34
CO	-1.90	-0.32
$CONH_2$	-2.18	-1.26
C_6H_5	1.90	

5.3 The Hydrophobic Fragmental Constant - f

Rekker investigated the reservations he had about the π system and found that he could replace the basic equations (5.iv and 5.v) by equations 5.vi and 5.vii respectively.

$$\log P(SX) = f(S) + f(X) \qquad 5.vi$$

$$\log P(S'X_1 X_2 \ldots X_n) = f(S') + \sum_1^n f(X_n) \qquad 5.vii$$

$$\text{or } \log P = \sum_1^n \underline{a}_n f_n$$

where f represents the hydrophobic fragmental constant, the lipophilicity contribution of a constituent part of a structure to the total lipophilicity, and $\underline{a}$ is a numerical factor indicating the incidence of a given fragment in the structure.

Thus:

1. $C_6H_5-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-C_6H_5$

2. $CH_3-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}-CH_2-O-\overset{\displaystyle O}{\overset{\|}{C}}-CH_3$

$$\text{Log } P(1) = 2f(C_6H_5) + f(CH_3) + f(CH_2) + f(CH)$$

$$\log P(2) = 4f(CH_3) + f(CH_2) + f(C) + f(COO)$$

The fact that CH_3 attached to C is not differentiated from CH_3 attached to COO and that the oxycarbonyl group (COO) is left intact and not split further into O and CO was deduced from later work. By utilising multiple regression analysis he obtained sets of hydrophobic fragmental constants.

5.3.1 Aliphatic Structures

Primary Set of f Values

Rekker selected a representative set of structures, including only directly measured octanol-water partition

values, and no pre-selection took place so that doubtful values were included until identified later as outliers.

Originally, eleven f values were computed from 128 log P values referring to 87 different structures distributed over 40 structural types.

The f values obtained were for: CH_3,CH_2,CH,NH_2,NH,N,C_6H_5, OH,O,COOH and COO. The phenyl group was included to prove or disprove any folding of a number of structures. Care was taken however to exclude structures in which C_6H_5 was connected directly with a functional group because any resonance interaction between the functional group and phenyl nucleus would enhance lipophilicity (see Table 9).

Since aliphatic organic structures always contain CH_3 and other hydrocarbon fragments, more values are available for these fragments and so standard deviations will be lower those of non-C fragments. Other important functional groups were omitted since there were few partitioning data on them and inclusion of the available data would have reduced the significance of the statistical data. A fairly high standard error of estimate was allowed, since to reduce it would have meant removal of an undesirable number of structures from the computation and would suggest an unduly high accuracy of partition measurement, unlikely since reproducibility of log P values between laboratories is difficult.

Therefore, by means of multiple regression analysis, a series of f values was obtained which when used to calculate

log P gave log P values which differed less from experimental values than those based on π values.

Amongst the outliers which were removed from the main data set, a systematic anomaly was observed which was proposed to be attributable to a proximity effect. Placed close together, two groups with distinctly electronegative properties (more or less hydrophilic in nature) will bind fewer water molecules from their surroundings than if each group was present alone. The resulting increase in lipophilicity is a function of both the distance between the two groups concerned and of their hydrophilic capacities, the former apparently being the more important.

A value of 0.80 ±0.08 for a 1C separation and 0.46 ±0.08 for a 2C separation was found to apply for the proximity effect. For 3C and higher separations the proximity effect disappears.

Allowance for the proximity effect in the regression analysis improved the significance levels of the f values.

Not all the outliers were capable of explanation, indicating that complete understanding of factors governing partitioning behaviour is yet to be attained.

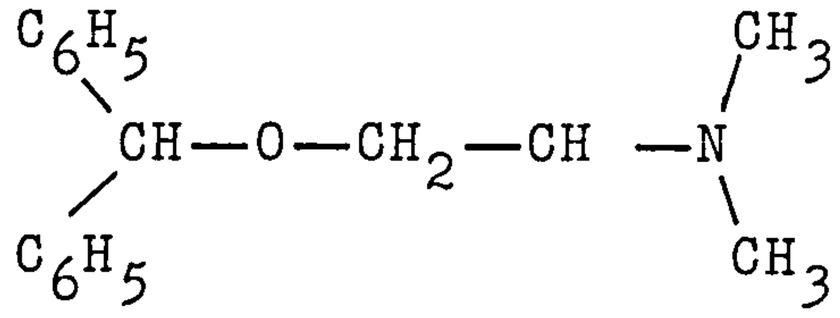
Secondary Set of f Values

A number of functional groups were excluded from the regression because of the lack of experimental data, but by using equation: $f(X) = \log P(SX) - \Sigma f(S)$ a secondary set of f values could be calculated. Here, f(X) is the hydrophobic constant of the desired fragment that forms part of structure SX and $\Sigma f(S)$ is the summation of the hydrophobic fragmental constants of the structure part S.

Lipophilicity and Folding

Using the values obtained above, there is no longer any need for a folding correction as applied by Hansch and Anderson (174).

i.e. Calculated from f values:



$\log P = 2f(C_6H_5) + f(CH) + f(O) + 2f(CH_2) + f(N) + 2f(CH_3) + pe2$

$= 3.792 + 0.236 + (-1.536) + 1.054 + (-2.133) + 1.404 + 0.46$

$= 3.28$

Calculated from Hansch and Anderson f values:

$\log P = 2f(C\ H\ ) + f(CH) + f(O) + 2f(CH\ ) + f(N) + 2f(CH\ )$

$+ 6b + 2cbr$

$= 3.80 + 0.43 + (-1.81) + 1.32 + (-2.16) + 1.78$

$+ (-0.72) + (-0.26)$

$= 3.38$

Experimental values = 3.27 and 3.40 (71)

The correction applied for folding by Hansch can be accounted for by the average difference between π and f values for individual fragments, plus the difference between $\log P(C_6H_6)$ and $f(C_6H_5)$.

5.3.2 Aromatic Structures

Calculation of log P of simple alkyl-substituted benzenes from f values previously derived shows good agreement with experimental values, indicating that in the f system, aliphatic and aromatic carbon fragments need not be differentiated. Application of the 'phenoxyacetic acid'

π values as recommended by Fujita et al (145) shows marked deviation from experimental values if branching occurs in the side-chain and use of aliphatic π values with branching corrections does not improve the situation although if the branching correction is omitted the agreement between calculated and experimental values improves.

By utilising the f value of C_6H_5, f(X), where X is a substituent on an aromatic ring can be calculated.

Monosubstituted Benzene Derivatives with the Functional Group Directly Connected to the Ring; Di- and Trisubstituted Benzene Derivatives

For the calculation of aromatic f values of functional groups, the initial set of compounds included mono-, di-para- and di-meta-substituted derivatives. Di-ortho-substituted benzene derivatives were all excluded as it was assumed that the accompanying steric factors would interfere with the results of the regression analysis.

The initial investigation revealed:-

1. for $f(C_6H_5)$ a value of 1.880 was obtained which agreed well with 1.896 obtained for C_6H_5 attached to aliphatic hydrocarbon fragments.
2. the value for f(COOH) did not differ much from zero.
3. meta- and para-derivatives did not show different behaviour.
4. the values obtained could be successfully applied to many di-ortho-substituted derivatives.

For the final regression analysis therefore, the set was increased to include di-ortho- and tri-substituted derivatives.

Thus a primary set of aromatic f values was produced, as shown in Table 14.

Table 14. Primary Set of Aromatic 'f' Values

Fragment	f	
F	0.412	
Cl	0.943	
Br	1.168	
I	1.460	
OH	-0.359	
O	-0.454	
NH_2	-0.897	
NO_2	-0.077	
OCH_2COOH	-0.588	
(ar)OCONH(al)	-1.370	
(ar)CO(al)	-0.869	
SO_2NH_2	-1.506	
$CONH_2$	-1.120	
C_6H_3	1.440	
C_6H_4	1.719	
C_6H_5	1.90	} Pre-fixed values
COOH	0.00	

Several outliers existed, possible reasons for their different behaviour being that suppression or reinforcement of mesomeric and/or inductive effects by (or in) the substituting group is operative. In addition, steric factors and hydrogen-bonding are known to play an important role in ortho-substituted structures.

Secondary Set of Aromatic 'f' Values

As in the aliphatic series, a number of functional groups belong to structures only occasionally examined in partition experiments. Thus a secondary set of aromatic f values was derived. Any additional information which could improve the statistical reliability of a secondary value could promote that value to primary status. (Table 15)

Table 15. Secondary Set of Aromatic 'f' Values

Fragment	f
COO	-0.43
NH	-0.93
N	-1.06
CF_3	1.25
CN	-0.23
SH	0.62
S	0.11
SO	-2.05
SO_2	-1.87

Differences Between Aliphatic and Aromatic f Values

Leo et al (257) pointed out the differences between aliphatic and aromatic structures in lipophilic behaviour and accounted for such differences by the definition of the substituent constant and consequential construction of the π system.

In the Rekker system, all C_6H_5 were equalised with each other, no matter what the character of the substituent on the ring. However, the lipophilic behaviour of the major functional groups shows that all indicate increased lipophilicity of the aromatic structure. In a number of cases this increase is not significant, but for groups such as COOH,O,N,OH and NO_2 significance is clear.

Leo et al (257) assumed that the relatively low aromatic $\pi(NH_2)$ value results from the better hydrogen bonding of the two hydrogen atoms, thus increasing the affinity of the aromatic NH_2 group for the water phase. Removal of these hydrogen atoms will offset this effect, so that the behaviour of a tertiary N approaches normal. The characteristics of OH and —O—(al), however, are not consistent with this reasoning.

Differences Between Aromatic π and f Values

For a set of six common substituents, the mean difference between f and π is expressed by:

$$f(X) = \pi(X) + f(H)$$

the difference 0.25 can be said to agree with the f(H) value. Inaccuracies in π can account for fluctuations in the difference between f and π. The π system, originally derived for aromatics, consists of eight different systems. Variations in the π values are greatest when two groups with strong mutual interactions are placed together on the aromatic ring e.g. NO_2—NH_2 and NO_2—OH. Fujita et al (144,145) attempted to account for these variations by including additional electronic parameters in the equations, but this was not altogether successful. Hansch et al (255) in subsequent studies used π values obtained from the phenoxy-acetic acid system as these values were considered to be a good compromise between those from electron-withdrawing and electron-releasing systems.

Rekker's system can also be extended to include condensed ring systems and hetero-atomic structures. This will not be discussed in detail here since none of the compounds investigated is of this type. However, the system used is the same as previously described, suitable fragmental constants being derived from multiple regression analysis and then used to build up the structure desired.

Lipophilicity of H Attached to an Electron-Withdrawing Centre

The value of f(H) has been stated to be about 0.20. However, it appears that this value must not be applied to a hydrogen atom which is attached to an electron-withdrawing centre such as C=O, COOH, COOR and CON. Examples quoted by Rekker (325) indicate that H atoms attached to these centres should be included in calculations with f = 0.47.

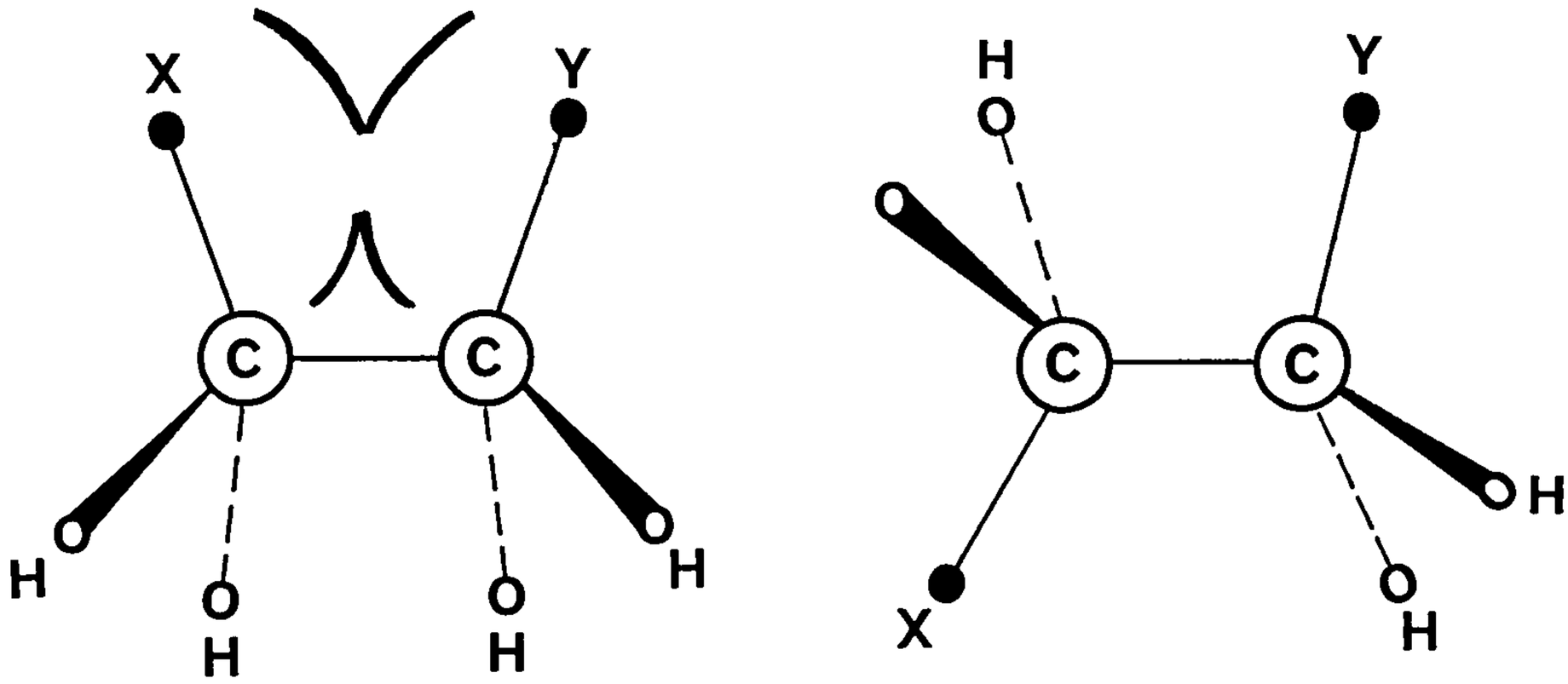
Proximity Effect

As stated earlier, in the presence of certain functional groups separated from each other by one or two carbon atoms, enhanced lipophilicity is observed. This increase is 0.80 ±0.08 for a IC separation and 0.46±0.08 for a 2C separation. For 3C and higher separations, the proximity effect disappears or lies below the limit of detectability (Δ0.20).

Initially it was thought that the proximity effect would be due to the fact that hydrophilic groups in the closest possible connection would lose part of their hydration mantle or perhaps even share it to a certain extent. This assumption presents no problems for structures such as $X—CH_2—Y$ since X and Y lie within each others hydration peripheries. However, with a structure such as $X—CH_2—CH_2—Y$, an eclipsed conformation would be essential to bring X and Y close enough together, when in practise a staggered conformation is common. Figure 22.

It is also notable that in benzene derivatives substituted with an electronegative group in ortho positions the proximity effect does not operate although there is a 2C separation.

Figure 22. Eclipsed and Staggered Conformations of $X—CH_2—CH_2—Y$

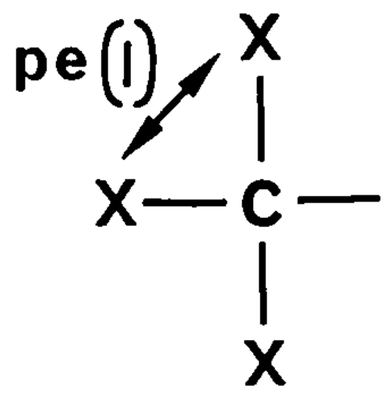


Eclipsed Staggered

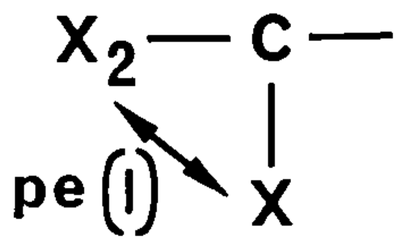
The eclipsed conformation is shown with an indication of the intersecting hydration peripheries.

Thus there is no specific, reliable measure of the proximity effect for structures in which there are several electronegative centres with separations of one and/or two carbon atoms. However, the following guidelines have been recommended.

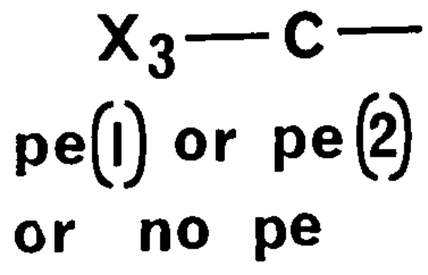
A.



B.



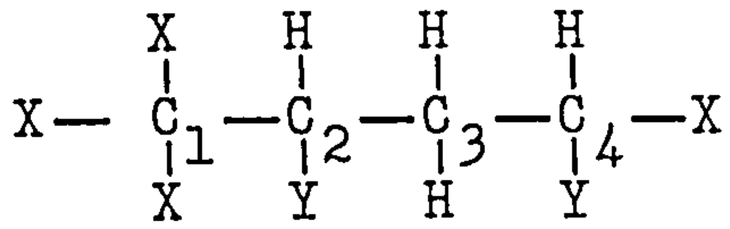
C.



In A, the two X atoms indicated by ⟷ are separated by 1C atom, for which 1 x pe(1) should be assigned. In B, these two X atoms are combined to X_2 and this X_2 combination is

again separated from the remaining X by 1C atom. Therefore, 1 x pe(1) is assigned again. The X_3 combination C is considered as a whole relative to what is attached to the remaining C valencies: either pe(1), pe(2) or zero, depending on the substitution pattern.

General example:



where X and Y are electronegative groups.

$$\text{Log P} = f(C) + 2f(CH) + f(CH_2) + 4f(X) + 2f(Y)$$
$$+ 2pe(1) \quad (\text{effect around } C_1)$$
$$+ 1pe(1) \quad (\text{effect around } C_4)$$
$$+ 1pe(2) \quad (\text{effect over } C_1—C_2)$$

When the value $f(CX_3)$ is available the calculation is simplified to:

$$\text{Log P} = f(CX_3) + 2f(CH) + f(CH_2) + f(X) + 2f(Y)$$
$$+ 1pe(1) \quad (\text{effect around } C_4)$$
$$+ 1pe(2) \quad (\text{effect over } C_1—C_2)$$

'Magic Constant' and 'Key Number'

In the calculations of the f constants, it was sometimes necessary to use certain correction terms. When listed, all of these corrections appear to share a greatest common divisor of 0.28. (Table 16.)

The frequent recurrence of the value 0.28, or a multiple of it, suggests that this figure could have major significance in partitioning.

Table 16. The Constant 0.28

Source	Value
proximity effect	
(2C)	2 x 0.23
(1C)	3 x 0.27
H attached to a negative group	Δ = 0.27
condensed aromatic units	0.31
Ar-Ar conjugation	0.28
cross-conjugation	0.28
aromatic-aliphatic differences	3 x 0.28
incorrectly presumed folding	2 x 0.29
abnormal proximity correction for phenoxyacetic acid	Δ = 2 x 0.23

The value 0.28 has therefore been designated by Rekker the 'magic constant', symbolized by C_M. The number that indicates how many times the factor 0.28 is operating in a given instance is designated the 'key number'. The key numbers of certain fragments are given in Table 17. Consideration of these factors allows calculation of log P.

Table 17. Hydrophobic Fragmental Constants (Rekker)

Fragment	f(Oct-Water)	Key Number	f(Cycl-Water)
C_6H_5	1.886	0	2.312
C_6H_4	1.688	0	2.123
C_6H_3	1.431	0	1.881
CH_3	0.702	0	0.805
CH_2	0.530	0	0.646
CH	0.235	0	0.397
H	0.175	0 or 1[a]	
(ar)COOH	-0.093	3*	
(ar)CO	-0.842	3*	
(ar)O	-0.433	4*	-0.754
(ar)OH	-0.343	4*	-3.157
(ar)COH	-0.380		-1.058
(ar)Cl	0.922	3*	0.856
(ar)NH_2	-0.854	2*	-2.301
(ar)NO_2	-0.939	3	-0.578
(ar)NH	-0.964	3*	
C_M	0.287		

Key to Table 17.

a - Depending on whether H is attached to a 'neutral' C (key number = 0) or to an electronegative centre, for instance N,C=O (key number = 1)

* - key number already included in numerical value of f.

In going from the octanol-water system to a system where the organic phase contains less water at saturation, the positive f values tend to become even more positive and the negative f values even more negative, in other words, there is a distinctly greater discriminative effect.

5.4 Correlation Between Log P Values in Different Solvents

Hydrophobicity depends heavily on the structure of water and thus there is a linear correlation between the log P values of compounds between various solvent systems. (77,256) For compounds partitioned between water and various alcohols, an average correlation coefficient of 0.99 with a standard deviation of estimate of 0.132 was observed between the octanol-water log P and that between oleyl alcohol, primary butanols, secondary and tertiary pentanols, cyclohexanol, or primary pentanols and water. There was also a linear statistically significant relationship between the octanol-water log P and any other solvent-water log P for which enough compounds had been measured to evaluate such a relationship.

For solvents which do not contain a polar group (cyclohexane) the log P values of 'hydrogen bond donors' and 'hydrogen bond acceptors' are correlated by significantly different equations. (256) Within each type of compound however,

there is an almost perfect correlation between the octanol-water log P and that of the other solvent-water system. Hansch and Dunn (402) reported that for a series of phenols the difference between the octanol-water log P and that between cyclohexane-water is quantitatively explained by hydrogen bonding.

Seiler (338) studied this problem by means of a regression analysis of the difference between the octanol-water and cyclohexane-water log P values of 230 compounds. He found that this difference is totally explained by the substituents present in the molecules. Accordingly, a new additive-constitutive substituent constant I_H is defined:

$$I_H = \log P_{octanol} - \log P_{cyclohexane} - 0.16$$

The intercept of -0.16 is the result of the regression analysis. Values of I_H are listed in Table 18. The value of I_H for a substituent seems to be proportional to the hydrogen bonding ability of the substituent. Compounds which are better hydrogen bond formers have a relatively higher log P in octanol-water than in cyclohexane-water.

Leo and Hansch (256) also proposed an equation relating the difference between the octanol-water and cyclohexane-water log P values. They found that one equation was not adequate for both 'hydrogen bond donor' and 'hydrogen bond acceptor' solutes and so after having divided the solutes into groups of donors and acceptors, equations A and B were derived for the cyclohexane-water system :

A. $\log P_{cyclohexane} = 0.675 \log P_{octanol} - 1.842$

$n = 26 \quad r = 0.761 \quad s = 0.503$

B. $\log P_{cyclohexane} = 1.063 \log P_{octanol} - 0.734$

$n = 30 \quad r = 0.957 \quad s = 0.360$

Thus, of the compounds studied in this thesis, acids, phenols and amides are correlated with equation A (H donors) and ketones and compounds with intramolecular hydrogen bonds (e.g.o-NO_2phenol) are correlated with equation B (H acceptors).

These two methods of calculating log P in the cyclohexane-water system were used to give approximate values of log P which were used to estimate the phase volume ratios necessary for the thermodynamics experiment. (Chapter 9) The results are shown in Table 19 and Table 20.

Table 18. Substituent Constants for the Difference Between Log P octanol and Log P cyclohexane

Substituent	I_H
—COOH (aliphatic)	2.88
—COOH (aromatic)	2.87
—OH (aromatic)	2.60
—CONH—	2.56
—OH (aliphatic)	1.82
—NH_2 (aliphatic)	1.33
—NH_2 (aromatic)	1.18
—NHR, R not H	0.61
—NO_2	0.45
C=O	0.31
—O—	0.11
Substituent ortho to —OH,—COOH	-0.62
Per unit change in pK of phenols	0.30
Per unit change in pK of anilines	0.16
Per unit change in pK of —COOH	0.00
Cl	0.00

Table 19. Partition Coefficients in the Cyclohexane-Water System. Calculated from I_H Values

Compound	Calc. from Octanol LogP Shake Flask	Calc. from Octanol LogP Filter Probe	Exp.
Phenol	-1.27	-1.30	-0.74
o-Clphenol	-0.02	-0.09	+0.82
m-Clphenol	-0.28	-0.37	-0.05
p-Clphenol	-0.35	-0.45	-0.30
o-NO_2phenol	-0.68	-0.68	+1.40
m-NO_2phenol	-1.04	-1.15	-1.57
p-NO_2phenol	-1.12	-1.17	-1.90
2-NO_2resorcinol	-3.11	-3.04	+1.02
o-OHbenzaldehyde	-0.78	-0.81	+1.38
m-OHbenzaldehyde	-1.75	-1.69	-1.96
p-OHbenzaldehyde	-1.72	-1.69	-2.29
Benzoic acid	-1.15	-1.24	-0.85
o-OHbenzoic acid	-2.66	-2.79	-1.42
m-OHbenzoic acid	-3.94	-4.12	---
p-OHbenzoic acid	-4.06	-4.13	---
2,6-OH_2benzoic acid	-5.34	-5.34	-3.35
3,5-OH_2benzoic acid	-7.74	-7.19	---
3-Me-2-NO_2phenol	-0.44	-0.44	+1.32
4-Me-2-NO_2phenol	-0.34	-0.37	+1.99
5-Me-2-NO_2phenol	-0.24	-0.27	+2.66
6-Me-2-NO_2phenol	+0.55	+0.59	+1.95
o-Mephenol	-0.15	-0.28	+0.15
m-Mephenol	-0.80	-0.86	-0.16
p-Mephenol	-0.74	-0.84	-0.15
2,3-Me_2phenol	+0.19	+0.13	+0.49
2,4-Me_2phenol	+0.19	+0.18	+0.66
2,5-Me_2phenol	+0.19	+0.17	+0.72
2,6-Me_2phenol	+0.70	+0.72	+0.97
3,4-Me_2phenol	-0.51	-0.54	+0.21
3,5-Me_2phenol	-0.39	-0.46	+0.26
2,3,5-Me_3phenol	+0.57	+0.54	+1.08
2,3,6-Me_3phenol	+1.02	+1.o8	+1.72
2,4,6-Me_3phenol	+1.09	+1.07	+1.69
2,3,5,6-Me_4phenol	+1.30	+1.24	+1.79
o-Mebenzoic acid	-0.37	+0.09	+0.98
m-Mebenzoic acid	-0.44	-0.36	+0.36
p-Mebenzoic acid	-0.75	-0.70	-0.53
2,6-Me_2benzoic acid	-0.14	+0.23	-0.98
3,5-Me_2benzoic acid	-0.17	-0.19	+0.06
Acetanilide	+0.07	+0.15	-1.37
o-Meacetanilide	+0.43	+0.41	-1.25
m-Meacetanilide	+0.54	+0.52	-0.99
p-Meacetanilide	+0.54	+0.53	-0.90
2,6-Me_2acetanilide	+1.12	+1.15	-1.48
3,5-Me_2acetanilide	+1.00	+0.95	-0.31

Table 20. Partition Coefficients in the Cyclohexane-Water System Estimated from Leo-Hansch Regression Equations

Compound	Reg Eqn	Calc. from Octanol LogP Shake Flask	Calc. from Octanol LogP Filter Probe	Exp.
Phenol	A	-0.84	-0.86	-0.74
o-Clphenol	A	-0.41	-0.46	+0.82
m-Clphenol	A	-0.17	-0.23	-0.05
p-Clphenol	A	-0.22	-0.28	-0.30
o-NO_2phenol	B	+1.13	+1.13	+1.40
m-NO_2phenol	A	-0.49	-0.56	-1.57
p-NO_2phenol	A	-0.53	-0.57	-1.91
2-NO_2resorcinol	B	+0.85	+0.89	+1.02
o-OHbenzaldehyde	B	+1.04	+1.01	+1.38
m-OHbenzaldehyde	A	-0.95	-0.91	-1.96
p-OHbenzaldehyde	A	-0.93	-0.91	-2.29
Benzoic acid	A	-0.57	-0.63	-0.85
o-OHbenzoic acid	B	+1.77	+1.63	-1.42
m-OHbenzoic acid	A	-0.70	-0.82	---
p-OHbenzoic acid	A	-0.78	-0.83	---
2,6-OH_2benzoic acid	B	+1.02	+1.02	-3.35
3,5-OH_2benzoic acid	A	-1.51	-1.34	---
3-Me-2-NO_2phenol	B	+1.55	+1.55	+1.32
4-Me-2-NO_2phenol	B	+1.66	+1.63	+1.99
5-Me-2-NO_2phenol	B	+1.77	+1.73	+2.66
6-Me-2-NO_2phenol	B	+1.95	+1.88	+1.95
o-Mephenol	A	-0.50	-0.59	+0.15
m-Mephenol	A	-0.52	-0.56	-0.16
p-Mephenol	A	-0.48	-0.54	-0.15
2,3-Me_2phenol	A	-0.27	-0.31	+0.49
2,4-Me_2phenol	A	-0.27	-0.27	+0.66
2,5-Me_2phenol	A	-0.27	-0.28	+0.72
2,6-Me_2phenol	A	-0.34	-0.33	+0.97
3,4-Me_2phenol	A	-0.32	-0.35	+0.21
3,5-Me_2phenol	A	-0.24	-0.29	+0.26
2,3,5-Me_3phenol	A	-0.01	-0.04	+1.08
2,3,6-Me_3phenol	A	-0.13	-0.14	+1.72
2,4,6-Me_3phenol	A	-0.08	-0.10	+1.69
2,3,5,6-Me_4phenol	A	+0.04	+0.02	+1.79
o-Mebenzoic acid	A	-0.57	-0.34	-0.38
m-Mebenzoic acid	A	-0.28	-0.23	+0.36
p-Mebenzoic acid	A	-0.30	-0.27	-0.53
2,6-Me_2benzoic acid	A	-0.73	-0.48	-0.98
3,5-Me_2benzoic acid	A	+0.09	+0.08	+0.06
Acetanilide	A	-1.06	-1.01	-1.37
o-Meacetanilide	A	-1.24	-1.26	-1.25
m-Meacetanilide	A	-0.75	-0.76	-0.99
p-Meacetanilide	A	-0.75	-0.76	-0.90
2,6-Me_2acetanilide	A	-1.19	-1.18	-1.47
3,5-Me_2acetanilide	A	-0.43	-0.48	-0.31

5.5 Discussion of the Methods

Despite the attempt to rationalize log P calculation by means of fragments and factors, the original hydrophobic substituent constant, π, remains the simplest method of calculating log P. It is also reasonably accurate and produces good agreement with experimental values, particularly for simple aromatic compounds. However, Table 10 shows that π for any given function varies with the solute so that accuracy appears to be difficult to attain. Fortunately, the range over which π varies with environment is not great, with the deviation from normal occurring when two strongly interacting groups such as nitro and amino are placed on the ring together. For inert groups such as alkyl (CH_3), the values are essentially constant from system to system as well as in the meta and para positions. However, this last statement has highlighted one area where agreement is difficult to obtain, that of substitution in the ortho position, such that prediction of log P for many compounds is still difficult.

Table 21. π from Benzene System

		Observed	Calculated
3Cl	4Cl	1.25	1.42
3Cl	4OH	0.02	0.04
$3CH_3$	$4CH_3$	0.99	1.12
$3CH_3$	$4NO_2$	0.17	0.28
$3CH_3$	4Cl	1.29	1.27
$3CH_3$	4OH	-0.18	-0.11
$3NO_2$	4OH	-0.34	-0.95
$3NO_2$	5OH	-0.13	-0.95
3Cl	5OH	0.37	0.04
$3CH_3$	5OH	-0.17	-0.11
$1,3,5(CH_3)_3$		1.29	1.68
$1.3(OH)_2 2NO_2$		-0.57	-1.62

The most pronounced effects occur when strong electron-withdrawing groups are placed on the ring with groups having lone pair electrons (—OH,—NH_2). The NO_2 function or even halogen functions when combined with —OH and —NH_2, give higher than expected π values. (see Table 21)

The variation in π derived from different systems means that more than one set of values is necessary, but fortunately, since the variation between most systems is relatively small, three sets of values (possibly: π_n, π^-, and π^+) should suffice for most analyses necessary in correlations of biological activity with chemical structure. π_n constants are those derived from benzene (normal values); the π^- constants would be those from phenols, needed when strong electron-releasing groups such as —OH,—NH_2,—NHR or —NR_2 are attached to a conjugated system; and the π^+ constants would be necessary when strong electron-attracting groups such as cyano or nitro are conjugated with the variable function.

Since the study compounds were largely substituted phenols, log P values were calculated from the π^- system (Table 11) as follows:

$$\log P_{\text{o-Mephenol}} = \log P_{C_6H_5} + \pi_{Me} + \pi_{OH}$$

$$= 1.96 + 0.56 + (-0.67)$$

$$= 1.85$$

$$\text{Observed log P} = 1.90$$

Table 22 shows the calculated log P's of the study compounds and reasonable agreement occurs between calculated and experimental values provided no interaction occurs between

Table 22. Calculated Log P - Octanol-Water System

Compound	π	f(Rekker)	f(Leo)	Exp.
Phenol	1.29	1.54	1.50	1.49
o-Clphenol	1.98	2.30	2.21	2.12
m-Clphenol	2.33	2.30	2.21	2.45
p-Clphenol	2.22	2.30	2.21	2.36
o-NO_2phenol	1.01	1.28	1.25	1.75
m-NO_2phenol	1.83	1.28	1.25	2.00
p-NO_2phenol	1.79	1.28	1.25	1.91
2-NO_2resorcinol	0.35	0.92	1.62	1.50
o-OHbenzaldehyde	0.64	0.95	2.06	1.66
m-OHbenzaldehyde	0.64	0.95	1.06	1.35
p-OHbenzaldehyde	0.64	0.95	1.06	1.35
Benzoic acid	1.64	1.80	1.87	1.87
o-OHbenzoic acid	0.97	1.54	2.24	2.30
m-OHbenzoic acid	1.26	1.54	1.24	1.60
p-OHbenzoic acid	1.34	1.54	1.24	1.55
2,6-OH_2benzoic acid	0.30	1.18	1.61	1.65
3,5-OH_2benzoic acid	0.88	1.18	0.61	0.80
3-Me-2-NO_2phenol	1.57	1.71	2.91	2.15
4-Me-2-NO_2phenol	1.58	1.71	2.91	2.24
5-Me-2-NO_2phenol	1.53	1.71	2.91	2.33
6-Me-2-NO_2phenol	1.57	1.71	2.91	2.54
o-Mephenol	1.85	2.07	2.16	1.90
m-Mephenol	1.85	2.07	2.16	1.93
p-Mephenol	1.85	2.07	2.16	1.98
2,3-Me_2phenol	2.41	2.49	2.82	2.30
2,4-Me_2phenol	2.41	2.49	2.82	2.32
2,5-Me_2phenol	2.41	2.49	2.82	2.32
2,6-Me_2phenol	2.41	2.49	2.82	2.23
3,4-Me_2phenol	2.41	2.49	2.82	2.24
3,5-Me_2phenol	2.41	2.49	2.82	2.34
2,3,5-Me_3phenol	2.97	3.02	3.48	2.70
2,3,6-Me_3phenol	2.97	3.02	3.48	2.56
2,4,6-Me_3phenol	2.97	3.02	3.48	2.60
2,3,5,6-Me_4phenol	3.53	3.54	4.14	2.77
o-Mebenzoic acid	2.20	2.30	2.53	2.10
m-Mebenzoic acid	2.16	2.30	2.53	2.35
p-Mebenzoic acid	2.06	2.30	2.53	2.30
2,6-Me_2benzoic acid	2.76	2.82	3.19	1.82
3,5-Me_2benzoic acid	2.72	2.82	3.19	2.85
Acetanilide	0.72	0.79	1.20	1.20
o-Meacetanilide	1.28	1.48	1.86	0.88
m-Meacetanilide	1.28	1.48	1.86	1.61
p-Meacetanilide	1.28	1.48	1.86	1.61
2,6-Me_2acetanilide	1.84	1.73	2.52	0.97
3.5-Me_2acetanilide	1.84	1.73	2.52	2.06

substituents. Thus ortho compounds show marked disagreement and indeed, when any strong electron interaction occurs between substituents (strong electron donating and strong electron attracting substituents on the same ring) simple additivity fails.

e.g. $\log P_{\text{o-OHbenzoic acid}} = \log P_{C_6H_5} + \pi_{COOH} + \pi_{OH}$

$= 1.96 - 0.32 - 0.67$

$= 0.97$

Observed logP = 2.30

In this case, intramolecular hydrogen bonding between the 2-OH and COOH groups increases log P, as well as that produced by other possible proximity effects.

The π method of calculating log P therefore has the major limitation that it is not suitable for ortho-substituted compounds unless a separate set of ortho π values is available. It is therefore necessary to understand interactions between functional groups of a set of congeners before attempting to calculate log P so that the correct π system is used and also to assess the reliability of the calculated values. Multi-substitution may present problems since more than one parent molecule is possible. The phenoxyacetic acid π values may be chosen as a good compromise between electron-withdrawing and electron-releasing factors.

Rekker considered that the π system imposed serious restrictions on accuracy since no allowance was made for any contribution to lipophilicity by the hydrogen atom. The hydrophobic fragment method is essentially very straight-forward since it involves breaking the molecule down into

constituent fragments and finding the contribution to lipophilicity of each fragment. Unknown log P's can then be calculated by summing constitutive fragments of the test molecule. However, again, interactions within the molecule cause anomalies so a set of corrective constants was introduced which included a correction for the proximity effect and the magic constant, as has been previously discussed. Log P values calculated from the hydrophobic fragmental constant of the study compounds are shown in Table 22. Again, agreement with experimental values is excellent for certain compounds, but interactions within the molecule cause additivity to fail.

Rekker also investigated many other solvent systems, including cyclohexane-water and he has produced a set of fragmental constants for this system. (Table 17) The calculated log P's of the study compounds are given in Table 23 and again accuracy depends on the interactions within the molecule. If ortho-effects are ignored, agreement with experimental values is good considering that the f values have been calculated from a restricted number of compounds. The percentage of error emphasises the need for actual measurement of log P for precise work, but the calculated value is useful for experimental work since it allows solvent volume ratios to be predicted which in itself saves much time and effort.

This method has the advantage of simplicity. The method of calculation is fairly straightforward and can be mastered reasonably quickly.

Table 23. Calculated Log P - Cyclohexane-Water System

Compound	f(Rekker)	Exp.
Phenol	-0.84	-0.74
o-Clphenol	-0.18	+0.82
m-Clphenol	-0.18	-0.05
p-Clphenol	-0.18	-0.30
o-NO_2phenol	-1.61	+1.40
m-NO_2phenol	-1.61	-1.57
p-NO_2phenol	-1.61	-1.90
2-NO_2resorcinol	-5.01	+1.02
o-OHbenzaldehyde	-2.09	+1.38
m-OHbenzaldehyde	-2.09	-1.96
p-OHbenzaldehyde	-2.09	-2.29
Benzoic acid	---	-0.85
o-OHbenzoic acid	---	-1.42
m-OHbenzoic acid	---	---
p-OHbenzoic acid	---	---
2,6-OH_2benzoic acid	---	-3.35
3,5-OH_2benzoic acid	---	---
3-Me-2-NO_2phenol	-0.81	+1.32
4-Me-2-NO_2phenol	-0.81	+1.99
5-Me-2-NO_2phenol	-0.81	+1.74
6-Me-2-NO_2phenol	-0.81	+1.95
o-Mephenol	-0.23	+0.15
m-Mephenol	-0.23	-0.16
p-Mephenol	-0.23	-0.15
2,3-Me_2phenol	+0.33	+0.49
2,4-Me_2phenol	+0.33	+0.66
2,5-Me_2phenol	+0.33	+0.72
2,6-Me_2phenol	+0.33	+0.97
3,4-Me_2phenol	+0.33	+0.21
3,5-Me_2phenol	+0.33	+0.26
2,3,5-Me_3phenol	+1.14	+1.08
2,3,6-Me_3phenol	+1.14	+1.72
2,4,6-Me_3phenol	+1.14	+1.69
2,3,5,6-Me_3phenol	+1.94	+1.79
o-Mebenzoic acid	---	+0.98
m-Mebenzoic acid	---	+0.36
p-Mebenzoic acid	---	-0.53
2,6-Me_2benzoic acid	---	-0.98
3,5-Me_2benzoic acid	---	+0.06
Acetanilide	---	-1.37
o-Meacetanilide	---	-1.25
m-Meacetanilide	---	-0.99
p-Meacetanilide	---	-0.90
2,6-Me_2acetanilide	---	-1.48
3,5-Me_2acetanilide ,	---	-0.31

Leo et al took Rekker's method a stage further by reducing the whole molecule to even more basic fragments i.e.H and C, and then applying factors to account for the structural relationships between the fragments. This system was designed for use with computers and is in fact very cumbersome to manipulate by hand, as illustrated by the calculation of log P for CCl_4 :

$$CCl_4 = f(C) + 4f(Cl_{al}) + 3(\text{alk chain bonds}) + 4(\text{Gem hal})$$
$$= 0.20 + 4(0.06) + 3(-0.12) + 4(0.70)$$
$$= 2.88$$

Observed = 2.83

The use of correction factors attempts to account for interactions within the molecule and to a certain extent this has been achieved since this is the only method which produces reasonable agreement with experimental values for ortho compounds. However, Table 22 shows that in general the agreement between calculated and experimental values is not as good as for either the π system or Rekker's f system. Rekker calculated his fragment constants from a large number of data which may have statistically reduced errors and produced a more accurate system.

One problem with the calculation methods described is that of the approach to anomalies. In each case a numerical deviation has been observed and a constant introduced to adjust the partition coefficient without reference to the cause of the anomaly. This thesis will attempt to rationalise

the interactions producing the anomaly since in many cases combinations of interacting factors will complicate the choice of correction constant.

Equations for calculating log P for solvent pairs other than octanol-water have also been discussed. Most experimental work with π and fragmental constants has been conducted in the octanol-water system so constants for other systems are limited. However, a linear relationship was observed between log P values in different solvents which allowed log P's in different systems to be calculated from the octanol log P. The results from the two methods discussed are shown in Table 19 and Table 20 . Comparison with experimental values shows reasonable agreement but again, interactions within the molecule cause anomalies which cannot be accounted for by the conversion factors. The Leo-Hansch Regression equations show greater agreement with experimental values than do I_H estimations. A further factor which has to be considered when converting partition coefficients from one solvent to another is that interactions between the solvent and solute may alter the intramolecular interactions and thus affect the solvent log P relationship.

5.6 Hydrophobic Substituent Constants

In order to investigate the anomalies in partition coefficient calculation it is necessary to identify their causes. Since partition coefficients are calculated by the summation of constitutive fragments or groups, then the anomaly must be present in the contribution made by one or more of those groups or in the interaction between them i.e. the proximity

effect. Thus it is necessary to ascertain the contribution made by each group in various positions within the molecule. Therefore substituent constants have been calculated from the experimental partition coefficients obtained for the study compounds. The compounds were chosen for their simple structure so that it could be assumed that changes in partition coefficient which occurred after the addition or removal of a group were due solely to the influence of that group on the molecule and its interaction with the solvent. Calculations of hydrophobic substituent constants were made in both octanol and cyclohexane.

Octanol

Table 24 shows hydrophobic substituent constants for the octanol-water system. These constants are compared with literature values and agreement can be seen to be good for Cl, NO_2, and Me but some discrepancy is apparent in the values for CHO and COOH. In this sort of comparison it must be remembered that literature values are derived from analysis of many compounds and represent an average, whereas the values found in the present work are obtained from a single compound so slight variations may be expected.

The study compounds however provide figures for ortho-substituents which are not always available in the literature.

Substituted phenols were the compounds chosen for investigation so the following observations refer to substitution with respect to the phenolic OH.

Table 24. Hydrophobic Substituent Constants Derived From the Octanol-Water System

Parent Compound: Phenol

Substituent	Log P	π	π(Literature)
p-H	1.49	0.00	0.00
o-Cl	2.12	+0.64	+0.69
m-Cl	2.48	+0.99	+1.04
p-Cl	2.41	+0.92	+0.93
o-NO_2	1.75	+0.26	---
m-NO_2	2.01	+0.52	+0.54
p-NO_2	1.94	+0.45	+0.50
o-CHO	1.67	+0.18	+0.24
m-CHO	1.32	-0.17	-0.08
p-CHO	1.35	-0.14	-0.06
o-COOH	2.35	+0.87	---
m-COOH	1.69	+0.20	+0.04
p-COOH	1.57	+0.09	+0.12
o-Me	1.99	+0.51	+0.49
m-Me	1.96	+0.47	+0.50
p-Me	2.02	+0.53	+0.48

Table 25. Hydrophobic Substituent Constants Derived From the Cyclohexane-Water System

Parent Compound: Phenol

Substituent	Log P	π
p-H	-0.74	0.00
o-Cl	+0.82	+1.55
m-Cl	-0.05	+0.68
p-Cl	-0.30	+0.44
o-NO_2	+1.40	+2.14
m-NO_2	-1.57	-0.84
p-NO_2	-1.91	-1.17
o-CHO	+1.38	+2.11
m-CHO	-1.96	-1.22
p-CHO	-2.29	-1.55
o-COOH	-1.41	-0.68
m-COOH	---	---
p-COOH	---	---
o-Me	+0.15	+0.89
m-Me	-0.16	+0.57
p-Me	-0.15	+0.59

It can be seen that the position of substitution has little effect on the lipophilicity of a single methyl group, producing an average π value of +0.50. Ortho-substitution of either the Cl or NO_2 group however causes a reduction in the lipophilicity of both groups. The Cl substituent causes an increase in lipophilicity of the molecule of +0.96 when in the meta- or para-position, but this is reduced to +0.64 when in the ortho-position. Similarly the NO_2 group increases lipophilicity by +0.48 in either the meta- or para-position, but by only +0.26 in the ortho position.

Conversely, the lipophilicity of both the COOH and CHO group is increased by ortho-substitution. Substitution of CHO in either the meta- or para-position causes a decrease in lipophilicity of the molecule of -0.16. In the ortho-position CHO produces an increased lipophilicity of +0.18. Ortho-substitution of COOH causes a large increase of +0.87. This is reduced to +0.15 if the COOH group is in either the meta- or para-position.

These molecules are all capable of forming hydrogen bonds both intra- and inter-molecular. Ortho-substitution of the groups mentioned allows the formation of intramolecular hydrogen bonds and these are responsible for the changes in lipophilicity of the molecule. However, it is seen that an intramolecular bond with the Cl or NO_2 groups causes a reduction in lipophilicity while intramolecular hydrogen bonding with the CHO or COOH group causes an increase in lipophilicity. This difference is not seen in cyclohexane and may be due to interactions with the solvent.

Intermolecular hydrogen bonding between solute and solvent is possible in octanol but not cyclohexane. This is discussed in Chapter 6 where the partition coefficients are discussed in greater detail.

In addition to looking at the π values for single groups in the ortho-, meta- or para-positions, Table 26 shows the π values obtained for methyl groups on different parent molecules and the effect of multi-substitution. It has already been seen that substitution on phenol whether in the ortho-, meta- or para-position does not alter significantly the lipophilic contribution of the methyl group, and a π value of +0.50 is obtained. This is in agreement with most published data which gives a π value of between 0.4 and 0.6 for an unhindered methyl or methylene group. However, π values for a methyl group in the ortho-position are much more varied, values reported ranging from -0.30 to +0.79, when the solute system is altered. This is confirmed by the present study which found that substitution of a methyl group in either benzoic acid or acetanilide in the ortho position causes decreased lipophilicity. In the case of benzoic acid, the methyl group still causes an overall increase in lipophilicity but by the reduced amount of +0.24 as compared with +0.44 for p-substitution. In the case of acetanilide a methyl group ortho to the $NHCOCH_3$ group causes a decrease in lipophilicity of -0.32 whereas a methyl group in the meta- or para- positions increases lipophilicity by +0.41. These figures also illustrate the variation in π caused by different solute systems since even in the meta- or para- positions the contribution to lipophilicity of the

Table 26. Hydrophobic Substituent Constant Values for Additional Methyl Groups in the Octanol-Water System

Parent Compound: Phenol

Substituent	Log P	π(Additional Me)			
o-Me	1.99	+0.50			
m-Me	1.96	+0.47			
p-Me	2.02	+0.53			
2,3-Me_2	2.30	+0.31	(3)	+0.34	(2)
2,4-Me_2	2.32	+0.33	(4)	+0.36	(2)
2,5-Me_2	2.32	+0.33	(5)	+0.36	(2)
2,6-Me_2	2.23	+0.27	(6)	+0.27	(2)
3,4-Me_2	2.24	+0.28	(4)	+0.22	(3)
3,5-Me_2	2.34	+0.38	(5)	+0.38	(3)
2,3,5-Me_3	2.70	+0.40	(5)	+0.38	(3) +0.36(2)
2,3,6-Me_3	2.56	+0.26	(6)	+0.33	(3)
2,4,6-Me_3	2.60	+0.28	(6)	+0.37	(4)
2,3,5,6-Me_4	2.77	+0.07	(6)	+0.20	(5)

Parent Compound: Benzoic Acid

Substituent	Log P	π(Additional Me)
p-H	1.86	0.00
o-Me	2.10	+0.24
m-Me	2.35	+0.49
p-Me	2.30	+0.44
2,6-Me_2	1.82	-0.30 (6)
3,5-Me_2	2.85	+0.52 (5)

Parent Compound: Acetanilide

Substituent	Log P	π(Additional Me)
p-H	1.20	0.00
o-Me	0.88	-0.32
m-Me	1.61	+0.41
p-Me	1.61	+0.41
2,6-Me_2	0.97	+0.09 (6)
3,5-Me_2	2.06	+0.45 (5)

Parent Compound: Orthonitrophenol

Substituent	Log P	π(Additional Me)
p-H	1.75	0.00
3-Me	2.15	+0.40
4-Me	2.24	+0.49
5-Me	2.33	+0.58
6-Me	2.54	+0.79

methyl group in benzoic acid or acetanilide appears to be less than in phenol.

Referring back to substituted phenol, Table 26 also shows the lipophilic contribution of additional methyl groups. These figures show that in a dimethyl phenol the second methyl group causes an increase in log P of +0.34 on average, although when two ortho substituents are present, as in 2,6-Me_2phenol, the lipophilicity of the second methyl group is reduced to +0.27, showing a possible steric effect. This effect is also seen in the trimethylphenols where 6-Me only contributes +0.26 to lipophilicity but 3-,4- or 5-Me contribute +0.37. This effect is probably due to steric shielding (112) and is further demonstrated by 2,3,5,6-Me_4phenol where the addition of 5-Me to 2,3,6-Me_3phenol increases log P by only +0.20 and the addition of 6-Me to 2,3,5-Me_3phenol increases log P by only +0.07. This illustrates that even with an otherwise inert group such as CH_3, a single π value cannot be used for multi-substitution since steric effects result in reduction of the lipophilic character of the additional groups.

The steric effect can be seen most markedly with methyl substituted acetanilide. The 2-methyl derivative is rather unusual in that the log P value is actually lower than that of the parent compound resulting in a negative π value of -0.32. This is close to the value of -0.20 obtained by Draber (117) for the same compound. This result may be explained in terms of a model consistent with that postulated to explain the ultraviolet spectra in the present study,

which was first used by Dearden and O'Hara (112). This was that a single ortho methyl group caused rotation about the $N\text{-}C_{(CO)}$ bond resulting in partial loss of conjugation between the nitrogen atom and the acetyl moiety. In this situation the lone pair electrons on the nitrogen atom will become more available for conjugation with the benzene ring, leaving the oxygen lone pair on the carbonyl group to become progressively more localised and more available for hydrogen bonding with the solvent. Although octanol is also a hydrogen bonding solvent, the effect would be assumed to be greater with water, leading to a reduced log P value. This particular effect seems to be unique to the acetanilides (304) and seems to depend on steric hindrance being able to induce a secondary effect; in this case increased electron localization.

An alternative method of viewing this effect is to consider that, with loss of coplanarity of the acetyl group and concomitant resonance interaction with the benzene ring, the acetyl group itself becomes more 'aliphatic' and thus will have a lower π value, contributing to the overall lowering in log P for the molecule.

The m-Me and p-Me derivatives however, have a π value of +0.41, which is much closer to the 'average' value of +0.50.

The $3,5\text{-}Me_2$ derivative again shows a π methyl value close to the expected value of +0.50. (117)

In the case of the $2,6\text{-}Me_2$ derivative, although the second ortho-methyl group increases lipophilicity it it by the much reduced amount of +0.09. This may be considered in

terms of rotation about the N-aryl bond in order to relieve steric strain which results in considerable loss of conjugation between the acetamido group and ring, leading to a chromophore approaching that of benzene. The evidence for this additional twisting in the case of 2,6-Me_2 substitution is given in the chapter on spectra and indicates loss of conjugation between the whole acetamido group and the ring, as compared with loss of conjugation between the acetyl and aniline groups in the case of 2-Me substitution. A partial contribution to the effect of diortho-methyl substitution may also be viewed as a case of shielding by the methyl groups preventing access by octanol to the acetamido moiety, also, since there is decreased conjugation of the ring and acetamido group, the increased localization of electrons on the heteroatoms may promote hydrogen bonding, particularly with water, and result in a lower than expected partition value.

The benzoic acids exhibit a similar ortho effect, with the meta-, para- and dimeta- methyl π values being close to the average value of +0.50, but the ortho-methyl group values being reduced to the extent that the second o-Me has a negative π value. This can be due to either shielding of the carboxylic acid group by the methyl groups or to steric hindrance causing twisting of the carboxylic acid group. Ultraviolet data indicates steric twisting which leads to greater solvent interaction, particularly with water, thus causing an additional decrease in log P, seen in the 2,6-Me_2 derivative where twisting is at a maximum.

The methyl ortho-nitrophenols do not demonstrate this relationship to the substituted methyl groups. In each case, the methyl group increases lipophilicity, with the 4-Me showing an expected π value of +0.49, but the 3-Me group having the slightly reduced value of +0.40. The ultraviolet data suggests that the 3-Me group weakens the intramolecular hydrogen bond and this π value supports that theory.

The methyl group in the 5- or 6- position however has an increased π value of +0.58 and +0.79 respectively. This indicates that from either of these positions the intramolecular hydrogen bond is strengthened. This is discussed in greater detail in Chapter 6.

Cyclohexane

Table 25 shows hydrophobic substituent constants for the cyclohexane-water system. In each group studied it can be seen that ortho substitution causes an increase in log P. The presence of an intramolecular hydrogen bond produces a large increase in the π value, indicating the increased solubility in cyclohexane allowed by the presence of the bond. The strength of the hydrogen bond seems to be reflected in the magnitude of the difference between π_o- and π_p- values. The difference is greater for the nitrophenols and hydroxybenzaldehydes than for the chlorophenols.

Table 27 shows the effect of methyl substitution in phenol. Here, shielding of the hydroxyl group, as in 2,6-Me_2phenol results in a higher π value than in the non-shielded case. This can be interpreted as simple steric hindrance to solvation by water favouring partition in the non-aqueous

Table 27. Hydrophobic Substituent Constant Values for Additional Methyl Groups in the Cyclohexane-Water System

Parent Compound: Phenol

Substituent	Log P	π(Additional Me)					
o-Me	+0.15	+0.99					
m-Me	-0.16	+0.68					
p-Me	-0.15	+0.69					
2,3-Me_2	+0.49	+0.34	(3)	+0.65	(2)		
2,4-Me_2	+0.66	+0.51	(4)	+0.81	(2)		
2,5-Me_2	+0.72	+0.57	(5)	+0.88	(2)		
2,6-Me_2	+0.97	+0.82	(6)	+0.82	(2)		
3,4-Me_2	+0.21	+0.37	(4)	+0.36	(3)		
3,5-Me_2	+0.26	+0.42	(5)	+0.42	(3)		
2,3,5-Me_3	+1.08	+0.59	(5)	+0.36	(3)	+0.82	(2)
2,3,6-Me_3	+1.72	+1.23	(6)	+0.75	(3)		
2,4,6-Me_3	+1.69	+1.03	(6)	+0.72	(4)		
2,3,5,6-Me_4	+1.79	+0.71	(6)	+0.07	(5)		

Parent Compound: Benzoic acid

Substituent	Log P	π(Additional Me)	
p-H	-0.85	0.00	
o-Me	+0.98	+1.83	
m-Me	+0.36	+1.21	
p-Me	-0.53	+0.37	
2,6-Me_2	-0.98	-0.13	(6)
3,5-Me_2	+0.06	-0.30	(5)

Parent Compound: Acetanilide

Substituent	Log P	π(Additional Me)	
p-H	-1.37	0.00	
o-Me	-1.25	+0.12	
m-Me	-0.99	+0.38	
p-Me	-0.90	+0.47	
2,6-Me_2	-1.48	-0.23	(6)
3,5-Me_2	-0.31	+0.70	(5)

Parent Compound: Ortho-Nitrophenol

Substituent	Log P	π(Additional Me)
p-H	+1.40	0.00
3-Me	+1.32	-0.08
4-Me	+1.99	+0.59
5-Me	+1.74	+0.34
6-Me	+1.95	+0.55

phase, since the relatively more hydrophobic, hydroxyl-shielded molecule will be more attracted to the hydrophobic cyclohexane phase than in the case of a molecule with a more dominant hydroxyl group.

Methyl substitution in benzoic acid produces results which are more difficult to explain, but as discussed in Chapter 6 it would appear that the two effects suggested by the octanol-water partition coefficients are both in operation. That is, o-Me substitution causes steric shielding which reduces interaction of the carboxylic acid group with water and thus increases log P, whilst 2,6-Me_2 substitution causes steric twisting which increases interaction of the carboxylic acid group with water and hence lowers log P.

The steric effect of the methyl group in acetanilide is reflected by the reduced π value of the o-Me group which becomes negative for 2,6-Me_2 substitution. This indicates that twisting of the acetamido group causes increased interaction with water.

In the cyclohexane-water system, the substitution of a methyl group in positions 4-,5- or 6- in o-nitrophenol appears to have little effect on partition other than that expected by the addition of a lipophilic group. However in the 3-position the methyl group has a negative π value and in fact causes log P to be less than that of the parent compound o-NO_2phenol. This is indicative of the 3-Me group weakening the intramolecular hydrogen bond.

These points are all discussed in detail in Chapter 6.

CHAPTER SIX

PARTITION COEFFICIENTS OF STUDY COMPOUNDS

6.1 Free-Energy Based Substituent Constants

The effect of substitution on an aromatic ring system is to cause changes in the physicochemical character of the ring, and of other groups attached to the ring. A study by Katrisky and Topsom (218) concluded that in non-crowded systems there are only three important effects on a benzene ring:

1. The conjugative or mesomeric effect, felt mainly at the ortho and para positions, which results in the transfer of electron density from the ring to the substituent or vice-versa.
2. Repulsion of the π electrons of the ring by pi electrons on the substituent. This is felt mainly at the ortho and para positions, although the net result is a redistribution of the existing π density of the ring.
3. A direct field effect of the substituent potential on a reaction or measurement site elsewhere on the ring.

A substituent group is generally considered as a whole, but where such a group contains more than one atom, much of the charge distribution may be located in bonds other than that connecting it to the ring. Leffler and Grunwald (253) showed that it is convenient to divide substituents into two classes, rigid substituents in which no internal motions are excited at ordinary temperatures and mobile substituents in which internal motions or rotations of the substituent about the bond connecting it to the host molecule are excited.

When a rigid substituent e.g. halogen, is introduced in a position where it is crowded by other groups, the lengths and angles of its bond to the host molecule, and the contribution of that bond to the zero-point potential energy of the molecule may be altered. In mobile substituents, such as alkyl or nitro groups, both crowding and electron demand can have a direct effect on the internal mobility of the molecule.

6.2 Hydrophobic Substituent Constants

π is a measure of the relative hydrophobicity of one group to another. Hydrophobicity is the description of the ability of a function to form a hydrophobic bond which although recognised as complex, consists of both polar and apolar interactions (137).

Assuming each atom of a substituent experiences an average effect due to its surroundings (i.e. solvent) it has been shown (60) that π is related to the electronic polarizability and the Hammett sigma value of a substituent (an important premise of the Hansch π constant is that π has little dependence on σ). Thus at one extreme substituents may be said to have their lipophilicity determined primarily by their polarizability. The polarizability of a substituent is essentially independent of its mode of substitution on an aromatic moiety (287) so that such substituents will have π values which are also independent of their position. Thus meta and para substituent π values are often of the same order of magnitude.

At the other extreme, a set of substituents may be said to

have their lipophilicities determined primarily by their charge i.e. magnitude of charge on the substituent determines the ease with which water is stripped off the substituent as it enters a lipophilic phase such as 1-octanol. This applies to such groups as nitro which are strongly electron-withdrawing. Such groups have similar σ_m and σ_p values.

Therefore, it can be said that partitioning into an aqueous phase may be considered as 'charge controlled' while partitioning into a non-polar phase may be considered as 'polarizability controlled'. It is the relative contribution of each controlling factor which therefore determines the partition coefficient observed for a compound.

The relationship between π and σ is complex, and involves several interactive forces comprising π, e.g. solvation by H-bonding, hydrophobic bonding in a structured solvent and permanent induced dipole-dipole interactions. (92)

Theoretically π is additive and constitutive in nature. However, experimental data has shown (145) that when interactions between groups occur, whether by inductive electronic effects or by hydrogen bonding, this causes non-additivity of group π values.

6.3 Causes of Non-Additivity of π Values

a. Steric Effects

Steric effects often account for breakdown in π additivity, especially in large drug molecules containing non-planar ring systems. It is possible to overcome this by selecting literature π values appropriate to the system under consideration. However, caution is necessary if small

molecules are considered due to the fact that the steric effect will overlap with other intramolecular interactions. In small conjugated cyclic aromatic systems, because of the coplanarity of aromatic rings and the fact that substituents are always equatorial to the ring, alicyclic steric effects are not evident and can be ignored, however, steric effects of a type do exist in non-alicyclic systems, the most common of these being the ortho effect.

The Ortho Effect

When polar groups are introduced into a molecular structure adjacent or ortho to an established grouping, it is possible that intramolecular bonding between the two groups will occur. Such an effect has been termed the ortho effect. The effect can be shown (355) to be mainly a polar one resulting from inductive and/or mesomeric effects. In attempting to elucidate the electrical composition of π constants, Cammarata (60) has suggested that there can exist two conditions under which non-additivity of π constants will be evident. These are:

i. When mutual electrical interaction occurs between functional groups, and,

ii. When a given group can no longer be desolvated to its maximum potential because of the physical effect of an adjacent or ortho group.

Empirically the type (i) effect can be overcome by using π values which would be expected to have similar electrical effects. This is achieved by choosing from the literature values obtained from related solute systems and values when the studied substituent is in a similar environment. Type

(ii) effects will occur because of competition between two adjacent groups for the same solvated water, which is thought to exist around the molecule when in solution. Upon transfer of the substituted molecule from an aqueous to a non-aqueous phase, the desolvation process is changed, hence the entropy contribution to the transfer is altered and will result in a change in the value of the free energy term. This has the net effect, for example in a pair of isomers, in one of which the ortho effect is present, of reducing the π value in the molecule having ortho interactions compared to its isomer.

The ortho effect can be reinforced by intramolecular hydrogen bonding.

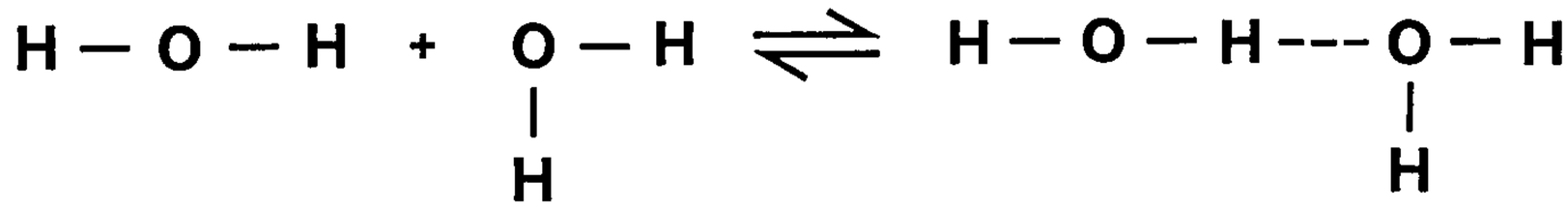
The total effect of ortho substitution can be composed of three components:

1. an electronic effect, considered as equal to that of meta or para.
2. a negative effect, which may in part stem from an adjacent bulky group twisting the heteroatom attaching a polar group to the ring and decoupling its lone pair(s) from the π bonding system, and in part to a reversal of the field effect if two polar substituents are in close proximity.
3. a positive effect when certain types of intramolecular hydrogen bonds can occur.

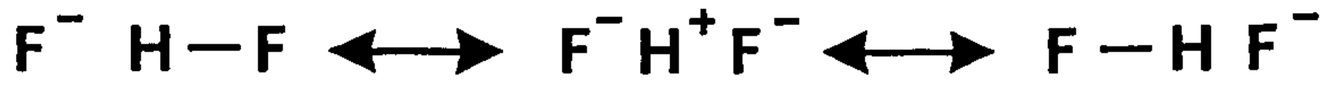
b. <u>Intramolecular Hydrogen Bonding</u>

In the covalent bond between a hydrogen atom and an electro-negative atom such as F,O or N, the attraction of the

electrons by the electronegative atom means that the hydrogen atom gains a partial positive charge. This partially positive atom can in turn interact with the partially negative atom of another molecule or an atom involved in a different covalent bond in the same molecule. Thus:



In this description the hydrogen bond is electrostatic. A covalent interpretation is also possible. Consider the HFH^- ion:



The hydrogen bond is unique to hydrogen because it is the only atom which can carry a partial positive charge while covalently bonded in a molecule and which is also small enough to allow a close approach of a second electronegative atom.

In aqueous solution, solute-solute hydrogen bonds are effectively masked by the competing solute-solvent hydrogen bonds (228). They may contribute in a co-operative manner. In nonpolar solvents and in the hydrophobic interior of globular proteins however, hydrogen bonds may contribute appreciably to the energy of inter- or intra-molecular interactions.

Changes in structure change the strength of a hydrogen bond

to a common donor or acceptor in direct proportion to the effect of the structural modification on the charge of the electronegative atom which is involved in the hydrogen bond. Such substituent effects are well parameterized by Hammett sigma values, or they may be estimated by molecular orbital calculations.

If phenol is considered as a hydrogen bond donor, substituents which make the oxygen more positive decrease its affinity for the proton and correspondingly increase the strength of the hydrogen bond.

When intramolecular hydrogen bonding occurs, the size of the deviation between $\Sigma\Delta\pi$ and log P values is influenced by the strength of the intramolecular hydrogen bond and its free energy of formation. For example, F,Cl or cyano groups will be expected to have no ortho effect due to hydrogen bonding, whereas hydroxyl and amino groups will (363). If π values are strongly dependent upon the nature of the solvent system in which they are measured, this normally indicates that strong intramolecular bonding is taking place. (271)

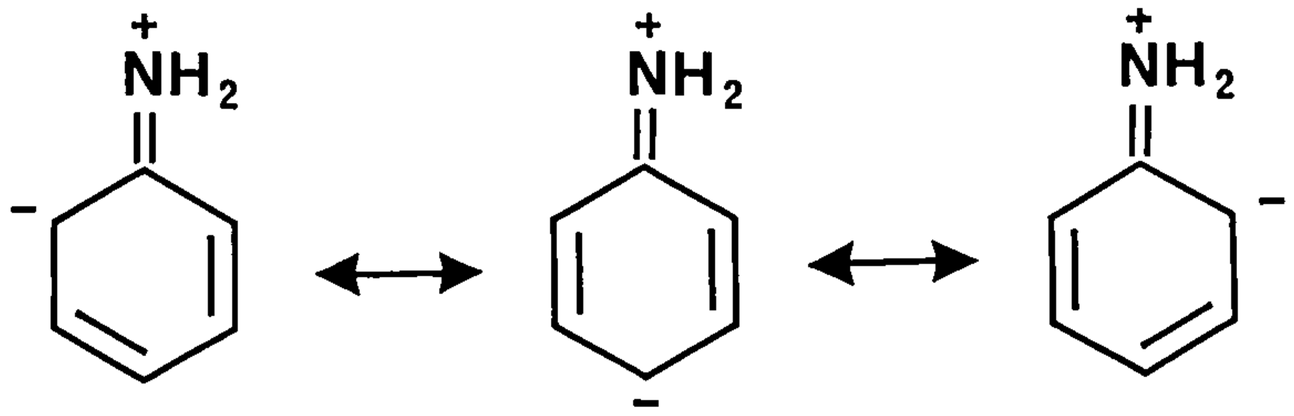
c. Electronic Effects

Substitution into any particular system can be expected to alter the general electronic distribution of the molecule. If such disruption is great, then non-additivity may result. This may be because of the actual electronic distribution of the substituent or the effect this has on the character of the original electronic displacements in the parent molecule (156). In aromatic systems where permanent charge separations are possible, such effects may often dominate any steric effects and will result in even larger deviations

from the $\Sigma\Delta\pi$ - logP relationship.

The greatest variation from normal will arise when two strongly interacting groups such as $-NO_2$ and $-NH_2$ are placed in the ring together.

e.g. The amino function has a very negative substituent value.

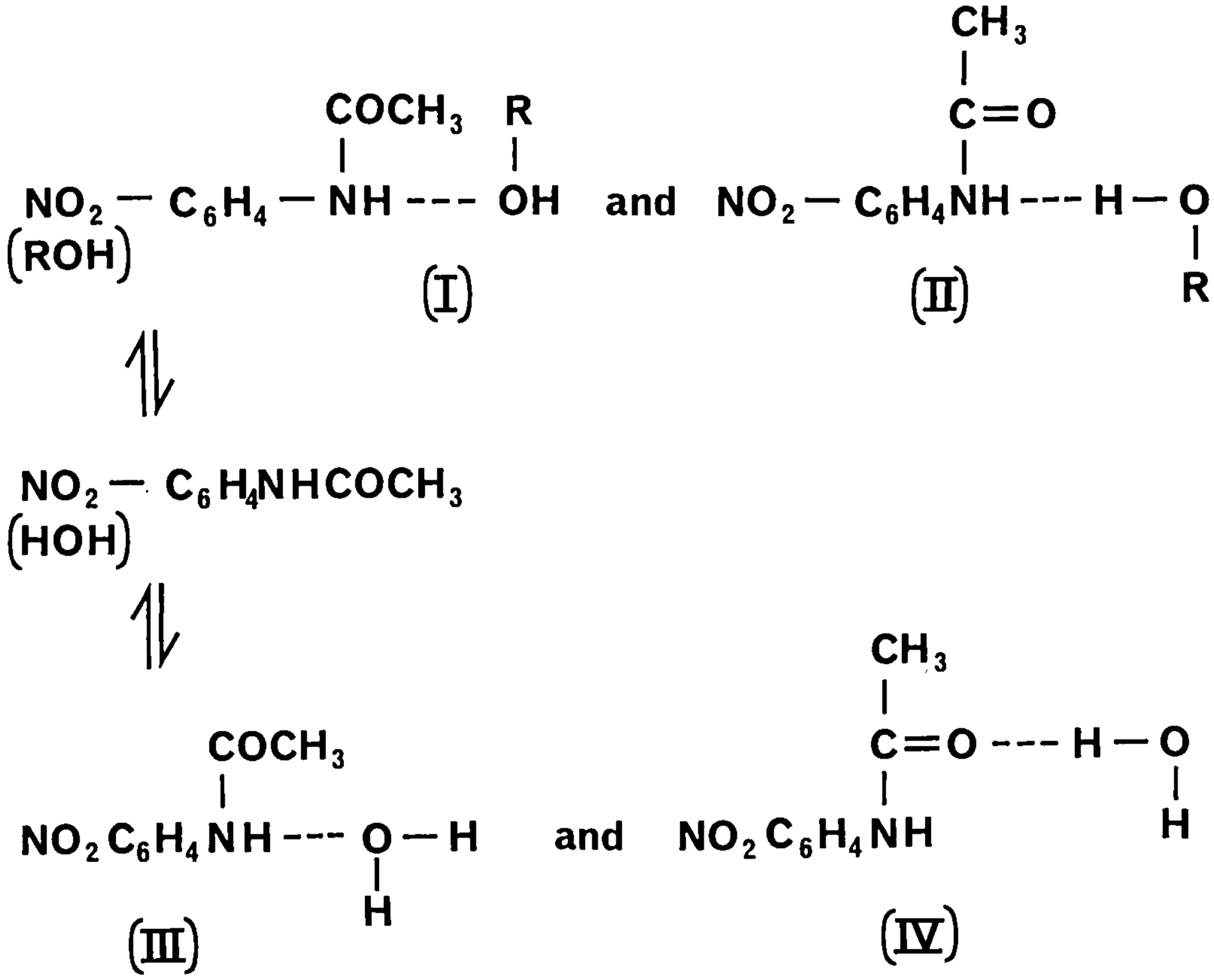


Canonical Forms of Aniline

The pair of electrons on N interact with the pi electron system of the ring, leading to their delocalisation and enhanced electron density in the ring. The amino substituent therefore transfers charge to the ring in opposition to its inductive effect. This charge transfer reduces the terminal electronic density and thus the π electron system is altered to reduce the relative hydrophobicity of the substituent. In amino acetanilide this increases the hydrogen bonding ability of the -NH- function giving the molecule a greater preference for the more polar phase and reducing its partition coefficient. Similar arguments apply to p-hydroxyacetanilide

Similarly, delocalisation of the π electrons of the benzene ring could occur into a substituent such as $-NO_2$ or -CO when

free orbitals are available. Doubt has been expressed (125) as to whether a single electron-withdrawing substituent attached to a benzene ring does show a mesomeric effect. The effect of the nitro group and other similar electron-withdrawing substituents on the π electron system of the benzene ring is solely due to a π-inductive effect in the ground state. It has been shown (144) that the $-NO_2$ group itself has no preference for either the octanol or the aqueous phase. The partition coefficient of acetanilide is increased from 14.3 to 73.4 for p-nitroacetanilide. Since the chief electronic effect of the nitro group is electron withdrawal, and since increased polarization should result in increased water solubility, these values must be related to changes in the hydrogen bonding ability of the molecule.



Electron withdrawal by the nitro group (or other similar function) inhibits the type of bonding shown by II and IV, but conversely promotes bonding of the type shown in I and III.

Octanol (ROH) is more nucleophilic than water and so competes more effectively in bonding of types I and III. Pure electron withdrawal by this means is able to raise the partition coefficient (ROH/H_2O) of acetanilide. To a lesser extent, electron withdrawal from the carbonyl oxygen would inhibit the water solubilising forces indicated by IV.

d. Intramolecular Hydrophobic Bonding

Examination of partition coefficient data of aromatic molecules with aliphatic side chains revealed that for polar aliphatic substituents the π values for the polar grouping depend upon the distance of the group from the aromatic ring. (211,174). Also, π for polar substituents, determined from completely aliphatic structures have higher positive values than π values determined for aromatic structures with aliphatic side chains, where the polar group is separated from the ring by three methylene groupings. Such an effect indicates that a polar group such as hydroxy, fluoro, chloro etc. has a higher hydrophobic nature when in a completely aliphatic system than when placed terminal to an aliphatic side-chain in an aromatic system.

Hansch and Anderson (174) proposed that this effect was due to folding of the side-chain over the phenyl ring (the effect being assisted by the tendency of the strong dipole of the polar group to interact with the π electrons of the ring),

in such a way that the polar substituent group projects away from the interaction. This would result in a more compact structure of greater water solubility and hence a lower logP value than expected. This postulate has been questioned (303) on the geometric basis that any interaction below an aliphatic chain of 4-C length would result in unfavourable strain on the structure. However, non-additivity occurs and direct measurement of P is needed.

e. Chain-branching

Non-additivity is found in aromatic ring systems with a branched substituent chain.

In this thesis, octanol/water and cyclohexane/water partition coefficients, in the free-energy based form of log P (168) are analysed in order to identify the effect of substituent types and their position on an aromatic ring. The purpose of the analysis is two-fold; first, to predict log P values more accurately from structure and second, to understand better the nature of the solvation-desolvation forces as a small solute passes from an aqueous phase to a lipid-like phase. As a basis for solvation theory, this analysis can only be tentative, because the basic forces which determine 'hydrophobicity' (that is the preference for a lipid phase over water) are still the subject of an intense debate (80)

6.4 The Effect of Different Solvents on Partition Coefficient

Two solvent systems are examined in this study, octanol/water and cyclohexane/water. These two systems were chosen because in one, octanol/water, the organic phase is polar and capable of hydrogen bonding and in the other, cyclohexane/

water, the organic phase is non-polar and not capable of hydrogen bonding.

If hydrogen bonding can occur between the solute and solvent, passage of the solute from the aqueous phase to the organic phase is assisted and so the partition coefficient is higher. For hydrocarbon solvents such as cyclohexane, the partition coefficients of ortho-substituted phenols are greater than those of the meta or para substituted isomers. However, for hydrogen bonding solvents, the coefficient for the ortho isomer is less than that for the meta or para isomers due to hydrogen bonding which can occur between solute and solvent.

The type of hydrogen bonding in a system can be determined by infra-red spectroscopy. The sharp band associated with a free hydroxyl group occurs at $\sim 3600 cm^{-1}$, while a hydroxyl group involved in intermolecular hydrogen bonding gives either a sharp band at $\sim 3450 cm^{-1}$ (dimeric) or a broader band between 3200 and $3400 cm^{-1}$ (polymeric). West has shown (380) that no self-association of phenols (i.e. intermolecular hydrogen bonding) occurs in inert solvents at concentrations of 0.02M or less.

Meta and para halogenophenols show intermolecular hydrogen bonding between phenol and solvent ($3450 cm^{-1}$), therefore transfer of the phenol is assisted. Ortho halogenophenols show a strong band at $3523 cm^{-1}$ (intramolecular hydrogen bond) and also a weaker band at $3603 cm^{-1}$ (free OH). For inter-molecular hydrogen bonding the <u>intra</u>molecular hydrogen bond

must first be broken, therefore, the band at 3440cm^{-1} only occurs with a suitable solvent.

In partition experiments phenol molecules will be hydrogen bonded to water molecules in the aqueous phase. This association should be extensive for meta and para substituted phenols but much less for ortho substituted phenols in which intramolecular hydrogen bonding occurs. When an organic solvent is used which can compete favourably in hydrogen bonding to the hydroxyl group of the meta and para substituted phenols, the transfer of the molecules to the organic phase will be favoured relative to the transfer of the molecules of the ortho substituted phenols. Therefore the ortho isomers will have a smaller log P. A hydrocarbon solvent cannot however compete in this manner in attracting the molecules of the meta and para substituted phenols away from their associations with water molecules and when using such solvents all partition coefficients are lower, but the ortho isomers which will be least involved in such intermolecular associations give the highest values.

Therefore, for example, for cresols the partition coefficients are the same in octanol because intramolecular hydrogen bonding between the OH group and the o-Me group is insignificant.

6.5 Experimental Details

Shake Flask Method

Twenty-five 250ml conical flasks of the same size and shape were selected and carefully washed, rinsed and dried. Five samples of each compound were measured each time, so five compounds could be tested simultaneously. An amount of compound, sufficient to produce a concentration of 10^{-3}M was accurately weighed on a Beckman LM 500 microbalance and transferred to a 100ml volumetric flask. Initial dissolution was achieved if necessary with 2ml of purified ethanol. Previous determinations had ascertained that this amount of ethanol did not affect P values. The samples were made up to volume with octanol-saturated distilled water. If the partition coefficient of a strongly acidic solute was to be measured, the solution was acidified to pH2.0 with hydrochloric acid. This solution was then transferred to a conical flask and purged with nitrogen. A suitable volume of water-saturated octanol was added, the volume having been calculated prior to the experiment to give equal weight of solute in each phase at equilibrium.

e.g. Log P (phenol) = 1.49

P = 30.9

$$P = \frac{\text{Vol.aq.phase}}{\text{Vol.oct.phase}} \times \frac{\text{Wt.solute in oct.phase}}{\text{Wt.solute in aq.phase}}$$

Therefore: $30.9 = \frac{100\text{ml}}{x} \times \frac{z}{y}$ where x = vol.oct.phase

Therefore: 30.9 x (x) = 100 when z = y

Therefore volume of octanol phase is 3.2ml; 3.0ml being used for convenience.

If the partition coefficient had not been previously measured, a calculated value was used to obtain an estimate of the appropriate volume of octanol. This was repeated for each solute.

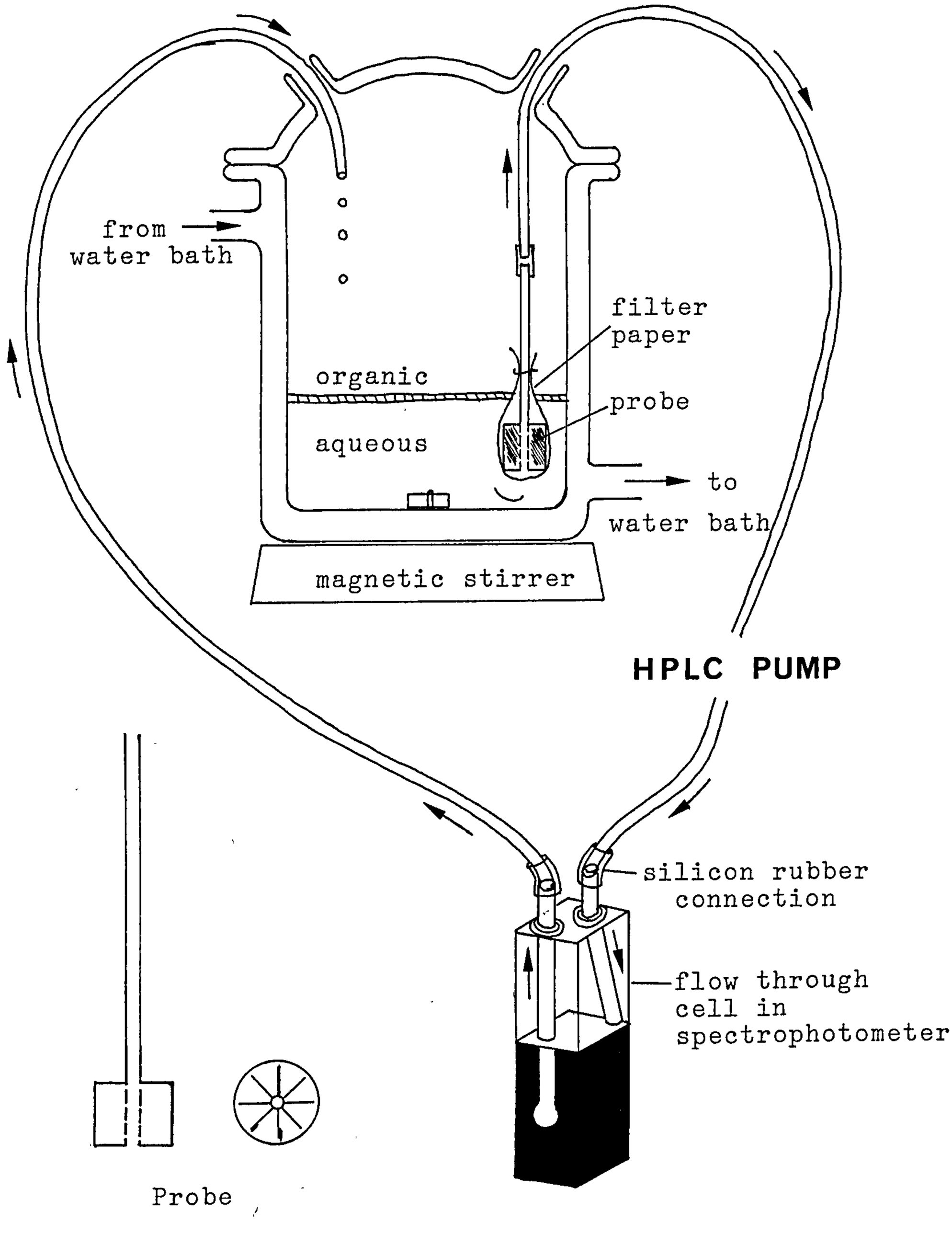
The conical flasks were capped with an aluminium foil cap and placed in the orbital incubator thermostatted at 25°C. A mixing speed of 80rpm and a mixing time of 2 hours were used.

At the end of this time the flasks were removed from the incubator and the aqueous phase separated from the octanol phase by removing a sample with a pipette. The volume of octanol used was usually quite small so that a complete layer was not formed and aqueous phase removal was quite simple. However, to ensure complete separation, the aqueous sample was then centrifuged at 2000rpm for 5 minutes and a portion of this carefully transferred to a U.V. cell so that the concentration of solute in the sample could be determined by reference to a previously measured calibration curve. The partition coefficient of each compound was then calculated. At least five measurements were made for each compound.

Filter Probe Method. Octanol/Water System

The samples were prepared as described for the shake flask method. The apparatus was assembled as illustrated in Figure 23 and the two phases placed in the beaker. Whatman No.1 filter paper was placed over the end of the probe and firmly fixed in position by securing to the probe stem. Care had to be taken to ensure that the filter paper was not damaged as it was secured, but providing this was done, the

Figure 23. Diagram of Thermodynamic Experiment Flow Cell Assembly



Probe

fact that all parts of the probe in the experimental system were covered by filter paper meant that leakage of octanol into the sampling system was eliminated. The speed of the magnetic follower was adjusted so that both phases were intimately mixed. Separation was achieved by the filter paper so centrifugation was not necessary. The aqueous phase was pumped through the U.V. cell and the absorbance recorded until no further change occurred. The apparatus was maintained at a temperature of 25°C ±0.2°C and the partition coefficient calculated from equilibrium concentration.

The flow cell used was a 2-port self-masking cell with a 10mm pathlength and 80µl optical volume.

The aqueous phase was returned to the partitioning vessel so that a constant volume was maintained.

Filter Probe Method. Cyclohexane/Water System

In this system, Whatman Phase Separating paper, which allowed the passage of cyclohexane, was used to separate the two phases. This presented certain problems with respect to the materials used in the pump and tubing, but these were overcome by using an HPLC pump with Teflon tubing. Connections were made with small sections of silicone tubing which was attacked by cyclohexane so had to be changed frequently.

Since cyclohexane was the circulating phase there had to be sufficient volume for circulation, a minimum of 30ml cyclohexane being used. The volume of water was calculated as described for the shake flask method. The solute was initially dissolved in cyclohexane, and heat was used if necessary to aid dissolution. Care was taken to check that

this did not cause solute degradation.

The temperature was maintained at 25°C ±0.2°C and the absorbance recorded until no further change occurred. The partition coefficient was calculated using previously measured calibration curves.

For both systems the partition coefficient of each solute was measured twice and the average calculated. If large discrepancies between the two measurements were observed, the experiment was repeated until two similar results were obtained.

6.6 Comparison of Methods of Measuring Partition Coefficients Octanol/Water System

The two methods of partition coefficient measurement chosen for further investigation were the Shake Flask method and the Filter Probe method. The results obtained from these experiments are given in Table 28 . The literature values are given for comparison. When more than one log P value was reported for a given solute, the choice for inclusion in Table 28 was made after considering the following: a) limits of error, if given; b) need to suppress ionization; c) probable precision of the analytical method; d) agreement with a third determination. Sometimes a choice was not warranted and an average value was taken, in which case, the standard deviation ($\bar{s}$) has been calculated.

