EQUILIBRIUM AND SPECTROSCOPIC ISOTOPE AND SOLVATION EFFECTS

ABSTRACT

The thesis describes experiments on isotope effects on steric and other interactions that may be used to test, for example, the validity of empirical potential functions for non-bonded interactions involving hydrogen. The earlier serendipitous observation of shifts in $\pi \rightarrow \pi^*$ UV absorption bands on deuteriation[1] is exploited as a possible probe for examination of the electronic and geometric changes accompanying deuteriation.

The effect of deuterium substitution on the free energy differences of conformational equilibria with hydrogen in two possible environments is studied for a series of 2-substituted-1,3-dioxanes and 3-phenyl-3,5,5-trimethylcyclohexanone. The time-averaged NMR chemical shifts of suitable protons in the deuteriated and undeuteriated molecules are used to indicate the positions of the equilibria in several solvents, and model compounds are used to provide shift values from which free energy differences for the equilibrating systems may be calculated with the same systematic errors in both the undeuteriated and deuteriated systems. Possible sources of error are discussed, and the largest is shown to be the standard deviation of measurement of the time-averaged NMR chemical shift differences, leading to errors of 1 to 2 cal/mole in isotope effects from -2 to +16 cal/mole on strain energies ~ 4 kcal/mole. The measured effects were not always of a sign compatible with a reduction of "size" on changing from an undeuteriated to a deuteriated group.

Preliminary investigations of systems for which isotope effects on intermolecular proton exchange may be measured, are reported, and isotope effects on the free energy differences between the two hydroxyl groups of partially deuteriated 3,5-di-t-butyl-2,6-dihydroxyacetophenone and of partially deuteriated 3,5-di-t-

butyl-2,6-dihydroxybenzaldehyde O-methyloxime of 50 and 20 cal/mole respectively, protium being favoured in the intramolecularly hydrogen bonded group, are measured by the NMR chemical shifts of hydroxyl proton signals. These chemical shift values are used in conjunction with the hydroxyl proton chemical shifts of suitable models to determine the quoted isotopic changes in free energy differences.

Isotopic changes in the intensities of UV absorption bands are measured for cis- and trans-3,5-di-t-butylcyclohexane-1,3-diones and interpreted in terms of changes in the free energy difference between keto-enol and diketo tautomers. No such change was detected on deuteriation of acetylacetone, contrary to the results reported by Allred and Thompson.[2]

Isotopic shifts in the frequency position of the centroids of UV bands are measured by "difference" spectrometry, a technique whose applicability and accuracy is illustrated by the reproducibility and insensitivity to calibration errors of shifts in the centroids of the IR carbonyl stretching bands between a series of cyclohexanone derivatives measured in the same way. The UV band shifts are interpreted as isotopic changes of differences in the potential energy between the ground state and geometrically different excited states for the enol forms of the above diketones and a series of 4-aminopyridine derivatives.

All the observed effects are interpreted in terms of the reduction of zero-point vibrational energy accompanying substitution of deuterium for hydrogen.[3] However the effect of the smaller "size" of deuteriated groups on steric strain energies appears to be accompanied by isotope effects on other contributions, for example those caused by bond polarities, to non-bonded interactions which may enhance or diminish the "size" effect.

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A Thesis submitted to the

Board of the Faculty of Physical Sciences

in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

in the

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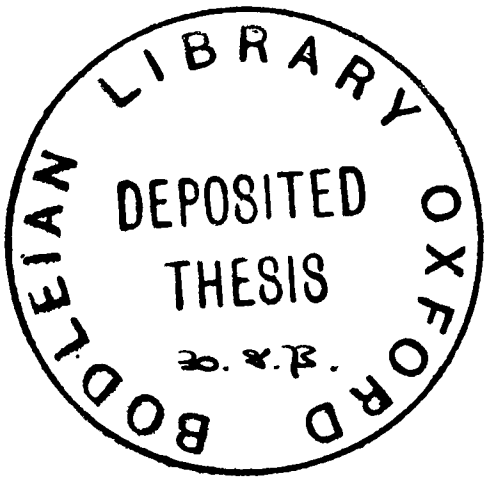
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INTRODUCTION

Increases in molecular weight resulting from the substitution of deuterium for light hydrogen ("protium") in a molecule are proportionally greater than from any other isotopic substitution; hence the resulting isotope effects upon many physical and chemical properties of a system are largest for "deuteriation".

An examination of the consequences of deuteriation has become almost routine in studies of molecular structure involving the assignment of IR and microwave absorption bands.[1,2] Kinetic isotope effects have become a common tool for the elucidation of reaction mechanisms.[3,4] However, in spite of the large amount of attention that isotope effects on transition state thermodynamics have received, relatively little has been paid to ground state effects. Recent surveys of the use of isotopes[5] and isotope effects[4] in chemistry indicate that interest in the effect of isotopic substitution on equilibria has been centred around an analysis of the problems of isotopic separation.

Deuterium bonded to other atoms has a lower frequency of vibration than protium, owing to the former's greater mass. This leads to a smaller average amplitude of anharmonic vibration. Hence sterically repulsive forces might be expected to be greater for a group of atoms containing protium in a crowded environment than for the same group with deuterium replacing protium. This and other consequences of deuteriation may lead to a change in the environmental preference of a bound hydrogen atom which equilibrates between different environments (examined in Chapter One). Partial deuteriation of molecules with hydrogen atoms in different, but rapidly exchanging, environments may lead to a non-statistical distribution of deuterium and protium between these environments (examined in Chapter Two). Also deuteriation may affect the position of a tautomeric equilibrium with hydrogen atoms

in different environments in the different tautomers (examined in Chapter Three).

All the above isotope effects may be expressed as isotopic changes of free energy differences, and must be evaluated from measurements of free energy differences in undeuteriated and deuteriated systems. As will be shown, many of the previous attempts to measure such isotope effects have led to values with uncertainties of the same order of magnitude as the effects. In this work, properties which can be measured with great precision are chosen for the determination of isotopic changes on systems selected for their sensitivity to small changes in equilibrium compositions. The free energy changes which control the value of these properties may only be determined approximately for the individual undeuteriated and deuteriated systems. However, provided that the systematic uncertainties in the value of the free energy changes are the same for the two systems, the effect of such uncertainties on the isotopic difference between the free energy changes is small.

If isotopic substitution affects the potential energy of the ground state of a molecule by changes in vibrational energies, then changes in the energy of electronically excited states may also be expected. Electronic excitation will alter the potential surface for vibrational distortions of the molecule and so the isotopic changes of energy may be different in the ground and excited states. Such differences are investigated as frequency shifts of UV absorption bands on deuteriation, and measured by the use of "difference" spectroscopy (Chapter Three).

CHAPTER ONE

ISOTOPE EFFECTS ON CONFORMATIONAL EQUILIBRIA

DISCUSSION

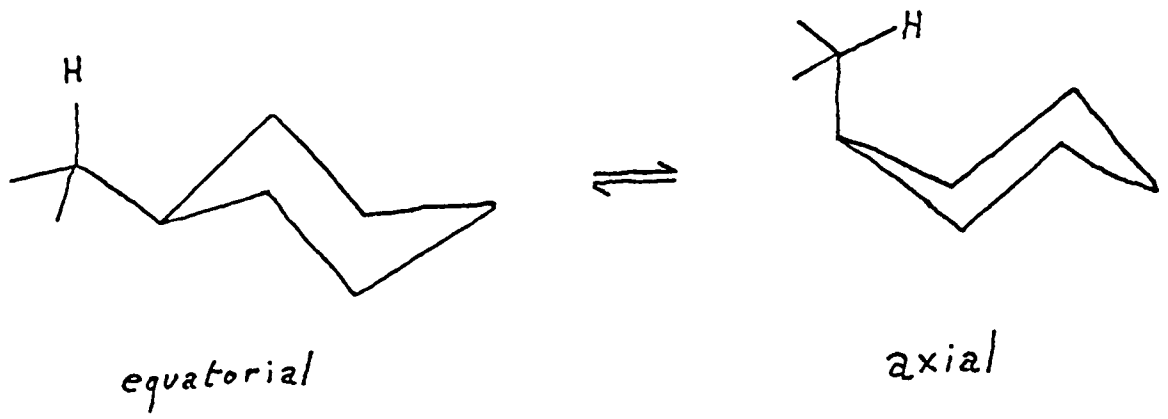


Figure 1.

SYSTEMS FOR NMR SPECTROSCOPIC INVESTIGATION OF CONFORMATIONAL ISOTOPE EFFECTS ON EQUILIBRIA

Increases in reaction rate on deuteriation close to the centre of reaction have been observed for reactions with crowded transition states: the racemisation of optically active 9,10-dihydro-4,5-dimethylphenanthrene[6] and the reaction of methyl iodide with 2,6-dimethylpyridine (2,6-lutidine).[7] These observations are consistent with a decrease in bulk of deuteriated groups resulting from a lower amplitude of anharmonic vibration for bonds to deuterium than for bonds to protium. An attempt to measure such a steric isotope effect upon an equilibrium, the interconversion of cis- and trans-2-methyl-4-t-butylcyclohexanone[8] led to values for the equilibrium isotope effect which were less than the experimental uncertainty (~ 50 cal/mole) resulting from the low precision in measurement of the composition of equilibrium mixtures. The precision in such measurements could be improved by the use of more modern GLC apparatus and the choice of a system with an equilibrium constant closer to unity. However the precision requirements (~ 1 cal/mole) which will be shown to be necessary could still not be met in this way. Therefore, for further attempts to determine steric isotope effects on equilibria, systems, and a form of measurement, which offer a greater degree of precision must be chosen.

A suitable system for the investigation of such effects must be simple enough for analysis in terms of a model. The kind of conformational equilibrium most easily analysed is that between only two conformations (or sets of conformations). Compounds with six-membered rings in two chair conformations provide many examples as in Figure 1. The hydrogen of the 1-substituent is in a more crowded environment in the axial conformation, as a result of steric repulsion from the 3,5-diaxial ring

hydrogen atoms, than in the equatorial conformation.

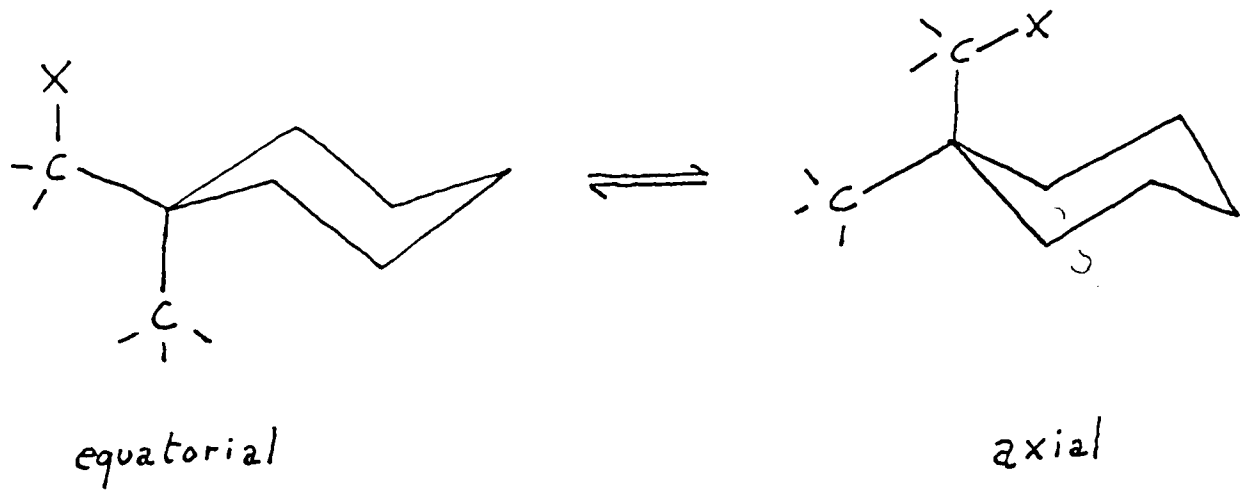
The choice of rings and substituents for the compounds used in this work leads to boat conformations being very disfavoured. Estimates of conformational energy differences between skew-boat and chair forms of 1,3-dioxanes from NMR studies of highly strained dioxanes[9] and of equilibria at high temperatures[10] are that the chair forms are favoured by at least 8 kcal/mole. The strain energy of the 1,3-dioxane derivatives in this study is about 4 kcal/mole greater than in the chair form of 1,3-dioxane, and so skew-boat forms are, at least, 4 kcal/mole greater in conformational energy than chair forms. Hence population of boat conformational forms is not significant for such compounds.