A graphical representation of the comparison of the two methods is given in Figure 24 and Figures 25 & 26 show the relationship between literature values of log P and values obtained from the shake flask method and filter probe method

Table 28. Octanol/Water Partition Coefficients

Compound	Shake Flask		Filter Probe		Literature	
	Log P	$\bar{s}$	Log P	$\bar{s}$	Log P	$\bar{s}$
Phenol	1.49	0.019	1.46	0.003	1.49	0.019
o-Clphenol	2.12	0.040	2.05	0.005	2.16	0.030
m-Clphenol	2.48	0.011	2.39	0.013	2.50	0.025
p-Clphenol	2.41	0.057	2.31	0.009	2.42	0.068
o-NO_2phenol	1.75	0.033	1.75	0.030	1.77	0.028
m-NO_2phenol	2.01	0.055	1.91	0.001	2.00	0.000
p-NO_2phenol	1.94	0.048	1.89	0.023	1.95	0.075
2-NO_2resorcinol	1.46	0.047	1.53	0.006	1.46	0.141
o-OHbenzaldehyde	1.67	0.076	1.64	0.025	1.70	0.083
m-OHbenzaldehyde	1.32	0.048	1.38	0.008	1.38	---
p-OHbenzaldehyde	1.35	0.018	1.39	0.013	1.37	0.029
Benzoic acid	1.88	0.024	1.79	0.042	1.95	0.113
o-OHbenzoic acid	2.35	0.015	2.23	0.011	2.24	0.022
m-OHbenzoic acid	1.69	0.010	1.52	0.001	1.49	0.014
p-OHbenzoic acid	1.57	0.017	1.50	0.041	1.58	0.007
2,6-OH_2benzoic acid	1.65	0.023	1.65	0.033	2.20	---
3,5-OH_2benzoic acid	0.49	0.021	1.04	0.006	--	---
3-Me-2-NO_2phenol	2.15	0.033	2.15	0.049	--	---
4-Me-2-NO_2phenol	2.25	0.023	2.23	0.006	--	---
5-Me-2-NO_2phenol	2.35	0.047	2.32	0.030	--	---
6-Me-2-NO_2phenol	2.52	0.083	2.56	0.013	--	---
o-Mephenol	1.99	0.027	1.86	0.067	1.99	0.047
m-Mephenol	1.96	0.047	1.90	0.052	1.97	0.032
p-Mephenol	2.02	0.064	1.92	0.035	1.95	0.029
2,3-Me_2phenol	2.33	0.103	2.27	0.035	--	---
2,4-Me_2phenol	2.33	0.103	2.32	0.054	2.30	---
2,5-Me_2phenol	2.33	0.076	2.31	0.034	2.33	---
2,6-Me_2phenol	2.22	0.060	2.24	0.018	2.36	---
3,4-Me_2phenol	2.25	0.058	2.22	0.059	2.23	---
3,5-Me_2phenol	2.37	0.086	2.30	0.017	2.33	---
2,3,5-Me_3phenol	2.71	0.031	2.68	0.051	--	---
2,3,6-Me_3phenol	2.54	0.017	2.52	0.018	--	---
2,4,6-Me_3phenol	2.61	0.035	2.59	0.039	--	---
2,3,5,6-Me_4phenol	2.78	0.044	2.76	0.078	--	---
o-Mebenzoic acid	1.88	0.075	2.23	0.030	--	---
m-Mebenzoic acid	2.32	0.023	2.40	0.002	2.37	---
p-Mebenzoic acid	2.28	0.021	2.33	0.003	2.27	---
2,6-Me_2benzoic acid	1.65	0.040	2.02	0.001	--	---
3,5-Me_2benzoic acid	2.86	0.027	2.85	0.042	--	---
Acetanilide	1.15	0.041	1.23	0.003	1.21	0.102
o-Meacetanilide	0.89	0.040	0.87	0.131	--	---
m-Meacetanilide	1.62	0.024	1.60	0.011	--	---
p-Meacetanilide	1.62	0.024	1.61	0.004	1.39	---
2,6-Me_2acetanilide	0.96	0.030	0.99	0.003	--	---
3,5-Me_2acetanilide	2.09	0.036	2.03	0.036	--	---

Figure 24. Log P Shake Flask Method vs Log P Filter Probe Method. Octanol/Water System

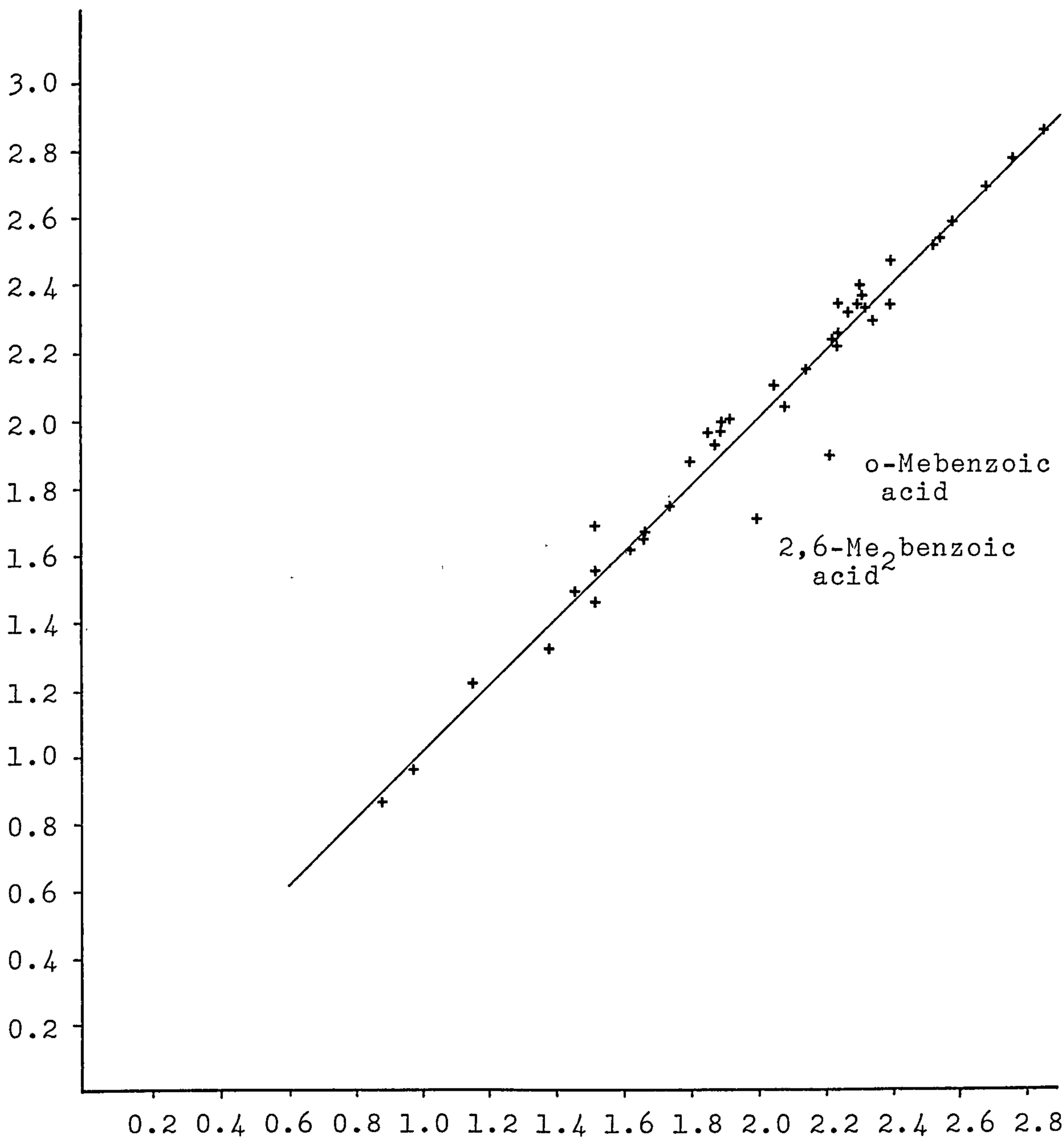


Figure 25. Log P Shake Flask Method vs Log P Literature Octanol/Water System

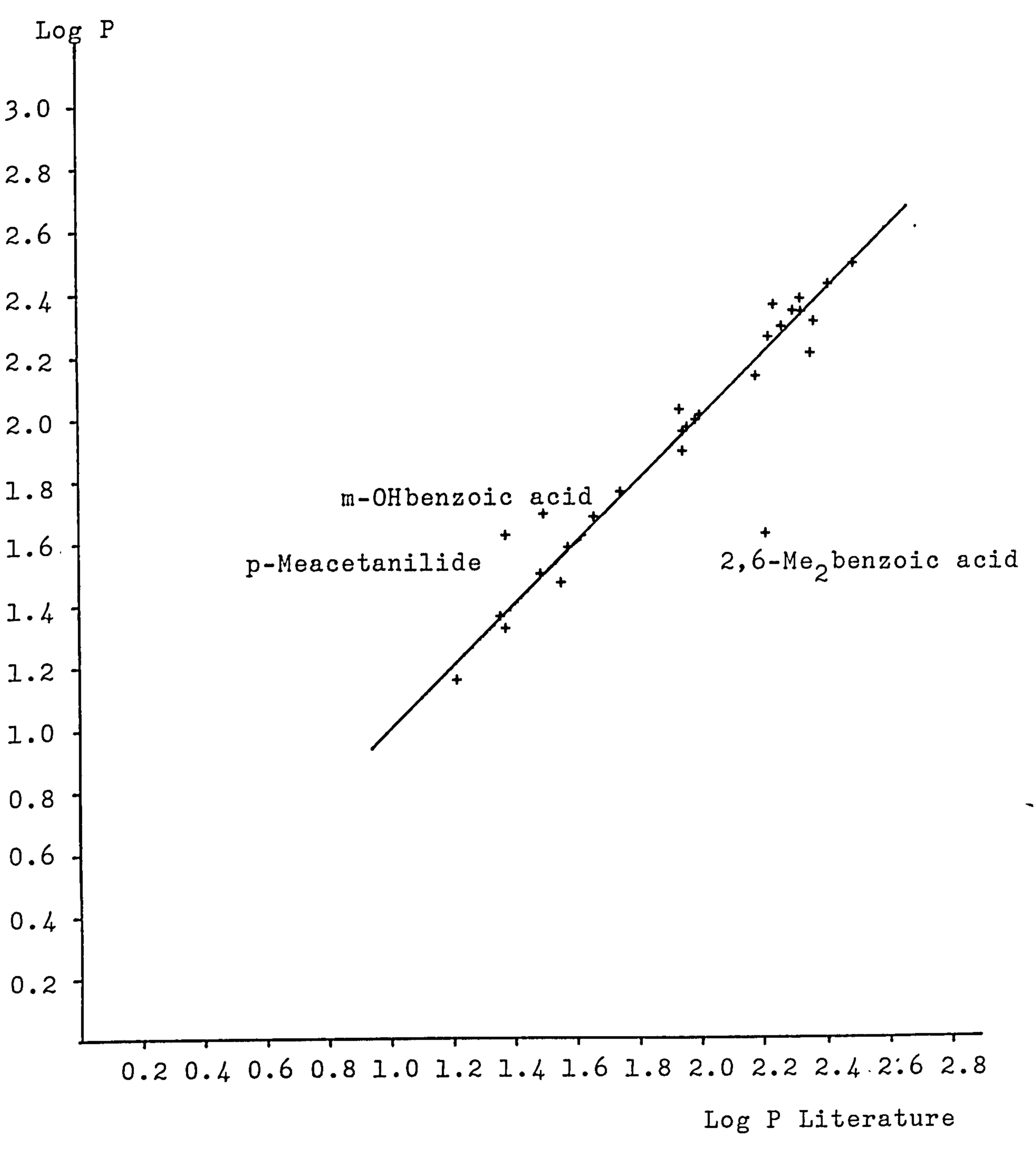
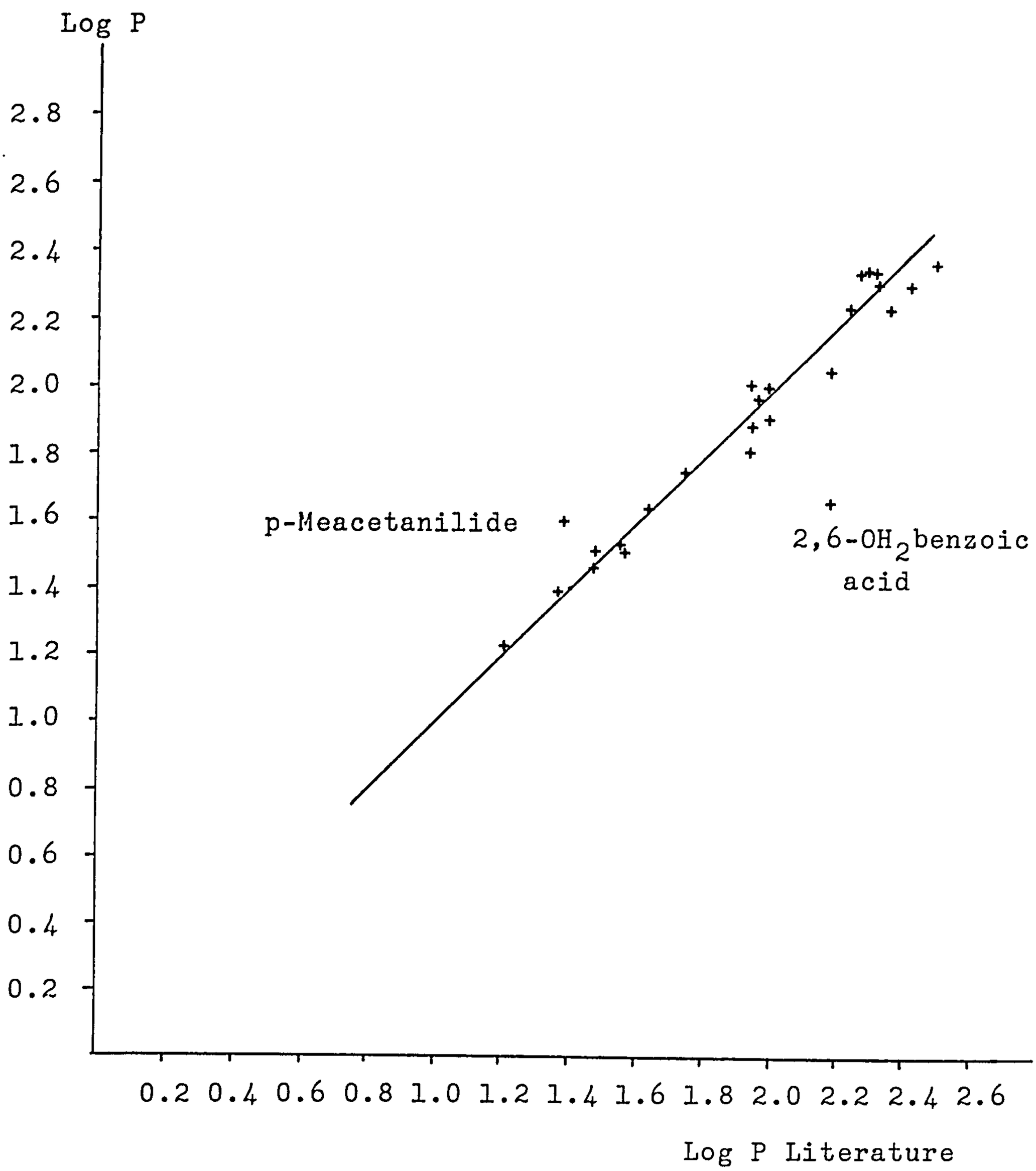


Figure 26. Log P Filter Probe Method vs Log P Literature Octanol/Water System



respectively.

The agreement obtained between the two experimental methods is good and supports the use of these figures as accurate representatives of the partition coefficient which may be subjected to rigorous analysis. Figures 25 and 26 show the disagreement which was found with literature values and probably reflect the importance of specifying the conditions of measurement; pH, temperature etc.

The partition coefficient values reported for the shake flask method are the result of at least five measurements, and sometimes as many as twenty, whereas the values reported for the filter probe method are an average of only two or three measurements. Therefore, the shake flask results may be expected to be more precise.

Cyclohexane/Water System

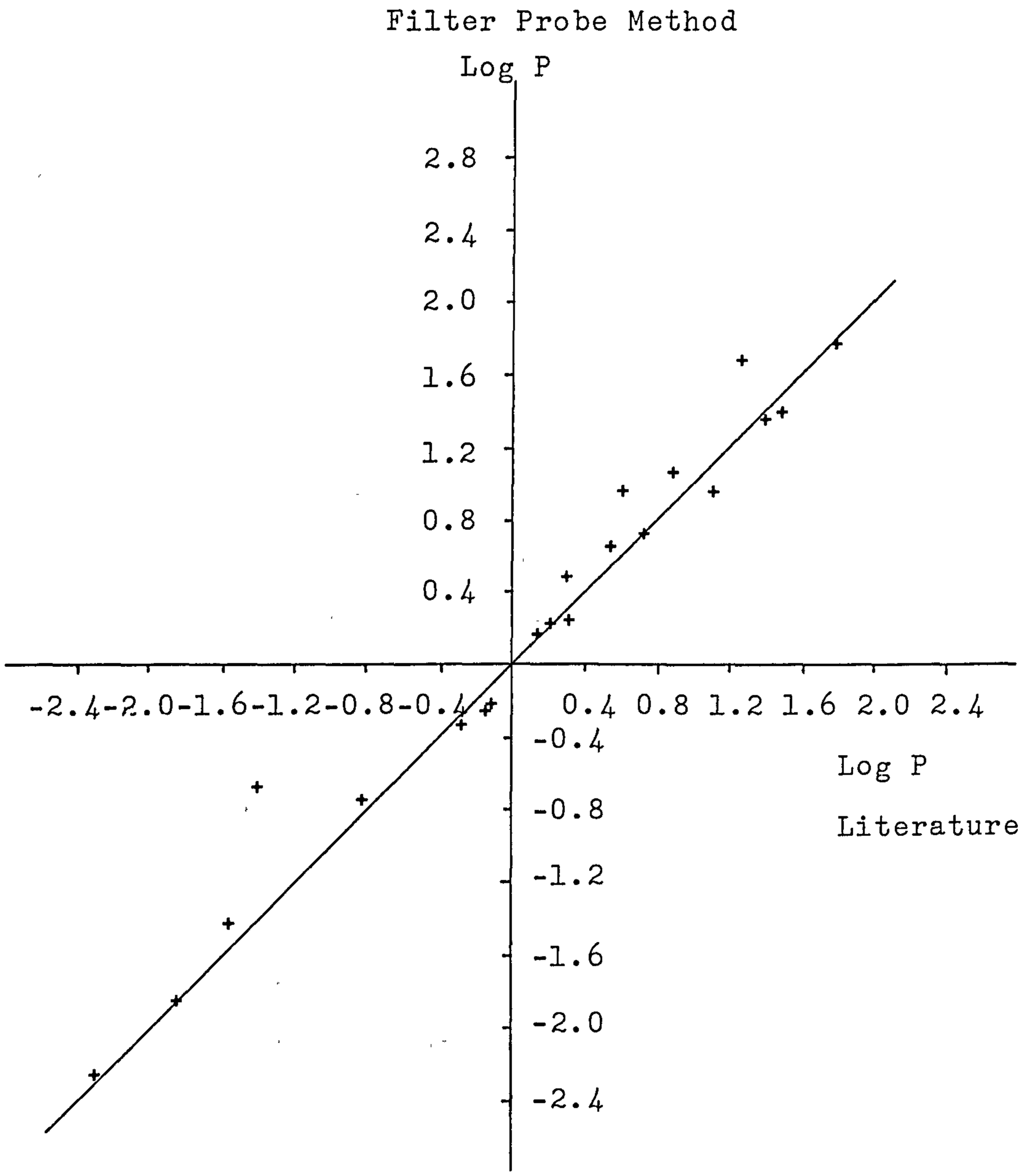
Time limited the measurement of partition coefficients in the cyclohexane/water system by only one method, the filter probe method. The results for this are given in Table 29, with the literature values for comparison. As with the literature values for the octanol/water system, when more than one log P value was reported for a solute, the same considerations were given to the choice of value. The graphical representation of the relationship between experimental and literature log P values is given in Figure 27. Agreement can be seen to be fairly good, in some cases the experimental values agree exactly with literature values, but in other cases the reported log P's have a large degree of scatter which causes disagreement with experimental values.

Table 29. Cyclohexane/Water Partition Coefficients

Compound	Filter Probe		Literature	
	Log P	$\bar{s}$	Log P	$\bar{s}$
Phenol	-0.74	0.000	-0.82	0.114
o-Clphenol	0.82	0.037	0.86	---
m-Clphenol	-0.05	0.023	0.08	---
p-Clphenol	-0.30	0.012	-0.31	0.202
o-NO_2phenol	1.40	0.003	1.51	0.067
m-NO_2phenol	-1.57	0.020	-1.44	0.189
p-NO_2phenol	-1.91	0.008	-1.86	0.139
2-NO_2resorcinol	1.02	0.002	---	---
o-OHbenzaldehyde	1.38	0.000	1.37	---
m-OHbenzaldehyde	-1.96	0.046	-1.93	---
p-OHbenzaldehyde	-2.29	0.030	-2.24	0.431
Benzoic acid	-0.85	0.049	0.05	---
o-OHbenzoic acid	-1.42	0.042	-0.66	0.309
m-OHbenzoic acid	---	---	-2.04	---
p-OHbenzoic acid	---	---	-1.77	---
2,6-OH_2benzoic acid	-3.35	0.000	---	---
3,5-OH_2benzoic acid	---	---	---	---
3-Me-2-NO_2phenol	1.32	0.000	---	---
4-Me-2-NO_2phenol	1.99	0.042	---	---
5-Me-2-NO_2phenol	1.72	0.182	---	---
6-Me-2-NO_2phenol	1.95	0.064	---	---
o-Mephenol	0.16	0.001	0.15	0.042
m-Mephenol	-0.16	0.026	-0.23	0.096
p-Mephenol	-0.15	0.038	-0.21	0.127
2,3-Me_2phenol	0.49	0.009	0.33	0.262
2,4-Me_2phenol	0.66	0.004	0.55	0.210
2,5-Me_2phenol	0.72	0.005	0.72	0.190
2,6-Me_2phenol	0.97	0.016	1.11	0.175
3,4-Me_2phenol	0.21	0.091	0.24	0.057
3,5-Me_2phenol	0.26	0.021	0.33	0.136
2,3,5-Me_3phenol	1.08	0.012	0.97	---
2,3,6-Me_3phenol	1.72	0.000	---	---
2,4,6-Me_3phenol	1.69	0.009	1.24	---
2,3,5,6-Me_4phenol	1.79	0.025	1.77	---
o-Mebenzoic acid	0.98	0.119	0.60	---
m-Mebenzoic acid	0.36	0.082	---	---
p-Mebenzoic acid	-0.53	0.080	---	---
2,6-Me_2benzoic acid	-0.98	0.017	---	---
3,5-Me_2benzoic acid	0.06	0.053	---	---
Acetanilide	-1.37	0.000	---	---
o-Meacetanilide	-1.25	0.042	---	---
m-Meacetanilide	-0.99	0.025	---	---
p-Meacetanilide	-0.90	0.011	---	---
2,6-Me_2acetanilide	-1.47	0.035	---	---
3,5-Me_2acetanilide	-0.31	0.022	---	---

Figure 27. Log P Filter Probe Method vs Log P Literature

Cyclohexane/Water System



6.7 Identification of Structures Producing Anomalies Between Calculated and Measured Partition Coefficients

The aim of this thesis is to explain the anomalies which cause disagreement between calculated and measured log P values. Therefore it is necessary to identify those structures which produce such anomalies. The partition coefficients of the solutes studied were carefully measured to ensure the values obtained were as accurate as possible. These can then be compared to the calculated values. Chapter 5 gives a detailed account of the methods available for calculating log P and from this it can be seen that much work has been done which attempts to produce π or f values which accurately describe the contribution to hydrophobicity of all substituents in whatever the environment. This has resulted in a number of methods which produce calculated log P's in good agreement with measured values, but which can of themselves produce problems since no method is universal and each one has a particular type of structure for which it is most accurate. This makes it important that the correct calculation method is chosen since if not, large errors will be introduced. The reason for this situation is that the mechanisms and interactions causing the anomalies are not understood. It is the aim of this thesis to further this inderstanding.

Since many calculation methods produce good agreement with measured logP values, particularly for the simple molecules such as those studied in this work, the original π method of calculation will be used to identify anomalous calculated partition coefficients. This will give some idea of the

type of structures which cause anomalous interactions with solvents.

Inaccuracies in the π system are apparent from Table 30 particularly in the cyclohexane/water system, and of course, it is because of this that other calculation systems have been developed. However, the main compounds showing discrepancies have been indicated by an asterisk and this reveals, as expected, that intramolecular bonding and steric hindrance cause anomalies between calculated and measured partition coefficients.

One possible reason for π values not producing accurate partition coefficients is that they have been calculated originally from inaccurate measured log P's. This is particularly likely for the cyclohexane/water system since less work has been reported in this system. Chapter 5 gives the π constants calculated from the present work.

A detailed discussion of individual solute partition coefficients is given in the following section of this chapter.

Table 30. Comparison of Experimental and Calculated Log P Values

Compound	Octanol		Cyclohexane		
	π	Exp.	π	Exp.	
Phenol	1.29	1.49	-0.84	-0.74	
o-Clphenol	1.98	2.12	-0.18	+0.82	*
m-Clphenol	2.33	2.45	-0.18	-0.05	
p-Clphenol	2.22	2.36	-0.18	-0.30	
o-NO_2phenol	1.01	1.75	-1.61	+1.40	*
m-NO_2phenol	1.83	2.00	-1.61	-1.57	
p-NO_2phenol	1.79	1.91	-1.61	-1.90	
2-NO_2resorcinol	0.35	1.50	-5.01	+1.02	*
o-OHbenzaldehyde	0.64	1.66	-2.09	+1.38	*
m-OHbenzaldehyde	0.64	1.35	-2.09	-1.96	*
p-OHbenzaldehyde	0.64	1.35	-2.09	-2.29	*
Benzoic acid	1.64	1.85	---	---	
o-OHbenzoic acid	0.97	2.30	---	---	*
m-OHbenzoic acid	1.26	1.60	---	---	
p-OHbenzoic acid	1.34	1.55	---	---	
2,6-OH_2benzoic acid	0.30	1.65	---	---	*
3,5-OH_2benzoic acid	0.88	0.80	---	---	
3-Me-2-NO_2phenol	1.57	2.15	-0.81	+1.32	*
4-Me-2-NO_2phenol	1.58	2.24	-0.81	+1.99	*
5-Me-2-NO_2phenol	1.53	2.33	-0.81	+2.66	*
6-Me-2-NO_2phenol	1.57	2.54	-0.81	+1.95	*
o-Mephenol	1.85	1.90	-0.23	+0.15	*
m-Mephenol	1.85	1.93	-0.23	-0.16	
p-Mephenol	1.85	1.98	-0.23	-0.16	
2,3-Me_2phenol	2.41	2.30	+0.33	+0.49	
2,4-Me_2phenol	2.41	2.32	+0.33	+0.66	
2,5-Me_2phenol	2.41	2.32	+0.33	+0.72	
2,6-Me_2phenol	2.41	2.23	+0.33	+0.97	*
3,4-Me_2phenol	2.41	2.24	+0.33	+0.21	
3,5-Me_2phenol	2.41	2.34	+0.33	+0.26	
2,3,5-Me_3phenol	2.97	2.70	+1.14	+1.08	
2,3,6-Me_3phenol	2.97	2.56	+1.14	+1.72	*
2,4,6-Me_3phenol	2.97	2.60	+1.14	+1.69	*
2,3,5,6-Me_4phenol	3.53	2.77	+1.94	+1.79	*
o-Mebenzoic acid	2.20	2.10	---	---	
m-Mebenzoic acid	2.16	2.35	---	---	
p-Mebenzoic acid	2.06	2.30	---	---	
2,6-Me_2benzoic acid	2.76	1.82	---	---	*
3,5-Me_2benzoic acid	2.72	2.85	---	---	
Acetanilide	0.72	1.20	---	---	*
o-Meacetanilide	1.28	0.88	---	---	*
m-Meacetanilide	1.28	1.61	---	---	
p-Meacetanilide	1.28	1.61	---	---	
2,6-Me_2acetanilide	1.84	0.97	---	---	*
3,5-Me_2acetanilide	1.84	2.06	---	---	

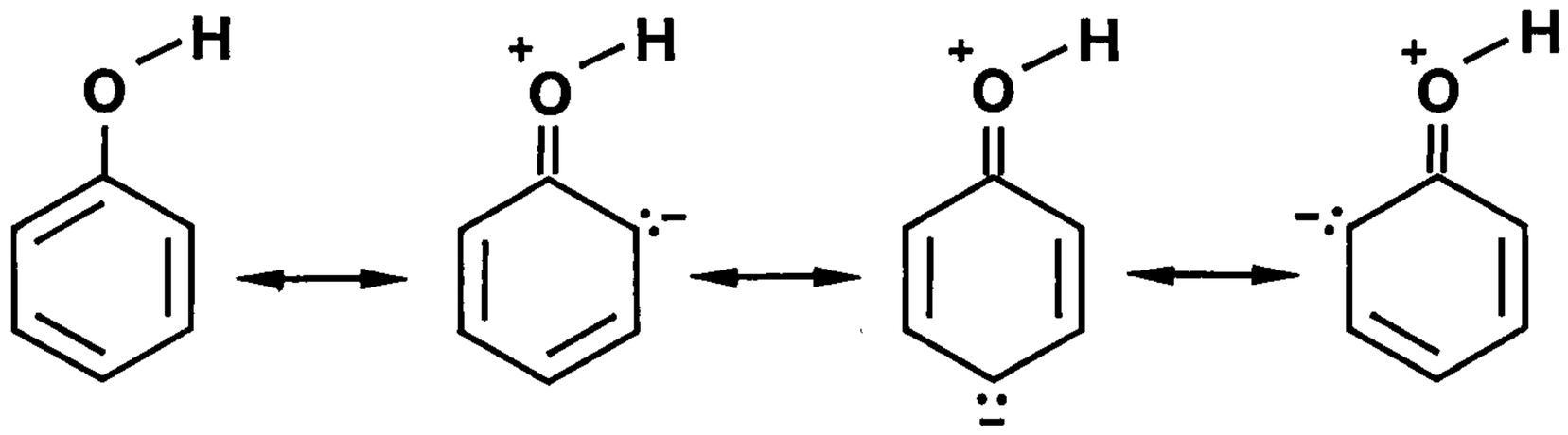
6.8 Interpretation of Partition Coefficients

Phenol

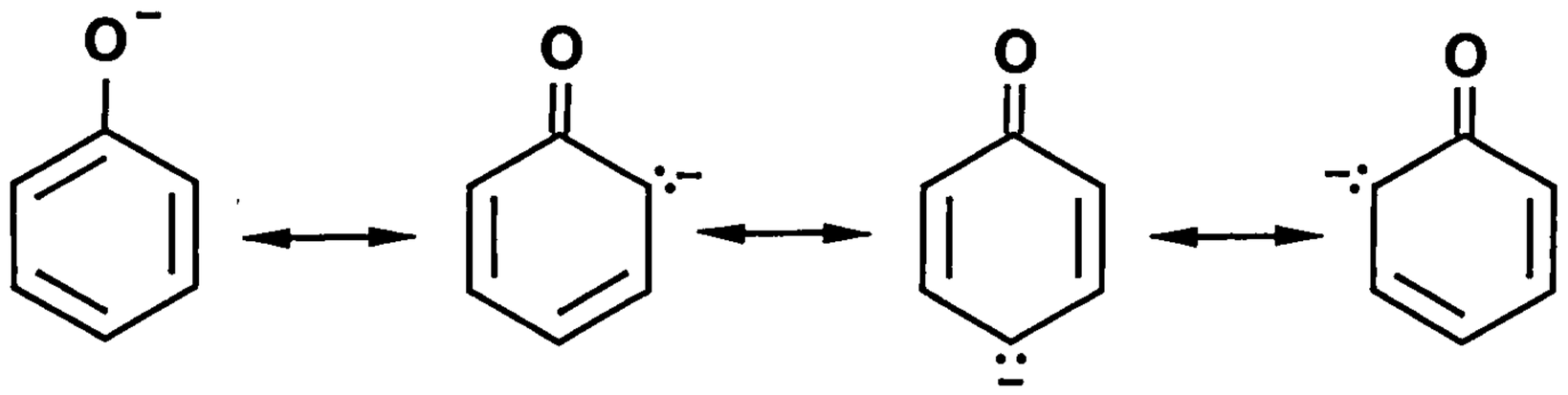
Most of the compounds studied in this thesis were substituted phenols, therefore the following discussion will be introduced by a description of phenol itself.

Generally phenols are more polar in character than the corresponding saturated alcohols which means they have a higher melting point and boiling point, are more soluble in water and more acidic. The acid nature of the phenol is due to the greater acidity of the hydroxyl group which means in the undissociated form the O—H bond is more strongly polarised as $\overset{\delta-}{O}—\overset{\delta+}{H}$ than for alcohols. Thus phenols form stronger hydrogen bonds.

One of the unshared electron pairs on oxygen is delocalised over the aromatic ring, increasing acidity.



The oxygen aquires a positive charge because of resonance and thus proton release is facilitated. Loss of the hydroxyl proton converts phenol to the phenoxide anion and leads to substantially greater delocalisation of the unshared electron pair because no charge separation is involved of the type apparent above - only the negative charge is spread.



Therefore the anion is stable and the ionization process is energetically favourable.

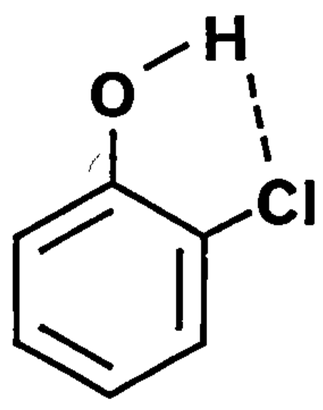
The excess of electrons in the π-orbital systems of phenol and the phenoxide ion makes them susceptible to electrophilic substitution in the ortho and para positions.

The effect of a ring substituent on the acid strength of phenols depends on whether the group is electron attracting or releasing, its ability to enter into resonance with the hydroxyl group, and its position. Effects in the ortho position are similar to those in the para position, but there are added complications due to the steric effect and to hydrogen bonding (when this is possible).

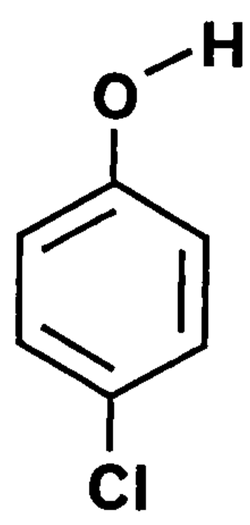
A. Intramolecular Hydrogen Bonding Compounds

i. Chlorophenols

In these molecules, an intramolecular hydrogen bond occurs in the ortho isomer so this compound has different physical properties to the meta or para isomers.



intramolecular hydrogen bond



only intermolecular hydrogen bonds

Isomer	Boiling Point	Melting Point	Solubility @ 20°C(aq)
ortho	175.6°C	-----	2.85g/100ml
meta	214°C	32.8°C	2.60g/100ml
para	217°C	43(39-40)°C	2.71g/100ml

The intramolecular hydrogen bond gives rise to ring formation or chelation. This usually produces a lower boiling point than expected, and this is demonstrated by the above figures.

Intermolecular hydrogen bonding gives rise to association which produces a higher boiling point than expected for example from the molecular weight of the compound.

Hydrogen bonds occur only with atoms with high electronegativity - fluorine, oxygen and nitrogen (decreasing in this order) and to a lesser extent chlorine and sulphur. The energy of a hydrogen bond varies:

H—F ..... H	41.84 $kJmol^{-1}$
H—O ..... H	29.29 $kJmol^{-1}$
H—N ..... H	8.37 $kJmol^{-1}$

The geometry of Z—H and Y is little changed when the hydrogen bond produces the complex Z—H....Y. Hence the bond length of Z—H is almost the same in both Z—H and Z—H....Y. A number of factors contribute, but the most important one is electrostatic. In bond Z—H, if Z has high electronegativity there will be a relatively large amount of polarity i.e. the state of affairs will be $Z—H^{\delta-\ \delta+}$, where $\delta+$ is relatively large. Since the H atom has a tiny volume, the $H^{\delta+}$ will exert a large electrostatic force and so can attract atoms with a relatively large $\delta-$ charge, providing these atoms have a small atomic radius: Fluorine, oxygen and nitrogen are of

this nature. If the atom has a greater radius, the electrostatic forces are weaker; thus, chlorine, although it has approximately the same electronegativity as nitrogen, forms very weak hydrogen bonds since its atomic radius is greater. The weak intramolecular hydrogen bond is easily broken, as indicated by the solubility of the o-isomer in water. Solubility would be much lower with a strong intramolecular hydrogen bond. This degree of solubility indicates the solute can form hydrogen bonds with the solvent.

Acid Strength

	pKa		
Phenol	o-Cl	m-Cl	p-Cl
9.98	8.48	9.02	9.38

Groups with a +R effect, e.g NH_2, MeO, weaken the phenol when in the para position, but strengthen it when in the meta position. In the latter case only the -I effect can operate. Halogens have both a -I and +R effect. Since halogenophenols are stronger acids than phenol, it follows that the -I effect of a halogen atom is greater than its +R effect.

Partition Coefficient

In the octanol/water system, the addition of a chloro-group to the phenol molecule produces an increase in log P, that is, it makes the molecule more lipophilic. The ortho effect can be seen quite clearly in this group, the meta and para isomers have similar values of log P and the ortho effect has lowered log P. This is a typical result when active extractants (higher alcohols, acetic esters) are used which form intermolecular hydrogen bonds with aromatic hydroxy

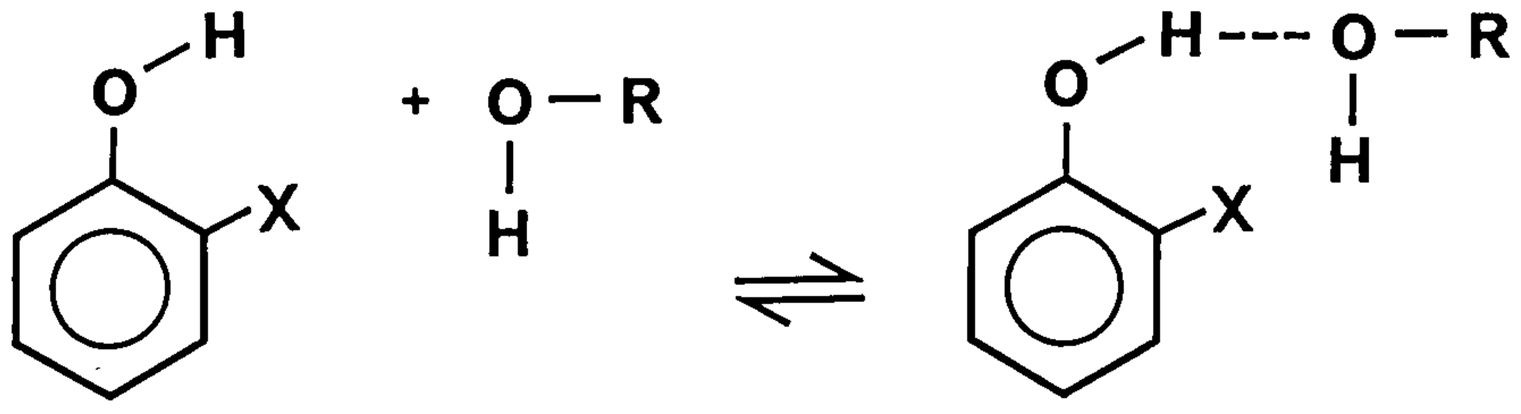
compounds.

In the cyclohexane/water system, the addition of the chloro group also increases log P, but in this case the ortho effect causes a further increase in log P of the ortho isomer.

This shows that the type of intermolecular interaction determines the character of the influence of the ortho effect on the partition coefficient. With low activity solvents the ortho effect raises the partition coefficient and with active solvents it lowers log P.

The presence of a substituent in the ortho position makes solvation of organic compounds by active solvents difficult. In this situation it is necessary to overcome an intramolecular hydrogen bond.

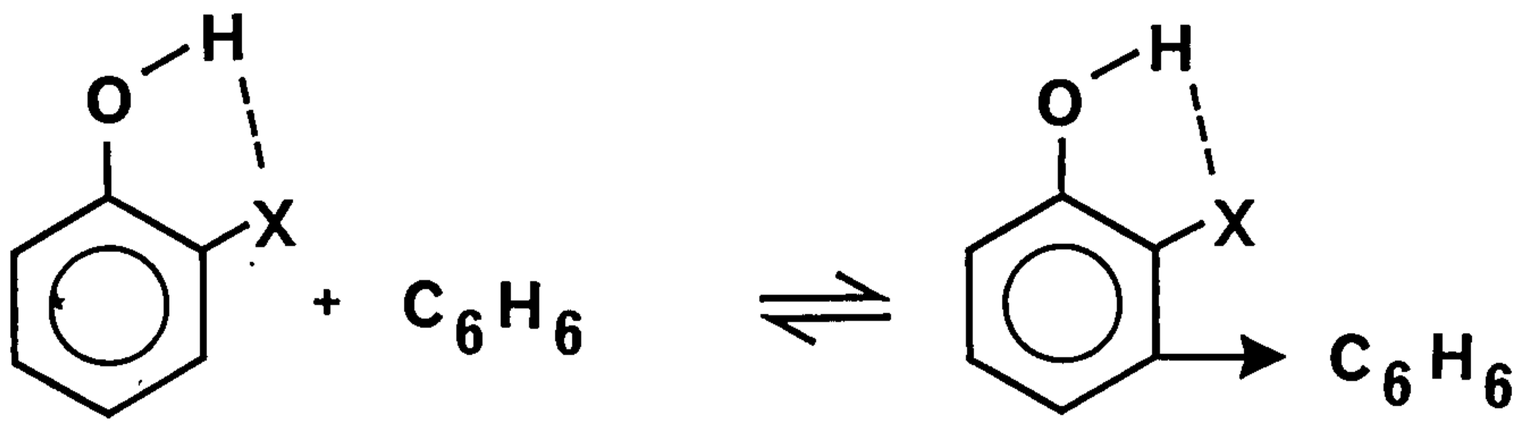
e.g Extraction of halophenols by higher alcohols



Such a phenomenon is not observed in the extraction of meta or para isomers.

Solvation by less active solvents goes by a different mechanism in which the intramolecular hydrogen bond is not affected. For example, extraction by aromatic hydrocarbons

goes by a scheme with the formation of a π complex.



It has been shown by Davis et al (94,93) that the thermodynamic properties of drug molecules in solution can be assumed to be an additive-constitutive property of the various functional groups. Group values for the free-energy term can be determined and used in a priori predictions of solution properties, including solubility and partition behaviour, and in analyses of structure-activity relations of congeneric drug series.

In connection with this theory, Davis et al (96) examined partition coefficient data for halogenated aromatic solutes and group contributions to log P were determined by comparing the partition behaviour of a substituted compound with that of the parent compound. This may be compared with the present work.

<u>Halogen group contributions (ringsystems) to partition between water and organic solvent</u>

Solvent & Group X	Log P_X	System	Ref
<u>Cyclohexane</u>			
2Cl	0.80	Phenol	58
	1.56	"	This work
3Cl	0.17	"	58
	0.69	"	This work
4Cl	0.39	"	58
	0.44	"	This work

Solvent & Group X	Log P_X	System	Ref
Octanol			
2Cl	0.69	Phenol	145
	0.63	"	This work
3Cl	1.04	"	145
	0.99	"	This work
4Cl	0.93	"	145
	0.92	"	This work

Group contributions are dependent on both the solvent system and the position of the group (94). Halogen substitution in the ortho position results in a pronounced interaction of the halogen with the functional group of the parent compound (electronic and steric effects) and the group values are very different to those for the same substituent in the meta or para positions. In general, ortho substitution results in a larger log P value than for other positions if the organic solvent is non-polar and the converse if the solvent is polar. This can be associated with dominant solute-solute interactions in the first case and dominant solute-solvent interactions in the second case since solutes containing halogen groups will exhibit a dipole. For polar solvents there will be the chance for marked dipole-dipole interactions between solute and solvent.

In general, the group values for polar solvents are higher than those for the non-polar solvents, indicating that the presence of dipole-dipole solvent-solute interactions results in a significantly higher value for log P.

Davis (95) compared group values (log P) in polar and non-polar solvents with other group values and parameters describing group size. The best fit was obtained with group area as determined from atomic models. This close dependence

of the halogen group contributions on group area is in line with the suggestion that the energy for hole formation in the solvent (into which the solute is placed) is dependent on the surface area of the solute rather than its volume. (193,252,288)

Halogen groups should act to withdraw electrons from the ring allowing it to interact more strongly with nearby solvent molecules (water or polar organic solvent) and a relationship has been found between polar and non-polar group values and the Hammett electronic substituent constant (165) This approach is similar to that described by Wulfert et al (389) who suggested that the electronic properties of a substituent can affect solubility. Davis (94) therefore examined the correlation between non-polar and polar group values using regression analysis.

$$\log P_{polar} = 0.99 \log P_{non\text{-}polar} + 0.96\sigma + 0.04$$

$$n = 5 \quad r = 0.995 \quad s = 0.07$$

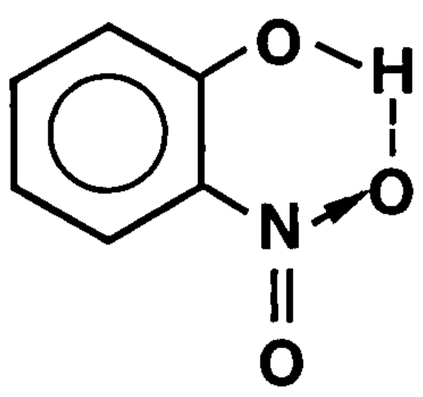
(n=no.of data, r=correlation coefficient, s=standard deviation)

Since the two coefficients are almost unity and the constant is almost zero, the equation could be written:

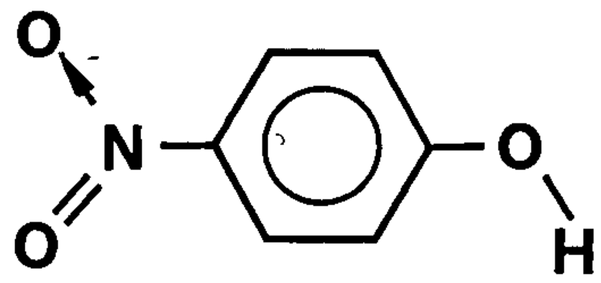
$$\log P_{polar} = \log P_{non\text{-}polar} + \sigma$$

ii. Nitrophenols

Orthonitrophenol, in which the hydroxyl group is ortho to the nitro group with which it can form a hydrogen bond, has exceptional physical properties compared with the meta or para isomers. Since the formation of intra- rather than intermolecular hydrogen bonds reduces intermolecular attraction, boiling point is reduced and solubility in non-polar solvents is increased.



intramolecular hydrogen bond



only intermolecular hydrogen bonds

Isomer	Boiling Point	Melting Point	Volatility with Steam
ortho	216°C	45°C	+
meta	194°C @70mm	97°C	-
para	-----	114°C	-

Many substances which can form intramolecular hydrogen bonds are volatile with steam, whereas the corresponding meta and para isomers are much less so.

Acid Strength

Since the nitro group can enter into resonance (-R) with the hydroxyl group from both the ortho and para positions, there is increased resonance in the contributing structures and so o- and p-NO_2phenol are stronger acids than phenol.

	pKa		
Phenol	o-NO_2	m-NO_2	p-NO_2
9.98	7.23	8.40	7.15

A m-NO_2 group cannot enter into resonance with the hydroxyl group, but it can exert a -I effect from this position. Hence, m-NO_2phenol is a stronger acid than phenol, but is not so strong as the ortho and para isomers.

Solubility

Solubility in hydroxylic solvents depends on, among other things, the power to form hydrogen bonds with the solvent. Phenol can form these bonds and hence a certain solubility in water can be expected. This argument also applies to substituted phenols since the hydroxyl group is still present, but in the ortho compounds, because chelation is possible, hydrogen bonding with the solvent water molecules is hindered and hence solubility is lowered.

Isomer	Solubility in Water
ortho	0.21g in 100ml @ 20°C
meta	1.35g in 100ml @ 25°C
para	1.60g in 100ml @ 25°C

Partition Coefficient

In the octanol/water system, the nitro group causes an increase in the hydrophobicity of the phenol molecule, but this is less than expected in the case of o-NO_2phenol.

In the cyclohexane/water system, the nitro group in either the meta or para positions produces a reduction in hydrophobicity, but in the ortho position a large increase is seen.

Nitro Group Contributions (Ring System) to Partition Between Water and Organic Solvents

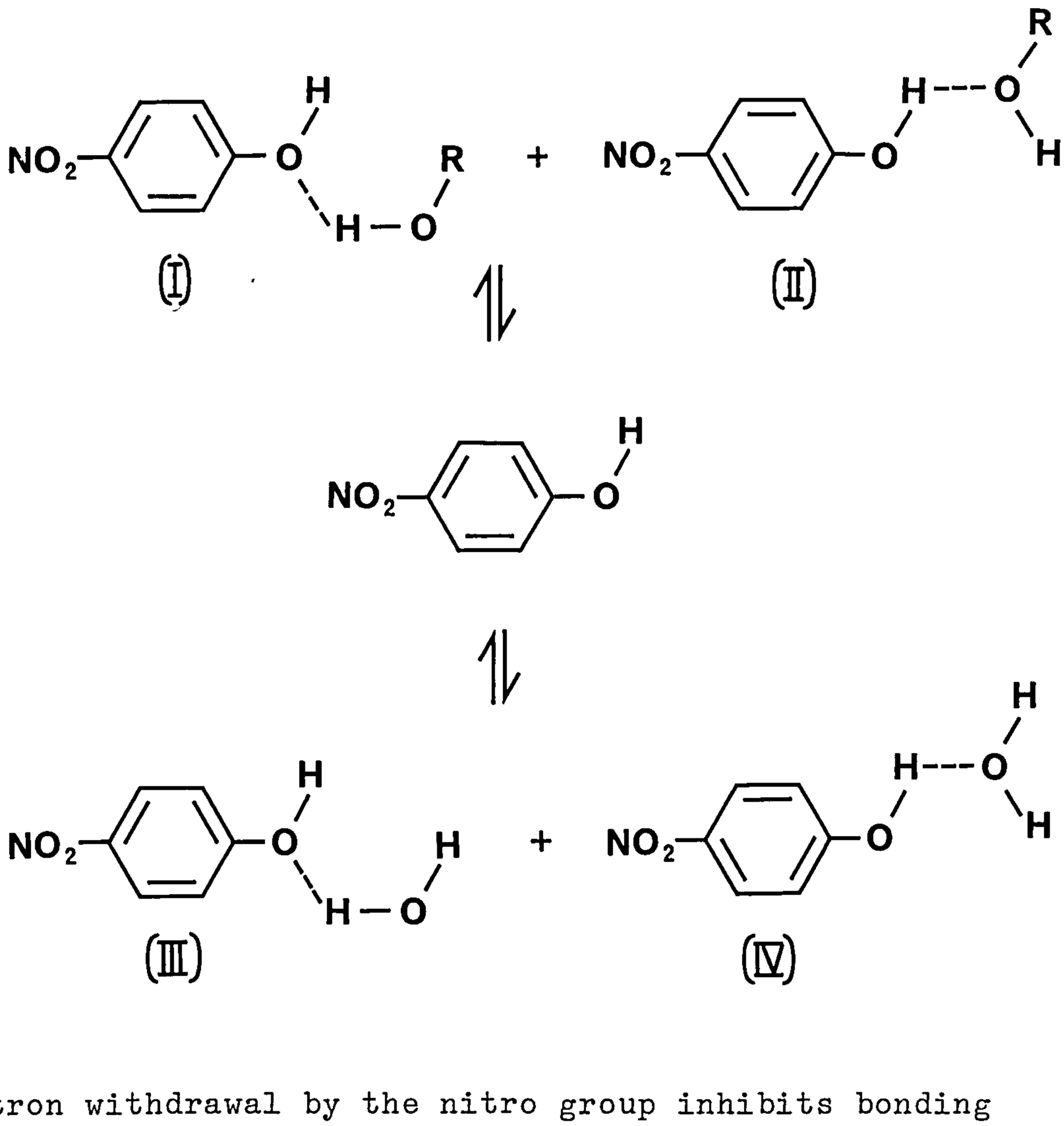
Solvent	$\log P_{NO_2}$	Solute System
Octanol	0.26	o-NO_2phenol
	0.51	m-NO_2phenol
	0.45	p-NO_2phenol
Cyclohexane	2.14	o-NO_2phenol
	-0.83	m-NO_2phenol
	-1.16	p-NO_2phenol

The nitrophenols occupy a special position among substituted phenols. Phenol and the majority of its derivatives are very weak acids (ionization constants of order of 10^{-10} - 10^{-11}) whereas nitrophenols have comparatively high ionization constants (10^{-9} - 10^{-8}) (238). The partition coefficients measured at different concentrations do not vary, therefore nitrophenols do not undergo dimerization.

The introduction of a nitro group into phenol lowers water solubility, especially for o-NO_2phenol. One litre of water dissolves 0.66mole of phenol (at 16°C) and only 0.016mole of o-NO_2phenol (at 25°C). However, the reduction in aqueous solubility is not the explanation of the partitioning results since the partition coefficients do not reflect the relative aqueous solubilities.

The effect of the nitro group and other similar electron withdrawing substituents on the π electron system of the benzene ring is thought to be due to a π-inductive effect in the ground state. It has been shown (145) that the nitro group itself has no preference for the octanol or the aqueous

phase. Therefore, it is surprising that the partition coefficient of phenol is increased to 1.94 in the case of p-NO_2phenol. Since the chief electronic effect of the nitro group is electron withdrawal, and since increased polarization should result in increased water solubility, the observed octanol/water partition values must be related to the hydrogen bonding ability of the molecule.



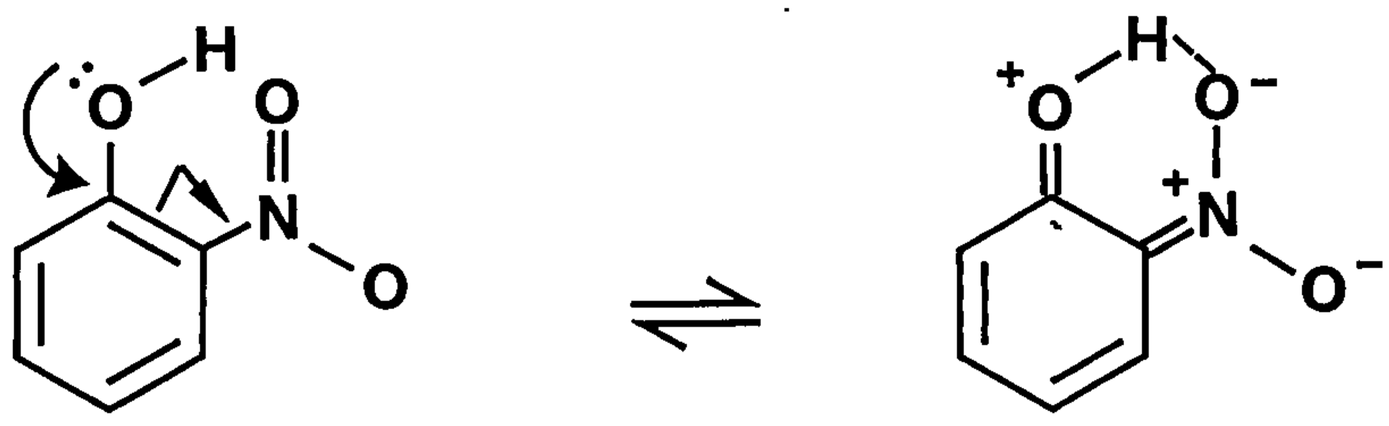
Electron withdrawal by the nitro group inhibits bonding shown by I and III, but conversely, promotes bonding of the type shown in II and IV. Octanol (ROH), being more nucleophilic than water, would compete more effectively in bonding of types II and IV. Electron withdrawal by this means can raise the partition coefficient of phenol (octanol/

water). To a lesser extent, electron withdrawal from the hydroxyl oxygen would inhibit the water-solubilising forces indicated by III.

The inductive effect of the nitro group is however responsible for the reduced partition coefficients of meta- and para-nitrophenol in the cyclohexane/water system.

In both solvent systems substitution of the nitro group ortho to the hydroxyl group has an effect on the partition coefficient. This is due to the formation of an intra-molecular hydrogen bond. The presence of this bond is revealed by I.R and U.V spectra. (Chapter 7).

In comparison with other ortho-substituted phenols, the intramolecular bond in o-NO_2phenol is of unusual strength as a result of the contribution of resonance structures V and VI leading to the formation of a 6-membered chelate ring having optimum size for stability. (20) as opposed to a 5-membered ring. Proton donation by OH and proton acceptance by NO_2 occur simultaneously and this is reflected in a spectral shift of 300 to 500 cm^{-1} from the standard stretching frequency for the free OH group (3610cm^{-1}) in phenol itself.



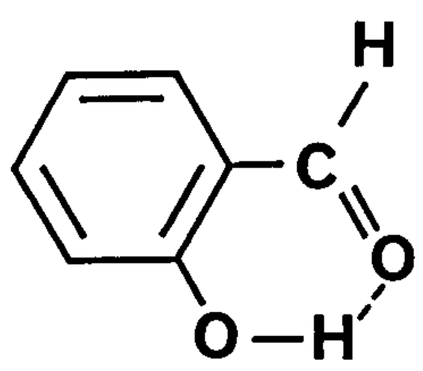
The effect of the intramolecular hydrogen bond is very marked when the lipophilic phase is cyclohexane. A small part of the effect may be accounted for by shielding which is discussed in detail with reference to the methylphenols and methylacetanilides, but the greater part of the higher partition coefficient of the ortho isomer can be attributed to intramolecular hydrogen bonding. This has the effect of increasing the partition coefficient of the ortho isomer relative to the meta isomer by 1000 times. Therefore it is a very powerful effect. The intramolecular hydrogen bond reduces polarity and therefore encourages dissolution in non-polar solvents.

In contrast, there appears to be little effect of intramolecular hydrogen bonding on octanol/water partition. This suggests that the affinities of water and octanol for the polar groups of the solute are about the same. The partition coefficients of the intramolecularly hydrogen bonded phenols are lower than those of their isomers, which suggests that steric and field effects are operating.

The ortho effect which operates in o-NO_2phenol is explained in the section dealing with the Chlorophenols.

iii. Hydroxybenzaldehydes

An intramolecular hydrogen bond is present in the ortho isomer which means its physical properties are different to the meta and para isomers. The presence of this bond is also revealed by the spectral data discussed in Chapter 7.



intramolecular hydrogen bond

Isomer	Boiling Point	Melting Point	Volatility with Steam	Solubility in Water
ortho	196.5°C	-7°C	+	1.72g/100ml @ 86°C
meta	240°C	108°C	-	2.78g/100ml @ 43°C
para	------	117°C	-	1.38g/100ml @ 30.5°C

The intramolecular hydrogen bond reduces intermolecular interaction. Therefore the boiling point and melting point of the ortho isomer are reduced. However, it also reduces interaction with polar solvents and so the solubility in water is also reduced.

Partition Coefficient

Substitution of the aldehyde group in phenol has the same effect on hydrophobicity in both the octanol/water system and the cyclohexane/water system. In the meta or para position the aldehyde group reduces P, but in the ortho

position it increases P. Both effects are larger in the non-polar system; i.e. negative values are more negative and vice-versa.

Aldehyde Group Contributions (Ring System) to Partition Between Water and Organic Solvents

Solvent	$\log P_{CHO}$	Solute System
Octanol	0.18	o-OHbenzaldehyde
	-0.17	m-OHbenzaldehyde
	-0.14	p-OHbenzaldehyde
Cyclohexane	2.12	o-OHbenzaldehyde
	-1.22	m-OHbenzaldehyde
	-1.55	p-OHbenzaldehyde

These results indicate that the aldehyde group causes the solubility of the solute to increase in water. The aldehyde group is electron withdrawing and this causes increased polarization of the molecule which results in increased water solubility and hence a lowered partition coefficient. The effect on solvent bonding as described in the section on Nitrophenols probably occurs with the hydroxybenzaldehydes thus accounting for the less negative P values in octanol than in cyclohexane, but the aldehyde group is not as powerful an electron withdrawer as the nitro group so that the effect is not as marked. Thus, whereas in the nitro-phenols the influence of electron withdrawal on solute-solvent interactions is dominant, in the hydroxybenzaldehydes the influence of electron withdrawal on polarization of the molecule is dominant, so that $\log P_{CHO}$ is negative.

The increased partition coefficient brought about by ortho substitution is due to intramolecular hydrogen bonding.

Substitution of the aldehyde group ortho to the hydroxyl group promotes the formation of a strong intramolecular hydrogen bond (314) formed by a 6-member ring characterized by great stability and the prevailing presence of one dominating form in equilibrium.

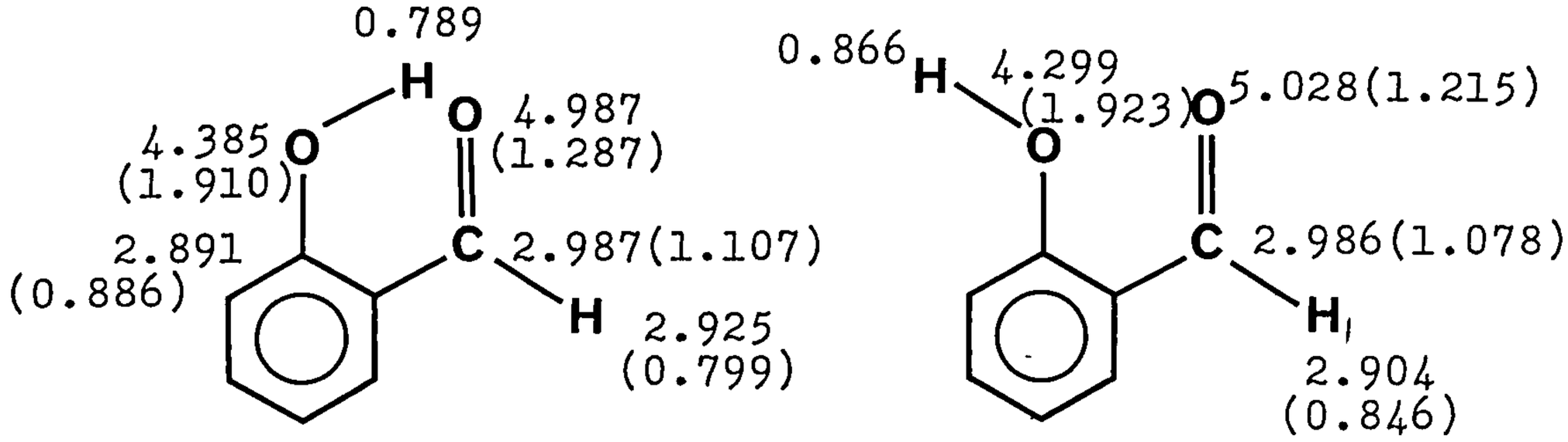
According to NMR and IR spectral data, the change in chemical shift in the NMR spectra and the frequency change of IR valence vibrations of the OH group are caused by the formation of a strong intramolecular hydrogen bond, O—H....O=C.

The 6-member rings formed are characterized by a planar π electron system joining both functional groups and the charge transfer along the H-bond is compensated by the reverse flow of π electrons.

According to the results of charge distribution studies of the σ and π electron density of the cis and trans isomers of o-OHbenzaldehyde shown in Figure 28 (326) the influence of the formation of the intramolecular hydrogen bond may be characterized quantitatively as follows. The formation of the hydrogen bond polarises the σ 'core' in such a way that the σ electron density for the carbonyl oxygen is reduced by 0.041 while for the hydroxyl oxygen it is increased by 0.087.

This shift of the σ electron density from the carbonyl oxygen towards the hydroxyl oxygen causes a reverse shift of π electrons ('back-donating'). According to Figure the result of the formation of the H-bond is lowering of

Figure 28. σ and π Charge Distribution (in parenthesis) of Electron Density of the cis and trans Isomers of 2-hydroxybenzaldehyde



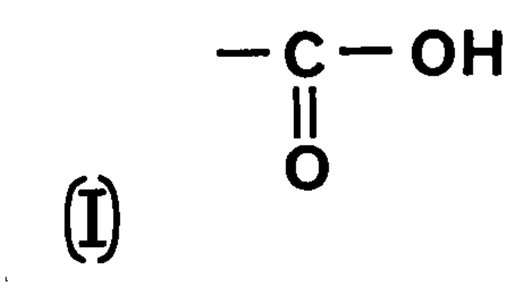
the electron charge by 0.077 for the hydrogen atom, an increase of 0.031 for the carbonyl oxygen and of 0.074 for the hydroxyl oxygen. Further, the electron charge decreases for both carbon atoms joined with oxygen atoms. This results in a bond of energy 33 - 44 kJ/mol (7.8 - 10.5 $kcalmol^{-1}$).

Again, the effect of the intramolecular hydrogen bond in o-OHbenzaldehyde on partitioning may be compared to that in o-NO_2phenol. The intramolecular hydrogen bond reduces the polarity of the molecule and hence reduces aqueous solubility. This has a marked effect on partitioning in cyclohexane, since cyclohexane is non-polar, the now much less polar molecule will have a far greater affinity for cyclohexane compared to water. The effect is not so dramatic in octanol. This solvent is polar and solubility is governed by interactions between the polar molecule and solvent, in a similar manner to that which occurs in water. Thus once the polarity of the solute is reduced it is only marginally more attracted to the octanol phase than the water phase.

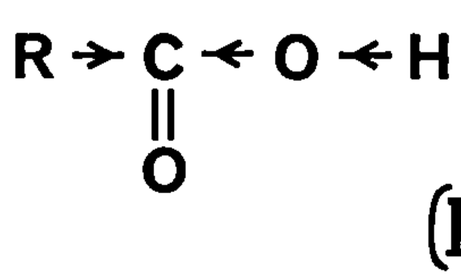
Benzoic Acid

Acid Strength

If the structure of the carboxyl group were simply (I) then because of the -I effect of the carbonyl group (as shown in II), proton release would be facilitated as compared with alcohols.

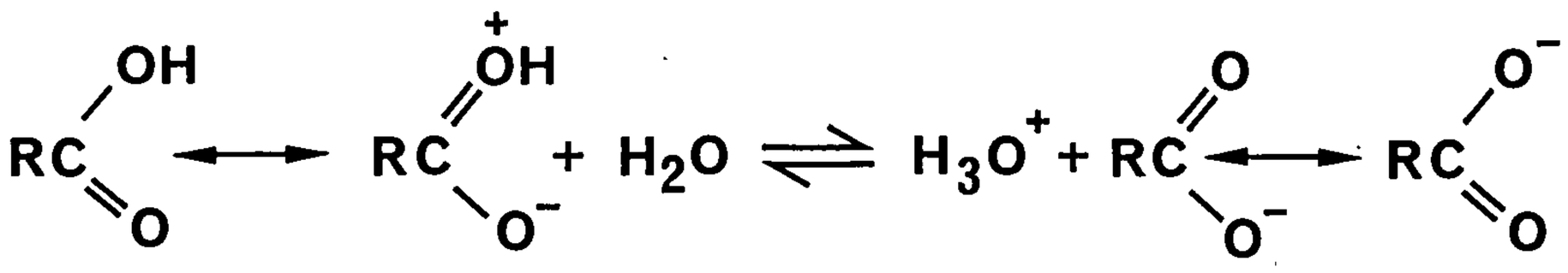


(I)



(II)

Thus carboxylic acids would be stronger acids than phenols. However, the inductive effect alone cannot account for the very large difference in acid strength. One explanation is that the carboxyl group is a resonance hybrid and because of the positive charge on the oxygen atom of the hydroxyl group, proton release is facilitated; this positive charge is absent in alcohols since they are not resonance hybrids.



This argument can be extended as follows. When the acid ionises, i.e. donates its proton to water (solvent), the conjugate base of the acid i.e. the carboxylate ion, is a resonance hybrid of two equvalent resonating structures. At the same time, since the negative charge is spread, the resonance energy of this anion will be greater than that of the un-ionised acid, which is a resonance hybrid of two different resonating structures, one with separation of unlike charges. Therefore, the internal energy of the anion

is lower than that of the un-ionised acid. In the case of alcohols, there is no resonance stabilisation in the alkoxide ion. It therefore follows that ΔG^{θ} for the ionisation of monocarboxylic acids will be more negative than that of alcohols. Hence the equilibrium constant of the former will be greater than that of the latter i.e. the pKa values of the acids will be lower than those of the alcohols. Thus alcohols are weaker acids than the monocarboxylic acids.

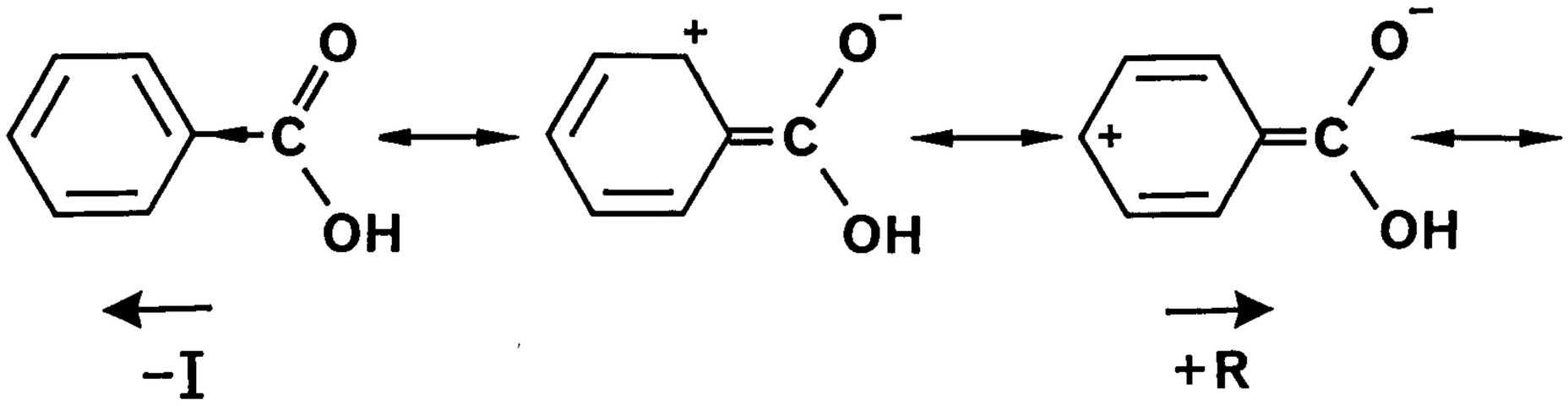
X-ray and electron diffraction measurements of the carboxylate ion have shown that the two C-O bonds are of equal length. This is in keeping with the ion being a resonance hybrid.

Strength of Aromatic Acids

Replacement of the hydrogen atom in formic acid by an alkyl group weakens the strength of the acid, and the greater the +I effect of the R group, the weaker the acid. Phenylacetic acid ($PhCH_2CO_2H$), pKa 4.31, is stronger than acetic acid (pKa 4.76) and therefore the phenyl group has a -I effect. On the other hand, benzoic acid (pKa 4.17) is weaker than formic acid (pKa 3.75). In this case the phenyl group has a +I effect (which is smaller than that of a methyl group). These apparently contradictory results may be explained as follows. When the carboxyl group is directly attached to the nucleus, the resonance effect (+R) overcomes the -I effect. In phenylacetic acid the phenyl group is insulated from the carboxyl group by a CH_2 group and so the +R effect is not possible.

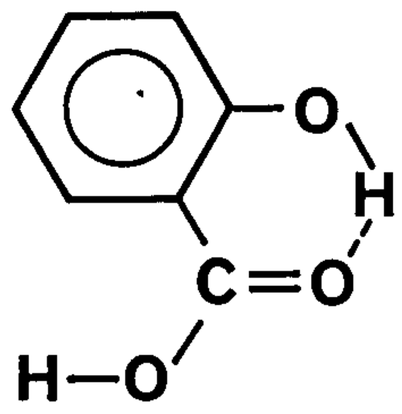
This prevents to a large extent, the lone pair on the oxygen atom of the hydroxyl group from entering into resonance with

the carboxyl group. The result is a smaller positive charge on the oxygen atom of the hydroxyl group, and so proton release is more difficult than in formic acid. The fact that benzoic acid is stronger than acetic acid means that {-I + (+R)} < +I of the methyl group.



iv. Hydroxybenzoic Acids

An intramolecular hydrogen bond is present in the ortho isomer which means it has different physical properties to the meta and para isomers.



intramolecular hydrogen bond

Isomer	Boiling Point	Melting Point	Volatility with Steam	Solubility in Water
ortho	211°C@20mm	158°C	+	0.18g/100ml @ 20°C
meta	-----	201°C	-	0.92g/100ml @ 18°C
para	subl 76°C	anh 167d	-	0.79g/100ml @ 15°C
$2,6\text{-}OH_2$	-----	232 - 3°C		
$3,5\text{-}OH_2$	-----	(anh 237)		

The ortho isomer behaves as a phenol and an acid. It is a atronger acid than its meta and para isomers.

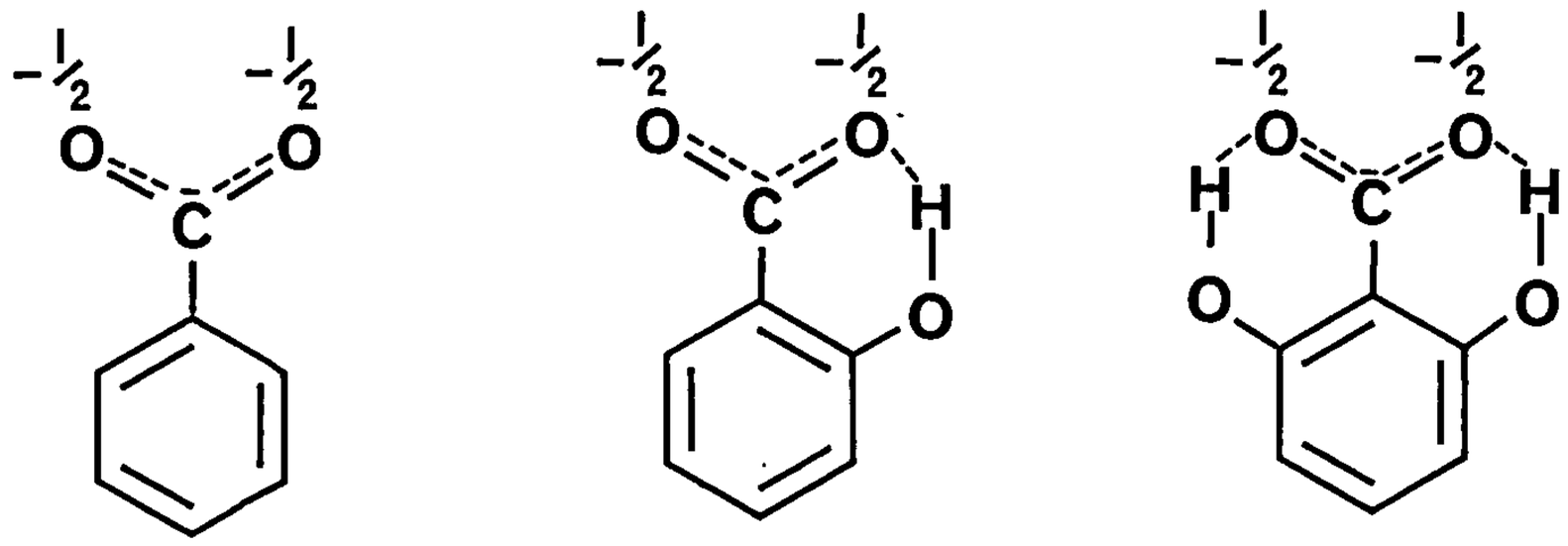
	pKa				
Benzoic acid	o-	m-	p-	$2,6\text{-}OH_2$	Phenol
4.17	2.98	4.08	4.58	2.30	9.98

The hydroxyl group has a strong -I effect and therefore tends to draw electrons from the ring. The 'key' atom however in this group has a lone pair of electrons and so the +R effect is possible, which is stronger than the I effect. This group can enter into resonance with the carboxyl group in the para position, but not from the meta position, in which case it is the -I effect which operates. Thus the para substituted acid will be expected to be weaker and the meta substituted acid stronger than benzoic acid.

The ortho effect operates such that o-OHbenzoic acid is far stronger than the corresponding meta and para isomers. Steric inhibition of resonance cannot explain this very large increase, since the corresponding methoxybenzoic acids all have similar strengths. The explanation offered is hydrogen bonding. The hydrogen of the o-OH group can form a hydrogen bond with the carboxyl group, whereas the Me of OMe cannot. The carboxylate ions of o-OHbenzoic acids are stabilised by intramolecular hydrogen bonding and support for this is given by the following order of acid strength:

2,6-diOH(pKa 2.30) > 2-OH(pKa 2.98) > Benzoic acid(pKa4.17)

It can be seen that two hydrogen bonds would be expected to bring about more stabilization than one hydrogen bond:



Partition Coefficient

Hydroxyl and Carboxylic Acid Group Contributions

(Ring System) to Partition Between Water and Organic Solvents

Solvent	$\log P_{OH}$	$\log P_{COOH}$	Solute System
Octanol	0.45	0.86	o-OHbenzoic acid
	-0.25	0.10	m-OHbenzoic acid
	-0.31	0.08	p-OHbenzoic acid
	-0.10	----	2,6-OH_2benzoic acid
	-0.54	----	3,5-OH_2benzoic acid
Cyclohexane	-0.57	-0.68	o-OHbenzoic acid
	-1.25	----	2,6-OH_2benzoic acid

Comparison of the two solvent systems is difficult with this group of compounds since they are virtually insoluble in cyclohexane. However, the fact that the two ortho substituted compounds were the only soluble isomers indicates that the intramolecular hydrogen bond increases the lipophilicity of the hydroxybenzoic acids.

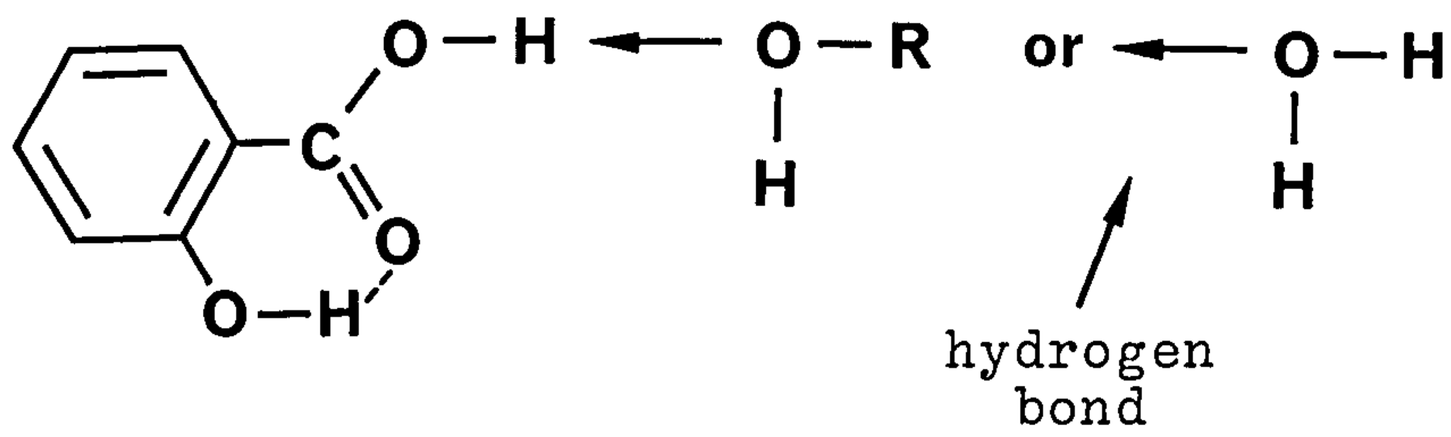
Both the hydroxyl group and the carboxylic acid group are in general considered to give negative group contributions (97, 237) indicating that the solubility of the solute in the aqueous phase has been increased by the presence of the group, through a hydrogen bonding interaction mechanism. However,

although this is seen to be true of the hydroxyl group in both the octanol/water and cyclohexane/water systems, it is not true of the carboxylic acid group in the octanol/water system. The log P value is small, but nevertheless, positive. Thus it would appear that the carboxylic acid group is also able to interact with octanol through hydrogen bonding.

The hydroxyl group values are smaller (less negative) for the octanol/water system than for the cyclohexane/water system, because in the former system strong hydrogen bonding interactions can take place between the OH group attached to the solute and the OH group in the solvent. This will result in an enhanced lipid solubility compared to an inert solvent such as cyclohexane.

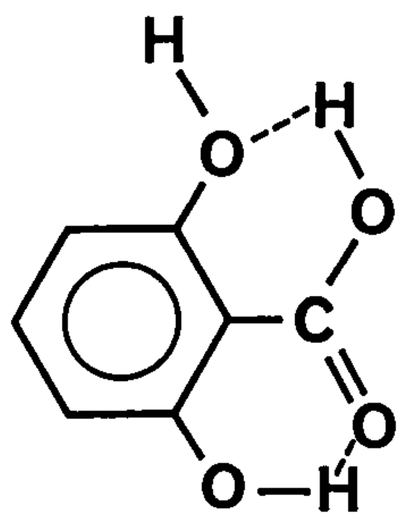
The partition coefficients of the meta and para hydroxy benzoic acids are of the same order of magnitude, however, if the groups are ortho to each other an intramolecular hydrogen bond can be formed which results in a higher partition coefficient for o-OHbenzoic acid in comparison with phenol and other hydroxybenzoic acid isomers. This also follows from a comparison of the solubilities of hydroxy-benzoic acids in water: at 15-20^{o}C 100ml of water dissolves 0.92g of m-OHbenzoic acid, 0.79g of p-OHbenzoic acid and only 0.18g of o-OHbenzoic acid. The log P values of both hydroxyl and carboxylic acid groups increase to become positive in the ortho position, indicating a greater affinity for the octanol phase. The extent of this seems unusual, since although one hydrogen bonding group is removed, the carboxylic acid moiety has a spare hydroxyl group also capable of hydrogen bonding.

Thus:



Octanol, being more nucleophilic than water can compete more effectively to bond with the hydroxyl hydrogen and thus a positive log P is found. This does not occur in cyclohexane and bonding between the benzoic acid and water is dominant so log P remains negative.

The addition of a second hydroxyl group in position 6, produces a reduction in log P and log P_{OH} in both octanol and cyclohexane systems becomes negative. This indicates that the introduction of the second hydroxyl group destroys solute-octanol interactions.



The second hydroxyl group can also form an intramolecular hydrogen bond and it is possible that this produces a structure which restricts the proximity of approach of either the octanol or the cyclohexane molecules. Only water molecules

are small enough to approach and they may form hydrogen bonds so increasing water solubility of 2,6-OH_2benzoic acid relative to o-OHbenzoic acid.

B. Sterically Hindered Compounds

i. Methylphenols

In the octanol/water system, the addition of a single methyl group to the phenol molecule, in any position, causes an increase in hydrophobicity of +0.50 as expected. A further increase in log P is observed with each additional methyl group but a smaller than expected increase in hydrophobicity is seen if two methyl groups are ortho to the hydroxyl group. The contribution of each additional methyl group also falls as the molecule becomes more crowded.

In the cyclohexane/water system, the methyl substituent again increases hydrophobicity, but in this case a single methyl group ortho to the hydroxyl group produces a greater than expected increase in log P. This increased hydrophobicity is maintained by di-ortho substitution.

The ways in which the methyl group may affect hydrophobicity are described in the section on the Methylacetanilides, and as there, the conclusion that the methyl group simply increases the non-polar section of the molecule, thereby enhancing hydrophobicity may be applied to the methylphenols. However, as with the methylacetanilides, this property is influenced by the proximity of other substituents.

This will be discussed by considering the individual methyl group contributions to partition.

Methyl Group Contributions (Ring Systems) to Partition Between Water and Organic Solvents

Solvent	log P_{CH_3}	Solute System	Ref
Cyclohexane	0.89	2-Mephenol	This work
	0.85	"	152
	0.85(a)	2,6-Me phenol	This work
	0.82(a)	2,3,6-Me phenol	"
	0.81(a)	2,4,6-Me phenol	"
	0.73(a)	2,5-Me phenol	"
	0.74(a)	"	152
	0.70(a)	2;4-Me phenol	This work
	0.74(a)	"	152
	0.63(a)	2,3,5,6-Me phenol	This work
	0.61(a)	2,3-Me phenol	"
	0.61(a)	2,3,5-Me phenol	"
	0.59	4-Mephenol	"
	0.71	"	198
	0.62	"	152
	0.58	3-Mephenol	This work
	0.57	"	152
	0.50(a)	3,5-Me phenol	This work
	0.63(a)	"	152
	0.47(a)	3,4-Me phenol	This work
Octanol	0.57	3-Mephenol	145
	0.53	4-Mephenol	This work
	0.50	2-Mephenol	"
	0.48	4-Mephenol	145
	0.47	"	269
	0.47	3-Mephenol	269
	0.47	"	This work
	0.45(a)	3,5-Me phenol	269
	0.44(a)	"	This work
	0.42(a)	2,3-Me phenol	"
	0.42(a)	2,4-Me phenol	"
	0.42(a)	2,5-Me phenol	"
	0.41(a)	2,3,5-Me phenol	"
	0.38(a)	3,4-Me phenol	"
	0.37(a)	2,6-Me phenol	"
	0.37(a)	2,4,6-Me phenol	"
	0.35(a)	2,3,6-Me phenol	"
	0.32(a)	2,3,5,6-Me phenol	"
	0.30(a)	2,6-Me phenol	269

(a) = Value per CH_3

It was found that the mean preferred values of log P_{CH_3} fall as the solvent becomes more polar. Christian et al (69) noted that the solubility of water in various solvents was a good measure of their relative solvation ability and Leo et al (255) found that partitioning solvents could be ordered sensibly according to the amount of water they contained at saturation. Therefore the inability of a particular solvent to accommodate water is a good measure of its lipophilic behaviour to a wide range of solutes. Davis (94) shows that this is also the case for the functional groups $-CH_2-$ and $-CH_3$, for as the solubility of water in the solvent increases, group contributions fall. This can be seen clearly in the preceding table.

Therefore the CH_3 group cannot be ascribed a single group contribution value for a given solvent system. Its group value depends on its position in the molecule. A methyl group attached to a ring system has a group molar volume similar to that of the CH_3 group ($16.5 cm^3 mol^{-1}$) and similar thermodynamic group contribution to solubility and partition equilibria.

Crowding and possible electron release (favouring hydrogen bonding with water and hence lowering log P) by the methyl group may account for the lowering of partition coefficient. This will now be discussed in greater detail.

There are two general aspects of the lipophilic constant which must be kept in mind when comparing π from different solute systems. The value of π for a given function is, for the most part, determined by the intrinsic lipophilic or

hydrophilic character of the substituent. However, this intrinsic value can be influenced by the environment which includes both substrate and solvent; that is, π is defined operationally.

Boiling Point, Steric Hindrance and Partition Coefficient

Boiling Points and Partition Coefficients of Phenols - Cyclohexane/Water System

Compound	Boiling Point(at 760mm)	Part.Coeff.	Ref
Phenol	181.7	0.17	347
o-Mephenol	190.6	1.25	347
2,6-Me_2phenol	200.6	8.47	351
p-Mephenol	201.5	0.645	347
m-Mephenol	202.1	0.625	347
2,4-Me_2phenol	211.3	3.57	351
2,5-Me_2phenol	211.5	3.70	351
2,3-Me_2phenol	217.1	3.21	347
2,4,6-Me_3phenol	220.6	17.20	351
3,5-Me_2phenol	221.7	1.86	351
3,4-Me_2phenol	226.9	1.57	347
2,4,5-Me_3phenol	235.2	8.70	347
2,3,5-Me_3phenol	236.0	9.35	100
2,3,4-Me_3phenol	237.0		100
2,3,5-Me_3phenol	251.9	4.25	351

From the boiling points, a rough idea of the steric hindrance of groups present in the phenols may be visualised, on the assumption that intermolecular attraction will be less in the case of phenols whose substituents hinder one another sterically and hence possess a lower boiling point (334). Among isomers, the lowest boiling one would be the least soluble in the aqueous phase, i.e. it would have the highest partition coefficient because the lowest boiling

isomer has the greatest steric effect which prevents hydrogen bonding with water. The solubility of phenols in the aqueous phase is due mainly to their ability to form hydrogen bonds with water (385). Consequently, ortho substituted phenols, which are likely to have greater steric hindrance, will be less soluble in the aqueous solvent than the corresponding meta and para isomers. For the same reason, if the alkyl side chains increase in size, the steric effect will be predominant, more so in the case of ortho substituted phenols. In the case of ortho substituted and other alkyl substituted phenols, the solubility in the aqueous phase will decrease and hence partition coefficients increase as the size of the alkyl group increases. The partition coefficient of alkyl phenols will further increase owing to an increase in solubility in the cyclohexane phase with an increase in size of the alkyl groups. As these two factors operate simultaneously, the partition coefficients of alkyl phenols are likely to increase rapidly as the size of the alkyl group increases. Thus, in the case of homologous phenols, the partition coefficient will be decided by solubility in cyclohexane as well as in the aqueous phase, while in the case of isomeric phenols water solubility will be the deciding factor.

Effect of Substituents on Partition Coefficients

Size of Alkyl Substituents: A larger alkyl group in any position, ortho, meta or para, with respect to the phenolic hydroxyl gives a higher value of P. owing to decreased water solubility and increased cyclohexane solubility, associated with increased molecular weight. The increase

in size of an alkyl substituent will affect the partition coefficient of phenols nearly to the same extent whether they are ortho, meta or para substituted.

Position of Alkyl Substituents: As meta and para substituents are nearly equidistant from the phenolic group, their values of P will be close. This is found in the octanol/water system, but in the cyclohexane/water system p-phenols have invariably higher P values than the corresponding m-phenols; this is in keeping with the slightly lower boiling point of the para isomer and is also supported by the IR spectra of phenols. According to Friedel (140) alkyl substitution in the para position causes more intense OH bands than substitution in the corresponding meta position. Greater intensity of the OH band indicates greater steric hindrance of the compound. The remarkably higher partition coefficient of o-phenols can be explained by the very strong steric hindrance associated with the 'ortho effect'. (385)

Effect of Position of Side Chain on Partition Coefficient

Cyclohexane/Water System

	Isomer	Part.Coeff.	Boiling Point °C (334)
A.Cresols			
	m-cresol	0.692	202.1
	p-cresol	0.710	201.5
	o-cresol	1.450	190.6
B.Xylenols			
Gp.1	3,4-xylenol	1.62	226.9
	3,5-xylenol	1.82	221.7
Gp.2	2,3-xylenol	3.09	217.1
	2,4-xylenol	4.57	211.3
	2,5-xylenol	5.24	211.5
Gp.3	2,6-xylenol	9.33	200.6

Isomer	Part.Coeff.	Boiling Point °C (334)
C.Trimethyphenols		
3,4,5-Me_3phenol	4.25 *	251.9
2,4,5-Me_3phenol	8.70 *	235.2
2,3,5-Me_3phenol	12.02	236.0
2,4,6-Me_3phenol	49.00	220.6

* = Ref 334

The preceding table shows that for cresols, xylenols and trimethylphenols the higher boiling isomer has the lower partition coefficient. 2,3,5-Me_3phenol and 2,4,5-Me_3phenol appear to be exceptions, but this is understandable from consideration of the relative meta-para effects. These two compounds differ in the position of the second methyl group in the 3- or 4- position; probably the 3-Me confers a greater 'ortho effect' on the existing methyl group in the 2- position. In fact, according to Sears and Kitchen (337) a methyl group forces an adjacent o-Me group closer to the hydroxyl, increasing the steric hindrance. Similarly, 2,3,6-Me_3phenol has a greater partition coefficient than 2,4,6-Me_3phenol and 2,3,4-Me_3phenol will have a higher partition coefficient than 2,4,5-Me_3phenol. The 'hydrogen bonding index' of Sears and Kitchen (337)(zero for completely hindered and unity for unhindered phenols) is 0.29 for 2,3,6-Me_3phenol and 0.33 for 2,4,6-Me_3phenol.

Mono-substituent Ortho to the Hydroxyl Group: It has been pointed out that the cyclohexane/water partition coefficients of phenol and its alkyl derivatives are many times lower than their octanol/water partition coefficients, probably because octanol interacts strongly with the hydroxyl group of phenol.

The octanol/water π value for the o-methyl substituent in phenol is virtually identical with those for the meta and para methyl substituents. Therefore, either the ability of octanol to solubilise the hydroxyl group is not restricted in o-methylphenol, or the solubilising abilities of both octanol and water are restricted to the same extent. The first explanation is believed to be correct, since both the melting points and aqueous solubilities of o- and m-methyl phenol are similar (o-cresol 30°C; 3% @ 40°C: m-cresol 11-12°C; 2.5% @ 40°C (116)). These values indicate that the methyl substituent adjacent to the hydroxyl group does not appear to reduce the affinity of the hydroxyl group for water.

However, the cyclohexane/water π value for the o-methyl substituent in phenol is much greater than are those for m- and p-Me substituents. Since o-methylation does not reduce the affinity of the OH group for water, it follows that this high π value must be due to increased affinity for cyclohexane; i.e. the o-Me group shields the hydroxyl group from exposure to cyclohexane, thus reducing the effective polarity of the solute and permitting greater interaction with the solvent.

Disubstitution Ortho to the Hydroxyl Group: In the cyclohexane/water system, the shielding effect mentioned earlier for o-alkylphenols is almost as pronounced again on 2,6-dialkyl substitution, the π value of the second methyl group being +0.81 compared with +0.90 for the first. Again this cannot be due to reduced affinity for water, for the aqueous solubilities (0.66% and 0.51% w/v at 25°C (114)) and melting

points (49°C and 68°C) of 2,6-Me_2phenol and 3,5-Me_2phenol respectively, are similar whilst the cyclohexane solubility of the 2,6-isomer (82.5% w/v at 25°C) is far greater than that of the 3,5-isomer (5.2% w/v at 25°C) (114).

However, the octanol/water π value of a second identical alkyl group adjacent to the hydroxyl group is much lower than that of the first alkyl group ($\pi_{2\text{-Me}}$ = +0.50; $\pi_{6\text{-Me}}$ = +0.23) This indicates that the shielding of the hydroxyl group on both sides favours interaction with water. There may be a number of reasons for this:

1. The effect may be entropic. However, if this were so, 2,3-dialkyl substitution should give a similar hydrophobic effect to that of 2,6-dialkyl substitution. 2,6-Me_2phenol has log P = 2.22, 2,3-Me_2phenol has log P = 2.33 which is almost additive (1.46 + 0.49 + 0.50 = 2.45 (302)). Therefore it seems that if an entropic effect is present in the partition of these compounds, it is not strong and low logP values of 2,6-disubstituted compounds cannot be explained wholly in entropic terms.

2. Dearden and O'Hara (112) proposed that the effect arises from restricted solvent access to the hydroxyl group. It was pointed out earlier that such shielding gave rise to higher than expected partition coefficients in cyclohexane/water partition. However, in the octanol/water system, both solvents are competing, largely for the hydroxyl group. Mono-alkyl ortho-substitution does not restrict access of either solvent, but alkyl substitution on both sides of the hydroxyl group could well restrict access of the larger solvent molecule to a greater extent; such restriction has

been demonstrated in IR studies of 2,6-dialkylphenols (395) A shielding effect would be expected to increase with the size of the shielding groups and this is indeed the case.

Effect of Substituent Size on Partition Coefficient Octanol/Water System (112)

Substituent	Log P	ObsΣalkylπ	Calc.Σalkylπ	ΔΣalkylπ (obs-calc)
2-Et	1.306	0.995	-------	----
2,6-Et_2	1.874	1.563	2Et+2Et=1.990	-0.427
2-iPr	1.707	1.396	-------	----
2,6-iPr_2	2.671	2.360	2iPr+2iPr=2.792	-0.432
2-tBu	2.357	2.046	-------	----
2,6-tBu	3.180	2.869	2tBu+2tBu=4.097	-1.223

The shielding effect is particularly pronounced for 2,6-di-t-butyl substitution.

ii. Methylorthonitrophenols

Methyl Group Contributions (Ring System) to Partition Between Water and Organic Solvents

Solvent	$\log P_{CH_3}$	Solute System
Octanol	0.40	3-Me-2-NO_2phenol
	0.50	4-Me-2-NO_2phenol
	0.60	5-Me-2-NO_2phenol
	0.77	6-Me-2-NO_2phenol
Cyclohexane	-0.08	3-Me-2-NO_2phenol
	0.59	4-Me-2-NO_2phenol
	0.32	5-Me-2-NO_2phenol
	0.55	6-Me-2-NO_2phenol

The methyl group increases the hydrophobicity of o-NO_2phenol in both solvent systems in all positions except for the 3-position in the cyclohexane/water system when it causes

a reduction in the partition coefficient.

In the discussion on the methylphenols it was found that the methyl group contribution fell as the solvent became more polar. This is not found in this group of solutes. The difference in the effect of the methyl group must therefore be caused by the change in its environment. In this series an intramolecular bond is present in the molecule and the partition values will reflect the influence of the methyl group on this bond.

In the cyclohexane/water system, the methyl group appears to have little effect in the 4-,5- or 6- positions and increases hydrophobicity by approximately its isolated group value (+0.50). However, in the 3-position a reduction in P is seen. This means that the affinity of the molecule for the lipid phase is reduced and increased for the aqueous phase. Cyclohexane is non-polar and solubility of the nitrophenol in this phase is governed by the presence of the intramolecular hydrogen bond which renders the molecule less polar. Thus a reduction in lipid solubility indicates an increase in polarity which can only be brought about by weakening of the intramolecular hydrogen bond. Thus in the 3-position the methyl group appears to exert a steric effect which weakens the intramolecular hydrogen bond. This can be confirmed by reference to the UV and IR spectra.

In the octanol/water system each position appears to produce a different methyl group contribution to hydrophobicity, with 3-Me having the lowest log P and 6-Me the highest log P. In general, the presence of an intramolecular hydrogen bond

results in a lower log P value than for other positions if the organic solvent is polar. Therefore, if the assumption that 3-Me substitution weakens the hydrogen bond is correct, then the increased log P value seen for 6-Me substitution indicates strengthening of the intramolecular bond. This effect is observed in the IR spectra, when 6-Me-2-NO_2phenol shows an increased lowering of the frequency of the intramolecularly bonded hydroxyl band compared with the parent o-NO_2phenol. Increasing the size or bulkiness of the 6-alkyl substituent progressively hinders free rotation of the OH bond about its axis, enhancing intramolecular bonding between the hydroxyl and nitro groups until the substituent group reaches a limiting size and/or shape when further increase in C number has no observable effect.

The 4- and 5- positions also show differing group contributions. This could be due to an effect of the methyl group in the 4- position. Hyperconjugation in alkyl groups has an important influence on the absorption frequencies of other groups attached to the molecule and both this and the inductive effect tend to weaken intramolecular bonding. (41) This would be apparent in a slight shift to lower frequency in the IR spectrum of 4-Me-2-NO_2phenol compared with the parent o-NO_2phenol. This does indeed occur as can be confirmed by reference to Chapter 7. Therefore it appears that the methyl group causes the strength of the intramolecular hydrogen bond to be weakened as it moves from position 6- to position 3- on the ring. This could account for the different log P_{CH_3} values.

iii. Methylbenzoic Acids

Isomer	Boiling Point	Melting Point
o-methyl	259°C	105°C
m-methyl	263°C	111°C
p-methyl	275°C	180°C
2,6-dimethyl	274.5°C	116°C
3,5-dimethyl	subl.	166°C

pKa

Benzoic acid	o-Me	m-Me	p-Me	$2,6\text{-Me}_2$
4.17	3.91	4.27	4.37	3.21

The methyl group decreases the strength of benzoic acid from both the meta and para positions. The methyl group has a +I effect and so the net result is to increase the +R effect in the meta or para tolyl group. Thus the two acids are weaker than benzoic acid. Resonance of the carboxyl group with the ring is from 'ring to group' and not the other way round.

Another way of explaining the strength of these acids is to consider the stabilising effect of the substituent on the anion. The more stable the anion is with respect to the unionised acid, the stronger will be the acid. +I and/or +R groups will oppose, whereas -I and/or -R groups will assist (by dispersing the negative charge),the +I effect of the carboxylate ion group. Hence the former substituents weaken and the latter strengthen the acid.

Irrespective of the polar type, nearly all ortho substituted benzoic acids are stronger than benzoic acid. Benzoic acid is a resonance hybrid and so the carboxyl group is coplanar

with the ring. An ortho substituent tends to prevent this coplanarity. Thus resonance is diminished (or prevented) and so the oxygen atom of the hydroxyl group has a greater positive charge, resulting in increased acid strength. Therefore it follows that the greater the steric inhibition of resonance, the stronger is the acid:-

$2,6\text{-}Me_2$(pKa 3.21) > 2-t-Bu(pKa 3.46) > 2-Me(pKa 3.91)

Here again, if the stability of the anion is considered, steric inhibition of resonance prevents the +R effect of the ring coming into operation, and since this weakens acid strength, its absence results in increased acid strength.

The actual geometry of highly substituted benzenes is an interesting problem. Ferguson et al (129,130)examined various substituted benzoic acids by X-ray analysis and found that steric strain is relieved by small out-of-plane displacements of the exocyclic valency bonds in addition to the larger in-plane displacements of these bonds away from one another. This type of deformation is referred to as molecular overcrowding. A molecule becomes overcrowded when at least one pair of non-bonded atoms are closer to each other than the sum of their van der Waals radii. Two extremes are now possible. If the atoms were to remain very close to each other and the rest of the molecule remained unaltered, then there would be large steric repulsion. On the other hand, if the molecule were deformed so that the overcrowded atoms were separated to the sum of their van der Waals radii, there would be a large strain energy. In practice, a compromise is reached, the steric

repulsion and the strain being relieved by bending of substituent bonds and buckling of the molecule as a whole.

Partition Coefficient

Methyl Group Contributions (Ring System) to Partition Between Water and Organic Solvents

Solvent	$\log P_{CH_3}$	Solute System
Octanol	0.20	o-Mebenzoic acid
	0.51	m-Mebenzoic acid
	0.45	p-Mebenzoic acid
	0.00(a)	2,6-Me_2benzoic acid
	0.51(a)	3,5-Me_2benzoic acid
Cyclohexane	1.83	o-Mebenzoic acid
	1.21	m-Mebenzoic acid
	0.32	p-Mebenzoic acid
	-0.065(a)	2,6-Me_2benzoic acid
	0.46(a)	3,5-Me_2benzoic acid

(a) = average CH_3 value

In both the octanol/water and cyclohexane/water systems, the methyl group has a positive log P value for all isomers except the 2,6-dimethyl isomer. Thus the methyl substituent increases the hydrophobicity of benzoic acid except when it is placed on both sides of the carboxylic acid group.

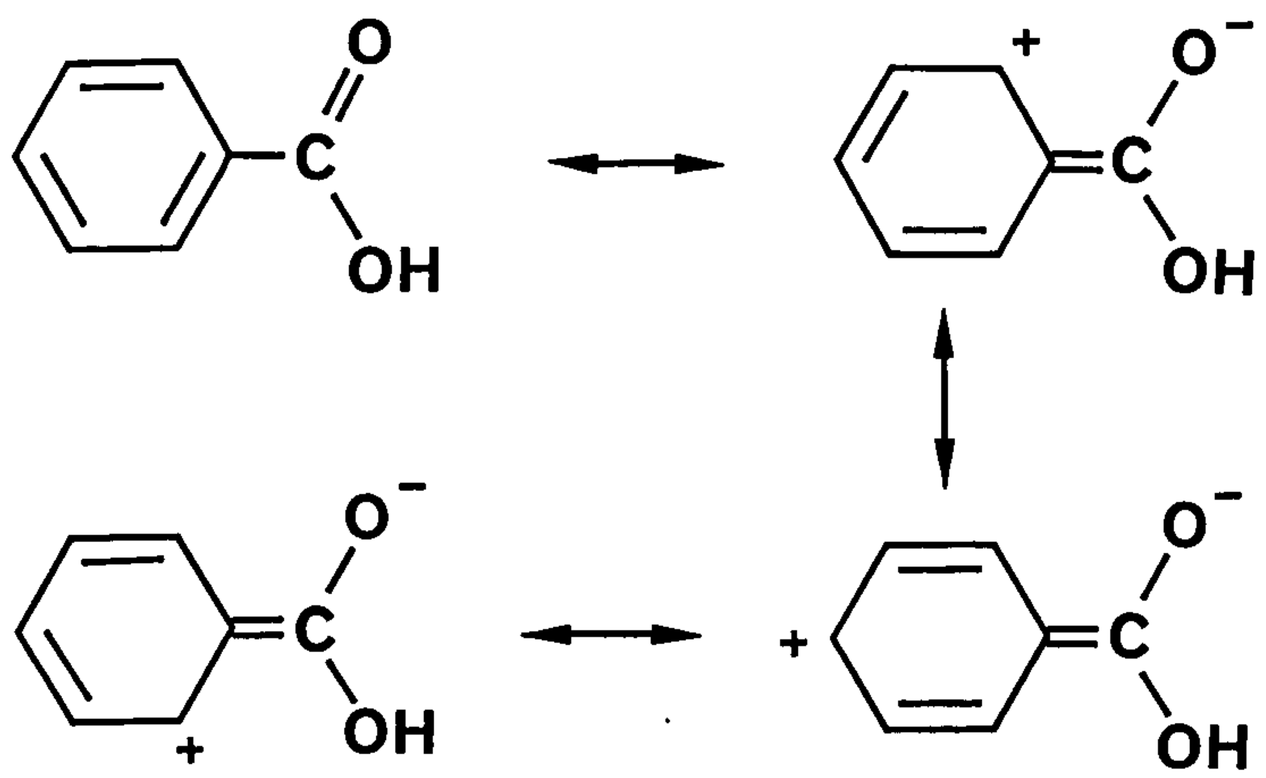
In the octanol/water system, the value of $\log P_{CH_3}$ in positions 3-, 4- or 5- is that expected of the methyl group and may be attributed to its intrinsic lipophilicity. However, in the ortho position the contribution to lipophilicity of the methyl group is reduced, and further reduced by di-ortho substitution. This can be due to two things; either shielding of the hydroxyl group by the methyl group or steric hindrance causing the carboxylic acid group to twist out of

plane.

If the hydroxyl group is shielded from interaction with octanol by the proximity of the methyl group, then solubility in octanol will be reduced. The water molecule is much smaller than the octanol molecule and so will still be able to approach, therefore solubility in water will increase relative to the other isomers. Two methyl groups will have double the effect and so octanol solubility will be further decreased.

Alternatively the methyl group could cause the carboxyl group to twist and this would also make it less lipophilic. This effect is reflected in the acidity of the methylbenzoic acids. Ortho-methylbenzoic acid has a higher acidity than the p-isomer. This has been attributed in solution to steric inhibition of resonance. In solution, resonance structures such as I stabilize the free acid and reduce acidity.

(I)



However, in the ortho position, bulky substituents cause the carboxyl group to twist out of the plane of the aromatic ring and thus prevent resonance stabilisation (285).

In cyclohexane, the position is slightly different. Solubility is not dependent on solvent interaction, except indirectly in partition by solubility in water. Thus if water solubility is reduced then the relative solubility in cyclohexane will increase. This is observed for o-Mebenzoic acid, but 2,6-Me_2benzoic acid shows an increased water solubility. This suggests that two mechanisms are in operation. A single methyl group could shield the hydroxyl group from interactions with water and thus increase log P, whilst two ortho methyl groups could twist the carboxyl group out of the plane of the aromatic ring and so make it more accessible to interactions with water molecules and thus lower the partition coefficient.

The steric effect of 2,6-dimethyl substitution can be confirmed by the UV spectra in cyclohexane which show a hypsochromic shift and lower absorbance (ε_{max}) for 2,6-Me_2benzoic acid compared with 3,5-Me_2benzoic acid.

iv. Methylacetanilides

Methyl Group Contributions (Ring System) to Partition Between Water and Organic Solvents

Solvent	log P_{CH_3}	Solute System
Octanol	-0.26	o-Meacetanilide
	0.47	m-Meacetanilide
	0.47	p-Meacetanilide
	-0.07(a)	2,6-Me_2acetanilide
	-0.47(a)	3,5-Me_2acetanilide
Cyclohexane	0.12	o-Meacetanilide
	0.38	m-Meacetanilide
	0.47	p-Meacetanilide
	-0.05(a)	2,6-Me_2acetanilide
	0.52(a)	3,5-Me_2acetanilide

The experimental data shows that in the octanol/water system substitution of a methyl group in the meta or para position of acetanilide causes an increase in log P of +0.47. Addition of a second methyl group in the 5-position (3,5-Me_2acetanilide) increases log P by the expected value of +0.47. However, in the ortho position log P is decreased by -0.26 and although substitution of a second methyl group in the 6-position causes an increase in log P it is by the much reduced amount of +0.07.

In the cyclohexane/water system, substitution of a single methyl group in the meta or para position produces an increase in log P of +0.38 and +0.47 respectively while the addition of a second methyl group in the meta position produces a further increase in log P of +0.68. A methyl group placed in the ortho position also produces an increase in log P in this system, but by the reduced amount of +0.12. However, a second ortho methyl group reduces the partition coefficient of o-Meacetanilide by -0.22 and of acetanilide by -0.10.

Cammarata (60) suggested that additivity of π values will break down when there is:

1) Mutual interaction between substituents (e.g. intra-molecular hydrogen bonding, steric and field effects) or,

2) When a substituent cannot be desolvated to its maximum potential.

Since no intramolecular hydrogen bonding is involved with methyl groups and field effects are minimal, the only significant effects are likely to be steric and desolvation effects.

1) Steric effects may be considered to take two main forms: a) a direct twisting or distortion effect and b) a shielding effect in which a group is protected from interaction with the solvent.

a) Leo,Hansch and Elkins (255) proposed that the former gave rise to loss of additivity of π values through steric inhibition of conjugation. An 'aromatic' value is generally higher than that for the same substituent in an aliphatic system (368) because of electron delocalisation. An ortho substituent in a benzene ring may twist an adjacent group out of the plane of the ring, therefore reducing its conjugation with the ring and rendering the twisted group less 'aromatic'. This gives rise to a lower log P and hence apparently low π value for the ortho substituent.

e.g. 1,2,3-trimethoxybenzene is a highly hindered compound as shown by the ultraviolet spectrum (397) and the octanol/water log P value of 1.53 is lower than the calculated value. (255). Since 1,3-dimethoxybenzene has a log P of 2.09, $\pi_{2\text{-}OCH_3}$ is -0.56 which is closer to the aliphatic methoxy π value of -0.47 than to the aromatic π value of -0.02. The fact that the observed π value is more negative than -0.47 may indicate some additional loss of planarity of the 1- and 3- methoxy groups.

b) The shielding effect is exemplified by the data of Saha et al (334) which shows that for the cyclohexane/water partition of alkyl phenols, $\pi_{2\text{-}CH_3}$ is 0.87 whilst $\pi_{3\text{-}CH_3}$ is normal at 0.57. This data is confirmed by the present work. Almost as dramatic is the effect of 2,6-dialkyl substitution for $\pi_{2,6(CH_3)_2}$ is 1.70 in contrast to the expected value of 2 x 0.57 = 1.14. This effect is not due to steric twisting,

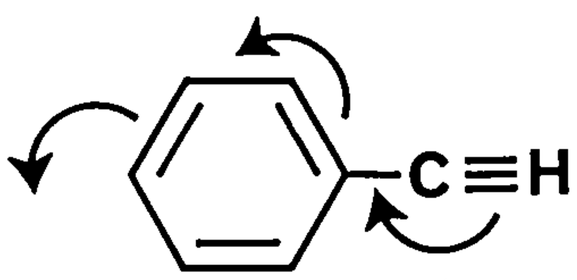
for the hydroxyl group remains planar in 2,6-dialkylphenols (103,28). Furthermore, as said above, twisting leads to lower than expected π values.

Leo et al (255) suggested that high octanol /water π values are found in ortho methyl substitution because of shielding and use as their example o-methylphenoxyacetic acid. However, Dearden and Wootton (113) ascribed the o-methyl phenoxyacetic acid value to steric enhancement of conjugation; such a phenomenon may be unique to an aromatic methoxy group adjacent to an alkyl group. Dearden and O'Hara (112) demonstrated that shielding generally gives rise to lower than expected π values in the octanol/water system.

2) The desolvation effect has been described by Tute (368) and Leo et al (255). It is generally acknowledged that structured water around a non-polar group or molecule in aqueous solution provides an entropic driving force encouraging the molecule to leave the aqueous solution. If two non-polar groups are adjacent to one another in a molecule then, it is argued, the total amount of structured water surrounding them both must be less than if they were separated; hence entropic encouragement to leave aqueous solution is less. An example of this effect, from Saha et al (334) may be 3,4,5-Me_3phenol which has a log P of 0.63 in the cyclohexane/water system. These authors data also give $\pi_{3\text{-}CH_3} = 0.57$, $\pi_{4\text{-}CH_3} = 0.58$ and $\log P_{phenol} = -0.77$. Hence the calculated value for 3,4,5-Me_3phenol is +0.95 and it may be that the observed value of 0.63 is due in part to an entropic ortho effect.

With regard to the methyl group itself, in the meta or para positions it produces an increase in log P. This can be regarded either as a consequence of the electronic interactions of the alkyl group with the aromatic ring, or simply as an increase in the non-polar portion of the molecule, so increasing relative hydrophobicity over acetanilide.

Although any alkyl group is neutral when attached to hydrogen or an aliphatic carbon atom, it is subject to polarisation when attached to a conjugated or aromatic system. This usually manifests itself in the direction of the ring, thus, compared to hydrogen the alkyl group is considered to donate electron density to the ring. It would appear that there are two possible orders of electron release by the methyl group, one being a general inductive effect (19) and the other being hyperconjugation - which is regarded as being due to the conjugation of single C-H bonds with an aromatic ring. i.e.:-



Hyperconjugation effects in toluene

This effect is in inverse proportion to the inductive effect for a series of alkyl substituents, resulting in permanent polarisation in alkyl benzenes. Both mechanisms have been rationalised by Berliner and Bondhus (30) as resonance effects. It would seem that such polarisations, being the result of hyperconjugation, cause a relative 'loosening' of the hydrogen atoms attached to the carbon atom involved

in the delocalisation. Therefore, this should result in increased hydrogen bonding, theoretically decreasing P. This is not what is observed, when methyl substituent values are seen to be higher than the -H value.

This suggests that the important property imparted to the molecule by the methyl group is one of simply increasing the non-polar portion of the molecule, thus enhancing hydrophobicity. A spatial effect may also be evident; it is known that aromatic rings participate directly in hydrogen bonding, by means of their π bonds and the relative large molar volume of the methyl group may impair this effect.

However, methyl groups substituted adjacent to the acetamido group in acetanilide do not produce this increase in hydrophobicity.

Dearden and O'Hara (112) investigated the partition coefficients of a series of alkyl derivatives of 4-hydroxyacetanilide in the octanol/water system. The conclusions from this study are relevant to the results from the present study.

As Table 31 shows 3-Me substitution in 4-acetamidophenol has a drastic effect, the π value being -0.14 instead of the normal π_{Me} value of $\sim$ +0.48. 3,5-Me_2 substitution increases this effect, the total π value of both groups being only 0.003 instead of 2 x 0.48. Draber (117) found similar behaviour with acetanilide ($\pi_{2\text{-Me}}$ = -0.20, $\pi_{2\text{-Et}}$ = 0.16 and $\pi_{2\text{-iPr}}$ = 0.54). Both of these studies confirm the results found in the present work, which found a value of $\pi_{2\text{-Me}}$ = -0.26, $\pi_{3\text{-Me}}$ = +0.47 and $\pi_{4\text{-Me}}$ = +0.46 while $\pi_{2,6\text{-Me}_2}$ = -0.18 and

Table 31. Log P,π and Δπ Values of Alkyl Derivatives of 4-Hydroxyacetanilide

Substituent	Log P	Observed Σalkyl π	Calculated Σalkyl π	ΔΣalkylπ (Obs-Calc)
H	0.311	0.000		
2-Me	0.793	0.482		
3-Me	0.173	-0.138	2-Me= 0.482	-0.620
$2,3\text{-Me}_2$	0.573	0.262	2-Me+3-Me=0.344	-0.082
$2,5\text{-Me}_2$	0.597	0.286	2-Me+3-Me=0.344	-0.058
$2,6\text{-Me}_2$	1.108	0.797	2-Me+2-Me=0.964	-0.167
$3,5\text{-Me}_2$	0.314	0.003	3-Me+3-Me=-0.276	+0.279
$2,3,5\text{-Me}_3$	0.749	0.438	2-Me+$3,5\text{-Me}_2$=0.485	-0.047
			3-Me+$2,3\text{-Me}_2$=0.124	+0.314
$2,3,6\text{-Me}_3$	0.816	0.505	3-Me+2.6-Me_2=0.659	-0.154
			2-Me+$2,3\text{-Me}_2$=0.744	-0.239
$2,3,5,6\text{-Me}_4$	0.951	0.640	$2,6\text{-Me}_2$+$3,5\text{-Me}_2$=0.800	-0.160
			$2,3\text{-Me}_2$+$2,3\text{-Me}_2$=0.524	+0.116
4-Me	0.768	0.457		

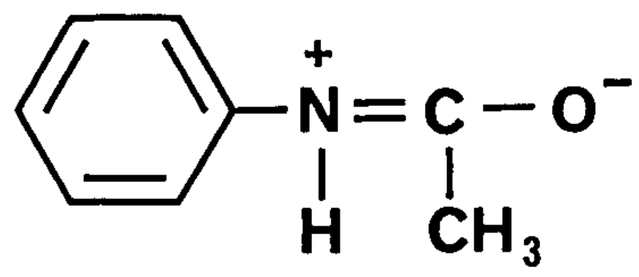
$\pi_{3,5\text{-Me}_2} = +0.91.$

This behaviour is quite different from that of ortho substituted anilines; π values for 2-Me substitution in aniline, N-methylaniline and N,N-dimethylaniline are +0.50 in each case (336). It is therefore tempting to ascribe the different behaviour to differences in the extent to which loss of conjugation between ring and substituent occurs. Such loss of conjugation can readily be detected by UV spectroscopy; however, o-methylation of acetanilide and of N,N-dimethylaniline produces similar decreases in molar absorptivity, 57% and 52% respectively. (371,249)

A search of the Hansch Database (189) reveals that no aromatic system other than acetanilide has been reported as

displaying a decrease in partition coefficient on o-methylation. Therefore, we have the situation in which a simple steric effect is apparently normal according to ultraviolet spectroscopic evidence and yet is markedly abnormal - even perhaps unique - in its effect on partition. However, a close look at the spectroscopic data reveals that these too are abnormal, in the sense that a large hypsochromic shift (12nm) accompanies o-methylation of acetanilide but not o-methylation of N,N-dimethylaniline (4nm) (249)

A normal steric effect manifests itself spectroscopically by a reduction in absorptivity of a band which remains at approximately constant wavelength, together with a concomitant increase in another band at a shorter wavelength arising from the residual chromophore after loss of conjugation (57) The marked hypsochromic shift observed with acetanilide suggests rather a transformation to another species - it is significant that the absorption maximum of o-Meacetanilide in ethanol is 230nm, close to that of o-methylaniline (233nm) (131). Therefore it has been suggested by Dearden and O'Hara (112) that o-methylation causes virtually complete loss of conjugation of the acetyl group only and this is all the more significant spectroscopically because of the predominance in non-hindered acetanilides of canonical forms such as:-



Skulski (341) has suggested that such canonical forms account for the unexpectedly long wavelength of the primary absorption band of acetanilide and it follows that loss of conjugation of the acetyl group should bring about a marked hypsochro ic effect. Support for this hypothesis comes from the work of Szepes et al (352) who showed by photo - electron spectroscopy that o-methyl substitution in trifluoroacetanilide did not cause any twisting of the aryl-N bond, although 2,6-dimethyl substitution did so. Therefore, the primary UV absorption band of 2,6-Me_2acetanilide is similar to that of 3,5-dimethylphenol and 3-Me-4acetamido-phenol in ethanol (231nm) is similar to 3-Me-4-aminophenol (233nm). Wepster (379) has similarly shown from UV data that the conjugative interaction between the amide nitrogen and the benzene ring in acetanilide is almost entirely eliminated by 2,6-dimethyl substitution. Thus it appears that the negative π value for methyl substitution ortho to the acetamido group may be explained by twisting of the acetyl group and the very small positive (+0.08) π value of a second methyl group ortho to acetamido may be caused by loss of conjugation of the amide nitrogen.

The markedly different effects of the first and second methyl substituents adjacent to the acetamido group account for the large discrepancy between observed and calculated log P values for 2,6-Me_2acetanilide.

CHAPTER SEVEN

SPECTRAL PROPERTIES OF THE COMPOUNDS

7.1 Spectroscopic Parameters

Linear free energy relationships deal only with relative reactivities in the form of reaction rate and equilibrium data, although this approach can be extended to include various physical measurements. Any theories of structural and solvent effects arising out of L.F.E.R's must be expressed ultimately in terms of interactions present within and between molecules. The interactions will influence other qualities which may be utilised as means of identifying irregularities in observed parameters. Prominant in such a role are the many and varied spectroscopic measurements that can be experimentally determined under conditions of relatively greater control, precision and variety, than those obtained from reactivity measurements. In this chapter, ultraviolet, infra-red and nuclear magnetic resonance spectroscopy will be utilised to reveal the existence of intramolecular hydrogen bonds and steric hindrance within molecules.

Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of most aromatic compounds consist of three absorption bands, all due to π-π^* transitions. In the simplest case - benzene - there are two strong bands at 184 (ε50,000) and 204mμ(ε7,400), the former being difficult to observe. The third band shows extensive fine structure in the vapour state and to a less degree in

solution; it is much weaker (forbidden transition), the 225mμ peak having ε∿200. These three bands are called the Rydberg transition band, π-π*allowed transition band, and the π-π*forbidden transition band respectively but for simplicity are referred to as Band A, Band B and Band C in the text.

In substituted derivatives of benzene and in aromatic compounds containing condensed nuclei these bands are modified, in general being moved to higher wavelengths. Some substituents such as alkyl groups and halogen atoms have a small bathochromic effect upon both bands B and C, but have little effect upon the intensity of either band. Other substituents, such as the hydroxyl, alkoxyl, sulphonamide and amino groups which are themselves transparent in the near-ultraviolet region, have more pronounced bathochromic effects and also raise the intensity of Band C.

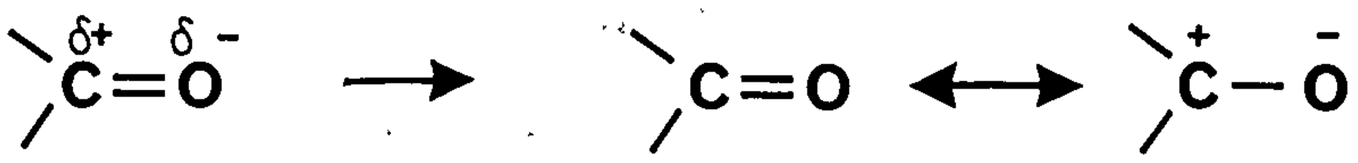
Substituents which are unsaturated (ethylenic and acetylenic, nitrile, aldehyde, carboxylic acid and nitro groupings) also have much the same effect, often to a greater degree. Two such chromophores conjugated to the benzene ring have a strong bathochromic and intensifying effect on Band B, which moves out sufficiently to obscure Band C. In several cases weak bands appear at longer wavelengths. For example in nitrobenzene and azobenzene there are bands at 330 and 443mμ respectively (which are responsible for the colour of these compounds). These bands, together with the bands around 320mμ in the carbonyl compounds, are due to n → π* transitions in the particular functional group (nitro, azo or carbonyl), modified by the remainder of the molecule.

Charge transfer both <u>to</u> and <u>from</u> the ring (exemplified by NH_2 and NO_2 substituents) is effective in causing a bathochromic shift and intensification of the absorption. In disubstituted benzenes, the combined effect of the groups depends on their electronic type and relative orientation; p-orientated substituents of opposite type often give unexpectedly large shifts, e.g.p-nitroaniline has λ_{max}375mμ (ε16,000).

Modification of bands will occur if the substituents are intramolecularly hydrogen bonded or sterically hindered.

<u>Infra-Red Absorption Spectra</u>

Two main factors affect the frequency and intensity of group absorptions- Physical State i.e. factors operating from outside the molecule containing the group, and Intramolecular Factors i.e. those operating from within. There are three important groupings - the carbonyl group, the hydroxyl group and the carbon-carbon double bond. The carbonyl group is polar and may be regarded in terms of relative contributions of the hypothetical canonical forms,



Thus, any effects which tend to increase the contribution of the single bond form will lower the force constant of the carbon-oxygen bond and will result in a fall in frequency of the stretching vibration νC=O.

In the case of the hydroxyl group, the main concern is with

the readily-identified O-H stretching mode, νOH. Most of the changes in νOH derive, firstly, from the ability of the slightly acidic hydrogen to form hydrogen bonds with electron-rich atoms outside (intermolecular) or inside (intramolecular) the molecule, and secondly, from the extent to which electrons are withdrawn from, or supplied to, the oxygen atom. Hydrogen bonding normally results in small to large frequency drops in νOH, together with proportionate broadening and intensification.

Much useful information can be obtained by comparing group frequencies which have been measured in solution. The dilution must be sufficient to eliminate solute-solute interactions. In non-polar solvents, such as carbon-tetrachloride, the changes in group frequencies from one compound to another give a measure of the various factors operating within the molecules. In polar solvents, such as chloroform, specific associations (e.g. hydrogen bonds and dipole interactions) occur, and the group frequencies of solute molecules no longer have the relationship to one another that they had in carbon tetrachloride.

Nuclear Magnetic Resonance Spectroscopy

When a system of atomic nuclei is placed in a uniform magnetic field and irradiated at the resonance frequency, transitions between adjacent energy levels result in a net absorption of energy by the nuclear system, because, although transitions from high energy states to low energy states have the same probability as those in the reverse direction, there is, initially, in the presence of the

applied magnetic field, a small but finite excess of nuclei in lower energy states.

The importance of nuclear resonance resonance spectroscopy derives from the fact that the apparent resonance frequency of a nucleus is to some extent dependent on molecular environment. This dependence is a consequence of the magnetic shielding of nuclei by the extra-nuclear electrons. The applied magnetic field causes induced electron circulations about one or more atomic nuclei and these circulations give rise to small secondary fields which may either augment or oppose the applied field at the nucleus, depending on the structure of the molecule.

7.2 Experimental Section

Solvents

Ethanol was spectrocopic grade (benzene-free) obtained from BDH Chemicals Ltd. Cyclohexane and chloroform were also spectroscopic grade from BDH Chemicals Ltd. Water was freshly distilled. All solvents were checked to ensure absence of extraneous absorption bands.

Preparation of Solutions

For quantitative ultraviolet spectroscopy, solutions were prepared in cyclohexane, ethanol and distilled water (or, where specified in the text, with 1% ethanol added) to concentrations in the region 10^{-3} to 10^{-4}M. All weighings were performed on a Beckman Microbalance Model LM 500. Each weighed sample was washed into a 100ml Quickfit stoppered volumetric flask, grade A, with the appropriate solvent.

For infra-red spectroscopy, solutions in chloroform were made to 10^{-3}M or saturation, whichever was the more dilute. Carbon tetrachloride, where used, was also spectroscopic grade obtained from BDH Chemicals Ltd. Again, solutions were made to concentrations of 10^{-3}M or saturation, which ever was the more dilute.

Methods

The ultraviolet spectra were recorded using a Perkin Elmer 550 continuous recording spectrophotometer, using 1cm silica cells at room temperature. The spectrophotometer was calibrated at intervals using the 241.5nm maximum on a holmium filter.

The infra-red spectra were recorded on a Perkin Elmer

spectrophotometer at room temperature, using variable path-length cells, with NaCl windows, set to give a path length of 0.5mm. Each spectrum was calibrated using a polystyrene film at specified known wavelengths.

The nuclear magnetic resonance spectra were kindly recorded by the Chemistry Department of Liverpool Polytechnic using a 90MHz instrument. The solvent used was Deuterochloroform and the reference substance was Tetramethylsilane.

7.3 Ultraviolet Absorption Spectra

7.3.1 Phenol

Phenol is the parent molecule of many of the compounds in this study, therefore the ultraviolet spectra of phenol itself will be discussed before considering the study compounds. This will allow a comparison to be made which will indicate the effect of different substituents.

Phenols generally exhibit two absorption bands between 200 and 360 nm. The band at longer wavelength is called the π-π^* forbidden transition, but will be referred to as the C band, and the band at shorter wavelength is the π-π^* allowed transition which will be referred to as the B band.

Table 32. Absorption Maxima of Phenol in Various Solvents

Solvent	B-band		C-band	
	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
Cyclohexane	217	3730	260	840
			264.5*	1460
			271	2210
			278	2050
Ethanol	218.5	5650	267	1410
			273	1790
			279	1420
Water	210	6910	265*	1270
			270	1560
			275	1280

The bands alter with a change in solvent and it has been shown by Coggleshall and Lang (73) that some of these spectral changes do not depend on stable hydrogen-bonded complexes. Various forms of intermolecular hydrogen bonding are possible involving both the hydrogen atom of the hydroxyl group and the oxygen atom.

The fine structure of the C-band for phenol in cyclohexane or similar inert media is fairly characteristic and can often be recognised in substituted, including sterically hindered, phenols. The fine structure disappears if the phenolic species no longer contributes appreciably to the spectrum; e.g. in the C-band of m-nitrophenol where the compound absorbs preferentially as nitrobenzene. The fine structure of the C-band also disappears for intramolecularly hydrogen bonded molecules but it is noticeable for a number of ortho substituted phenols even in ethanolic solution. This is probably because this fine structure is caused by vibrational sub-levels and a hydrogen bond may obscure this. Hence, the fine structure is frequently reduced if the phenol is associated with a solvent molecule by means of an intermolecular hydrogen bond (see Table 32) or if the phenol molecule is intramolecularly hydrogen bonded. However, the fine structure does become evident if steric interactions hinder the formation of a hydrogen bond.

Para-substituted Phenols

The B-band in phenol is displaced to longer wavelength on para-substitution. If the para-substituent is electron-withdrawing, this displacement is greater, qualitatively related to the electron-withdrawing effect of the substituent, and the usual high-intensity band corresponding to an extended conjugated system is obtained.

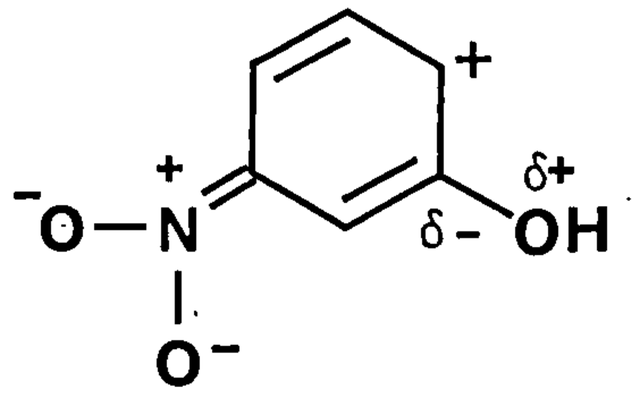
The C-band shows the expected changes if the para-substituent is electron-withdrawing i.e. with increase in mesomeric interaction the C-band usually becomes progressively less

pronounced. If the para-substituent is electron-donating, the fine structure of the C-band is frequently enhanced, the effect being most pronounced for p-methylphenol in cyclohexane. The fine structure of the C-band for this compound is considerably more pronounced than for m-methyl phenol. This is surprising since increased molecular symmetry is generally assumed to simplify the absorption spectra, therefore one would expect the fine structure of the C-band to be less pronounced in the para- than in the meta-iosmer.

Meta-substituted Phenols

In meta-disubstituted benzene derivatives direct resonance interaction is ruled out by definition. Therefore two bands corresponding to the two mono-substituted benzene derivatives would be expected. Both these bands may be modified because of the other substituent, either by means of secondary short range electronic interactions or possibly because of a buttressing effect. It is also possible that one of the B-bands may be obscured because of overlap with the other B-band or because of the A-band absorption (Rydberg transition). This means reliable band assignment is difficult for some examples, but characteristic properties like the fine structure of the phenolic C-band assist in band assignments.

If the non-phenolic substituent is electron-withdrawing, the non-phenolic B-bands are usually displaced to longer wave-length on account of a secondary interaction of the hydroxyl group. A displacement of the order of 7mμ suggests that the hydroxyl group tends to facilitate the absorption of the non-phenolic chromophore.



The effect of the second substituent on the phenolic B-band is difficult to see since the relevant absorption band often occurs at too short a wavelength to be readily identified. However, in m-nitrophenol the phenolic absorption is displaced to a longer wavelength - the nitro substituent facilitating phenolic absorption. This bathochromic displacement may be related to changes in integrated absorption intensities for infra-red hydroxyl absorption in phenols, since the integrated absorption intensity for m-nitrophenol is greater than for phenol and may be related to increased mesomeric interactions.

Some C-bands show expected changes - if the meta-substituent is electron-withdrawing the C-bands are enhanced compared with those for the para-isomer.

Ortho-substituted Phenols

The B-band in ortho-substituted phenols does not usually undergo a pronounced wavelength displacement even if the ortho-substituents are bulky. The B-bands of the ortho-isomers frequently resemble the B-bands of the meta-iosmers. However, steric interactions are indicated in the ultraviolet spectra by the absence of the usual wavelength displacements between solutions in cyclohexane and ethanol. That is, whereas between solutions of phenol in cyclohexane and ethanol a wavelength displacement of 8mμ is observed in the

B-band, no such displacement is observed for solutions of strongly sterically hindered phenols. Coggleshall and Lang (73) ascribed this reduced wavelength displacement to steric interactions inhibiting solvent-solute interactions between phenol and solvent molecules.

Table 33. Absorption Maxima of Orthosubstituted Phenols in Cyclohexane and Ethanol (103)

	Cyclohexane		Ethanol	
Orthosubstituents	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
H,H (B-band)	211	6200	218.5	6000
H,H (C-band)	264	1450	266*	1500
	270	2200	271	1900
	276	2100	276	1450
H,CH_3(B-band)	213.5	6500	215	6160
H,CH_3(C-band)	272	1950	273.5	1950
	278	1850	277	1820
CH_3,CH_3 (B-band)	215	7400	213*	7400
CH_3,CH_3 (C-band)	272	1600	273	1510
	278	1660	277*	1400
H,Cl (B-band)	213	6600	217	6460
H,Cl (C-band)	269*	1750	277	2400
	274	2570	283*	2090
	281.5	2690		

* denotes an inflection

Intramolecular hydrogen bonds are indicated by a bathochromic wavelength displacement of the B-band, accompanied by an increased absorption intensity. In ortho-substituted phenols which may contain an intramolecular hydrogen bond a bathochromic wavelength displacement of between 5 and 12mμ may be observed in the B-band compared with the B-band absorption in the corresponding meta-isomer. (The B-band referred to is the B-band associated with transitions involving predominantly that mono-substituted

benzene derivative in which the substituent is electron withdrawing).

The C-band in intramolecularly bonded compounds also shows bathochromic wavelength displacements and intensity is increased in the ortho-isomer. However, this also occurs in compounds with no intramolecular hydrogen bond so is not reliable for showing bond presence.

The absence of appreciable spectral change on determining the UV spectra of molecules in cyclohexane and ethanol (or ether) also provides support for the supposed presence of a strong intramolecular hydrogen bond. This argument is particularly convincing if the meta-isomer or other reference compounds shows an appreciable spectral change on altering the solvent in an identical manner.

Intermolecular Bonding

Phenols are capable of forming bonds with the solvent and spectral effects can be seen which are ascribed to intermolecular hydrogen bonding between solute and solvent. For example, ether frequently causes a bathochromic wavelength displacement in the B-band which is ascribed to intermolecular hydrogen bonding of type I because replacement of the relevant hydrogen atom by a methyl group tends to destroy this effect, (277) and also because parallel effects may be deduced from C-band and dipole moment data. (297)

(I)

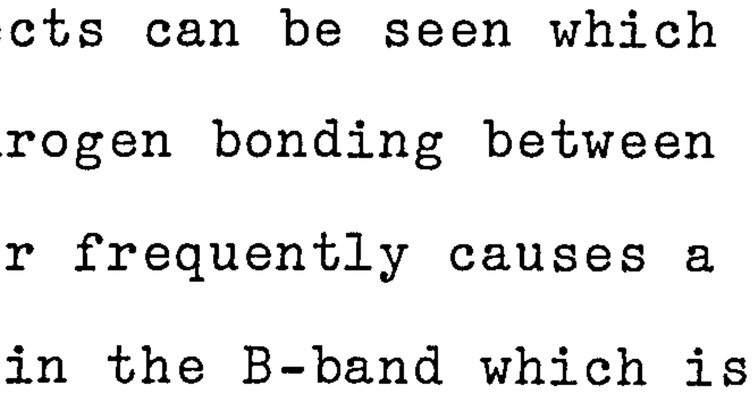


Table 34. Bathochromic Wavelength Displacements Between B-bands in Cyclohexane (non-bonded species) and Ether (hydrogen-bonded species)

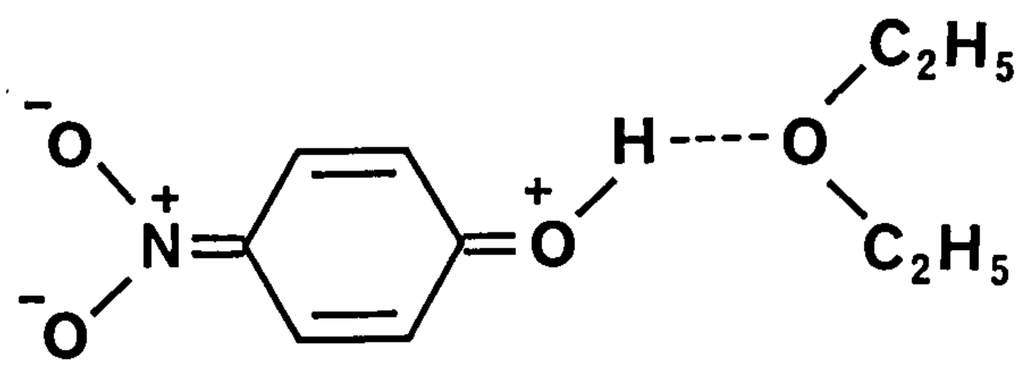
Solute	Cyclohexane λ_{max}(mμ)	Cyclohexane ε_{max}	Ether* λ_{max}(mμ)	Ether* ε_{max}	$\Delta\lambda$
Phenol	211	6200	218	6400	7
p-NO_2phenol	286	11000	299	11400	13
p-Clphenol	225	8500	228	12400	3
p-Mephenol	221	5800	223	6600	2
m-NO_2phenol	222	10550	226	10000	4
m-Mephenol	215	6200	221	5600	6
m-OHbenzaldehyde	246	10470	250	9800	4
	252	9620	255	9200	3
o-OHbenzaldehyde	252.5	11450	251*	10400	-1.5
	258.5	11950	255.5	10700	-3.0
o-Clphenol	213	6600	217	6200	4
o-Mephenol	213.5	6500	218	5350	4.5
o-NO_2phenol	271.5	7700	268.5	6900	-3.0

* Ref: 104

Table 34 indicates that for a number of para or meta-substituted compounds the observed wavelength displacements are similar to those in the parent compound, phenol; i.e. the bathochromic wavelength displacement, ascribed to the intermolecular hydrogen bond is comparable for compounds like p-NO_2phenol and phenol.

Interactions which can be represented by resonance structures of type II do not appreciably affect the intermolecular hydrogen bond since the total displacement, if expressed in wavenumbers, for p-NO_2phenol is only slightly smaller than the sum of the effects in phenol and nitrobenzene.

Other para-substituents such as -Cl and -CH_3 appear to have a similar effect and also tend to weaken the intermolecular



(II)

hydrogen bond slightly, as seen in the frequency displacements. Meta-substitution also appears to reduce the strength of the hydrogen bond. This is possibly because the meta or para substituent tends to interact with the π-electrons of the benzene ring and this interaction is competitive in that it reduces the benzene ring-hydroxyl group interaction which facilitates intermolecular hydrogen bonding.

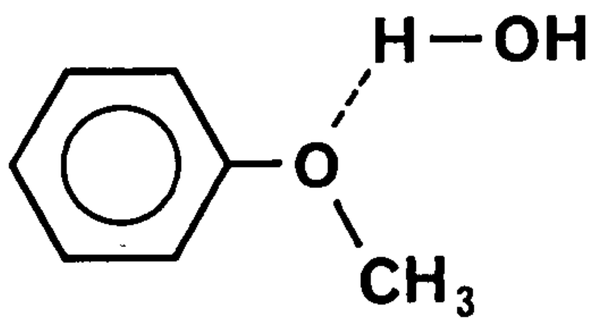
Ortho-substituted compounds cannot be used for comparison since steric interactions and intramolecular hydrogen bonding would be expected to interfere with the conformations of the molecule and with the solvent-solute interactions. However, the spectra of intramolecularly hydrogen bonded molecules such as o-nitrophenol, o-hydroxybenzaldehyde etc. are almost identical in ether and cyclohexane - this can be assumed to show that intramolecular hydrogen bonds prevent the formation of intermolecular hydrogen bonds since these usually give rise to spectral changes.

Intermolecular hydrogen bonding may also be observed in hypsochromic wavelength displacements of the B-band on changing solvent from cyclohexane or ethanol to water. (see Table 35)

However, the bonding may be of type III since wavelength

Table 35. Wavelength Displacements Between B-bands in Cyclohexane (non-bonded species) and Ethanol or Aqueous Solutions

Solute	Cyclohexane		Ethanol		Water	
	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
Phenol	211	6200	219	6000	210	6000
p-NO_2phenol	286	11000	314	10960	317	9300
p-Clphenol	225	8500	228	9120	225	8600



(III)

displacements also occur for the methyl analogues (anisoles) (104). This structure can decrease electronic interaction between the oxygen atom and the benzene ring and so cause hypsochromic wavelength displacement.

However, with these solvents other factors are also involved. Water or ethanol molecules can attach themselves to the nitro group in p-nitrophenol and this accounts partly for the bathochromic shift between cyclohexane, ethanol and water. Also, dipole-dipole interactions are expected to cause a bathochromic shift which may hide any hypsochromic effect caused by solute-solvent interaction. Hydrogen bonding involving the hydrogen atom of the phenolic hydroxyl group would be expected to have a similar effect.

In addition, the change to a more polar solvent (water) decreases the fine structure of the phenolic B-band.

7.3.2 Chlorophenols

The ultraviolet spectra of the chlorophenols in cyclohexane show the characteristic phenolic fine structure in the C-band. However, this is less pronounced for the ortho isomer which probably indicates the presence of an intramolecular hydrogen bond, since the fine structure is caused by vibrational sub-levels and a hydrogen bond may obscure this. An intra-molecular hydrogen bond is usually identified by the absence of spectral change on altering the solvent from cyclohexane to ethanol. However, with the chlorophenols a similar wavelength displacement of 3mμ is observed for all the isomers. Alternatively an intramolecular hydrogen bond may be indicated by a bathochromic wavelength displacement of both the B-band and the C-band of the ortho isomer compared with the corresponding meta isomer, accompanied by increased band intensity for the ortho isomer. This is not observed for o-Clphenol and in fact, a hypsochromic shift is seen relative to both the meta and para isomers. This has been shown to be characteristic of the type of intramolecular hydrogen bond found in this compound (101). This data suggests that although the hydrogen bond is present in cyclohexane, it is broken when the molecule is in either ethanol or water, hence the wavelength displacement on solvent change. This indicates that the chlorine atom forms weak hydrogen bonds which is confirmed by other physical data.

Intermolecular hydrogen bonds are responsible for the wavelength changes which occur when the solvent is changed from cyclohexane to ethanol. Para or meta substituents such

Table 36. Ultra-Violet Absorption Spectra - Chlorophenols

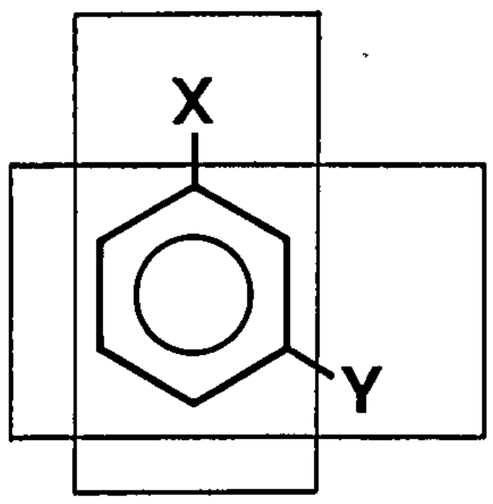
Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
Benzene			204	7400	254	200
Phenol			217	3730	260*	840
					264.5*	1460
					271	2210
					278	2050
o-Clphenol	205	7320	213	6600	269*	1760
					274	2580
					281.5	2690
m-Clphenol	205	7600	217.5	6860	268	1350
					274.5	2140
					282	2100
p-Clphenol			225	8510	275.5	1470
					281.5*	1960
					283*	1910
					287*	1380
					290	1810
Solvent - Ethanol						
Benzene			204	7400	254*	200
Phenol			218.5	5650	267*	1410
					273	1790
					279	1420
o-Clphenol	205	8650	217	6500	277*	2390
					283*	2100
m-Clphenol	206.5	8880	220	6390	277	2050
					283.5	1790
p-Clphenol	203	5640	228	9220	283.5*	1810
					289*	1520
Solvent - Water						
Benzene			200		256*	
Phenol			210	6910	265*	1270
					270	1560
					275	1280
o-Clphenol	202	11,850	214*	7800	274	1950
					279.5	1720
m-Clphenol	203	13,200	216	7210	274	1760
					280.5	1540
p-Clphenol			225	10,230	280*	1550
					285*	1340

* denotes an inflection

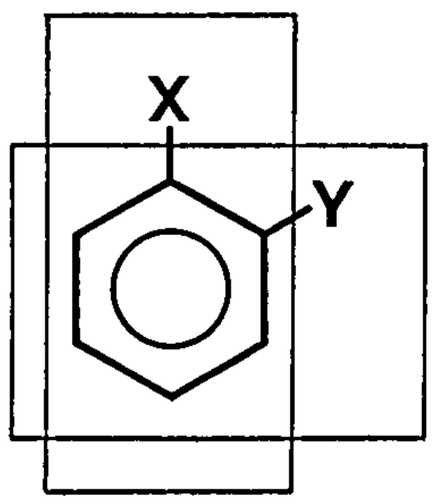
as -Cl or $-CH_3$ appear to weaken the intermolecular hydrogen bond which is reflected in the small frequency displacement on solvent change. If the substituent is electron donating it will reduce the δ^+ charge on the hydrogen of the OH group from either the meta or para position and therefore reduce hydrogen bonding. The converse is true for electron attracting substituents.

p-Chlorophenol shows the displacement to longer wavelength expected of para substitution in phenol. Since the chloro group is electron withdrawing and can form an extended conjugated system, the B-band is also of higher intensity. The C-band however is less pronounced.

The mesomeric interactions of conjugated systems are largely responsible for the characteristics of their ultraviolet absorption spectra and therefore, if a molecule contains two or more absorbing species or 'partial chromophores' that are not conjugated with each other, its spectrum will be the sum of the separate absorptions of the partial chromophores. In disubstituted benzenes, conjugation is at a maximum in the para isomer and the molecule generally absorbs as a single chromophore. Partial isolation of the chromophores, leading to local excitation, occurs in meta-disubstituted benzenes (I) because of the absence of classical conjugation, and in ortho-disubstituted benzenes (II) largely because of steric effects. (134). Although some features of the absorption of both chromophores in an ortho- or meta-disubstituted benzene will be retained, the spectrum of such a compound will not be a simple summation of the spectra of



(I)



(II)

the two constituent monosubstituted benzenes. This occurs because it has generally been shown (403) that separation of two chromophores by a single carbon atom, which is analogous to the separation of groups X and Y in structure I, does not result in complete loss of interaction; secondly because short-range interactions, such as inductive effects and hydrogen bonding, may affect the spectrum of an ortho-disubstituted benzene (II).

This means that in m-Clphenol two bands corresponding to the two mono-substituted benzene derivatives would be expected. However, it is rarely easy to identify two bands and m-Clphenol is no exception, the phenolic B-band probably being masked by the other band or by A-band absorption. Since the chloro-group is electron withdrawing the non-phenolic B-band will be displaced to longer wavelength on account of secondary interaction of the hydroxyl group.

The spectrum of a disubstituted benzene generally resembles the spectrum of the parent monosubstituted compound that absorbs at the longer wavelength. Thus the B-band of o-chlorophenol resembles that of phenol, in its lack of structure, rather than that of chlorobenzene, although the latter is at longer wavelength than that of phenol. But

the B-band of phenol is at a shorter wavelength than would be expected from mesomeric and other considerations.

Chlorobenzene: (Cyclohexane)	Band B		Band C	
	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
	211	7500	245	70
	215	7500	251	120
	219*	6000	257	180
			261	170
			264	250
			270	190

Similarly in m-chlorophenol the bands resemble those of phenol.

A bathochromic shift is seen on changing the solvent from cyclohexane to ethanol due to intermolecular hydrogen bonding however, a hypsochromic shift occurs when the solvent is changed to water. This occurs because water is a proton donor and will therefore induce a hypsochromic shift.

7.3.3. Nitrophenols

The ultraviolet spectra of the nitrophenols in cyclohexane do not show the characteristic fine structure of the phenolic C-band. Neither is it seen in the spectrum of 2-NO_2resorcinol. In o-NO_2phenol and 2-NO_2resorcinol this is due to the presence of an intramolecular hydrogen bond. As has previously been explained this is probably because the fine structure is caused by vibrational sub-levels which may be obscured by the hydrogen bond. In m-NO_2phenol the fine structure is absent because the phenolic species no longer contributes appreciably to the spectrum since the molecule absorbs preferentially as nitrobenzene. In the case of p-NO_2phenol the nitro group is electron withdrawing and causes a considerable increase in mesomeric interaction such

Table 37. Ultra-Violet Absorption Spectra - Nitrophenols

Solvent - Cyclohexane

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Benzene			204	7400	254	200
Phenol			217	3730	260*	840
					264.5	1460
					271	2210
					278	2050
o-NO_2phenol	213	12,800	230*	4100	347.5	3970
			271.5	7720		
m-NO_2phenol	209	11,140	222	10,550	313	2220
			259	6450		
p-NO_2phenol	204.5	9200	286	10,800		
	220	10,100				
	225	9900				
2-NO_2resorcinol	216*	12,800	315	11,000	410	1830
	250	2630				

Solvent - Ethanol

Benzene			204	7400	254*	200
Phenol			218.5	5650	267	1410
					273	1790
					279	1420
o-NO_2phenol	212	12,880	230*	4100	347	3350
			273	6260		
m-NO_2phenol	212	12,590	230	9220	332	2130
			270	6260		
p-NO_2phenol	205	8430	313.5	10,980		
	228.5	7320				
2-NO_2resorcinol	206	12,020	213*	10,710	281*	2400
					311	3290

Solvent - Water

Benzene			200		256*	
Phenol			210	6910	265	1270
					270	1560
					275	1280
o-NO_2phenol	210	13,070	230*	3900	350.5	3190
			278	6520		
m-NO_2phenol	208.5	13,000	229.5	7820	331	2060
			273.5	6060		
p-NO_2phenol	200	9550	318	9700		
	226	6800				
2-NO_2resorcinol	212	11,660	313	5370		

313

* denotes an inflection

that the C-band is so reduced in intensity as to disappear. However, in connection with this, because the nitro group has a strong electron withdrawing effect, the B-band is displaced to a longer wavelength than otherwise expected and is of high intensity, corresponding to an extended conjugated system.

The spectrum of m-NO_2phenol may be explained in terms of partial chromophores as described in the section on the Chlorophenols (7.3.2). The ultraviolet absorption spectrum of m-NO_2phenol consists of three bands ascribed to nitrobenzene absorption (107) and one band ascribed to phenol absorption (133,103,104), the spectrum is not the sum of the spectra of the two parent compounds as can be seen from Table 38.

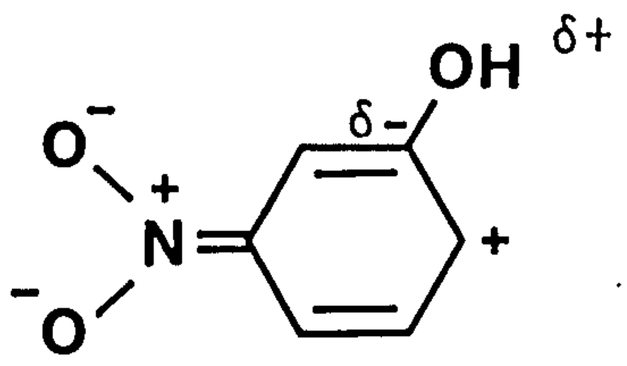
Table 38. Absorption Maxima of Nitrobenzene, Phenol and m-Nitrophenol in Cyclohexane

Bands in nitrobenzene spectrum		Bands in Phenol spectrum		Corresponding bands in m-NO_2phenol spectrum	
λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
206.2	13000@			209	11140
253	9000			259	6450
287	1500			313	2220
		217	3730	222	10550
		260	840		
		264.5*	1460		
		271	2210		
		278	2050		

* Ref Present work
@ Ref 56

Four bands are apparent in m-NO_2phenol since phenolic absorption is displaced to longer wavelength. However, in addition, since the non-phenolic substituent, NO_2, is electron withdrawing, the non-phenolic B-band, i.e.the

nitrobenzene band is also displaced to longer wavelength on account of secondary interaction of the OH group. This consists of a shift of 12mμ in ethanol and 7mμ in cyclohexane in the nitrobenzene band of m-nitrophenol (The nitrobenzene B-band in ethanol occurs at 258mμ ε_{max}= 8000, compared with 270mμ ε_{max}=6260 for m-NO_2phenol). Displacement of the order of 7mμ suggests that the OH group tends to facilitate the absorption of the non-phenolic chromophore. The NO_2 group also facilitates phenolic absorption and hence the phenolic B-band is displaced to longer wavelength. This bathochromic displacement may be related to changes in integrated absorption intensities for infra-red absorption of OH in phenols since the integrated absorption intensity of m-NO_2phenol is greater than for phenol and may be related to increased mesomeric interactions.



When the meta-substituent is electron withdrawing as in the case of the nitro group, the C-band is often enhanced compared with the para-isomer. This can be seen to occur with the nitrophenols since in the para isomer the C-band can no longer be seen. The para-isomer does not show two B-band peaks due to the fact that p-NO_2phenol absorbs as a single chromophore.

The ultraviolet spectral data confirm the presence of an intramolecular hydrogen bond in o-NO_2phenol. The phenolic B-band is hardly discernible, but the nitrobenzene B-band, i.e. the B-band associated with transitions involving predominantly the mono-substituted derivative in which the substituent is electron withdrawing, is bathochromically displaced compared with the corresponding band in m-NO_2phenol. The C-band in the ortho isomer also shows a bathochromic wavelength displacement accompanied by increased band intensity. In addition to these effects, the absence of wavelength displacement on changing the solvent from cyclohexane to ethanol also indicates the presence of an intramolecular hydrogen bond. These spectral effects which are ascribed to the intramolecular hydrogen bond can be adequately explained in terms of an electrostatic model. That is, it may be assumed that the NO_2 group, because of its negative charge, merely attracts the H-atom of the OH group. An appreciable wavelength displacement is observed for both the meta and para isomers on solvent change which further supports the presence of an intramolecular hydrogen bond in o-NO_2phenol.

Intermolecular hydrogen bonding is responsible for the wavelength displacement observed on changing the solvent from cyclohexane to ethanol, cyclohexane cannot form hydrogen bonds whilst ethanol can. However, a further slight bathochromic wavelength displacement is observed when the solvent is changed from ethanol to water. This is also seen with the ortho isomer, suggesting greater interaction with the water molecules, probably due to their small size,

which allows access to the free oxygen atom of the nitro group.

The compound 2-NO_2resorcinol may be examined here to observe the effect of a second hydroxyl group adjacent to the nitro group. Two intramolecular hydrogen bonds are thought to be formed. This seems to be the case in cyclohexane since a large bathochromic shift is observed in the B- and C-bands of 2-NO_2resorcinol compared with o-NO_2phenol. In addition, the B-band of 2-NO_2resorcinol has a much larger ε_{max}. However, a hypsochromic shift is seen when the solvent is changed from cyclohexane to ethanol or water. This suggests loss of planarity of the nitro group and therefore rupture of at least one intramolecular hydrogen bond.

7.3.4 Hydroxybenzaldehydes

In cyclohexane these compounds show the characteristic benzaldehyde doublet in the B-band, but the phenolic C-band fine structure is absent. In the ortho isomer this is again due to the presence of an intramolecular hydrogen bond which obscures the vibrational sub-levels responsible for the fine structure. In the meta isomer the absence of C-band fine structure indicates that the phenolic species does not contribute appreciably to the spectrum and the molecule absorbs preferentially as benzaldehyde. Reduction of the fine structure can be an indication of some sort of interaction between the two groups, but steric effects are unlikely since the formyl group is very small. In the case of the para isomer, the para-hydroxy substituent can act as an electron donor and considerable interactions can occur. This accounts for the large displacement to longer wavelength

Table 39. Ultra-Violet Absorption Spectra - Hydroxybenzaldehydes

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
Benzene			204	7400	254*	200
Phenol			217	3730	260*	840
					264.5	1460
					271	2210
					278	2050
o-OHbenzaldehyde	222	13,080	252.5	11,470	329	3920
			258.5	11,940		
m-OHbenzaldehyde	218.5	12,500	246	10,470	304*	3180
			252	9620	310*	2890
p-OHbenzaldehyde	213.5	15,100	262	17,000	C-band as	
	219	13,820	271	12,360	fine structure	
			278	7380	on B-band.	
			287.5	2940		
Solvent - Ethanol						
Benzene			204	7400	254*	200
Phenol			218.5	5650	267*	1410
					273	1790
					279	1420
o-OHbenzaldehyde	217	13,350	255.5	11,700	327	3950
m-OHbenzaldehyde	220	19,000	255	9600	317.5	2940
p-OHbenzaldehyde	222.5	12,360	285.5	16,600		
Solvent - Water						
Benzene			200		256*	
Phenol			210	6910	265*	1270
					270	1560
					275	1280
o-OHbenzaldehyde	212.5	15,650	256	11,840	324	3230
m-OHbenzaldehyde	217	16,540	254.5	10,100	313	2640
p-OHbenzaldehyde	221	11,460	284	14,870		

* denotes an inflection

of the para-isomer B-band compared to the B-bands of either the ortho or meta isomers. Since the formyl group is electron withdrawing this displacement is greater and band intensity increases. This has the effect of submerging the C-band which can be seen as fine structure on the B-band. The para isomer absorbs as a single chromophore so if it were not for the B-band masking the C-band, the characteristic benzaldehyde doublet would be expected in the cyclohexane spectrum.

The doublet is also seen in the cyclohexane spectrum of m-OHbenzaldehyde but here it is likely that two bands corresponding to the two mono-substituted benzene derivatives would appear. Therefore one of the bands appears to be obscured. This is possibly the phenolic B-band which is obscured by the A-band.

In cyclohexane the B-band of o-OHbenzaldehyde resembles that of m-OHbenzaldehyde but the lack of appreciable wavelength displacement on changing the solvent from cyclohexane to ethanol indicates the presence of an intra-molecular hydrogen bond in the ortho isomer. A slight bathochromic displacement of the B-band of o-OHbenzaldehyde in cyclohexane compared with that of m-OHbenzaldehyde as well as an increase in band intensity is also indicative of an intramolecular hydrogen bond. As is the longer wavelength and greater ε_{max} of the ortho isomer's C-band. The fact that there is a slight solvent effect probably means that the carbonyl group participates in both intra- and inter-molecular hydrogen bonding.

7.3.5 Hydroxybenzoic Acids

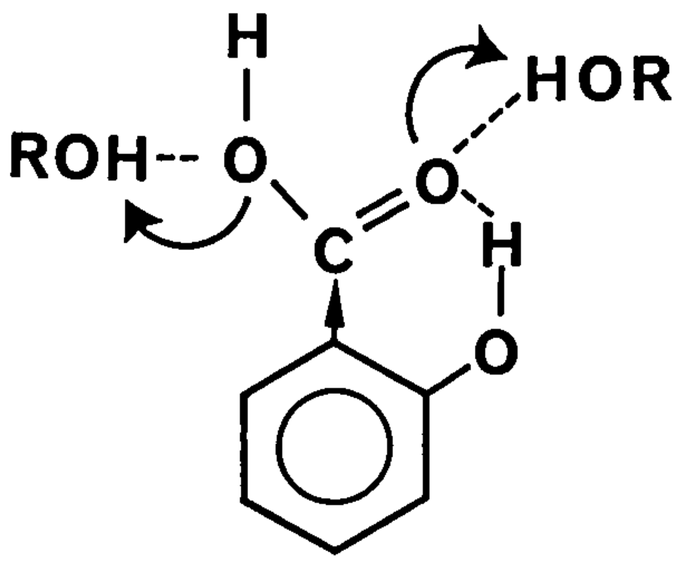
These compounds consist of two chromophores; phenol and benzoic acid, therefore it would be expected that their spectra show characteristics of both these compounds.

The meta and para hydroxybenzoic acids were not soluble in cyclohexane so comparison of spectra is not possible. However, it is apparent that the phenolic C-band fine structure is absent in both the ortho and diortho isomers. This is indicative of an intramolecular hydrogen bond, however, this is usually accompanied by an absence of wavelength displacement on changing solvent, but in the case of the hydroxybenzoic acids a hypsochromic shift occurs on changing from cyclohexane to ethanol to water. This indicates either:

a) the intramolecular hydrogen bond is broken, or

b) solvent interaction reduces conjugation.

i.



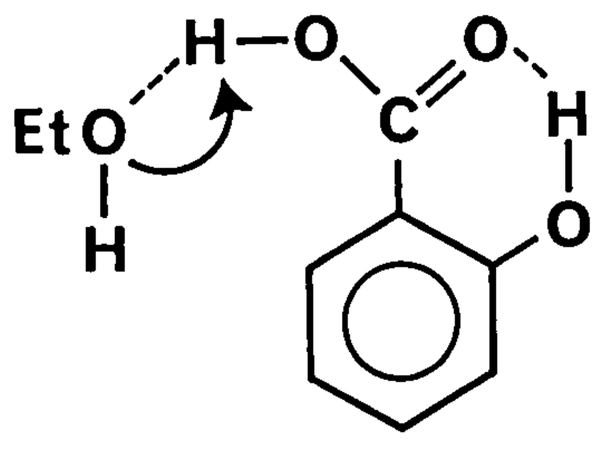
Both these hydrogen bonds would increase conjugation. They are more likely with water, as water is a better proton donor than is ethanol. But this would give a bathochromic shift.

Table 40. Ultra-Violet Absorption Spectra - Hydroxybenzoic Acids

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
Phenol			217	3730	260*	840
					264.5	1460
					271	2210
					278*	2050
Benzoic Acid	201.5	9590	231*5	13,210	267*	980
			237*	11,500	274	1200
					282	1000
o-OHbenzoic acid	213	12,250	240.5	9360	312	4600
			243.5	9340		
m-OHbenzoic acid	INSOLUBLE					
p-OHbenzoic acid	INSOLUBLE					
2,6-OH_2benzoic acid	220	10,450	256	6200	330	2540
3,5-OH_2benzoic acid	INSOLUBLE					
Solvent - Ethanol						
Phenol			218.5	5650	267*	1410
					273	1790
					279*	1420
Benzoic Acid	202.5	4240	228	11,350	265*	700
					272.5	850
					280	660
o-OHbenzoic acid	212	12,880	236	7790	304	4090
m-OHbenzoic acid	214	13,930	235	7050	298	2820
p-OHbenzoic acid	209	13,800	255	15,300		
2,6-OH_2benzoic acid	218	15,840	248	5960	309	3120
3,5-OH_2benzoic acid	215	15,000	250.5	5250	310	2400
Solvent - Water						
Phenol			210	6910	265*	1270
					270	1560
					275	1280
Benzoic Acid	202		228	9700	270	850
o-OHbenzoic acid	202	39,830	230.5	7140	297	3570
m-OHbenzoic acid	200	26,430	231	6500	293	2280
p-OHbenzoic acid			250.5	13,220		
2,6-OH_2benzoic acid	207* 213	21,850 20,700	245.5	6260	307	3060
3,5-OH_2benzoic acid	203	28,120	245	4960	299	2090

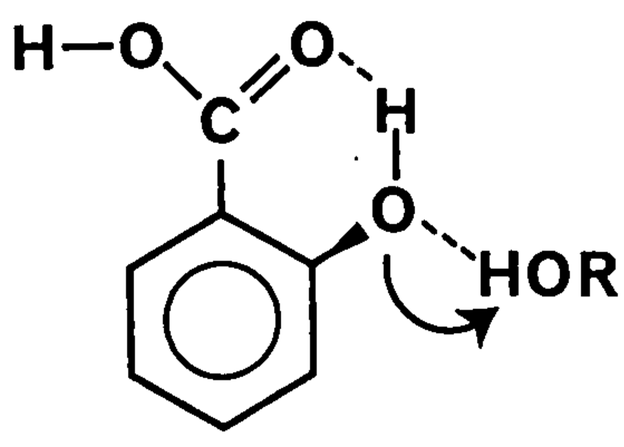
* denotes an inflection

ii.



In ethanol this is also possible, which would reduce conjugation.

iii.



This type of interaction would also reduce cnjugation.

The type (iii) interaction is unlikely to occur in ethanol because ethanol acts largely as a proton acceptor, but may account for the additional hypsochromic shift seen on going from ethanol to water. This shift also occurs with the meta and para isomers which again is due to water being a proton donor.

The para isomer absorbs as a single chromophore and therefore a single B-band is observed but this is displaced to longer wavelength than in the ortho or meta isomer. Since the carboxylic acid group is electron withdrawing this band is also of increased intensity due to the extended conjugated system. With the increase in mesomeric interaction however, the C-band becomes less pronounced such that in p-hydroxy benzoic acid the C-band has disappeared.

In the meta-substituted derivative, direct resonance interaction is ruled out by definition and two bands may be expected corresponding to the two mono-substituted benzene derivatives. Only a single B-band is seen but the A-band is of quite high intensity and it is probable that this obscures the phenolic B-band. With an electron withdrawing substituent such as COOH, the non-phenolic B-band is displaced to longer wavelength on account of secondary interaction of the OH group. The electron withdrawing meta-substituent causes the C-band to be enhanced compared with that of the para-derivative. Thus m-hydroxybenzoic acid has a relatively strong C-band whereas p-hydroxybenzoic acid has no C-band.

Di-meta hydroxy substitution causes a further bathochromic shift of the non-phenolic B-band but a reduction in ε_{max}. A slight bathochromic shift is observed in the A-band, but this is accompanied by an increase in intensity. This is probably indicative of the submerged phenolic B-band since in the 3,5-dihydroxy isomer two phenolic chromophores may be expected to contribute to the spectrum. The shift of the non-phenolic B-band suggests that the two hydroxyl groups facilitate the absorption of the benzoic acid chromophore.

7.3.6 Methylphenols

The substitution of a single methyl group in the phenol molecule causes a slight bathochromic shift in band wavelength in all three solvents which were investigated; cyclohexane, ethanol and water. In each case the shift was most pronounced for the para-isomer. The fine structure of the C-band in cyclohexane is apparent for all isomers, but since the methyl group is electron donating, in the para position it enhances this fine structure. This is despite the fact that increased molecular symmetry is supposed to simplify the absorption spectra.

The wavelength displacements between solutions in cyclohexane and ethanol are similar for phenol and the methylphenols, indicating that even in the ortho position the methyl group has little steric effect. However, if diortho-methyl substitution is considered, it can be seen that a hypsochromic shift occurs in the B-band. This indicates steric hindrance of the hydroxyl group. A similar hypsochromic shift can also be seen in the 2,3-dimethyl isomer. This suggests that the 3-Me group has a buttressing effect which intensifies the ortho effect of the 2-Me group. Evidence of steric hindrance is also given by the reduction in absorption magnitude of the C-band in the sterically hindered molecules.

Para-methyl substitution causes a bathochromic displacement of the B-band, but a similar wavelength displacement is not observed in the di-, tri- or tetra-methyl isomers.
However, a bathochromic wavelength displacement is observed in the C-band of those isomers with a para-substituent i.e.

Table 41. Ultra-Violet Absorption Spectra - Methylphenols

Solvent - Water

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Benzene			200		256*	
Phenol			210	6910	265*	1270
					270	1560
					275	1280
o-Mephenol			211	6950	271*	1680
					274	1550
m-Mephenol			212.5	6750	271.5	1470
					277*	1320
p-Mephenol			214	6920	277	1840
			220	7190	283*	1560
2,3-Me_2phenol	203	11,270	212*	8700	272	1310
					277	1250
2,4-Me_2phenol	200	20,300	215*	8150	278*	2030
					284	1750
2,5-Me_2phenol	201	20,700	215*	9390	274.5	2030
					279	1930
2,6-Me_2phenol	203	10,950	212*	8880	269.5	1330
					275	1270
3,4-Me_2phenol	199.5	20,180	216*	8310	277*	2020
					282	1860
3,5-Me_2phenol			215*	8070	272.5	1300
					279	1280
2,3,5-Me_3phenol			216	9360	275*	1280
					279.5*	1300
2,3,6-Me_3phenol			220	10,480	269.5*	1000
					273	1020
					277	990
2,4,6-Me_3phenol			215*	8990	277	1640
2,3,5,6-Me_4phenol			215*	12,740	270.5*	830
					273.5*	810
					278*	740

* denotes an inflection

Table 41. Ultra-Violet Absorption Spectra - Methylphenols

Solvent - Ethanol

Compound	Rydberg transition Band A		π-π*allowed transition Band B		π-π*forbidden transition Band C	
	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
Benzene			204	7400	254*	200
Phenol			218.5	5650	267	1410
					273	1790
					279	1420
o-Mephenol	204.5	6160	215	6160	273*5	1950
					277	1820
m-Mephenol	205	6760	216.5	5750	274	1660
					280	1510
p-Mephenol	203.5	5010	223.5	6460	280*	1950
					285	1660
2,3-Me_2phenol	207.5	7940	215*	6390	274	1420
					280	1360
2,4-Me_2phenol	206	8310	217*5	6000	280.5	2100
2,5-Me_2phenol	207	8200	217	6300	277*	1900
					283	1780
2,6-Me_2phenol	207	8520	213*	7430	273	1500
					277	1400
3,4-Me_2phenol	206	7080	218	5830	280*	1820
					283	1710
3,5-Me_2phenol	208	9770	220*	6760	275	1510
					281.5	1520
2,3,5-Me_3phenol	210	10,440	219*	7140	275*	1530
					282*5	1630
2,3,6-Me_3phenol	208.5	9590	215*	7760	272	1240
					276	1270
					279	1270
2,4,6-Me_3phenol	208	9690	218*	6820	280	1790
2,3,5,6-Me_4phenol	210	11,700	220*	8300	273*	810
					278	790

* denotes an inflection

Table 41. Ultra-Violet Absorption Spectra - Methylphenols

Solvent - Cyclohexane

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Benzene			204	7400	254*	200
Phenol			217	3730	260*	840
					264.5*	1460
					271	2210
					278	2050
o-Mephenol			213.5	6520	272	1970
					278.5*	1850
m-Mephenol	204	6160	215	6210	268*	1210
					273	1710
					280	1780
p-Mephenol	214*	5500	221	5860	270.5	1490
					273.5	1660
					276.5	1920
					279.5	2220
					286	2030
2,3-Me_2phenol	207	7340	216	7140	267*	1100
					271	1470
					275	1500
					279.5*	1740
2,4-Me_2phenol	205	7300	216	6720	277*	2130
					279.5	2200
					286	2010
2,5-Me_2phenol	206	7620	216	7130	276	2030
					282	1950
2,6-Me_2phenol	205	7000	215	7440	272	1600
					278*	1670
3,4-Me_2phenol	205	6970	216.5	6800	272*	1600
					276	2090
					279	2140
					285*	2020
3,5-Me_2phenol	207.5	7770	218	6890	267*	950
					271	1270
					273.5	1520
					276.5	1320
					281	1780
2,3,5-Me_3phenol	209	9700	216*	8270	273	1410
					278	1430
					282*	1630
2,3,6-Me_3phenol	208.5	8930	215*	8420	267*	1060
					271	1410
					275	1450
					280*	1550
2,4,6-Me_3phenol	208	8820	215*	7710	276*	1960
					280*	1980
					284.5	2050
2,3,5,6-Me_4phenol	211	10,880	219*	9390	269*	1000
					272	1190
					277	1210
					281	1330

2,4-Me_2phenol, 3,4-Me_2phenol and 2,4,6-Me_3phenol. The para-substituent also causes enhancement of the C-band fine structure in cyclohexane which is apparent in the increased ε_{max} values.

2,4,6-Me_3phenol is an interesting compound because although it has two ortho methyl groups which have been said to cause steric hindrance of the hydroxyl group and therefore reduce the absorption magnitude of the C-band, comparison with 2,3,6-Me_3phenol shows that this is not the case. It appears that the enhancement caused by the para-methyl substituent dominates the steric effect.

2,3,5,6-Me_4phenol shows a further reduction in ε_{max} of the C-band which is expected since both ortho methyl groups are buttressed by an adjacent methyl group. This causes increased steric hindrance of the hydroxyl group. Steric hindrance is also apparent in the absence of wavelength displacement on changing solvent from cyclohexane to ethanol, both for the tetramethyl isomer and 2,3,6-Me_3phenol where again the buttressing effect of the 3-Me group is apparent. However, it is possible that rather than steric hindrance being responsible for the reduction in fine structure of the C-band which is observed for the ortho-substituted methylphenols, it could be due to shielding. Fine structure is related to vibrational levels and shielding will reduce the number of available vibrational states.

Meta-methyl substitution appears to produce reduction in the magnitude of the C-band, this is particularly noticeable with the 3,5-dimethyl isomer. This may be attributed to the

electron donating character of the methyl group. Electron withdrawing groups cause enhancement of the C-band in the meta-position and electron donating groups have the opposite effect.

7.3.7 Methylbenzoic Acids

The methylbenzoic acids may be used as an example of the de-coupling of resonance. This discussion may also be applied to the methylphenols and methylacetanilides.

The consequence of de-coupling the resonance between two moieties of a given aromatic structure can be studied very well by analysis of the conjugative absorption band in the UV spectrum (B-band).

This B-band usually has a high intensity (ε = 10,000 or greater) in benzene derivatives, where the substituent is in conjugation with the ring, and can therefore easily be recognised in the spectral pattern. It reflects the interaction of mobile electrons of the benzene system. An essential requirement for optimal interaction (maximum resonance) in a conjugated system is a coplanar configuration of all of the bonds concerned.

When structural changes, e.g. the introduction of an ortho-substituent, reduce the coplanarity of the conjugated system (steric hindrance of resonance), a transformation of the UV absorption spectrum becomes observable. When these changes remain limited in size, the characteristic transition will be confined to vibrational states in which the appropriate bonds are sufficiently extended to allow for a

reasonable degree of residual coplanarity.

The various energy levels will not change appreciably, so that the transition energy remains virtually the same and is reflected spectroscopically in unchanged λ_{max} values. The intensity of absorption is, however, decreased considerably as the transition is restricted to an obviously smaller number of vibrational states.

Enhanced steric hindrance, which may ultimately result in complete de-coupling of the conjugative interaction of the chromophores, increases the energy content of the excited state relative to that of the ground state. This effect implies a higher excitation energy and absorption at lower wavelengths.

Between the two extremes - full coplanarity and complete perpendicularity of the chromophoric moieties - there is a continuous range of possiblities.

Owing to the presence of two ortho CH_3 groups in 2,6-Me_2benzoic acid, the resonance between the benzene ring and the carboxyl group is partially decoupled as revealed by the much reduced ε_{max} but slightly reduced λ_{max} (compared with that of the meta isomer). The spectral pattern reveals that de-coupling is by no means complete, because the absorption in the 230mμ region is far too intense to be neglected. The B-band of 2,6-Me_2benzoic acid is at a slightly longer wavelength than that of benzoic acid which suggests conjugation with the methyl groups, but the ε_{max} value shows pronounced steric twisting. This can be seen to become worse in ethanol and the band disappears

Table 42. Ultra-Violet Absorption Spectra - Methylbenzoic Acids

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
Benzoic acid	201.5	9590	231.5	13,210	267*	980
			237	11,500	274	1200
					282	1000
o-Mebenzoic acid	206	10,110	233	10,890	280*	2300
					286*	2000
m-Mebenzoic acid	208	10,410	235	12,360	281	1690
					289	1520
p-Mebenzoic acid	204	19,660	242	17,750	272	1190
					283	900
2,6-Me_2benzoic acid	206	19,900	233	3800	277	1100
3,5-Me_2benzoic acid	207	26,770	240	11,150	287	1600
					295.5	1590
Solvent - Ethanol						
Benzoic acid	202.5	4240	228	11,350	265*	700
					272.5	850
					280	660
o-Mebenzoic acid	205.5	10,010	229.5	8400	279*	1230
					286*	1000
m-Mebenzoic acid	206.5	10,850	232	10,300	279	1200
					286*	1040
p-Mebenzoic acid	206	10,850	237	14,300	270*	960
					281	660
2,6-Me_2benzoic acid	207.5	11,320	234*	1820	273.5	890
3,5-Me_2benzoic acid	209	13,750	236	9500	285	1520
					293	1390
Solvent - Water						
Benzoic acid	202		228	9700	270	850
o-Mebenzoic acid			229	5140	272	660
m-Mebenzoic acid			232	8440	278	910
p-Mebenzoic acid			237	12,150		
2,6-Me_2benzoic acid	202	17,620			266	470
					268	470
					272.5	460
3,5-Me_2benzoic acid	202	38,700	237	8020	287	1180

* denotes an inflection

altogether in water as the twisting becomes greater due to increased solvent interaction with the carboxyl group.

Slight inhibition of resonance is seen with the ortho isomer in the form of a slight reduction in ε_{max} compared with benzoic acid. Again, this effect becomes more pronounced in ethanol and then water, but the single methyl group does not cause the B-band to disappear altogether in water, showing that the twisting is limited.

In benzoic acid itself, a negative mesomeric effect operates. Whenever a para-substituent can act as an electron donor, appreciable interactions occur and displacements to longer wavelengths are seen. The methyl group can act as such an electron donor and thus should strengthen the H-bond acceptor capability of the carboxyl group, but weaken the H-bond donor ability. The effect of this on λ_{max} and ε_{max} can be seen in Table 42. The meta substituent has little influence in this way. The meta-methyl group causes a bathochromic displacement of the benzoic acid bands in all solvents which is accompanied by a reduction in band intensity. This effect is increased by the addition of a second meta-methyl group.

7.3.8 Methylacetanilides

As with the methylbenzoic acids these compounds show evidence of solvent-assisted steric hindrance causing de-coupling of resonance. The B-band of o-methylacetanilide is at the same wavelength as that of acetanilide, but the intensity is reduced which is indicative of steric twisting. This twisting will be limited so that the appropriate bonds

Table 43. Ultra-Violet Absorption Spectra - Methylacetanilides

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
Acetanilide	204.5	20,000	240	16,040	274.5	1070
					282	840
o-Meacetanilide	206.5	24,450	240	11,820	280*	800
m-Meacetanilide	211	15,050	244	13,500	273	1290
					283	740
p-Meacetanilide	205	22,480	243	16,600	278	1200
					282	1100
					288	1000
2,6-Me_2acetanilide	INSOLUBLE					
3,5-Me_2acetanilide	212	30,160	244.5	13,950	285	640
Solvent - Ethanol						
Acetanilide	207	11,800	242	14,300	280	580
o-Meacetanilide	208	13,150	229.5	6420	270*	720
					280*	340
m-Meacetanilide	211	15,050	244	13,560	273	1290
					283*	740
p-Meacetanilide	208	13,700	245.5	15,340	275	1430
					286	760
2,6-Me_2acetanilide	209	12,960			263	420
					271*5	350
3,5-Me_2acetanilide	215	17,770	247	13,000	285	500
Solvent - Water						
Acetanilide			239	10,870		
o-Meacetanilide			225*	5220		
m-Meacetanilide	203	30,280	241	10,340		
p-Meacetanilide			242	11,760		
2,6-Me_2acetanilide	202	18,620			259*	350
					262*5	400
					267*	360
					271	320
3,5-Me_2acetanilide	207	31,920	244	10,220		

* denotes an inflection

are extended to allow for a certain amount of residual coplanarity. Thus the band wavelength is unaltered. However, the spectrum in ethanol shows a hypsochromic wavelength shift for the ortho isomer as well as a hypochromic shift. This indicates pronounced steric twisting which becomes greater in water such that the band is seen only as an inflection. This occurs because of increased solvent interaction with the acetamido group.

When water is used as the solvent, 2-methyl substitution causes a hypsochromic shift of the B-band with respect to the parent compound, acetanilide. The band maximum for the o-methyl derivative at 225mμ is very close to that of aniline at 224mμ (313) indicating that twisting about the $N\text{-}C_{CO}$ bond probably occurs, relieving steric strain between the o-Me and carbonyl groups, and resulting in loss of resonance between the bulk of the molecule and the aldehydic function, and reversion of the spectrum to the aniline type. Twisting about this bond will cause the least loss of resonance energy concomitant with relief of steric strain as the nitrogen lone pair will still be available for resonance with the ring as long as coplanarity is maintained. This twisting is also reflected in the decreased intensity of the band. The introduction of a second o-methyl group into the 6-position would greatly increase the strain between the methyl and acetamido groups to the extent that the maintenance of coplanarity between the ring and nitrogen would no longer be possible, resulting in the energetically preferred situation of further twisting about the $C_{ring}\text{-}N$ bond with subsequent loss of resonance

energy. The transition, involving only π electrons, would now be expected to resemble that of benzene, and indeed, the value for λ_{max} at 202mμ does approach that of benzene at 200mμ. The very small difference between the two suggests that either the two methyl groups do not completely destroy conjugation, or that there is a very slight residual migration of the lone pair nitrogen electrons into the ring.

The spectrum of 2,6-Me_2acetanilide in ethanol is similar to that in water, but the compound was found to be insoluble in cyclohexane.

The absence of significant spectral change in the meta-, di-meta-, or para-derivatives suggests that the methyl group has little effect on the acetamido group from these positions. The methyl group is small and would therefore exert no further steric effect once moved from close proximity to the acetamido group.