Chair conformations of 3-phenyl-3,5,5-trimethylcyclohexanone are strained as a result of di-axial substituent interaction. However, the presence of four substituents on two of the ring carbon atoms contributes to the strain energy of skew-boat forms to a greater extent which overrides any relief of chair di-axial strain. Allinger[11] has calculated that the skew-boat form of 1,1,3,3-tetramethylcyclohexane is less favoured than the chair form by 4.8 kcal/mole.*

For the greatest accuracy in measurement, the isotope effects should be as

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* Interpretation of the properties of 3-phenyl-3,5,5-trimethylcyclohexanone, by Balasubramanian and Souza, in terms of a significant boat population[12,13] was based upon the erroneous belief that an axial phenyl substituent is unacceptable. Shapiro et al.[14] have shown that the NMR spectrum, as well as the measurements made by Balasubramanian and Souza, are consistent with a simple two-conformation chair system.



equatorial

axial

$K \simeq 1$

X = H or D

Figure 2

large as possible and should be upon systems whose equilibrium constants are close to unity (see the discussion of the calculation of equilibrium constants). A system in which two substituents of approximately equal "size", conveniently, but not necessarily, on the same ring atom, occupy sterically hindered and unhindered positions, their positions being reversed as the result of a change from one conformation to another is used, with and without deuteriation of one of the two substituents as shown in Figure 2.

The property of the equilibrium mixtures that is measured must naturally be sensitive to their composition. Basically two types of measurement are possible, one of which involves observation of the sum of the effects of individual conformers, while the other involves measurement of a time-averaged property of the inter-converting conformers.

If exchange between the two conformations is slow relative to the time scale of measurement, for example in the case of measurement of the intensity of well-separated individual spectroscopic absorptions of the two conformations in the mixture, then

$$n_a = CP_a$$

$$n_e = C'P_e$$

where n_a and n_e are respectively the mole fractions of the axial and equatorial conformations in the equilibrium mixture, P_a and P_e are the absorption intensities, and C and C' are proportionality constants (or functions). For measurement of the intensities of NMR signals $C = C'$ (or, if signals corresponding to different proton groups for the two conformations are used, $C = NC'$ where N is a ratio of two known integers) and measurement of P_a and P_e leads directly to n_a and n_e with no systematic

errors (for measurements on a suitably adjusted instrument!). Many conformational equilibria can be studied in this direct way at low temperatures ($T < -80^{\circ}$), since the activation energy for inversions is usually greater than 10 kcal/mole for saturated six-membered rings.[15] Often the low abundance of the less stable conformation makes integration an inaccurate technique, restricting it to systems of low conformational energy difference, in which cases the precision of measurement is still only two or three per cent, that is, an error of four to six per cent in equilibrium constants, even though they be close to unity.

If exchange between the two conformations is fast relative to the time scale of measurement, a "time-averaged" property, P, must be measured for the mixture where

$$P = n_a P_a + n_e P_e \qquad (1)$$

The NMR chemical shift difference between protons whose magnetic environments are exchanged in the conformational change being studied (or the shift difference between a single group, whose magnetic environment changes greatly from one conformation to another, and an internal standard) is suitable because, in favourable instances, it can be large relative to the precision of measurement. In the examples studied chemical shift differences are measured to an accuracy better than one tenth of one per cent. However, the calculation of n_a and n_e from equation (1) requires values for P_a and P_e corresponding to the chemical shift differences of the individual conformations. These may not be measured directly at room temperature, and must be estimated from measurements upon a "frozen" equilibrium at low temperatures and/or derived from measurements on conformationally biased model compounds for which n_a or $n_e \simeq 1$, and, perhaps, some assumed relation between P_a and P_e. All

of these approaches to the values of P_a and P_e bring systematic errors into the determination of equilibrium constants by measurements of time-averaged properties.

Slow-exchange measurements, then, offer low precision without systematic errors for the determination of conformational equilibrium constants, whilst fast-exchange measurements offer much higher precision with systematic errors. Therefore, provided that the systematic errors are the same for undeuteriated and deuteriated systems, measurement of a time-averaged property would be expected to be a more fruitful approach to the determination of conformational isotope effects. If the chemical shift difference between suitable protons is chosen as the property P, any effect of deuteriation on the systematic errors may be sought as a change in the value of P_a (or P_e) between the deuteriated and undeuteriated systems.

1,3-Dioxanes are particularly suitable for measuring conformational effects on NMR spectra. Their spectra, generally showing protons in fewer and more widely different magnetic environments than, for example, cyclohexanes, are readily understood and the positions of some signals may be precisely measured. 2-Methyl-1,3-dioxane has a conformational energy of more than 4 kcal/mole[16] compared with 1.7 kcal/mole in methylcyclohexane.[15] Deuteriation changes in forces between axial groups in the 2-position and the axial 4,6-hydrogen atoms should then be more easily detected since they should have a correspondingly greater effect than in cyclohexanes.

Methyl substituents at the 5-carbon of 1,3-dioxanes provide protons with a suitable chemical shift difference between the axial and equatorial environments for the determination of conformational equilibrium constants from time-averaged measurements, as described above. The use of 5,5-dibenzyl compounds provides

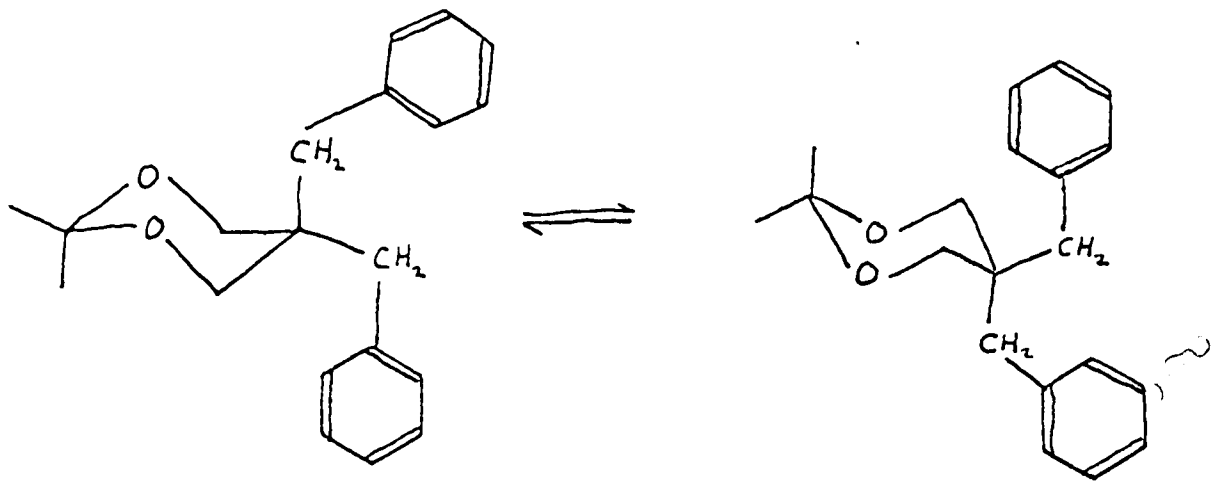
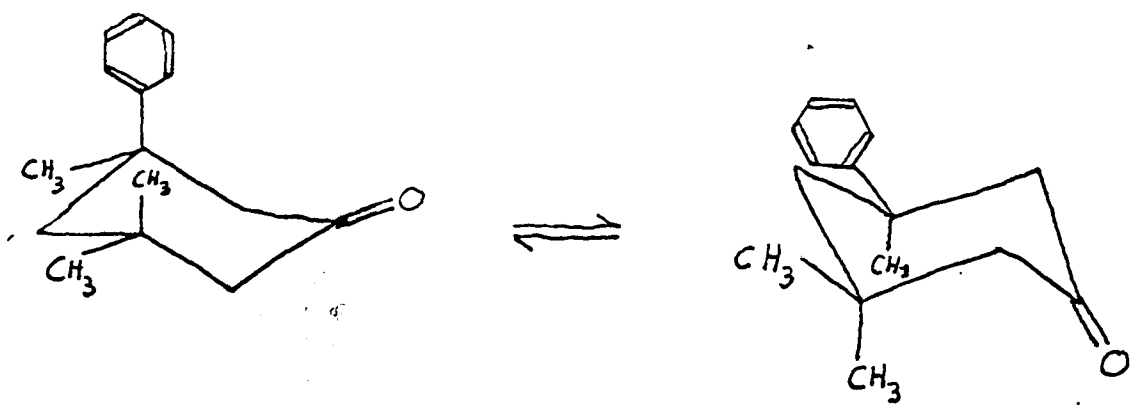
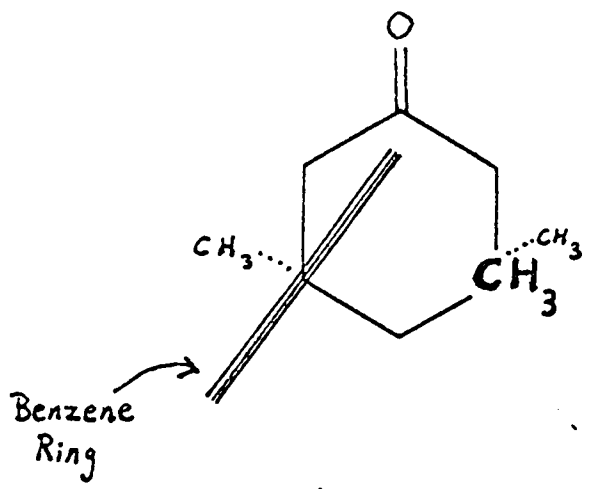


Figure 3



IA IE

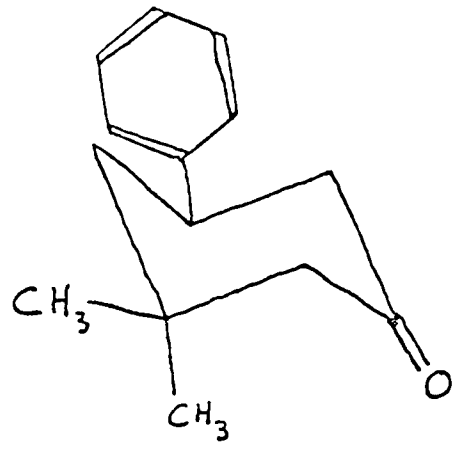


II (IA viewed from above)

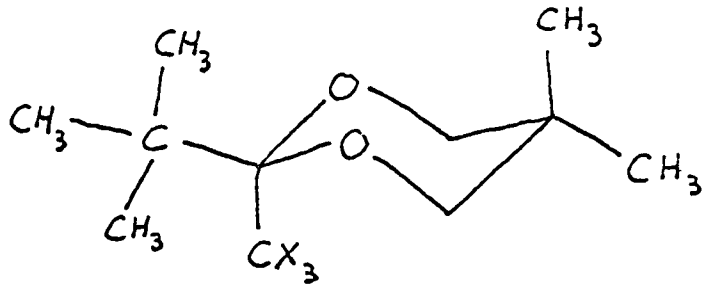
even greater sensitivity, taking P in equation(1) as the chemical shift difference between the methylene group protons of the 5-axial and 5-equatorial benzyl groups. Steric restrictions of the population of rotamers of the conformations shown in Figure 3 lead to extra shielding of 5-equatorial methylene protons by the 5-axial benzyl aromatic rings. This, in addition to the greater deshielding of 5-axial methylene protons by the oxygen atoms, provides a large chemical shift difference between 5-equatorial and 5-axial benzyl methylene groups.

Accordingly 2,2,5,5-tetrasubstituted-1,3-dioxanes of two types were prepared. The first type had 2-substituents of approximately equal "size" with and without specific deuteriations. The second type had 2-substituents chosen to provide conformationally biased dioxanes as models.

3-Phenyl-3,5,5-trimethylcyclohexanone offers an equilibrating two-conformation system in which 3,5-diaxial methyl-methyl repulsion in one conformation is balanced by 3,5-diaxial methyl-phenyl repulsion in the other. This compound was prepared with and without deuteriation in the 3-methyl group and around the ring, and the equilibrium was studied using the chemical shift difference between the 5-methyl proton group cis- to the 3-phenyl group and tetramethylsilane as the property P in equation (1). This property is particularly sensitive to the equilibrium composition since the cis-5-methyl group protons are shielded by the benzene ring in conformation IA, but no such shielding effect would be expected in conformation IE. In IA, the absence of a 1-axial ring hydrogen (which would be present in the analogous cyclohexane derivative) permits the benzene ring to lie in a plane coplanar (or close to coplanar) with the carbonyl group, as in II, thus minimising steric interaction between the cis-5-methyl group and the benzene ring whilst maximising the shielding effect.