7.3.9 Methylorthonitrophenols

The UV spectrum of 3-Me-2-NO_2phenol in cyclohexane initially indicates that the 3-Me group causes a slight twist of the nitro group away from the plane of the benzene ring as judged by the reduced ε_{max} value of the nitrobenzene B-band. However, the ε_{max} value of 3-Me-2-NO_2phenol is slightly greater than that of o-methylnitrobenzene in iso-octane solution (133) whereas in cyclohexane solution the ε_{max} of o-NO_2phenol is less than that of nitrobenzene (133) and this may imply that the intramolecular hydrogen bonding tends to keep the NO_2 group in a position coplanar with that of the benzene ring. In ethanol solution the ε_{max} values of all

three bands in 3-Me-2-NO_2phenol are drastically reduced. Alteration of the solvent from cyclohexane to ethanol gives rise to appreciable wavelength displacements which indicates competing solvent-solute interactions.

The hypothesis of a coplanar conformation facilitating hydrogen bonding receives support from the fact that this coplanarity is disturbed in ethanol or ether solution, as judged by the greatly reduced ε_{max} values of the 3-methyl substituted compound, although ether probably interacts predominantly with the OH group.

Table 44. Absorption Maxima of o-Nitrophenol and 3-Methyl-2-Nitrophenol in Ether

		o-NO_2phenol		3-Me-2-NO_2phenol	
		λ_{max}(mμ)	ε_{max}	λ_{max}(mμ)	ε_{max}
Nitrobenzene	B-band	271	6900	278.5	3300
second	B-band	234*	3000	236*	2600
	C-band	346*	3550	348	1400

* denotes an inflection

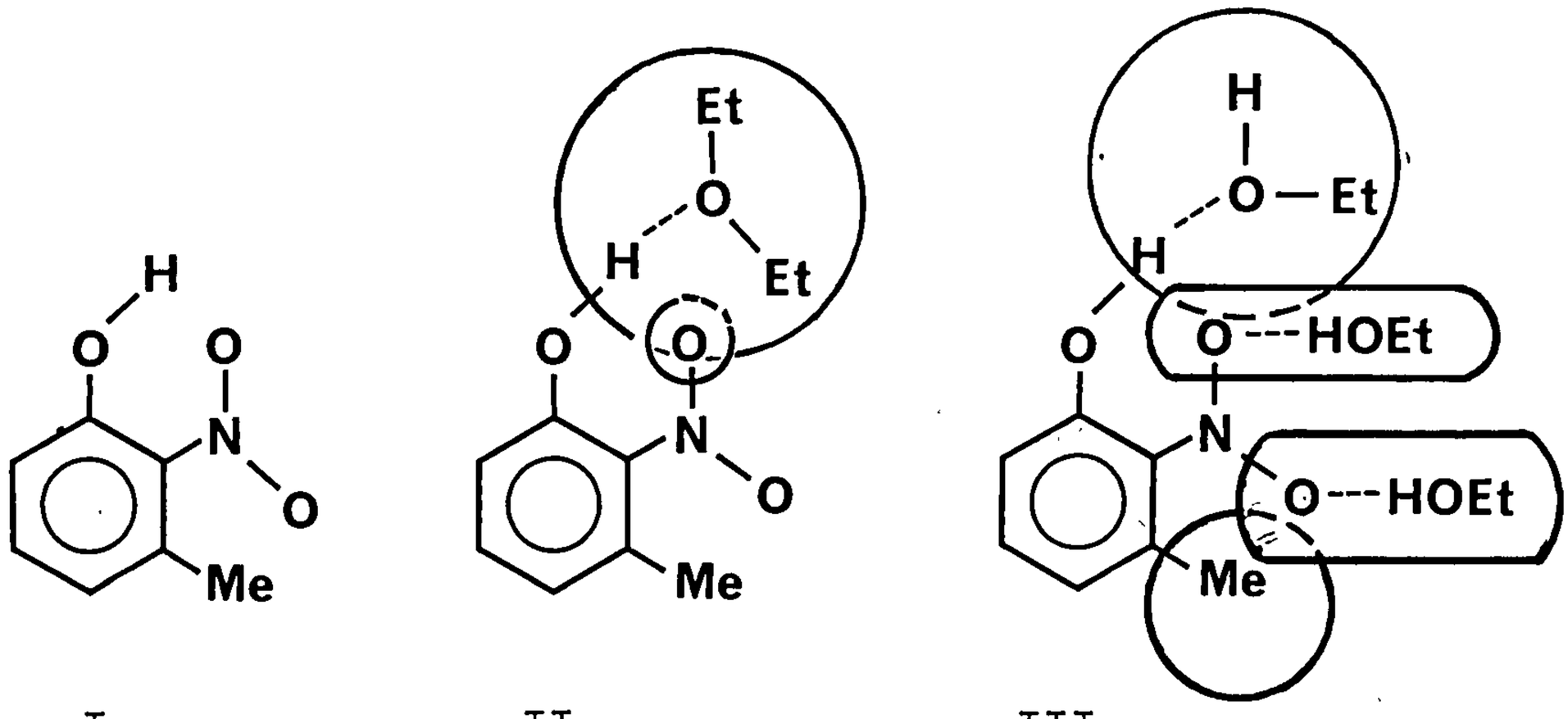
It may thus be assumed that ether interacts competitively with the OH group and in this way weakens the intramolecular hydrogen bond, and that as a result of this weakening, there occurs the above-mentioned non-planarity between the nitro group and the benzene ring. This is further illustrated by structures I,II and III, which schematically indicate the effect of competing intermolecular hydrogen bonding on the intramolecular hydrogen bond and on the steric interactions present in 3-Me-2-NO_2phenol. The steric facilitation of hydrogen bonding relative to o-NO_2phenol, which is observed

Table 45. Ultra-Violet Absorption Spectra - Methylorthonitrophenols

Compound	Rydberg transition Band A λ_{max}(mμ)	ε_{max}	π-π*allowed transition Band B λ_{max}(mμ)	ε_{max}	π-π*forbidden transition Band C λ_{max}(mμ)	ε_{max}
Solvent - Cyclohexane						
o-NO_2 phenol	213	12,800	230*	4100	347.5	3970
			271.5	7720		
3-Me-2-NO_2 phenol	217	11,880	236*	3660	354	3050
			279*	6820		
4-Me-2-NO_2phenol	216.5	14,360	235*	4300	362	3900
			274*5	7780		
5-Me-2-NO_2phenol	215	13,320	231*	4100	346	4720
			283	9250		
6-Me-2-NO_2phenol	215	13,000	281	7980	356	3630
Solvent - Ethanol						
o-NO_2phenol	212	12,880	230*	4100	347	3350
			273	6260		
3-Me-2-NO_2phenol	214	10,340	243*	1810		
			270*	1810		
			295*	1150		
4-Me-2-NO_2phenol	214	12,020	234*	2790	363	1960
			277*	4780		
5-Me-2-NO_2phenol	214	12,980	231*	4160	347	4370
			287	8030		
6-Me-2-NO_2phenol	214	12,540	285	7150	357	3240
Solvent - Water						
o-NO_2phenol	210	13,070	230*	3900	350.5	3190
			278	6520		
3-Me-2-NO_2phenol	210	10,780	268*5	2310	344	1220
4-Me-2-NO_2phenol	212	15,580	235*	4120	369	3010
			282*	6520		
5-Me-2-NO_2phenol	212	12,620	230*	4210	347	4300
			295	8080		
6-Me-2-NO_2phenol	211	12,840	291	6920	362	2900

* denotes an inflection

for 3-Me-2-NO_2phenol as judged from the IR O-H bands, may be ascribed to the NO_2 group - OH group distance being decreased probably through the NO_2 group being bent towards the OH group.



I II III

In Cyclohexane In Ether In Ethanol

If a molecule contains two or more absorbing species or 'partial chromophores' which are not conjugated with each other, its spectrum will be the sum of the separate absorptions of the partial chromophores. Ortho and meta disubstituted benzenes exhibit partial isolation of the chromophores, but it is to be expected that strong absorption by the chromophore containing an electron-attracting substituent will often completely submerge the residual absorption of the other chromophore. However, if absorption by the former chromophore is in some way prevented or reduced, this residual absorption should become apparent. This is what occurs in 3-Me-2-NO_2phenol and it can be seen by comparison of the spectra in different solvents. In cyclohexane the spectrum is very similar to that of o-NO_2phenol

except for a slight decrease of molar absorptivity due to steric hindrance.

In ether a marked decrease in molar absorptivity occurs, which is attributed to an increase in the effective size of the hydroxy group due to competitive intermolecular hydrogen bonding (II). In ethanol, at least two inter-molecular hydrogen bonds can be formed (III) and the resulting steric interactions force the nitro group to be far from coplanar with the benzene ring. Absorption by the nitrobenzene chromophore is thus reduced to such an extent that the residual phenol B-band absorption becomes evident as fine structure.

Alkyl substitution ortho to the hydroxyl group, as in 6-Me-2-NO_2phenol, causes the UV maximal wavelengths to be displaced to longer wavelengths, suggesting that the intra-molecular hydrogen bond has been strengthened. This may be assumed to provide an example of steric facilitation of hydrogen bonding. Increased intensity of the B-band also supports the theory of strengthening of the hydrogen bond. In addition, the C-band is displaced bathochromically with increased intensity which is also indicative of a strengthened hydrogen bond.

The methyl group in position 5- appears to have little effect on the molecule, producing a spectrum which is very similar to that of o-NO_2phenol, although the nitrobenzene B-band is displaced to longer wavelength with increased intensity, indicating that the methyl group enhances nitrobenzene absorption.

Substitution of a methyl group in the 4-position has little effect on the spectrum in cyclohexane and the spectrum is very similar to that of o-NO_2phenol, however, in ethanol the ε_{max} value is considerably lower. This indicates either steric inhibition of resonance or a reduction in the strength of the intramolecular hydrogen bond. Since the methyl group is relatively small and is one carbon removed from the nitro group it is unlikely that this effect is due to steric hindrance. However, hyperconjugation and an inductive effect can weaken intramolecular bonding and it appears that this is the case in this molecule. Infra-red spectral evidence supports this theory.

Thus, 3- and 4-methyl substitution in o-NO_2phenol tend to weaken the intramolecular hydrogen bond, whilst 6-methyl substitution seems to strengthen it.

7.4 Infra-Red Absorption Spectra

7.4.1 Chlorophenols

Table 46. Infra-red Absorption Maxima in Chloroform and Carbon Tetrachloride

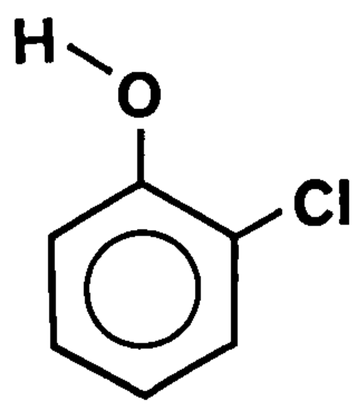
Compound	Chloroform $\upsilon OHcm^{-1}$	Carbon Tetrachloride $\upsilon OHcm^{-1}$
Phenol	3595 (s)	3611 (s)
o-Clphenol	3540 (s) & 3595 (w)	3550 (s) & 3611 (w)
m-Clphenol	3595 (s)	3611 (s)
p-Clphenol	3595 (s)	3611 (s)

s = strong band
w = weak band

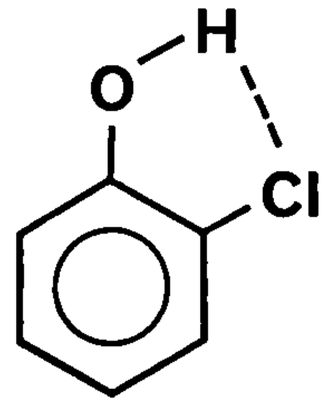
The vibrational spectra of the o-halophenols were first investigated in the near infrared region by Wulf et al (386) who found two hydroxyl stretching frequencies at concentrations low enough to prevent intermolecular association. It can be seen from the table above that this investigation has found the same effect.

Table 46 shows that one of the bands occurs at the normal phenolic position while the other, which is always much more intense, is shifted to lower frequencies by an amount which is dependent on the halogen (18). Since comparable effects do not occur in the spectra of phenols o-substituted with other types of basic atoms such as oxygen, nitrogen or sulphur, the doubling is somewhat anomalous and a complete explanation has not yet been found.

Pauling (311) and subsequently others (396,90), ascribed this doubling to an equilibrium involving cis and trans structures of the type:



trans



cis

According to this hypothesis, the O-H bond is partially constrained to the aromatic plane because of resonance interaction between the aromatic π-molecular orbitals and one of the non-bonding electron pairs on the oxygen. The bond type of the oxygen consequently tends towards sp^2 hybridization with two minimum potential energy configurations as shown. In the cis position, a hydrogen bond is formed to the halogen thus making the cis configuration more stable than the trans by 2 to 3kcal/mole (18).

The hydrogen bond is strongly indicated by the shift to lower frequency of the O-H stretching band in o-Clphenol. The meta and para isomers which do not possess an intramolecular hydrogen bond have the hydroxyl stretching frequency in the same position as phenol. It can also be seen that changing from a polar solvent (chloroform) to a non-polar solvent (carbon tetrachloride) produces a frequency displacement of $16cm^{-1}$ for the non-bonded species which is reduced to $10cm^{-1}$ for the ortho isomer where an intramolecular hydrogen bond is present. The trans isomer shift however is of the same magnitude as the non-bonded species.

7.4.2 Nitrophenols

Table 47. Infra-Red Absorption Maxima in Chloroform and Carbon Tetrachloride

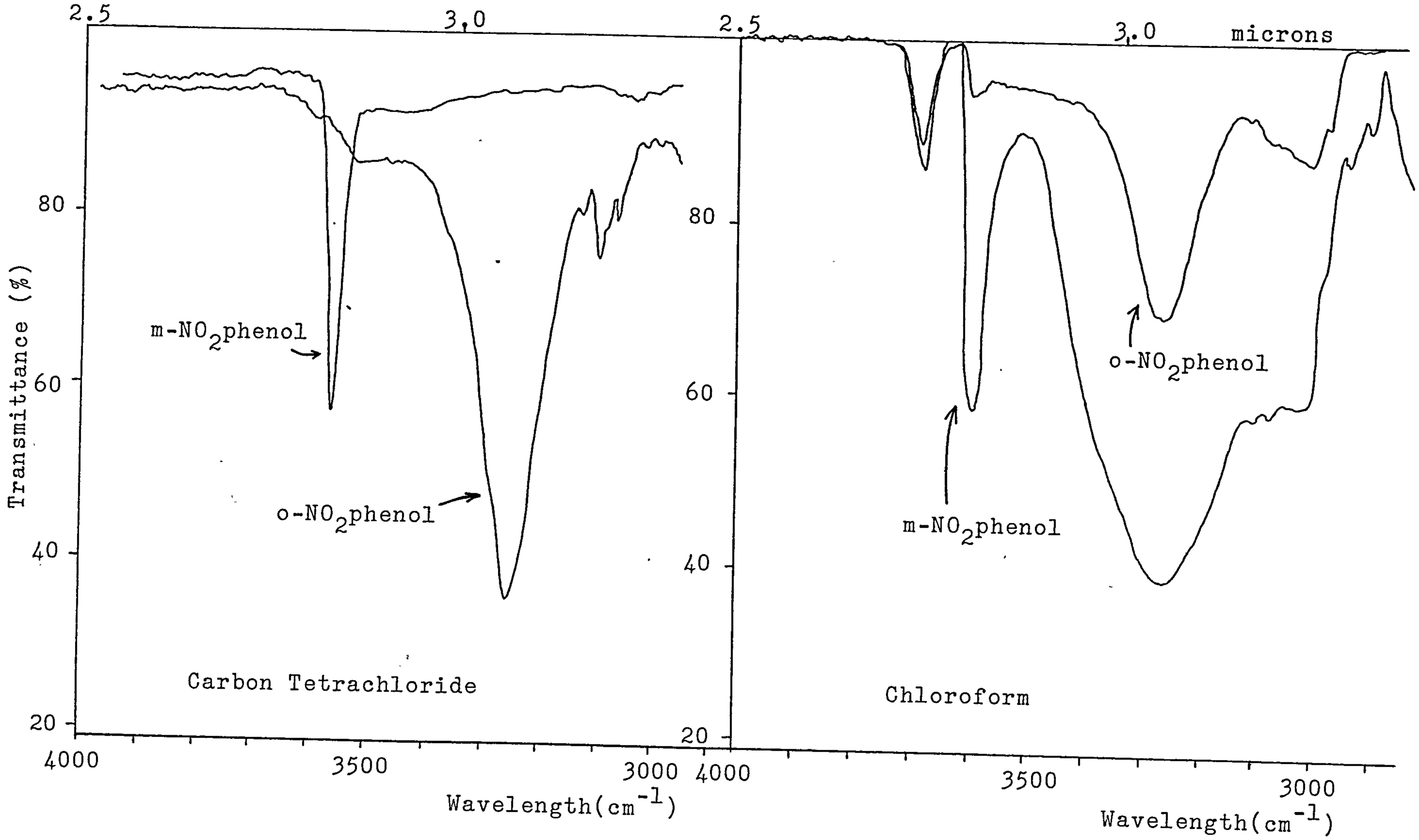
Compound	Chloroform			Carbon Tetrachloride		
	νN-Ocm^{-1}	νN-Ocm^{-1}	νO-Hcm^{-1}	νN-Ocm^{-1}	νN-Ocm^{-1}	νOHcm^{-1}
Phenol	---	---	3595	---	---	3611
o-NO_2phenol	1530	1330	3260(s)	1530	1330	3250(s)
m-NO_2phenol	1535	1350	3590(s)	1533	1358	3610(s)
p-NO_2phenol	1500	1340	3585(s)	1526	1348	3600(s)
2-NO_2resorcinol	1550	1340	3260(s)	1560	1350	3250(s)

s = strong band

The hydroxyl stretching band if o-NO_2phenol shows an intense band at 3260cm^{-1} with only low intensity absorption at higher frequency. The position of this band indicates that it corresponds to an intramolecular hydrogen bond. The meta and para isomers do not show this band although 2-NO_2resorcinol does. The meta and para isomers instead have a distinct free hydroxyl stretching band at 3590cm^{-1} in chloroform and 3610cm^{-1} in carbon tetrachloride. This frequency displacement when the solvent is changed also demonstrates the absence of an intramolecular hydrogen bond since the displacement does not occur for o-NO_2phenol or for 2-NO_2resorcinol. The intensity of the hydroxyl stretching band is increased in 2-NO_2resorcinol indicating the presence of two hydroxyl groups and thus two intra-molecular hydrogen bonds.

It can be seen that although the O-H stretching vibration is affected by the intramolecular hydrogen bond, the N-O stretching vibrations remain virtually unaffected by the hydrogen bond or related interactions since the frequencies

Figure 28. Infra-Red Spectra of o-Nitrophenol and m-Nitrophenol in Chloroform and Carbon Tetrachloride



are similar in all isomers. Therefore the data provides no evidence for other than an electrostatic linkage in o-NO_2phenol, that is, covalent-type hydrogen-bonded structures are excluded. A covalent-type linkage would be expected to affect the N-O bond order and consequently the IR stretching bands. The fact that the N-O bond order remains unaffected in o-NO_2phenol represents somewhat exceptional behaviour which cannot be explained readily interms of a covalent linkage, but can be explained in terms of an electrostatic linkage if it is assumed that the relatively large negative charge on the NO_2 oxygen atom is only slightly affected by the comparatively smaller positive charge on the neighbouring hydrogen atom of the OH group.

7.4.3 Hydroxybenzaldehydes

Table 48. Infra-Red Absorption Maxima in Chloroform and Carbon Tetrachloride

Compound	Chloroform		Carbon Tetrachloride	
	νOHcm^{-1}	νC=Ocm^{-1}	νOHcm^{-1}	νC=Ocm^{-1}
Phenol	3595(s)	----	3611(s)	----
o-OHbenzaldehyde	3190(w)	1667(s)	3185(w)	1670(s)
m-OHbenzaldehyde	3595(s)	1700(s)	3611(s)	1708(s)
p-OHbenzaldehyde	3590(s)	1680(s)	3611(s)	1708(s)

s = strong band
w = weak band

m-Hydroxybenzaldehyde and p-hydroxybenzaldehyde do not form intramolecular hydrogen bonds and therefore the phenolic OH stretching frequency is the same as that of the parent phenol and also a frequency displacement of 16 and 21cm^{-1} respectively occurs when the solvent is changed from the polar chloroform to the non-polar carbon tetrachloride.

o-Hydroxybenzaldehyde however does form an intramolecular hydrogen bond as can be seen from the move to lower frequency of both the O-H and C=O stretching bands when compared to phenol or the meta and para isomers. The lack of frequency displacement on solvent change is also indicative of an intramolecular hydrogen bond. The hydrogen bonded hydroxyl group has a reduced intensity and the band is rather broader than the band for an unbonded hydroxyl.

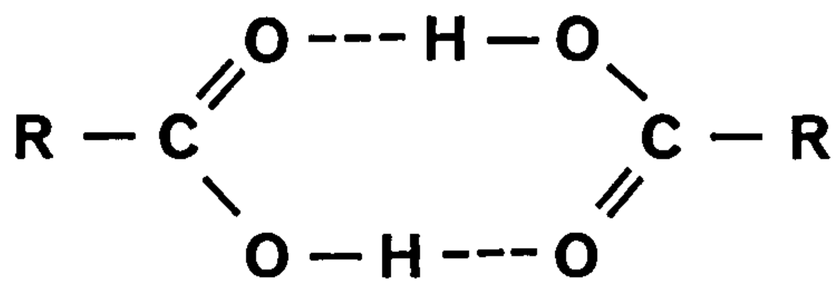
7.4.4 Hydroxybenzoic Acids

Table 49. Infra-Red Absorption Maxima in Chloroform and Carbon Tetrachloride

Compound	Chloroform			Carbon Tetrachloride	
	νO-H cm^{-1} (acid)	νC=O cm^{-1} (acid)	νO-H cm^{-1} (phenol)	νO-H cm^{-1}	νC=O cm^{-1}
Phenol	----	----	3595	3611(phenol)	
Benzoic acid	3520	1734	----	3545	1744
o-OHbenzoic acid	3505	1668	3190		1691
2,6-OH_2benzoic acid	3410	1678	3050		

Due to solubility problems it was only possible to record the spectra of o-OHbenzoic acid and 2,6-OH_2benzoic acid.

Hydroxy compounds in solid and liquid states normally exist as polymeric aggregates held together by hydrogen bonds. Carboxylic acids form dimers such as:



which can be picked out by the very broad hydroxyl νOH band

at ∿3400-2500cm^{-1}. When this band is examined under higher resolution, it is found to be composed of a number of subsidiary maxima which are generally held to be due to Fermi resonance of the νOH vibration with overtone and combination bands from lower frequency fundamentals and not to a variety of hydroxyl groups. The other principal absorptions which may be assigned to the acid dimer group are:

s 1710cm^{-1}		νC=O
w 1420cm^{-1}	}	coupled C-C, C-O stretch and C-OH
ms 1300cm^{-1}		in plane bending modes

monomeric forms of carboxylic acids have νOH ∿3530 sharp and νC=O ∿1760s cm^{-1} in CCl_4.

Dimer formation occurs through intermolecular hydrogen bonding but in o-hydroxybenzoic acid, intramolecular hydrogen bonding is possible which has a similar effect on the absorption maxima. It can be seen that the acid νC=O band and the phenolic νOH band are at much reduced frequency in the ortho isomer compared with either of the parent molecules. This indicates the presence of a hydrogen bond between these two groups. The acid νOH band is only slightly changed in comparison indicating that this is unbonded. A similar lowering of band frequency is found for the acid νC=O band and the phenolic νOH band in 2,6-OH_2benzoic acid, but in this case the acid νOH band also has a lowered frequency indicating its participation in an intramolecular hydrogen bond with the second hydroxyl group.

The frequency change observed for o-OHbenzoic acid on changing solvent is probably indicative of the free hydroxyl group.

7.4.5 Methylphenols

Table 50. Infra-Red Absorption Maxima in Chloroform

Compound	νOH cm^{-1} (free OH)	νOH cm^{-1} (inter-Hbond)
Phenol	3595 (s)	3330 (s)
o-Mephenol	3600 (s)	3340 (m)
m-Mephenol	3595 (s)	3330 (s)
p-Mephenol	3595 (s)	3340 (s)
2,3-Me_2phenol	3600 (s)	3350 (m)
2,4-Me_2phenol	3600 (s)	3350 (m)
2,5-Me_2phenol	3600 (s)	3340 (m)
2,6-Me_2phenol	3605 (s)	3400 (w)
3,4-Me_2phenol	3595 (s)	3340 (s)
3,5-Me_2phenol	3595 (s)	3340 (s)
2,3,5-Me_3phenol	3600 (s)	3350 (m)
2,3,6-Me_3phenol	3605 (s)	3420 (w)
2,4,6-Me_3phenol	3605 (s)	3400 (w)
2,3,5,6-Me_4phenol	3605 (s)	3420 (w)

Band intensity: w = weak, m = medium and s = strong

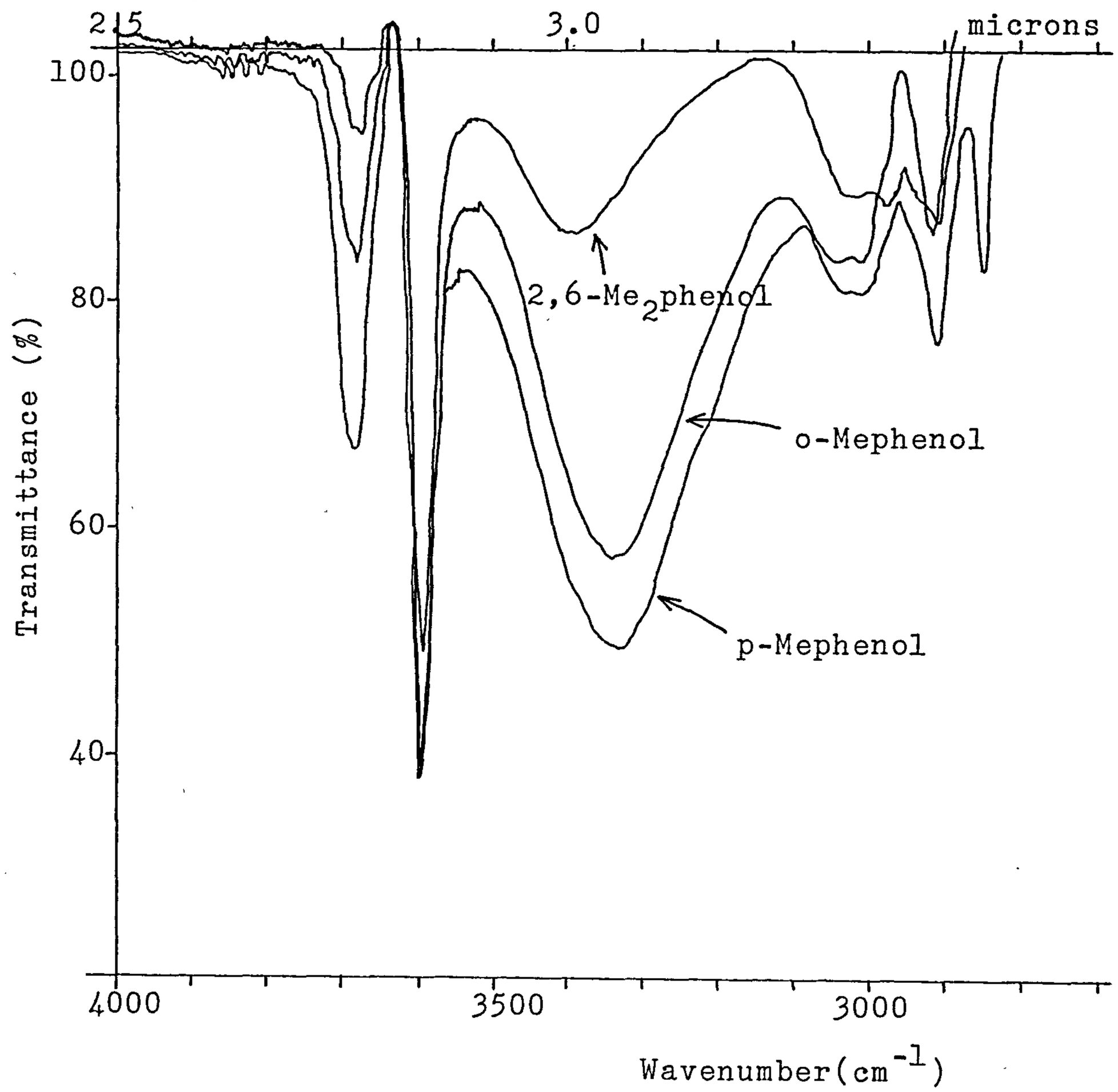
Two νOH stretching bands can be seen in the methylphenols which indicate the effects of methyl substitution.

The first band at ν_{max}3595cm^{-1} represents the free O-H stretching vibration and the second band at ν_{max}3330cm^{-1}, which is also much broader, represents the intermolecularly hydrogen bonded O-H stretching frequency.

The free νOH band is moved to a slightly higher frequency when a methyl group is substituted in the ortho position. A single ortho-methyl group produces a frequency displacement of 5cm^{-1} and diortho-methyl substitution produces a frequency displacement of 10cm^{-1}. This indicates steric hindrance of the OH group which restricts its stretching vibration.

The effect of steric hindrance can be seen more clearly in the band representing intermolecular hydrogen bonding. A

Figure 29. Infra-Red Spectra of o-Methylphenol, p-Methylphenol and 2,6-Dimethylphenol in Chloroform



single ortho-methyl group has little if any effect on intermolecular bonding. However, diortho-methyl substitution produces a fairly large displacement to higher frequency as well as a reduction in band intensity. This indicates that the methyl groups shield the hydroxyl group and prevent bonding between the two molecules.

7.4.6 Methylbenzoic Acids

Table 51. Infra-Red Absorption Maxima in Chloroform and Carbon Tetrachloride

Compound	Chloroform		Carbon Tetrachloride	
	νOH cm^{-1}	νC=O cm^{-1}	νOH cm^{-1}	νC=O cm^{-1}
Benzoic acid	3520	1695(1734)	3545	1744
o-Mebenzoic acid	3520	1695	3540	1740
m-Mebenzoic acid	3520	1695	3545	1740
p-Mebenzoic acid	3530	1690	3545	1740
2,6-Me_2benzoic acid	3500	1725	3520	1750
3,5-Me_2benzoic acid	3525	1695	3545	1740

The methylbenzoic acids infrared spectra give a good example of the effect of dimer formation between carboxylic acid groups.

At first glance it appears that the methyl group has little effect on the IR spectrum of benzoic acid unless two groups are substituted in the ortho positions. This appears to increase the frequency of the νC=O stretching band and decrease the frequency of the νOH stretching band. This may be attributed to a steric effect of the methyl groups which causes the carboxylic acid group to twist out of the aromatic ring plane. However, the COOH group is electron attracting and if twisted it cannot attract much electron density and therefore the band orders would be expected to

be less and the frequencies lower. Thus this explanation does not seem to be viable. However, if the formation of dimers is considered a different explanation may be offered. The carboxylic acid group dimer may be seen as a very broad hydroxyl νOH band at ∿3400-2500cm^{-1}. This band can be clearly seen in the spectra of the methylbenzoic acids. As explained in section 7.4.4, when examined under higher resolution this band is found to be composed of a number of subsidiary maxima which are probably due to Fermi resonance of the νOH vibration with overtone and combination bands from lower frequency fundamentals. In addition to the broad νOH band, the νC=O band is displaced to a lower frequency of approximately 1710cm^{-1}. The monomeric acid has a sharp hydroxyl νOH at ∿3530cm^{-1} and a strong carboxyl νC=O band at ∿1760cm^{-1}. Thus the carbonyl band in 2,6-Me_2benzoic acid appears to represent the monomer whilst the carbonyl bands in the other isomers represent the dimers. This indicates that steric hindrance by the two ortho methyl groups prevents dimer formation. The presence of a weak but sharp band at ∿3530cm^{-1} in the spectra of all the isomers indicates that the molecules are present as both monomers and dimers.

7.4.7 Methylacetanilides

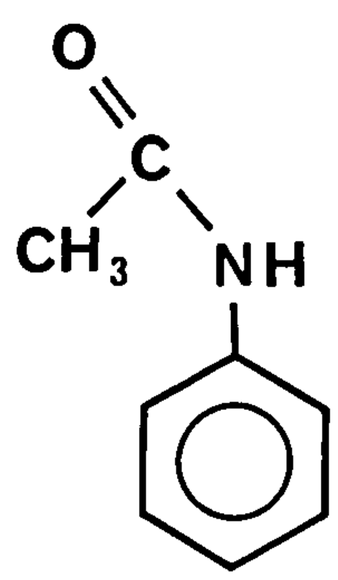
As can be seen from Table 52 two N-H stretching vibrations are observed for the methylacetanilides. Similar peaks were originally observed for acetanilide and certain derivatives by Dyall and Kemp (120) and Suzuki et al (350) Since all the solutions were dilute, of the order of 10^{-3}M, the lower frequency band is unlikely to be an association

Table 52. Infra-Red Absorption Maxima in Chloroform

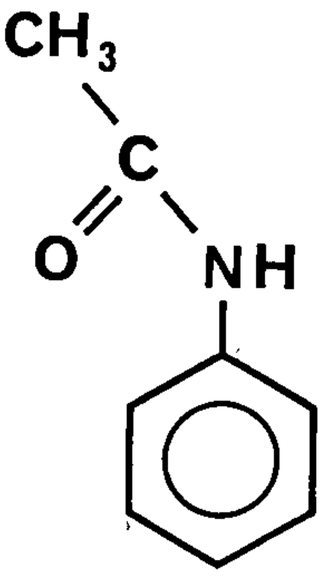
Compound	νNHcm^{-1} (endo)	νNHcm^{-1} (exo)	νC=Ocm^{-1} (aldehyde)
Acetanilide	3440(s)	3390(w)	1690
o-Meacetanilide	3436(s)	3385(m)	1690
m-Meacetanilide	3440(s)	3390(w)	1690
p-Meacetanilide	3440(s)	3390(w)	1685
2,6-Me_2acetanilide	3425(s)	3380(m)	1675
3,5-Me_2acetanilide	3440(s)	3390(w)	1685

Band intensities: w = weak, m = medium and s = strong.

band. Thus the two have been assigned to the endo- and exo-forms, the higher frequency band being the endo conformer stretching frequency in view of its greater intensity and the reasoning that this will be the preferred conformation by analogy with acetanilide (304)



exo



endo

The derivatives with methyl groups in the meta or para positions show values for both νN-H stretching bands equal to those of the parent acetanilide. This is consistent with the analysis of the ultraviolet spectra of these compounds which suggested coplanarity of the ring and nitrogen lone pair electrons and sp^2 hybridization at the nitrogen atom. In the o-methyl isomer the frequency is slightly lower, supporting the theory that twisting about

the N-C_{CO} bond occurs to relieve steric strain and resulting in loss of resonance between the benzene ring and the acetyl function, but without loss of coplanarity of the ring and nitrogen lone pair electrons. The frequency is reduced further for 2,6-dimethyl substitution, again supporting the theory that further twisting about the C_{ring}-N bond occurs causing loss of resonance and suggesting that without the preference for the lone pair to occupy a p-orbital for optimal resonance stabilization, steric requirements direct an approach to sp^3 hybridization, the reduced s character of the bonds causing a reduction in stretching frequency of the N-H bond.

The C=O stretching vibrations show a similar reaction to methyl subsitution, the 2,6-dimethyl derivative producing a marked frequency reduction.

7.4.8 Methylorthonitrophenols

Table 53. Infra-Red Absorption Maxima in Chloroform and Carbon Tetrachloride

Compound	Chloroform			Carbon Tetrachloride		
	$\nu NO cm^{-1}$	$\nu NO cm^{-1}$	$\nu OH cm^{-1}$	$\nu NO cm^{-1}$	$\nu NO cm^{-1}$	$\nu OH cm^{-1}$
Phenol	----	----	3595	----	----	3611
o-NO phenol	1530	1330	3260	1530	1330	3250
3-Me-2-NO phenol	1530	1340	3200	1535	1350	3200
4-Me-2-NO phenol	1535	1323	3260	1540	1330	3225
5-Me-2-NO phenol	1530	1328	3250	1540	1330	3220
6-Me-2-NO phenol	1535	1320	3230	1540	1330	3210

The infrared νOH stretching vibration bands of all o-nitrophenols containing a methyl group vicinal to the nitro group are different from those bands in other o-nitrophenols. (106). 3-methyl substituted 2-NO_2phenols afford a broad

O-H absorption band in the 3350-2900cm^{-1} region as opposed to the more intense, sharp bands observed for the other o-NO_2phenols. This broadness of the band suggests that 3-Me-2-NO_2phenol gives rise to a strong intramolecular hydrogen bond, presumably because of steric facilitation of the hydrogen bond. Apart from the diffuse O-H stretching band, 3-Me-2-NO_2phenol also affords a sharp band which occurs at lower frequency and which is ascribed to -C-H stretching bands. The displacement of the IR νOH stretching band for 3-Me-2-NO_2phenol with respect to o-NO_2phenol ($\Delta\nu$ = +45cm^{-1}) again indicates steric facilitation of hydrogen bonding. This indicates the importance of the solvent for this molecule since UV evidence indicates that ethanol or ether weaken the intramolecular hydrogen bond by means of competitive intermolecular bonding.

The IR νNO_2 symmetrical stretching band of 3-Me-2-NO_2phenol shows some signs of band splitting, the most obvious cause of which is conformational heterogenicity, but it is difficult to conceive of two different conformational isomers in 3-Me-2-NO_2phenol. However, the very broad band suggests a number of possible intramolecular hydrogen bonds which may be possible because of a twisting effect of the methyl group. This could give rise to the different νNO values. There is also some evidence that in sterically hindered o-nitrophenols the intensity of the symmetrical NO_2 stretching band decreases slightly relative to the same band in o-NO_2phenol, presumably because of steric interactions (215).

The steric facilitation of hydrogen bonding relative to o-NO_2phenol which is observed for 3-Me-2-NO_2phenol as judged from the IR hydroxyl bands may be ascribed to the NO_2 group - OH group distance being decreased through the nitro group being bent towards the hydroxyl group.

The νOH band in 5-Me-2-NO_2phenol occurs fairly close to the νOH band in o-NO_2phenol. Relative to the νOH band in o-NO_2phenol, the νOH band of 3-Me-2-NO_2phenol is displaced by $60cm^{-1}$ and the relative band in 5-Me-2-NO_2phenol by $10cm^{-1}$ when chloroform is the solvent. No displacement is observed for this band in 4-Me-2-NO_2phenol when measured in chloroform, but the displacements become $50cm^{-1}$, $30cm^{-1}$ and $25cm^{-1}$ respectively in carbon tetrachloride. These displacements in the 5-Me and 4-Me isomers are due to mesomeric interactions and not steric interactions which cause a larger effect.

A substituent in the 4-position causes absorption at a slightly higher frequency whereas a substituent in the 5-position causes absorption at a slightly lower frequency than for o-NO_2phenol.(105) This may in general terms be related to the ability of an electron donating substituent in the 5-position to enhance the negative charge on the nitro group by means of mesomeric interaction which tends to facilitate the intramolecular hydrogen bond.

Related, though less pronounced effects are observed for the NO_2 stretching vibrations: 4-Me-2-NO_2phenol absorbs at $1535cm^{-1}$ which is slightly higher than for o-NO_2phenol, but 5-Me-2-NO_2phenol absorbs at the same frequency.

Similar effects are observed for the symmetrical NO_2 stretching vibration near 1330cm^{-1} except that comparisons are more difficult because of band splitting. Thus the effect of both electronic and steric interactions on the NO_2 vibrational stretching bands are small but there is a tendency for electron-donating substituents in the 4- or 5-positions to cause absorption at lower frequency.

As judged by the IR OH stretching band of 6-Me-2-NO_2phenol an alkyl substituent ortho to the hydroxyl group in o-NO_2phenol apparently strengthens the intramolecular hydrogen bond, seen in the displacement to lower frequency. Thus introduction of an alkyl group vicinal to the OH group in o-NO_2phenol may be assumed to provide an example of steric facilitation of hydrogen bonding.

7.5 Nuclear Magnetic Resonance Spectra

The NMR spectra of these compounds did not reveal a great deal of information, therefore this discussion will be brief.

The presence of the intramolecular hydrogen bond in o-Clphenol was indicated by a shift in the hydroxyl band from 5.58 for p-Clphenol to 6.38 for o-Clphenol. Similar shifts are seen for the nitrophenols and hydroxybenzaldehydes although the shifts are much larger, possibly indicating the increased strength of the hydrogen bond. The aldehyde band of hydroxybenzaldehyde does not appear to be affected by the intramolecular hydrogen bond since no shift is observed from the meta to the ortho isomer. The hydroxyl band shifts from 6.05 in m-OHbenzaldehyde to 11.10 in o-OHbenzaldehyde and from 5.45 in m-NO_2phenol to 10.60 in o-NO_2phenol. The hydroxyl band is in the same place in 5-Me-2-NO_2phenol as it is in o-NO_2phenol, but in 3-Me-2-NO_2phenol the band is at the slightly lower position of 10.20, possibly indicating that the methyl group in this position weakens the intramolecular hydrogen bond.

The acetyl methyl proton and ring methyl proton shifts in the methyl acetanilides were observed to see the effect of methyl substitution in acetanilide. This revealed that 2,6-Me_2acetanilide showed three acetyl methyl proton shifts at 2.25, 1.71 and 2.13 and one ring methyl proton shift at 2.18 compared with single shifts at about 2.11 and 2.28 respectively for the other methyl derivatives. The signal at 1.7Hz is possibly from the acetyl group when in the exo position. Support for this theory is given by the work of

Kessler and Rieker (222) who found the presence of both exo and endo conformers for diortho-alkyl substituted acetanilides at room temperature, the exo peak occurring at 1.71 and the endo at 2.13. They also studied mono-alkyl derivatives, obtaining only one acetyl methyl signal at room temperature, separation into two peaks becoming apparent with decreasing temperature. The present study also found this, the 2-Me substituent alone being insufficient to slow down rotation of the amido moiety to the extent whereby the separate conformer signals would register, thus giving only one acetyl methyl signal. For the non-2-methyl substituted derivatives, the frequency of the acetyl methyl signal is only slightly lower than that of the pure endo conformer signal. This indicates in a qualitative manner that the endo form must predominate in these cases since the single signal, being a weighted average of the contributions from the exo and endo signals, is close to that of the endo signal, indicating the greater contribution from this conformer. This agrees with infrared data.

CHAPTER EIGHT

COMPUTER GRAPHICS

Current interest in drug conformation rests on the postulate that a single preferred conformer binds to the receptor productively. If this conformer can be identified, it can then be fixed, or at least its population can be enhanced, by chemical means. In most areas of interest in drug design, however, drug-receptor complexes are not available for detailed molecular study, so the active conformation must be inferred. The usual procedure has been to seek correlations between conformation, determined experimentally or theoretically for a drug series, and biological response.

The dominant conformer was assumed to be the biologically active agent (223) but recent work initiated by the widely variable responses that can occur even when the physical conformation is unambiguous (280) pursued the idea that a minor conformer may sometimes be responsible for activity (329,327,150). Thus, besides an interest in the nature of the active conformation, there is an interest in the fraction of active conformer in solution and in how it varies with chemical structure (327,150).

The multiparameter approach to drug design is entirely different and is based on correlations of biological response with partition coefficients and other physical properties. These two different approaches have in the past been pursued in isolation, but groups such as Davies et al (91) have shown that there is a relationhsip between them.

A flexible molecule behaves as many different molecules, depending on conformation. Different conformations have their own physical, chemical, biological and thermodynamic properties. Davies et al (91) demonstrated an interdependence between the conformer balance and partition coefficient. Variations in the active conformer fraction between phases will be accompanied by variations in macro- and micro-partition coefficients. The micropartition coefficient is defined as the partition coefficient attaching to an individual conformer. This result has consequence only to the extent that shifts in conformer balance actually occur, but conformer populations can vary considerably with solvent and this must mean that micropartition coefficients can vary sharply with conformation. Evidence for this comes from work by Parker et al (309) on hydroxyureas. Discrepancies between calculated and observed log P values for hydroxy-urea and some of its substituted analogues could be explained by solvent-dependent conformational preferences of various analogues which may have an influence on biological action in vivo. Similarly, the 5.6-fold increase in the octanol/water partition coefficient between p- and o-hydroxybenzoic acids is almost certainly due to internally hydrogen-bonded species in the latter having greater micropartition coefficients than the open forms (255)

Since a relationship between conformation and partition coefficient has been established, it was decided to investigate the conformations of the compounds included in the present study to observe the effect, if any, on the partition coefficient.

8.1 The Graphic Modelling System

Computer graphics is a visual system which allows molecular data to be represented three-dimensionally. The molecule can be assembled on the computer screen and relationships within the molecule or between the molecule and a receptor can be observed. This is often a useful tool since it allows unsuspected relationships as well as errors resulting from agorithmic or software mistakes to be observed. The connection of the computer to the graphic system enables relationships to be explored since the computer can rapidly examine hundreds of minute positional changes for their effects on energetic and spatial relationships. The system was utilised for this investigation to provide information about the conformation of the molecules and interactions within the molecule which may help to explain anomalies in the partition coefficients.

The system used was molecular mechanics using software developed at The Wellcome Laboratories Ltd, Beckenham, for energy minimisation by the quasi Newton-Raphson method. The computer was a DEC PDP 11/34 with a GT40 display; 34 kilobytes of memory and a further 34 kilobytes of virtual memory.

The structure of each compound was entered into the data system. The input programme creates a standard molecular structure representation, which may be stored on the computer and which may be subjected to geometry refinement, to structure display and comparison and to the calculation of various structural properties.

The method used for generating three-dimensional structures involves sketching a two-dimensional structural diagram on the terminal screen by means of a light pen, and using a rough strain energy minimization procedure to create a stereochemically correct model. Thus, with the computer in the DRAW mode, a benzene ring was entered on the screen, then each substituent bond placed in position and the correct atom assigned to each bond.

e.g. O_{sp3} for OH
C_{sp3} for CH_3
O_{sp2} for O=
N_{tri} for NH_2

The MINIMISE mode placed each bond in its correct position relative to its neighbour. Atoms were positioned without their respective hydrogen atom complement so that when all substituent atoms were in place, the hydrogen atoms were added to complete the structure.

For many drug molecules that are sterically strained or that contain one or more rings, a structure built from standard fragments is inaccurate. For such structures preferred conformation was determined by 'rigid rotation' about various bonds.

A range of molecular properties may be calculated. These include: Crystal volume (van der Waals volume)
Solution volume (molecular volume)
Collision diameter
Approach diameter (diameter of closest approach)

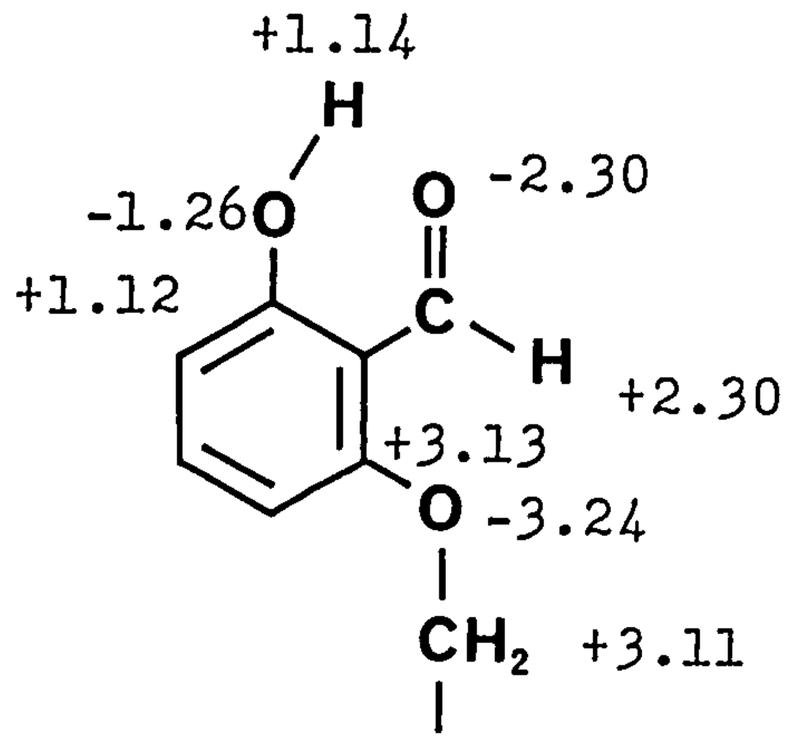
It was also possible to calculate the surface area of each molecule. The surface area of each constituent atom was calculated and then its actual area contribution to the molecular area with respect to the other atoms. From these values it was possible to ascertain the proximity of atoms within the molecule. This has been utilised to illustrate interactions within the molecule. The total surface area of the molecule is also calculated.

Each single bond could be rotated and thus groups could be moved relative to one another. The positional energy for each movement is calculated and the position of least energy found. This was the position favoured by the molecule and retained. Once all the atoms were in the most energetically favourable position, the bond lengths and angles could be calculated. This gave information about the existence of hydrogen bonds and torsional angles revealed if the molecule was planar. This could also be seen on the visual screen. The torsional angles also indicated whether certain substituent positioning placed the molecule under strain.

In order that hydrogen bonding could be detected, it was necessary to include charges on those atoms likely to form a hydrogen bond e.g. N,O,Cl etc.

Each group was considered separately and must have an overall charge of zero, therefore the sum of charges within the group must be zero. Each group is identified by an integer prefix so that the program can identify and interact individual groups.

Thus, H (of OH) has a charge of +0.14 but this is entered as +1.14.



Charge assignment is shown in Table 54.

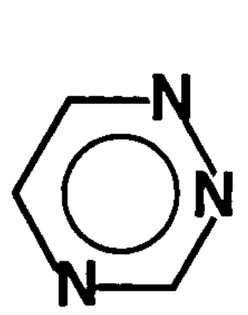
Once the atoms have a charge, attraction or repulsion will occur when the bonds are rotated. Electrical attraction which is energetically favourable will govern the positions of atoms in the molecule and thus its structure can be determined.

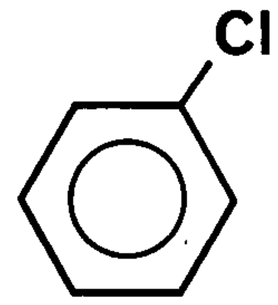
Details of the most favourable conformations are given in section 8.2. The diagrams and details of bond angles and lengths show the occurrence of hydrogen bonds and also the effect of steric hindrance. This can distort the shape of the molecule and could present different parts of the molecule to the solvent, so affecting solvent-solute interactions and therefore the partition coefficient. The molecular distortion could also cause bonds within the molecule or between the molecule and the solvent to break and new bonds to form, again affecting the partition coefficient.

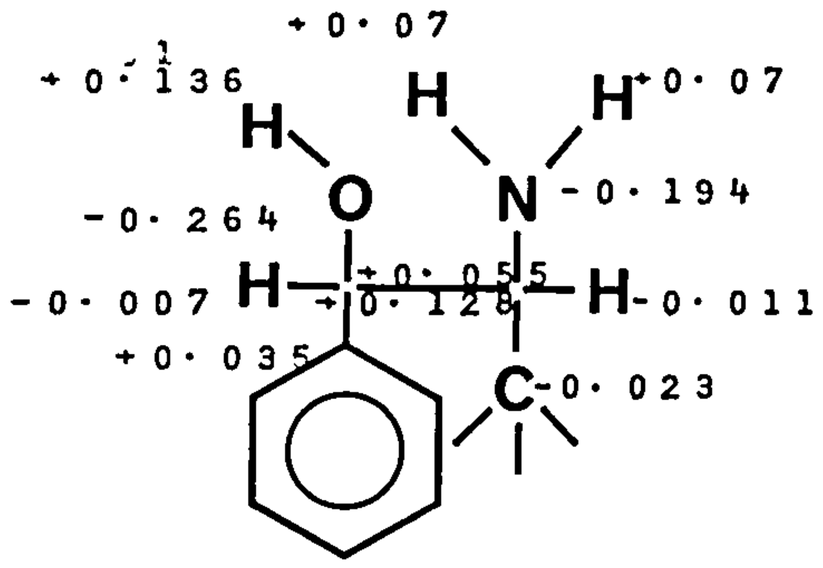
Figure 30 shows the 2,3,5,6-tetramethylphenol molecule as

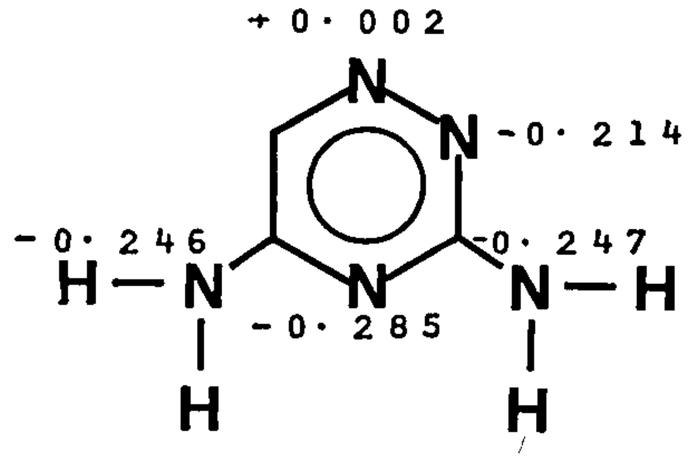
Table 54. Charge Assignment

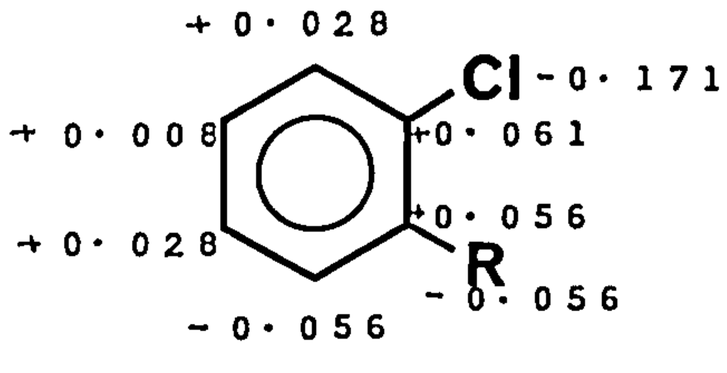
Groups covered : -OH
-NH_2
-COOH
-CONHR
-C-O-C-

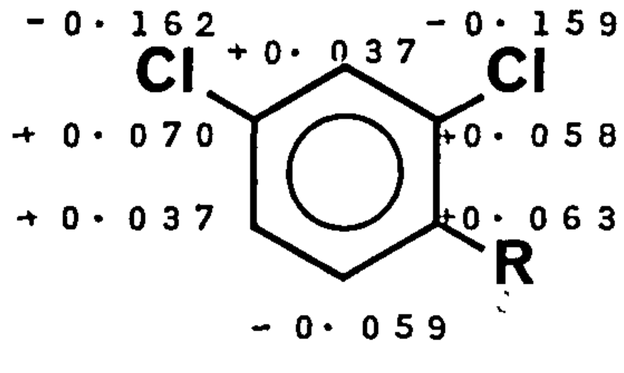


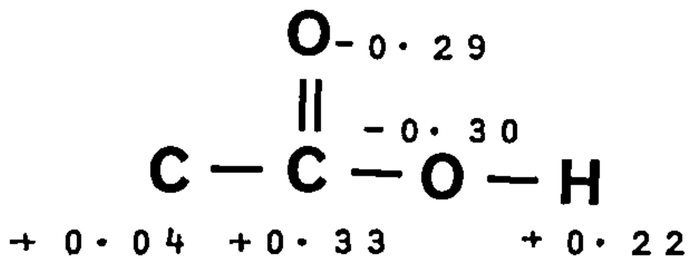


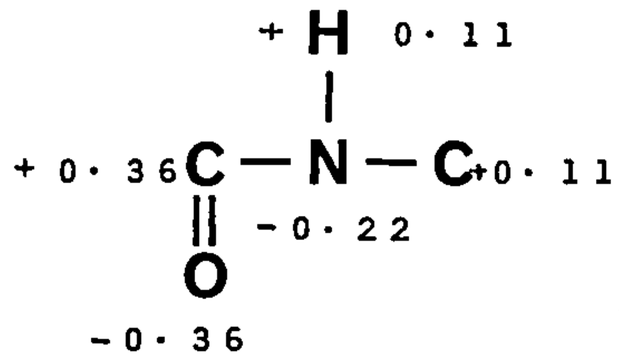




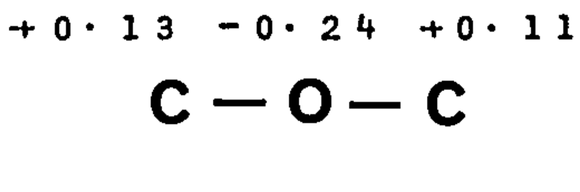


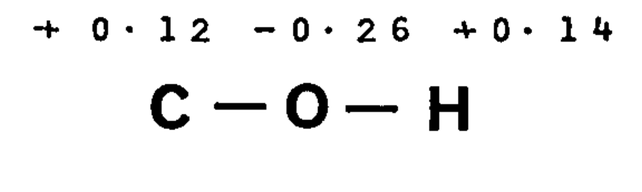


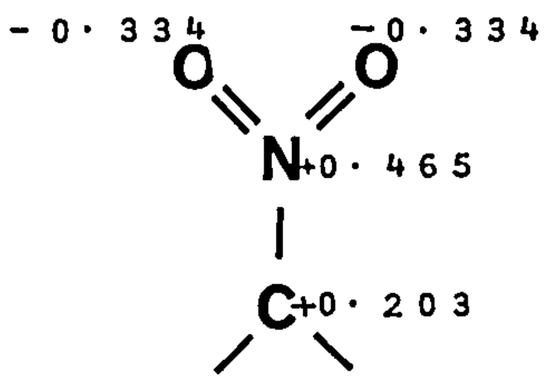




sugars

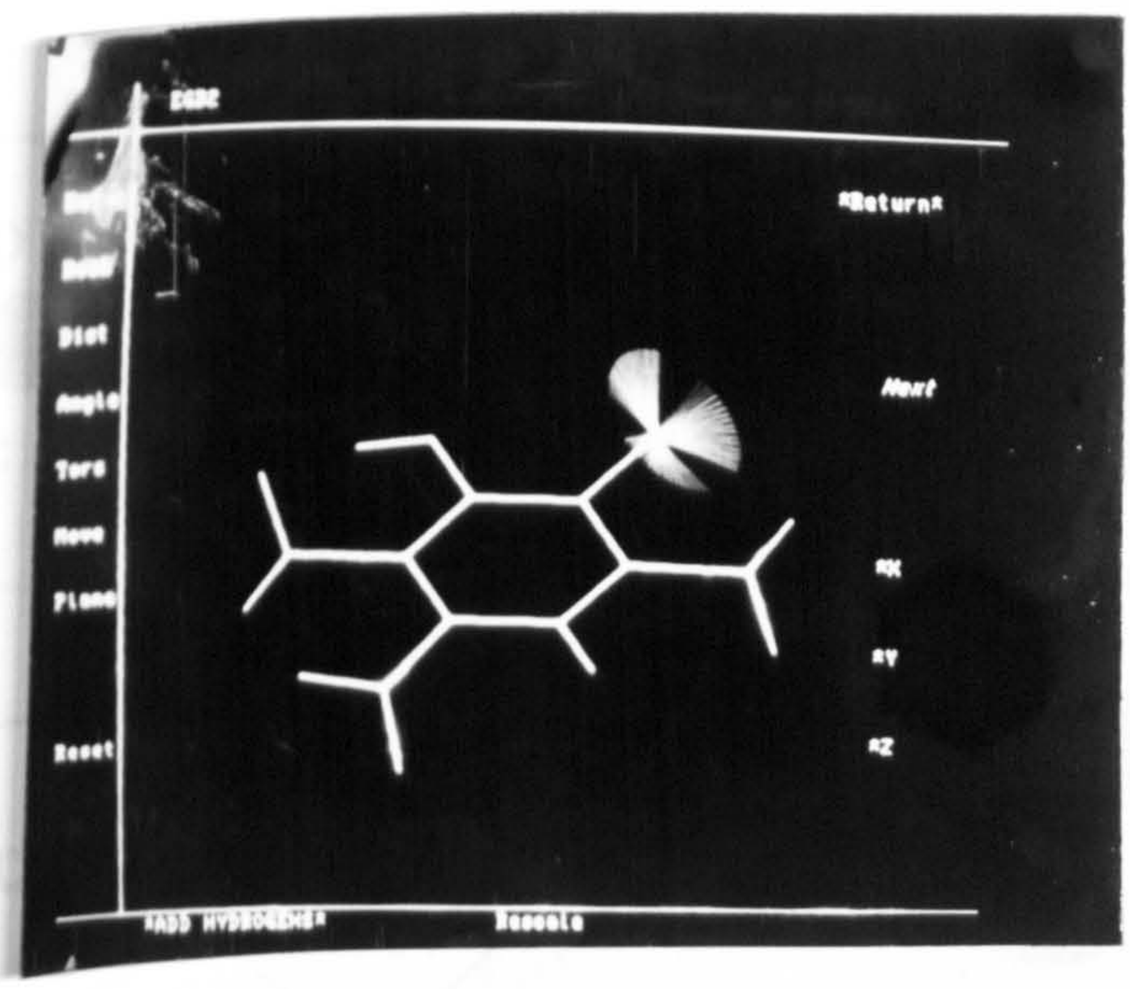






represented graphically, with one methyl group rotating about the C-C bond. The groups could be rotated singly or together. The other methyl groups appear to only have two hydrogen atoms because of the position relative to the ring C-methylC bond. This causes one of the hydrogen atoms to be masked.

Figure 30. 2,3,5,6-Tetramethylphenol



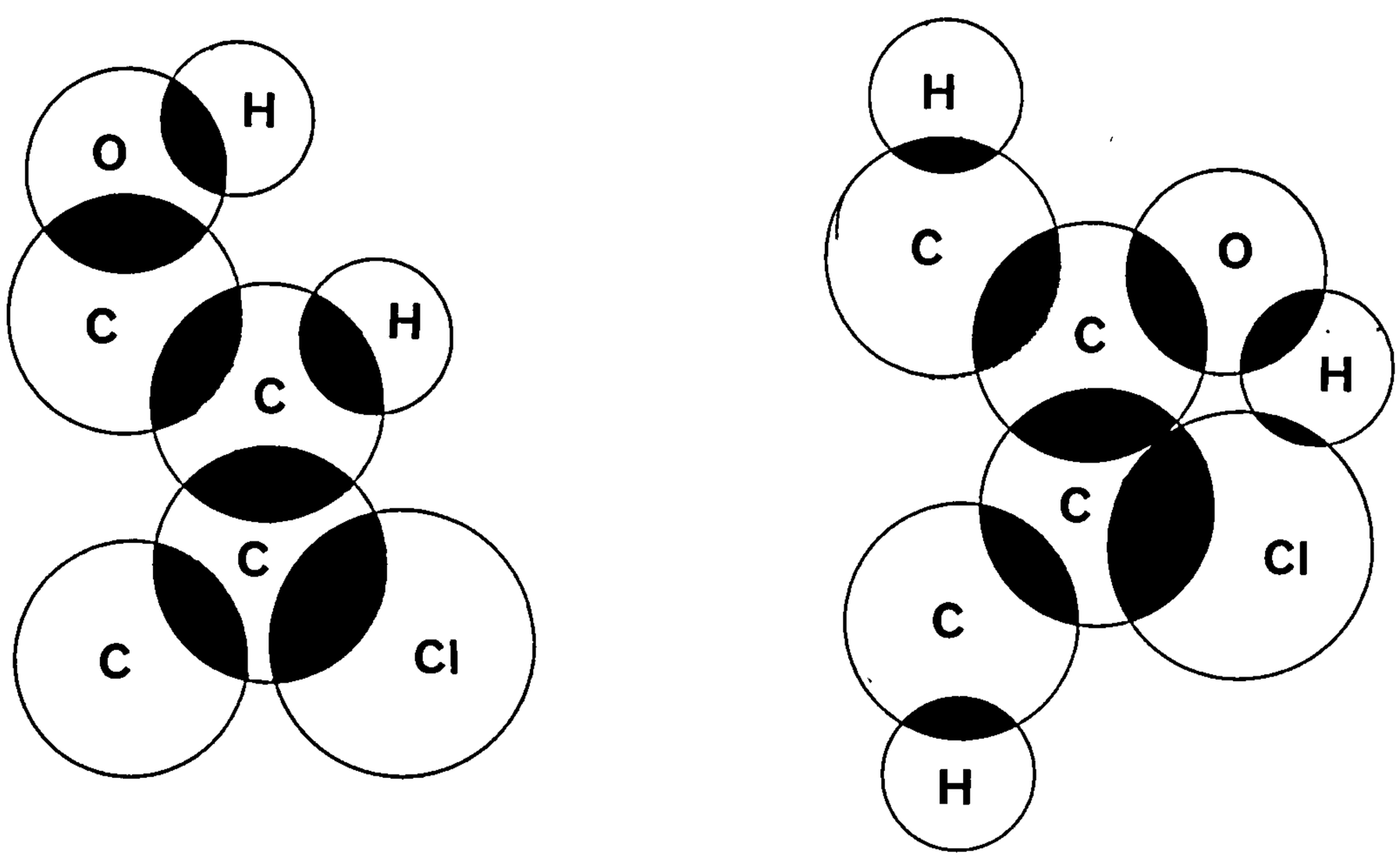
The program used for this graphic system calculated the contribution made by each individual atom to the total area. This took into account the amount of electron overlap occurring due to bond formation and the proximity of neighbouring atoms. Thus changes in the area of atoms could indicate their position in the molecule. This can be used to confirm suspected intramolecular interactions.

1. Chlorophenols

The chlorine atom contributes 27.26 $\mathring{A}^2$ to the molecular area in the ortho isomer, but 28.92 $\mathring{A}^2$ in both the meta and

para isomers.

The oxygen atom of the hydroxyl group contributes 13.49 $\overset{o}{A}^2$ in each isomer, but the hydroxyl hydrogen has a value of 7.98 $\overset{o}{A}^2$ in both the meta and para isomers and only 6.60 $\overset{o}{A}^2$ in the ortho isomer. These figures indicate an overlap of the chlorine and hydrogen atoms in the ortho isomer which allows the formation of a hydrogen bond:



2. Nitrophenols

The nitrogen atom contributes 4.4 $\overset{o}{A}^2$ to the molecular area in each isomer, and the oxygen from each hydroxyl contibutes 13.38 $\overset{o}{A}^2$. However, the value for the oxygen in the nitro group is 18.5 $\overset{o}{A}^2$ except for one oxygen atom in the ortho isomer which has a value of 15.96 $\overset{o}{A}^2$. Again, in the ortho isomer the hydrogen of the hydroxyl group has a value of 4.56 $\overset{o}{A}^2$ compared with 7.6 $\overset{o}{A}^2$ in the meta and para isomers. This indicates overlapping of the nitro and hydroxyl groups in the ortho isomer - representing a hydrogen bond. The

reduction in area of the hydroxyl hydrogen is greater in o-nitrophenol than o-chlorophenol which indicates a stronger hydrogen bond since the atoms are closer to one another.

3. 2-Nitroresorcinol

The value of both nitro oxygens is reduced from 18.5 $\overset{o}{A}^2$ to 15.7 $\overset{o}{A}^2$ and the hydrogen from each hydroxyl is reduced to 4.06 $\overset{o}{A}^2$ from 7.9 $\overset{o}{A}^2$. This indicates the presence of two hydrogen bonds of reasonable strength.

4. Hydroxybenzaldehydes

The area contribution of each hydroxyl oxygen is approximately 13.45 $\overset{o}{A}^2$, but the ortho hydroxyl oxygen has a value of 4.09 $\overset{o}{A}^2$ compared with approximately 7.9 $\overset{o}{A}^2$ for the meta and para isomers.

In the aldehyde group the hydrogen atom has a value of 7.9 $\overset{o}{A}^2$ in each isomer but the oxygen atom has an area of 16.61 $\overset{o}{A}^2$ in the meta position and 17.25 $\overset{o}{A}^2$ in the para position, but only 14.26 $\overset{o}{A}^2$ in the ortho position. This indicates overlapping of the oxygen of the aldehyde group and the hydrogen of the hydroxyl group in the ortho isomer. As with the nitrophenols, the extent of overlap indicates a fairly strong hydrogen bond.

The reduction in area of the meta isomer aldehyde oxygen indicates some sort of interaction which is difficult to explain, but which reflects the cyclohexane/water partition coefficients of the hydroxybenzaldehydes, where lop P_{m-} is greater than log P_{p-} (-1.96 vs -2.29). This relationship does not hold for other compounds.

5. Hydroxybenzoic Acids

The free oxygen atom in the carboxylic acid group has an area of 16.81 $\mathring{A}^2$ in the meta isomer and 17.18 $\mathring{A}^2$ in the para isomer. This is reduced to 15.3 $\mathring{A}^2$ in the ortho isomer. The ortho hydroxyl hydrogen has a reduced value of 5.27 $\mathring{A}^2$ so again an intramolecular hydrogen bond is indicated. The same difference in meta and para isomers is observed as was seen in the hydroxybenzaldehydes, but unfortunately solubility problems prevented the measurement of cyclohexane/water partition coefficients of these compounds in this study and literature values of log P give log P_{m-} = -2.04 and log P_{p-} = -1.77 which is the opposite way round to the hydroxybenzaldehydes i.e. log P_{m-} < log P_{p-}.

In 2,6-OH_2benzoic acid, the acid group is slightly out of plane and the carbonyl oxygen has a reduced area contribution of 14.7 $\mathring{A}^2$. This indicates the presence of an intramolecular hydrogen bond, but surprisingly the hydroxyl hydrogens have values of 8.79 $\mathring{A}^2$ and 8.49 $\mathring{A}^2$ which do not indicate any great interaction. However, the acid hydroxyl group and the neighbouring ring hydroxyl appear to interact to form a hydrogen bond since the acid hydrogen has an area of 6.13 $\mathring{A}^2$ and the hydroxyl oxygen an area of 11.13 $\mathring{A}^2$ when it is usually 13.38 $\mathring{A}^2$. Thus two intramolecular hydrogen bonds seem to be present. No interaction is seen in the 3,5-dihydroxy isomer.

6. Methylorthonitrophenols

The nitrogen molecular area is the same as in the nitrophenols as is the oxygen of the hydroxyl group. However, in nitrophenol the nitro oxygens have a value of 18.5 $\mathring{A}^2$ which

is reduced to 16.3 $\mathring{A}^2$ and 15.9 $\mathring{A}^2$ in 3-Me-2-NO_2phenol. The nitro oxygen adjacent to the hydroxyl has an area of 15.9 $\mathring{A}^2$ and the hydrogen an area of 4.45 $\mathring{A}^2$ which is indicative of an intramolecular hydrogen bond. Steric hindrance of the second nitro oxygen by the adjacent methyl is indicated by the reduced area of 16.3 $\mathring{A}^2$ and also the reduced area of the methyl carbon. This carbon has a molecular area of 9.97 $\mathring{A}^2$ in the 4-Me isomer and 8.57 $\mathring{A}^2$ in the 3-Me isomer. The movement of the methyl group away from the nitro group also releases the nitro oxygen so that it has the expected molecular area of 18.4 $\mathring{A}^2$. The hydrogen bond is present in all the isomers and 5-Me-2-NO_2phenol is similar to 4-Me-2-NO_2phenol. However, in 6-Me-2-NO_2phenol there is indication of interaction between the methyl and the hydroxyl group since the oxygen atom area is reduced from 13.5 $\mathring{A}^2$ to 12.77 $\mathring{A}^2$ and the carbon area from 9.97 $\mathring{A}^2$ to 9.37 $\mathring{A}^2$.

7. <u>Methylphenols</u>

There is indication of steric shielding in o-methylphenol since one hydrogen atom of the methyl group and the hydroxyl hydrogen show reduced areas of 5.81 $\mathring{A}^2$ and 6.26 $\mathring{A}^2$ respectively as opposed to 8.23 $\mathring{A}^2$ and 8.1 $\mathring{A}^2$. The meta and para isomers do not show this effect.

In the dimethylphenols steric shielding is indicated by reduced areas of hydrogen atoms on the methyl and hydroxyl groups. In 2,3-dimethylphenol the 2-CH_3 carbon atom is also reduced in area due to the closeness of all three groups. Overlap still occurs between the 2-CH_3 and OH groups in all isomers containing this arrangement, but the

additional effect of the second methyl group is eliminated in the 2,4- and 2,5-isomers. In 2,6-dimethylphenol the second methyl in the ortho position causes reduction of the molecular area of the hydroxyl oxygen from 13.4 $\mathring{A}^2$ to 12.35 $\mathring{A}^2$, indicating additional steric shielding. In 3,4-dimethylphenol the hydroxyl group is not affected, but there is slight interaction between the two methyl groups. No interaction at all is observed in the 3,5-Me_2 isomer.

In the trimethylphenols, the CH_3 group next to the hydroxyl group again causes shielding which is increased by the addition of a second ortho methyl so that the hydroxyl oxygen area is reduced. The third methyl group has the expected shielding effect, as does the fourth methyl group in 2,3,5,6-Me_4phenol.

8. Methylbenzoic Acids

No real interaction is observed in these molecules which is slightly surprising since the partitioning and UV data seems to indicate steric hindrance. However, the acid group does appear to be pushed out of the plane of the ring by the proximity of an ortho methyl group and this supports the theory of steric twisting which is described in Chapter 7.

9. Methylacetanilides

Steric hindrance is observed in the ortho isomer, with interaction occurring between the methyl carbon and the nitrogen and oxygen atoms of the acetanilide group. This is indicated by the nitrogen molecular area being reduced from 5.28 $\mathring{A}^2$ to 4.77 $\mathring{A}^2$ and the oxygen from 18.11 $\mathring{A}^2$ to 15.53 $\mathring{A}^2$.

No steric effect is observed in the m-, p- or 3,5-Me_2 isomers, but in 2,6-Me_2acetanilide the molecular area of the nitrogen atom is reduced further to 3.85 $\overset{o}{A}^2$ which indicates that both methyl groups hinder the acetanilide group. However, the oxygen atom has an increased area. This group is pushed out of the plane of the ring by the second methyl group and so is no longer hindered. This supports the evidence of the partition, UV and thermodynamic data.

The size and shape of drug molecules are generally acknowledged to be important factors in the molecular interactions which govern structure-activity relationships. Because molecular volume depends upon size and shape, various measures of size and shape have been used as variables in SAR studies. (305,387). For example, Moriguchi and Kenada (291,292,293) attempted to show the significance of correlations using geometrically calculated van der Waals volume and the relation of such volume to other SAR variables. Their work is based on the proposition that hydrophobicity may be related to the volume of apolar molecules. For polar molecules however, empirical hydrophilicity constants must also be invoked.

It is generally true that biological activity depends upon molecular structure: the structure of the bioactive molecule as well as the structure of macromolecular biological tissues such as membranes, receptors, enzymes. For a series of drug molecules which elicit the same biological response - in varying degrees - it is generally assumed that all the molecules experience the same sequence of biological events and, if there is a limiting step, all

molecules are limited by the same step. Because of these assumptions it is possible in QSAR studies to limit attention to drug molecules alone. If, then, the measured biological response is determined by kinetic factors, the activity depends on molecular structure in such a way as to reflect rate-determining factors. If, on the other hand, the response is determined by thermodynamic factors, then the activity depends upon structure in such a way that interaction and equilibrium effects are expressed. This is the basic view point of QSAR methods which seek to relate biological response to molecular structure.

Geometric properties of molecules, volume and surface area, are accepted as attributes influencing physical properties and biological activity (305,387); however, the volume and surface area of a molecule are indefinite because of the probability nature of electron density. Practically, size measures such as the van der Waals radii of atoms can be used to define a molecular surface from which molecular volume and surface area can be computed.

Viewed at the molecular level, drug activity may depend upon tissue distribution characteristics related to volume. A manifestation of this geometric influence is the measured partition coefficient between immiscible liquids. Activity may also depend upon geometric characteristics governing the orientation or fit of a molecule to a macromolecular cavity associated with the receptor, possibly reflected as steric factors. Finally, the degree of intermolecular interaction of a drug with a receptor may be

directly influenced by the molecular volume or surface area of the drug, which govern the relationship of interacting electron fields, described by electronic factors. The recognition of these possibilities has led investigators to attempt to compute theoretically or obtain experimentally molecular volume structure descriptors for SAR analyses.

Experimental estimates of molecular volume have been made from density measurements of solutions with extrapolations to infinite dilution (122). These are referred to as partial molar volumes. Molecular volume determined in this manner includes a fraction of free space associated with each molecule.

Several investigators have approached the calculation of molecular volume by dissecting a molecule into constituent atoms or groups in various valence states and assigning increment values to the fragments (35,126). The presumption is that the volume of a molecule can be computed approximately as a sum of the volume of properly quantitated fragments. Bondi (35) has demonstrated this by using X-ray diffraction data to derive a set of 'recommended' group and atom volume values. Limitations arise due to the interaction of adjacent atoms or groups, necessitating correction factors to account for the molecular environment of an atom.

Other approaches to the calculation of molecular volume depend upon the use of scale models of molecules. Computed values may include a water molecule envelope as used by Hermann (193) who computed a molecular cavity

surface area, the use of a model enclosed in plastic wrap followed by the measurement of the liquid volume displaced (258), or the use of a model to measure several axial radii to estimate steric influence (372).

The computer program used in this work allowed the calculation of a number of size parameters. These included molecular surface area, crystal volume and solution volume, collision diameter and approach diameter. The results of these calculations are shown in Table 55. It can be seen that positional changes within the molecule do not alter the crystal or solution volume or the collision diameter. However, the diameter of closest approach is altered by isomeric changes as is the molecular surface area. These values have therefore been plotted against log P and the results can be seen in Figures 31 and 32 and 33.

Approach diameter gives a general relationship with log P and certain class relationships have been illustrated. Approach diameter seems to be of most importance in the cyclohexane/water system with a relationship appearing to exist between the log P and approach diameter of the alkyl phenols. The relationship between log P and molecular surface area is also illustrated (Figs 32&33) but although class members have been joined, there is no evidence of any relationships except perhaps again between the alkyl phenols. This possibly illustrates that such simple relationships cannot be applied to compounds where steric and electronic factors are involved, but may be used for groups influenced by steric factors alone.

Table 55. Computer Graphics - Parameters

Compound	LogP Octanol	Surface Area A^{o2}	Volume (A^{o3}) Crystal	Volume (A^{o3}) Solution	Diameter (A^{o}) Collision	Diameter (A^{o}) Approach	LogP Cycl
o-Clphenol	2.12	135.06	128.8	167.4	6.3	3.2	+0.815
m-Clphenol	2.40	137.42	128.8	167.4	6.3	2.2	-0.051
p-Clphenol	2.36	136.36	128.8	167.4	6.3	2.1	-0.290
o-NO_2phenol	1.75	141.71	135.5	176.2	6.4	3.2	+1.400
m-NO_2phenol	2.00	146.00	135.5	176.2	6.4	2.5	-1.570
p-NO_2phenol	1.91	146.77	135.5	176.2	6.4	3.0	-1.910
2-NO_2resorc	1.50	146.06	141.7	184.2	6.5	2.7	+1.020
o-OHbenzald	1.66	138.96	133.6	173.7	6.3	4.3	+1.380
m-OHbenzald	1.35	141.78	133.6	173.7	6.3	3.1	-1.960
p-OHbenzald	1.35	143.99	133.6	173.7	6.3	3.8	-2.290
o-OHbenz.ac	2.30	150.95	139.8	181.7	6.4	0.3	-1.410
m-OHbenz.ac	1.60	151.84	139.8	181.7	6.4	4.4	------
p-OHbenz.ac	1.55	149.05	139.8	181.7	6.4	2.8	------
2,6-OH_2b.a.	1.65	160.37	146.0	189.8	6.5	3.0	-3.350
3,5-OH_2b.a.	0.80	165.21	146.0	189.8	6.5	3.4	------
3-Me-2-NO_2p	2.15	160.02	160.9	209.2	6.7	4.2	+1.320
4-Me-2-NO_2p	2.24	163.72	160.9	209.2	6.7	4.5	+1.990
5-Me-2-NO_2p	2.33	163.20	160.9	209.2	6.7	4.0	+1.740
6-Me-2-NO_2p	2.54	162.65	160.9	209.2	6.7	4.7	+1.950
o-Mephenol	1.90	137.75	139.6	181.5	6.4	3.6	+0.150
m-Mephenol	1.93	141.89	139.6	181.5	6.4	3.5	-0.160
p-Mephenol	1.98	140.20	139.6	181.5	6.4	3.6	-0.150
2,3-Me_2ph	2.30	155.52	165.0	214.5	6.8	4.4	+0.490
2,4-Me_2ph	2.32	157.81	165.0	214.5	6.8	4.9	+0.660
2,5-Me_2ph	2.32	158.47	165.0	214.5	6.8	4.0	+0.720
2,6-Me_2ph	2.23	162.34	165.0	214.5	6.8	3.9	+0.970
3,4-Me_2ph	2.24	158.5	165.0	214.5	6.8	4.4	+0.210
3,5-Me_2ph	2.34	162.59	165.0	214.5	6.8	4.6	+0.260
2,3,5-Me_3ph	2.70	177.21	190.4	247.5	7.1	4.4	+1.080
2,3,6-Me_3ph	2.56	176.11	190.4	247.5	7.1	4.9	+1.720
2,4,6-Me_3ph	2.60	179.37	190.4	247.5	7.1	5.2	+1.690
2,3,5,6-Me_4	2.77	196.25	215.8	280.5	7.4	6.0	+1.790
o-Mebenz.ac	2.10	164.19	159.0	206.5	6.7	4.6	-0.380
m-Mebenz.ac	2.35	162.15	159.0	206.5	6.7	4.5	+0.360
p-Mebenz.ac	2.30	165.51	159.0	206.5	6.7	4.5	-0.530
2,6-Me_2b.a.	1.82	175.88	184.4	239.7	7.1	4.3	-0.980
3,5-Me_2b.a.	2.85	182.69	184.4	239.7	7.1	5.4	+0.060
o-Meacetan.	0.88	186.91	196.7	255.7	7.2	5.7	-1.250
m-Meacetan.	1.61	191.61	196.7	255.7	7.2	5.4	-0.990
p-Meacetan.	1.61	190.58	196.7	255.7	7.2	5.2	-0.900
2.6-Me_2acet	0.97	211.54	222.1	288.7	7.5	5.5	-1.470
3,5-Me_2acet	2.06	213.33	222.1	288.7	7.5	5.6	-0.310

Key to Figures - 31,32 and 33.

■ Chlorophenols

□ Nitrophenols

△ Benzaldehydes

● Hydroxybenzoic Acids

▲ Methylorthonitrophenols

◇ Methylphenols

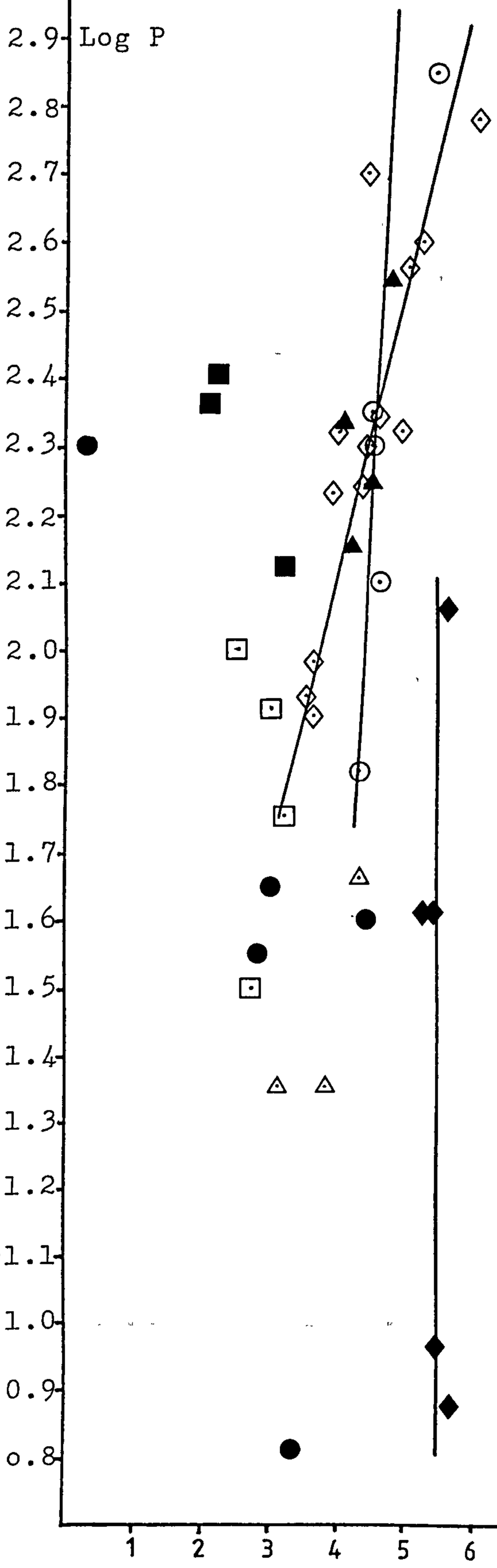
○ Methylbenzoic Acids

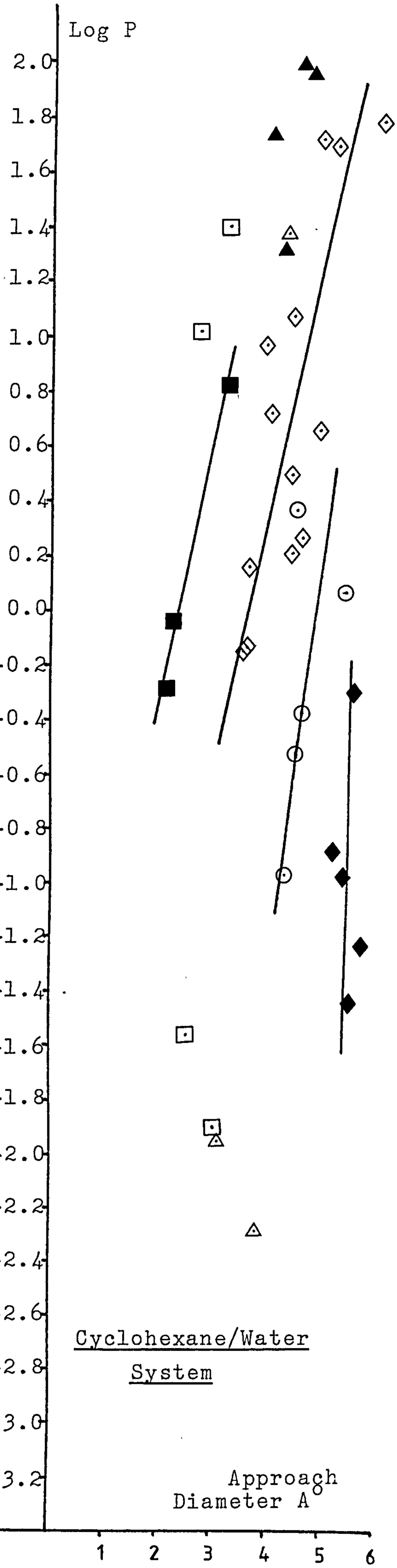
◆ Methylacetanilides

Figure 31.

The Relationship of Log P to Approach Diameter A°

Octanol/Water System

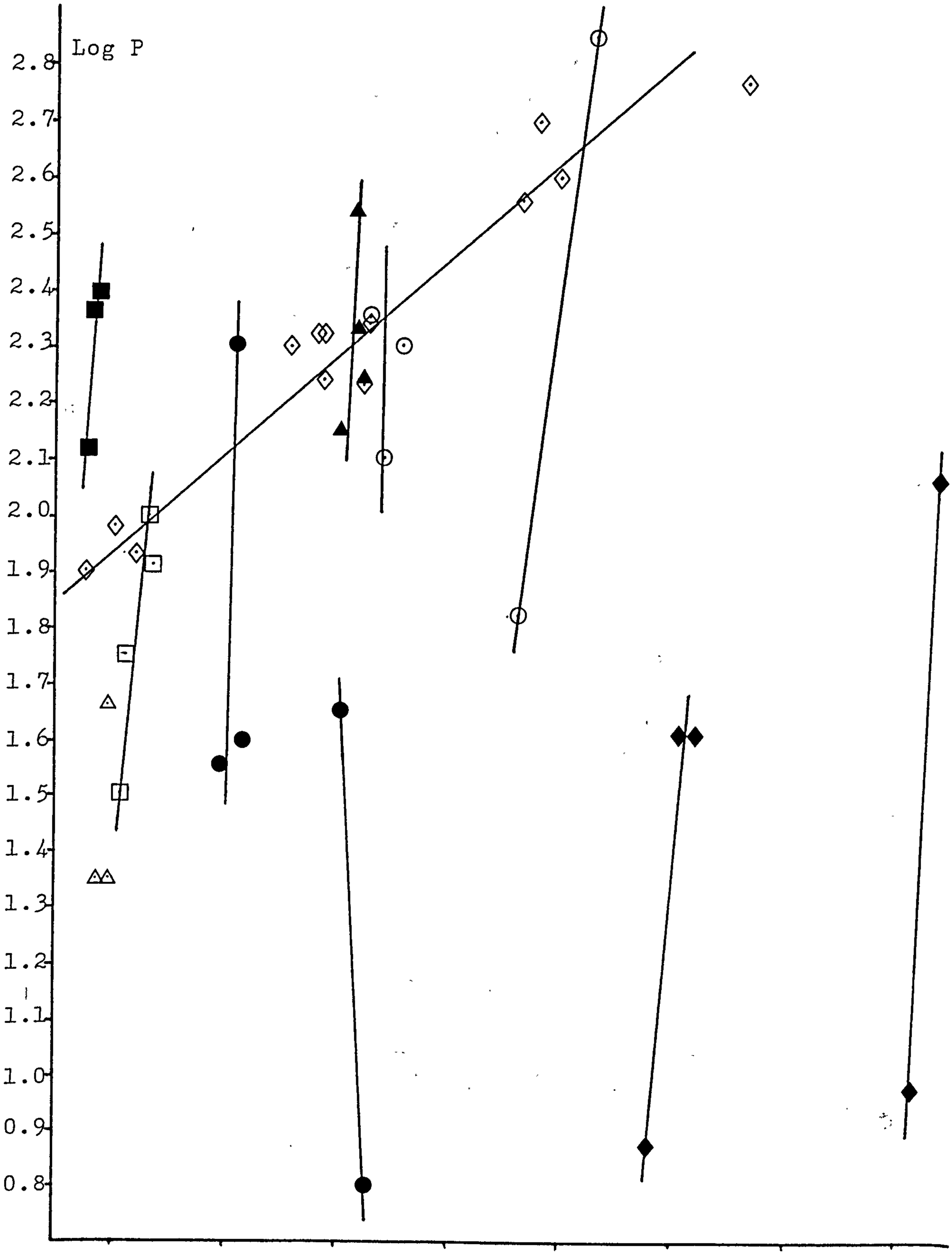




Cyclohexane/Water System

Approach Diameter A°

Figure 32. The Relationship Between Log P and Molecule Surface Area A^{o2} Octanol/Water System



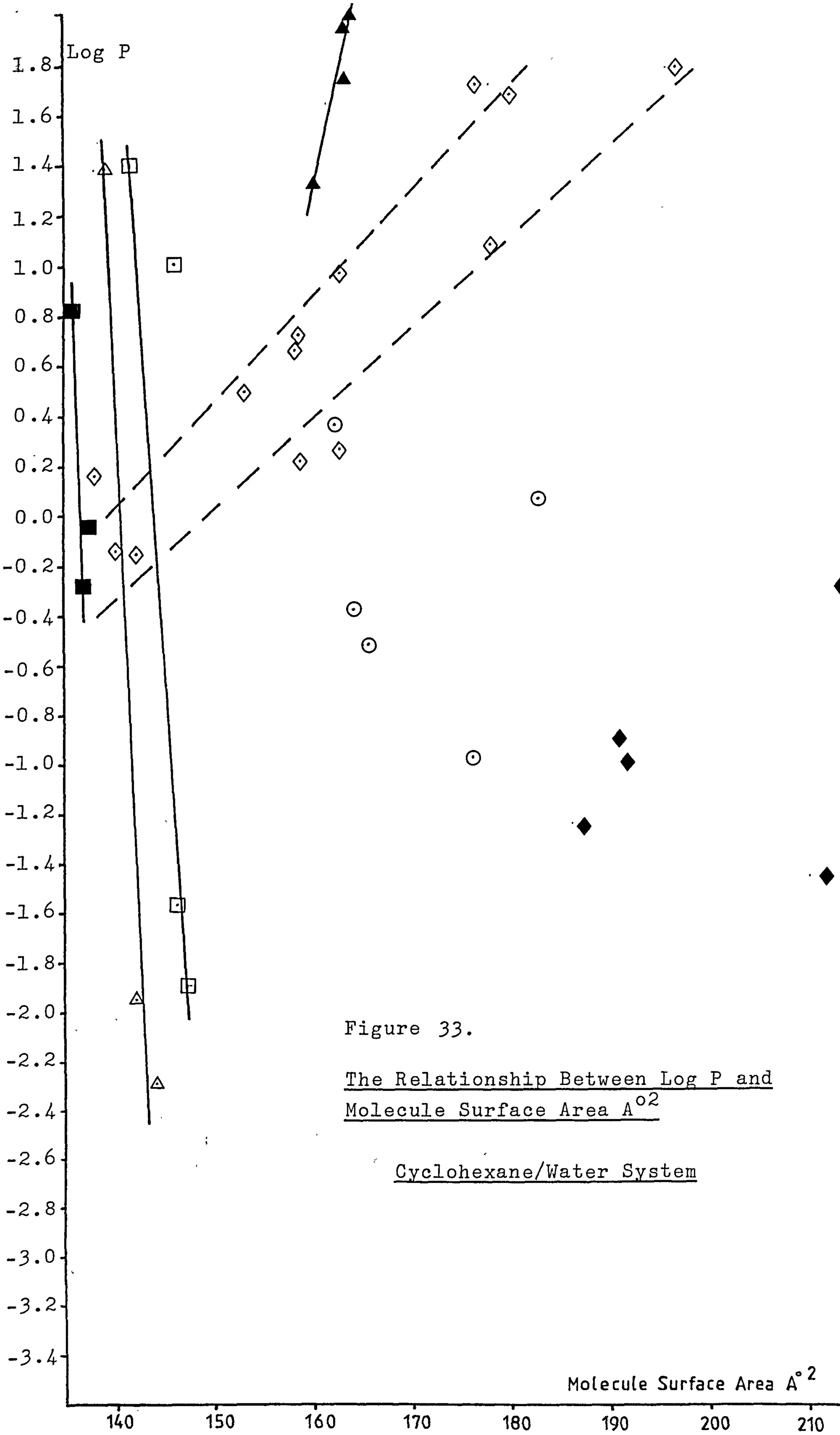


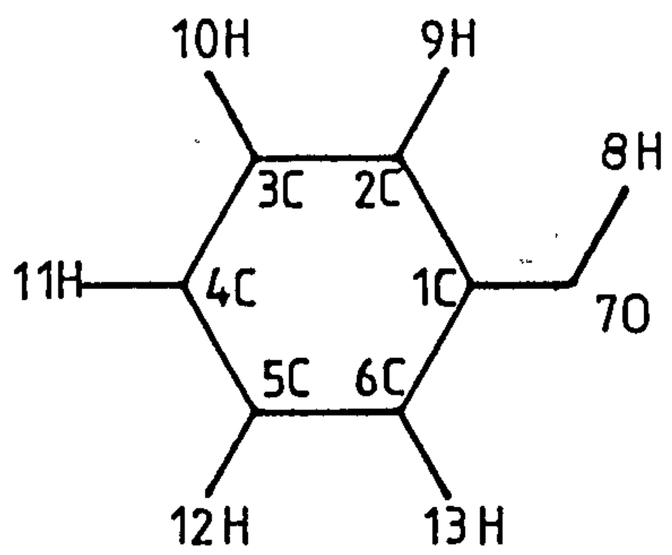
Figure 33.

The Relationship Between Log P and Molecule Surface Area A^{o2}

Cyclohexane/Water System

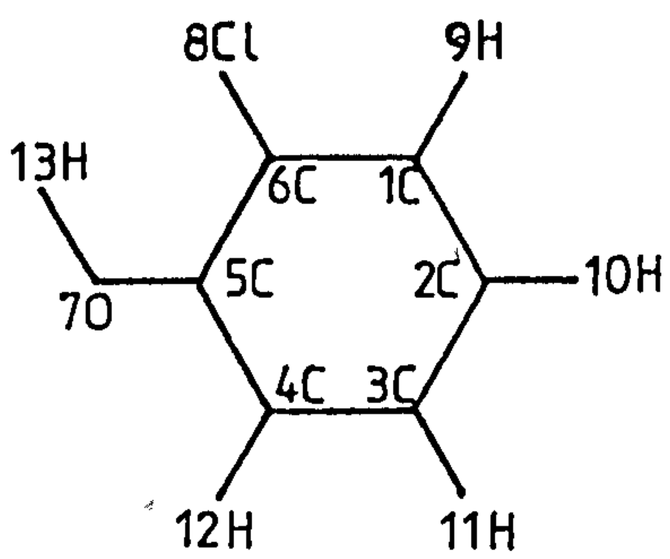
8.2 Molecular Conformations

1. Phenol



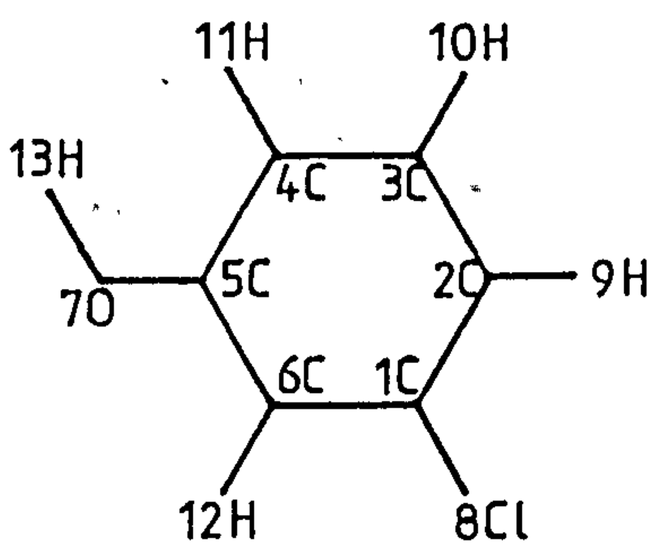
Planar molecule. Free rotation about 1C - 7O Bond angles 120° except for 1 7 8 = 109.5°.

2. o-Cl phenol



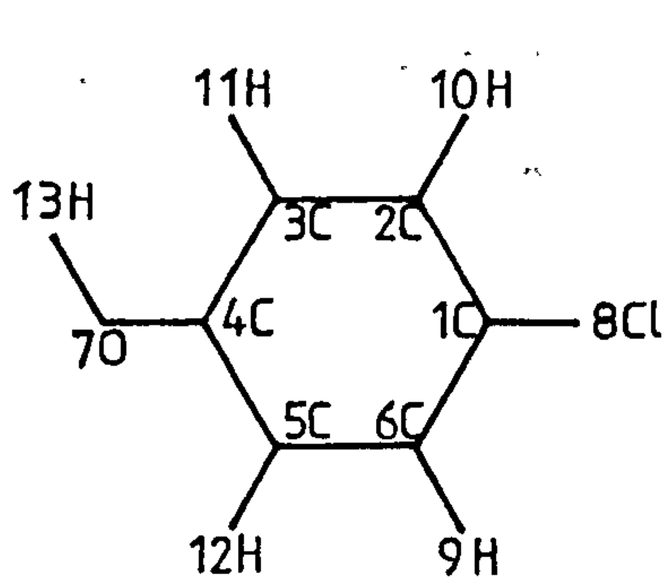
Planar molecule. All angles same (120°) except for 5 7 13 = 109.4°. Hydrogen bond 8 13 = 2.35A° Not very strong. Free rotation about 5 7.

3.m-Cl phenol



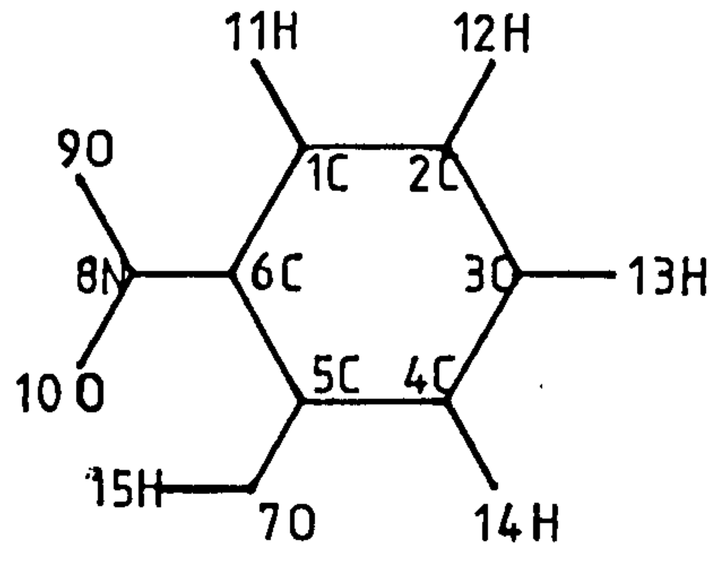
Planar molecule. Free rotation about 5 7. No hydrogen bond. Bond angles 120° except for 5 7 13 = 109.5°.

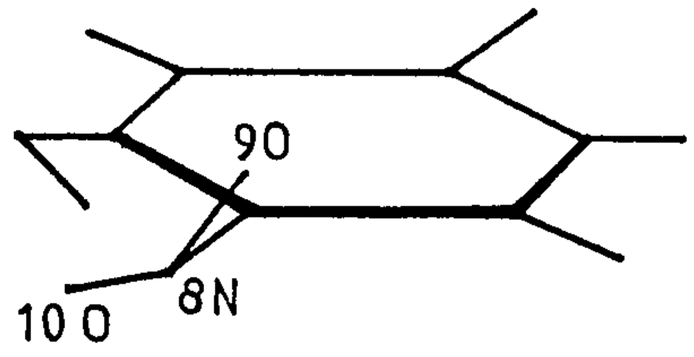
4.p-Cl phenol



Planar molecule. Free rotation about 4 7. No hydrogen bond. Bond angles 120° except for 4 7 13 = 109.5°.

5.o-NO_2 phenol



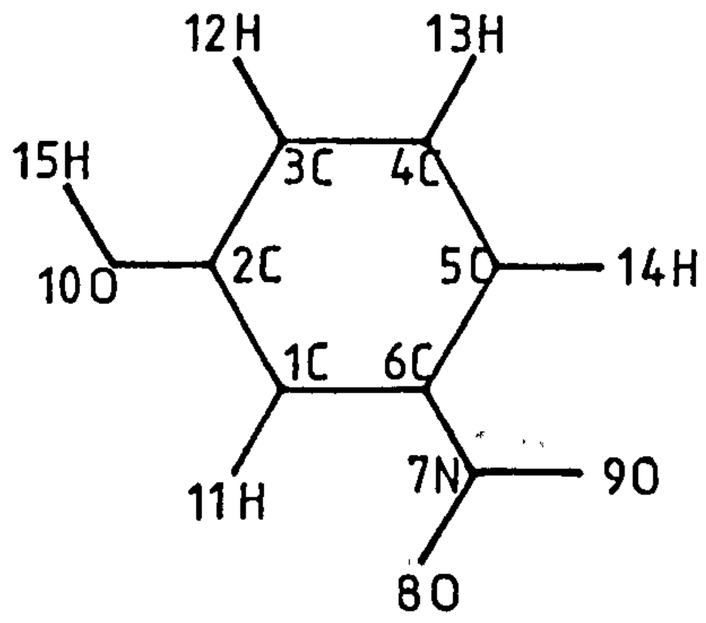


Bond angles 120° except for 9 8 10 = 119.1°
5 7 15 = 110.3°

Ring very slightly twisted. NO_2 group twisted slightly out of plane of ring.
i.e. Torsional angles : 1 6 8 9 = 11.5°
1 6 8 10 = -172.8°

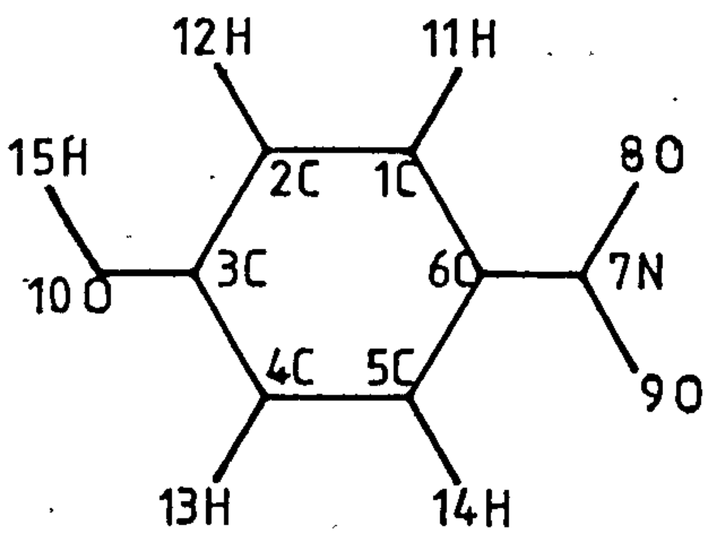
Hydrogen bond 10 15 = 1.67A° - bond length indicates strong bond.

6.m-NO_2 phenol



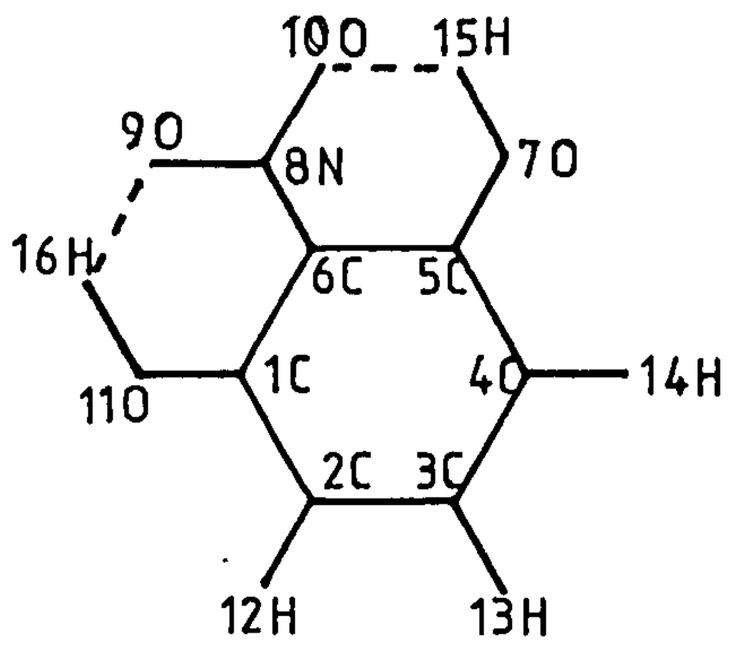
Planar molecule. NO_2 group twisted very slightly out of plane.
Free rotation about 2 10
and 6 7
No hydrogen bonds
All bond angles 120° except for 2 10 15 = 109.5°

7.p-NO_2 phenol



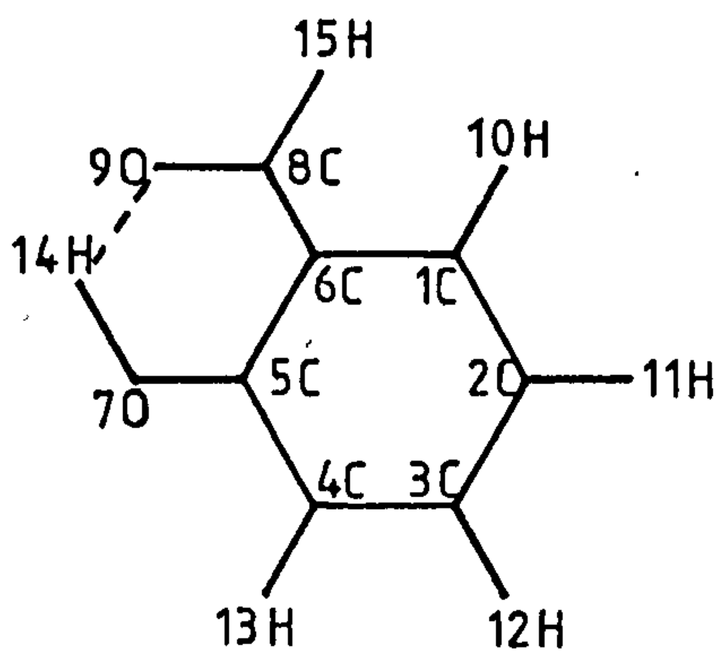
Planar molecule - no twisting out of plane of NO_2 group at all.
Free rotation about 3 10
and 6 7
No hydrogen bonds
All bond angles 120° except for 3 10 15 = 109.5°

8. 2-NO_2resorcinol



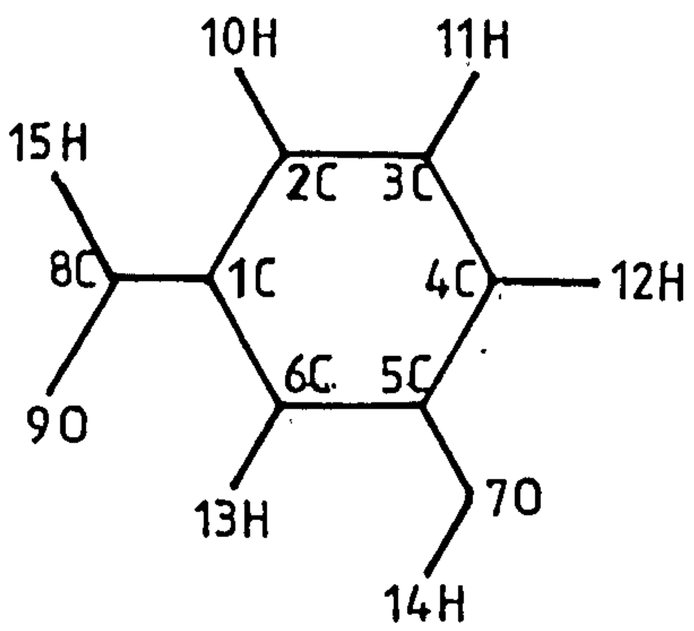
Planar molecule. No twisting of NO_2 group.
All bond angles 120° except
5 7 15 = 110.3°
1 11 16 = 110.2°
Two hydrogen bonds :
9 16 = 1.62A°
10 15 = 1.64A°

9. o-OHbenzaldehyde



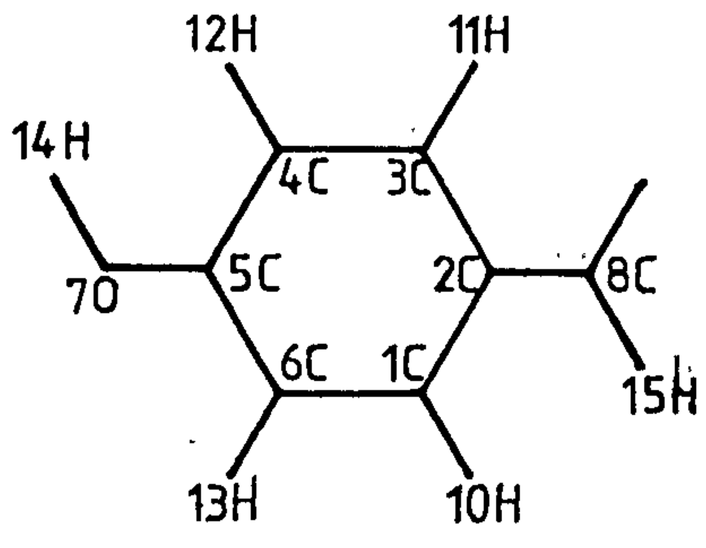
Planar molecule - no torsional strain at all.
Held rigid by hydrogen bond.
All bond angles 120° except
5 7 14 = 109.5°
1 6 8 = 122.0°
5 6 8 = 118.0°
Therefore COH group moved over slightly towards OH group and held by hydrogen bond
Hydrogen bond 9 14 = 1.57A°
Bond length indicates strong bond.

10. m-OHbenzaldehyde



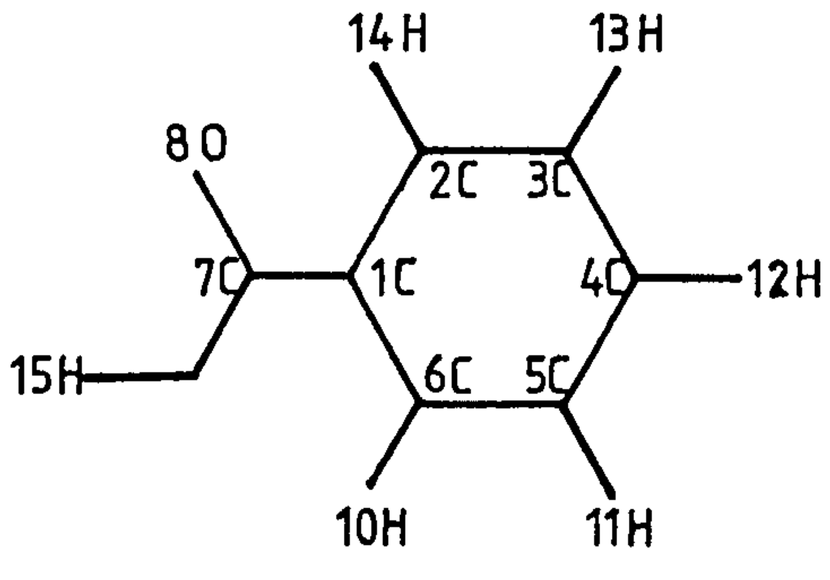
Planar molecule, although not held in position by intramolecular bonding.
Therefore molecule free to move.
All bond angles 120° except
5 7 14 = 109.1°
2 1 8 = 127.6°
6 1 8 = 113.4°
No hydrogen bonds

11. p-OHbenzaldehyde



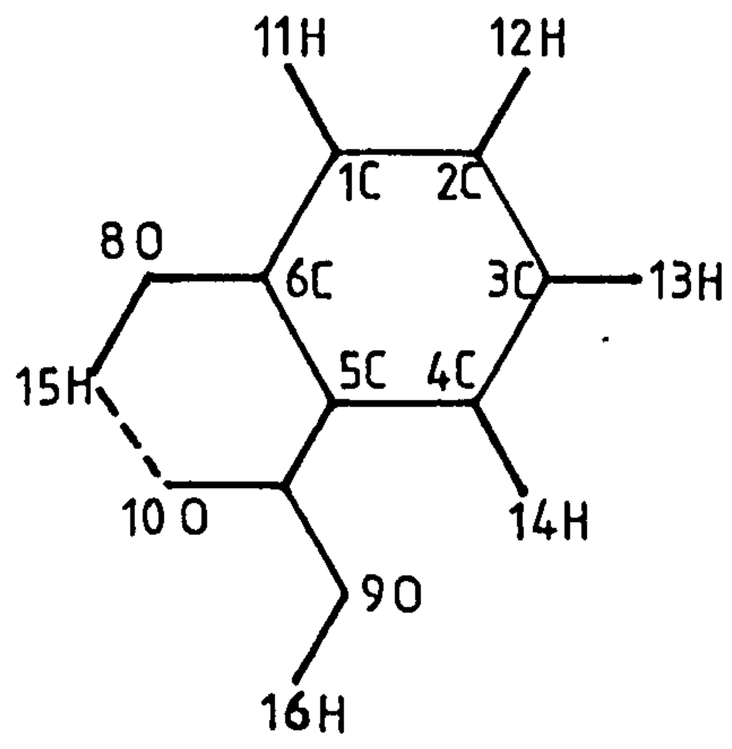
Planar molecule - no movement out of plane at all.
All bond angles 120° except
5 7 14 = 109.5°
No movement by CHO group
No hydrogen bonds
Free rotation about 5 7
and 2 8

12. Benzoic Acid



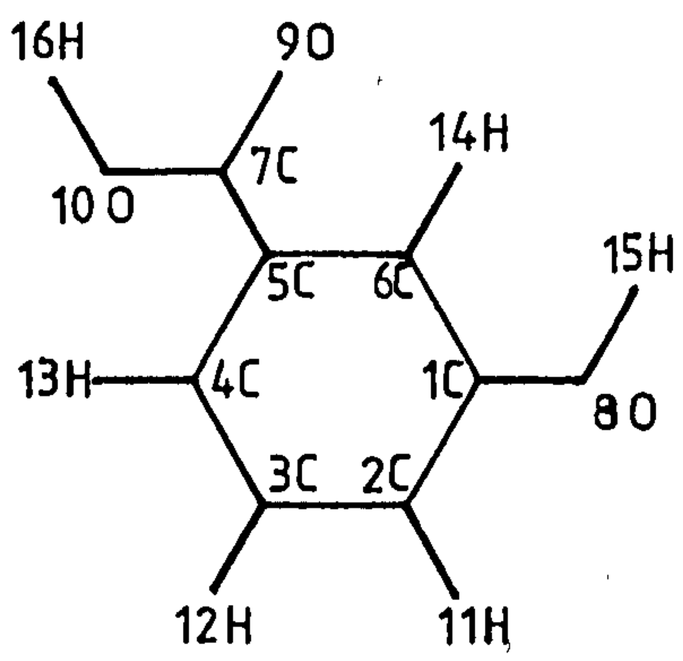
Planar molecule. All bond angles 120° except
7 9 15 = 109.5°

13. o-OHbenzoic acid



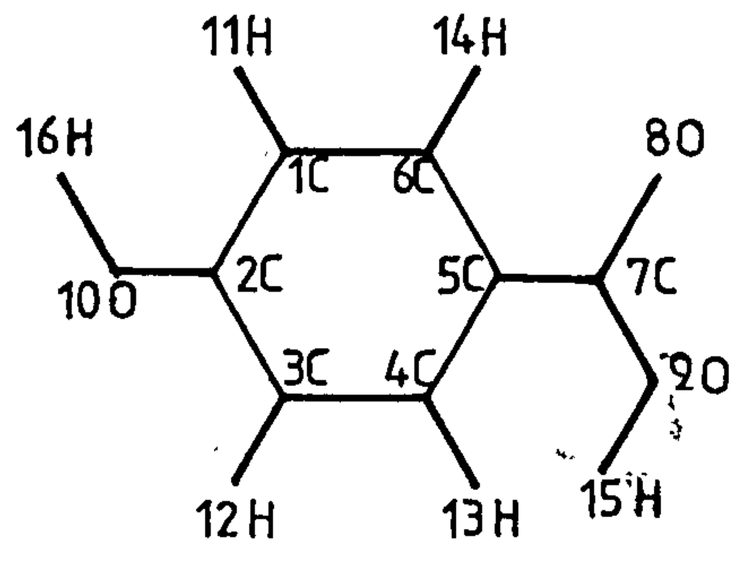
Planar molecule. All bond angles 120° except
5 7 9 = 113.9°
9 7 10 = 122.8°
5 7 10 = 123.2°
6 8 15 = 109.5°
7 9 16 = 109.5°
Hydrogen bond 10 15 = 1.89A°

14. m-OHbenzoic acid



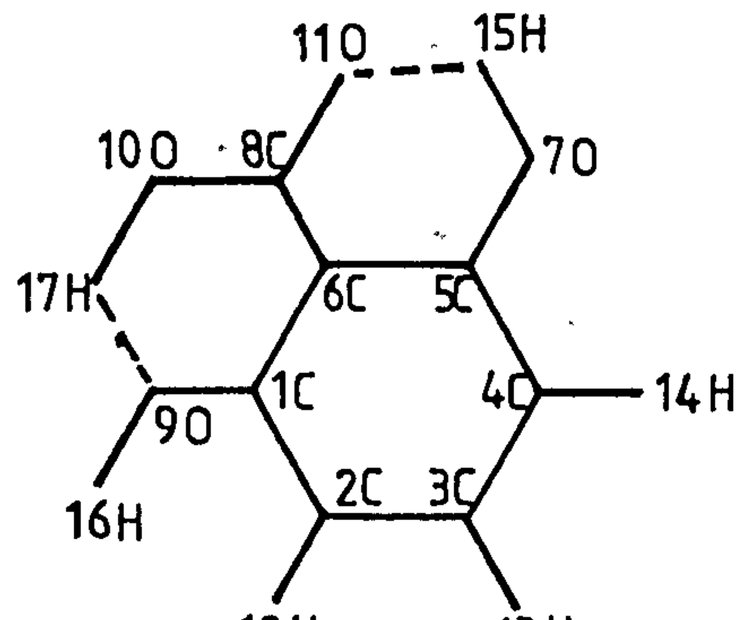
Planar molecule. All bond angles 120° except
1 8 15 = 109.5°
7 10 16 = 109.4°
No hydrogen bond
Free rotation about 1 8
and 5 7
and 7 10
C-COOH bond shorter than in o-OHbenzoic acid

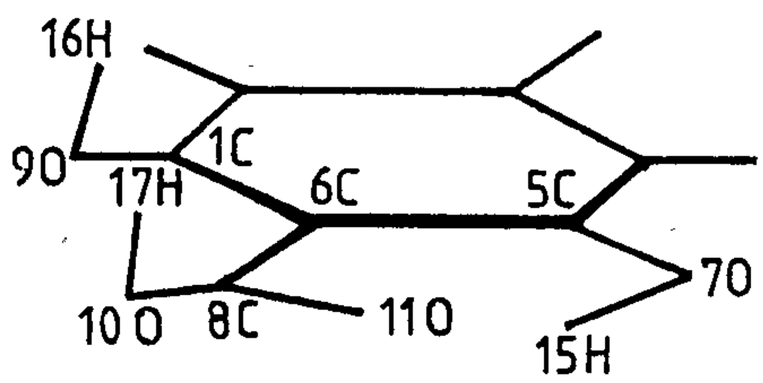
15. p-OHbenzoic acid



Planar molecule. All bond angles 120° except
7 9 15 = 110.0°
2 10 16 = 109.4°
No hydrogen bond
Free rotation about 2 10
5 7
7 9
Again, as with m-isomer, C-COOH bond shorter than in o-isomer

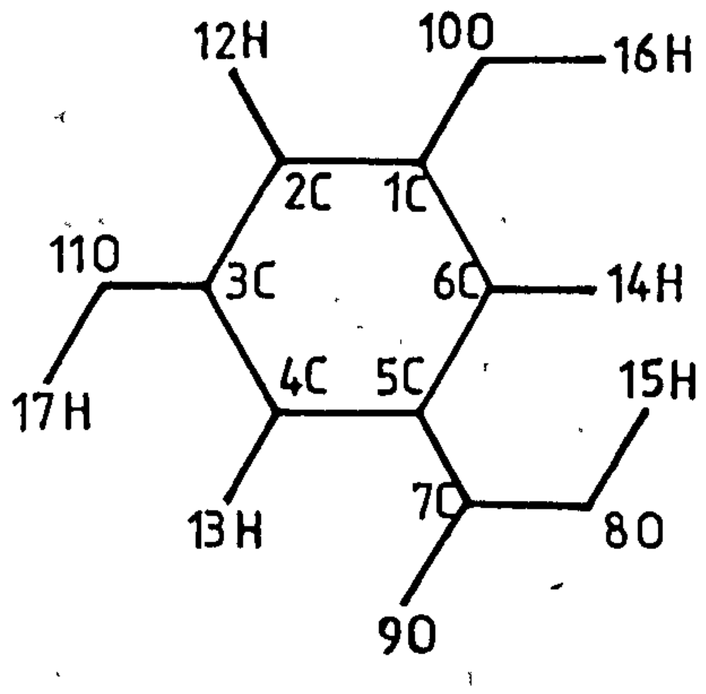
16. 2,6-OH_2benzoic acid





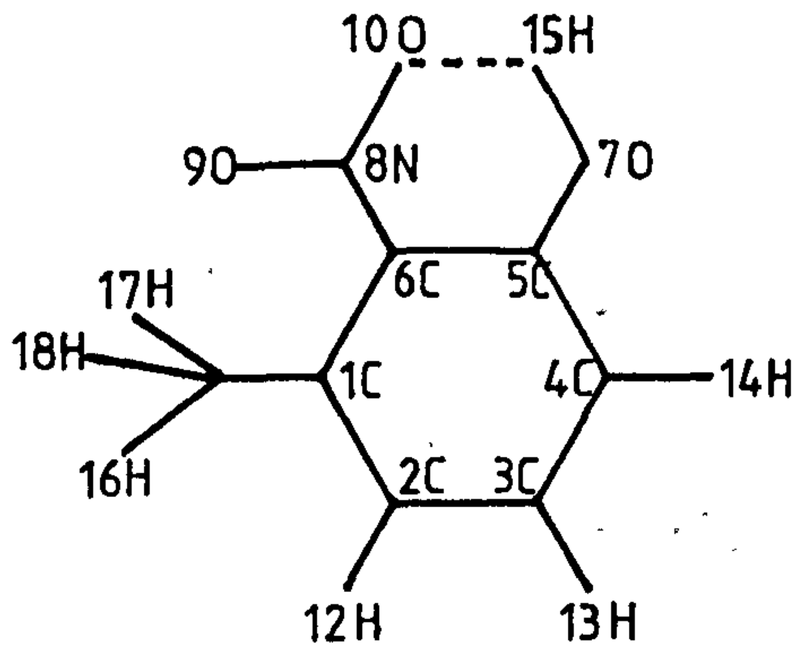
Ring planar, but C-OH twisted out of plane
i.e. Torsional angles : 6 5 7 15 = -110.0°
4 5 7 15 = + 70.0°
All bond angles 120° except 1 6 8 = 131.3°
5 6 8 = 108.7°
6 8 10 = 117.0°
5 7 15 = 109.5°
1 9 16 = 109.5°
8 10 17 = 109.5°
Therefore the COOH group appears to be pushed away from one neighbouring OH group towards the other which is pushed out of the plane of the ring. Therefore the hydrogen bonds are not as strong as expected and in fact only one is recorded.
Hydrogen bond 9 17 = 2.01A°

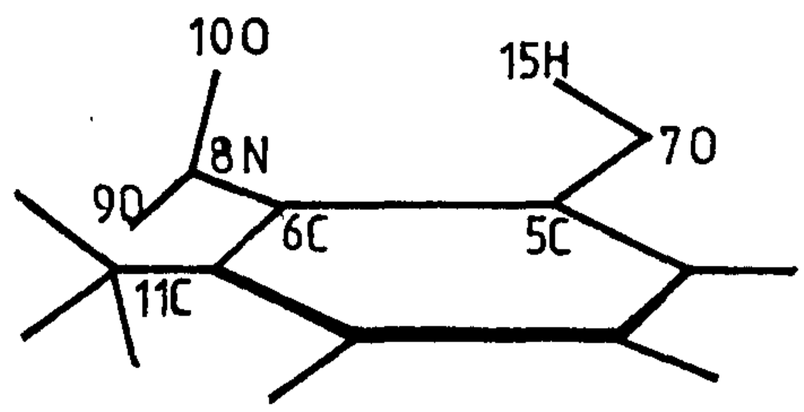
17. 3,5-OH_2benzoic acid



Planar molecule.
All bond angles 120° except
6 5 7 = 140.1°
4 5 7 = 99.9°
5 7 8 = 107.7°
8 7 9 = 125.7°
5 7 9 = 126.6°
1 10 16 = 109.5°
3 11 17 = 109.5°
7 8 15 = 109.5°
No hydrogen bond
C-COOH bond longer than in other isomers. COOH group pushed to one side.

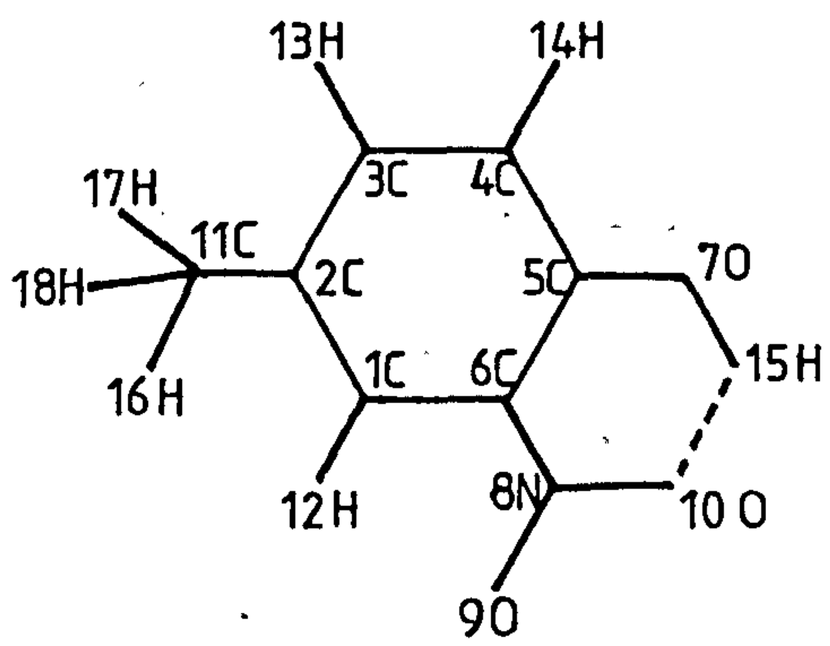
18. 3-Me-2-NO_2phenol





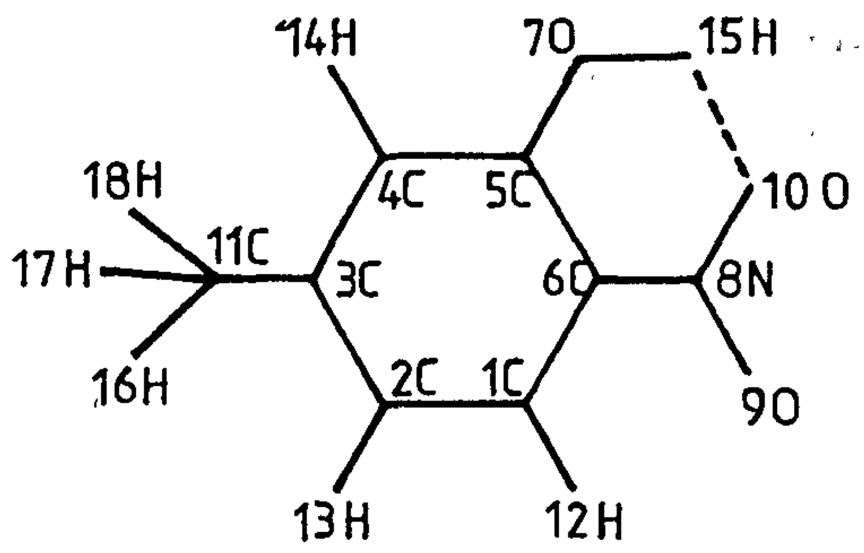
Molecule slightly twisted out of flat plane. NO_2 group slightly out of plane.
i.e. Torsional angles : 5 6 8 9 = 158.8°
5 6 8 10 = -13.6°
Possibly due to presence of methyl group
Hydrogen bond 10 15 = 1.66A°
All bond angles 120° except 5 7 15 = 109.2°
1 11 16 = 109.8°
16 11 17 = 108.8°
16 11 18 = 109.3°
1 11 17 = 109.8°
17 11 18 = 109.7°
1 11 18 = 109.3°

19. 4-Me-2-NO_2phenol



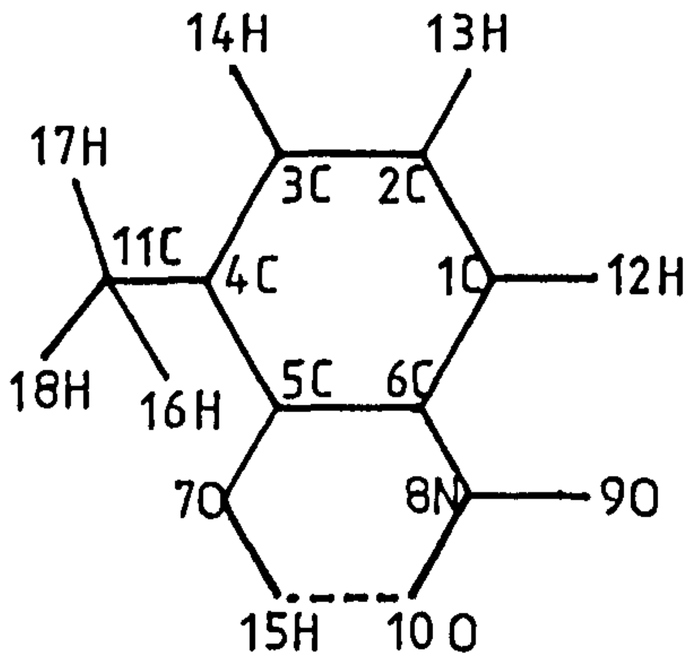
Planar molecule
All bond angles 120° except
5 7 15 = 109.5°
2 11 16 = 109.6°
16 11 17 = 109.3°
16 11 18 = 109.4°
2 11 17 = 109.8°
17 11 18 = 109.4°
2 11 18 = 109.3°
Hydrogen bond 10 15 = 1.68A°
Free rotation about 2 11

20. 5-Me-2-NO_2phenol



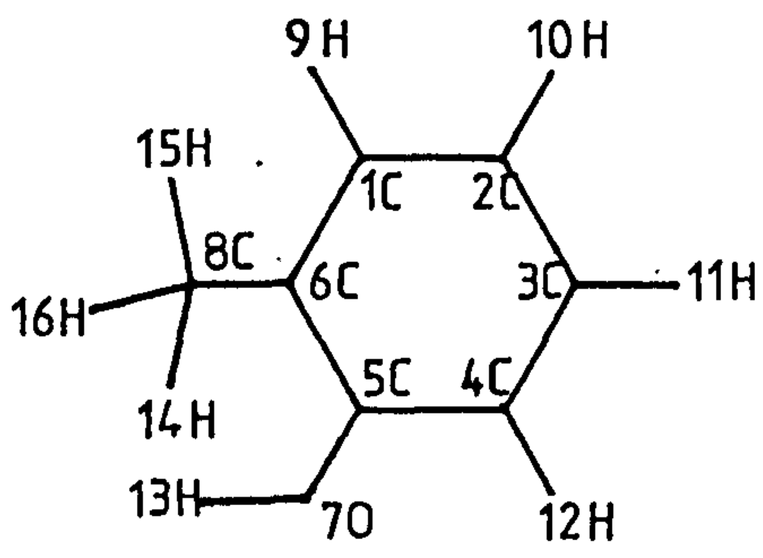
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
Hydrogen bond 10 15 = 1.68A°
Free rotation about 3 11
Flexible molecule, therefore slight movement out of plane

21. 6-Me-2-NO_2phenol



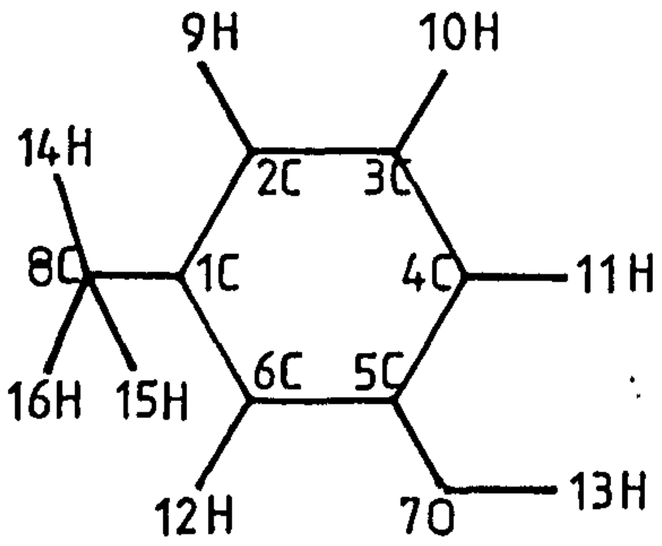
Planar molecule but flexible therefore slight movement out of plane. NO_2 group very slightly twisted out of plane.
i.e. Torsional angles :
1 6 8 9 = 4.0°
1 6 8 10 = -177.9°
Hydrogen bond 10 15 = 1.67A°
All bond angles 120° except C-C-H and C-O-H = 109.5°

22. o-Mephenol



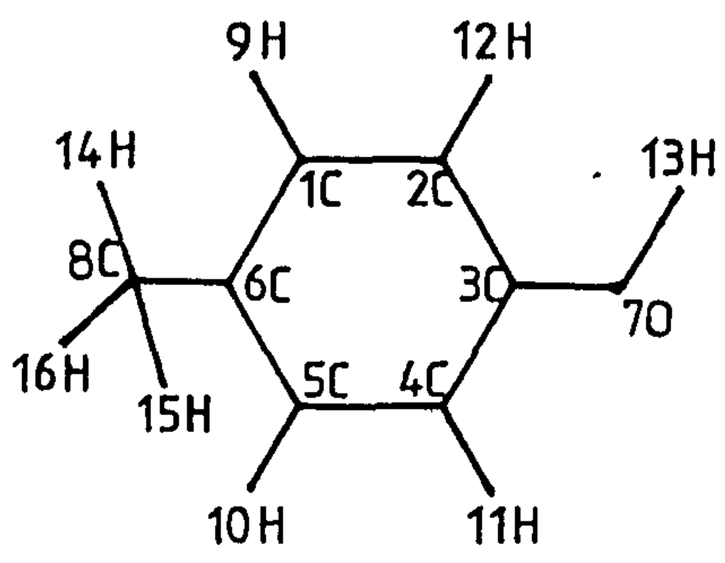
Planar molecule
Bond angles 120° except C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 5 7 and 6 8

23. m-Mephenol



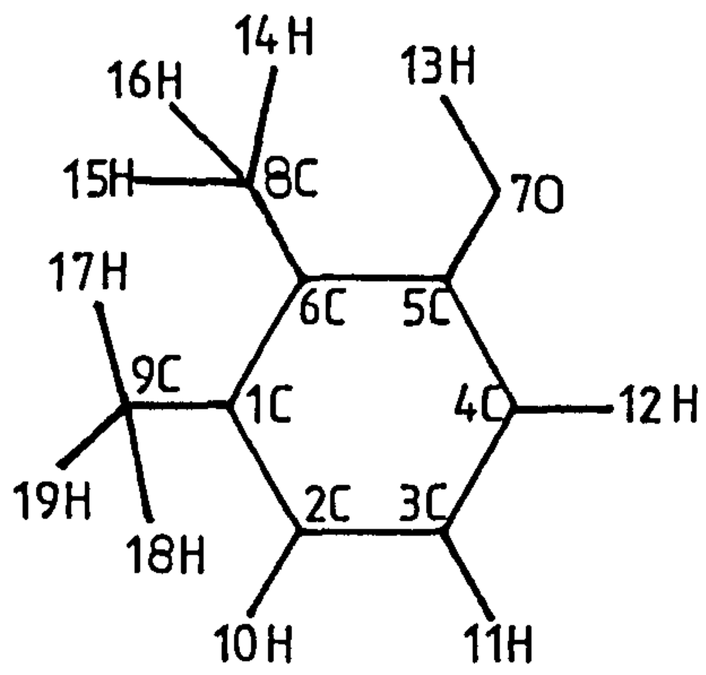
Planar molecule
All bond angles 120° except C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 1 8 and 5 7

24. p-Mephenol



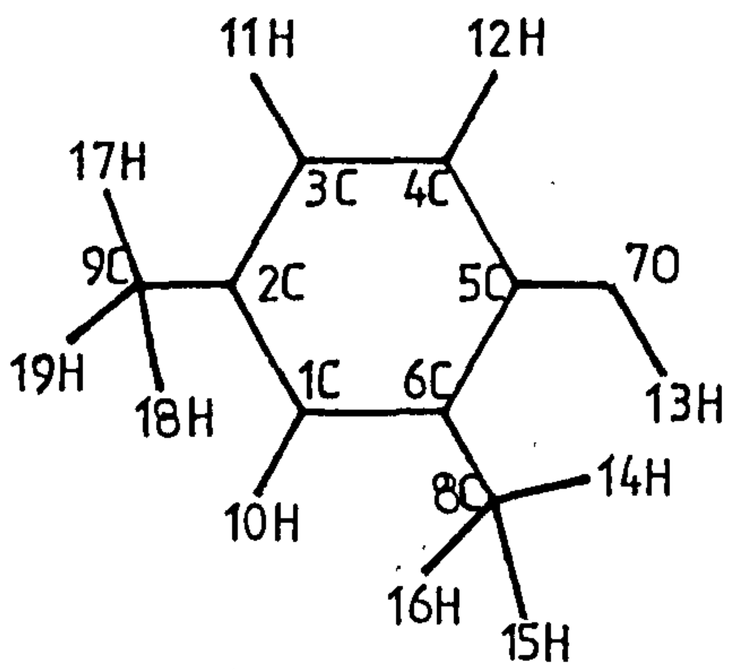
Planar molecule
All bond angles 120° except C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 3 7 and 6 8

25. 2,3-Me_2phenol



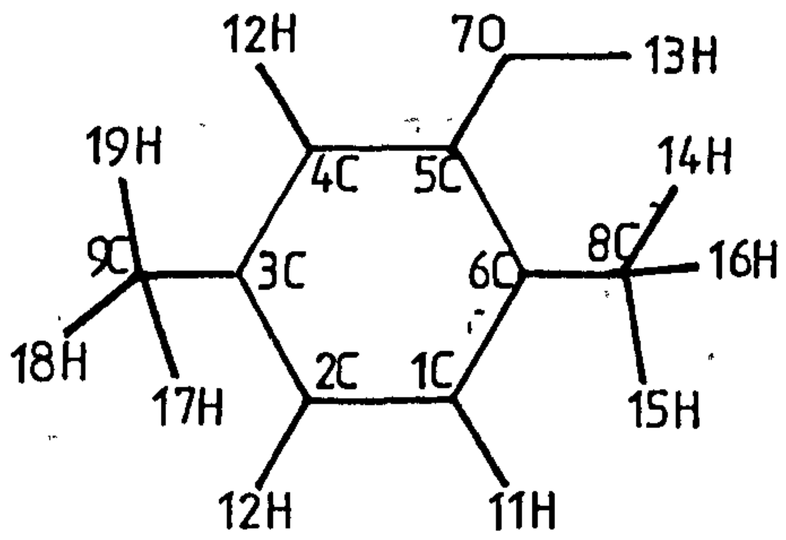
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 5 7
and 6 8
and 1 9

26. 2,4-Me_2phenol



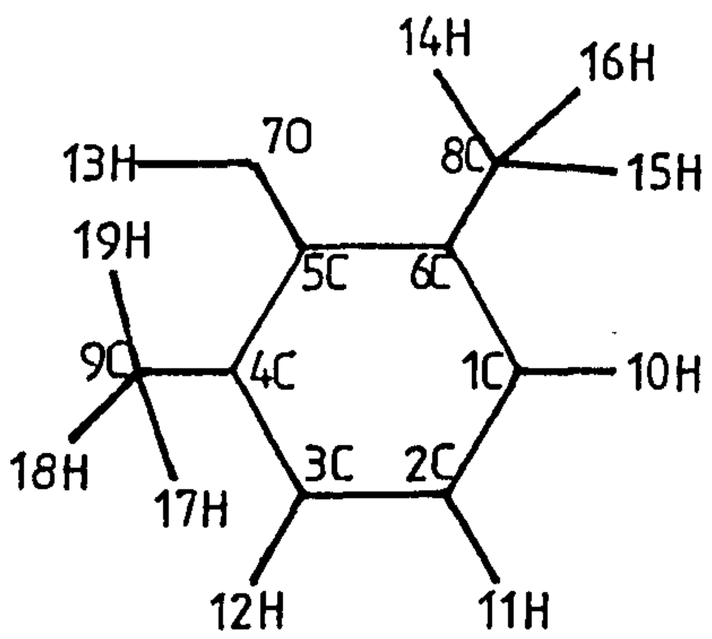
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 5 7
and 6 8
and 2 9

27. 2,5-Me_2phenol



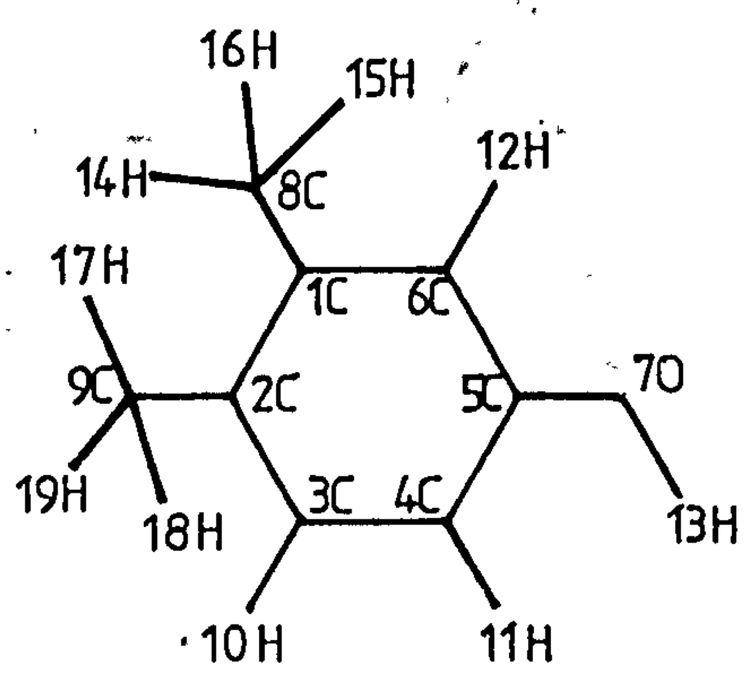
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 5 7
and 6 8
and 3 9

28. 2.6-Me_2phenol



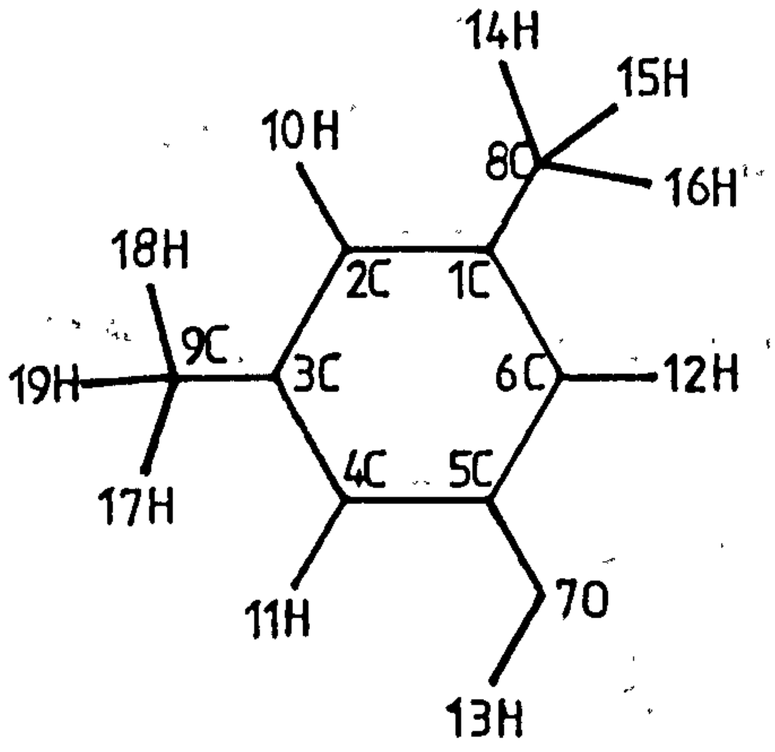
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
No hydrogen bond
Free rotation about 5 7
and 6 8
and 4 9

29. 3,4-Me_2phenol



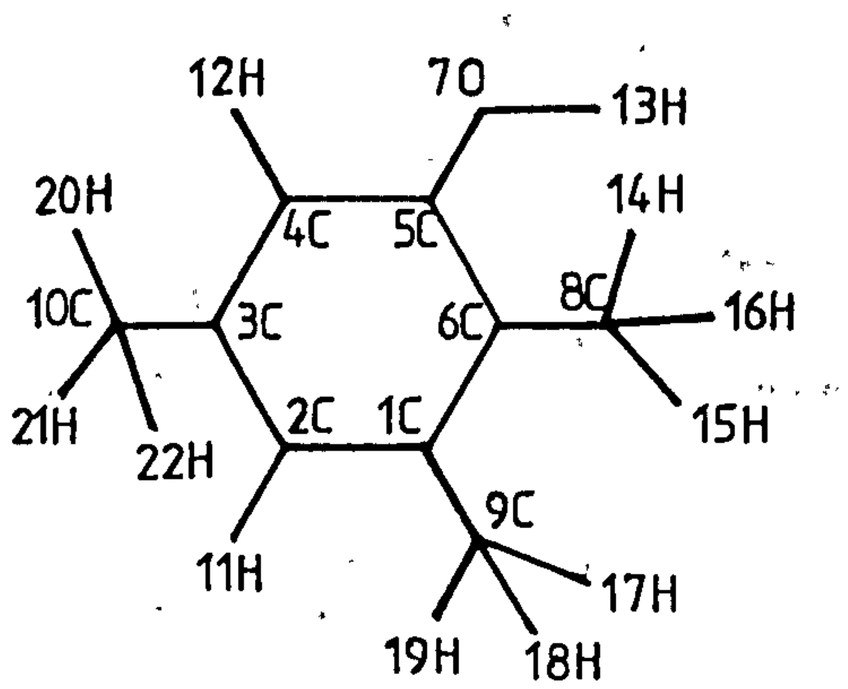
Planar molecule
All bond angles 120° except
2 1 8 = 123.5°
1 2 9 = 122.7°
Methyl groups pushing away from each other
No hydrogen bond
Restricted rotation about
1 8 and 2 9

30. 3,5-Me_2phenol



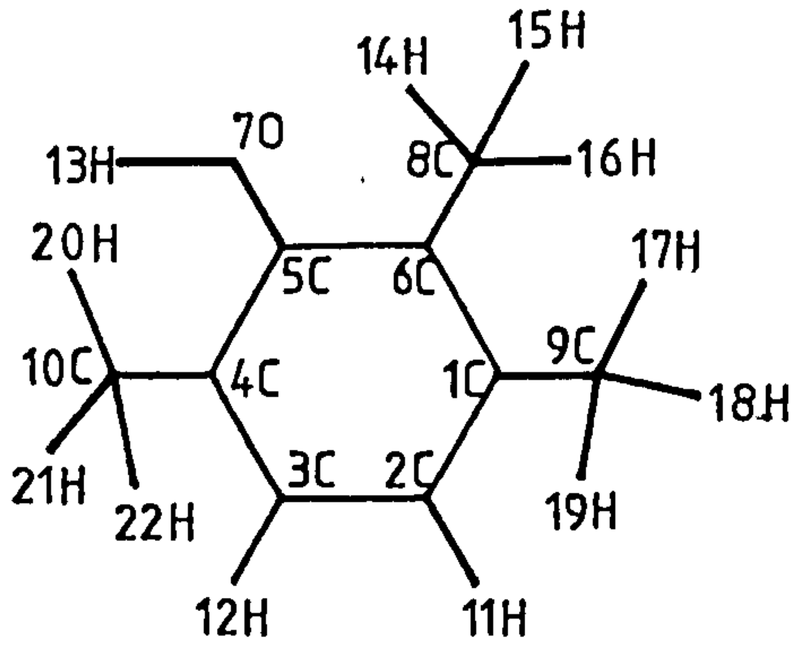
Planar molecule
All bond angles 120° except
C-C-H (methyl) and C-O-H (hydroxyl) = 109.5°
No hydrogen bond
Free rotation about 1 8
3 9
5 7

31. 2,3,5-Me_3phenol



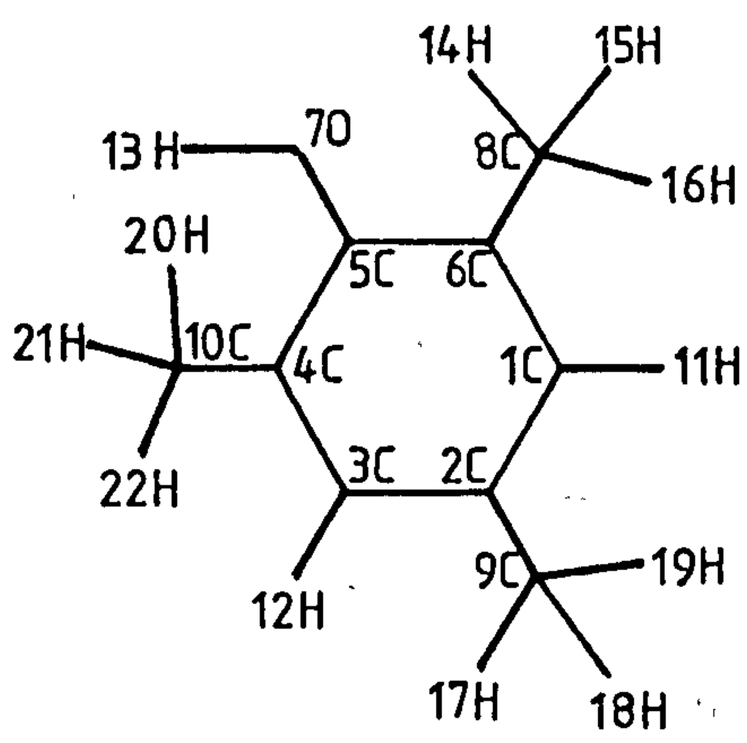
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
and 6 1 9 = 121.4°
Methyl groups pushing away from each other slightly.
No hydrogen bond

32. 2,3,6-Me_3phenol



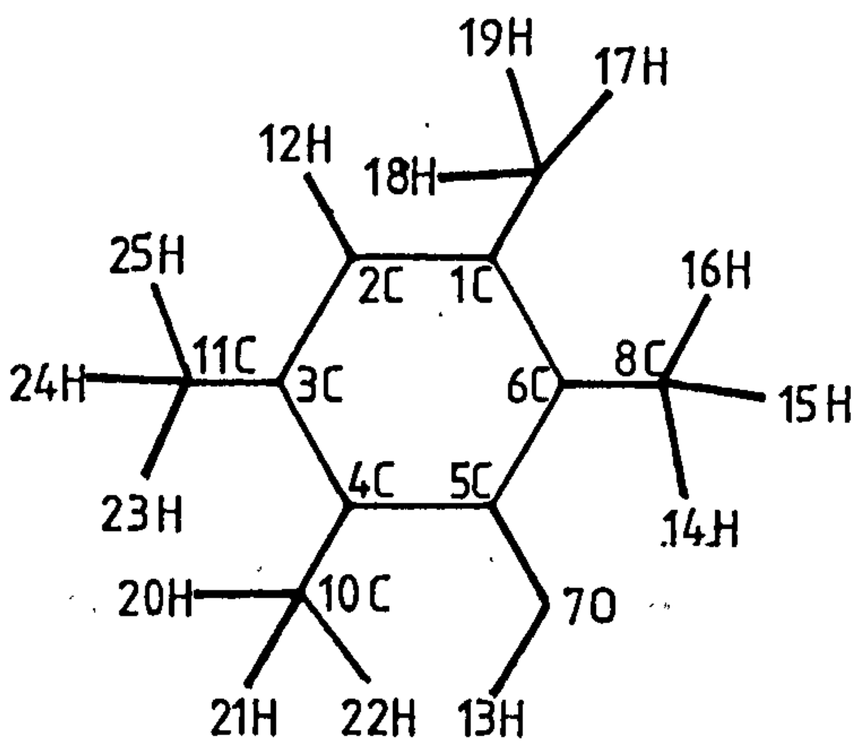
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
and 6 1 9 = 121.2°
Methyl groups adjacent to
one another push away
slightly
No hydrogen bond

33. 2,4,6-Me_3phenol



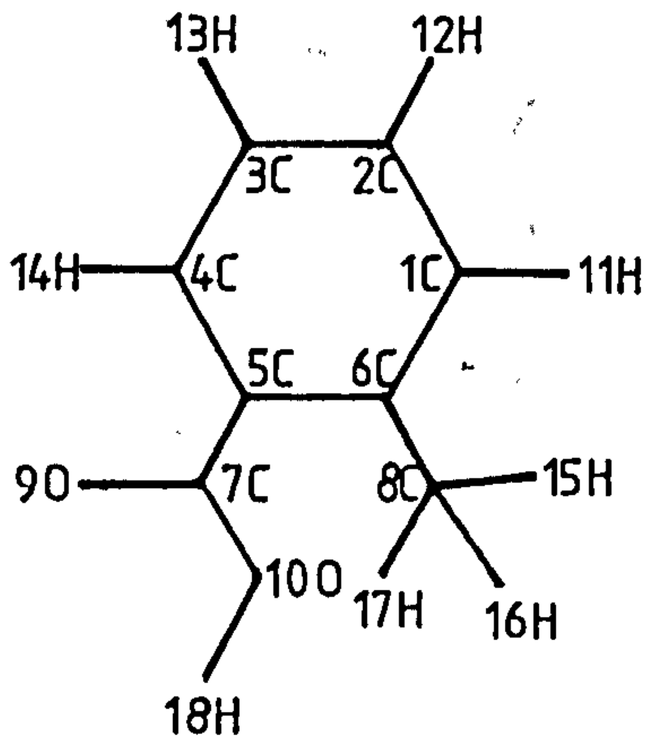
Planar molecule
All bond angles 120° except
C-C-H and C-O-H = 109.5°
No hydrogen bond.
No steric hindrance so no
change in bond angle

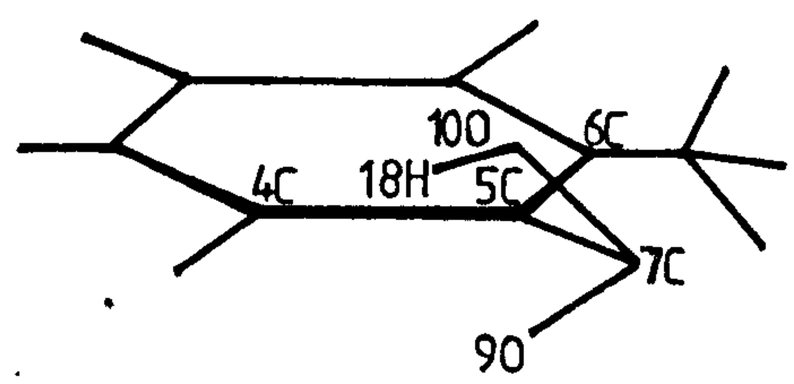
34. 2,3,5,6-Me_4phenol



Planar molecule
Adjacent methyl groups
push away from each other
and alter position of
hydrogen atoms relative to
carbon
No hydrogen bond

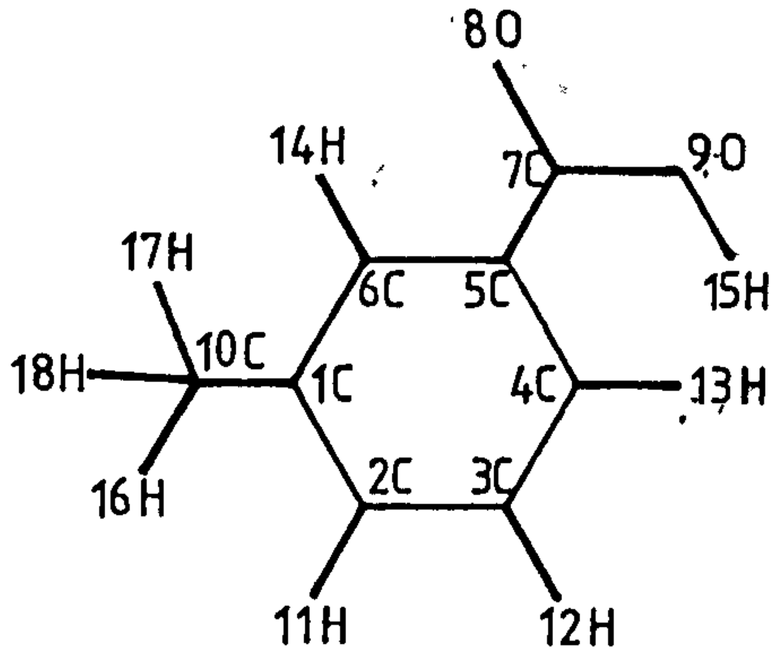
35. o-Mebenzoic acid





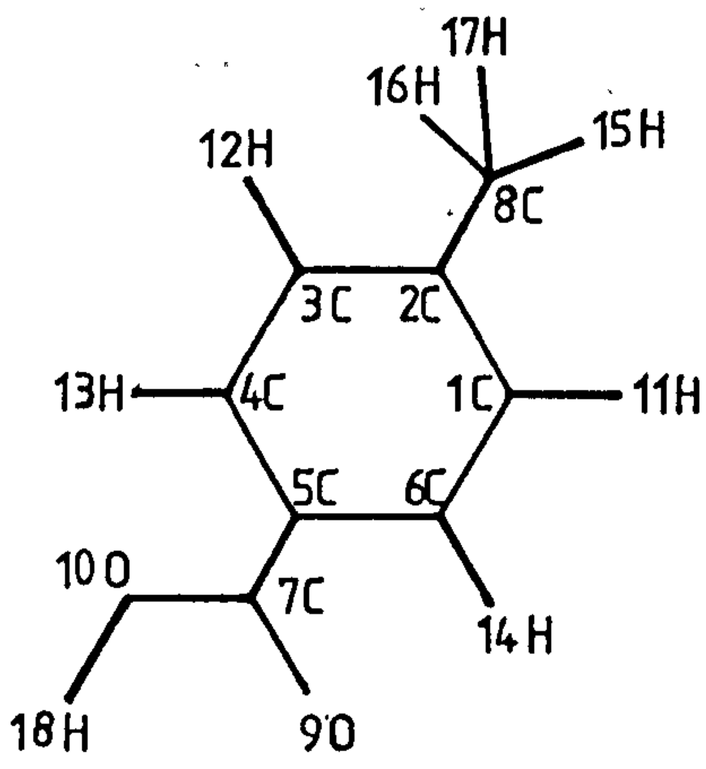
Non-planar molecule. Benzene ring under torsional strain, and acid group pushed out of ring plane by close proximity of methyl group, unless atom 90 is rotated next to methyl, which relieves strain. No hydrogen bond.

36. m-Mebenzoic acid



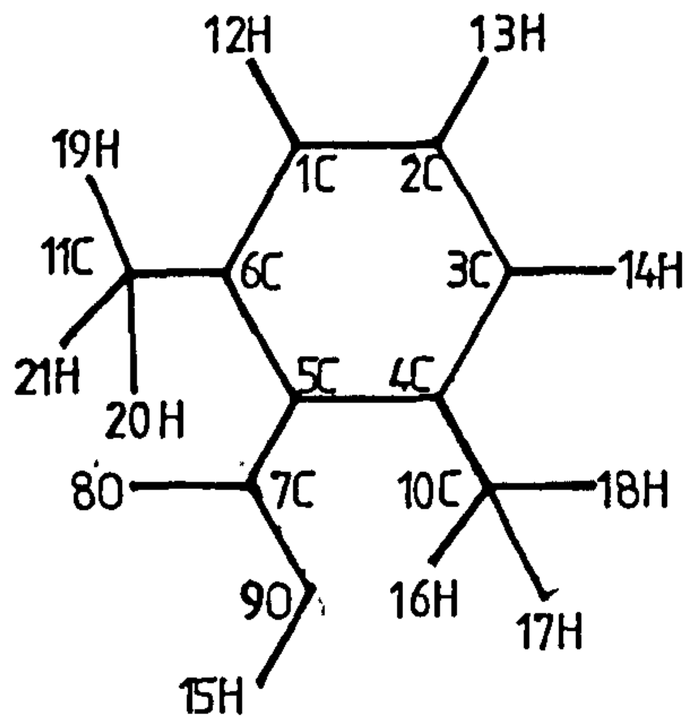
Planar molecule. Strain removed as methyl and carboxylic acid groups move apart.
Bond angles 120° except C-C-H (methyl) and C-O-H (carboxyl) = 109.5° and 8 7 9 = 118.3°

37. p-Mebenzoic acid



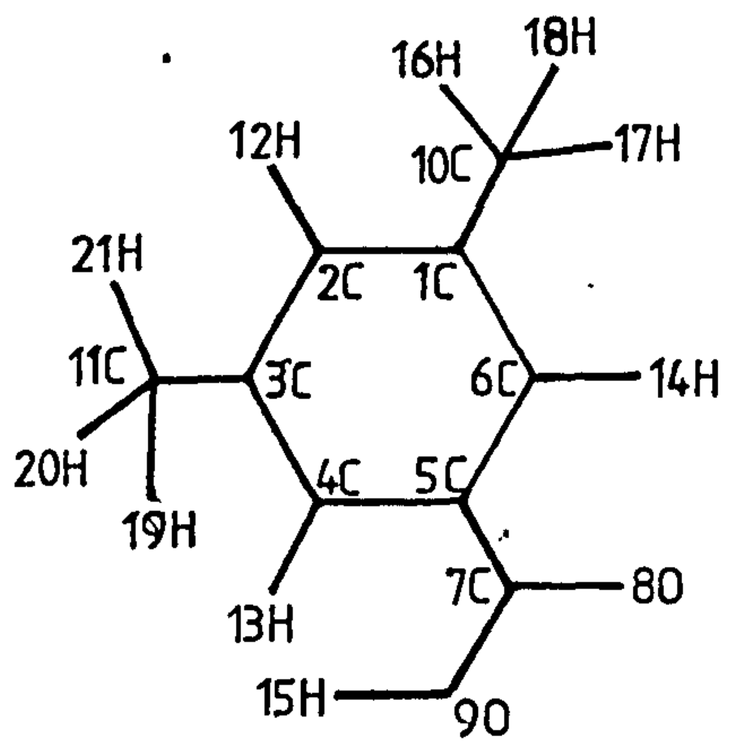
Planar molecule
Free rotation about 5 7
and 2 8

38. 2,6-Me_2benzoic acid



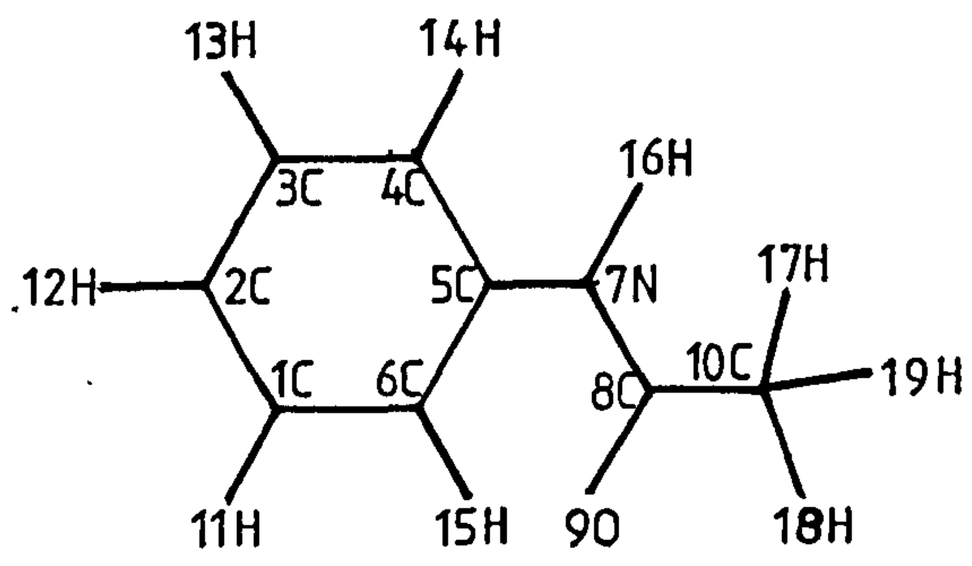
Planar molecule, but proximity of carboxyl and methyl groups causes them to move away from each other.
5 4 10 = 125.1°
4 5 7 = 124.5°
6 5 7 = 117.9°
5 6 11 = 121.9°
Atoms 9 and 16 are 2.09A° apart.

39. 3,5-Me_2benzoic acid



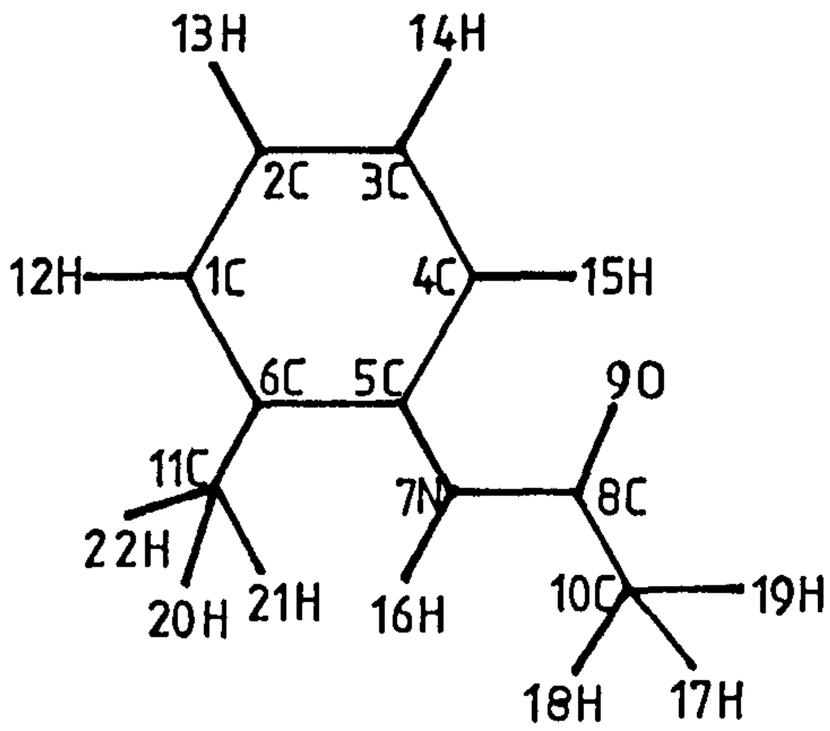
Planar molecule
Bond angles 120° except for C-C-H (methyl) and C-O-H (hydroxyl) = 109.5° and 4 5 7 = 125°, allowing free movement about bond 5 7 and 7 9

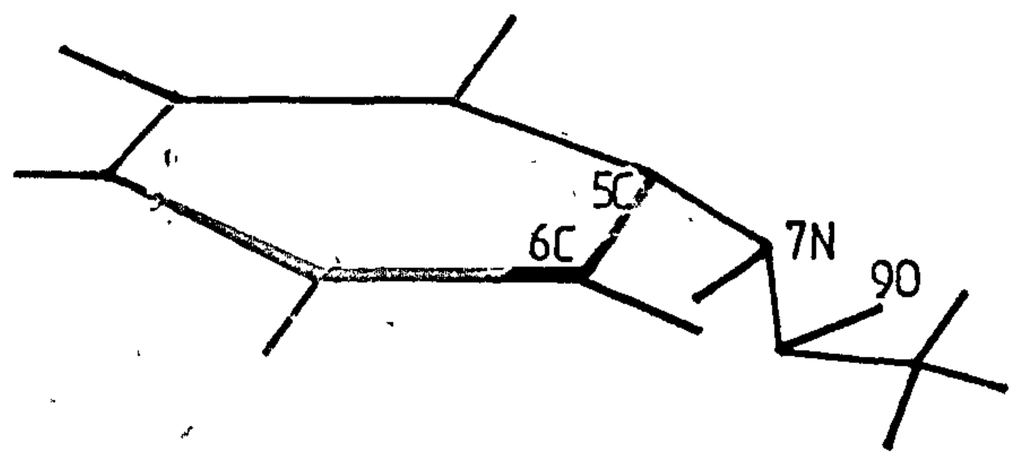
40. Acetanilide



Planar molecule. Acetanilide group free to rotate freely out of plane of molecule

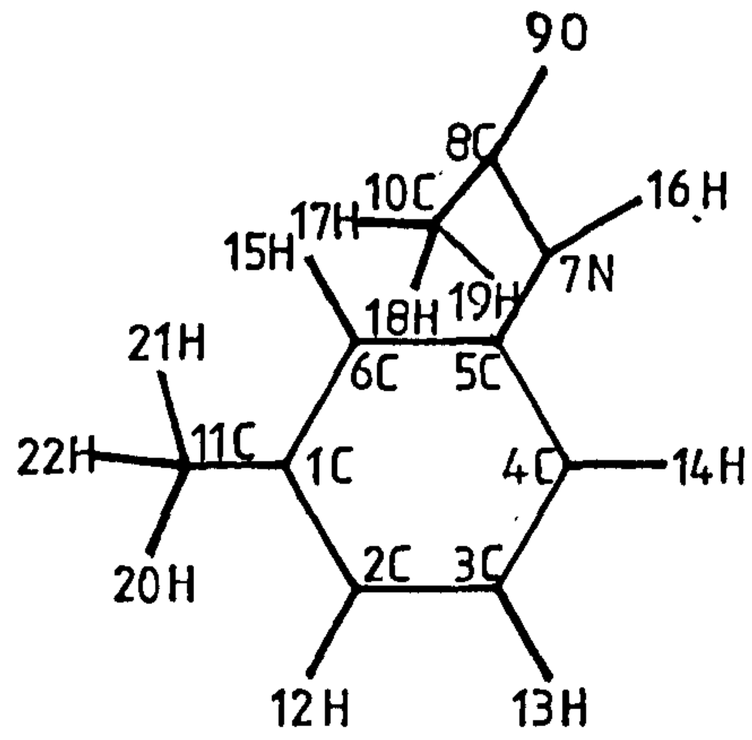
41. o-Meacetanilide





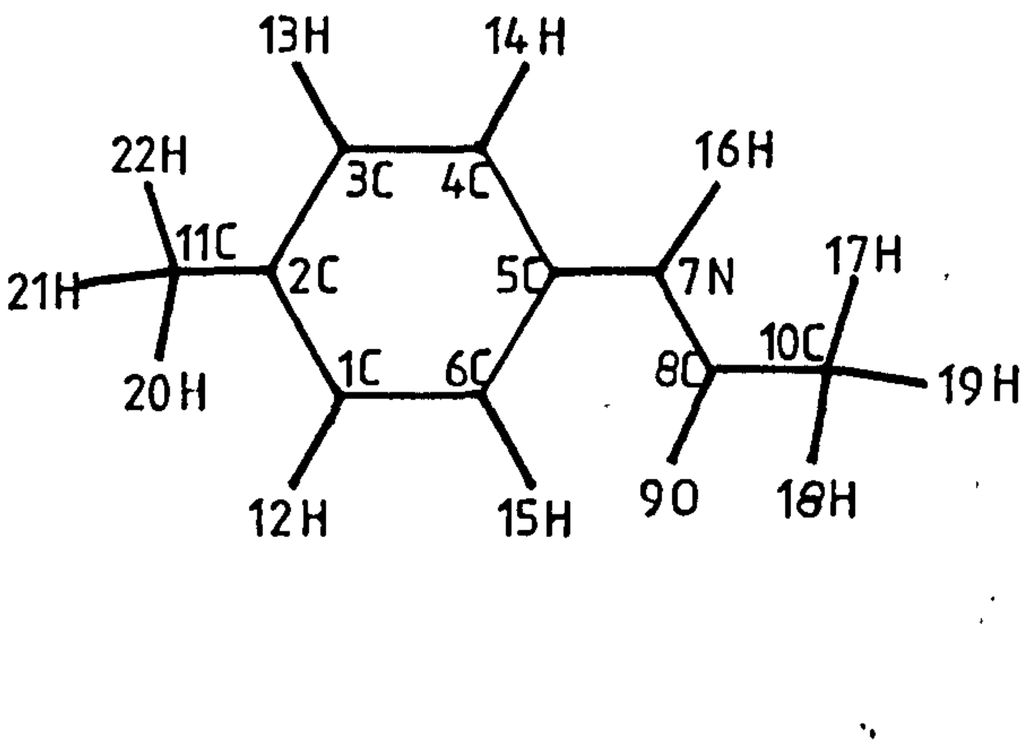
Proximity of methyl and acetanilide groups causes torsional strain within the molecule and pushes the acetanilide group out of the plane of the molecule. Acetanilide group then can rotate freely about bond 7 8.

42. m-Meacetanilide



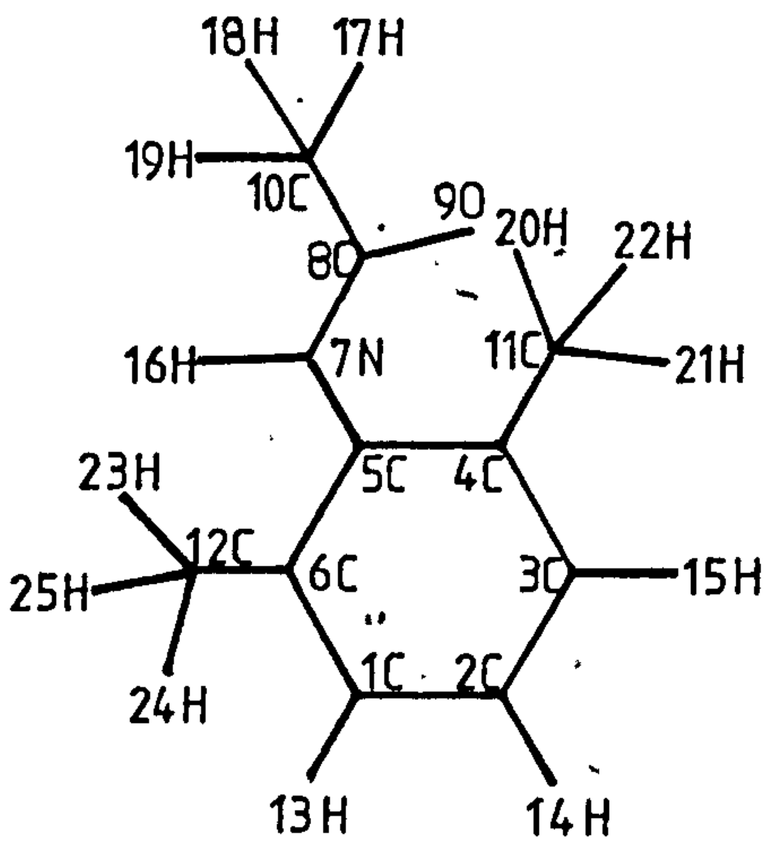
Molecule more or less planar, torsional tension removed by placing the methyl group one carbon away from the acetanilide group. Acetanilide group very flexible and rotates freely.

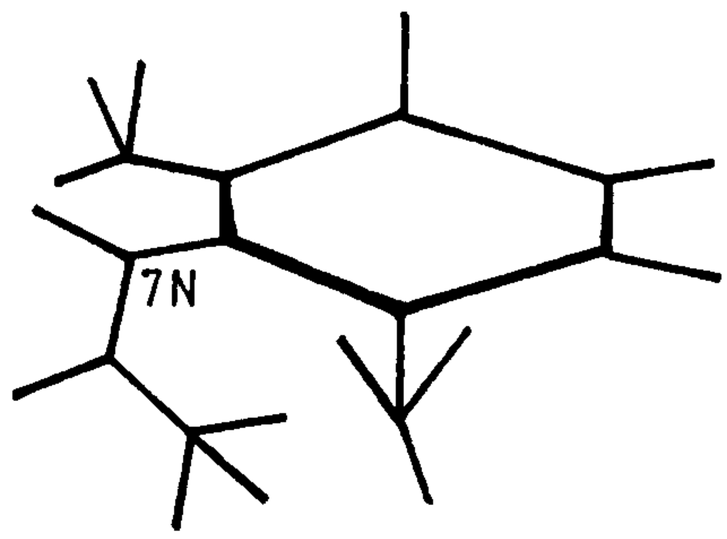
43. p-Meacetanilide



Planar molecule, but acetanilide group free to rotate so frequently out of plane of molecule.