III



IV, X = H

V, X = D

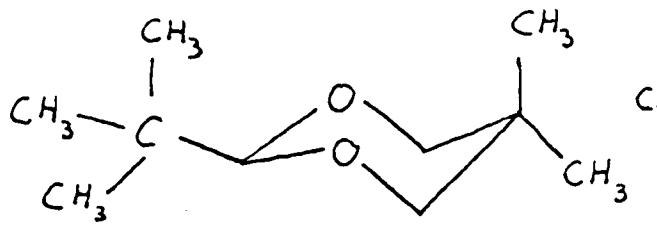
3,3-Dimethyl-5-phenylcyclohexanone, III, was prepared as a model of IE. A chemical shift value for IA was obtained from extrapolation of measurements from spectra recorded in low temperature studies (see the discussion of the isotope effect).[10]

THE CALCULATION OF CONFORMATIONAL EQUILIBRIUM CONSTANTS FROM NMR SPECTRA

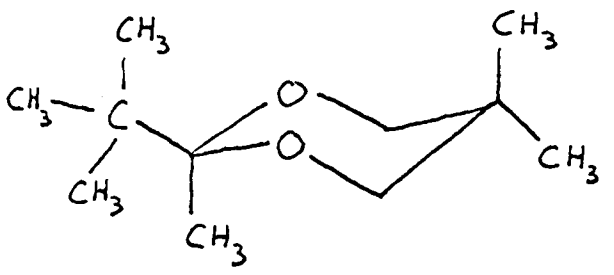
Rearrangement of equation (1) gives

$$\frac{1}{K} = \frac{n_e}{n_a} = \frac{P_a - P}{P - P_e} \qquad (2)$$

where K is the conformational equilibrium constant. The validity of the use of this equation for the determination of changes in K between undeuteriated and deuteriated systems depends upon the assumption that the systematic errors involved in the estimation of P_a and P_e, usually from measurements on model compounds, are the same for the undeuteriated and deuteriated systems. As chemical shift differences are to be used as the property P, the assumption involved is that the deuteriations studied will have no significant effect on the values of P_a and P_e. Justification for ignoring any such isotope shift[17] is provided by the equality of the chemical shift difference between 5-axial and 5-equatorial methyl protons in 2-t-butyl-2,5,5-trimethyl-1,3-dioxane, IV, and 2-t-butyl-5,5-dimethyl-2-trideuteriomethyl-1,3-dioxane, V,[10] models for the estimation of the chemical shift difference in a single conformation of an equilibrating system for undeuteriated and deuteriated systems respectively.



VI



VII

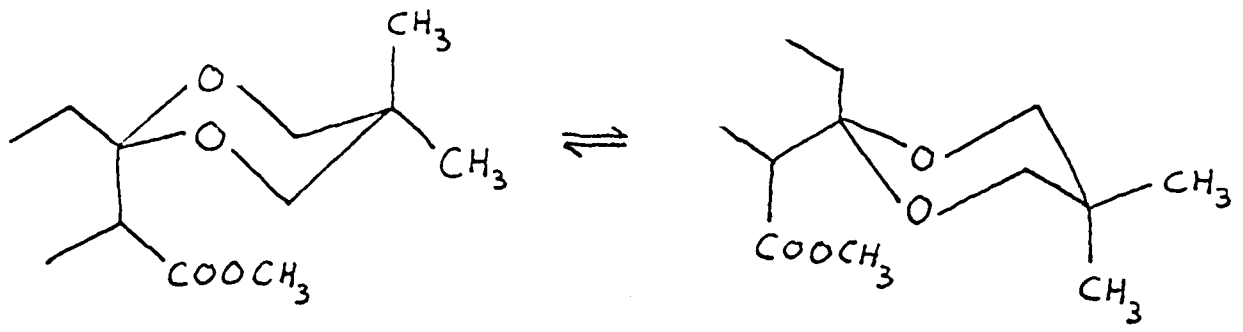
THE USE OF LOCKED MODEL COMPOUNDS

As a source of systematic error in the calculation of K, the use of locked compounds for the derivation of P_a and P_e values requires analysis.

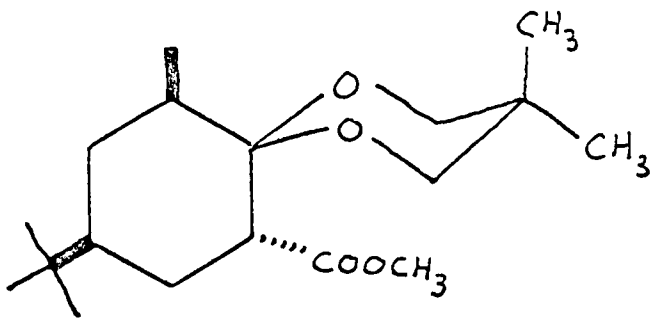
The use of the adjective "locked" implies the assumption that only the one conformation is present. In the case of, for example, t-butyl groups locking in the 4-position of a cyclohexane, this assumption is justifiable since the conformational energy is greater than 4.4 kcal/mole[15] and will be even larger in the case of 2-t-butyl-1,3-dioxane (compare methylcyclohexane and 2-methyl-1,3-dioxane, discussed earlier). Robinson[10] has observed a parallel temperature dependence for the chemical shift difference between equatorial and axial 5-methyl protons in 2-t-butyl-5,5-dimethyl-1,3-dioxane, VI, and 2-t-butyl-2,5,5-trimethyl-1,3-dioxane, VII, up to 180° indicating no significant population of conformations of VII other than that shown in the diagram for the range of temperature over which it was studied in this work.

A further problem in the use of model compounds is the reproduction of the magnetic environments of, for this work, the 5-methyl or 5-benzyl methylene protons in the individual conformations by a suitable choice of 2-substituents for locked models. It will be necessary to assume that the locking groups do not exert any peculiar effects on the chemical shifts of the protons under discussion, other than by changing the equilibrium. Much evidence from direct comparison of the low-temperature spectra of compounds with "frozen" equilibria with those of the locked compounds at the same temperature indicates that this assumption is often unjustified.[18,19,20,21]

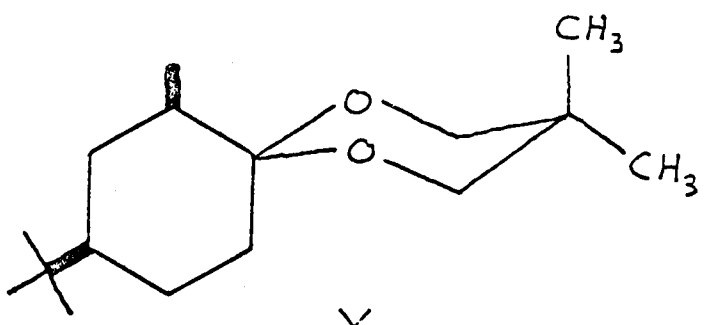
Robinson[10] has studied 5,5-dimethyl-1,3-dioxanes in this way with the examination of the chemical shift difference between 5-axial and 5-equatorial methyl



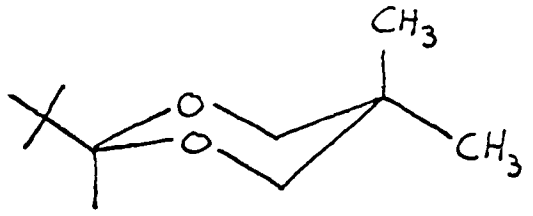
VIII A ⇌ VIII E



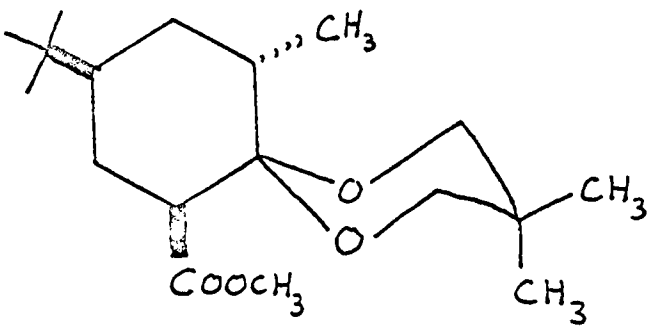
IX



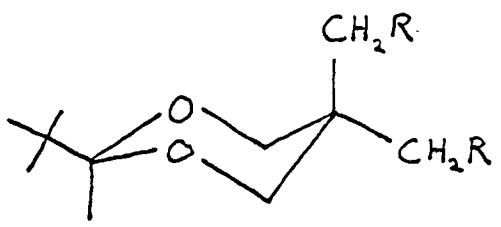
X



XI

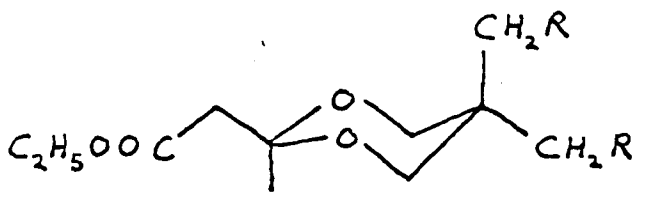


XII



XIII, R = H

XIV, R = C_6H_5



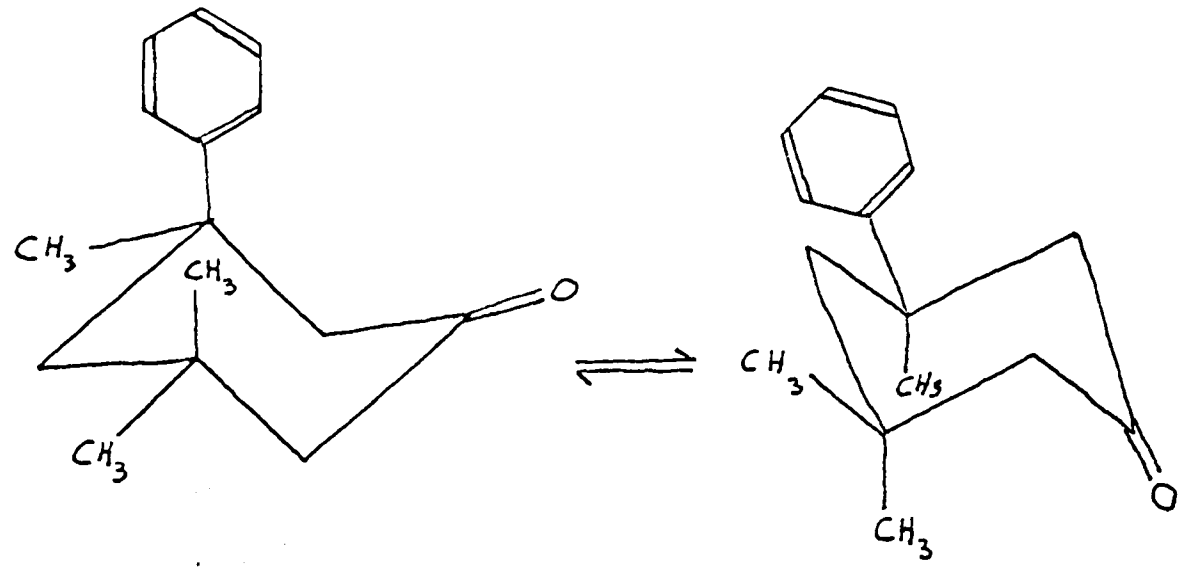
XV R = H

XVI R = C_6H_5

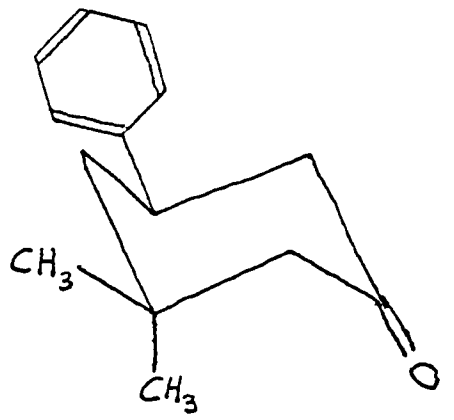
protons. The P values obtained give $P_e = P_{locked}$ for several 2-alkyl compounds locked in different ways, which suggests the relation $P_e = -P_a$ is applicable as a first order of approximation for compounds with alkyl 2-substituents.