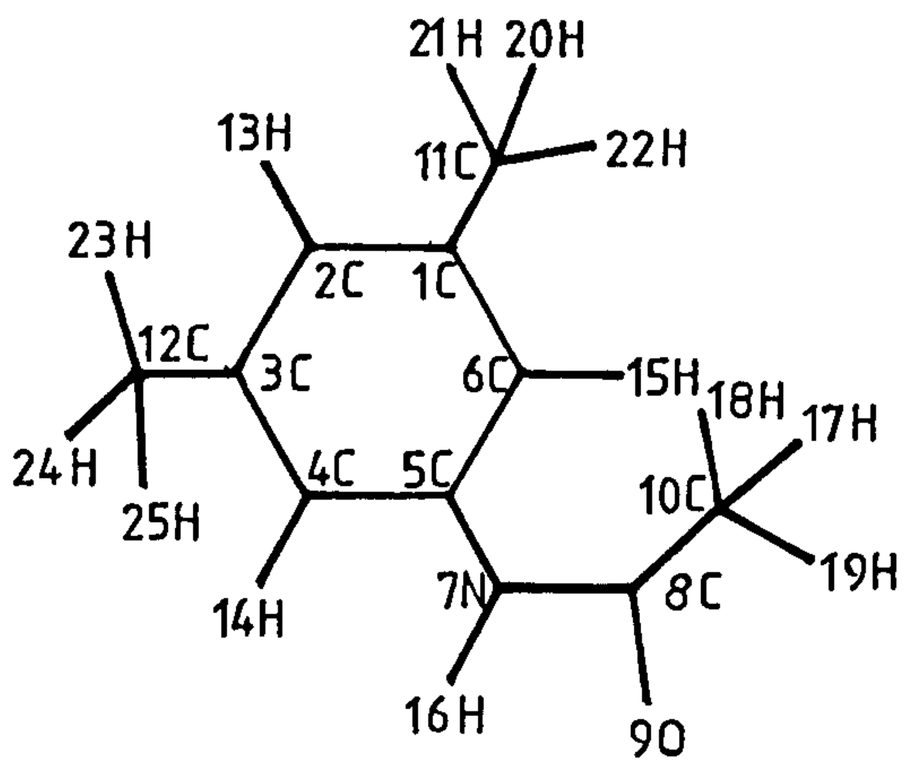
44. 2,6-Me_2acetanilide





Molecule not planar, ring system under strain and acetanilide group held in restricted position by adjacent methyl groups Acetanilide group not free to rotate.

45. 3,5-Me_2acetanilide



Planar molecule
Ring strain removed.
Acetanilide group free to rotate.

N.B. The molecules described above have been referred to as planar unless the ring is distorted or a substituent group is twisted out of the plane of the ring. This ignores the fact that strictly speaking the methyl group is planar only as regards the carbon atom. Planarity with respect to the methyl group refers to the bond - $-C_{Me}-C_{ring}$.

CHAPTER NINE

THE THERMODYNAMICS OF THE TRANSFER PROCESS

9.1 The Role of Thermodynamics of Solution Behaviour in Pharmacy and Biology

As the study of biology and biological processes has developed from a primarily descriptive science to an analytical science inνolved with molecular structure, the role of quantitative physical concepts and methods has become increasingly useful and important. The use of various physical techniques such as X-ray diffraction, spectroscopy, electron microscopy etc. has played an essential role in elucidating the molecular structures of many components of biological systems. The principles of kinetics and thermodynamics have been invaluable in understanding and describing many biological processes.

The application of the principles of thermodynamics has long been recognised as the most fundamental approach to the study of physical and chemical changes. Since biological processes are essentially physical and chemical by nature and are therefore controlled by the exchange of energy, it has become apparent that thermodynamic concepts can be fruitfully applied to biological systems.

The influence of physical comcepts in general and thermodynamics in particular in pharmaceutical sciences has also been significant. Physical concepts provide the basis for predicting solubility, rate of absorption, duration of action, specificity of action and other parameters

essential in the rational design of new drugs and appropriate dosage forms.

9.2 Drug Activity

In general terms, drug activity (defined as the response to interaction of the drug with a receptor site) may be thought of as occurring as two types : (a) structurally specific and (b) structurally non-specific. The former depends on the presence of certain groups in the drug molecule, particular intermolecular distances and/or molecular shape. This type of interaction is determined by the structure or 'fit' of the drug molecule to the receptor site. The latter type of activity is largely non-specific in nature and is not dependent on the 'fit' of the drug to the receptor site, but rather on the physical properties i.e. thermodynamic activity of the drug molecule. Essentially it suggests that if the drug reaches the receptor site in sufficient concentration, a biological response is observed. This type of activity appears to depend to a large extent on the distributive tendencies of the drug molecule between aqueous phases and biological phases with varying degrees of lipophilicity. Modifications of a drug molecule which affect the distributive properties of the molecule can thus have a profound effect on the biological activity of the molecule. This second type of interaction is primarily controlled by the solution thermodynamics of the drug molecule in various environments and provides one of the impetuses to examine relationships between solution thermodynamics and biological activity.

However, although solute oil/water distribution has been studied since the last century and a large collection of partition coefficients exists (189) most work has concentrated on the determination of the free-energy based coefficient itself, with few workers even reporting the temperature of measurement. Although such information indicates the extent of distribution, it yields little information on why the process occurs. This can be afforded by a more complete thermodynamic description of the system to include, for example, the enthalpic and entropic contributions towards the distribution. Some workers have reported either these particular quantities or the effect of temperature on the partition coefficient for various solutes and solvent pairs, but only those reports by Harris et al (191) on ion-pair transfer and two studies using octanol as the oil phase (Beezer et al{ 25 } and Rogers and Wong{330}) can be considered as attempts at a systematic examination of the thermodynamics of solute distribution. All reported studies save those of Breslauer et al (37), Kinkel et al (225) and Beezer et al (404) have used the temperature dependence of the distribution process to determine enthalpies and thence entropy changes. Kinkel et al (225) attempted to determine the thermodynamics of organic solute distribution from knowledge of the temperature dependence of the process using as simple a two-phase system as possible (water/2,2,4-trimethylpentane). This group found a single unique relationship between partition coefficients and enthalpies of distribution which indicated linear enthalpy entropy compensation which could be accredited to the properties of water. However, they also

suggested that it was inappropriate to obtain thermodynamic quantities from a study of the temperature dependence of distribution when the solvent pair studied have (1) high mutual solubility and (2) various intermolecular and molecular states which are temperature sensitive. Such a system is exemplified by the octanol-water system and they concluded that any previously reported linear enthalpy-entropy compensation for this system was due to probable statistical and experimental effects. However, the recent report of James (399) found that his van't Hoff results agreed with some calorimetric results and Beezer et al (404) measured enthalpies of solution calorimetrically in each solvent separately, and then combined the two and showed that the results they got for ΔH and ΔS agreed pretty well with previously recorded van't Hoff data. These two reports therefore suggest that linear enthalpy-entropy compensation can be found in the octanol-water system.

A group of workers which has contributed extensively to thermodynamics of partitioning is that of Davis, Higuchi and Rytting (99). This group considered group contributions to partitioning thermodynamics. Many group contribution approaches to structure-activity relationships of drugs have been discussed and as with these, one of the primary goals of solution thermodynamics has been the development of a priori methods for the prediction of solution behaviour and then using these methods in various physical and biological studies. Ideally one should be able to estimate the behaviour of a solute in a given solvent simply from the physical properties of the pure components. However,

attempts to do this have been limited almost entirely to mixtures of non-polar species (9 9). The semi-empirical group method is however a predictive method which has been relatively successful.

The basic assumption made in this method is that the free energy of the solution process is additively composed of independent contributions from the constituent functional groups. By calculating group contribution values it should be possible to use them in a priori fashion to predict solution behaviour for a wide range of drug molecules since the activity coefficient, excess free energy and partiton coefficient could be found by summing contributions for the different groupings comprising the molecule. In cases where two polar groupings are close to each other on a solute molecule, some modification of the additivity concept will be necessary but once the interactions are understood it should be possible to calculate correction factors.

If the enthalpic and entropic contributions to the free energy were found to be additive, prediction of all three terms would be possible and knowledge of such data would help clarify the physical processes occurring upon solution and the causes of non-ideal behaviour.

This chapter of the thesis aims to utilise both approaches described above to provide additional information about the internal and external interactions which affect the relationship of a molecule with its environment as seen in the study compounds.

9.3 Theoretical Significance of Partition Coefficients

Since partitioning is a process involving molecular equilibria, a partition coefficient is an equilibrium constant of fundamental significance in terms of free energies. It can be seen from equation 9.i.

$$\Delta G^{o} = -RT\ln K_{eg} = -RT\ln K_{p} \qquad (9.i)$$

that the partition coefficient is directly relatable to the free energy change in the system when a mole of the partitioning agent is transferred between the phases. A mounting body of evidence indicates that when two structurally closely related molecules are partitioned between the same two immiscible phases, the differences in the free energies of transfer calculated by equation 9.i. are directly relatable to the specific structural modification (283). This generality is at least applicable to solutes that form regular solutions in both phases or for which entropy of mixing is maximised. Thus:

$$\Delta G_{\text{alcohol}} = -RT\ln K_{p\ \text{alcohol}}$$

$$\Delta G_{\text{ester}} = -RT\ln K_{p\ \text{ester}}$$

and: $$\Delta G_{\text{acetate moiety}} = \Delta G_{\text{alcohol}} - \Delta G_{\text{ester}} = RT\ln \frac{K_{p\ \text{ester}}}{K_{p\ \text{alcohol}}}$$

$$= \text{constant}$$

or in general:

$$\Delta G_{\substack{\text{functional group free energy} \\ \text{of partitioning}}} = \Delta G_{GFE}$$

$$= -RT\ln \frac{K_{p\ \text{derivative}}}{K_{p\ \text{parent molecule}}}$$

$$= \text{constant}$$

If these relationships are valid, by working from the reverse direction it is possible to generate a system whereby partition coefficients can be calculated from knowledge of the functional group free energy of partitioning. This is what Hansch and Stewart (171) accomplished with the octanol-water system. The π values are directly related to group free energy change:

$$\pi = \frac{\Delta G_{GFE}}{2.303RT}$$

Under isothermal conditions, the group free energy of partitioning can be generated by simply multiplying the π value times the constant factor of 2.303RT.

9.4 Thermodynamics of Partitioning Systems

Solvent systems which are almost completely immiscible (e.g.alkanes-water) are fairly well behaved and lend themselves to more rigorous thermodynamic treatment of partitioning data than solvent systems which are partially soluble in each other. (14) The following development can be applied more strictly to the former systems, but the departures from ideality exhibited by the more polar solvent systems are not so great as to render this approach valueless. It should be noted here that the thermodynamic partition coefficient is a ratio of mole fractions ($P' = X_o/X_w$) and it should not be confused with the more common expression of P which is a dimensionless ratio of concentrations.

Cratin (84) has presented a discussion of some of the aspects of the thermodynamics of the partitioning process. The following discussion is drawn from his analysis which relies

heavily on extrathermodynamic assumptions.

For each of the 'i' components comprising an ideal solution, the following equation is assumed to hold:

$$\mu_i(T,P,X) = \mu_i^{\theta}(T,P) + RT\ln X_i \qquad (9.ii)$$

where μ_i^{θ} is the chemical potential of pure 'i' in the solution under specified conditions, and X_i is its mole fraction. μ_i^{θ} is not the actual chemical potential of pure 'i' but the value it would have if the solution remained ideal up to $X_i = 1$. It can be shown (84) that for dilute solutions, the chemical potential based on mole fractions is larger than that based on molar concentrations by a factor of $RT\ln\bar{v}_s^{o}$, where $\bar{v}_s^{o}$ is the molar volume of solvent and therefore:

$$\mu_i(T,P,X) = \mu_i^{\theta}(T,P) + RT\ln\bar{v}_s^{o} + RT\ln C \qquad (9.iii)$$

Cratin then considered the thermodynamic implications of the concept that P is an additive-constitutive property of a molecule; that is, he approached the study of the intermolecular forces involved in partitioning by assuming that the free energy of transfer of a molecule can be factored into the contributions of its various parts. Assuming that the free energy of a molecule (μ_t) is made up of a lipophilic component (μ_l) and n hydrophilic groups (μ_h) it may be written:

$$\mu_t(w) = \mu_l(w) + n\mu_h(w)$$

$$\mu_t(o) = \mu_l(o) + n\mu_h(\varpi)$$

Assuming ideal behaviour:

$$\mu_t(w) = \mu_1^{\theta}(w) + n\mu_h^{\theta}(w) + RT\ln X(w)$$

$$\mu_t(o) \quad \mu_1^{\theta}(o) + n\mu_h^{\theta}(o) + RT\ln X(o)$$

Converting from mole fractions to concentration terms, the above equations become:

$$\mu_t(w) = \mu_1^{\theta}(w) + n\mu_h^{\theta}(w) + RT\ln\bar{\upsilon}^{o}(w) + RT\ln C(w)$$

$$\mu_t(o) = \mu_1^{\theta}(o) + n\mu_h^{\theta}(o) + RT\ln\bar{\upsilon}^{o}(o) + RT\ln C(o)$$

At equilibrium $\mu_t(w) = \mu_t(o)$; hence equating equations, collecting terms and replacing C(o)/C(w) by P, we obtain:

$$\{\mu_1^{\theta}(w) - \mu_1^{\theta}(o)\} + RT\ln\{\bar{\upsilon}^{o}(w)/\bar{\upsilon}^{o}(o)\} + n\{\mu_h^{\theta}(w) - \mu_h^{\theta}(o)\}$$

$$= +RT\ln P \qquad (9.iv)$$

Setting $\Delta\mu^{\theta} = \mu^{\theta}(w) - \mu^{\theta}(o)$ we obtain:

$$\log P = \frac{n\Delta\mu_h^{\theta}}{2.3RT} + \frac{\Delta\mu_1^{\theta}}{2.3RT} + \log\{\bar{\upsilon}^{o}(w)/\bar{\upsilon}^{o}(o)\} \qquad (9.v)$$

If equation 9.v. holds, a plot of log P against n will be linear with a slope equal to $\Delta\mu_h^{\theta}/2.3RT$ and an intercept of $\Delta\mu_1^{\theta}/2.3RT + \log\{\bar{\upsilon}^{o}(w)/\bar{\upsilon}^{o}(o)\}$

Equation 9.v. defines the necessary conditions for additivity of log P values. The standard free energy of transfer of solute in the partitioning process is given by:

$$\Delta G_{tr}^{\theta} = \Delta\bar{\mu}^{\theta} = RT\ln P'$$

If it is assumed that the standard molar enthalpy change is not temperature dependent in the range studied (84) then

$$\frac{\delta \ln P'}{T} = \frac{\Delta \bar{H}^{\theta}}{RT^2}$$

where $\Delta \bar{H}^{\theta}$ is equivalent to the standard enthalpy of transfer between the two solvents. It is thus possible to calculate this enthalpy of transfer by measuring P' over a range of temperatures. In practice this is rather imprecise because of two implied assumptions: first, that the levels of each solvent dissolved in the other remain constant over the temperature range; second, if P is measured in terms of concentrations, that the ratio of solvent molar volumes remains constant also. Therefore, it is preferable to obtain the enthalpy of transfer by measuring the heats of solution in two separate solvents so that:

$$\Delta \bar{\mu}^{o} = \Delta \bar{H}_{tr}{}^{o} = \Delta H^{o}(w) - \Delta H^{o}(o)$$

The entropy of transfer can be calculated from:

$$\Delta G_{tr}{}^{o} = \Delta H_{tr}{}^{o} - T\Delta S_{tr}{}^{o}$$

The changes in miscibility of more polar solvent systems as a function of solute concentration have been studied in only a few systems. (135,157) However, experience has shown that the partition coefficient at low solute concentrations is usually not highly dependent on this effect. Even with solvent pairs as miscible as isobutyl alcohol-water, the effect is small with solutes at 0.01M or less, and solvent pairs less miscible than chloroform-water will easily tolerate 0.1M solute without appreciable miscibility changes. (255)

9.5 Energy Requirements for Phase Transfer

The relative roles of the various binding forces which determine the way a solute distributes itself between two phases has been examined by a number of authors, notably Kauzmann (219) on whose work the following discussion is based.

The study of the hydrocarbons in water shows that although the ΔH of solution is negative (indicating a favourable enthalpy change by the evolution of heat), such compounds are insoluble in water. This is due to a large ΔS for the process. The large energy of re-ordering the hydrocarbon solute and the water solvent molecules keeps them in separate phases when placed together. The same phenomenon regulates the distribution of apolar solute molecules in an apolar solvent-water system.

A variety of work supports the conclusion that the entropic component of ΔG plays a large role in the position of equilibrium (partition coefficient) taken by nonpolar compounds in nonpolar water-solvent systems. Kauzmann put forward the following facts:

1. Mixtures of lower aliphatic alcohols with water show positive deviations from Raoult's law, indicating an increase in unitary free energy ($\Delta G_u > 0$) for the transfer of alcohol from alcohol to water phase, despite the fact that heat is evolved ($\Delta H<0$) on the addition of these alcohols to water. Therefore $\Delta S_u = (\Delta H_u - \Delta G_u)/T<0$ when an alcohol molecule is transferred to water.

2. The solubilities of many liquid aliphatic compounds e.g.butanol, in water decrease with increase in temperature.

Hence, ΔH for the transfer process must be < 0, and ΔS_u for mixing must be negative.

3. The formation of micelles from detergent molecules in water is accompanied by very small heat changes, hence it is assumed that this reaction is controlled by a large negative ΔS rather than a large positive ΔH.

The origin of the large negative unitary entropy change and the small negative enthalpy change involved in partitioning between aqueous and nonaqueous phases was first clearly appreciated by Frank and Evans (137). They concluded that when organic compounds are placed in water, the water molecules arrange themselves around the apolar parts in what was termed 'iceberg structures'. These structures were later referred to as 'flickering clusters' to indicate their lack of stability.

The Frank-Evans point of view is that the stripping of the water molecules from the apolar part of the solute results in a large entropy change in the randomization of the water molecules. An alternative view is that of Aranow and Witten (8) who reason that in the aqueous phase the apolar chain of a solute molecule is rigidly held in a favoured rotational configuration by the structured layer of water molecules surrounding it. In the organic solvent its rotational oscillations are relatively unrestricted.

Partitioning data however favour the Frank-Evans hypothesis (255)

The next factor to consider in studying the energy

requirements for phase transfer is hydrogen bonding. This factor is important in determining the character of both the solute and the organic solvent phase. Compounds such as alcohols, esters, and ketones have quite different properties from those of hydrocarbons. Moreover, as solvents, it is not simply the hydrogen bonding character of the pure compound which must be considered, but also the water-saturated phase which is actually involved in the partitioning process. Beezer et al (404) measured the ΔH of solution in pure solvents and in water-saturated solvent and found appreciable differences. The transfer of an alcohol or acid from the water phase to a hydrocarbon phase may involve complete 'dehydration' of the polar OH or COOH function. It is unlikely that such complete 'dehydration' would occur in, say, butanol, which is 9M with respect to water content at saturation. Even in octanol, which is 2.3M with respect to water at saturation, it is likely that most highly polar functions would be more or less solvated by water and/or the hydroxyl function of the alcohol.

Certain solvents such as alcohols and amines can act as both donors and acceptors in hydrogen bonding. This increases their versatility as solvents.

Other intermolecular forces which must be considered in the partitioning process are dispersion forces arising out of electron correlation. It seems that these would play a minor role in setting equilibrium positions of solutes. Dispersion forces involved in complex formation in solution will largely cancel out since, when a solute molecule leaves one

phase and enters a new phase, it exchanges one set of London interactions for another (294).

The energy required to transfer from the aqueous phase to the organic phase any solute which contains two or more formal charges will depend on the dielectric constant of the organic phase in question. Most of the water-immiscible organic solvents have dielectric constants much lower than that of water, and thus charged solutes must contain rather large hydrocarbon residues to have positive log P values. This combination makes them very surface active and usually results in difficulties of measurement.

9.6 Temperature Dependence of the Equilibrium Constant

Invariant as it is at constant temperature, the equilibrium constant K is well known to vary with temperature. The origin of this variability is easily identified. That ΔG^{o} is a function of temperature is evident from the equation:

$$\Delta G^{o} = \Delta H^{o} - T\Delta S^{o}$$

ΔG^{o} = Difference of standard free energies
ΔH^{o} = Standard enthalpy change
T = Temperature ^{o}K
ΔS^{o} = Standard entropy change

Substituting for ΔG^{o} from the equation $\ln K = -\Delta G^{o}/RT$ we obtain:

$$-RT\ln K = \Delta H^{o} - T\Delta S^{o}$$

$$\ln K = \frac{-\Delta H^{o}}{RT} + \frac{\Delta S^{o}}{R}$$

If K is compared at two temperatures spanned by an interval over which ΔH^{o} may be regarded as constant, we obtain:

$$\ln K_2 = \frac{-\Delta H^{o}}{RT_2} + \frac{\Delta S^{o}}{R} \quad \text{and} \quad \ln K_1 = \frac{-\Delta H^{o}}{RT_1} + \frac{\Delta S^{o}}{R}$$

This requires that ΔC_p(overall change in heat capacity of system) be negligibly small and ΔS^o is constant, but,

$$\ln K_2 - \ln K_1 = -\frac{\Delta H^o}{RT_2} + \frac{\Delta H^o}{RT_1} \quad \text{or} \quad \ln\frac{K_2}{K_1} = 2.303\text{Log}\frac{K_2}{K_1}$$

$$= -\frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

This integral form of van't Hoff's law applies to a great many systems.

$-\Delta H^o/2.303R$ represents the slope of a plot of logK vs 1/T. If ΔH is effectively constant over the span of temperature concerned, the slope of the plotted line will be constant and the line therefore straight. By actually measuring the line's slope, it is possible to establish $-\Delta H^o/2.303R$ and thence ΔH^o.

Any significant variation of ΔH^o over the temperature range will give a curvature of the plot logK vs 1/T. At a given point on the line a tangent can be constructed and ΔH^o determined at that temperature.

9.7 Experimental Determination of the Free Energy, Enthalpy and Entropy of Transfer

1. Free Energy of Transfer. ΔG_{tr}

The free energy of transfer, ΔG_{tr}, is related to the partition coefficient by the equation:

$$\Delta G_{tr} = -RT\ln K_d$$

where R = gas constant, $8.314 JK^{-1}mole^{-1}$

T = temperature, units of Kelvin

Thus the free energy of transfer can be obtained from the partition coefficient at a particular temperature.

2.Enthalpy of Transfer. ΔH_{tr}

The enthalpy of transfer can be determined indirectly by the van't Hoff method as described in section 9.6.

3.Entropy of Transfer. ΔS_{tr}

The entropy term cannot be determined experimentally, but must be obtained by substitution in the equation:

$$\Delta G_{tr} = \Delta H_{tr} - T\Delta S_{tr}$$

where the free energy and enthalpy of transfer are obtained experimentally.

9.8 Apparatus and Method

Liquid-liquid partition coefficients were determined using a rapid-mix/filter probe system (as shown in Figure 23). This was a modification of that developed by Cantwell and Mohammed (62) and used by Kinkel et al (226). It consisted of a thermostatted (±0.1°C) mixing chamber of 200ml volume, whose contents could be vigorously stirred using a magnetic stirring bar and motor. Using filters of hydrophilic (Whatman No.1) or hydrophobic (Whatman PS) material, the water or oil phases respectively could be probed by linking the filters to an HPLC pump and then examining the phase under study by on-line detection using an ultraviolet spectrophotometer (Perkin Elmer 550) fitted with an 80µl flow-through cell thermostatted at 25°C. Flow exiting from this cell was returned to the mixing chamber. The pump membrane was constructed of stainless steel and all connecting tubes were constructed of Teflon. Two partitioning systems were investigated; in the octanol-water system the

aqueous phase was probed and in the cyclohexane-water system the hydrophobic phase was probed. Using this arrangement to determine partition coefficients, first both solvents were pre-equilibrated at 20°C by gentle shaking for 24 hours. The aqueous phase was added to the mixing chamber and probed to give a blank reading, A_b, then a second addition of aqueous phase containing solute was made to obtain an unextracted sample reading, A_u. The organic phase was then added and the absorbance of the relevant phase monitored continuously (A_e). To ensure adsorption was not occurring within the system, solvent containing solute was pumped through the apparatus prior to commencing experimental readings. It was found that solute levels fell initially during pumping and then levelled off. Checks revealed that no adsorbed material returned to solution, even when fresh solvent was pumped through. Therefore the system was primed before each experimental solute change. To examine the effect of temperature on the solute liquid-liquid partition coefficient, the temperature of the mixing chamber was raised over a range of 20°C to 50°C and the A_e values determined at equilibrium.

Thus: $$K_p = (A_u - A_e).(A_e - A_b)^{-1} R^{-1}$$

where R is the oil/water phase volume ratio. The ultraviolet absorbance of each solute was examined in the system at the different mixing chamber temperatures in the absence of the oil phase. In no case did the absorbance in the flow-through cell change. All measurements were carried out in the linear portion of the Beer-Lambert plots. All determinations were carried out in duplicate.

9.9 Results and Discussion

Assuming simple Nernst law distribution of a neutral solute, N, between two immiscible solvents we may write:

$$K_d = \{N_o\}/\{N_w\} \qquad (9.vi)$$

where subscripts o and w refer to the oil and water phases respectively. Equation 9.vi. holds only for systems which are totally immiscible and for which the solute is in the same molecular state in both phases; and where K_d is described as the thermodynamic liquid-liquid partition coefficient, K_d^x, when concentrations are expressed in mole fractions. For sufficiently dilute solutions (15):

$$\ln K_d^x = \ln K_d - \ln(V_w/V_o)$$

where V is the molar volume. Although the use of a molarity scale has been proposed for examining the thermodynamics of distribution (29) the mole fraction scale enjoys freedom from both temperature and density effects. (10) Thus, using mole fraction units, as the ratio (V_w/V_o) changes this is countered by an opposite and equal change in the nominal phase volume ratio. Thus:

$$RT\ln K_d^x = -\Delta G^x \qquad (9.vii)$$

where ΔG^x is the free energy change upon solute distribution, and R and T are the gas constant and absolute temperature. Enthalpies of distribution, ΔH^x, may be obtained from the slopes of $\ln K_d^x$ versus T^{-1} plots via the van't Hoff equation and entropy changes upon distribution, ΔS^x, from the Gibbs equation.

9.9.1 The Thermodynamics of Partitioning Between Aqueous Solution and n-Octanol

Much use has been made of the aqueous/n-octanol partitioning system as a model for the biological membrane due to its similarity to the lipid bilayer. (255) The hydroxyl group of n-octanol provides a hydrogen bonding acceptor group similar to that of the phosphate and acetyl groups of the polar head region of the bilayer, and a degree of polarity to the molecule. Hydrophobicity is provided by the nonpolar octyl group and weak van der Waals interactions between adjacent octyl chains may occur. Some degree of ordering is found in the n-octanol phase due to hydrogen bonding between octanol molecules, and also between octanol and water molecules. (7) n-Octanol comprises (on a mole fraction basis) 27% water at 25°C. The solubility of water in n-octanol varies with temperature as does the solubility of n-octanol in water, although as reported in Chapter 3 whether solubility increases or decreases with rise in temperature is difficult to ascertain. However, it is known that the composition of the water/n-octanol system varies considerably with change in temperature.

Figures 34-40 give the van't Hoff plots for the solutes examined. In general, linearity is good and indicates that the enthalpies of distribution were constant over the studied temperature range. However, certain compounds, such as 2,4,6-Me_3phenol did not produce a straight line and so for these a tangent was constructed at a specific temperature (25°C) or the best straight line was used to determine the enthalpy of transfer. Comparison of the van't Hoff plots in

Figure 34. Van't Hoff Plots for Chlorophenols in the Octanol/Water System

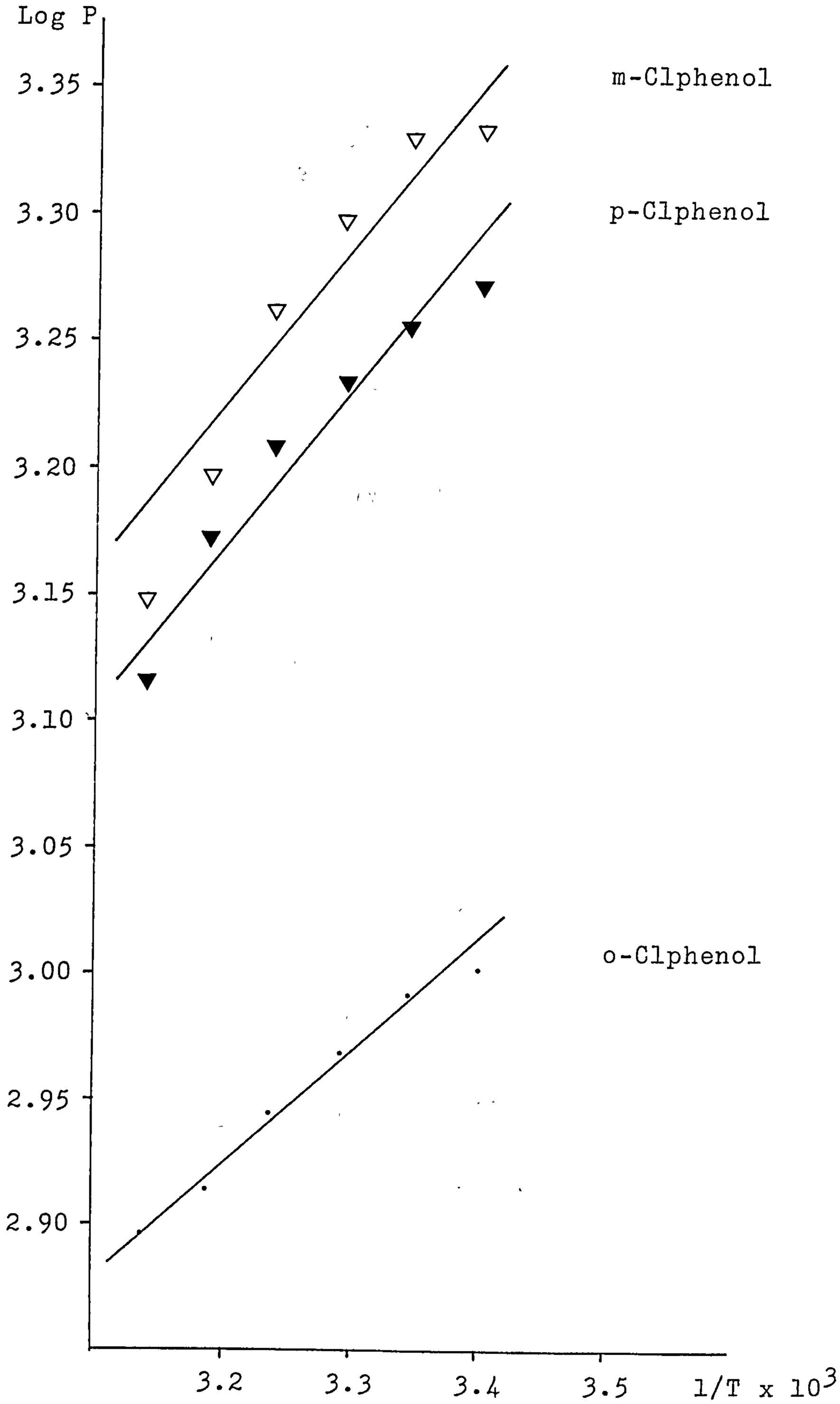


Figure 35. Van't Hoff Plots for Nitrophenols in the Octanol/Water System

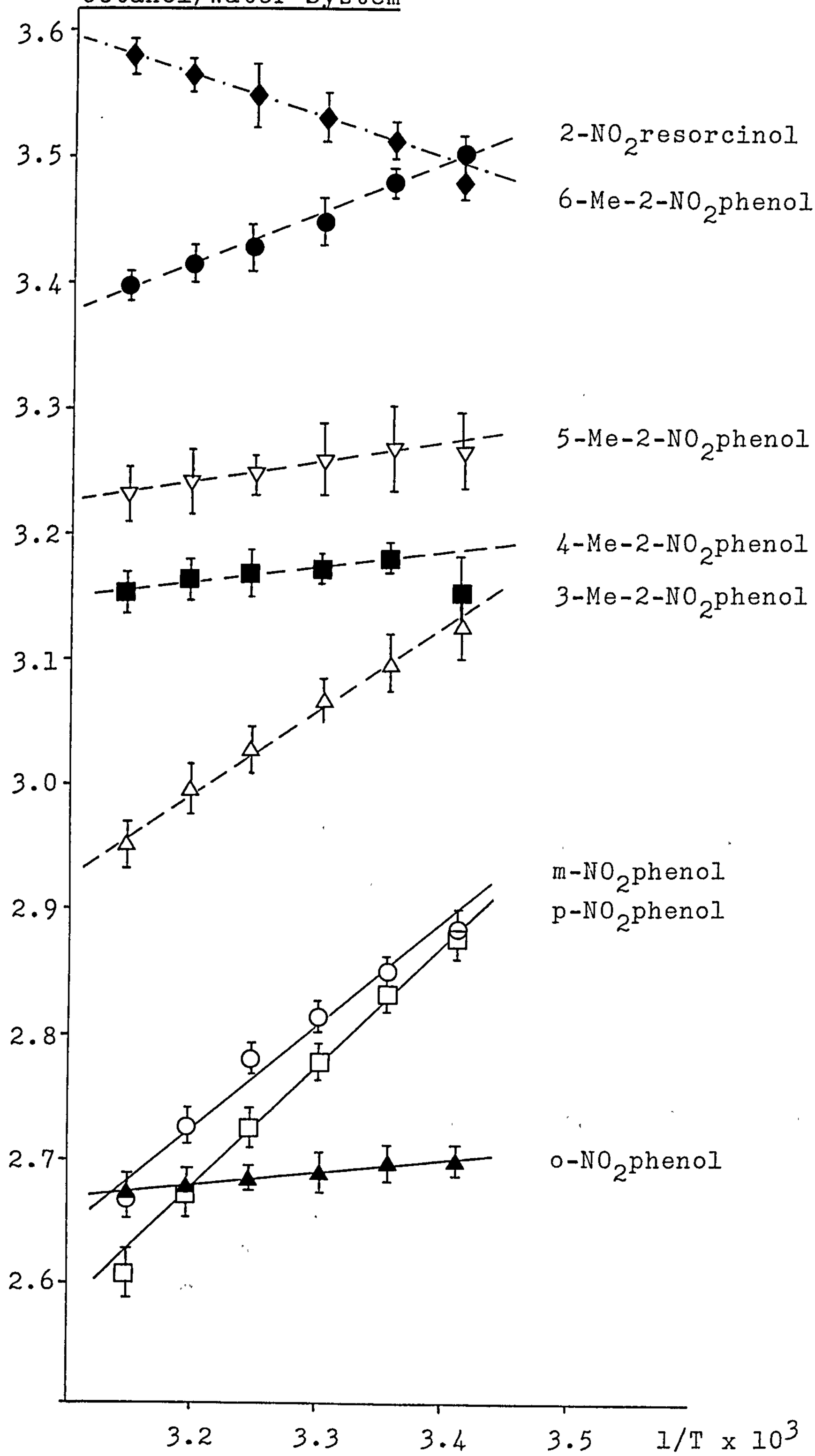


Figure 36. Van't Hoff Plots for Hydroxybenzoic Acids in the Octanol/Water System

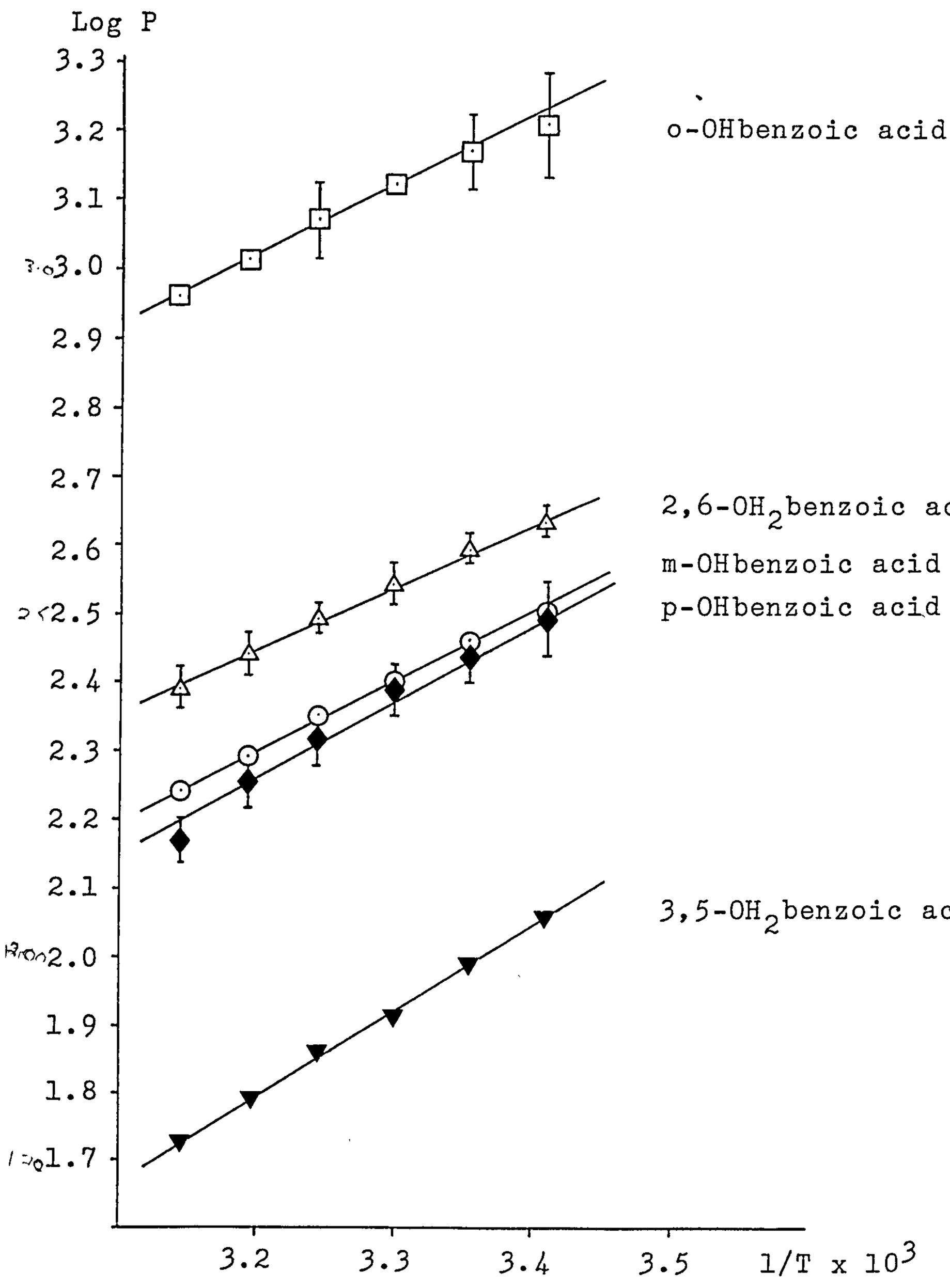


Figure 37. Van't Hoff Plots for Hydroxybenzaldehydes in the Octanol/Water System

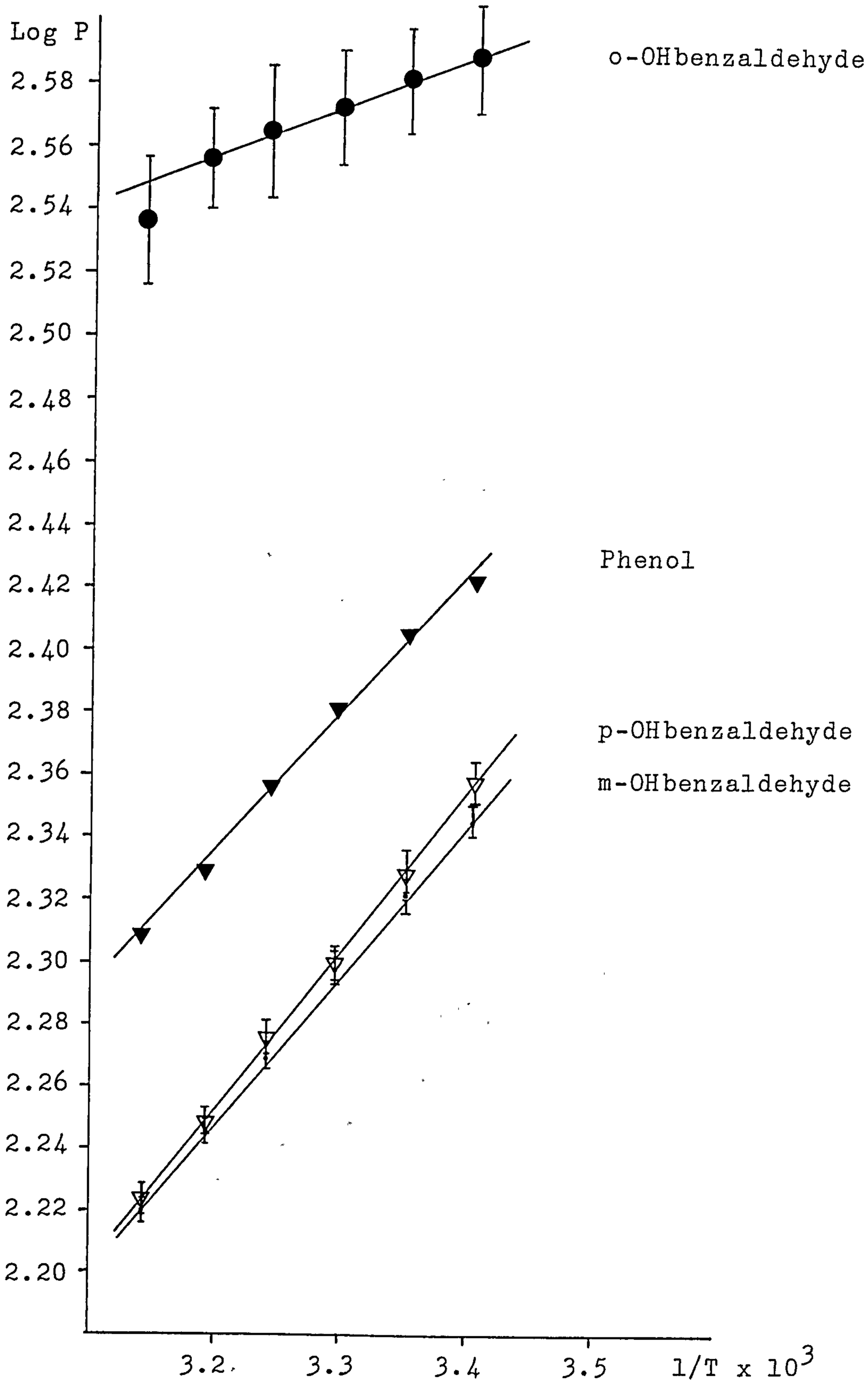


Figure 38. Van't Hoff Plots for Methylphenols in the Octanol/Water System

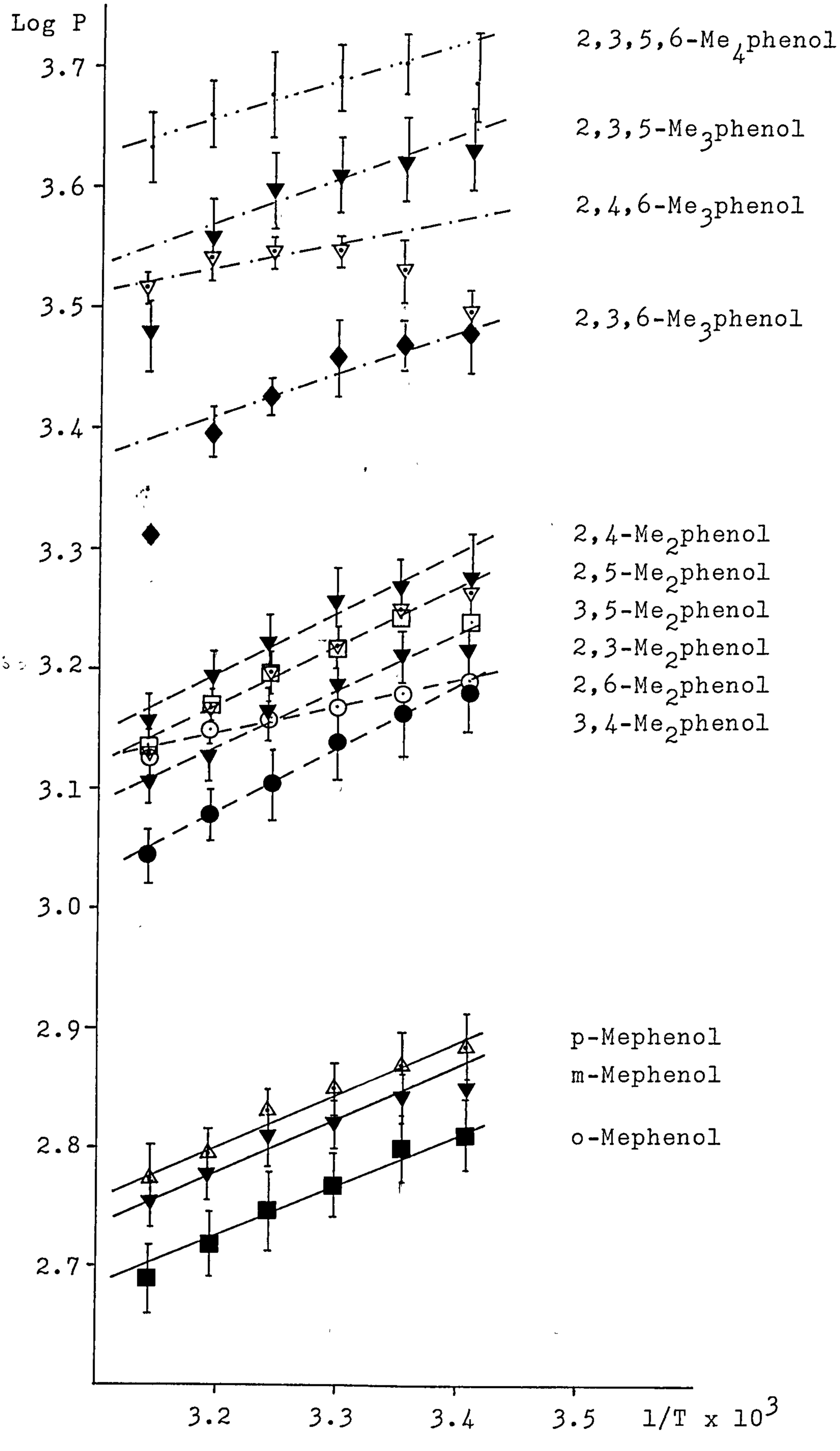
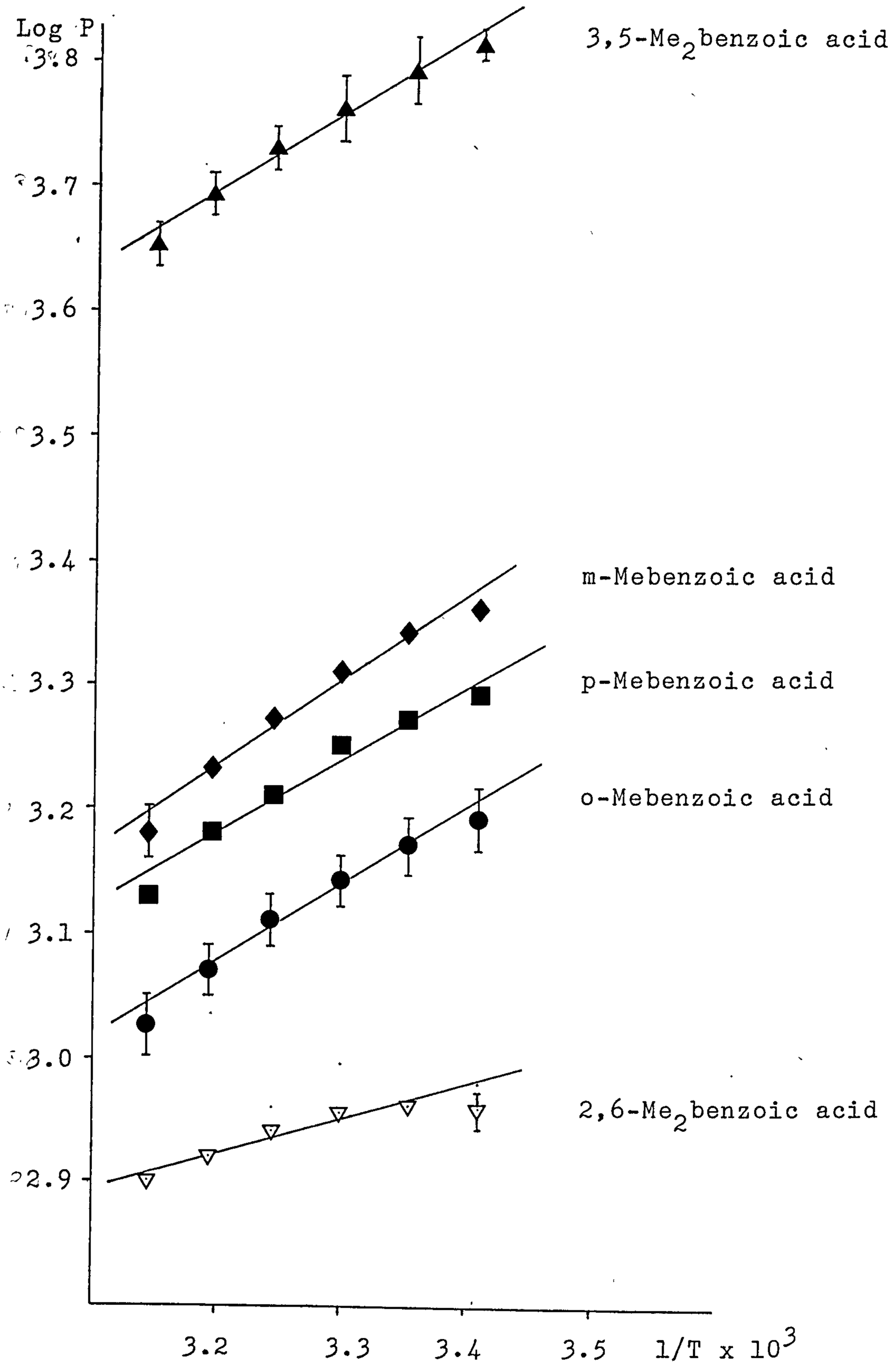


Figure 39. Van't Hoff Plots for Methylbenzoic Acids in the Octanol/Water System



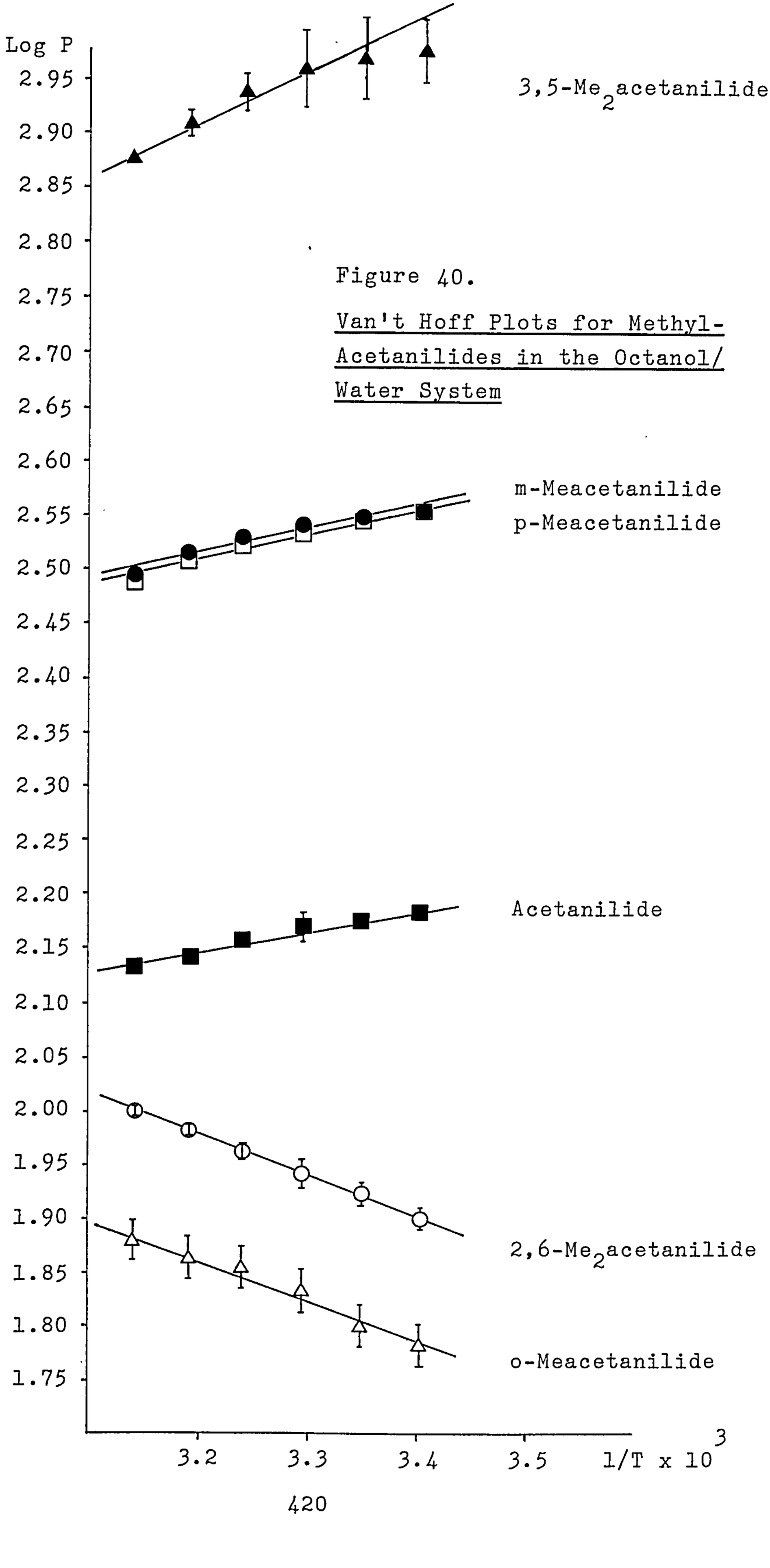


Figure 40.

Van't Hoff Plots for Methyl-Acetanilides in the Octanol/Water System

Table 56. Thermodynamic Parameters for the Octanol-Water System

Compound	Enthalpy $\Delta H kJ mole^{-1}$	Free Energy $\Delta G kJ mole^{-1}$	Entropy $\Delta S J mole^{-1} deg^{-1}$
Phenol	- 8.42	-13.69	+17.68
o-Clphenol	- 8.42	-17.03	+28.88
m-Clphenol	-10.85	-18.57	+26.35
p-Clphenol	-10.53	-18.55	+26.91
o-NO_2phenol	- 2.46	-15.35	+43.25
m-NO_2phenol	-15.32	-16.22	+ 3.00
p-NO_2phenol	-19.15	-16.09	-10.27
2-NO_2resorcinol	- 7.31	-19.79	+41.89
o-OHbenzaldehyde	- 3.66	-14.72	+37.10
m-OHbenzaldehyde	- 8.94	-13.23	+14.40
p-OHbenzaldehyde	- 9.57	-13.28	+12.44
Benzoic acid	- 8.42	-15.61	+24.13
o-OHbenzoic acid	-17.85	-18.04	+ 0.64
m-OHbenzoic acid	-19.15	-14.13	-16.85
p-OHbenzoic acid	-22.98	-13.92	-30.40
2,6-OH_2benzoic acid	-17.87	-14.79	-10.34
3,5-OH_2benzoic acid	-22.98	-11.32	-39.13
3-Me-2-NO_2phenol	-12.76	-17.61	+16.27
4-Me-2-NO_2phenol	- 2.20	-18.10	+53.35
5-Me-2-NO_2phenol	- 3.35	-18.62	+51.23
6-Me-2-NO_2phenol	+ 6.80	-19.97	+89.83
o-Mephenol	- 7.66	-15.94	+28.26
m-Mephenol	- 8.14	-16.18	+27.45
p-Mephenol	- 8.42	-16.07	+26.68
2,3-Me_2phenol	- 9.09	-18.31	+30.94
2,4-Me_2phenol	- 9.50	-18.77	+31.39
2,5-Me_2phenol	- 9.85	-18.52	+29.10
2,6-Me_2phenol	- 3.64	-18.13	+48.63
3,4-Me_2phenol	-10.05	-18.01	+27.17
3,5-Me_2phenol	-10.53	-18.56	+26.93
2,3,5-Me_3phenol	- 6.70	-20.64	+47.57
2,3,6-Me_3phenol	- 5.74	-19.77	+47.87
2,4,6-Me_3phenol	- 3.80	-20.12	+55.70
2,3,5,6-Me_4phenol	- 6.02	-21.13	+50.72
o-Mebenzoic acid	-12.45	-18.09	+18.92
m-Mebenzoic acid	-13.40	-19.06	+18.99
p-Mebenzoic acid	-12.13	-18.68	+21.98
2,6-Me_2benzoic acid	- 5.74	-16.86	+37.32
3,5-Me_2benzoic acid	-11.49	-21.61	+33.97
Acetanilide	- 3.51	-12.42	+29.89
o-Meacetanilide	+ 8.25	-10.27	+62.15
m-Meacetanilide	- 3.45	-14.53	+37.20
p-Meacetanilide	- 4.60	-14.52	+33.30
2,6-Me_2acetanilide	+ 7.18	-10.98	+60.94
3,5-Me_2acetanilide	- 3.83	-16.92	+43.92

octanol/water with those in cyclohexane/water (Fig 41 - 46) show that enthalpies of transfer in cyclohexane/water were rather more constant over the studied temperature range. This again illustrates the problems associated with using n-octanol as a model system.

The full thermodynamic parameters for the transfer of the solutes from aqueous solution to n-octanol are shown in Table 56.

The large negative free energies for all the solutes studied indicates the greater preference for the lipoidal phase over the aqueous environment. The transfer of a phenol from water to n-octanol occurs in two stages. Initially, disruption of water-phenol hydrogen bonds (positive enthalpy change) is followed immediately by the formation of new water-water hydrogen bonds (negative enthalpy change). Insertion in the lipid phase requires the disruption of octanol-octanol interactions and some water-octanol hydrogen bonds (positive enthalpy change) followed by the formation of octanol-phenol hydrogen bonds (negative enthalpy change). Since there is a substantial amount of water dissolved in the n-octanol phase, there may also be the reformation of water-phenol hydrogen bonds within the lipid phase (negative enthalpy change).

All the phenolic solutes studied, except 6-Me-2-NO_2phenol, have negative enthalpies of transfer, indicating that there is an overall increase in the strength and/or the number of molecular interactions on going from water to octanol. The transfer of the phenolic solutes between

aqueous solution and n-octanol is exothermic in nature. Thus the partition coefficient decreases with rise in temperature. This is a common phenomenon for solvent pairs having high mutual solubility (226). All the phenols, except p-NO_2phenol, m-, p-, 2,6-di-, and 3,5-di- hydroxy benzoic acids have positive entropy values, indicating that the transfer of the majority of phenols results in an overall increase in entropy or disorder.

The acetanilides do not contain the phenolic OH group and must therefore be considered separately. They have negative free energies as do the phenols, indicating a preference for the lipoidal phase over the aqueous environment. However, transfer from one phase to the other involves different mechanisms. Solubility in water is associated with the formation of a loosely held, but highly structured sheath of water molecules around the organic molecule. This sheath is disrupted on passage into a nonpolar phase which gives a positive entropy of transfer. The molecular size and shape determine the number of water molecules present in the structured sheath and therefore the magnitude of the positive entropy of transfer. Since octanol and water are mutually soluble, stripping of the water molecules may not be complete or restructuring of the sheath may occur after transfer.

The acetanilides, except for o-Meacetanilide and 2,6-Me_2acetanilide, have negative enthalpies of transfer which suggests there is an overall increase in the strength and/or the number of interactions except for the two ortho-substituted molecules. With the same two exceptions, the

partition coefficient decreases with rise in temperature, showing that transfer is exothermic in nature.

All compounds have a negative free energy term which comprises favourable contributions from both the negative enthalpy term and the positive entropy term. Individual entropy and enthalpy contributions to the overall free energy term may be calculated in order to determine the dominating parameter for the transfer process. This indicates that for those solutes whose substituent groups (not hydroxyl) are capable of increasing the strength of hydrogen bonding, e.g. p-NO_2phenol, the transfer process seems to be enthalpically dominated. Conversely, an entropy dominated process is found for those solutes whose substituent groups reduce hydrogen bonding and/or increase the hydrophobicity of the molecule e.g. p-alkyl phenol.

9.9.2 The Thermodynamics of Partitioning Between Aqueous Solution and Cyclohexane

The aqueous/cyclohexane system has advantages over the water/n-octanol system as a reference system for partition studies because its composition changes little with temperature and the organic phase has a low water content. Also, the relatively low solute-lipid interactions makes interpretation of results easier. For these reasons, many authors prefer it for studying the thermodynamics of partitioning. (333,405) (226).

Figures 41 - 46 give the van't Hoff plots for the solutes examined. Linearity is good and indicates that the enthalpies of transfer were constant over the studied

Figure 41. Van't Hoff Plots for Chlorophenols in the Cyclohexane/Water System

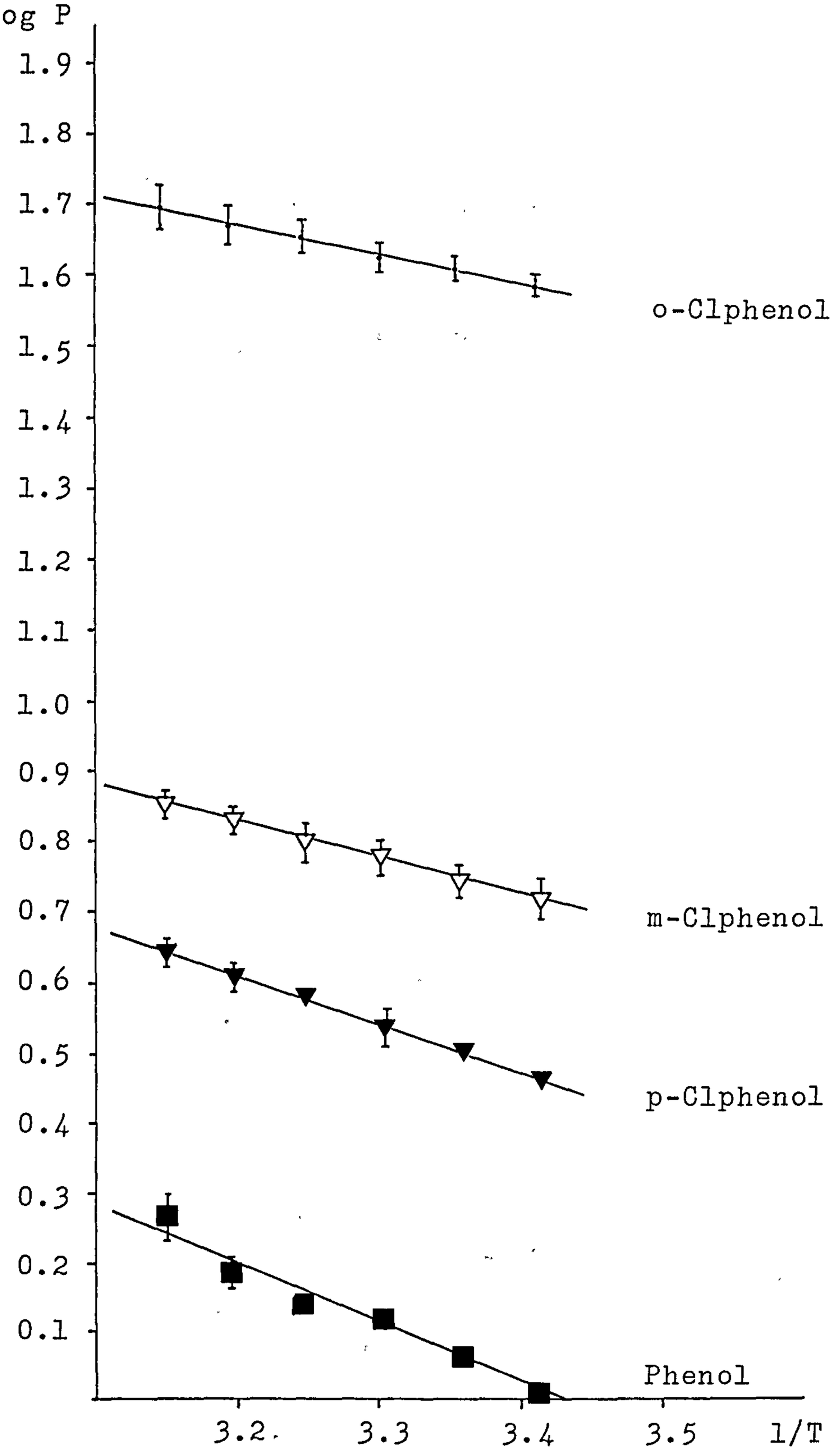


Figure 42. Van't Hoff Plots for Nitrophenols in the Cyclohexane/Water System

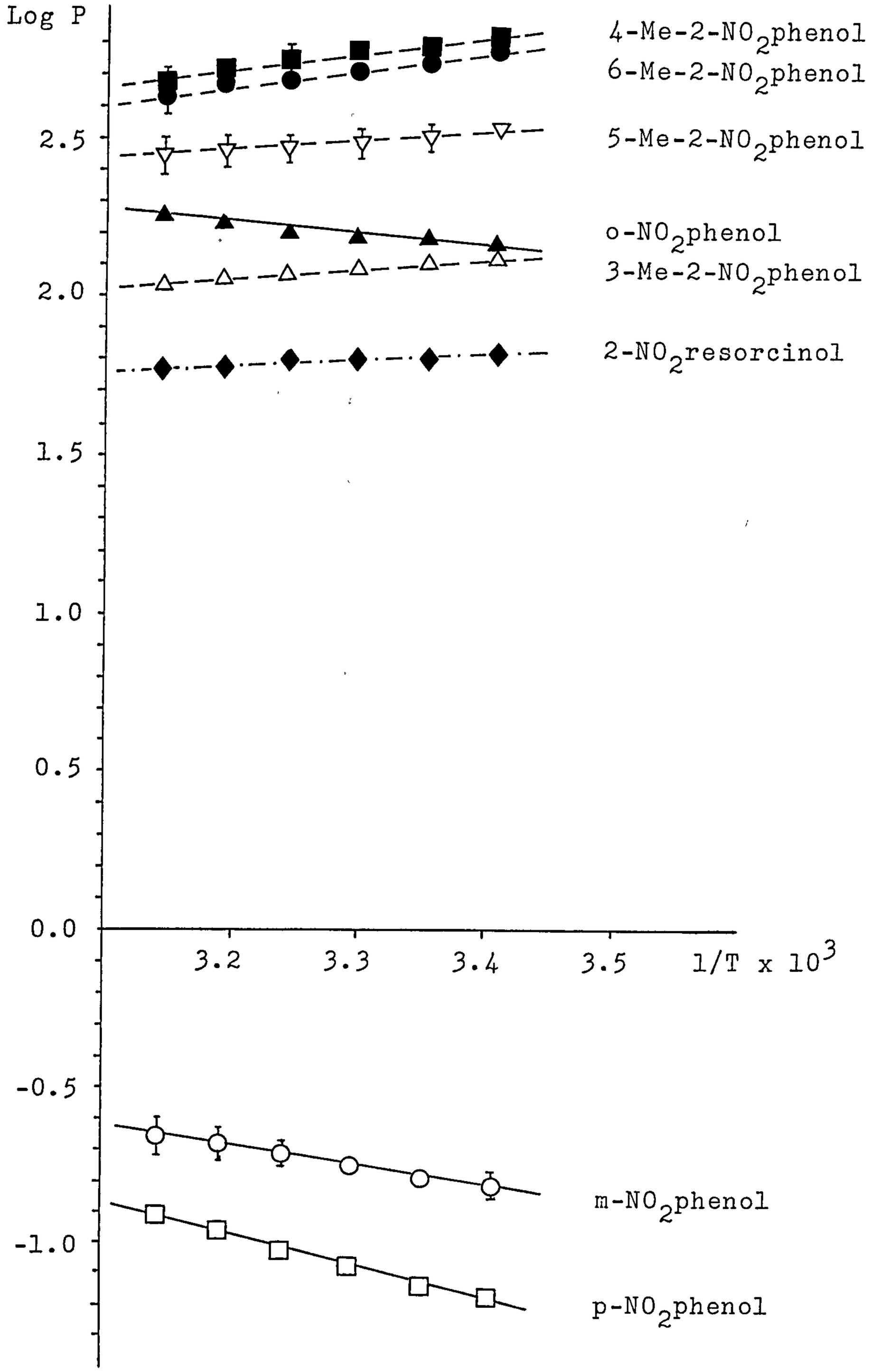


Figure 43. Van't Hoff Plots for Benzoic Acids in the Cyclohexane/Water System

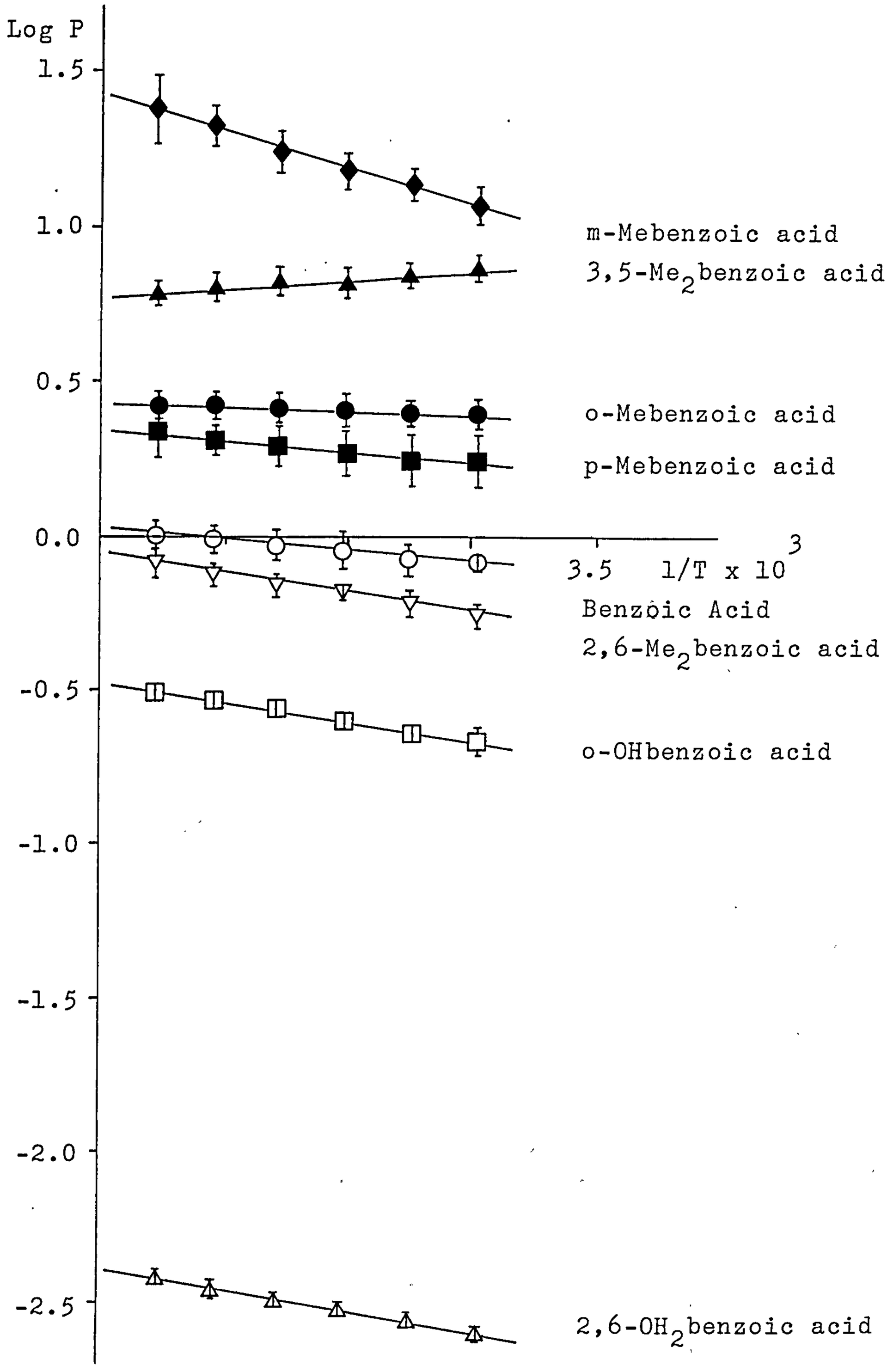


Figure 44. Van't Hoff Plots for Hydroxybenzaldehydes in the Cyclohexane/Water System

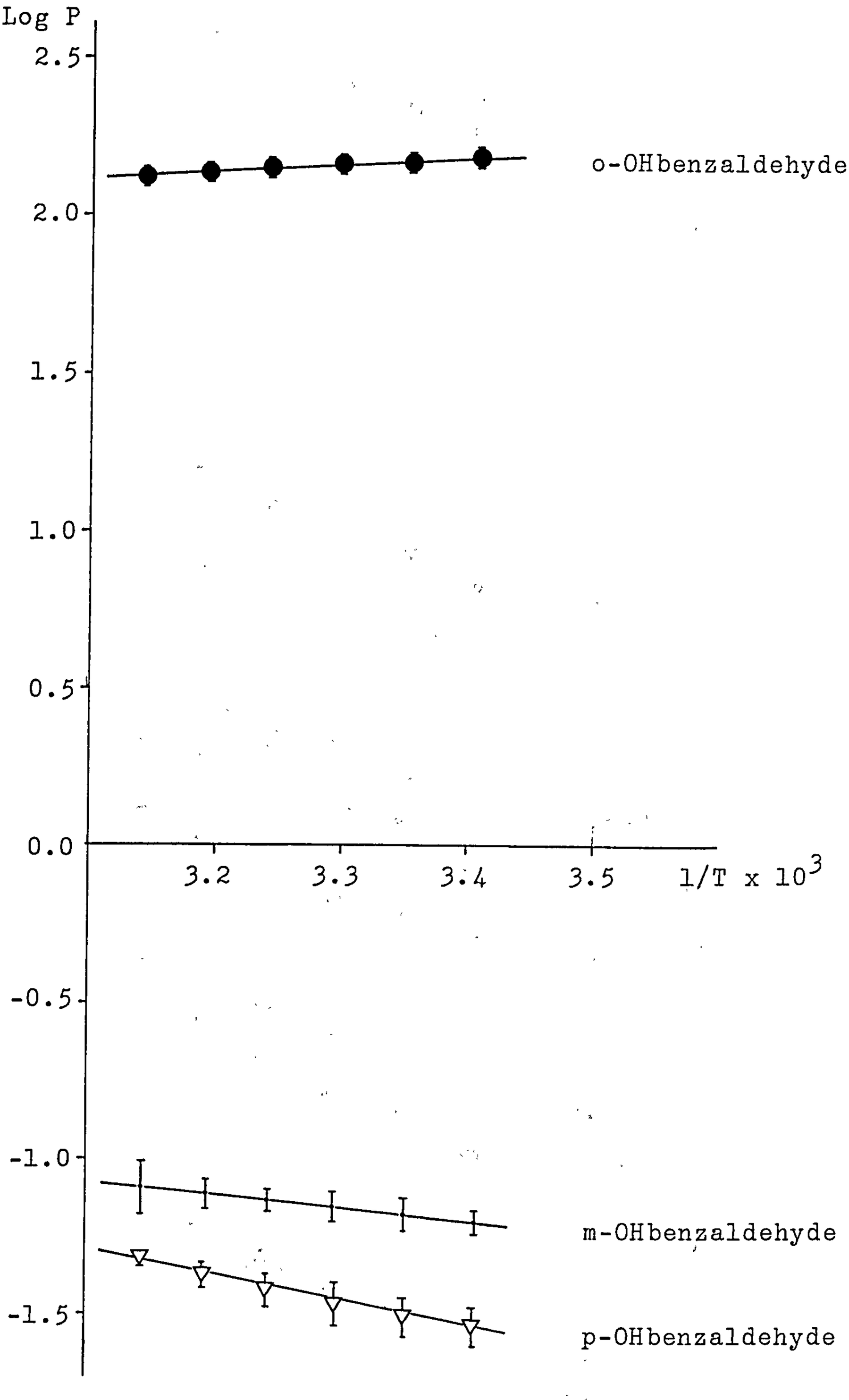


Figure 45. Van't Hoff Plots for Methylphenols in the Cyclohexane/Water System

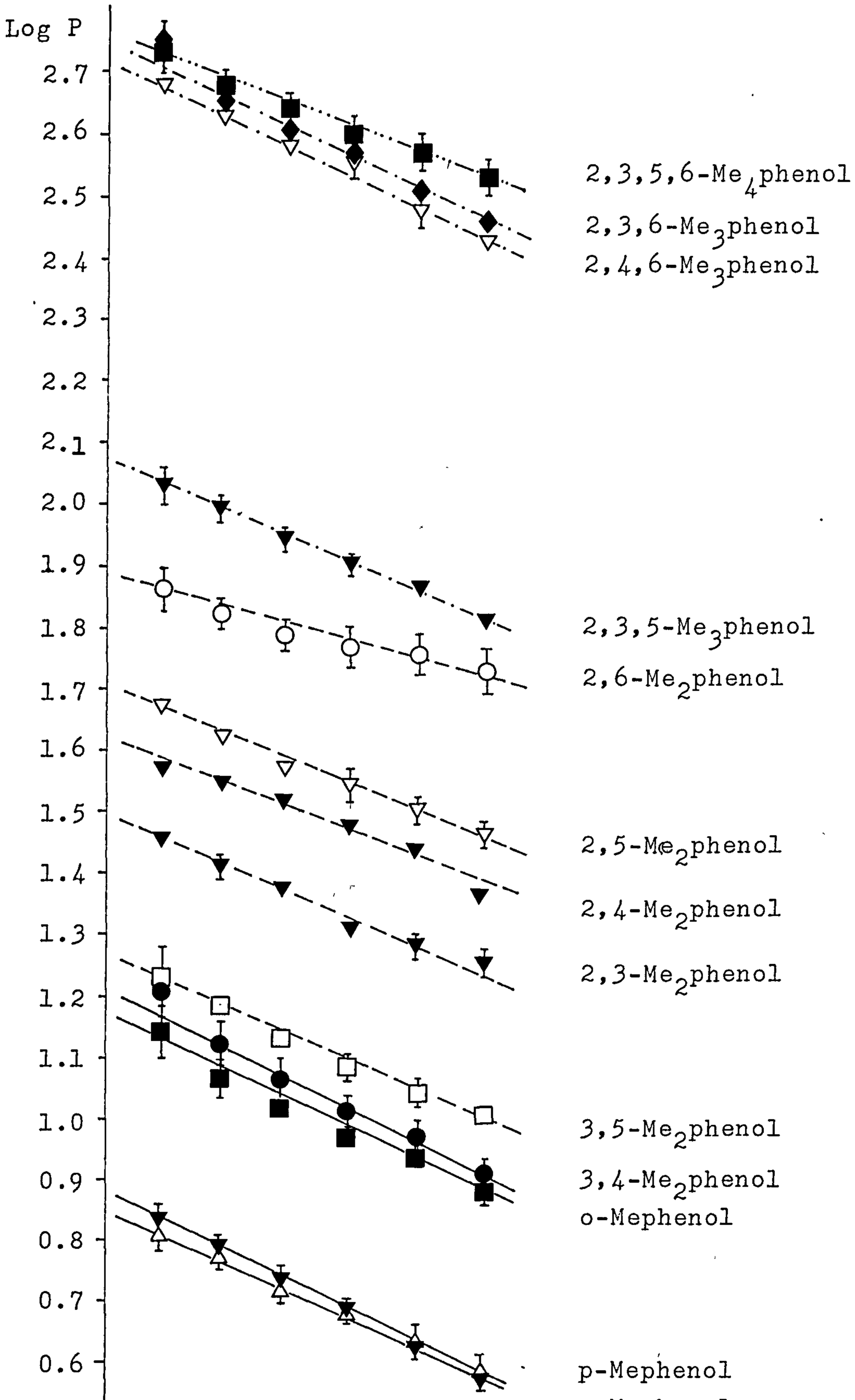
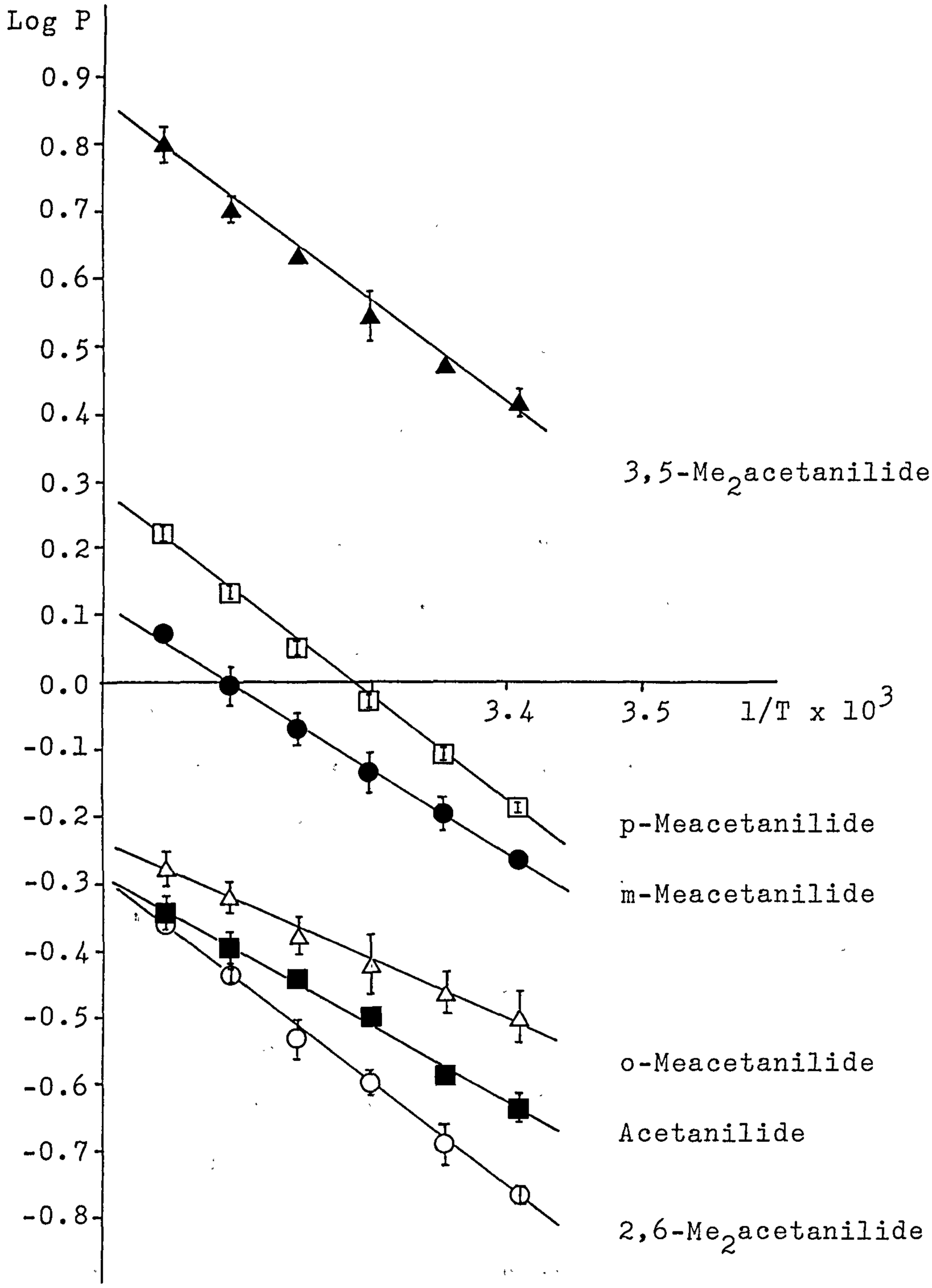


Figure 46. Van't Hoff Plots for Methylacetanilides in the Cyclohexane/Water System



temperature range. The improved linearity of the cyclohexane results as compared to the octanol results indicates that the water/cyclohexane system is less complex than is the water/octanol system.

The full thermodynamic parameters for the transfer of the solutes from aqueous solution to cyclohexane are shown in Table 57.

Both positive and negative free energy values were obtained, indicating that some solutes prefer the aqueous phase (those with positive ΔG) while the others prefer the organic phase (those with negative ΔG).

Enthalpy values are positive for all but six solutes, indicating that for most compounds the transfer process is endothermic in nature and as the temperature is raised the partition coefficient increases. This is a common phenomenon for solvent pairs having low mutual solubility. (226) A positive enthalpy value indicates that there is an overall reduction in the number or strength of molecular interactions. The transfer of a phenolic solute from water to cyclohexane requires the breaking of solute-water bonds (large positive enthalpy change) followed by the reformation of new hydrogen bonds between water molecules.(negative enthalpy change). Insertion of the solute in the lipid phase may require disruption of weak van der Waals interactions between cyclohexane molecules (small positive enthalpy change), followed by the formation of van der Waals and dipole-induced dipole interactions between phenol molecules and cyclohexane molecules (small negative enthalpy

Table 57. Thermodynamic Parameters for the Cyclohexane-Water System

Compound	Enthalpy $\Delta H kJmole^{-1}$	Free Energy $\Delta G kJmole^{-1}$	Entropy $\Delta S Jmole^{-1}deg^{-1}$
Phenol	+15.32	- 0.32	+52.48
o-Clphenol	+ 8.62	- 9.07	+59.37
m-Clphenol	+10.21	- 4.18	+48.30
p-Clphenol	+14.04	- 2 76	+56.38
o-NO_2phenol	+ 5.74	-12.45	+61.04
m-NO_2phenol	+11.49	+ 4.45	+23.62
p-NO_2phenol	+22.98	+ 6.44	+55.52
2-NO_2resorcinol	- 3.26	-10.24	+23.43
o-OHbenzaldehyde	- 3.51	-12.32	+29.58
m-OHbenzaldehyde	+ 7.85	+ 6.71	+ 3.82
p-OHbenzaldehyde	+15.32	+ 8.54	+22.77
Benzoic acid	+ 6.56	+ 0.36	+20.81
o-OHbenzoic acid	+11.49	+ 3.59	+26.49
m-OHbenzoic acid	----	----	----
p-OHbenzoic acid	----	----	----
2,6-OH_2benzoic acid	+12.45	+14.61	- 7.24
3,5-OH_2benzoic acid	----	----	----
3-Me-2-NO_2phenol	- 5.74	-11.95	+20.85
4-Me-2-NO_2phenol	- 9.85	-15.87	+20.21
5-Me-2-NO_2phenol	- 5.74	-14.26	+28.59
6-Me-2-NO_2phenol	- 1.02	-15.57	+48.81
o-Mephenol	+15.57	- 5.31	+70.06
m-Mephenol	+19.15	- 3.56	+76.21
p-Mephenol	+15.32	- 3.57	+63.40
2,3-Me_2phenol	+15.32	- 7.29	+75.88
2,4-Me_2phenol	+12.76	- 8.16	+70.20
2,5-Me_2phenol	+14.30	- 8.56	+76.71
2,6-Me_2phenol	+ 9.19	- 9.99	+64.35
3,4-Me_2phenol	+19.15	- 5.45	+82.55
3,5-Me_2phenol	+16.41	- 5.95	+75.02
2,3,5-Me_3phenol	+15.32	-10.58	+86.91
2,3,6-Me_3phenol	+17.87	-14.28	+107.87
2,4,6-Me_3phenol	+19.69	-14.01	+113.00
2,3,5,6-Me_4phenol	+13.79	-14.61	+95.29
o-Mebenzoic acid	+ 2.74	- 2.24	+16.70
m-Mebenzoic acid	+20.42	- 6.50	+90.35
p-Mebenzoic acid	+ 6.70	- 1.43	+27.29
2,6-Me_2benzoic acid	+14.04	+ 1.28	+42.83
3,5-Me_2benzoic acid	- 9.19	- 4.76	-14.87
Acetanilide	+22.98	+ 3.37	+65.82
o-Meacetanilide	+15.32	+ 2.66	+42.49
m-Meacetanilide	+22.98	+ 1.15	+73.25
p-Meacetanilide	+33.32	+ 0.65	+109.63
2,6-Me_2acetanilide	+30.64	+ 3.91	+89.70
3,5-Me_2acetanilide	+30.64	- 2.69	+111.86

change). The main contribution to the overall positive enthalpy value for the transfer of a phenolic solute from water to cyclohexane is clearly derived from the destruction of solute-water interactions.

All solutes except for two, have a positive entropy of transfer. This is due to the transfer from the ordered water environment, where translational and rotational motion is greatly reduced, to the organic phase, where structuring is absent and the solute is free to move unhindered. Also a loss in the structure of water will occur when the hydrophobic moiety of the phenol is removed. Another possible source of increase in entropy may be that the 'clusters' of water molecules may have greater motional freedom once the structuring influence of the phenolic solute has been removed. The overall process, either entropy or enthalpy dominated, depends on the type of substituent in the molecule. Generally, for those solutes whose substituents increase solute-water interactions (e.g. p-NO_2) either by increasing the strength of hydrogen bonding of the hydroxyl group of the phenol or by participating in hydrogen bonding themselves. the transfer process is enthalpically controlled, whilst for those solutes whose substituents reduce solute-water interactions due to the hydrophobic effect (e.g. p-alkyl) partitioning is entropically controlled.

The acetanilides have positive free energies except for the 3,5-dimethyl isomer. This indicates a preference for the aqueous phase. Transfer however involves a different mechanism from that associated with phenols and will be

discussed in greater detail in the following section.

The thermodynamics of partitioning for individual solutes will now be discussed.

9.9.3 The Thermodynamics of Partitioning

A. Intramolecular Hydrogen Bonding Compounds

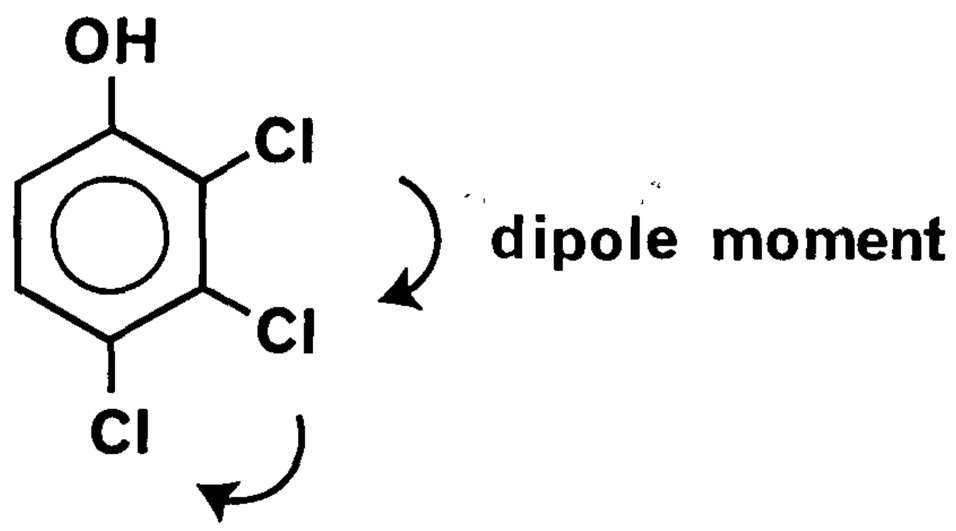
i. Chlorophenols

	Thermodynamic Parameters		
	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
o-Clphenol	- 8.42	-17.03	+28.88
m-Clphenol	-10.85	-18.57	+26.35
p-Clphenol	-10.53	-18.55	+26.91
Cyclohexane			
o-Clphenol	+ 8.62	- 9.07	+59.37
m-Clphenol	+10.21	- 4.18	+48.30
p-Clphenol	+14.04	- 2.76	+56.38

In both the n-octanol/water system and the cyclohexane/water system the chlorophenols have negative free energies i.e.they show a preference for the organic phase. In both systems the free energies are more negative than that of phenol due to an increase in hydrophobicity contributed by the chloro group. In the n-octanol/water system the free energy value of the ortho-isomer is slightly less negative than that of either the meta- or para- isomers but the values are so close (probably within experimental error) that they probably indicate that the intramolecular bond in o-Clphenol is broken in both water and octanol. In the cyclohexane/ water system however the ortho-isomer has a much larger negative free energy than either the meta- or para- isomer. This is indicative of the presence of an intramolecular

hydrogen bond which means that the molecule has either less interaction with the water molecules in the aqueous phase, or more interaction with the cyclohexane molecules. Thus reducing the affinity of the ortho-isomer for the aqueous phase. This also means that the enthalpy of transfer of the ortho-isomer is lower since there are fewer solute-water bonds to break. Although, as indicated in the water/n-octanol system, the intramolecular hydrogen bond may be at least partially broken in water but reformed in cyclohexane, leading to a less positive ΔH. There is a decrease in the enthalpy of transfer of the chlorophenols from that of phenol. This is due either to a restructuring of the water molecules on removal of the halogen group or to van der Waals interactions between the halogen group and the lipid solvent molecules. In this respect the halogen group behaves like an alkyl group and transfer of such a group results in a negative enthalpy change.

The enthalpy of transfer decreases o<m<p, which is similar to the change in dipole moment found in the different position isomers:



This trend suggests a dipole contribution to enthalpy. The same trend is not observed in the n-octanol/water system instead, the meta- and para- isomers have very similar ΔH

values with the value for the ortho-isomer being slightly less negative. This can be partly accounted for by the increased hydrogen bonding of the meta- and para- isomers with n-octanol. The intramolecular hydrogen bond in o-chlorophenol prevents bonding of the chlorine atom with octanol, but bonding of the O- of the hydroxyl group is still possible which means the enthalpy of transfer for o-chlorophenol is very similar to that of phenol. However, as with the free energy values, the ΔH values are so close that they indicate that the intramolecular hydrogen bond is at least partially broken in both water and octanol.

In the n-octanol/water system the entropy values are larger than that of phenol indicating a greater increase in disorder. This is characteristic of the hydrophobic effect and is due to increased water structuring relative to phenol because of increased hydrophobicity, therefore there is a greater loss of water structure on transfer to the organic phase. A slightly increased entropy is observed for the ortho-isomer which occurs because the intramolecular hydrogen bond makes the molecule less polar and so there is less solute/solvent interaction so a lower ΔH, but more structured water around the molecule so a greater ΔS. However, the values are again probably within experimental error and thus give further support to the possibility that the intramolecular hydrogen bond is broken. The entropy values in the cyclohexane/water system show no definite pattern although the ortho-isomer has a slightly higher ΔS which is indicative of an intramolecular hydrogen bond.

ii. Nitrophenols

	Thermodynamic Parameters		
	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
Phenol	- 8.42	-13.69	+17.68
o-NO_2phenol	- 2.46	-15.35	+43.25
m-NO_2phenol	-15.32	-16.22	+ 3.00
p-NO_2phenol	-19.15	-16.09	-10.27
Cyclohexane			
Phenol	+15.32	- 0.32	+52.48
o-NO_2phenol	+ 5.74	-12.45	+61.04
m-NO_2phenol	+11.49	+ 4.45	+23.62
p-NO_2phenol	+22.98	+ 6.44	+55.52

Substitution of phenol with a group such as the nitro group has a considerable effect on the partitioning characteristics. In the ortho-position the nitro group can form a strong intramolecular hydrogen bond and in other positions it can form intermolecular hydrogen bonds.

In the n-octanol/water system these compounds have a negative free energy, the value of which is more negative than that for phenol because of the increase in hydrophobicity, produced by the nitro group. However, in the cyclohexane/water system the ortho-isomer has a negative ΔG while the meta- and para- isomers have positive ΔG's.

The nitro group is elecron withdrawing and in both the ortho- and para- positions can enter into resonance with the hydroxyl group. Therefore, in the cyclohexane/water system a positive free energy is obtained for the para-isomer due to increased molecular interactions with water and thus a greater tendency to remain in the aqueous phase because water

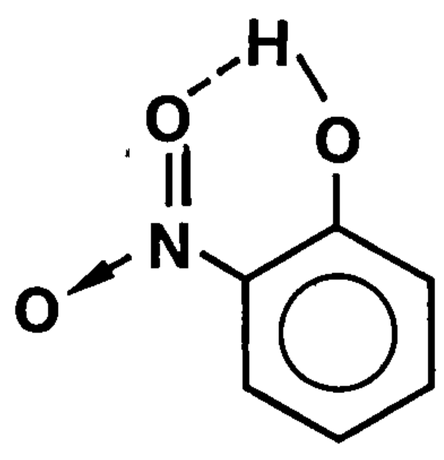
is a good proton donor. The high enthalpy value of p-NO_2phenol indicates the large amount of energy required to break the strong molecular interactions between the solute and water molecules. Similarly a high entropy of transfer is seen with p-nitrophenol. This molecule is strongly bound in the aqueous phase through increased hydrogen bonding of the hydroxyl group and the nitro group with water molecules and when it is transferred it undergoes a greater degree of motional freedom in the organic phase.

In contrast to this, m-nitrophenol, which does not have increased hydrogen bonding through the hydroxyl group because it cannot enter into resonance with the nitro group, has a much lower entropy and enthalpy of transfer. This indicates that less energy is required to break the bonds holding the molecule in the aqueous phase. However, ΔG is still positive, showing a preference for the aqueous phase. o-Nitrophenol on the other hand has a negative free energy of transfer in the cyclohexane/water system, showing a higher partition coefficient and thus a preference for the organic phase. The presence of an intramolecular hydrogen bond makes the molecule less polar, there is less solute-solvent interaction in the aqueous phase since intermolecular hydrogen bonding is prohibited and there are therefore fewer bonds to break when the molecule is transferred from the aqueous to the organic phase. Therefore the enthalpy of transfer is reduced. However, since the molecule is less polar, there is more structured water to be removed on transfer and so entropy is increased as the molecule moves into the organic phase.

As with the chlorophenols and hydroxybenzaldehydes, the trend of ΔH values between the isomers suggests a relationship with dipole moment although this is not necessarily a causal relationship, the basic factor remains the intramolecular hydrogen bond.

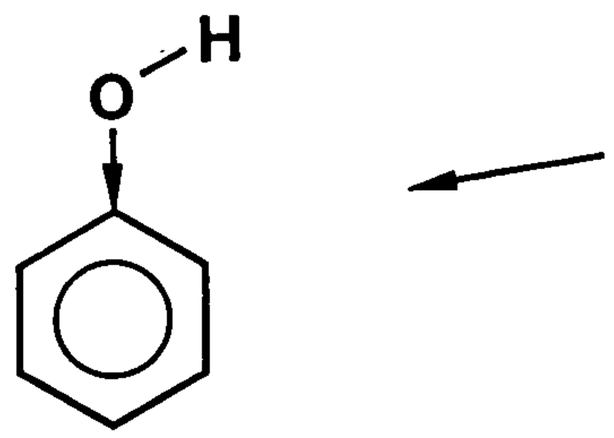
In the n-octanol/water system the enthalpy of transfer for p-nitrophenol is $-19.2 kJmole^{-1}$ compared with $-2.5 kJmole^{-1}$ for o-nitrophenol. This probably indicates that the para-isomer can bond to n-octanol far better than does the ortho-isomer. However, if this effect is due to hydrogen bonding then the ability of the ortho-isomer to bond with water will also be reduced as will the effective polarity. This will mean the ortho-isomer will contain a large hydrophobic portion which will aid interaction with octanol and should lead to an increased partition coefficient which is not found in practice.

In the ortho-isomer the OH group i.e. the H donor is tied up:



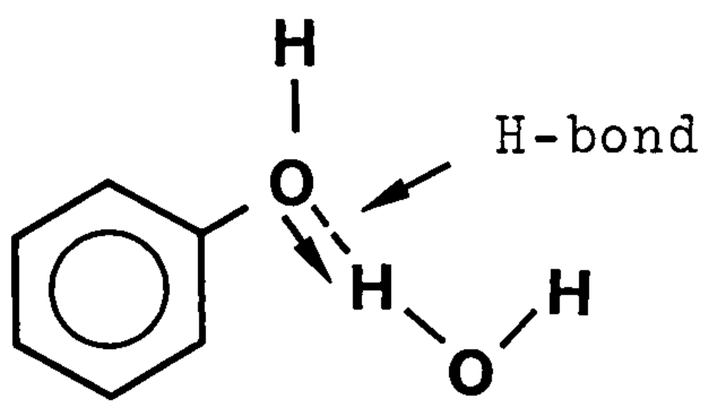
Water is a better H donor than acceptor, whereas alcohols i.e. octanol, are better H acceptors. This is demonstrated by, for example, the ultraviolet spectrum of phenol which shifts to shorter wavelength when the solvent is changed

from cyclohexane to water and to longer wavelength when the solvent is changed from cyclohexane to alcohol. (see Ch 7) Thus:

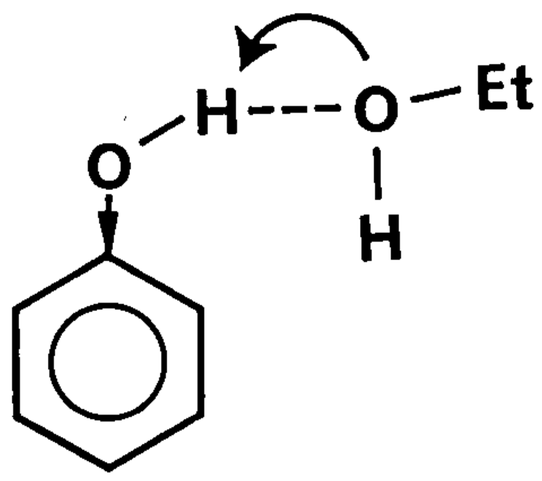


increase in donation gives Bathochromic shift

In water:



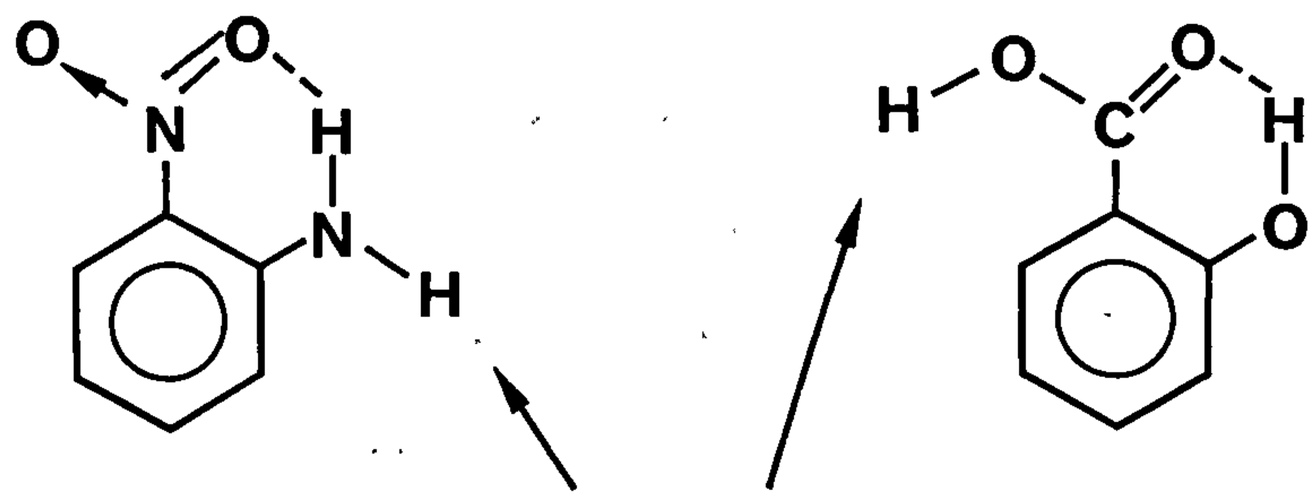
In alcohol:



Therefore, if the H donor in the phenol is tied up in an intramolecular hydrogen bond it should reduce the interaction with octanol more than with water. Therefore ΔH is less negative when an intramolecular hydrogen bond is formed.

However, a large positive entropy is obtained for ortho-nitrophenol which could mean much greater water-structuring, which is lost on transfer. But loss of water structure must mean hydrogen bonds breaking and therefore a positive ΔH. This could account for the low overall negative enthalpy for o-nitrophenol.

If the hydrogen bonding ability of the compound to octanol is reduced, then a reduced partition coefficient is expected and this is what was found, despite the fact that an intramolecular hydrogen bond is normally expected to increase the partition coefficient. In fact an increased partition coefficient is therefore only likely if there is spare H donor capacity as in o-nitroaniline or o-OHbenzoic acid:



spare H donor capacity

Therefore, if the H donor capacity is tied up, as in o-nitrophenol or o-chlorophenol, a lower partition coefficient may be predicted and there is less interaction with octanol relative to water hence a less negative ΔH.

p-Nitrophenol shows a negative entropy of transfer in the

n-octanol/water system, that is, an increase in solvation, which is also apparent in p-hydroxybenzoic acid but not p-chlorophenol or p-hydroxybenzaldehyde. This indicates an increase in order on transfer of these molecules. The mobility of these compounds in the lipid phase will be greatly reduced by strong interactions with n-octanol, producing an overall decrease in entropy.

iii. 2-Nitroresorcinol

	Thermodynamic Parameters		
	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
o-NO_2phenol	- 2.46	-15.35	+43.25
2-NO_2resorcinol	- 7.31	-19.79	+41.89
Cyclohexane			
o-NO_2phenol	+ 5.74	-12.45	+61.04
2-NO_2resorcinol	- 3.26	-10.24	+23.43

2-nitroresorcinol has a hydroxyl group on either side of the nitro group and theoretically possesses two intra-molecular hydrogen bonds. This should mean that the enthalpy of transfer is less negative than that of o-nitrophenol and the entropy more positive. However this is not the case in either of the two solvent systems investigated. The evidence in the octanol/water system suggests that only one hydrogen bond is present and this theory is supported by the ultraviolet data. The cyclohexane/water data show a negative free energy of transfer, indicating quite a pronounced preference for the organic phase. The enthalpy of transfer is also negative which suggests that solute-solvent interactions in the aqueous phase are small and

therefore there are few solute-water bonds to break. However, this suggests that the intramolecular hydrogen bond exists. Therefore another reason for ΔH being negative is that the second hydrogen bond could form in cyclohexane, whilst not existing in water. · The ΔS value is also relatively small, being less than that of phenol or o-nitrophenol which indicates there is less structured water around the 2-nitroresorcinol molecule than the o-nitrophenol molecule. The presence of two intramolecular hydrogen bonds would be expected to produce an increase in structured water and hence ΔS so again the thermodynamic results question the existence of two intramolecular hydrogen bonds in 2-nitroresorcinol.

iv. Hydroxybenzaldehydes

	Thermodynamic Parameters		
	ΔHkJmole^{-1}	ΔGkJmole^{-1}	ΔSJmole^{-1}deg^{-1}
Octanol			
Phenol	-8.42	-13.69	+17.68
o-OHbenzaldehyde	--3.66	-14.72	+37.10
m-OHbenzaldehyde	-8.94	-13.23	+14.40
p-OHbenzaldehyde	-9.57	-13.28	+12.44
Cyclohexane			
Phenol	+15.32	- 0.32	+52.48
o-OHbenzaldehyde	- 3.51	-12.32	+29.58
m-OHbenzaldehyde	+ 7.85	+ 6.71	+ 3.82
p-OHbenzaldehyde	+15.32	+ 8.54	+22.77

These compounds again have two groups which can form intra- and inter- molecular hydrogen bonds. In both solvent systems the ortho isomer has a more negative ΔG than the other isomers showing that the intramolecular hydrogen bond causes an increase in partition coefficient. This is due

to the increased hydrophobicity of the molecule created by the hydrogen bond. The molecule can no longer bond to water molecules and so has a greater affinity for the organic phase. In the cyclohexane/watersystem the meta- and para- isomers have positive ΔG values which indicate a preference for the aqueous phase. This occurs because of the ability to form intermolecular hydrogen bonds with the water molecules. Such bonds are not possible in cyclohexane and so the molecules show an aversion for this phase. Intermolecular hydrogen bonds are possible in n-octanol and so the partition coefficient is dependent on the inherent hydrophobicity of the molecule. Hence negative ΔG values are obtained.

In the cyclohexane/water system the enthalpy of transfer is less positive for the ortho-isomer than for the meta- or para- isomers, and in fact, becomes negative. This indicates that few if any solute-water bonds are broken for transfer, and possibly the main contribution to the enthalpy change comes from the formation of weak van der Waals forces between the compound and cyclohexane which produces a small negative enthalpy change, and the reformation of hydrogen bonds between the water molecules as the solute moves into the organic phase which also produces a negative enthalpy change. The relationship in enthalpy and entropy values for the isomers in this system is the same as for the nitrophenols and may be related to the polarity of the isomer. The presence of the aldehyde group in the para-position causes the hydroxyl group to become more polar and thus form stronger hydrogen bonds in the aqueous phase.

This means the molecule is held quite rigidly in the aqueous phase and so a substantial increase in entropy is experienced when the molecule is transferred to the organic phase. Also, a large amount of energy is required to break the bonds so the enthalpy of transfer is high.

The ortho isomer contains an intramolecular hydrogen bond and is therefore less polar so there are fewer solute-solvent interactions to disrupt so ΔH is lower, but because the molecule is less polar it is associated with more structured water in the aqueous phase. This gives it rigidity in the aqueous phase and so the combination of stripping of the structured water and resultant increase in mobility produces a large entropy on transfer into the organic phase.

The meta-isomer experiences neither of these phenomena.. It is less polar than the para-isomer because from the meta-position the aldehyde group cannot enter into resonance with the hydroxyl group, so ΔH is lower, and has neither the strong water- solute bonds nor structured water so ΔS is also lower than that of either the ortho- or the para-isomer.

In the n-octanol/water system, as with the other intra-molecularly bonded isomers, except salicylic acid, o-hydroxy-benzaldehyde has a large positive entropy value which can again be attributed to structuring of water molecules around the bonded groups which decreases on transfer from water to octanol where there will be less solvation. The meta- and para- isomers also have positive entropy values

but they are lower than that of phenol, reflecting the presence of the additional substituent which can interact with n-octanol to produce increased order. However, the increase in ordering is not of the magnitude observed with p-NO_2phenol or p-OHbenzoic acid. This is possibly due to the fact that NO_2 is a better proton-acceptor than CHO, and COOH is better still at interacting with octanol because it also contains a proton donating group.

v. Hydroxybenzoic Acids

	Thermodynamic Parameters		
	ΔHkJmole^{-1}	ΔGkJmole^{-1}	ΔSJmole^{-1}deg^{-1}
Octanol			
Phenol	- 8.42	-13.69	+17.68
Benzoic acid	- 8.42	-15.61	+24.13
o-OHbenzoic acid	-17.85	-18.04	+ 0.64
m-OHbenzoic acid	-19.15	-14.13	-16.85
p-OHbenzoic acid	-22.98	-13.92	-30.40
2,6-OH_2benzoic acid	-17.87	-14.79	-10.34
3,5-OH_2benzoic acid	-22.98	-11.32	-39.13
Cyclohexane			
Phenol	+15.32	- 0.32	+52.48
Benzoic acid	+ 6.56	+ 0.36	+20.81
o-OHbenzoic acid	+11.49	+ 3.59	+26.49
m-OHbenzoic acid	----	----	----
p-OHbenzoic acid	----	----	----
2,6-OH_2benzoic acid	+12.45	+14.61	- 7.24
3,5-OH_2benzoic acid	----	----	----

These compounds contain two groups which are both capable of acting as proton-donor and proton-acceptor. In addition, two dihydroxy benzoic acids were also investigated. The effects of both the carboxyl and hydroxyl groups could be determined since both phenol and benzoic acid could be considered as the parent molecule. These compounds are so hydrophilic that solubility problems were experienced in

cyclohexane for all but the ortho-isomers. This indicates the strength of intermolecular hydrogen bonding found between the water molecules and both the hydroxyl and carboxylic acid substituents. It is only when polarity is reduced by intramolecular hydrogen bonds that solubility in cyclohexane becomes appreciable.

In the cyclohexane/water system, both o-hydroxybenzoic acid and 2,6-dihydroxy benzoic acid have a positive ΔG, showing a preference for the aqueous phase. The enthalpy of transfer is also positive and slightly less than that of phenol, but greater than that of benzoic acid. Unlike 2-nitroresorcinol the addition of a second hydroxyl group ortho to the carboxyl group produces a slight increase in enthalpy, although the change is small and may be attributed to experimental error. However the effect of the second hydroxyl group on ΔG is the same for both dihydroxy compounds. ΔG for 2-nitroresorcinol is increased with respect to o-nitrophenol by becoming less negative and ΔG for 2,6-OH_2benzoic acid is increased with respect to o-OHbenzoic acid by becoming more positive. Therefore the second hydroxyl group increases the affinity of the compound for water. For 2-NO_2resorcinol it was suggested that the data supported the existence of one intramolecular hydrogen bond only and this suggestion is strengthened by the data for 2,6-dihydroxy benzoic acid. If the second hydroxyl group is not tied up in an intra-molecular hydrogen bond it will be free to interact with water molecules and thus increase water solubility. This theory is supported by the spectral data discussed in Ch If the theory is correct then an increase in ΔH is expected

because solute-water interactions are increased, but a decrease in entropy because the amount of structured water around the molecule is reduced. This is exactly what is observed, although ΔH is not as large as might be expected which can again be compared to 2-NO_2resorcinol where it was suggested that the negative (or in the case of 2,6-OH_2benzoic acid; reduced positive) ΔH was due to the second hydrogen bond forming in cyclohexane whilst not existing in water.

A positive entropy is seen for the transfer of o-OHbenzoic acid from water to cyclohexane which reflects the breaking of water-solute bonds and the disruption of structured water on transfer. However, a negative entropy is seen for 2,6-OH_2benzoic acid. The reduction in entropy has already been explained as being due to the formation of the second intramolecular hydrogen bond in cyclohexane, but an additional factor may account for the magnitude of the reduction in ΔS. This compound has the lowest log P of all the compounds studied. Therefore it is very polar and could be a structure breaker in water. This would mean an increase in water structure when it is transferred to cyclohexane and therefore a negative ΔS.

In the n-octanol/water system, the addition of a carboxyl group to the phenol molecule produces a reduction in ΔG. This indicates an increase in log P which does not agree with previous findings since the πvalue of COOH is -0.28 which means the carboxyl group reduces lipophilicity. A reduction in log P is only seen in the 3,5-dihydroxy isomer. This is expected since πOH = -0.67.

The expected reduction in log P due to the substitution of a hydroxyl group in benzoic acid is seen in all but the ortho isomer. This compound displays an increase in log P, i.e. a more negative ΔG, with respect to both phenol and benzoic acid and this is due to the formation of an intramolecular hydrogen bond between the carboxyl group and the hydroxyl group. In the hydrogen bond the H donor as represented by the OH group is tied up and this was stated as reducing the interaction with octanol more than with water in the case of o-nitrophenol. Therefore a large reduction in the negative ΔH value was observed for o-NO_2phenol. Although ΔH is also less -ve for o-OHbenzoic acid than for either the meta- or the para- isomer, the difference is not so great. This is because although one H donor is involved in the intramolecular hydrogen bond, there is of course, a second OH group and it is likely that it is this second proton donor group which confers a greater affinity for octanol and thus a more negative ΔG. Since there is also likely to be more interaction with octanol relative to water ΔH will be more negative than expected. The entropy of transfer of the ortho-isomer is slightly positive which indicates the loss of structured water on transfer, although since $+\Delta S$ is small there does not appear to be much structured water associated with this molecule

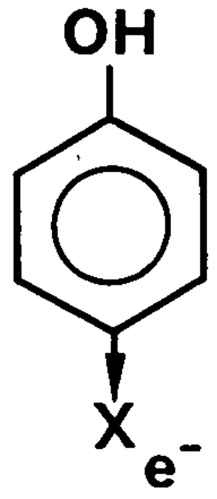
The addition of a second ortho-hydroxyl group produces a less negative free energy value, that is, the partition coefficient is decreased. This is the opposite effect to that observed on substituting a second hydroxyl group ortho to the nitro group. The second hydroxyl group is expected

to form a second hydrogen bond (although the cyclohexane/water data questions this) but if this were so then an increase in log P would be expected i.e. a more negative ΔG. Therefore, as in the cyclohexane/water system it seems unlikely that the second hydrogen bond exists. This is further supported by the entropy of transfer. ΔS is negative and smaller (more negative) for 2,6-OH_2benzoic acid than for o-OHbenzoic acid. This indicates less structured water is removed when the molecule is transferred from the aqueous phase to the octanol phase which is unlikely if two intramolecular hydrogen bonds are present. The enthalpy value for the 2,6-dihydroxy isomer is also negative with a magnitude similar to that of the meta- and para- isomers. This indicates a fair degree of interaction with water which again is unlikely if two intramolecular hydrogen bonds are present. The enthalpy figures reflect the fact that in this type of compound ΔH is less negative when an intramolecular hydrogen bond is formed since the interaction of the molecule with water is reduced more than its interaction with octanol.

Of the hydroxybenzoic acids, the ortho-isomer is the only one which has a positive entropy of transfer. The high negative entropies indicate an increase in order due to restricted mobility in the n-octanol phase brought about by hydrogen bonding with the octanol molecules which is of greater magnitude since two OH groups are available. The almost zero entropy of o-OHbenzoic acid shows that there is less octanol structuring than with the meta- or para- isomers relative to water structuring, because of the loss

of an available OH group to bond to.

If the compounds containing one hydroxyl group are considered i.e.Chlorophenols, hydroxybenzaldehydes and nitrophenols:



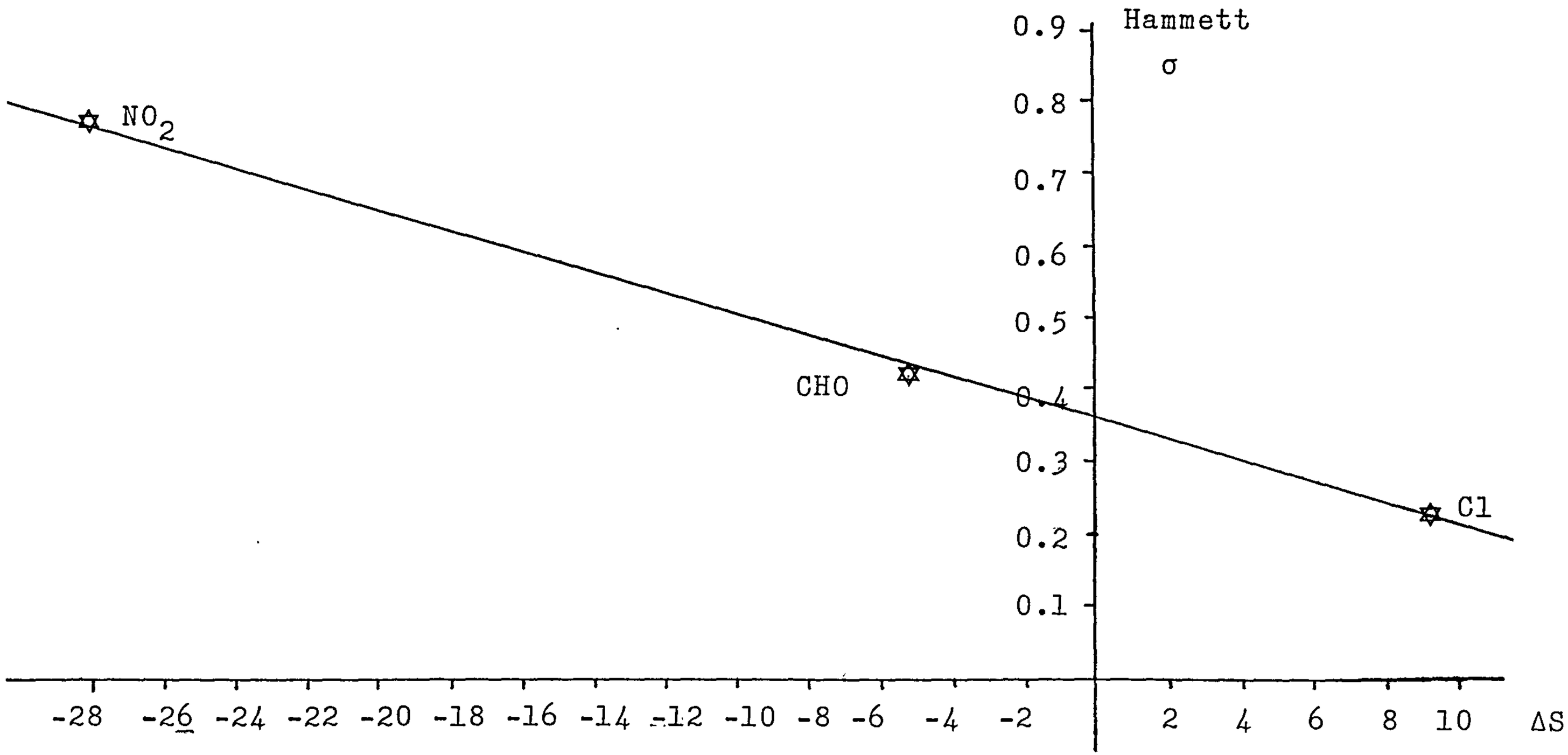
If X is electron withdrawing and pulls electrons out of the ring, the OH group becomes a better proton donor and there is a more negative entropy change. $—O—H^{\delta+}$. Therefore there is a better hydrogen bond with octanol than water so there is more solvation in octanol and increased ordering so ΔS is more negative.

It was considered that there might be a relationship between the degree of solvation of the para-substituent and its Hammett constant (i.e. the overall electronic character of the group).

Group	Hammett σ	$\Delta S_{p\text{-subst}}$(oct)	$\Delta S_{p\text{-subst}}$(cyc)
Cl	+0.23	+ 9.2	+ 3.9
CHO	+0.42	- 5.3	-29.7
COOH	+0.45	-48.1	---
NO_2	+0.78	-28.0	+ 3.0
CH_3	-0.17	+ 9.0	+11.0

Figure 47 shows the relationship which is obtained and it can be seen that a relationship between σ and ΔS exists for those substituents which are not H-bond donors. The 4-COOH group does not fit this relationship because it has H-bond

Figure 47. The Relationship Between the Hammett Constant and the Entropy of Transfer in the Octanol/Water System



donor capacity.

All the compounds examined show that the entropy of transfer is increased if an intramolecular hydrogen bond is present. This indicates that there is more disorder on going from water to octanol when an intramolecular hydrogen bond is present - that is, when the OH group is tied up. This reduces the ability of the molecule to interact with octanol but not with water to the same extent, since water molecules can still hydrogen bond with the —O atom of the OH group being a better proton donor than is octanol, and also smaller so that it is not so restricted of access by steric effects.

B. Sterically Influenced Compounds

i. Methylphenols

Thermodynamic Parameters

	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
Phenol	- 8.42	-13.69	+17.68
o-Mephenol	- 7.66	-15.94	+28.26
m-Mephenol	- 8.14	-16.18	+27.45
p-Mephenol	- 8.42	-16.07	+26.68
2,3-Me_2phenol	- 9.09	-18.31	+30.94
2,4-Me_2phenol	- 9.57	-18.77	+31.39
2,5-Me_2phenol	- 9.85	-18.52	+29.10
2,6-Me_2phenol	- 3.64	-18.13	+48.63
3,4-Me_2phenol	-10.05	-18.01	+27.17
3,5-Me_2phenol	-10.53	-18.56	+26.93
2,3,5-Me_3phenol	- 6.70	-20.64	+47.57
2,3,6-Me_3phenol	- 5.74	-19.77	+47.87
2,4,6-Me_3phenol	- 3.80	-20.12	+55.70
2,3,5,6-Me_4phenol	- 6.02	-21.13	+50.72

	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Cyclohexane			
Phenol	+15.32	- 0.32	+52.48
o-Mephenol	+15.57	- 5.31	+70.06
m-Mephenol	+19.15	- 3.56	+76.21
p-Mephenol	+15.32	- 3.57	+63.40
2,3-Me_2phenol	+15.32	- 7.29	+75.88
2,4-Me_2phenol	+12.76	- 8.16	+70.20
2,5-Me_2phenol	+14.30	- 8.56	+76.71
2,6-Me_2phenol	+ 9.19	- 9.99	+64.35
3,4-Me_2phenol	+19.15	- 5.45	+82.55
3,5-Me_2phenol	+16.41	- 5.95	+75.02
2,3,5-Me_3phenol	+15.32	-10.58	+86.91
2,3,6-Me_3phenol	+17.87	-14.28	+107.87
2,4,6-Me_3phenol	+19.69	-14.01	+113.00
2,3,5,6-Me_4phenol	+13.79	-14.61	+95.29

Any effect observed in these molecules is due to the steric effect of the methyl group. Intramolecular hydrogen bonding cannot occur but the presence of the methyl group may affect intermolecular hydrogen bonds.

In both systems, an increase in the negative free energy is observed as the hydrophobicity of the molecule is increased by the addition of substituent methyl groups. Ortho-methyl substitution causes a reduction in the free energy of transfer (ΔG becomes less negative) in the n-octanol/water system because of steric shielding of the OH group which restricts access of octanol molecules but not water molecules. This is supported by the lower than expected values of ΔG for the other ortho-substituted methylphenols, particularly the diortho-substituted isomers. The effect is not great since the methyl group is relatively small itself. A more pronounced effect would be expected with bulkier alkyl substituents (399)

This shielding of the hydroxyl group has the opposite effect in the cyclohexane/water system and causes an increase in

the free energy of transfer (ΔG becomes more negative). This is because the close proximity of the methyl group to the hydroxyl group probably reduces its hydrogen bonding capability and therefore solute-water interactions so that affinity for cyclohexane relative to water is increased. This effect is more pronounced than the effect in the water/n-octanol system and again is emphasised by diortho-substitution. It appears that in 2,3,5,6-Me_4phenol the steric effect of the two orthomethyl groups dominates the hydrophobicity of the molecule since 2,3,5,6-Me_4phenol is only slightly more hydrophobic than 2,3,6-Me_3phenol. This could be an example of Hansch's entropy effect operating (255). Adjacent methyl groups have less structured water around them therefore there is less entropic driving effect and thus a lower than expected log P and also a lower positive ΔS which is also observed.

The m-isomer has a similar ΔG value to the p-isomer, indicating that this position has little effect on the hydroxyl group.

The increased negative free energy of o-methylphenol has been attributed to shielding of the hydroxyl group and this explanation may also be used for the change in free energy of 2,6-Me_2phenol. However, unpublished work in 1976 by Dearden and Langton (114) showed that the solubility of 2,6-Me_2phenol in water was slightly higher than that of 3,5-Me_2phenol, which would not be anticipated if the 2,6-Me_2 isomer had a reduced affinity for water because of shielding of the hydroxyl group. Therefore, the higher ΔG (and hence partition coefficient) of 2,6-Me_2phenol must be due to an

increased affinity for cyclohexane and this is reflected in the much higher solubility of 2,6-Me_2phenol in cyclohexane. ($Sol_{3,5\text{-}Me_2ph}$ = 57.0gl^{-1} @ 25°C cyclohexane; 5.10gl^{-1} @ 25°C water: $Sol_{2,6\text{-}Me_2ph}$ = 850gl^{-1} @ 25°C cyclohexane; 6.6gl^{-1} @ 25°C water)

This shows that the polarity of a solute, as measured by dipole moment, is not always an adequate measure of affinity, or lack of affinity, for a solvent. Rather, the extent to which the polar group is exposed to the solvent must be the governing factor. It might be argued on this basis that the aqueous solubility of 2,6-Me_2phenol should be less than that of 3,5-Me_2phenol. However, the water molecule is small enough not to be restricted by adjacent methyl groups in its approach to the hydroxyl group. Larger polar solvent molecules such as n-octanol, will however be restricted, hence the slightly lower octanol/water partition coefficient of 2,6-Me_2phenol compared to 3,5-Me_2phenol. Therefore, the diortho- methyl groups are probably shielding the polarity of the hydroxyl group so that it is less polar to cyclohexane. This increased interaction with cyclohexane is probably also responsible for the lower positive ΔH for the ortho-isomers. The methylphenols all have positive enthalpies of transfer in the cyclohexane/water system which result mainly from the disruption of solute-water bonds. The transfer of the methylphenol from water to cyclohexane results in a decrease in the structure of water and a greater mobility of the molecule in the structureless organic phase. Thus the entropy of transfer is fairly large. Again, the entropy of transfer for ortho-methyl isomers is

slightly less than for the other isomers since the presence of the methyl group reduces the extent of structured water around the hydroxyl group.

In the n-octanol/water system a negative increase in the enthalpy of transfer may be expected as the number of methyl substituents increases. This would be explained by the restructuring of water molecules and increased hydrogen bonding resulting from the removal of an alkyl group from water. However, this is not observed for the orthomethyl-substituted isomers, instead, ΔH is less negative. This however can also be explained by the fact that ortho-substitution reduces octanol interaction more than water interaction.

The increase in entropy for the removal of alkylphenols from water to a lipid solvent is characteristic of the hydrophobic effect, and is due to an increase in mobility of the phenol in the less ordered n-octanol phase and a loss of water structure. The magnitude of the entropy increase is dependent on the size of the alkyl group (399). A second factor also influences the value of ΔS and that is shielding of the hydroxyl group. This causes an increase in ΔS. It is due to the fact that there is less interaction between solute and solvent in octanol than in water, and therefore there is less ordering in octanol. The unexpectedly low ΔS for 2,3,5,6-Me_4phenol has been previously explained as probably due to Hansch's entropy effect.

ii. Methylbenzoic Acids

	Thermodynamic Parameters		
	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
Benzoic acid	- 8.42	-15.61	+24.13
o-Mebenzoic acid	-12.45	-18.09	+18.92
m-Mebenzoic acid	-13.40	-19.06	+18.99
p-Mebenzoic acid	-12.13	-18.68	+21.98
2,6-Me_2benzoic acid	- 5.74	-16.86	+37.32
3,5-Me_2benzoic acid	-11.49	-21.61	+33.97
Cyclohexane			
Benzoic acid	+ 6.56	+ 0.36	+20.81
o-Mebenzoic acid	+ 2.74	- 2.24	+16.70
m-Mebenzoic acid	+20.42	- 6.50	+90.35
p-Mebenzoic acid	+ 6.70	- 1.43	+27.29
2,6-Me_2benzoic acid	+14.04	+ 1.28	+42.83
3,5-Me_2benzoic acid	- 9.19	- 4.76	-14.87

The effect to be observed here is the steric effect of the methyl group on the carboxylic acid group and its interaction with the solvents.

The expected negative increase in the free energy due to the hydrophobic effect of the methyl group is observed in both solvent systems for all isomers except 2,6-Me_2benzoic acid in the cyclohexane/water system. In the octanol/water system o-methyl substitution causes ΔG to be less negative and the effect is intensified by diortho-substitution. This reduction in free energy may be partly due to steric hindrance restricting the access of octanol molecules but not water molecules to the acid group, but is probably largely due to steric twisting of the carboxylic acid group. This deconjugates the COOH group and makes it less lipophilic (c.f. πCOOH aliph = -0.67 and πCOOH arom = -0.28) This theory is supported by the ultraviolet spectroscopic

data which reveals decoupling of resonance between the benzene ring and the carboxyl group owing to the presence of two ortho-CH_3 groups, although the decoupling is by no means complete. Steric twisting also affects the $^{11}\Delta G$ value in the cyclohexane/water system. A single ortho-methyl group has little effect on log P other than the intrinsic hydrophobic effect of the methyl group. However, the addition of a second ortho-methyl causes the free energy value to become positive i.e. the partition coefficient is reduced and the molecule has more affinity for the aqueous phase. The enthalpy of transfer is increased positively for 2,6-Me_2benzoic acid in both solvent systems and this is consistent with the carboxyl group being twisted out of the plane of the molecule. In the cyclohexane/water system more energy is required for transfer from the aqueous to the organic phase and this is associated with a high ΔS since the loss of solute-solvent interaction is greater and more water is lost on transfer. In the n-octanol/water system steric shielding of the carboxyl group by the two methyl groups means that octanol has less access than water and so once again more energy is required for the transfer. In this system the other isomers have a negative increase in ΔH as compared with benzoic acid. There are possibly two reasons for this, the first being due to restructuring of the water molecules and increased hydrogen bonding on removal of the methyl group from water and the second being due to more interaction of the methyl groups with the octanol alkyl chain when the molecule is transferred to the organic phase. This also accounts for the increase in ΔS.

The hydrophobic effect of the methyl group causes an increase in entropy of transfer but di-ortho methyl substitution causes a greater increase in ΔS than expected. This is due to the COOH group being shielded by the two methyl groups so that there is less interaction with octanol than for 3,5-Me_2benzoic acid. Therefore there is less order when 2,6-Me_2benzoic acid is in the octanol phase.

In the cyclohexane/water system, 3,5-Me_2benzoic acid has a negative enthalpy value which means that it gains energy on going into cyclohexane, therefore there is more solvation in cyclohexane than in water. This molecule also has a negative entropy value which indicates more ordering in the cyclohexane phase. This could be due to self-association of 3,5-Me_2benzoic acid in cyclohexane.

Meta-methylbenzoic acid has an odd set of thermodynamic data which are difficult to explain and can only be attributed for the moment to experimental error.

iii. Methylacetanilides

	Thermodynamic Parameters		
	$\Delta H kJmole^{-1}$	$\Delta G kJmole^{-1}$	$\Delta S Jmole^{-1}deg^{-1}$
Octanol			
Acetanilide	- 3.51	-12.42	+29.89
o-Meacetanilide	+ 8.25	-10.27	+62.15
m-Meacetanilide	- 3.45	-14.53	+37.20
p-Meacetanilide	- 4.60	-14.52	+33.30
2,6-Me_2acetanilide	+ 7.18	-10.98	+60.94
3,5-Me_2acetanilide	- 3.83	-16.92	+43.92
Cyclohexane			
Acetanilide	+22.98	+ 3.37	+65.82
o-Meacetanilide	+15.32	+ 2.66	+42.49
m-Meacetanilide	+22.98	+ 1.15	+73.25
p-Meacetanilide	+33.32	+ 0.65	+109.63
2,6-Me_2acetanilide	+30.64	+ 3.91	+89.70
3,5-Me_2acetanilide	+30.64	- 2.69	+111.86

This group was also included in the study to observe the steric effect of the methyl group. The acetamido group is somewhat larger than the other groups studied and is capable of greater movement.

Acetanilide was examined as the parent compound and as expected, meta-, para- and 3,5-di- methyl acetanilide showed the negative increase in ΔG attributable to the increase in hydrophobicity caused by the methyl group. The ΔG values in the octanol/water system are negative, showing a preference for the organic phase, while in the cyclohexane/water system ΔG values for all but the 3,5-dimethyl isomer are positive, indicating a preference for the aqueous phase.

Ortho-methyl substitution causes a reduction in log P, indicating a loss of affinity for each organic phase. This is due to twisting of the acetamido group by one or two methyl groups which causes loss of conjugation and therefore lower lipophilicity. This twisting is confirmed by the UV spectra. If steric twisting lowers lipophilicity, it is probably by increased interaction of the twisted group with water. Therefore ΔH should be more positive. This is the case in the octanol/water system but not in the cyclohexane/water system. Similarly, increased interactions with water in the octanol/water system mean more structured water associated with the ortho-isomers and therefore a more positive entropy of transfer. However, this does not occur in the cyclohexane/water system. These results indicate less structured water. Therefore, transfer would seem to be governed by reduction in interactions with cyclohexane

rather than an increase in the interactions with water. The methylphenol data showed that steric shielding produces a reduction in positive ΔH and positive ΔS so it appears that the acetanilides are affected by both types of steric effect.

iv. Methylorthonitrophenols

	Thermodynamic Parameters		
	ΔHkJmole^{-1}	ΔGkJmole^{-1}	ΔSJmole^{-1}deg^{-1}
Octanol			
o-NO_2phenol	- 2.46	-15.35	+43.25
3-Me-2-NO_2phenol	-12.76	-17.61	+16.27
4-Me-2-NO_2phenol	- 2.20	-18.10	+53.35
5-Me-2-NO_2phenol	- 3.35	-18.62	+51.23
6-Me-2-NO_2phenol	+16.80	-19.97	+89.83
Cyclohexane			
o-NO_2phenol	+ 5.74	-12.45	+61.04
3-Me-2-NO_2phenol	- 5.74	-11.95	+20.85
4-Me-2-NO_2phenol	- 9.85	-15.87	+20.21
5-Me-2-NO_2phenol	- 5.74	-14.26	+28.59
6-Me-2-NO_2phenol	- 1.02	-15.57	+48.81

The methylorthonitrophenols combine intramolecular hydrogen bonding with a steric effect. The intramolecular hydrogen bond is present between the nitro and hydroxyl groups and a methyl group is substituted in the remaining ring positions to observe its effect on the hydrogen bond.

In the n-octanol/water system ΔG is more negative for the methyl substituted compounds than for o-NO_2phenol because of the increase in hydrophobicity due to the methyl group. When placed in the 4- or 5- position the methyl group has no steric effect on the molecule and so ΔG is similar for both these isomers. Indeed, both enthalpy and entropy are similar for these isomers. The increase in entropy from

that of o-nitrophenol is characteristic of the hydrophobic effect for the removal of an alkyl phenol from water to a lipid solvent and due to an increase in mobility of the phenol in the less ordered n-octanol phase, and a loss of water structure. The enthalpy of transfer is very similar to that of o-nitrophenol, indicating that interactions between solvent and solute are not greatly affected by the methyl group in either of these positions, other than causing slight restructuring of the water molecules and increased hydrogen bonding resulting from the removal of an alkyl group from water which leads to a slight negative enthalpy change such as is observed.

The methyl group in the 3-position has a steric effect which reduces intramolecular hydrogen bonding. The intramolecular hydrogen bond is intact in cyclohexane, indeed it is very strong, to judge from the infra-red spectrum, but it is broken, at least partially, in hydrogen bonding solvents because solvent attack on the NO_2 and OH groups is supported by steric stress of the methyl group. ΔG is lower than for the other isomers because of loss of conjugation of the nitro group and possibly by shielding of the nitro group by the methyl group. The latter reason is not likely because ΔH is greatest for the 3-Me isomer. i.e. NO_2 and OH are accessible to octanol. This is supported by $+\Delta S$ being very low.

The methyl group in the 6-position also has a steric effect, but in this case it serves to strengthen the intramolecular hydrogen bond. Thus the entropy of transfer is increased due to increased structuring of water molecules around the

substituents. On the transfer of this molecule from water to n-octanol there is the expected decrease in water structure which allows greater motional freedom in the lipid phase. Since the intramolecular hydrogen bond is strengthened interaction of octanol with the NO_2 group is reduced (also octanol ---- O—H interaction reduced by shielding), therefore the enthalpy of transfer is less negative and in fact becomes positive.

In the cyclohexane/water system, all four methylorthonitrophenols have negative free energies which indicate a preference for the organic phase. This is to be expected since both o-NO_2phenol and methylphenol show a preference for the cyclohexane phase. With the exception of the 3-Me isomer, ΔG is more negative than for o-NO_2phenol, showing that the methyl group increases hydrophobicity. This does not occur with the 3-Me isomer and the less negative ΔG value apparently supports the theory that the methyl group in the 3-position causes steric hindrance of the intramolecular hydrogen bond by twisting the NO_2 and OH groups out of the plane of the molecule and weakening the bond. However, spectral data shows that the intramolecular hydrogen bond reforms in cyclohexane which should make ΔH more negative, which it is not. However, increased solute-water interaction in the 3-Me isomer which produces a less negative ΔG, means that hydrogen bonds are broken on transfer to cyclohexane. Clearly, this effect outweighs reforming of the intramolecular hydrogen bond.

The methylorthonitrophenols have negative enthalpies,

unlike either o-NO_2phenol or methylphenol. The negative ΔH indicates an overall increase in the number or strength of molecular interactions which seems to suggest that interactions with water molecules are few. The enthalpy value of 6-Me-2-NO_2phenol is less negative than that of the other isomers, and it has a larger ΔS. This is probably due to the fact that the 6-Me group increases the strength of the intramolecular hydrogen bond by a buttressing effect.

9.10 Enthalpy-Entropy Compensation

In order to study trends in the thermodynamic data it is convenient to use the extrathermodynamic approach. Leo and Hansch (255) have discussed the existence of linear-free energy relationships in partitioning solvent systems, as first formalised by Collander (76). These are examples of extrathermodynamic relationships, (253) where although the relationships themselves are outside the formal structure of thermodynamics their approach resembles that of thermodynamics in that the detailed microscopic mechanisms need not be explicitly identified. Availability of thermodynamic quantities other than the free energy enables the extrathermodynamic approach to be used to give information for a single system or process. Leffler and Grunwald (253) have argued that to identify a single unique mechanism for a series of solutes, if ΔH and ΔS are approximated as being constant, the $\delta\Delta H$ should be simply proportional to $\delta\Delta S$ (where δ denotes a change caused in the thermodynamic parameter by either a medium effect, or a change in solute structure. According to Lumry and Rajender (267) any enthalpy-entropy compensation phenomenon

for a chemical reaction or process appears to be a thermodynamic manifestation of the structure forming and structure breaking properties of water solutions. If an enthalpy-entropy compensation pattern is observed when the thermodynamics of partitioning of a series of related compounds e.g. substituted phenols, between aqueous solution and an organic phase is studied, it is reasonable to assume that the same mechanism is involved in the transfer process. Krug et al (244,245) have recommended that for thermodynamic quantities obtained using van't Hoff relationships, regressions between these quantities should be carried out in ΔH_{Thm}- ΔG_{Thm} coordinates (where Thm refers to the harmonic mean temperature of the experiments); for linear enthalpy-entropy compensation can arise as statistical artefacts, since ΔH and ΔS are derived from the same set of data and where the use of Thm values minimises any statistical bias in the subsequent analysis. Enthalpy-entropy compensation can be expressed (245) by:

$$\Delta H = \beta\Delta S + \Delta G(\text{at } T=\beta) = \beta\Delta S + \Delta G_{\beta} \qquad (9.viii)$$

where β is a proportionality factor having dimensions of absolute temperature (253). Using the Gibbs equation in rewriting equation 9.viii. to express the free energy change measured at a fixed temperature, T, (ΔG_T) we obtain:

$$\Delta G_T = \Delta H(1 - T/\beta) + T\Delta G_{\beta}/\beta \qquad (9.ix)$$

Using 35°C as the harmonic mean temperature, Kinkel et al (226) found that for a group of simple substituted benzenes in the water/2,2,4-trimethylpentane system good linear

enthalpy-entropy compensation correlation existed. They concluded that good linear correlation is found for solute distribution between solvent pairs having little mutual solubility e.g. cyclohexane/water and 2,2,4-trimethylpentane/water, but not for solvent pairs with great mutual solubility such as octanol and water.

Interpretation of the experimental determination of the temperature dependence of distribution by the van't Hoff equation to provide ΔH and ΔS quantities are common. However, as well discussed for the micellisation process (203), macroscopic operational van't Hoff relationships fail if the system itself changes. Considering the distributing system this could be interpreted (68,70) in terms of:

1. The water solubility in the oil and vice versa.
2. The state of any oil/water complexes.
3. The molecular state of the oil.
4. The effect of solute on 1 - 3.

The reported (255) mole fraction solubility of water in cyclohexane is 2.65×10^{-4} (ΔH;13.2kJmole^{-1}(322)) and water is considered (345) as being unassociated with the oil. Conversely, the mole fraction solubility of oil in water for cyclohexane at 25°C is 1.17×10^{-5} (ΔH;0.3kJmole^{-1} (322)). These data show that conditions 1 - 3 do not apply for the cyclohexane/water system. Condition 4 is of interest and could provide a suitable avenue for extension of this project.

However, these theories provide support for the assumption of good linear enthalpy-entropy compensation in the cyclohexane/water system. Therefore, using 25°C as the

Figure 48. ΔH vs ΔS Cyclohexane/Water System

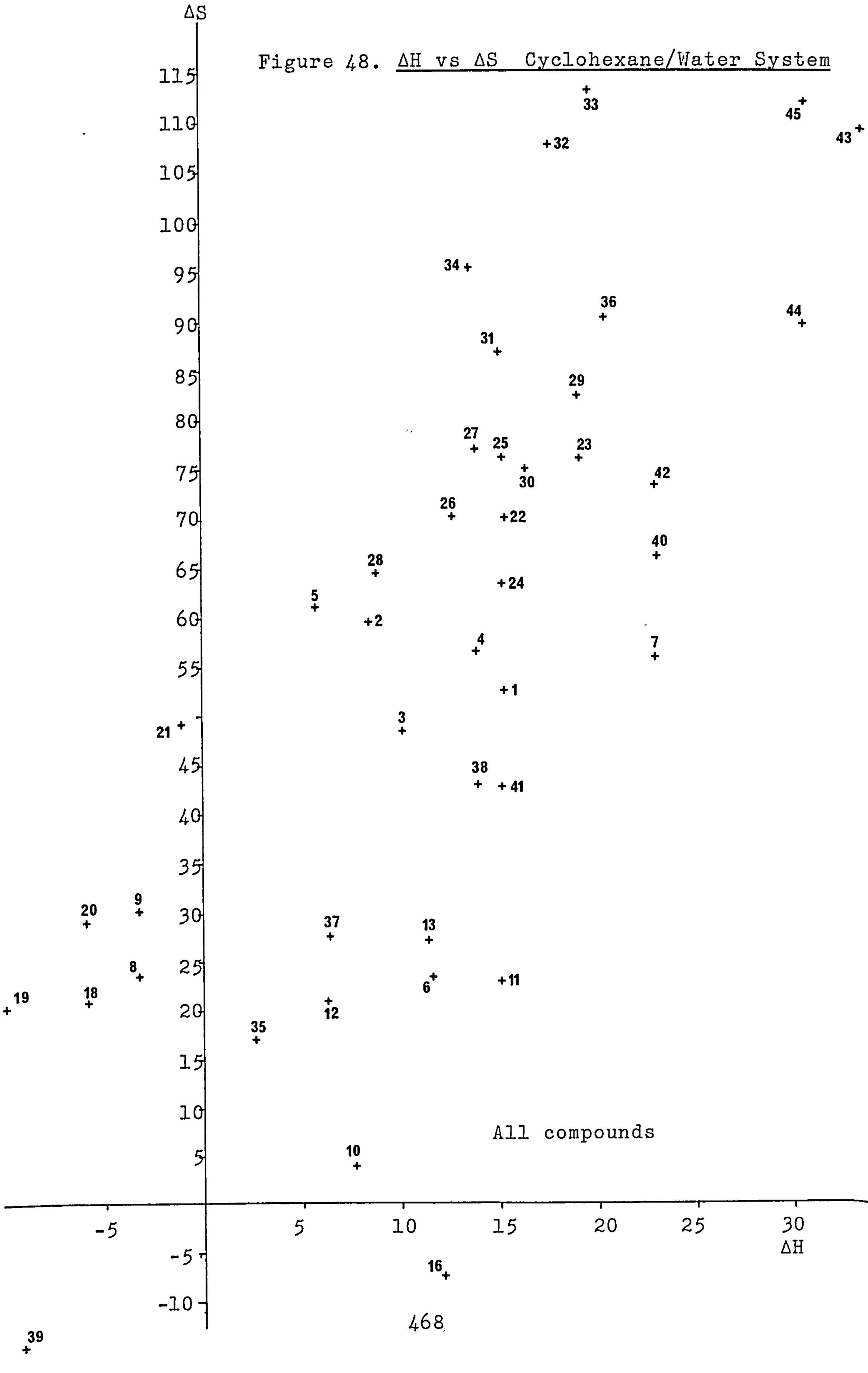


Figure 49. ΔH vs ΔG Cyclohexane/Water System

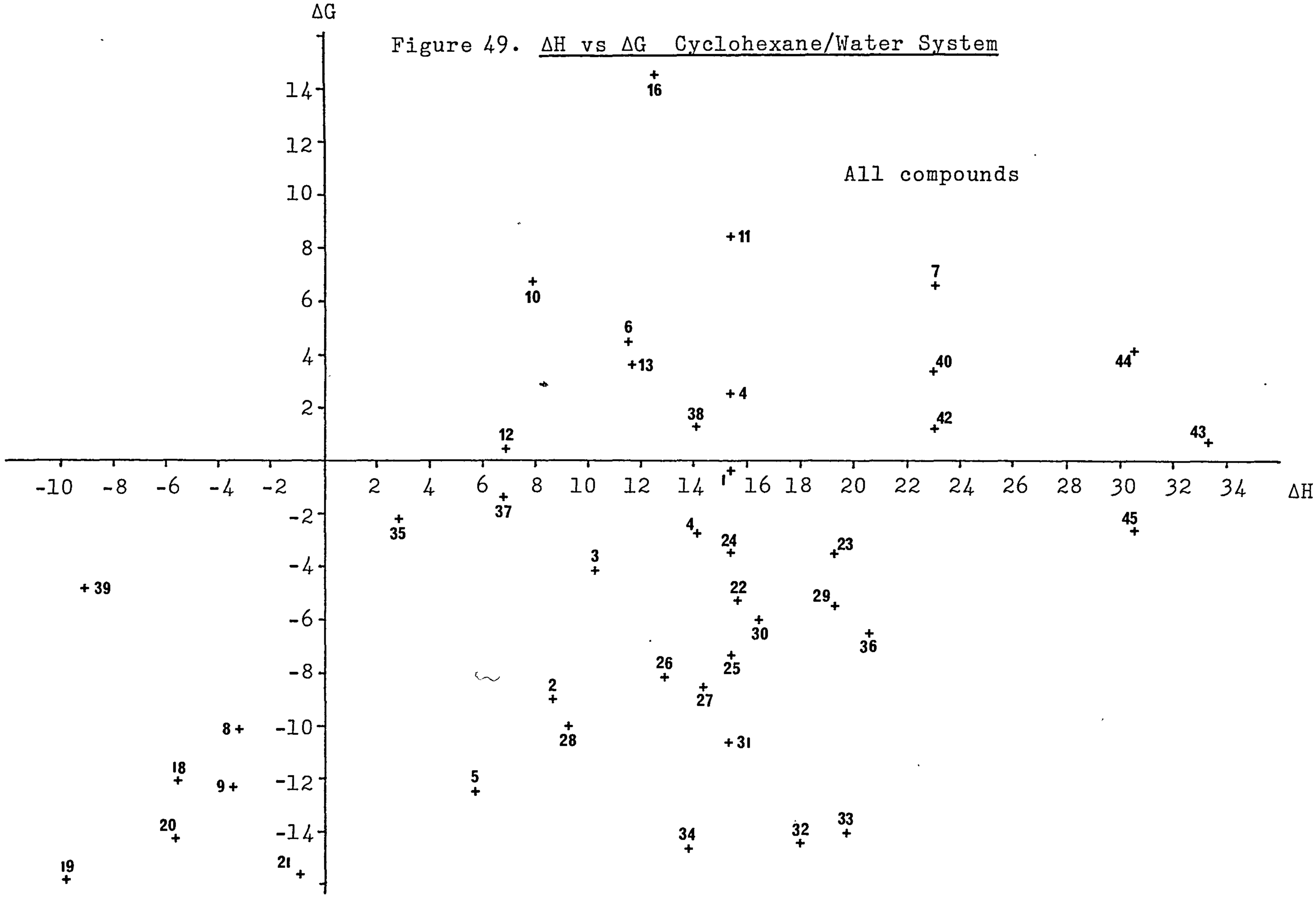


Figure 50. ΔG vs ΔH Cyclohexane/Water System

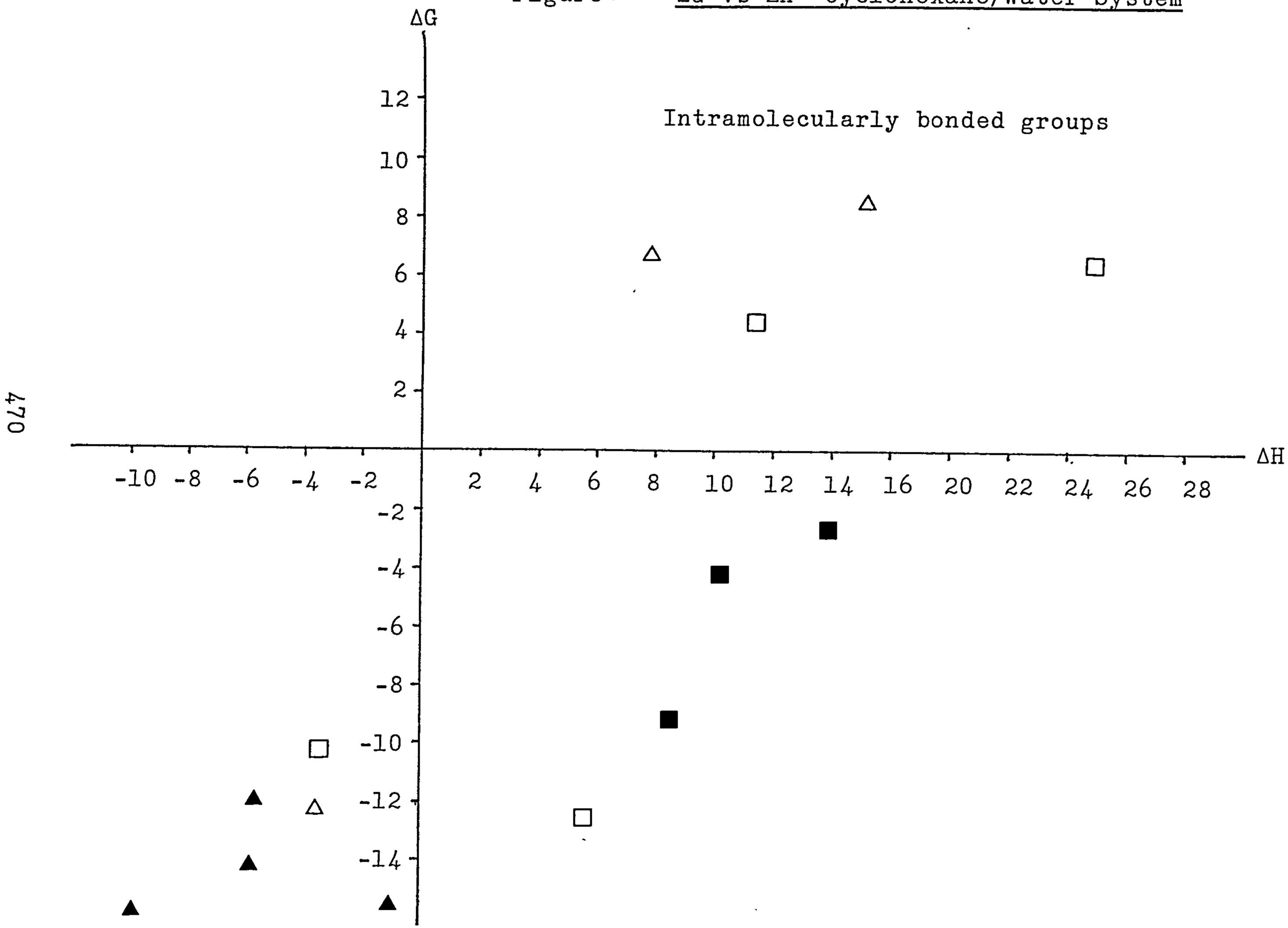


Figure 51. ΔG vs ΔH Cyclohexane/Water System

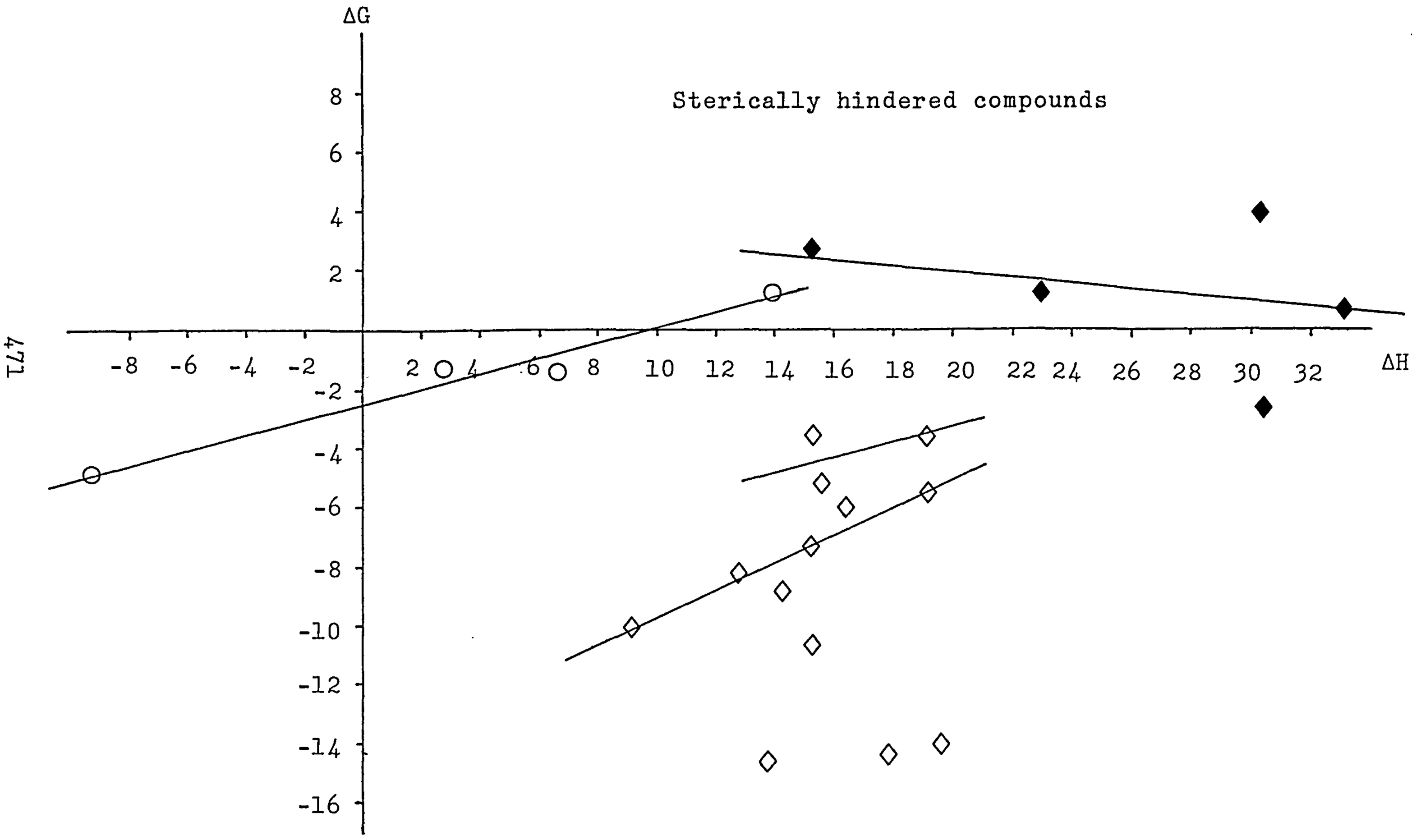
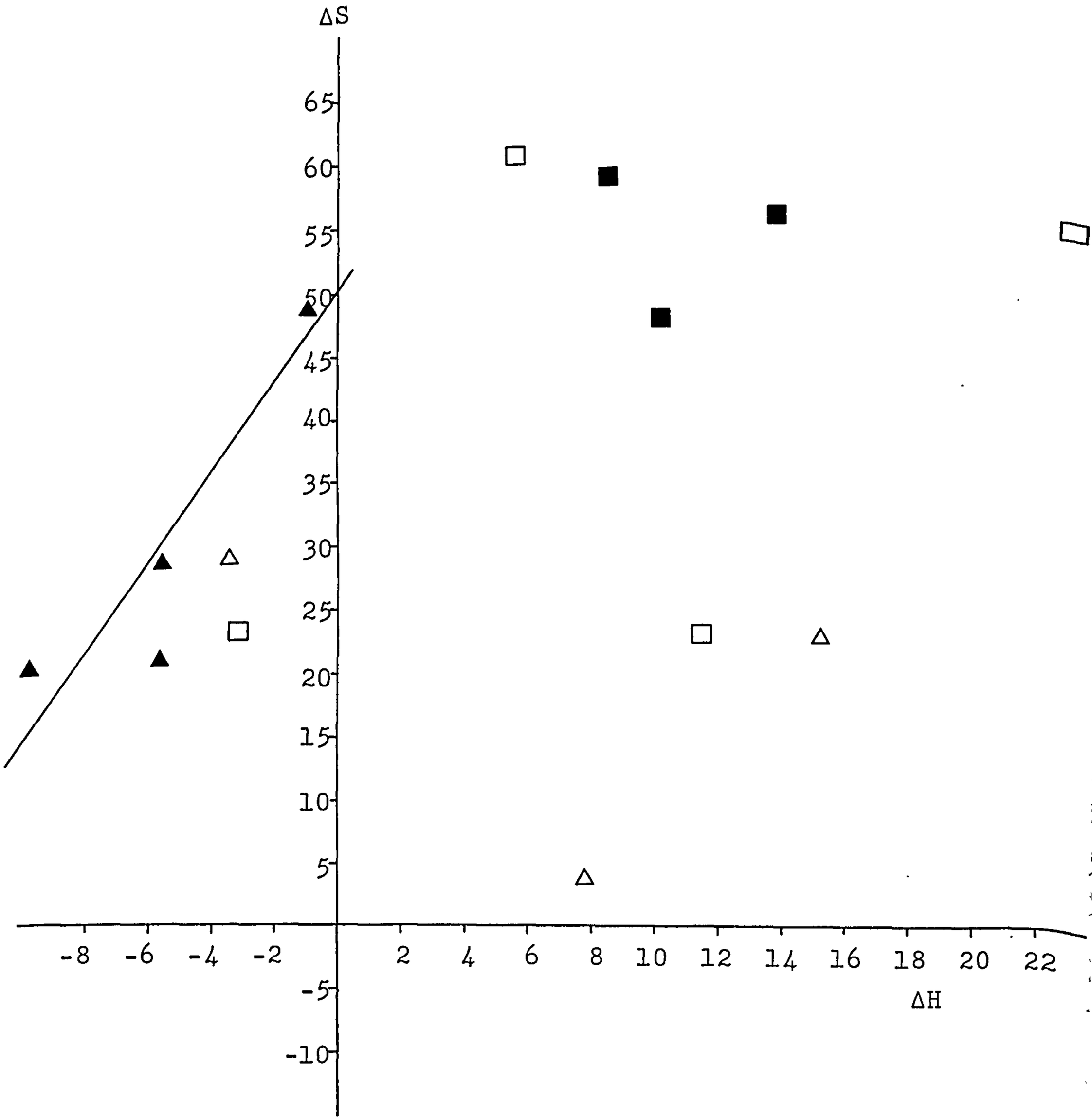


Figure 52. ΔH vs ΔS Cyclohexane/Water System

Intramolecularly bonded groups



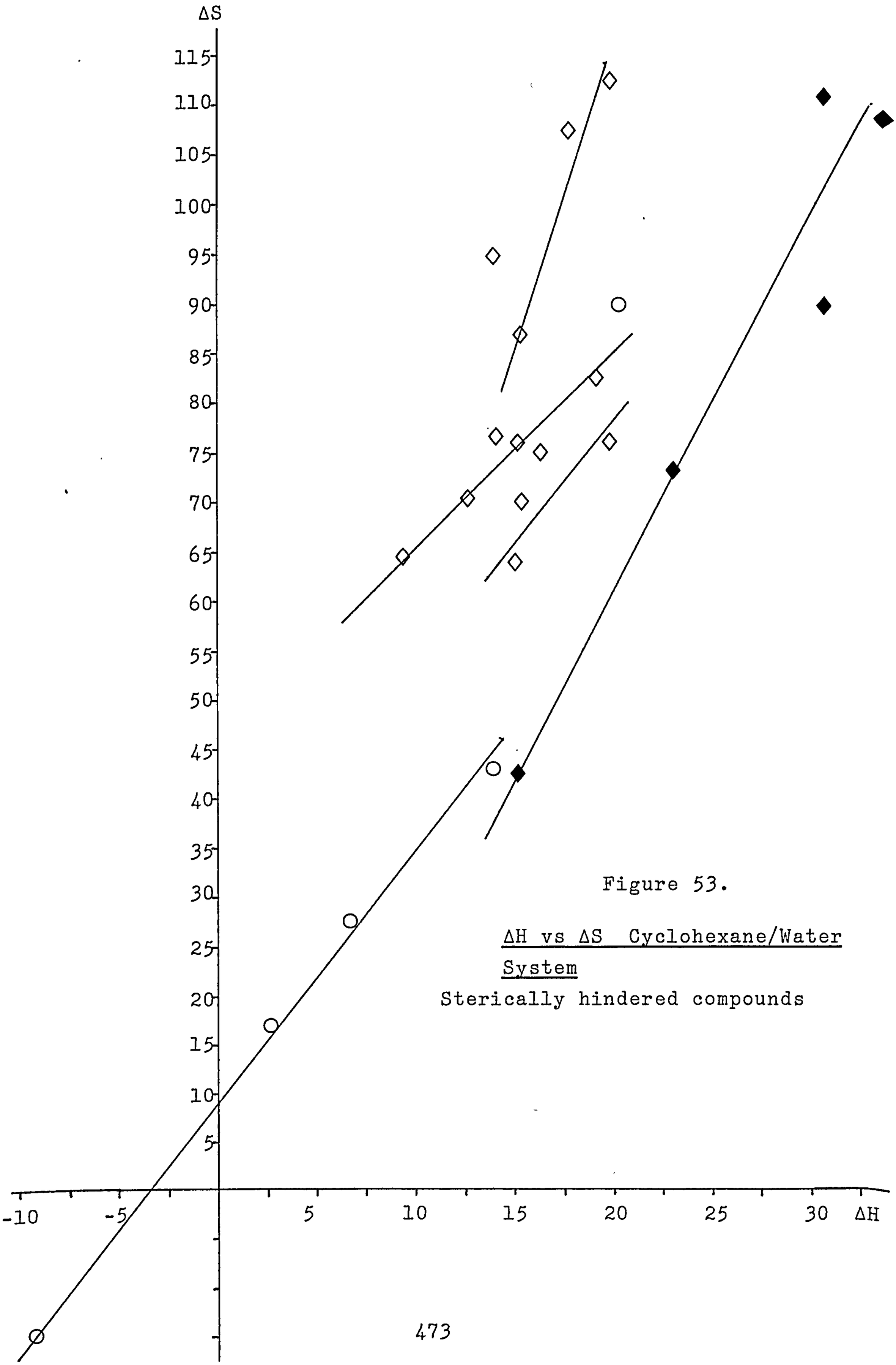


Figure 53.

ΔH vs ΔS Cyclohexane/Water System

Sterically hindered compounds

harmonic mean temperature, the partitioning of the compounds investigated in this study was examined for enthalpy-entropy compensation in the cyclohexane/water system. Figures 48 - 53 show the results. Both ΔG and ΔS versus ΔH were investigated, despite the reservations about the validity of ΔH vs ΔS correlations. It can be seen that no general correlation exists between all the compounds (Figures 48 & 49) and it is difficult to see any correlation between the compounds containing hydrogen bonding substituents. However, correlations can be seen in those groups containing methyl substituents, indicating that the methyl group has the same effect upon distribution in each case.

Enthalpy-entropy compensation is expected for a group of substituted phenols in the cyclohexane/water system since transfer involves the same basic mechanism, that is, the breaking of solute-water interactions, followed by a strong hydrophobic interaction and possibly weak van der Waals interactions between the phenol molecules and the cyclohexane molecules. However, the lack of compensation evidence indicates that for this group of compounds at least, notably those capable of steric effects and intra- or inter-molecular hydrogen bonding, different mechanisms of transfer may operate.

Distribution Between Solvent Pairs Having High Mutual Solubility

Water/octanol is a much studied solvent pair in distribution studies (189) and although as a medium describing solute hydrophobicity this system has its uses, the complex nature of the system has led to its criticism.as a standard state.(332)

It has been suggested (264) that water centred aggregates exist in octanol with a 4:1 alcohol:water ratio and that a solute transferring to this phase may replace one octanol in the complex (345). In addition, linear aliphatic alcohols (including octanol) have been shown to exist in monomeric and polymeric forms (7). For reference, the corresponding values (39) of solubility at 25°C (mole fraction) and enthalpies of solution for water in octanol and vice versa are 0.27; 5.7kJmole^{-1} and 7.29 x 10^{-5}; 3.9kJmole^{-1} respectively. This shows that at 25°C upon equilibration octanol comprises (on a mole fraction scale) 27% water which rises to 32% water at 50°C. In relation to this, Lippold and Adel (264) show that the dielectric constant of octanol decreases as both temperature and water content increase whereas the dielectric constant for cyclohexane at 25°C does not change upon saturation.

Thus arguments against the use of van't Hoff operators for obtaining ΔH and ΔS quantities would appear to hold for the water/octanol system and similar solvent pairs. Beezer et al (25) and Rogers and Wong (330) studied the temperature dependence of the distribution of resorcinol monoethers and substituted phenols between octanol and respectiviely water and 0.15moledm^{-3} aq.NaCl. Kinkel et al (226) compared these results with their own study of substituted benzenes and methylbenzoates in aq.phosphate buffer (pH7.0) and 2,2,4-trimethylpentane; to see whether linear enthalpy-entropy compensation exists in these systems. They found linear compensation behaviour absent, although Rogers and Wong (330) reported good correlation between ΔH and ΔS.(0.942,n=18)

This however, was thought to be due to statistical and experimental reasons, and therefore it has been assumed that true enthalpy-entropy compensation has not been exhibited for the octanol system which implies that the van't Hoff operators should not be used to obtain thermodynamic quantities in such systems.

Bearing these conclusions in mind, the compounds investigated in this study were observed for enthalpy-entropy compensation in the octanol/water system. As with the cyclohexane/water system, ΔH was plotted against ΔG and ΔS for comparison. Figures 54 - 60 show the resultant graphs and it can be seen that although no linearity is detectable in the ΔH vs ΔG plot, that of ΔH vs ΔS shows a definite linear trend.

Figures 59 and 60 show a breakdown of Fig. 55 into structural groups and enthalpy-entropy compensation can be seen to be present within the isomeric groups. Linearity is particularly apparent in Figures 56,57,58 which give the plot of ΔH vs ΔS. (Figure 54)

Figure 57 indicates that all the methylphenols are transferred in a similar manner but with the addition of a methyl group causing a slight change. It can be seen that four distinct groups are present: 1-Me, 2-Me's, 3-Me's and 4-Me. This indicates that hydrophobicity influences the mode of transfer of these molecules. The addition of a methyl group increases hydrophobicity and it would appear that although hydrogen bonding in both phases will be present, the transfer of these phenols is mainly governed by hydrophobic interactions.