The equilibrating 1,3-dioxanes studied in this work have alkoxycarbonyl groups in one of their 2-substituents: for example, 5,5-dimethyl-2-ethyl-2-(1-methoxycarbonylethyl)-1,3-dioxane, represented by the equilibrium VIIIA ⇌ VIIIE. The magnetic anisotropy of the methoxycarbonylethyl group may affect the values P_a for VIIIA and P_e for VIIIE. Compounds IX and X are models of conformation VIIIA which would be expected to provide a means of assessing the effect of the methoxycarbonyl group on P_a and were accordingly prepared. A 6% increase in the value of P_{locked} was observed on changing from IX to X. However, approximately the same change (5%) in the value of P_{locked} was observed on changing from XI to X. Compound XII is a model of the rotamer of conformation VIIIE in which the methoxycarbonyl group may be expected to have the maximum effect on P_e, and, if this effect is measured by the difference in P_{locked} between XI and XII and assumed to be three (or more) times the effect in conformation VIIIE with free (or slightly restricted) rotation of the 2-methoxycarbonylethyl substituent, then the effect in VIIIE was observed to decrease P_e from a value with no polar 2-substituent by up to 6%, a change at the most equal to that observed between the P_{locked} values for X and XI. So, for the compounds studied, it appears that the influence of polar 2-substituents on estimates of P_a and P_e is no greater than the uncertainties resulting from a variation of P_{locked} values with the choice of locking 2-alkyl substituent (±6%).

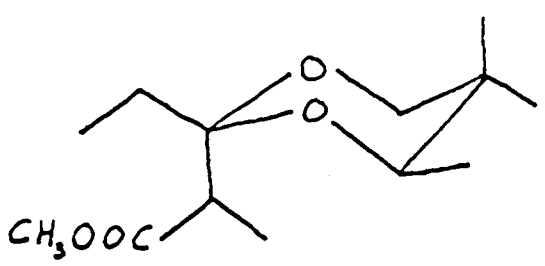
In the calculation of conformational equilibrium constants for 1,3-dioxanes in this work, IX was used as a model of VIIIA, XIII as a model of XV, and XIV as a



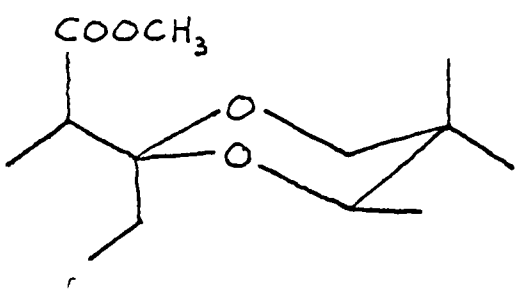
XVIIA ⇌ XVIIE



XVIII



XIX



XX

model of XVI. The relation $P_a = -P_e$ was applied, accepting an uncertainty of $8\frac{1}{2}\%$ ($\simeq\sqrt{2 \times (6\%)^2}$) in the estimated values derived for both P_a and P_e. For the calculation of the equilibrium constant for XVIIA $\rightleftharpoons$ XVIIE using the chemical shift of the cis-5-methyl protons as the property P, XVIII was used as a model of XVIIE, and a P_a value was obtained from extrapolation of low-temperature measurements, since the lower the temperature, the greater is the preference for XVIIA[10] (see the discussion of the measured isotope effect). In this case the uncertainty in the estimates of P_a and P_e was about 0.1 ppm for both values.

A possible series of useful model compounds are 2,2-dimethylbutane-1,3-diol condensates. For example, XIX and XX are models of VIIIA and VIIIE respectively. Comparison of 2-t-butyl-5,5-dimethyl-1,3-dioxanes with and without the equatorial 4-methyl group would provide a means of measuring the influence of the 4-methyl group on the chemical shifts of the 5-axial and 5-equatorial methyl group protons. Hence a suitable correction could be applied to the P_{locked} values of XIX and XX. There was not enough time to prepare and investigate these compounds.

THE EFFECT OF SYSTEMATIC ERRORS IN CALCULATED EQUILIBRIUM CONSTANTS UPON ISOTOPIC CHANGES IN CONFORMATIONAL FREE ENERGY DIFFERENCES

Conformational free energy differences, ΔG*, were calculated from the expression

$$\Delta G = -RT \ln K$$

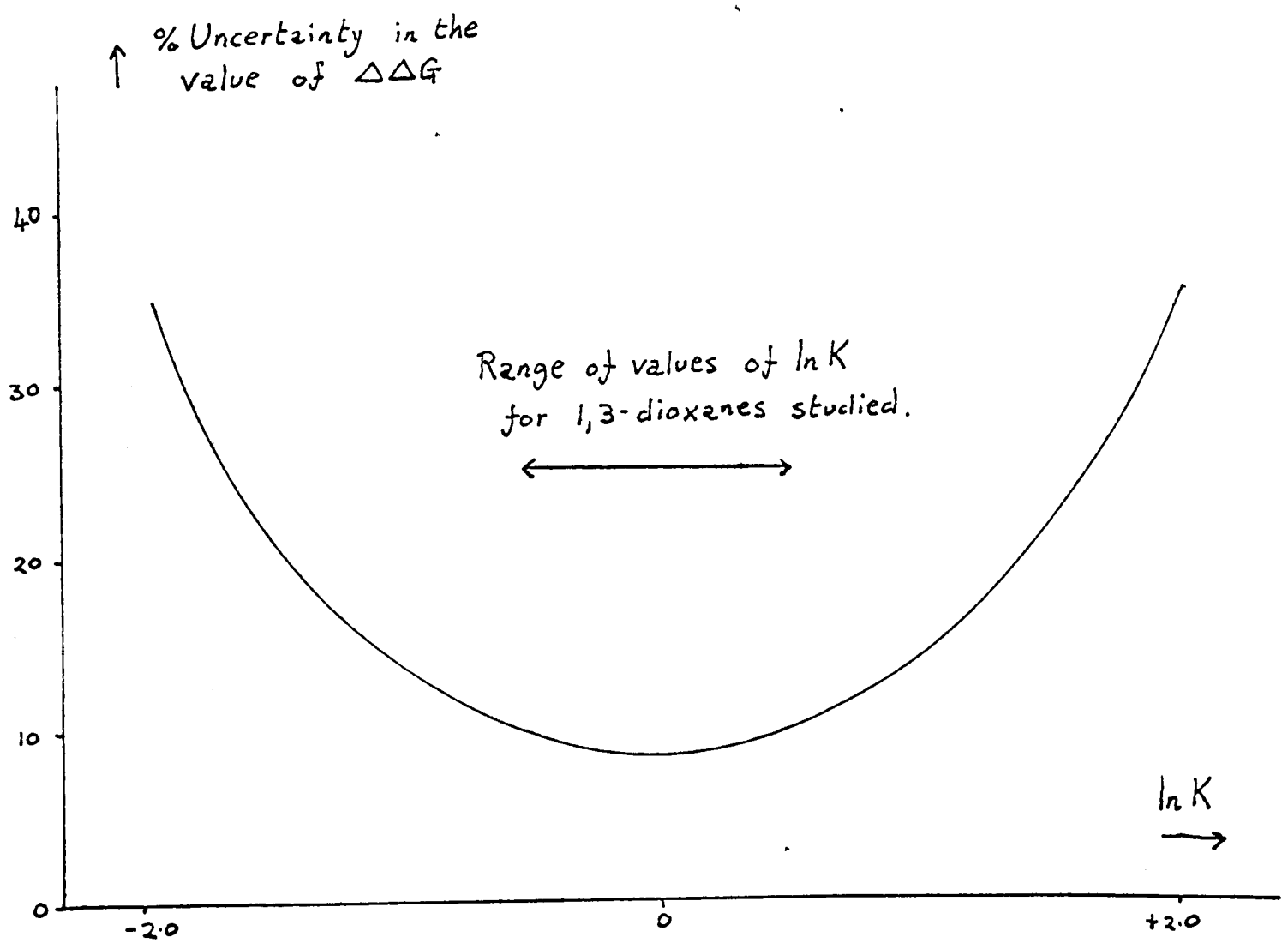
for undeuteriated and deuteriated compounds to obtain values for $\Delta\Delta G$, the changes in conformational free energy differences accompanying isotopic substitution.

The effect of the systematic errors in the calculation of K (and hence ΔG) upon the accuracy of $\Delta\Delta G$ values was investigated. Accepting that the errors in P_a

*As used here, ΔG refers to a free energy difference between [illegible] states, ΔG°.

Figure 4. A plot of the uncertainties of measured isotope effects against $\ln K$ for 8·5% systematic errors in P_a and in P_e.

$1 \text{ cal/mole} \leqslant \Delta\Delta G \leqslant 10 \text{ cal/mole}$ at 35°.

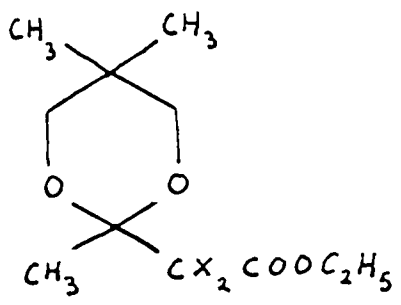


and P_e are the same for the undeuteriated and deuteriated systems, the errors in $\Delta\Delta G$ were computed for $8\frac{1}{2}\%$ uncertainties in P_a and P_e over a range of values of K for the undeuteriated system. Theoretical values for $\Delta\Delta G$ of between 1 and 10 cal/mole (typical of those measured in this work) were used and the temperature of measurement was taken as 35°. Figure 4 is a plot of the computed uncertainty of $\Delta\Delta G$, resulting from an $8\frac{1}{2}\%$ uncertainty in the value of P_a and of P_e, against ln K. The uncertainty of $\Delta\Delta G$ is at a minimum for ln K = 0, providing the reason for the desirability of systems for study with K close to unity, which was mentioned earlier. The double-headed arrow indicates the limits of the range of K values for the 1,3-dioxanes which were investigated. Thus in the worst case the error in the measured isotope effect will be ~ 10% of its value, which is less than the error resulting from the standard deviation of the measurement of time-averaged chemical shift differences.

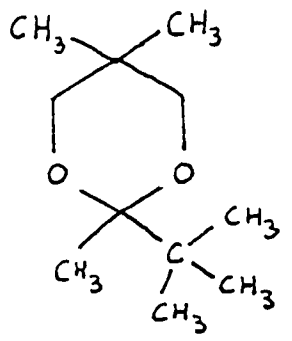
From the uncertainties in the value of P_a and P_e in the calculation of K for undeuteriated and deuteriated 3-phenyl-3,5,5-trimethylcyclohexanone, the resulting uncertainty in the value of $\Delta\Delta G$ was calculated to be 3.5%. As for the 1,3-dioxanes, this error is smaller than that resulting from the standard deviation of measurements of the time-averaged spectra.

THE MEASUREMENT OF CONFORMATIONAL FREE ENERGY DIFFERENCES

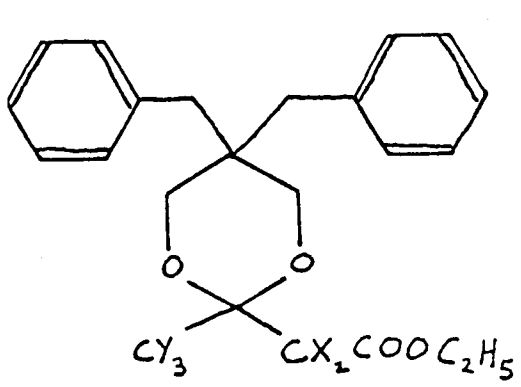
Chemical shift differences were measured for compounds XXI to XXXVIII. Using XXIII as a model for XXI and XXII, XXVIII as a model for XXIV to XXVII, XXIII and XXXIII to XXXV as models for XXIX to XXXII, and using XXXVIII as a model for a cis-5-methyl equatorial group in XXXVI and XXXVII, conformational free energy differences were calculated for the equilibrating compounds. (The axial cis-5-methyl conformation in XXXVI and XXXVII is favoured at low temperatures