Figure 54. ΔH vs ΔS Octanol/Water System

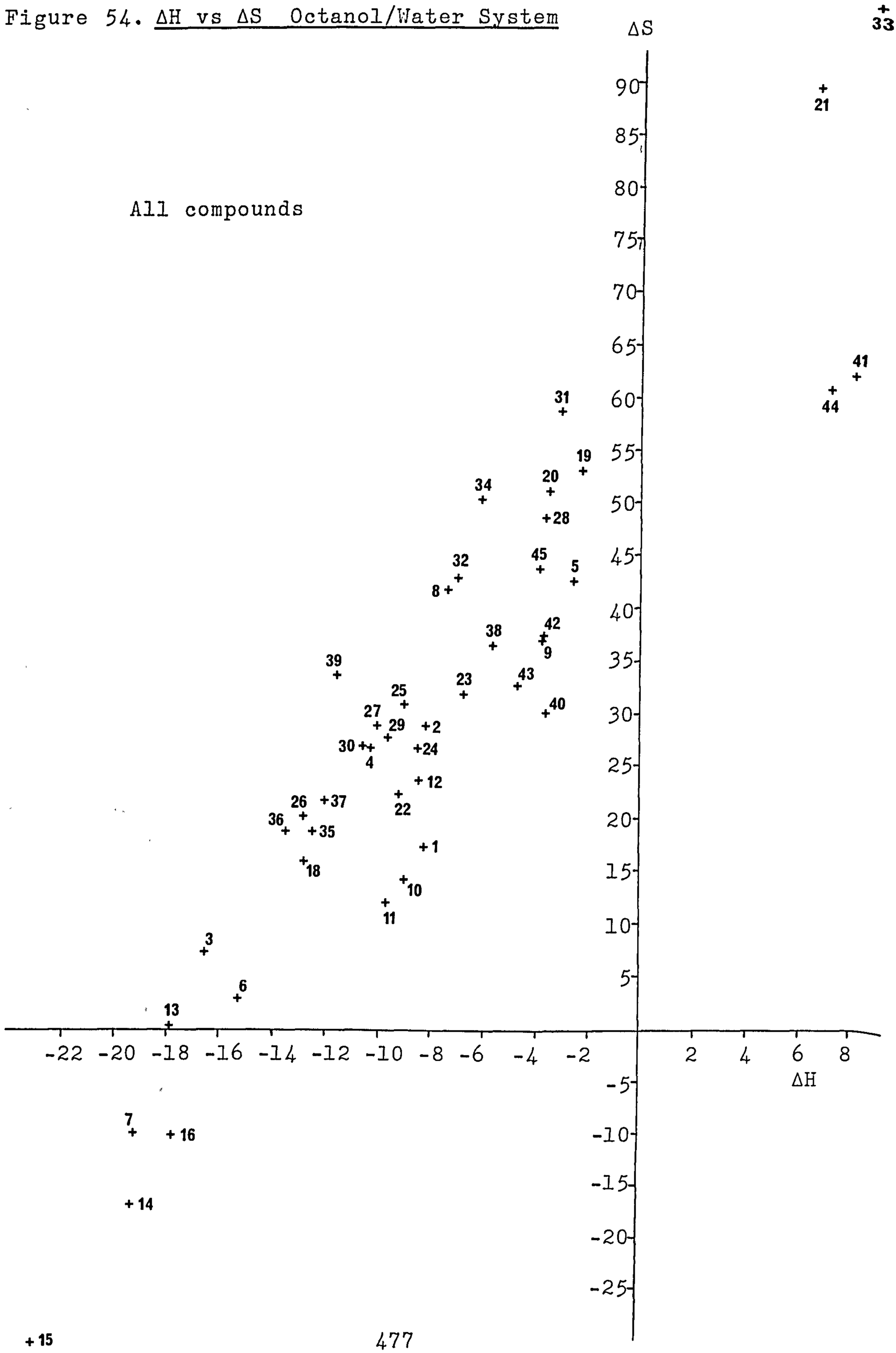


Figure 55. ΔG vs ΔH Octanol/Water System

All compounds

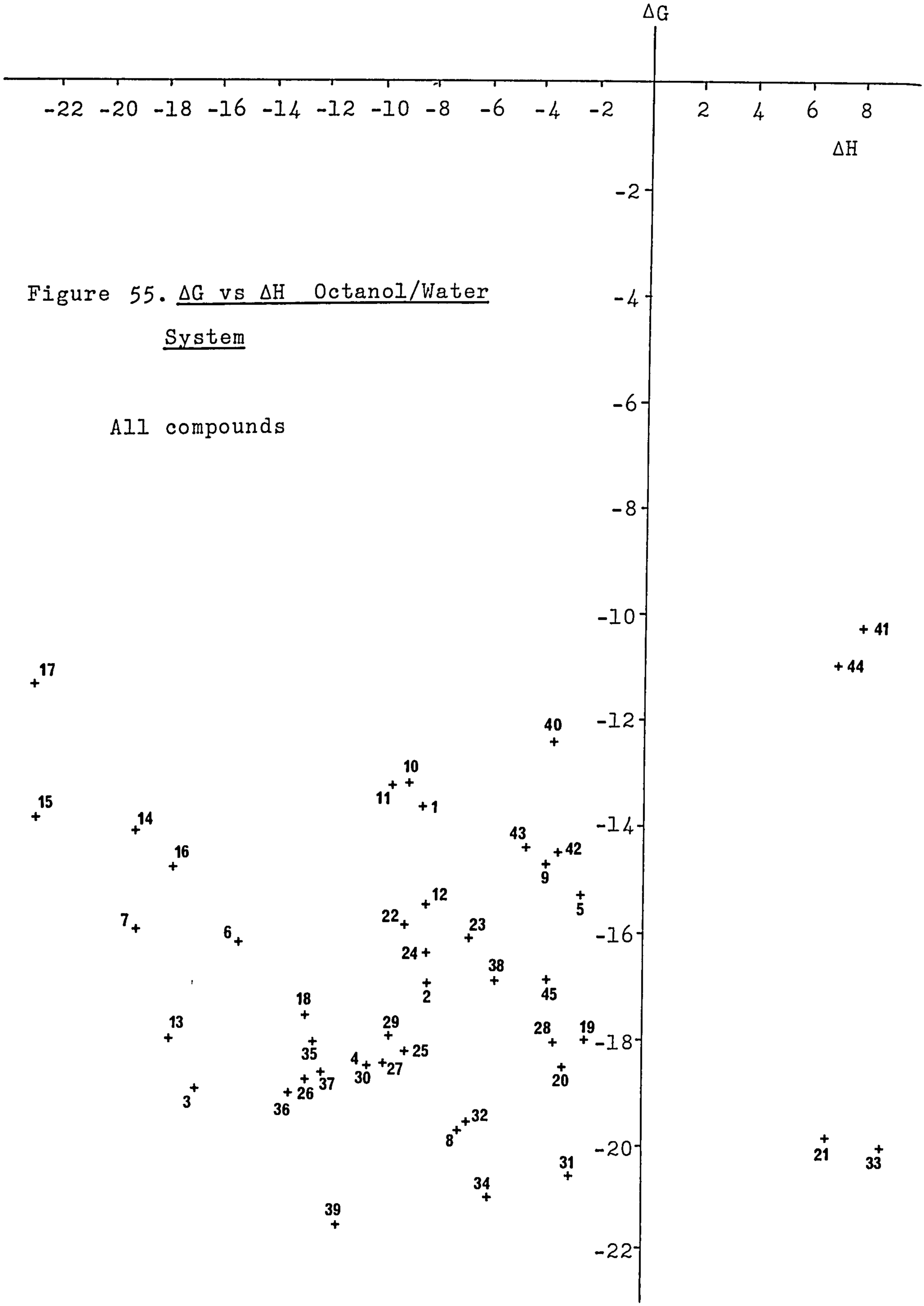


Figure 56. ΔS vs ΔH Octanol/Water System

Intramolecularly bonded groups

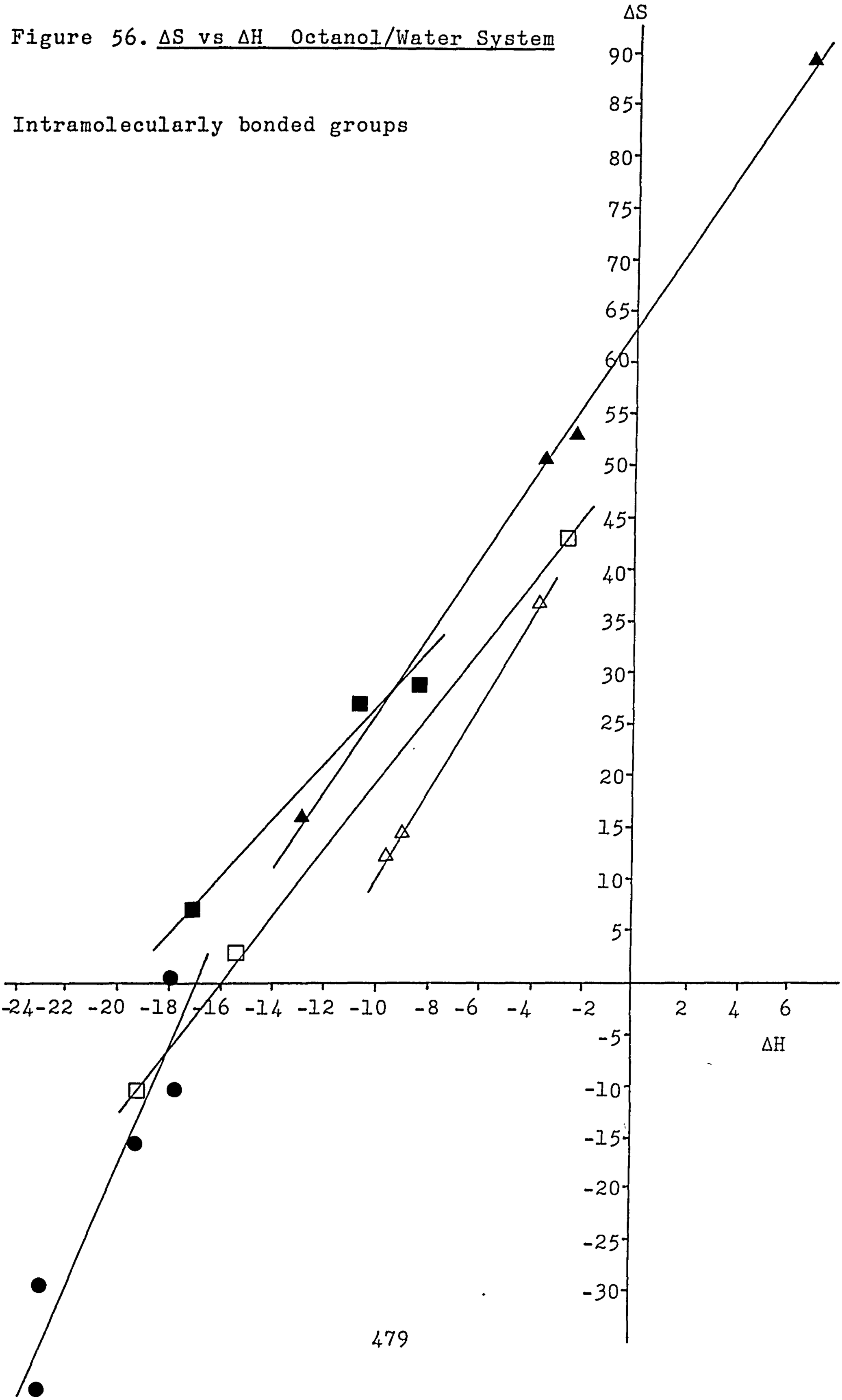


Figure 57. ΔH vs ΔS Octanol/Water System

Methylphenols

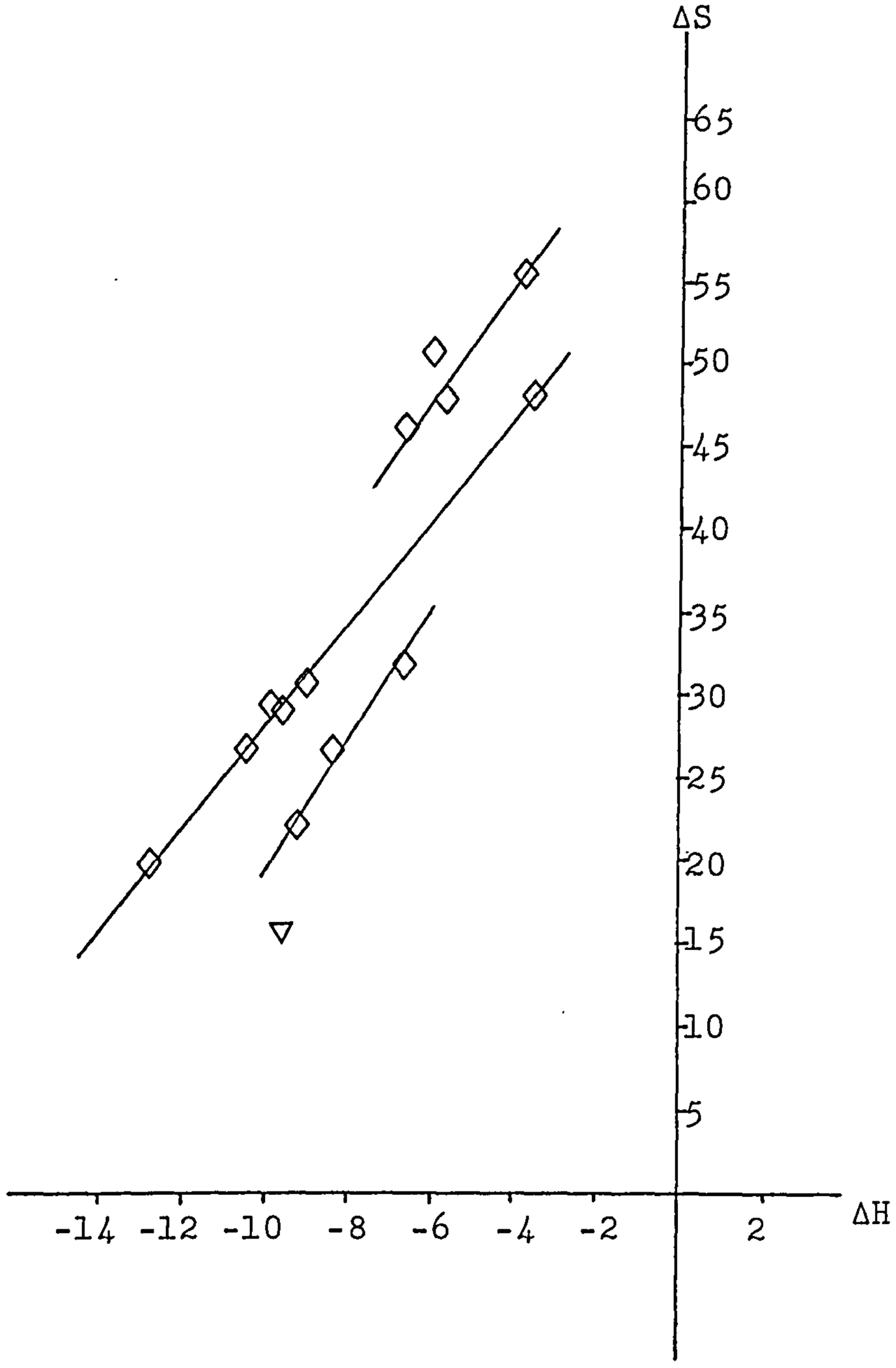


Figure 58. ΔH vs ΔS Octanol/Water System

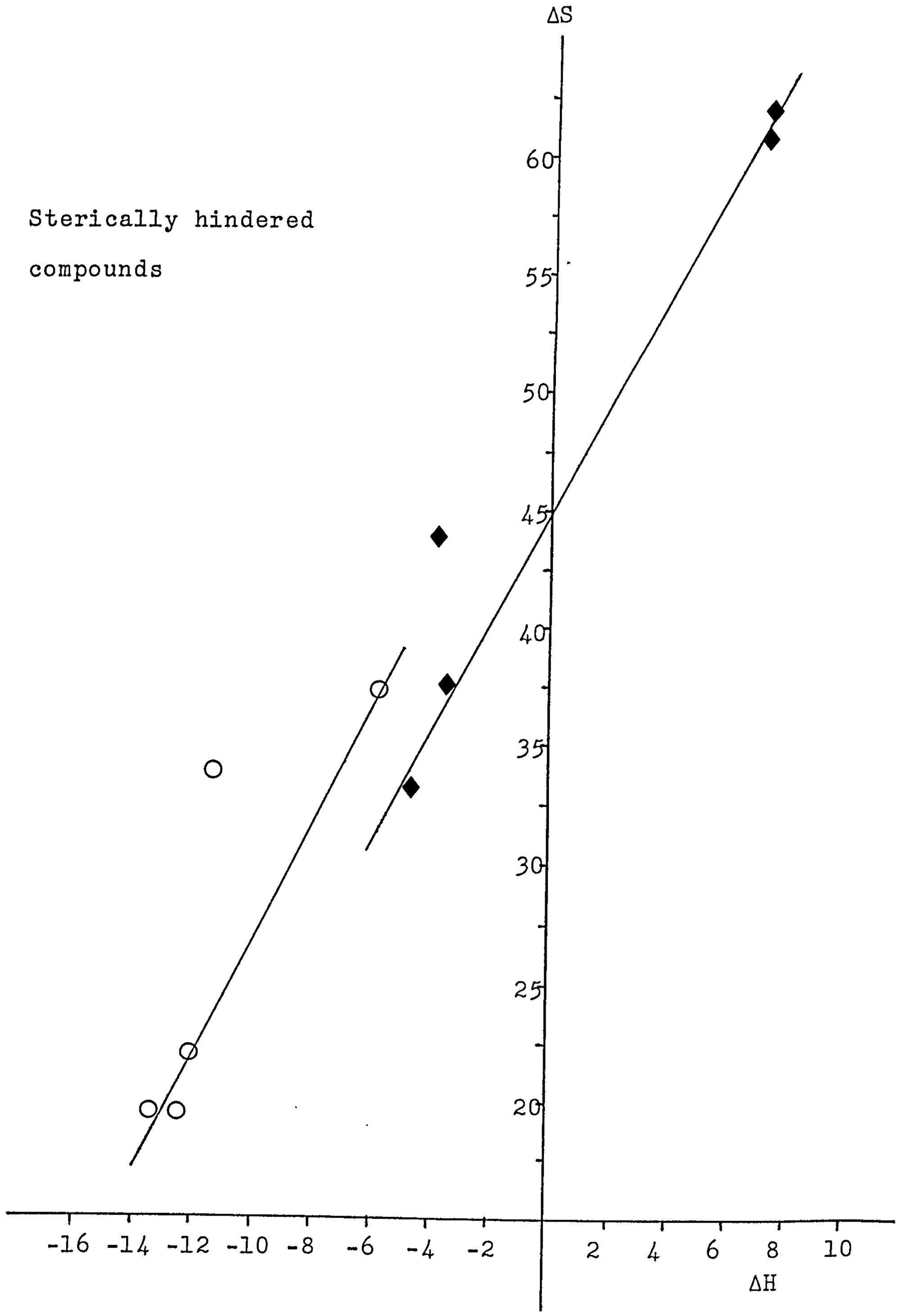


Figure 59. ΔG vs ΔH Octanol/Water System

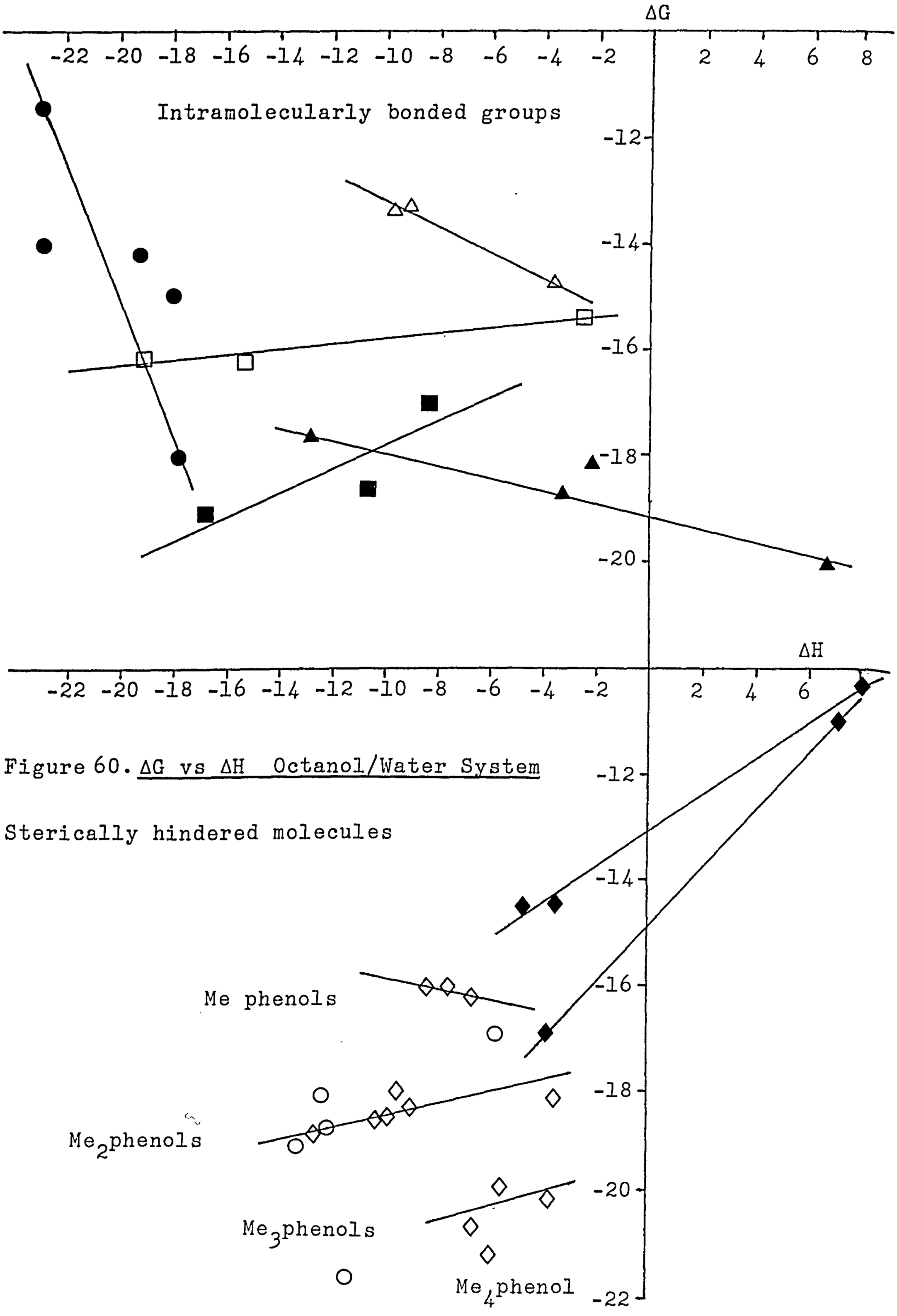


Figure 60. ΔG vs ΔH Octanol/Water System

Sterically hindered molecules

Those molecules containing hydrogen bonding groups display similar enthalpy-entropy compensation, indicating that they are transferred by a similar mechanism, that of increased hydrogen bonding with octanol.

Enthalpy-entropy compensation has been discussed here in terms of ΔH vs ΔS which has been questioned as to its validity. However, the same observations can be made from the ΔH vs ΔG plots, suggesting that both sets of co-ordinates can provide useful information. A second point where this discussion disagrees with the preceding suggestions is that linear enthalpy-entropy compensation has been found within the octanol/water system. This leads to the suggestion that although it is accepted that this system is difficult to work with, provided both phases are mutually saturated, any alterations within the system occurring due to temperature change will be consistent for each experiment and will not affect observed trends. A third point of disagreement with previous work is that linear enthalpy-entropy compensation has been sought within groups of isomers. Therefore, lack of compensation can be a good indication that interactions within the molecule affect the method of transfer from one phase to another.

CHAPTER TEN

PARTITIONING RATE CONSTANT AS A PARAMETER IN QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

It has previously been stated that for the design of optimally-active drugs an understanding of drug behaviour in biological systems and an understanding of the experimental relationships between chemical structure and chemical and physical properties of drugs are required. With the introduction of structure-activity relationships in quantitative terms by Hansch (169), hypotheses on the action of drugs as well as the optimisation of drug activity were tested statistically. Also at this period it was shown that the biological contributions of physico-chemical parameters are often linearly additive. Thus producing an equation such as:-

$$\log BR = a(\text{lipophilic parameters}) + b(\text{steric parameters}) + c(\text{electronic parameters})$$

where BR is the biological response which is mostly expressed as 1/C, where C is the concentration of drug producing a certain effect. The constants a, b and c are obtained from regression analysis.

Of the parameters used in QSAR's, the partition coefficient is the most common and many studies have revealed good correlations between biological activity and partition coefficient (183,246,247). However, within any set of congeners studied there are often so called 'outliers', compounds which do not show the same degree of correlation

as the rest of the series. These 'outliers' are usually explained as anomalies due to, for example, specific receptor binding or metabolism, and attempts are often made to account for these effects by including steric, electronic or other parameters in the correlation.

However, the aim of structure-activity correlations is to disclose possibilities for the rational design of drugs, so, to attain this aim rather than simply utilise the predictive value of such correlations, it is necessary to obtain more extensive knowledge of the drug in relation to the living organism, so that biological and physico-chemical parameters can be chosen or determined on a rational basis.

The partition coefficient is an equilibrium parameter, whilst the partitioning of drugs in the body is dynamic by nature. From the site of administration the drug is transported to certain active sites e.g.receptors. A part of the dose is metabolised and/or excreted as a 'side-reaction' with a rate constant, k_{el} (Figure 61)

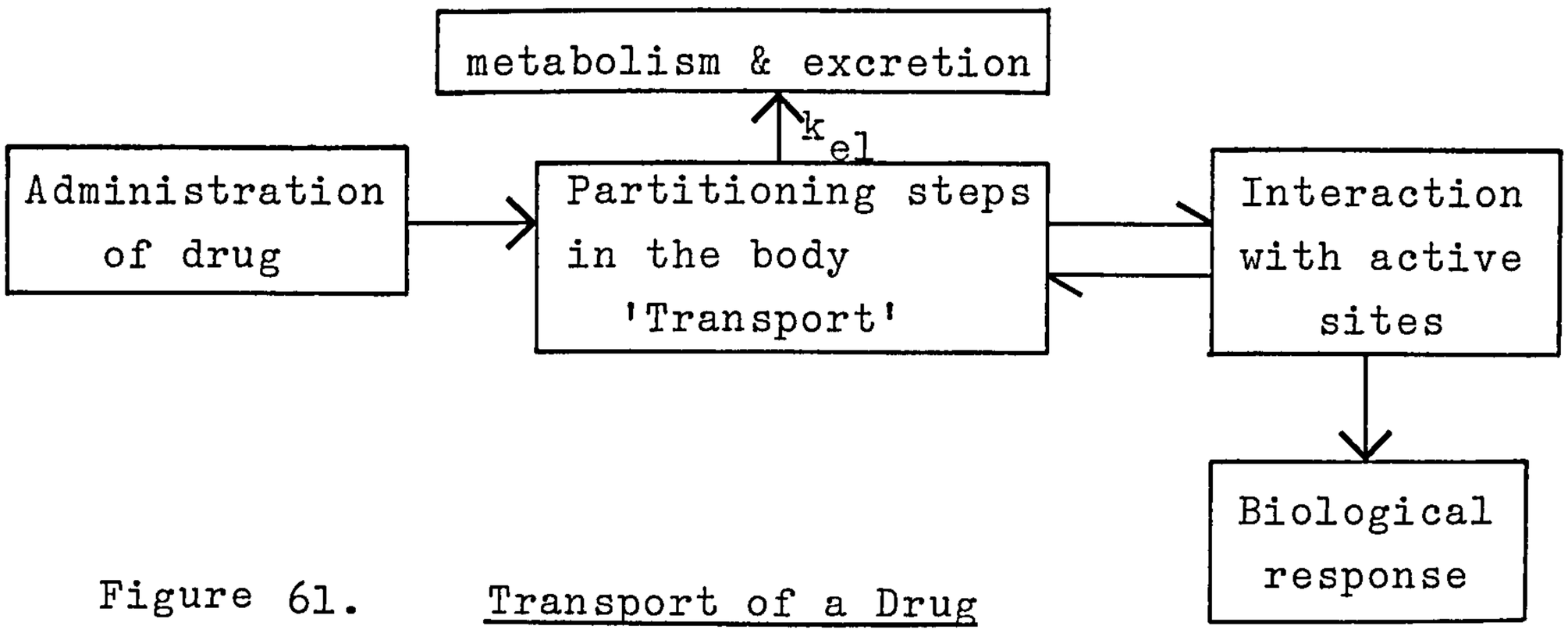


Figure 61. Transport of a Drug in the Body

Most pharmacological testing is carried out following administration of a single dose, and response is usually measured at a fixed time after dosage. Thus it is the rate of partitioning and not the partition coefficient per se, that will govern the concentration of drug at the receptor at a given time. It might be argued that since partition coefficient is the ratio of the forward and reverse partitioning rate constants, partition coefficient is a satisfactory parameter to use. However, it is known that some compounds have abnormal rates of partitioning whilst displaying normal or expected partition coefficients; such compounds could appear as outliers in structure-activity relationships. (123)

The first correlations between biological activity and partition coefficient (286,306) were recti-linear and Ferguson (128) expressed the relationship in the equation:-

$$\log BR = a \log P + b$$

However, it was later proved by Hansch and Fujita (170) that non-rectilinearity existed and for many series a better correlation was obtained if a parabolic relationship was presumed, described by the equation:-

$$\log BR = a(\log P)^2 + b \log P + c$$

where $(\log P)^2$ is explained as being derived from transport or solubility factors (406).

Mathematical explanations were sought for the parabolic relations and via a kinetic multicompartment model,

Penniston et al (312) calculated the concentration in the last compartment at a fixed time.

In a multicompartment system as illustrated in Figure 62, aq_o represents the aqueous phase where the drug is initially introduced; lip_1 represents the first lipid phase into which the drug with a transport rate constant k passes. From here the drug can either return to aq_o or enter aq_1 with a transport rate constant, l. The ratio k/l gives the partition coefficient P and the product k x l is assumed to be constant.

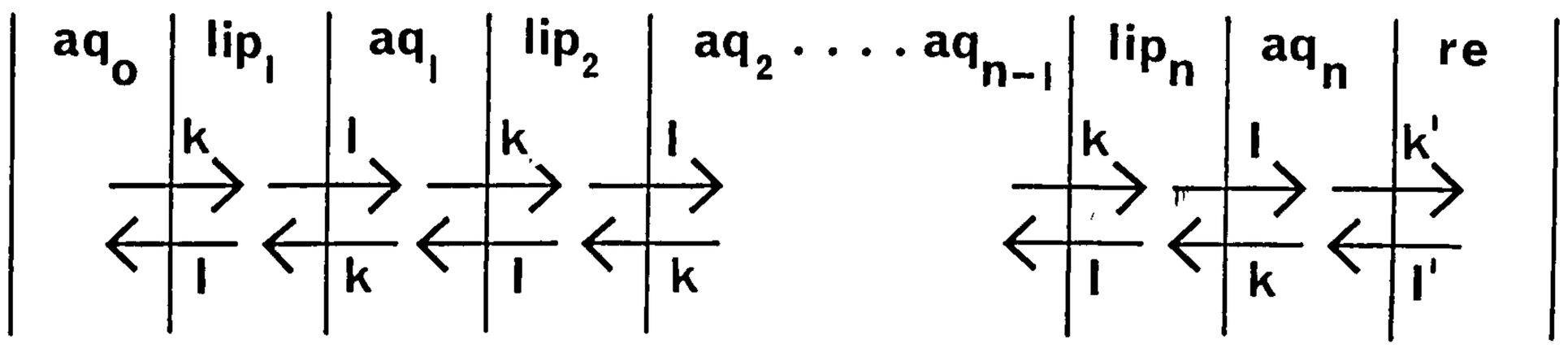


Figure 62. A Multicompartment System of Alternating Aqueous and Lipid Phases

Penniston et al (312) assumed for simplicity that all lipid and all aqueous phases were of equal shape and volume and obtained a parabolic relationship between the concentration in the last compartment and the partition coefficient. However, Dearden and Townend (111) developed a computer model based on that of Penniston' to predict the passive movement of drugs through living organisms. A differential equation was set up for each cell which could then be

integrated to give the concentration in each cell at different times. Figure 63 shows that at any given time, in this case t = 10, a graph can be plotted, for each cell, of concentration against partition coefficient. However, if one cell is taken, for example, cell 10 at t=20, although when the product k x l is 1 a parabolic relationship is obtained, if for any given partition coefficient the product k x l is altered, for example, k x l = 1.5,2.0,3.0, then the point moves away from the curve and becomes an outlier (Figure 63). Thus, for any series of compounds, differences in the product of forward and reverse rate constants may cause one or more to appear as outliers. In practice, k x l is not constant and seems to vary more or less parabolically with log P. However, outlier k and l values can still be spotted.

McFarland (284) devised an equation for determining the probability of a molecule reaching the last (n^{th}) compartment and assumed that this expression could be correlated with biological activity so that he produced the relationship:

$$\log BR = a \log P - b \log(P + 1) + c$$

This was combined with the equilibrium model of Higuchi and Davis (197) to give the expression:

$$\log BR = a \log P - b \log (\beta P + 1) + c$$

The definition of β is obscure, but it includes factors concerning volumes, differences in partition coefficients between the various compartments, the time lapse and the

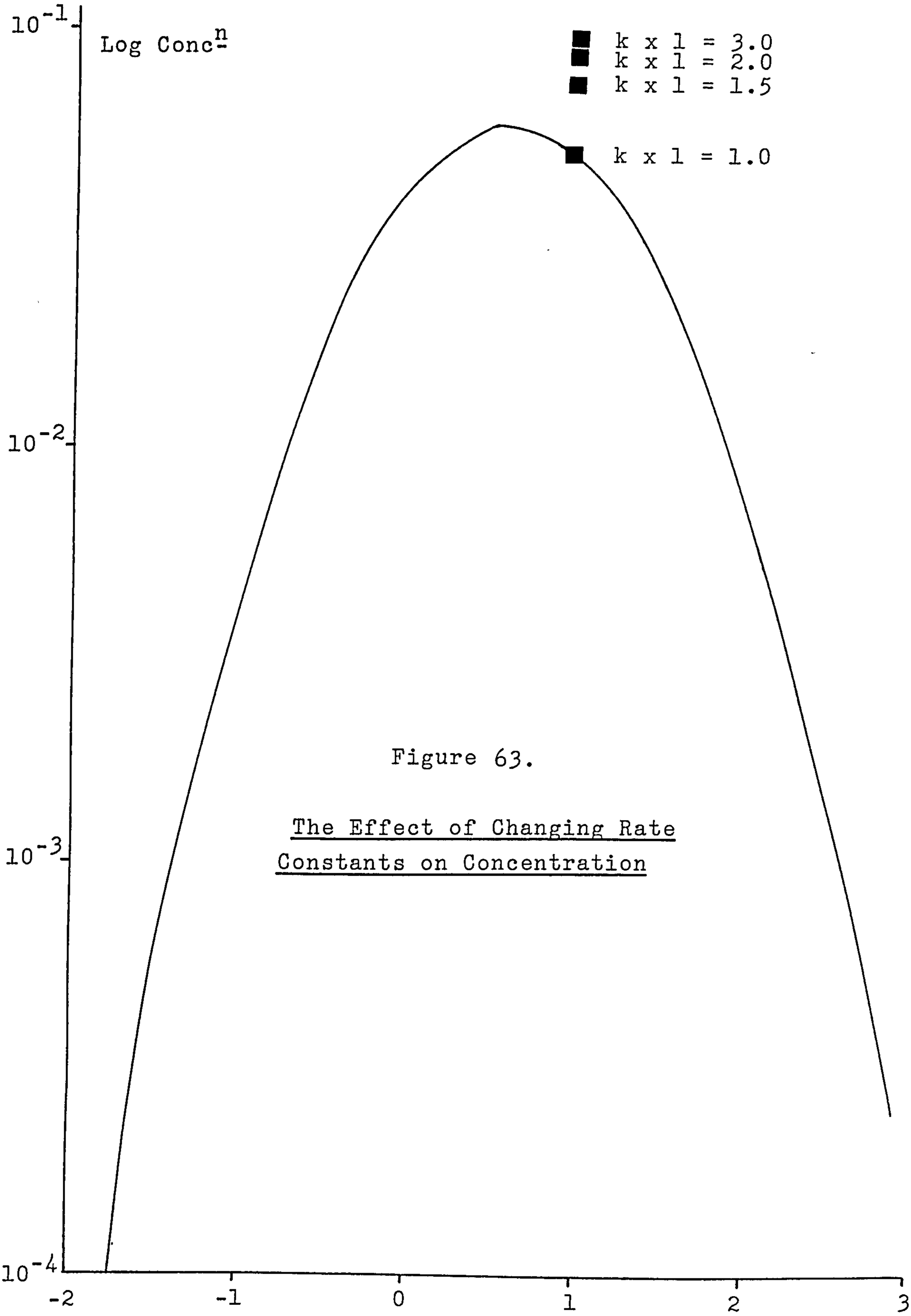


Figure 63.

The Effect of Changing Rate Constants on Concentration

number of compartments between administration site and receptor.

This so-called bi-linear model has produced good results with a few chosen series of biologically active compounds.

In 1937, Hober and Hober (200) correlated absorption rate with molecular size, and since then correlations have been made between partition coefficient, molecular weight and molecule size. From the different models, one formula was derived (274):

$$\log K_i = b \log P_i - \log(aP_i^c + d) + e$$

K is a transport rate constant of which the value is dependent on the model system in use.

The simplest representation of the transport of drugs in the body can be given by a three compartment model as described by van Waterbeemd (374). The first, aqueous, compartment represents the site where the drug becomes biologically available e.g. alimentary tract, blood vessel etc. The second compartment is an organic layer which represents the lipid layers which drugs must pass through to reach the third compartment. This last compartment is also of an aqueous nature and represents the blood, cell contents etc. This model can be used for calculations which demonstrate that the absolute magnitude of the transport rate constants, for every single partition step, determines the rate at which a certain concentration in the receptor compartment is reached.

From the third compartment the attachment of drugs to

receptors can eventually take place, in which case, lipophilic, steric and electronic factors are of importance, but this is not of relevance in this discussion.

The distribution kinetics of a drug over a three-phase system corresponds with the kinetics of a chemical equilibrium between three compounds, A,B and C (265)

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C$$

A,B and C represent the same drug present in each of the three compartments respectively, and k_1, k_2, k_3 and k_4 are the respective transport rate constants between these compartments.

Assuming that A and C are identical aqueous systems and B an organic system, it is simple to calculate the concentrations in the three compartments at any specific time if the k values are known and the following conditions are met:- the compartments are of equal volume, and at time t=0, the drug is present in A with a concentration of 1 mol/l.

Thus, information can be gained about the concentration at equilibrium and at the commencement of transport processes.

At equilibrium, $t=\infty$ and the concentrations in the different compartments are exclusively dependent on the partition coefficient of the drug, but it is necessary to know the transport rate of the compound so as to estimate the rate at which equilibrium is attainable, since this can differ considerably for two compounds with equal P values. So

that the rate at which equilibrium is reached is determined not by P, i.e. the ratio of k_1 and k_2, but by the absolute magnitudes of k_1 and k_2.

Thus transport rate seems to be closely allied to partition coefficients and hence biological activity and it was felt that correlation of biological activity with forward and reverse rate constants may account for compounds previously described as outliers. Thus the forward and reverse rate constants were measured for a series of substituted acetanilides with a previously reported QSAR (304).

The choice of the apparatus used can be explained as follows. The partition process of a drug in the body can be described with a multicompartmental model, which can be thought to be built up from many (different) two-compartment systems. For each two-compartment system transport rate constants can be determined in an apparatus in which the drug is partitioned in a two-phase system. The concentration at any time in any compartment of a multicompartmental system can be exactly calculated from the rate constants determined in a series of two-phase systems.

The interfacial transfer in a two-phase system can be described by a reversible kinetic scheme (393)

$$C_{aq} \underset{k_2^{obs}}{\overset{k_1^{obs}}{\rightleftharpoons}} C_{org}$$

In this scheme, C_{aq} and C_{org} are the concentrations of a solute in the aqueous and organic phase respectively; it is

assumed that only single molecules transfer from one phase to the other. The observed transport rate constant for the transfer of the aqueous to the organic phase is expressed as k_1^{obs} and for the reverse transfer as k_2^{obs}.

At equilibrium:

$$k_1^{obs} \cdot C_{aq} = k_2^{obs} \cdot C_{org}$$

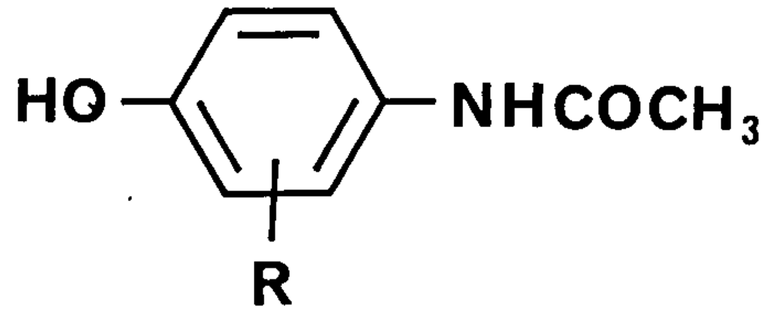
From the definition of the partition coefficient (P) it follows that the quotient of the observed rate constants equals the partition coefficient.

$$P = \frac{C_{org}}{C_{aq}} = \frac{k_1^{obs}}{k_2^{obs}}$$

10.1 Experimental Section

Compounds

Paracetamol Derivatives



R	Name	Molecular Weight
1. H	p-hydroxyacetanilide	151.16
2. 3-Me	3-methyl-4-hydroxyacetanilide	165.22
3. 3,5-Me	3,5-dimethyl-4-hydroxyacetanilide	179.24
4. 2-Me	2-methyl-4-hydroxyacetanilide	165.22
5. 2,3-Me	2,3-dimethyl-4-hydroxyacetanilide	179.24
6. 3-Et	3-ethyl-4-hydroxyacetanilide	179.22
7. 2,3,4-Me	2,3,5-trimethyl-4-hydroxyacetanilide	193.30
8. 2,5-Me	2,5-dimethyl-4-hydroxyacetanilide	179.24
9. 3-iPr	3-isopropyl-4-hydroxyacetanilide	193.20

Apparatus

All solutions were prepared by weighing the solute on a Beckman LM 500 Microbalance and transferring to Grade A volumetric flasks to be made up to volume.

All concentrations were measured spectrophotometrically with a Perkin Elmer 550 U.V. spectrophotometer. The tubing via which samples were obtained was Teflon with a section of silicone tubing on the delivery end to which was fitted a screw clip for regulating flow. · Each layer was stirred independently, the aqueous layer by means of a magnetic stirrer and follower and the octanol layer by means of a helical glass rod attached to a mechanical stirrer.

Transport Vessel

The transport vessel is shown in Figure 17 and described in Chapter 4. The inner compartment of the vessel was thermostatically controlled with a water jacket via A and B. Each solvent layer was stirred independently and samples were taken via a short length of Teflon tubing which was passed through the octanol layer into the aqueous layer. Gravity was used to obtain the sample which was delivered into a apectrophotometric cuvette at specified time intervals. The tubing held $0.5cm^3$ of solvent and each sample was obtained in 30 seconds.

Method

A two phase model was used, consisting of mutually saturated water and octanol. The water was distilled and the octanol supplied by Koch-Light of previously tested quality. The apparatus was assembled as in Figure 17 with 100ml of each phase being used. For forward rate constant determination a known quantity of solute ($\sim 10^{-3}$M) was dissolved in the aqueous phase and the decrease in concentration fron this phase was followed by U.V.spectrophotometry after mixing the two phases. For reverse rate constant determination the solute was dissolved in the octanol phase and for ease of measurement, the increase in concentration in the aqueous phase was measured, and from this the decrease in concentration in the octanol phase was calculated. Each phase was stirred independently stirring rates adjusted so that no vortex could be seen at the interface. The efficiency of stirring was tested before the experiment began by addition of a crystal of

potassium permanganate. The stirring rate was kept constant throughout the experiment. The transport process was followed for twenty minutes and each experiment was repeated. The temperature was maintained throughout at 25°C.

The sample used for U.V. absorption measurement was collected in a U.V. cell via a length of Teflon tubing. The tubing was placed in the aqueous layer before the addition of octanol so that there was no chance of octanol contamination. This then allowed an aqueous sample to be collected by capillary action. The length of tubing used contained 0.5ml liquid and a flow rate of 5ml per minute allowed an adequate sample to be collected in 30 seconds. After the absorbance had been read, the sample was returned to the transport vessel. Samples were taken at two minute intervals.

For each compound a graph was plotted of change in concentration in octanol and aqueous phase against time. From these rate graphs the forward and reverse rate constants were calculated which were then plotted against previously obtained biological activities (304).

6. 3-Ethyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.965	-3.948	0	-3.258	-3.263
2	-3.988	-3.968	2	-3.262	-3.266
4	-4.008	-3.984	4	-3.263	-3.267
6	-4.031	-4.005	6	-3.265	-3.268
8	-4.055	-4.028	8	-3.267	-3.270
10	-4.077	-4.049	10	-3.268	-3.271
12	-4.104	-4.072	12	-3.269	-3.272
14	-4.126	-4.086	14	-3.271	-3.273
16	-4.150	-4.107	16	-3.272	-3.274
18	-4.167	-4.126	18	-3.273	-3.275
20	-4.193	-4.146	20	-3.274	-3.276

7. 2,3,5-Trimethyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.974	-3.906	0	-3.297	-3.296
2	-4.008	-3.926	2	-3.303	-3.299
4	-4.031	-3.948	4	-3.306	-3.302
6	-4.048	-3.968	6	-3.308	-3.304
8	-4.061	-3.981	8	-3.310	-3.308
10	-4.085	-3.992	10	-3.313	-3.309
12	-4.099	-4.010	12	-3.315	-3.311
14	-4.113	-4.031	14	-3.316	-3.312
16	-4.134	-4.046	16	-3.318	-3.313
18	-4.146	-4.061	18	-3.320	-3.315
20	-4.162	-4.074	20	-3.323	-3.318

10.2 Results

1. Paracetamol (p-hydroxyacetanilide)

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-4.142	-4.128	0	-3.206	-3.200
2	-4.154	-4.142	2	-3.222	-3.210
5	-4.171	-4.156	4	-3.232	-3.221
8	-4.188	-4.167	6	-3.240	-3.230
11	-4.202	-4.179	8	-3.248	-3.240
14	-4.215	-4.193	10	-3.257	-3.246
			12	-3.265	-3.253

2. 3-Methyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-4.078	-4.089	0	-3.152	-3.171
1	-4.101	-4.101	1	-3.157	-3.186
2	-4.114	-4.114	2	-3.159	-3.189
4	-4.125	-4.135	4	-3.163	-3.194
6	-4.143	-4.157	6	-3.167	-3.201
8	-4.165	-4.173	8	-3.172	-3.204
10	-4.185	-4.191	10	-3.176	-3.208
12	-4.205	-4.209	12	-3.179	-3.211

3. 2,3-Dimethyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.967	-3.980	0	-3.593	-3.596
2	-3.984	-3.986	2	-3.596	-3.599
4	-3.993	-3.996	4	-3.599	-3.601
6	-4.000	-4.013	6	-3.603	-3.605
8	-4.010	-4.028	8	-3.608	-3.609
10	-4.020	-4.039	10	-3.612	-3.615
12	-4.033	-4.052	12	-3.615	-3.619
14	-4.044	-4.066	14	-3.620	-3.625
16	-4.057	-4.080	16	-3.625	-3.631
18	-4.066	-4.092	18	-3.631	-3.635
20	-4.072	-4.107	20	-3.635	-3.637

4. 3,5-Dimethyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.990	-3.951	0	-3.023	-3.042
1	-4.000	-3.952	1	-3.026	-3.046
2.5	-4.023	-3.953	2.5	-3.027	-3.047
4	-4.042	-3.974	4	-3.030	-3.049
6	-4.058	-3.998	6	-3.033	-3.052
8	-4.078	-4.023	8	-3.036	-3.055
10	-4.096	-4.050	10	-3.038	-3.058
12	-4.115	-4.075	12	-3.041	-3.060
14	-4.135	-4.106	14	-3.043	-3.062
16	-4.155	-4.128	16	-3.045	-3.064
18	-4.173	-4.152	18	-3.047	-3.066

5. 2-Methyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.980	-4.038	0	-3.357	-3.732
2	-3.993	-4.049	2	-3.378	-3.744
4	-4.009	-4.058	4	-3.386	-3.750
6	-4.022	-4.073	6	-3.396	-3.761
8	-4.036	-4.092	8	-3.406	-3.770
10	-4.045	-4.114	10	-3.411	-3.780
12	-4.057	-4.131	12	-3.417	-3.787
14	-4.078	-4.150	14	-3.430	-3.796
16	-4.107	-4.157	16	-3.436	-3.807
18	-4.139	-4.173	18	-3.439	-3.815

8. 2,5-Dimethyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.970	-3.868	0	-3.582	-3.265
2	-3.975	-3.880	2	-3.588	-3.268
4	-3.991	-3.893	4	-3.590	-3.274
6	-4.008	-3.917	6	-3.594	-3.280
8	-4.025	-3.921	8	-3.599	-3.286
10	-4.039	-3.929	10	-3.604	-3.292
12	-4.060	-3.944	12	-3.609	-3.298
14	-4.083	-3.955	14	-3.611	-3.302
16	-4.107	-3.975	16	-3.616	-3.305
18	-4.117	-3.986	18	-3.620	-3.309
20	-4.139	-3.998	20	-3.623	-3.313

9. 3-Isopropyl-4-Hydroxyacetanilide

Forward Rate Constant			Reverse Rate Constant		
Time(min)	Log Conc.H_2O(M)		Time(min)	Log Conc.Octanol(M)	
	Run 1	Run 2		Run 1	Run 2
0	-3.994	-4.003	0	-3.2904	-3.2915
2	-4.019	-4.028	2	-3.2915	-3.2926
4	-4.053	-4.058	4	-3.2926	-3.2934
6	-4.087	-4.090	6	-3.2939	-3.2934
8	-4.122	-4.128	8	-3.2948	-3.2952
10	-4.159	-4.162	10	-3.2957	-3.2961
12	-4.203	-4.203	12	-3.2966	-3.2970
14	-4.225	-4.245	14	-3.2974	-3.2979
16	-4.260	-4.277	16	-3.2982	-3.2988
18	-4.297	-4.299	18	-3.2986	-3.2996
20	-4.331	-4.332	20	-3.2990	-3.3006

Figure 64. Transfer of 3-Isopropyl-4-Hydroxyacetanilide in the Octanol/Water System

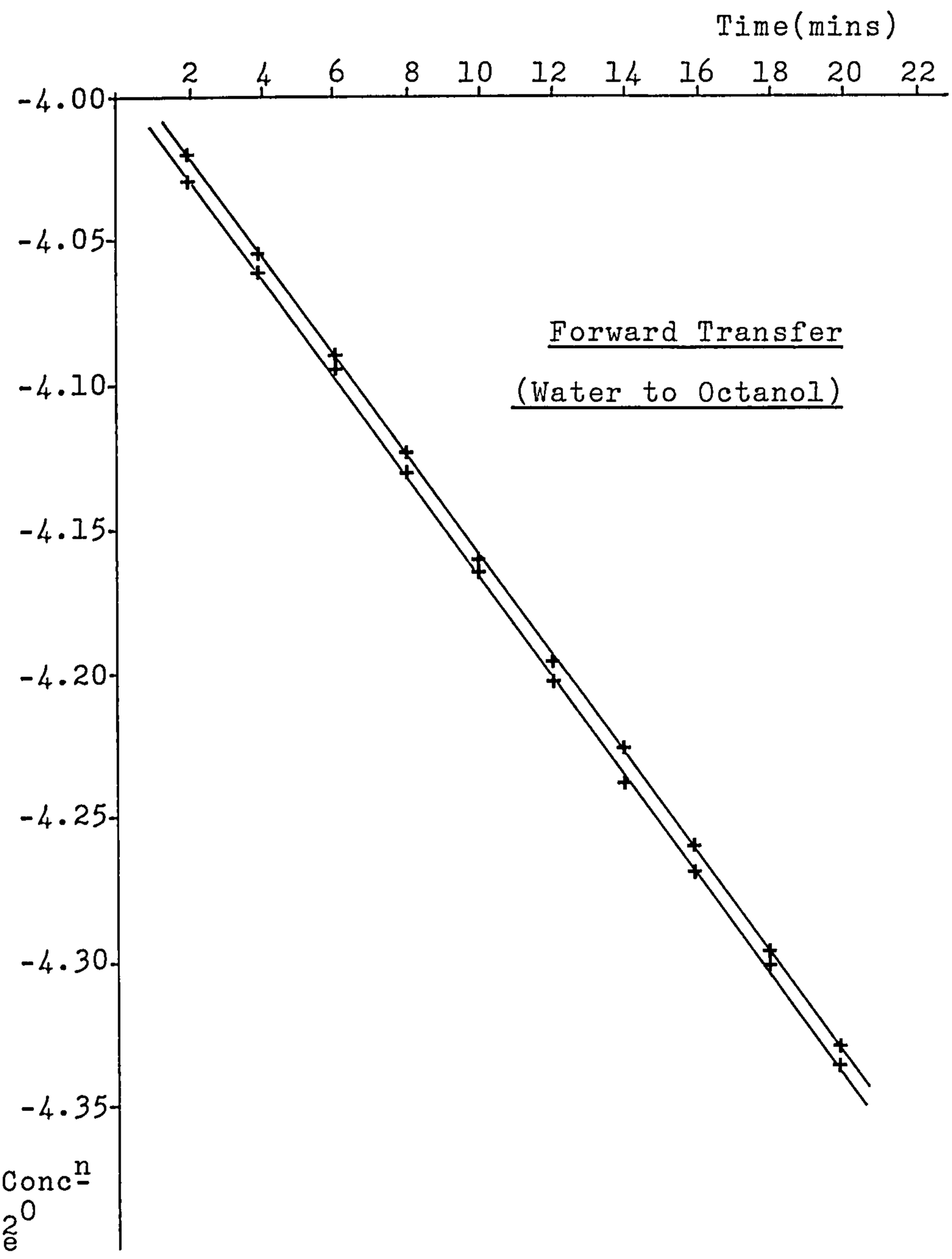


Figure 65. Transfer of 3-Isopropyl-4-Hydroxyacetanilide in the Octanol/Water System

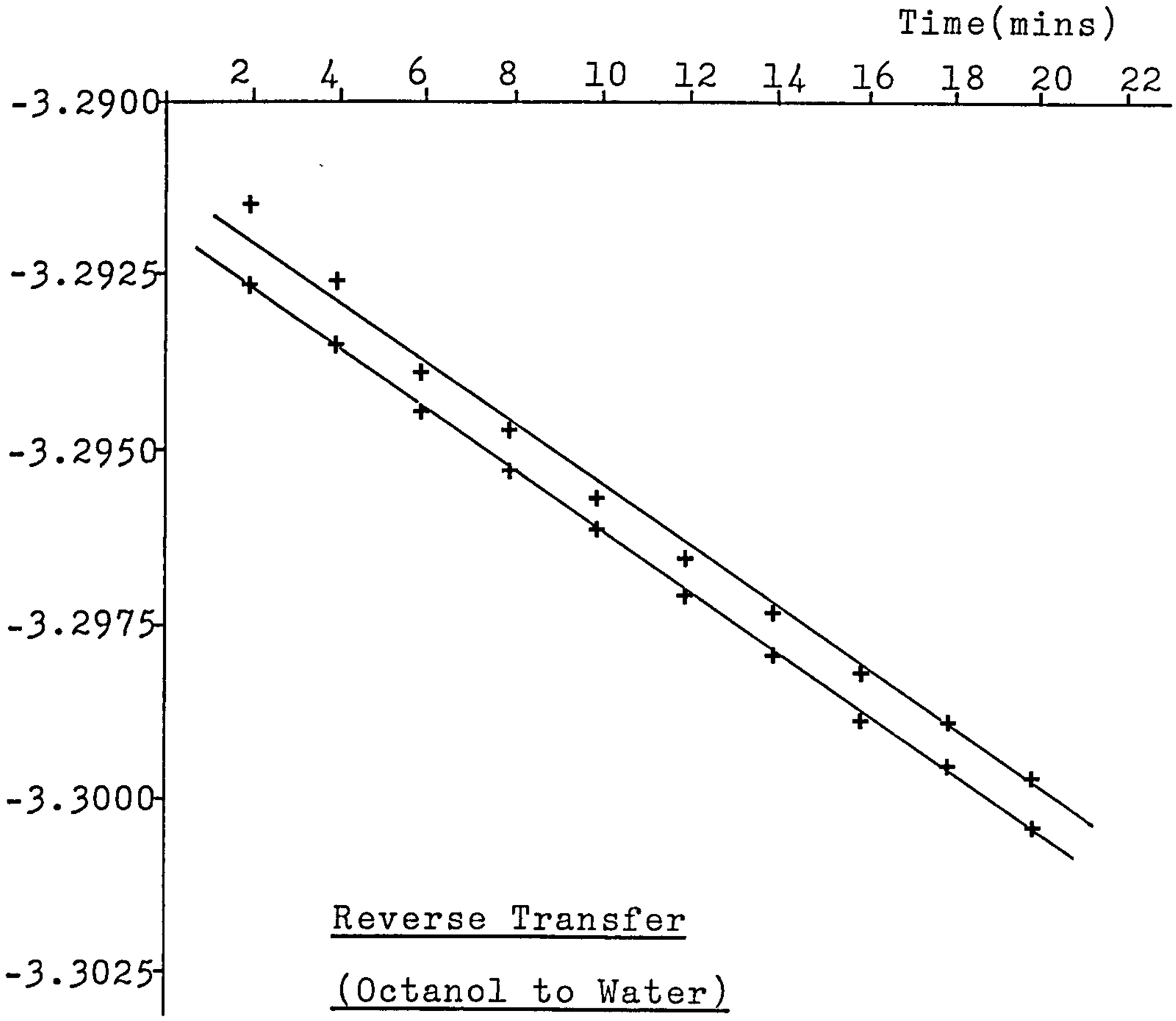
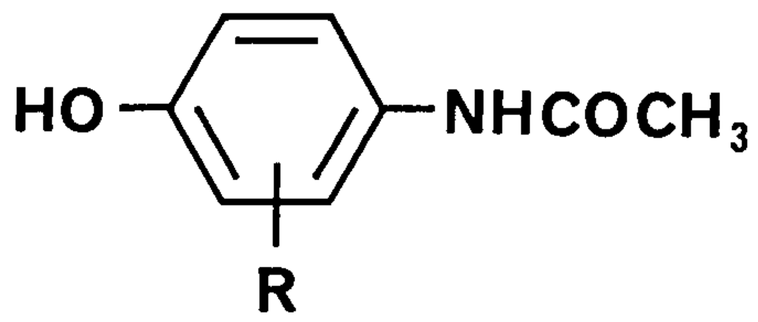


Table 58. Forward Rate Constants



R	Slope= $\frac{-k}{2.303}$	$k_{w\rightarrow o}$ $hr^{-1}{}_m{}^{-2}$
H	-0.0057	+261.3
3-Me	-0.00955	+437.1
3,5-Me_2	-0.0101	+516.6
2-Me	-0.0074	+340.2
2,3-Me_2	-0.0060	+274.5
3-Et	-0.0108	+494.7
2,3,5-Me_3	-0.0083	+379.5
2,5-Me_2	-0.0082	+375.5
3-iPr	-0.175	+274.5

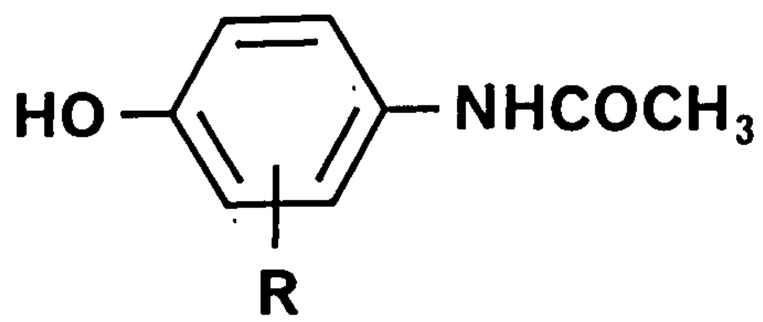
Compounds travel across a surface interface area of $30.3cm^2$ in x minutes. For the calculation of the forward and reverse rate constants therefore, k is multiplied by 60 x 331.13.

$$\text{Slope} = \frac{-k_{w\rightarrow o}}{2.303}$$

Therefore: $k_{w\rightarrow o} = -\text{Slope} \times 2.303 \times 60 \times 331.13\ hr^{-1}{}_m{}^{-2}$

where $k_{w\rightarrow o}$ = forward rate constant

Table 59. Reverse Rate Constants



R	Slope = $\frac{-k_{o\rightarrow w}}{2.303}$	$k_{o\rightarrow w}$ $hr^{-1}m^{-2}$
H	-0.0041	+187.6
3-Me	-0.0021	+ 95.2
3,5-Me_2	-0.0014	+ 64.0
2-Me	-0.0043	+199.0
2,3-Me_2	-0.0020	+ 93.8
3-Et	-0.0007	+ 32.3
2,3,5-Me_3	-0.00108	+ 49.5
2,5-Me_2	-0.00227	+103.8
3-isopropyl	-0.00045	+ 20.9

Compounds travel across a surface interface area of 30.3cm^2 in x minutes. Therefore k is multiplied by 60 x 331.13.

$$\text{Slope} = \frac{-k_{o\rightarrow w}}{2.303}$$

Therefore: $k_{o\rightarrow w}$ = -Slope x 2.303 x 60 x 331.13 $hr^{-1}m^{-2}$

where $k_{o\rightarrow w}$ = reverse rate constant.

Partition Coefficients

Compound	LogP(Rate)	LogP(Shake Flask)	LogP(Calc)	LogP(Lit)
H	0.164	0.311	0.311	0.36
2-Me	0.185	0.173	0.871	0.66
3-Me	0.682	0.793	0.871	1.28
$2,3\text{-Me}_2$	0.522	0.573	1.431	1.06
$2,5\text{-Me}_2$	0.607	0.597	1.431	1.09
$3,5\text{-Me}_2$	0.966	1.108	1.431	1.60
$2,3,5\text{-Me}_3$	0.845	0.816	1.991	1.30
3-Et	1.212	1.306	1.331	1.79
3-iPr	1.615	1.707	1.841	2.20

LogP(Rate) was calculated from the forward and reverse transfer rate constants:

$$\text{Partition Coefficient} = \frac{\text{Forward transfer rate}}{\text{Reverse transfer rate}}$$

LogP(Shake Flask) These results were taken from the work of O'Hara (304). The method used consisted of a stoppered round-bottomed flask containing accurately pipetted volumes of the two phases, the aqueous phase initially containing a known concentration of the drug (10^{-4} to 10^{-5}M). The flask, containing a Teflon-coated magnetic follower, was placed on a magnetic stirrer and the contents were stirred at a rate to give good interphase mixing but without the appearance of a visible vortex. The temperature was maintained at $25^{\circ}C \pm 1^{\circ}C$ and mixing was continued for 45 minutes.

LogP(Calc) These values were calculated from the experimental log P value of the parent compound 4-hydroxyacetanilide plus the appropriate π values taken from Hansch (189)

Analgesic Activity

Compound	Log $1/ED_{30}$
H	0.455
2-Me	0.156
3-Me	0.475
$2,3\text{-Me}_2$	0.522
$2,5\text{-Me}_2$	0.341
$3,5\text{-Me}_2$	0.659
$2,3,5\text{-Me}_3$	0.646
3-iPr	0.050
3-Et	0.476

These results were again taken from the work of O'Hara (304) The analgesic activity of the drugs was measured by means of the abdominal constriction response test. This is the most effective method for assessing non-narcotic analgesics and consists of inhibition of chemically induced writhing in mice.

The drugs were suspended in 0.7% methyl cellulose and 0.9% sodium chloride solution in a volume of 10ml/kg and were administered sub-cutaneously in a weight-related dose. Ten minutes after administration of the drug the writhing agent was administered. This consisted of a 0.2% solution of phenyl-1-4-benzoquinone containing 5% absolute alcohol. It was injected intraperitoneally in a standard dose of 0.2ml per mouse. Control mice were treated identically except that the sub-cutaneous injection was of suspending vehicle only. The number of writhes in a pre-determind time were then counted and the results used to calculate biological activity.

10.3 Discussion

The compounds used in this present work were taken from a study by O'Hara (304) of the physico-chemical parameters and biological activities of a series of alkyl-substituted 4-hydroxyacetanilides. Selection of these compounds was made on the basis that they had proven analgesic activity which could be correlated with the forward and reverse rate constants measured in the present study.

O'Hara found that the analgesic activities of these derivatives exhibited a parabolic dependence on log P as illustrated in Figure 66.

A number of equations were formulated to describe this relationship:

1) $\log 1/ED_{30} = -0.049\pi + 0.444$ n=9, r=0.115, s=0.217

2) $\log 1/ED_{30} = 0.885\pi - 0.757\pi^2 + 0.324$
n=9, r=0.881, s=0.112

3) $\log 1/ED_{30} = 1.365\log P - 0.761\log P^2 - 0.027$
n=9, r=0.881, s=0.114

A comparison of equations 1 and 2 shows the marked improvement of the correlation effected by the inclusion of the π^2 term and the vastly increased percentage of the variance in the data accounted for by equation 2 (77% i.e. r^2=0.77) since equation 1 accounts for only some 1.4% of the variance.

An interesting comparison may be made between the relationship illustrated in Figure 66 and that of Figure 67 which shows the correlation (or rather lack of correlation) between

Figure 66. The Correlation of Analgesic Activity (Log $1/ED_{30}$) with Log P

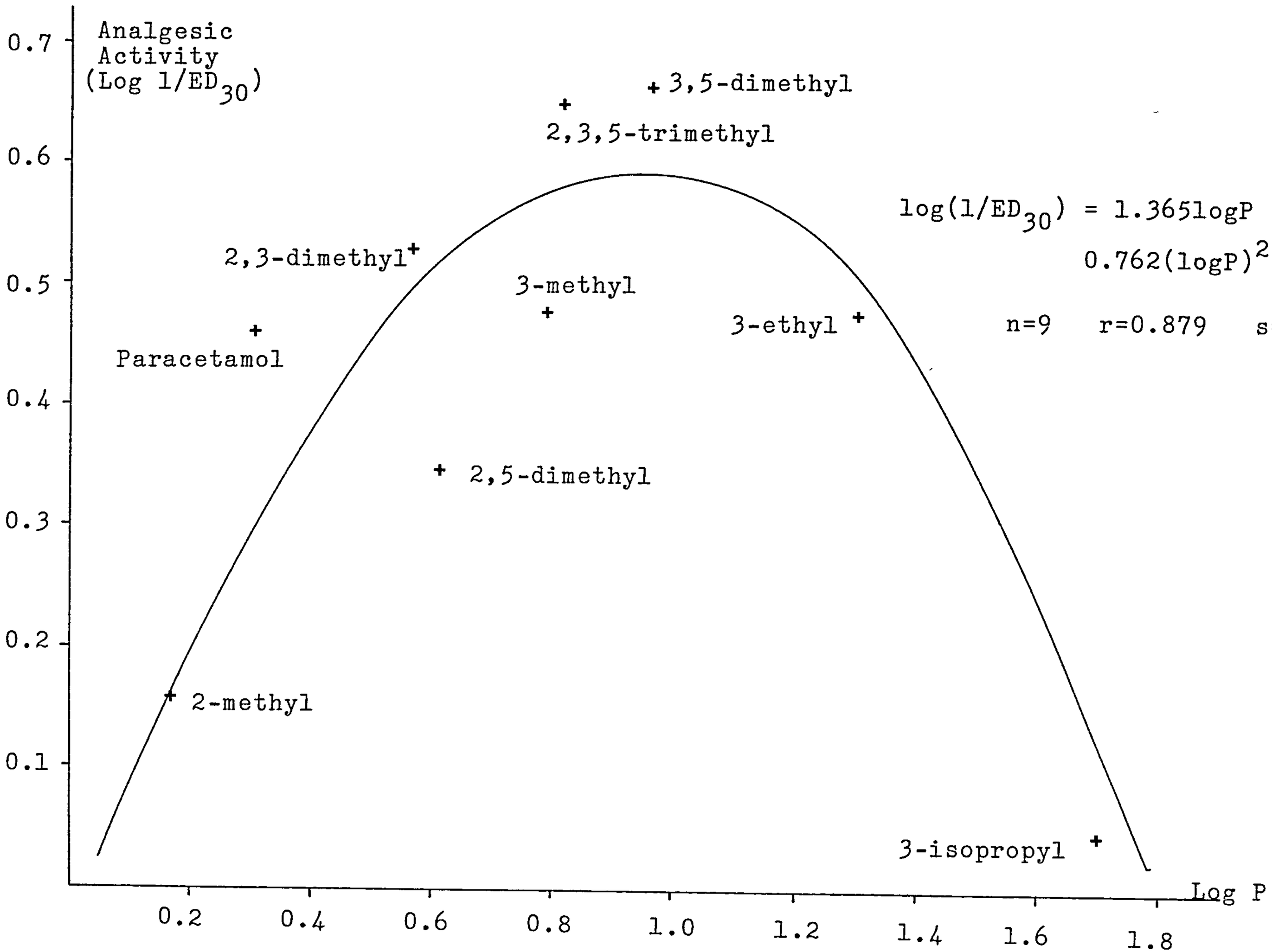


Figure 67. The Correlation of Analgesic Activity (Log $1/ED_{30}$) with Log P - Calculated from π Values

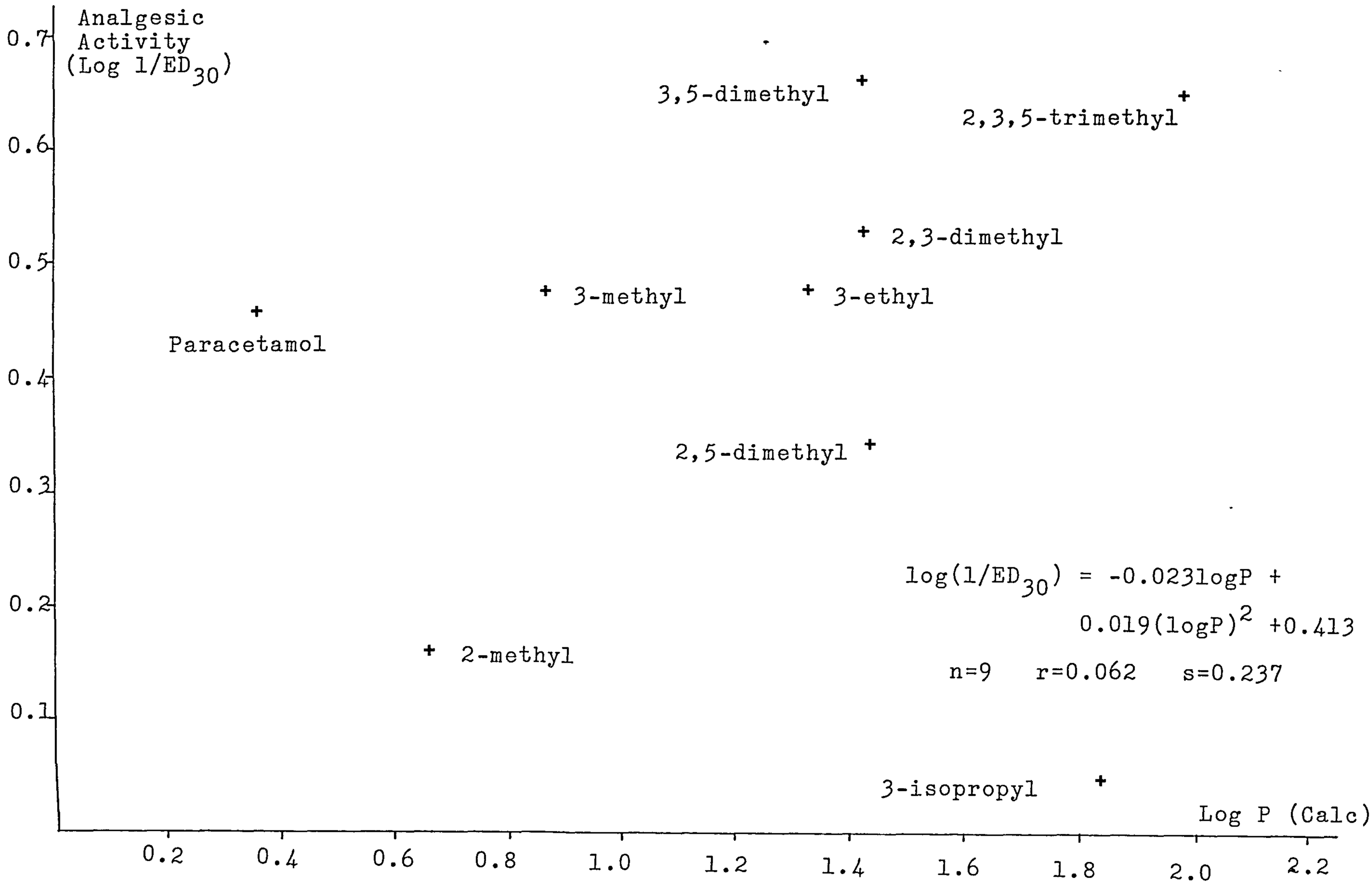
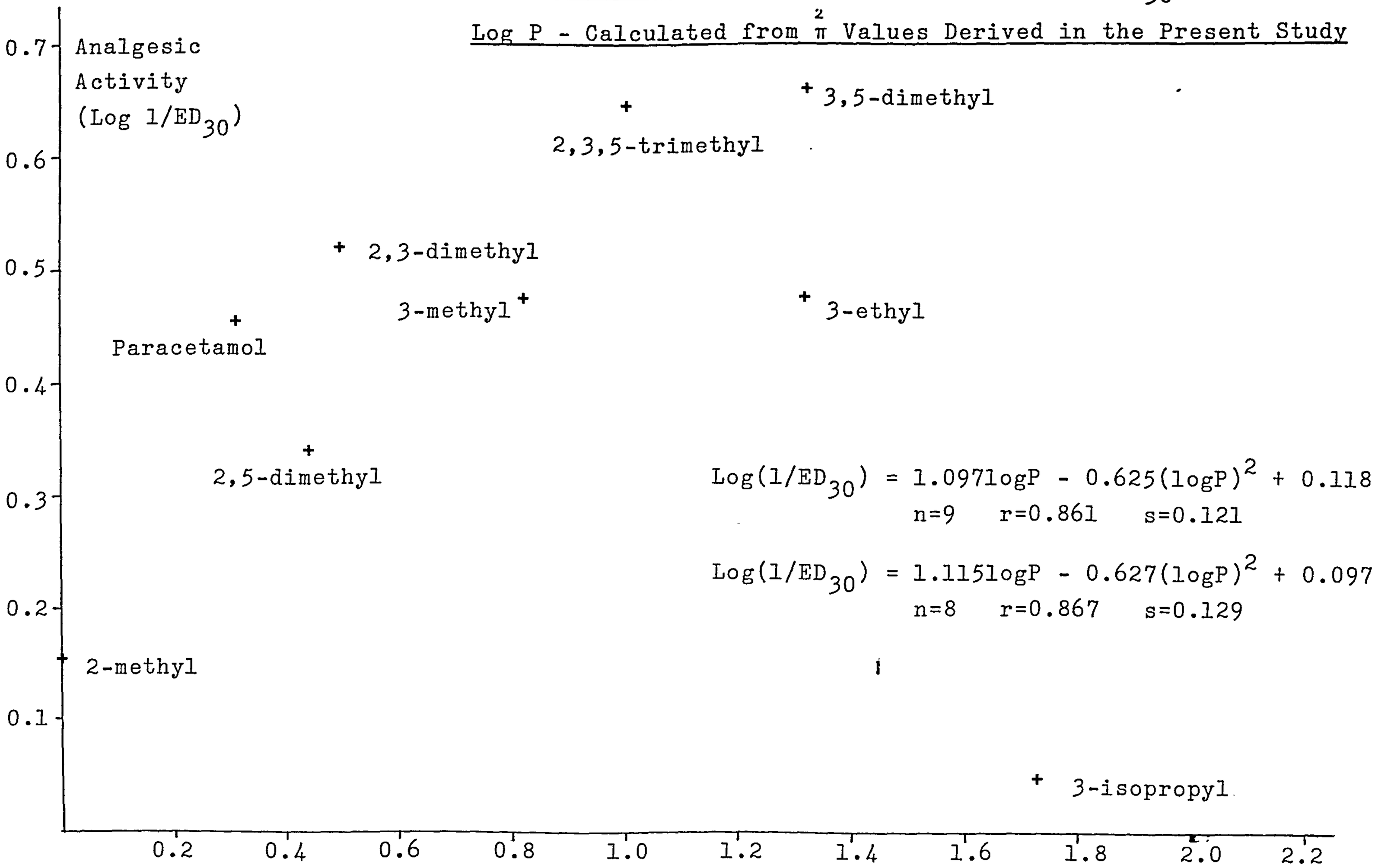


Figure 68. The Correlation of Analgesic Activity (Log $1/ED_{30}$) with Log P - Calculated from π^2 Values Derived in the Present Study



log $1/ED_{30}$ and log P using the principle of additivity for calculating log P values for the derivatives from the experimental value for the parent compound. The calculated log P values were obtained using π values taken from Hansch (189) of 0.56, 1.02 and 1.53 for Me, Et and iPr respectively. This illustration illustrates the need to use experimental rather than calculated values of partition coefficient in cases where ortho substitution is involved.

However, Figure 68 shows the relationship between log $1/ED_{30}$ and log P calculated from substituent constants measured in the present work and recorded in Table 24 and 26. This shows much better correlation and indicates the importance of group environment in the assessment of its π value. By using group values calculated from the acetanilide system for the substituents ortho to the $NHCOCH_3$ group and group values from the phenol system for those substituents ortho to the OH group, the accuracy of the calculated log P values is improved. This study did not examine the ethyl group or the isopropyl group so values for these substituents were taken from Fujita (149). They are however also calculated for the ortho position in the phenol system.

However, even the parabolic relationship shown in Figure 66. and described by equation 2, is far from perfect, some 23% of the variance in analgesic activity being unaccounted for. In order to improve correlation, O'Hara included various experimental parameters to provide some measure of electronic and/or steric effects. These were; ΔpKa, ΔνN-H (the difference between the frequencies of the parent and

the substituted compound for N-H endo stretch), σ^{o}, $\Delta\tau_{CH_3CO}$ (differences in acetyl proton chemical shift between the parent and substituted derivatives) and Taft's steric substituent constant, E_s.

However, none of these variables was found to significantly improve the correlation. This would seem to suggest that the most complete interpretation of the analgesic activity is given by the lipophilicity of the compound and that electronic and steric effects play little overall part. If this is the case, it may be that much of the variance not accounted for by equation 2 is due to experimental error, most probably in the estimation of analgesic activity. Alternatively, it was felt that the correlation could possibly be further improved by showing that activity could be related to transport rate constants. Thus the transport rate constants for both forward and reverse transfer in the octanol/water system were calculated and correlated with biological activity. The rate constants are given in Tables 58 and 59 .

The relationship between analgesic activity and the forward rate constant is illustrated in Figure 69 and described by the equation:

4) $\log(1/ED_{30}) = -30.409\log K_F + 6.100(\log K_F)^2 + 38.228$

$n = 9 \qquad r = 0.549 \qquad s = 0.199$

The relationship between analgesic activity and the reverse rate constant is illustrated in Figure 70 and described by the equation:

Figure 69. The Relationship Between Forward Rate Constants Measured in an Octanol/Water System and Analgesic Activity of Selected Hydroxyacetanilides

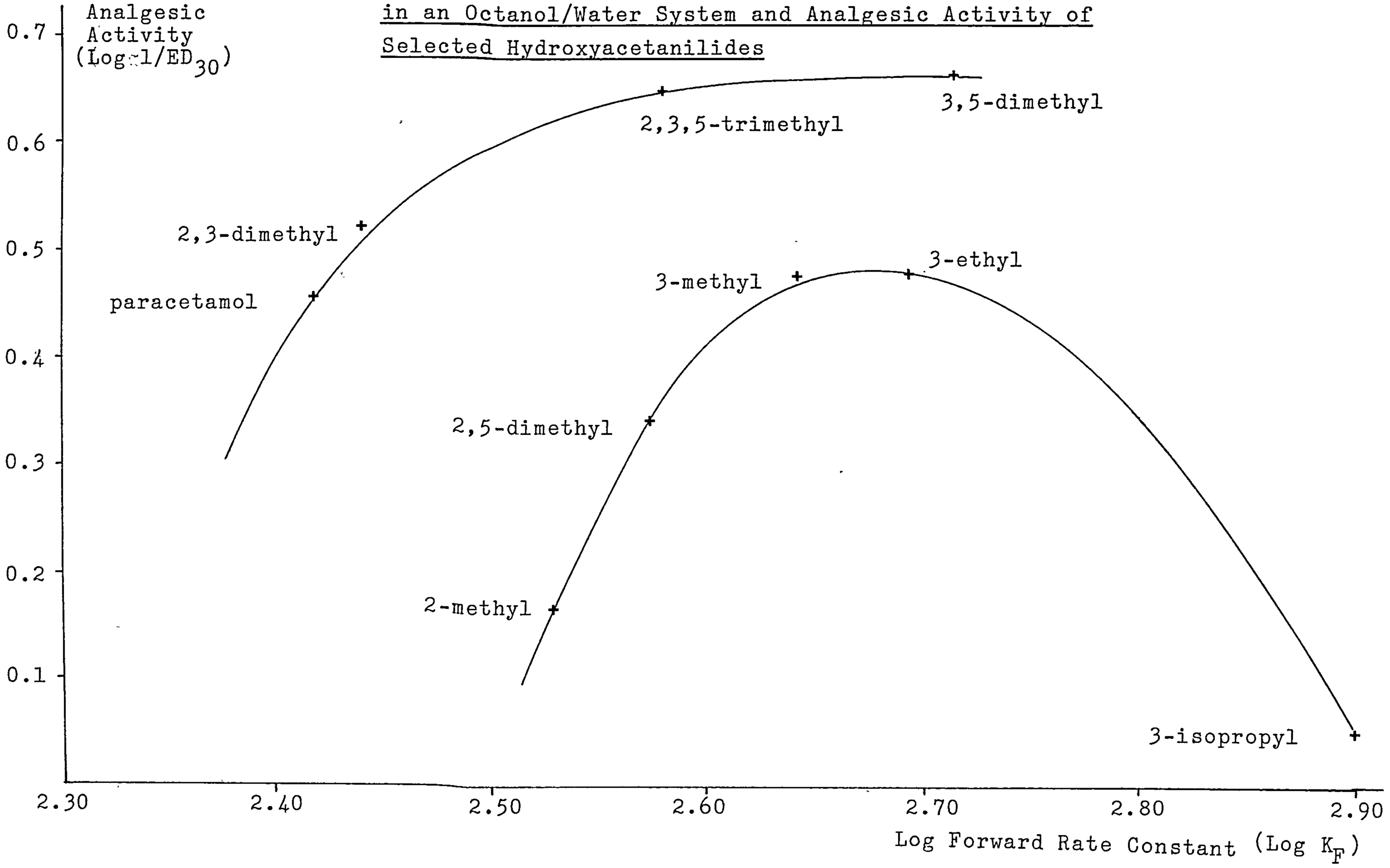
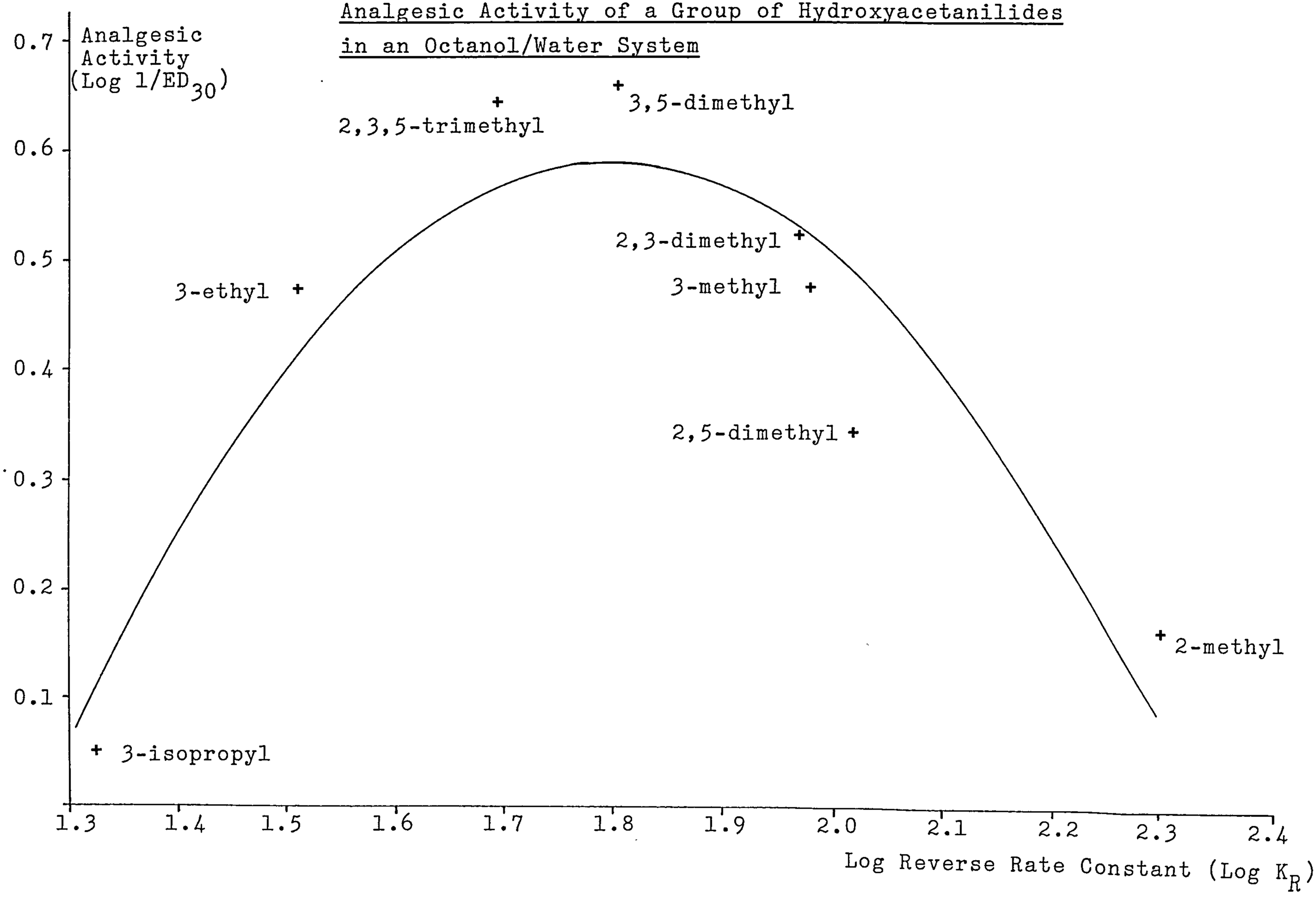


Figure 70. The Relationship Between Reverse Rate Constants and Analgesic Activity of a Group of Hydroxyacetanilides in an Octanol/Water System



5) $\log(1/ED_{30}) = +6.073\log K_R - 1.651(\log K_R)^2 - 5.006$

$n = 9 \qquad r = 0.819 \qquad s = 0.136$

The correlation between analgesic activity and the reverse rate constant is much better than that between analgesic activity and the forward rate constant, but it provides no improvement upon the correlation already found between logP and analgesic activity.

However, it has already been stated that the partition coefficient is equal to the quotient of the observed rate constants, such that, $\log P = \log K_F/K_R$. Therefore this value of logP was also used in the regression. This produced the equation: (see Figure 71)

6) $\log(1/ED_{30}) = 1.153\log(K_F/K_R) - 0.715(\log(K_F/K_R))^2 - 0.0963$

$n = 9 \qquad r = 0.806 \qquad s = 0.141$

Again it can be seen that unfortunately no improvement has been made to the correlation, although it has similar significance to that obtained with $\log K_R$.

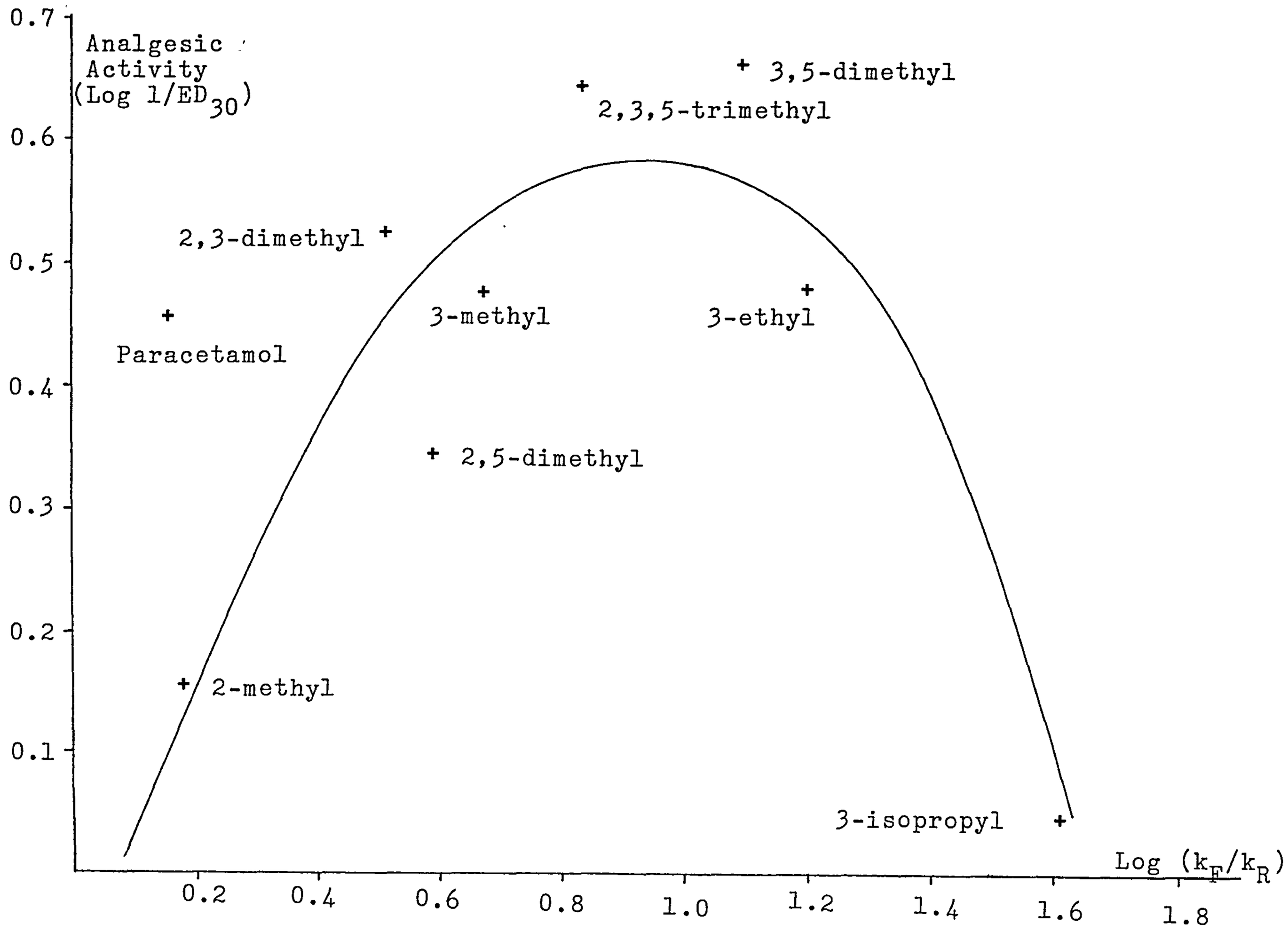
It was noted that there was a discrepancy between the value of logP for Paracetamol determined by the shake flask method and by the ratio of rate constants ($\log P_{\text{shake flask}} = 0.311$ and $\log P_{(K_F/K_R)} = 0.164$) therefore this compound was removed from the regression.

This produced the following set of equations:

7) $\log(1/ED_{30}) = 1.541\log P - 0.829(\log P)^2 - 0.133$

$n = 8 \qquad r = 0.919 \qquad s = 0.103$

Figure 71. The Correlation of Analgesic Activity (Log $1/ED_{30}$) with Log (k_F/k_R)



8) $\log(1/ED_{30}) = -14.338\log K_F + 3.016(\log K_F)^2 + 17.313$

n = 8 r = 0.585 s = 0.211

9) $\log(1/ED_{30}) = +7.388\log K_R - 2.052(\log K_R)^2 - 6.059$

n = 8 r = 0.922 s = 0.101

10) $\log(1/ED_{30}) = 1.669\log(K_F/K_R) - 0.946(\log(K_F/K_R))^2 - 0.156$

n = 8 r = 0.922 s = 0.100

This produced an improved correlation for all parameters, but the significant improvement for $\log K_R$ and $\log(K_F/K_R)$ suggests an error in the paracetamol data. However, as before, the correlation between the forward rate constant and analgesic activity is poor.

Correlations with various combinations of K_F and K_R were attempted but none improved the correlation significantly other than use of $\log(K_F/K_R)$ as already recorded. This does not imply that dynamically determined logP values will produce better results than the experimentally determined partition coefficient since the correlation is not significantly improved, but it does suggest that in cases where anomalies are apparent, the dynamic logP should be investigated. For this series of compounds however, these results suggest that partition coefficient is a satisfactory parameter to use in a regression analysis.

The fact that K_R correlates much better than does K_F with analgesic activity may in part be accounted for by the much wider range of the former ($\log K_R$ 1.309 - 2.299) than the latter rate constant ($\log K_F$ 2.439 - 2.904). However, the improved correlation indicates that the reverse rate of

transfer is the predominant factor governing <u>in-vivo</u> transport in this series of compounds. This illustrates the importance of the forward and reverse rates of transfer and the effect changes may have on the partition coefficient. The fact that K_R does not correlate significantly better than does P with biological activity in this series of compounds probably means that none of the compounds has anomalous partitioning rate behaviour, but the fact that the correlation with K_R is almost identical to that with P indicates the importance of transport rate in determining partition coefficient. The fact that a significant correlation can be found indicates that further investigation of partitioning rate behaviour as a possible parameter for the refining of quantitative structure activity relationships should be encouraged.

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Appendix

Published Work

Partitioning rate constant as a parameter in quantitative structure-activity relationships (QSARs)

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Many studies have shown that, within a series of congeners, biological activity correlates more or less well with partition coefficient. Any so-called "outliers" in such relationships are usually explained as anomalies due to, for example, specific receptor binding or metabolism, and attempts are often made to account for these effects by including steric, electronic or other parameters in the correlation.

It must be borne in mind, however, that partition coefficient is an equilibrium constant; as such, it is somewhat inappropriate for describing a biological response which is time-dependent (i.e. non-steady state). Most pharmacological testing is carried out following administration of a single dose, and response is usually measured at a fixed time after dosage. Under such conditions, it is the rate of partitioning, and not the partition coefficient, that will govern the concentration of drug at the receptor at a given time. It might be argued that since partition coefficient is the ratio of the forward and reverse partitioning rate constants, partition coefficient is a satisfactory parameter to use. However, it is known that some compounds have abnormal rates of partitioning (Elson 1978), whilst displaying normal or expected partition coefficients; such compounds could appear as outliers in QSARs.

We have therefore measured forward and reverse partitioning rate constants in the water-octanol system of a series of paracetamol derivatives for which we have already reported a QSAR (Dearden & O'Hara 1976). For the eight compounds examined, correlation of analgesic activity with octanol-water partition coefficient (P) gave:

$$\log(1/ED30) = -0.133 + 1.541 \log P - 0.829(\log P)^2 \qquad (1)$$

$$n = 8 \quad r = 0.919 \quad s = 0.103$$

Correlation with the reverse (i.e. octanol to water) rate constant k_R (m^{-2} h^{-1}) gave an equation of almost identical significance:

$$\log(1/ED30) = -6.050 + 7.379 \log K_R - 2.050(\log K_R)^2 \qquad (2)$$

$$n = 8 \quad r = 0.921 \quad s = 0.101$$

Correlation of biological activity with the forward rate constant k_F gave a poor correlation ($r = 0.601$); correlation with various combinations of k_F and k_R did not improve equation (2) significantly, although it is interesting to note that the use of $\log(k_F/k_R)$ (which is equivalent to $\log P$) gave a correlation coefficient of 0.931.

The fact that k_R correlates much better than does k_F with biological activity is possibly accounted for by the much wider range of the former ($\log k_R$ 1.309 - 2.299) than the latter rate constant ($\log k_F$ 2.439 - 2.904) and indicates that k_R is the predominant factor governing <u>in vivo</u> transport, in this series of compounds at least.

The fact that k_R does not correlate significantly better than does P with biological activity in this series of compounds probably means that none of the compounds has anomalous partitioning rate behaviour. Nonetheless, we commend the further investigation of partitioning rate behaviour as a possible parameter for the refining of quantitative structure-activity relationships.

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Thermodynamics of partitioning - some considerations of intramolecular hydrogen bonding and steric effects

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Intramolecular hydrogen bonding and steric effects are two factors that contribute to the non-additivity of hydrophobic substituent constants (π values) (Dearden & O'Hara 1975). As part of a study of such phenomena, we have investigated the thermodynamics of partitioning, in the octanol-water system, of the compounds shown in Table 1, using the probe method devised by Cantwell & Mohammed (1979).

Table 1. Thermodynamic parameters of transfer from water to 1-octanol

	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
o-Hydroxybenzoic acid[a]	-12.9	-24.9	-41.1
m-Hydroxybenzoic acid[a]	-8.9	-21.1	-41.6
p-Hydroxybenzoic acid[a]	-8.9	-23.0	-48.0
o-Chlorophenol	-11.6	-8.9	+9.2
m-Chlorophenol	-13.9	-19.9	-20.6
p-Chlorophenol	-13.4	-15.3	-6.6
o-Methylacetanilide	-4.8	+6.5	+38.5
m-Methylacetanilide	-9.1	-5.7	+11.6
p-Methylacetanilide	-9.1	-5.5	+12.4

[a]Aqueous phase at pH 1.

The low aqueous solubility of o-hydroxybenzoic acid (0.18% w/v at 20° compared with 0.92% w/v at 18° for the meta-isomer (Handbook 1953)) indicates that its intramolecular hydrogen bond is more or less intact in aqueous solution; the remarkably similar values for both the enthalpy and entropy of partitioning of all the hydroxybenzoic acids thus indicate that the intramolecular hydrogen bond remains intact in octanol, and that the extent of solvation consequent upon transfer from water to octanol is not significantly affected by the presence of the intramolecular hydrogen bond. This may be because salicylic acid, even when intramolecularly hydrogen bonded, still has a free -OH group available for solvation.

A very different situation is observed with the chlorophenols. The aqueous solubilities of all three isomers are very similar (Handbook 1953), indicating that the o-isomer is not intramolecularly hydrogen bonded in water. Octanol being less polar than is water, the intramolecular hydrogen bond re-forms upon transfer, with a consequent reduction in solvation and hence a positive entropy change. One might have expected a larger negative enthalpy of transfer as a result of the intramolecular hydrogen bond's re-forming, but this is more than counterbalanced by the consequent loss of solvation capability.

Dearden & O'Hara (1978) reported an unusual partitioning effect of steric hindrance in o-substituted acetanilides; such an effect is clearly demonstrated by the positive enthalpy of transfer in o-methylacetanilide, and explained by the fact that in this compound the acetamido group is twisted out of the plane of the ring, loses conjugation and becomes less lipophilic (more polar) and is thus readily hydrated. Upon transfer the solvated water is released, leading to a positive enthalpy change and also to the very high positive entropy of transfer.

The above results emphasise the value of thermodynamic considerations in the interpretation of partitioning behaviour.

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