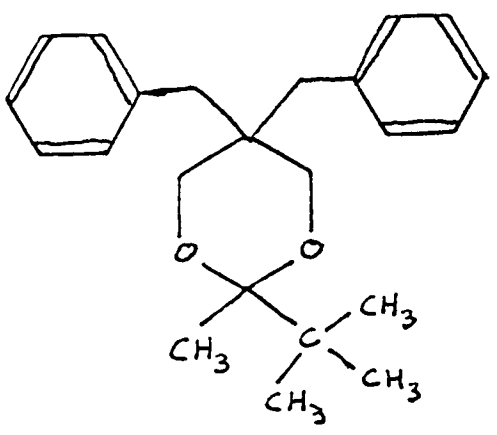
XXI X = H
XXII X = D



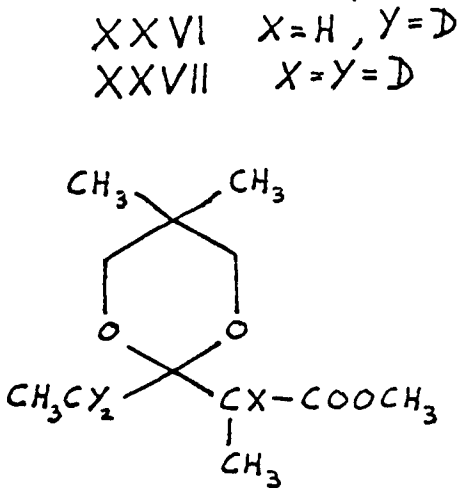
XXIII



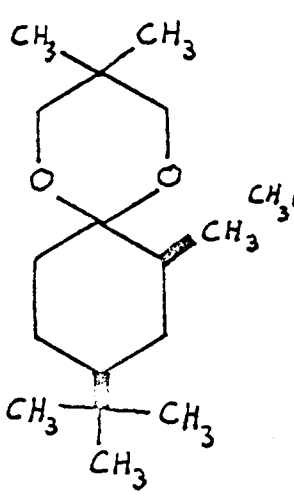
XXIV X = Y = H
XXV X = D , Y = H
XXVI X = H , Y = D
XXVII X = Y = D



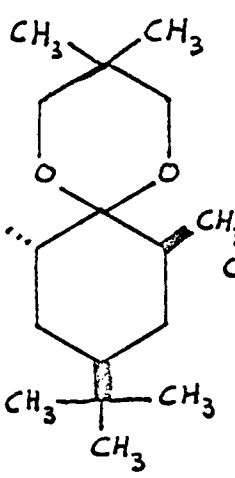
XXVIII



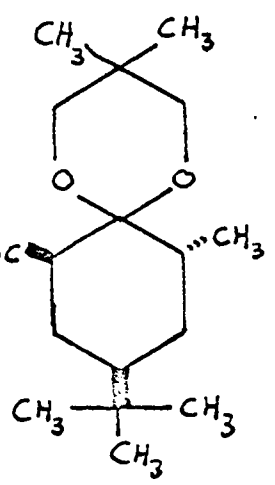
XXIX X = Y = H
XXX X = D , Y = H
XXXI X = H , Y = D
XXXII X = Y = D



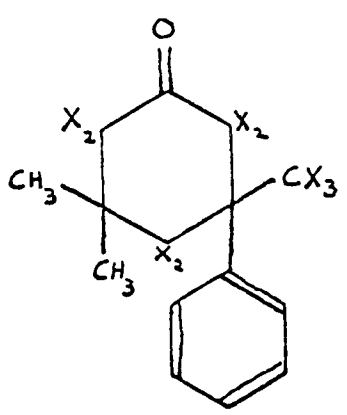
XXXIII



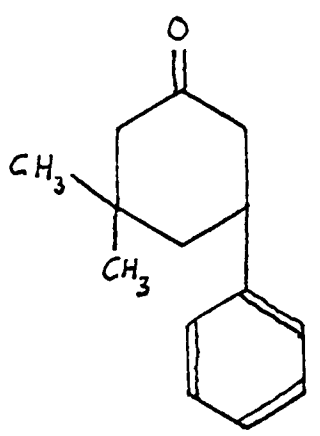
XXXIV



XXXV



XXXVI X = H
XXXVII X = D



XXXVIII

and an "axial" chemical shift value was obtained from the extrapolation of low temperature measurements[10]).

Spectra were run using solutions of molar concentration, except for XXIV to XXVII, which were examined as 0.5 M solutions and, when trideuterioacetonitrile was used as the solvent, XXVIII, as a 10% w/v solution (saturated at 30°). XXVIII was examined as a 0.5 M solution when trifluorochloromethane was used as the solvent. The chemical shift difference between the protons of the two methyl groups attached to the dioxane ring of XXXIV for 0.5 M and 1.0 M solutions varied by only 0.3 ± 0.1%, indicating that the measured free energy differences are relatively insensitive to concentration effects.

The temperature for all measurements was 35.2 ± 0.1° unless otherwise stated.

For compounds XXI to XXVIII and XXXVI to XXXVIII, fluorotrichloromethane was used as a solvent. The low melting point (-110°[22]) and low viscosity of trichlorofluoromethane will facilitate projected low temperature studies of the solutions. Carbon tetrachloride (dried over potassium hydroxide) was also used for measurements on compounds XXI to XXIII. 2% v/v tetramethylsilane was added to the solvent for use as an internal standard except for solutions of compounds XXXVI and XXXVII. For these two compounds the trans-5-methyl proton signal was used as a standard signal. Its position varies by less than 0.01 ppm, relative to tetramethylsilane over a range of temperature, -90 to +40°, corresponding to a fourfold change of equilibrium constant.[10]

Chemical shift differences for compounds XXIV to XXVIII were also measured using 0.5 M solutions in trideuterioacetonitrile (dried over BDH Molecular Sieve, type 3A) with 2% v/v tetramethylsilane in order to examine the effect of a change in solvent polarity.

The solvent for measurements on compounds XXIX to XXXV was a mixture of methanol (magnesium dried and stored over BDH Molecular Sieve, type 3A*), weight fraction 0.8744, benzene (dried over sodium), weight fraction 0.1106, and tetramethylsilane. This mixture was chosen to maximise the chemical shift difference between the two 5-methyl proton group signals, while giving chemical shift values for these groups which did not lead to any significant signal overlap with any part of the signal for a 2-ethyl methyl proton group (a triplet for XXIX and XXX, or a broad singlet for XXXI and XXXII).

Corrections to the observed isotope effects on conformational free energy difference values for imperfect deuteriation of samples were made using the method described in the section on the analysis of experimental errors (Page 80).

The conformational free energy differences with systematic uncertainties are shown for the undeuteriated compounds in Table 1. The sign of the ΔG values for 1,3-dioxanes is based upon an analysis of solvent effects on the equilibria and on model compounds, discussed below. The sign of the ΔG value for 3-phenyl-3,5,5-trimethylcyclohexanone is known since the phenyl-axial conformation will have a 5-methyl group with the highest chemical shift value (on the τ scale).

Table 2 shows the changes in conformational free energy difference, $\Delta\Delta G$, for the deuteriations indicated for the 1,3-dioxanes.

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* A few bubbles of ammonia gas were passed through the methanol before it was used to prevent any acid-catalysed hydrolysis or methanolysis of the 1,3-dioxanes.

TABLE 1: CONFORMATIONAL FREE ENERGY DIFFERENCES

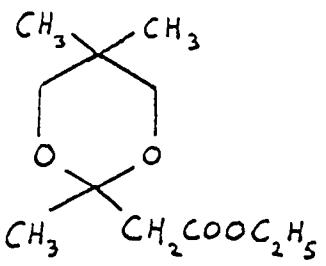
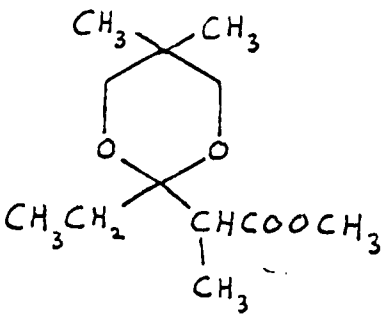
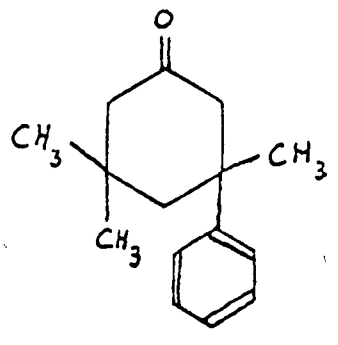
Compound Number	Compound Structure	Concentration and Solvent	Temperature	Conformational Free Energy Change ΔG (cal/mole) [Signs assigned on the basis of solvent effects]
XXI		1M CCl_4	41.4°	-205.3 ± 77
		1M CCl_3F	40.3°	-237.7 ± 78
XXIV		0.5M CCl_3F	35.2°	-71.5 ± 74
		0.5M CD_3CN	35.2°	-24.7 ± 74
XXIX		1M CH_3OH/C_6H_6	35.2°	-281.1 ± 78
XXXVI		1M CCl_3F	35.2°	-168.8 ± 175

TABLE 2: ISOTOPE EFFECTS ON CONFORMATIONAL FREE ENERGY DIFFERENCES OF 1,3-DIOXANES

Compound Numbers	2-Substituent (site of deuteriation underlined)	Solvent	ΔΔG (cal/mole)
XXIV, XXVI	$-C\underline{H}_3$	CCl_3F	2.4 ± 1.6
XXV, XXVII	"	"	1.5 ± 2.3
XXIV, XXVI	$-C\underline{H}_3$	CD_3CN	3.3 ± 2.2
XXV, XXVII	"	"	3.0 ± 2.3
XXI, XXII	$-C\underline{H}_2COOC_2H_5$	CCl_4	-12.4 ± 3.7
XXI, XXII	$-C\underline{H}_2COOC_2H_5$	CCl_3F	-12.5 ± 1.3
XXIV, XXV	"	"	-13.8 ± 2.3
XXVI, XXVII	"	"	-12.9 ± 1.6
XXIV, XXV	$-C\underline{H}_2COOC_2H_5$	CD_3CN	-8.3 ± 1.9
XXVI, XXVII	"	"	-8.0 ± 2.5
XXIX, XXXI	$-C\underline{H}_2CH_3$	CH_3OH/C_6H_6	2.4 ± 1.3
XXX, XXXII	"	"	2.6 ± 1.7
XXIX, XXX	$-C\underline{H}CH_3COOCH_3$	CH_3OH/C_6H_6	-4.8 ± 1.5
XXXI, XXXII	"	"	-5.0 ± 1.8

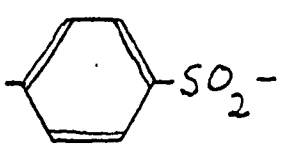
CONFORMATIONAL EQUILIBRIUM ISOTOPE EFFECTS

In Table 2, the agreement between ΔΔG values for the compound pairs XXI and XXII, and XXIV and XXV (for measurements on fluorotrichloromethane solutions), to within the standard deviations of the two ΔΔG values indicates that the isotope effects on the equilibria of 1,3-dioxanes are independent of the 5-substituents used to provide suitable NMR signals for the measurement of equilibrium constants, as well as being independent of the concentration of the solutions used for measurements.

For those groups of compounds (XXIV to XXVII and XXIX to XXXII) with deuterium distributed in four different ways between the two 2-substituents, the various changes in ΔG values indicate that the effect of deuteriation in one substituent is independent of the effect of deuteriation in the other to within half the standard deviation for any ΔΔG value.

Ball[23] has measured the following equilibrium isotope effects in 2,2-disubstituted-5,5-dimethyl-1,3-dioxanes:

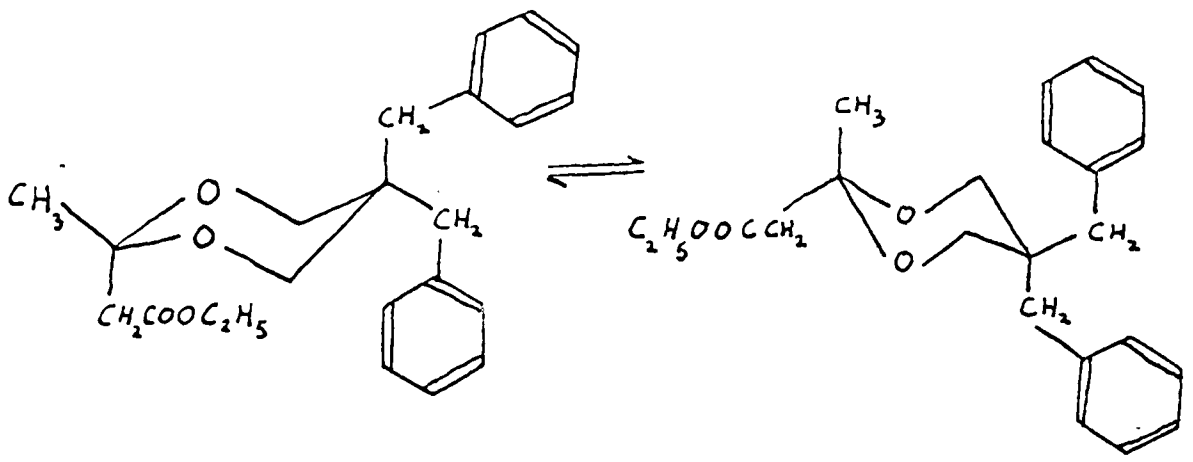
2-Substituent (site of deuteriation underlined)	Solvent	Temperature	ΔΔG (cal/mole)
$-C\underline{H}_2COOC_2H_5$	CCl_3F	37.0°	-17.0
"	"	34.0°	-17.4
"	"	-35.0°	-16.7
$-C\underline{H}_2CH_2OOCCH_3$	CCl_3F	32.5°	± 7.3
$-C\underline{H}_2CH_2OTos$*	CCl_3F	34.4°	∓11.0
$-C\underline{H}_2CH_3$	12% C_6H_6/88% CCl_4 v/v	35.0°	- 7.9
"	"	-32.0°	- 8.0

* Tos = 

Ball's results for the effect of deuteriation of a 2-ethoxycarbonylmethyl substituent agree with the values measured in this work in sign, although there is a 50% difference in size. Examination of the NMR spectra of the solution samples used by Ball indicated the presence of impurities. The same was found for the samples with 2-(2-acetoxyethyl) substituents. Remeasurement of the 2-(2-tosyloxyethyl) isotope effect on rerecorded spectra gave a value of $\mp 1.8 \pm 4.0$ cal/mole for $\Delta\Delta G$. A time-dependent variation of this last value is not surprising since the decomposition of 2-(2-tosyloxyethyl)-2,5,5-trimethyl-1,3-dioxane is probably autocatalytic.

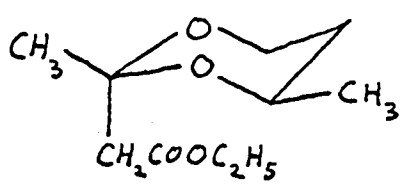
Ball measured the effect of deuteriation at the 1-carbon atom of an ethyl group by comparison of the chemical shift difference between the two 5-methyl proton signals of the spectra of 2-ethyl-2,5,5-trimethyl-1,3-dioxane and 2-(1,1-dideuterioethyl)-2,5,5-trimethyl-1,3-dioxane. In the spectrum of the undeuteriated compound each 5-methyl group signal overlaps with one of the two "outer" signals of the 2-ethyl methyl group triplet. The measurement of the distance between the area bisectors of the two "composite" signals would not, therefore, be expected to give a reliable value for the separation of the 5-methyl signals. This may account for the gross discrepancy between Ball's value for $\Delta\Delta G$ between CH_3CH_2- and CH_3CD_2-, and the value in Table 2.

In spite of these criticisms, Ball's results do indicate that the types of isotope effect on the conformational equilibria of 1,3-dioxanes studied in this work are, within the accuracy of measurement, independent of temperature over the range -35 to $+37^{\circ}$. Instrumental facilities for variable temperature work did not become available during the course of this study.

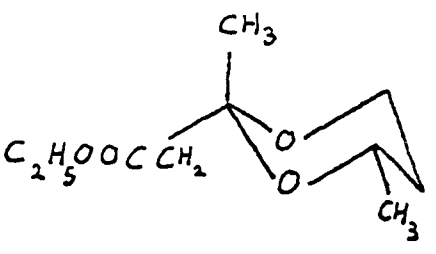


XXIV A

XXIVE



IXL



XL

The assignment of signs to the $\Delta\Delta G$ values in Table 2 depends upon the signs of the ΔG values, and hence upon a knowledge of which of the two conformations in any particular equilibrium is the preferred one for the conditions of measurement. The identification of the preferred conformer may be based upon the observation of solvation effects.

All the 1,3-dioxane equilibria studied have ΔG values which decrease algebraically with an increase in the polarity of the solvent. This is indicated in Table 1 for a change from trichlorofluoromethane to trideuterioacetonitrile as the solvent for XXIV. A parallel solvent effect has been observed for XXI.[23] XXIX has $|\Delta G| = 600$ cal/mole for a carbon tetrachloride solution and $|\Delta G| = 300$ cal/mole for a solution in the methanol-benzene mixture. For all these compounds, substitution of deuterium in a position α to the carbonyl group <u>increased</u> the preference for whichever conformation was preferred in the undeuteriated compound.

The conformationally biased compounds, IXL and XL, readily distinguishable by their NMR spectra, as explained in the discussion of the preparation, were prepared for investigation as a pair of models: IXL as a model for XXIVA and XL as a model for XXIVE. The ratio of the GLC retention time for IXL to that for XL decreased with an increase in the polarity of the stationary phase. The work of Gordon[24] illustrates the use of stationary phases in GLC as models for solvents. Therefore the increase in solvation from non-polar to polar media will be greater for XL than for IXL, and will lead to a relative decrease of the concentration of IXL in equilibrium mixtures. The equilibrium mixture of IXL and XL, which had been prepared by the acid-catalysed condensation of ethyl acetoacetate and butane-1,3-diol in boiling benzene, contained three parts of IXL to two parts of XL.

The parallel solvent effects on the equilibria IXL ⇌ XL and XXIVA ⇌ XXIVE indicate a preference for XXIVA in the latter and a general preference for axial 2-alkoxycarbonylalkyl groups in all the 1,3-dioxane equilibria studied.

The increase of this preference ($\Delta\Delta G$ = -5 to -13 cal/mole) on deuteriation α to the carbonyl group is explicable on the basis of considering size effects on steric repulsions. The lower amplitude of anharmonic vibration of C-D bonds in the 2-methylene group α to the carbonyl group, compared with C-H bond vibrations leads to a lessening of the repulsive interaction with the 4,6-diaxial ring hydrogen atoms for axial 2-alkoxycarbonylalkyl groups.

The substitution of deuterium into the 2-alkyl groups <u>also</u> leads to an increased preference, albeit smaller, for axial 2-alkoxycarbonylalkyl groups. Therefore, as indicated in Table 2, such isotope effects ($\Delta\Delta G$ = 2 to 3 cal/mole) are in the <u>opposite direction</u> to the "steric" isotope effects resulting from substitution α to the carbonyl group, an indication that a simple analysis of the effect of group "size" upon steric interactions is inadequate. The change in size and sign between the two "types" of isotope effect which have been observed suggests at least one contribution to the effect which may work against changes in steric repulsions. Any changes of the size of the individual isotope effects between solvents of different polarity are also inexplicable on a simple "steric" basis.

The "zero-point" vibration of a molecule will be expected to make a contribution to the dipole moment, if polar bonds within the molecule are distorted by vibration.[25] This contribution, which depends upon the amplitude of vibration, will vary with the polarity of the distorted bonds. If polar C-H bonds within the molecule are changed to C-D bonds, the mean amplitude of vibration within the same

anharmonic potential wells will decrease, and therefore the anisotropic vibrational contribution to the molecular dipole moment will be different. Hence for substitution of deuterium into C-H bonds, an isotope effect on molecular dipole moments,

$$\Delta\mu = \mu_D - \mu_H ,$$

the difference in dipole moment between the deuteriated and undeuteriated molecules, will be expected to vary with the environment of the carbon-hydrogen bond.

Values of $\Delta\mu$ have been measured by Muenter et al.[26,27] and are shown in the table below with the corresponding deuteriated and undeuteriated molecules.

Undeuteriated Molecule	Deuteriated Molecule	$\Delta\mu$ (Debye) for lowest vibrational states
$CH_3C{\equiv}CH$	$CH_3C{\equiv}CD$	-0.013 ± 0.001
$CD_3C{\equiv}CH$	$CD_3C{\equiv}CD$	-0.012 ± 0.001
CF_3H	CF_3D	$+0.001 \pm 0.001$
CH_4	CH_3D	$+0.005641 \pm 3 \times 10^{-6}$
$HC{\equiv}CCH_3$	$HC{\equiv}CCD_3$	-0.009 ± 0.001
$DC{\equiv}CCH_3$	$DC{\equiv}CCD_3$	-0.010 ± 0.001
FCH_3	FCD_3	$+0.011 \pm 0.001$

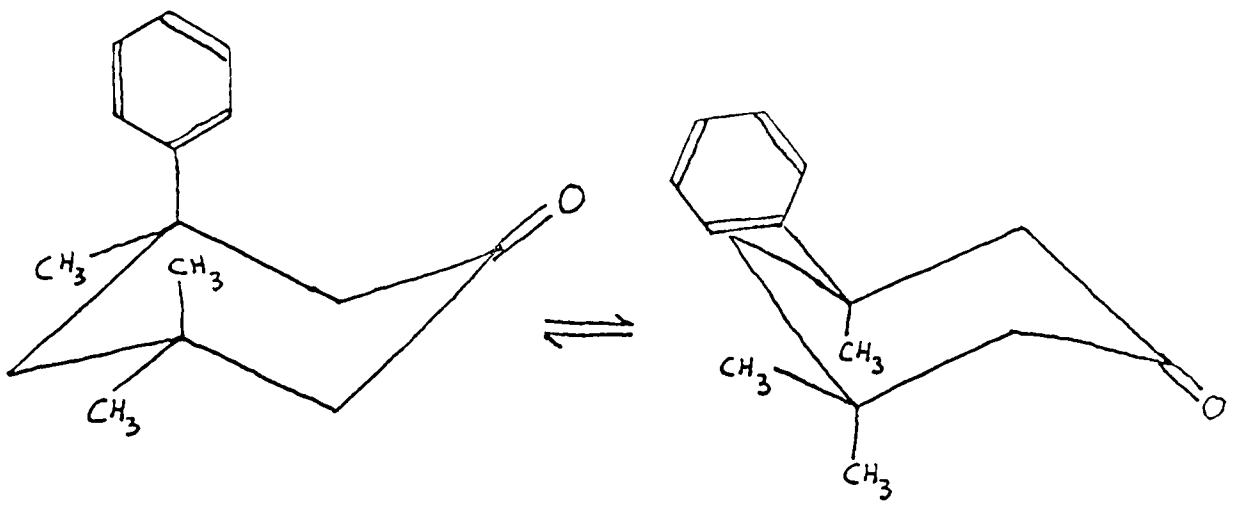
These results illustrate the strong dependence of $\Delta\mu$ on the chemical environment of the substitution site.

The two "types" of isotope effect on conformational equilibria which have been measured in this work correspond to deuteriation on the 1-carbon atom of a 2-alkyl substituent of a 1,3-dioxane for one type and deuteriation on the 1-carbon atom of a 2-(1-alkoxycarbonylalkyl)-substituent for the other type. The presence

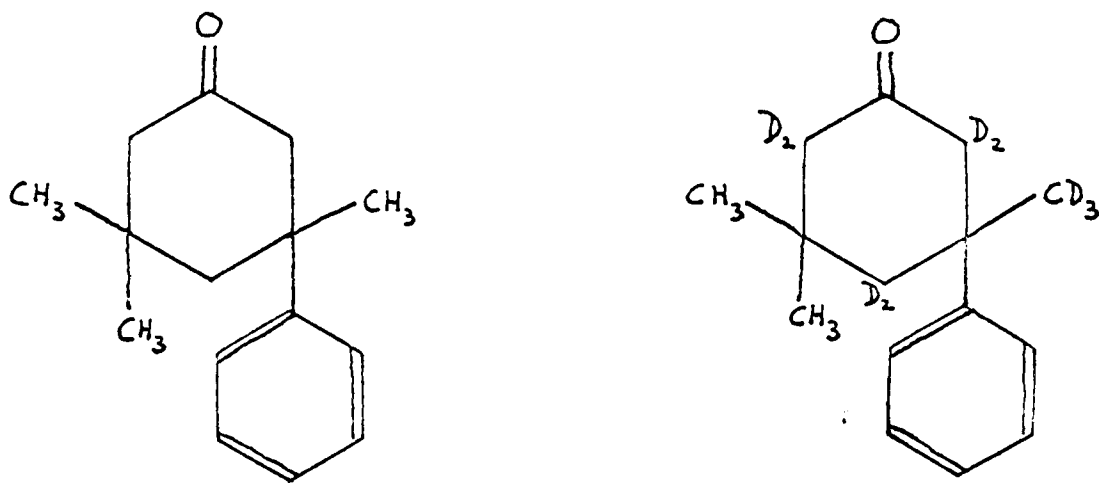
of an oxo group α to the site of deuteriation for the latter may well lead to a different chemical environment at the deuteriation site from the environment at the deuteriation site of the former. Such a difference could lead to different isotopic changes of the group dipole moments for the two kinds of 2-substituent and, in particular, the changes in bond polarity from C-H to C-D may be different.

In the 1,3-dioxanes studied, the non-bonded interactions between hydrogen atoms attached to the 1-carbon of a 2-substituent in the axial position and the 4,6-diaxial ring hydrogen atoms will include dipole-dipole interaction terms as well as a van der Waals repulsion resulting from overlapping electron clouds. The change in value of this dipole-dipole contribution on deuteriation may be different, even in sign, for substitution at chemically dissimilar sites. For the change of 2-substituent from methyl to trideuteriomethyl or ethyl to 1,1-dideuterioethyl, the dipole-dipole interactions appear to favour conformations with axial undeuteriated substituents more than conformations with axial deuteriated substituents to the extent that the overall isotope effect, including the effect of a change of "size" on steric repulsions, is positive (crowded hydrogen preferable to crowded deuterium). The conformational isotope effect of deuteriation of 2-(1-alkoxycarbonylalkyl)-substituents at the 1-carbon atom is negative, as would be expected on a "size" basis, indicating that dipole-dipole interaction changes are either smaller than those associated with the 2-alkyl deuteriations or of the opposite sign, enhancing the value of the "size" effect.

The conformational equilibrium between XXXVIA and XXXVIE in trichlorofluoromethane has been studied over a temperature range -90 to +40° by Robinson,[10] using the time-averaged NMR chemical shift of the cis-5-methyl proton signal. In conformation XXXVIA, this 5-methyl group is axial and strongly shielded by the



XXXVIA XXXVIE



XXXVI XXXVII

axial phenyl group which is forced to coplanarity or near coplanarity with the C=O bond in order to minimise the steric repulsion of the axial 5-methyl group. Therefore the chemical shift value (on the τ scale) of the cis-5-methyl group protons will be larger for XXXVIA than for XXXVIE in which no such shielding of the equatorial 5-methyl group protons is expected.

Robinson found that the time-averaged chemical shift of the cis-5-methyl proton signal, τ_H, increased with a decrease of temperature, indicating an increase of enthalpy ($\Delta H > 0$) in passing from XXXVIA to XXXVIE which may be ascribed to the 3,5-diaxial methyl groups in XXXVIE being more crowded than the 3-axial phenyl and 5-axial methyl groups in XXXVIA. Extrapolation of the low temperature τ_H values provided a value for the chemical shift of the cis-5-methyl proton group in XXXVIA, τ_a.

The equatorial methyl group in the conformationally biased compound 3,3-dimethyl-5-phenylcyclohexanone was used as a model to provide a chemical shift value corresponding to the equatorial methyl group in XXXVIE, τ_e.

At 35°, τ_H was closer to τ_a than to τ_e, indicating a preference for XXXVIA. A change from the undeuteriated compound, XXXVI, to the nonadeuteriated compound, XXXVII, gives an isotope effect, $\Delta\Delta G$, of 15.7 ± 6.1 calories per mole on the conformational free energy difference. That is, deuteriation decreases the preference for the phenyl-axial conformation.

The largest change in any non-bonded interactions from conformation XXXVIA to XXXVIE is the difference in crowding of the 3,5-diaxial substituents: methyl-phenyl in XXXVIA and methyl-methyl in XXXVIE. An examination of Dreiding models indicates that any change in the non-bonded interactions of the 2,2,4,4,6,6-

ring hydrogen atoms, with each other or with any other atoms, will be negligible between the two conformations in comparison with the effect of the change in 3,5-diaxial substituents. Therefore, the isotope effect on the conformational free energy change must be primarily attributed to the change from a 3-methyl group in XXXVI to a 3-trideuteriomethyl group in XXXVII.

The measured isotope effect is in the direction predicted on the basis of the increase of "size" from a trideuteriomethyl group to a methyl group owing to the greater amplitude of anharmonic vibration of protium over deuterium. Since the size of the effect (16 cal/mole) is algebraically greater than any isotope effect on the conformational equilibria of the 1,3-dioxanes studied, any contribution to this isotope effect from changes in dipole-dipole interactions is probably small in comparison with the "steric" contribution or is enhancing the steric effect rather than reducing it.

The results obtained in this work indicate that isotope effects on conformational equilibria may be attributed to a decrease in steric repulsions on substituting deuterium for protium in a crowded environment only as a crude approximation. The electrostatic consequences of the decreased amplitude of anharmonic vibrations on deuteriation may provide a contribution to the isotope effect which is in the opposite direction to a purely "steric" effect.

PREPARATION AND PROPERTIES OF THE COMPOUNDS

As intermediates in the synthesis of 1,3-dioxanes, methyl 4,4-dimethyl-3-oxopentanoate and 4-*t*-butyl-2-methoxycarbonyl-6-methylcyclohexanone were prepared by the Claisen condensation with dimethyl carbonate of pinacolone and

42

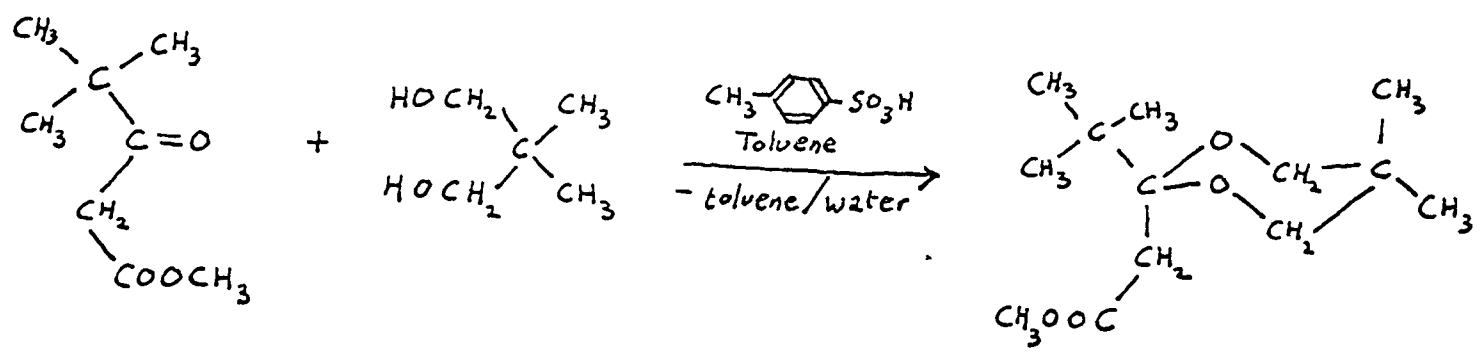

CH3
CH3
C
CH3
C=O
CH2
COOCH3
+
HOCH2
CH3
C
HOCH2
CH3
CH3
SO3H
Toluene
- toluene/water
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CH3
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O
CH2
CH2
CH3OOC

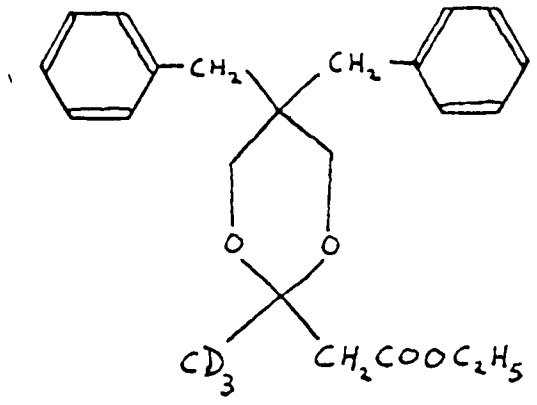
Figure 5

4-t-butyl-2-methylcyclohexanone respectively, using sodium hydride as base. The same base was used for the self-condensation of methyl propionate to prepare methyl 2-methyl-3-oxopentanoate.

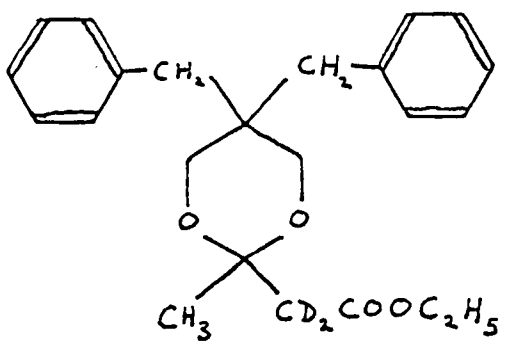
The Preparation of 1,3-Dioxanes

For the acid-catalysed condensation of β-keto esters or rarely, simple ketones, with diols, the method of Salmi[28] was used to prepare 1,3-dioxanes in yields of 50 to 90%. This involved the removal of water formed during the reaction as a benzene azeotrope (or a toluene azeotrope when considerations of steric "strains" suggested more forcing conditions), using a Dean and Stark apparatus. Attempts to condense 2,2-dimethylpropane-1,3-diol with methyl 4,4-dimethyl-3-oxopentanoate, however, produced the desired product, the model compound 2-t-butyl-5,5-dimethyl-2-methoxycarbonylmethyl-1,3-dioxane, in low yield (4%) and contaminated with starting material (Fig.5). The product decomposed in the course of distillation and hence the low yield may be attributed to its inherent instability. Non-bonded repulsion forces between the protons in the t-butyl group and either the protons in the 2-methylene position or the methoxycarbonyl group might be expected to be the cause of such low stability.

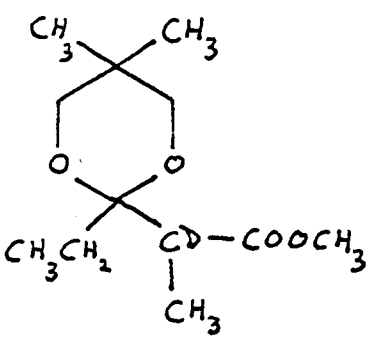
For the preparation of 2,2,5,5-tetrasubstituted 1,3-dioxanes with deuteriation of both 2-substituents a modification of the Salmi method was used. The β-keto ester starting material was deuteriated in all positions α to the keto group by acid catalysed exchange using the addition of portions of heavy water to a refluxing benzene solution of the keto ester and p-toluene sulphonic acid with azeotropic removal of water/heavy water mixtures after each addition. The resulting solution of deuteriated keto ester and acid was added to one of diol which had been treated in a similar way to deuteriate



XXVI



XXV



XXX

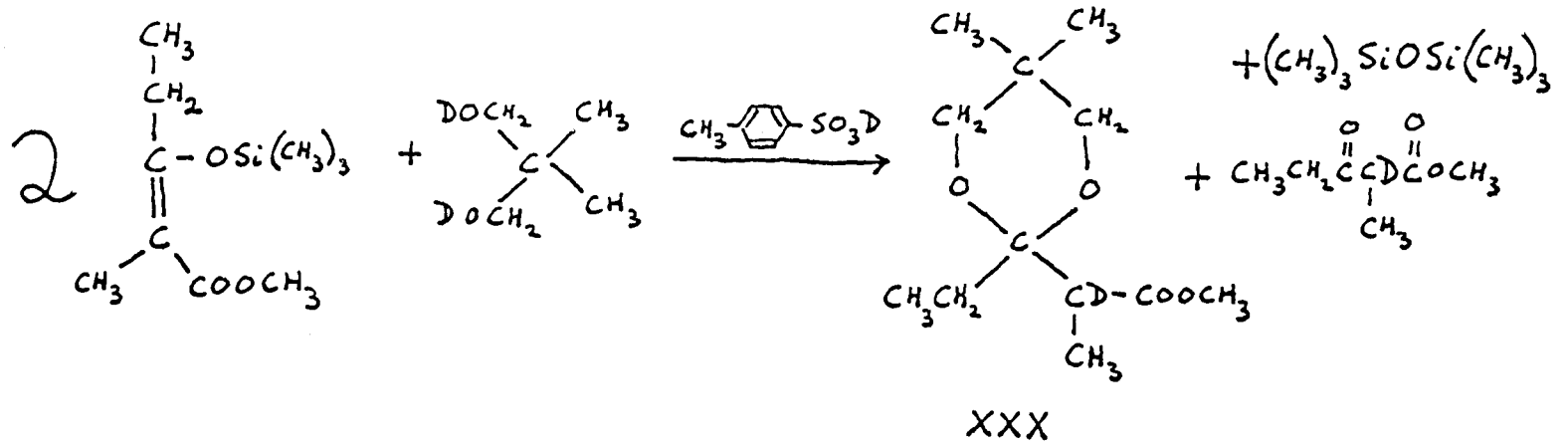


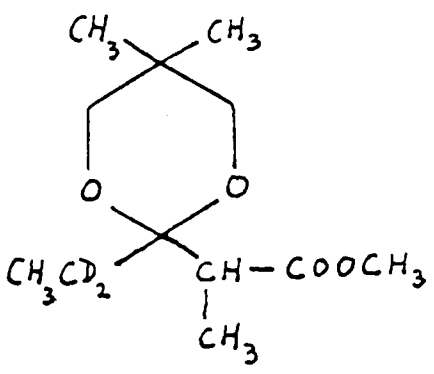
Figure 6.

the exchangeable hydrogens, and then the heavy water formed by the condensation was removed azeotropically. Acid-catalysed hydrolysis of the keto-ester starting material during the exchange process presumably limited the yields which were 30 to 40%. The use of a large excess of keto-ester relative to the diol was prohibited by the cost of heavy water.

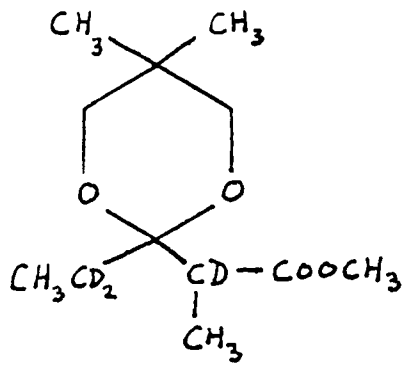
For the preparation of 5,5-dibenzyl-2-ethoxycarbonylmethyl-2-trideuteriomethyl-1,3-dioxane, XXVI, a sample of ethyl pentadeuterioacetoacetate which had been prepared in low yield earlier[29] by base-catalysed deuterium exchange, was used. Portions of "light" water were added to a solution of the deuteriated keto-ester and 2,2-dibenzylpropane-1,3-diol in benzene with azeotropic removal of water/heavy water mixtures after each addition in order to exchange protium for deuterium at the methylene positions of the keto-ester before addition of the acid catalyst and condensation in the usual way. The crude yield was 95%.

Attempts to prepare 5,5-dibenzyl-2-ethoxycarbonyldideuteriomethyl-2-methyl-1,3-dioxane, XXV, and 5,5-dimethyl-2-ethyl-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane, XXX, by deuterium exchange of the reactants before addition of the deuteriated acid catalyst gave products whose low resolution mass spectra corresponded with scrambling of deuterium between the two 2-substituents. This indicated too small a difference between the rate of the condensation reaction and the rate of acid-catalysed deuterium exchange between positions α to both carbonyl groups and positions α to the ketone carbonyl group and γ to the ester carbonyl group, in the β keto ester starting material for any further attempt to prepare 1,3-dioxanes with deuteriation in only one of the 2-substituents by this method.

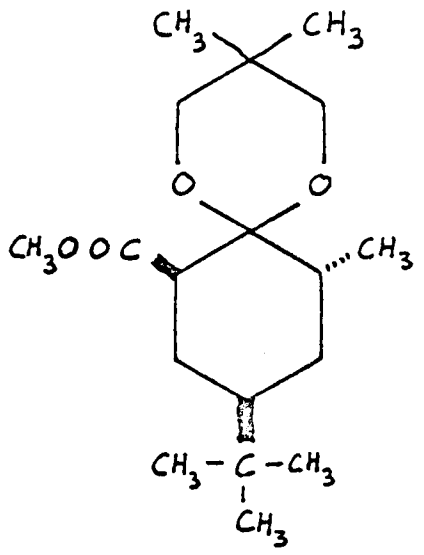
Preliminary investigations of the condensation reaction illustrated in Fig.6,



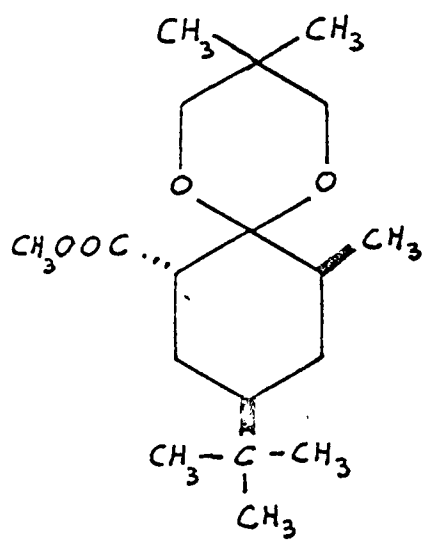
XXXI



XXXII



XXXV



XXXIV

using a trimethylsilyl enol ether of the β keto ester starting material, as a possible preparation of XXX, indicated a rate of condensation not significantly greater than that of the simple keto ester diol condensation. In addition, silylated by-products were formed in yields at least as great as that of the desired product and of similar volatility, presenting severe separation problems.

XXV and XXX were prepared successfully by the direct substitution of deuterium in the required position on the undeuteriated 1,3-dioxanes which had themselves been prepared using the normal condensation method. The undeuteriated starting material was sealed in a tube with a solution prepared from the reaction of sodium with a large excess of alcohol-OD. A little dialkyl carbonate was included to ensure the rapid conversion of any water present to alcohol. 5,5-Dimethyl-2-(1,1-dideuterioethyl)-2-(1-methoxycarbonylethyl)-1,3-dioxane, XXXI, was prepared from the trideuteriated starting material 5,5-dimethyl-2-(1,1-dideuterioethyl)-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane, XXXII, (a product of the condensation of deuteriated starting materials) by this direct substitution method using alcohol-OH in the place of alcohol-OD. The yields of these directly deuteriated and protiated products were from 50-85% and were primarily limited by the efficiency of distillation of the small quantities which were prepared.

Diastereoisomeric 1,3-dioxanes

Using a simple β keto ester-diol condensation followed by removal of residual keto ester starting material as an insoluble lanthanum trienolate salt, a mixture of c-9-t-butyl-r-7-methoxycarbonyl-3,3,t-11-trimethyl-1,5-dioxaspiro[5.5]undecane, XXXV, and t-9-t-butyl-r-7-methoxycarbonyl-3,3,t-11-trimethyl-1,5-dioxaspiro-[5.5]undecane, XXXIV, was prepared. In the NMR spectrum of the mixture (10%

Figure 7. NMR spectrum (100 MHz) of XXXV (10% w/v in benzene).

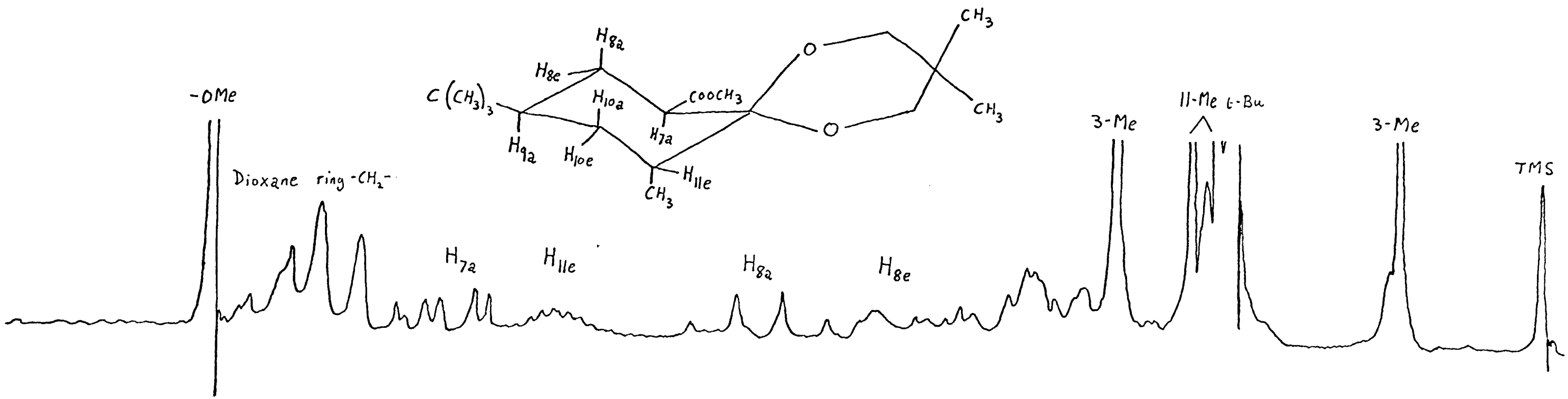
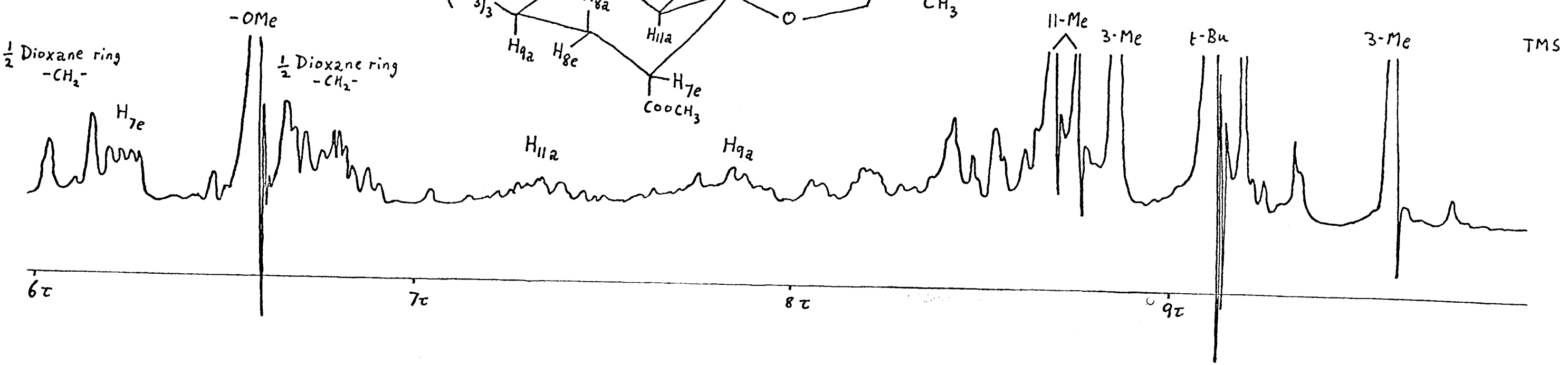


Figure 8. NMR spectrum (100 MHz) of XXXIV (10% w/v in benzene).



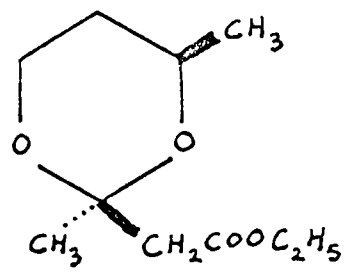
w/v in benzene) the relative intensities of the two methoxy proton singlet signals at 6.43 τ and 6.58 τ were 1:4.

Preparative thin layer chromatography separated the mixture, the more abundant isomer being more readily eluted by ether petroleum ether mixtures on Kieselgel. The 100 MHz NMR spectra of the separate isomers (10% w/v in benzene) are shown with some of the signal assignments which aided identification. Fig.7 is the spectrum of XXXV and Fig.8 is the spectrum of XXXIV. The strongest criterion for identification is the change in chemical shift of the proton attached to the 7-carbon atom between the two isomers. This proton is much closer to the oxygens of the dioxane ring in XXXIV with a consequent deshielding corresponding to a down-field signal shift of 0.83 ppm relative to its position in the spectrum of XXXV.

The condensation of butane-1,3-diol with ethyl acetoacetate gave a mixture of 2,c-4-dimethyl-r-2-ethoxycarbonylmethyl-1,3-dioxane, XL, and 2,t-4-dimethyl-r-2-ethoxycarbonylmethyl-1,3-dioxane, IXL. As described in the experimental section, analysis of various GLC chromatograms showed that whichever of the isomers was present in greater abundance was eluted first and accounted for 60% of the mixture.

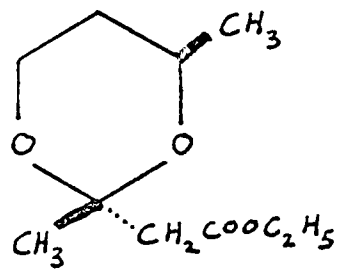
The NMR spectra (all of 20% w/v solutions in carbon tetrachloride) of the mixture and each of the two isomers, separated by preparative GLC, showed an AB quartet (J_{AB} = 12.5 Hz, τ_A = 7.19 ppm, τ_B = 7.33 ppm) in the spectrum of the more abundant isomer and a singlet (τ = 7.53 ppm) in the spectrum of the other. Both signals corresponded to the two 2-methylene protons.

Both isomers have a molecular structure with "locked" chair dioxane rings, with the 4-methyl substituent in the equatorial position, avoiding the strain of non-



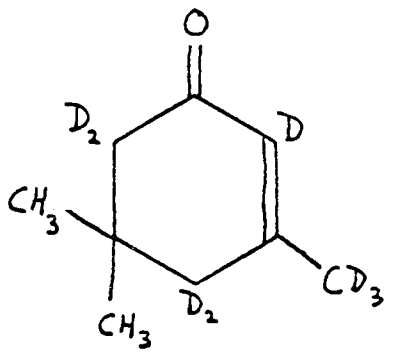
XL

cis

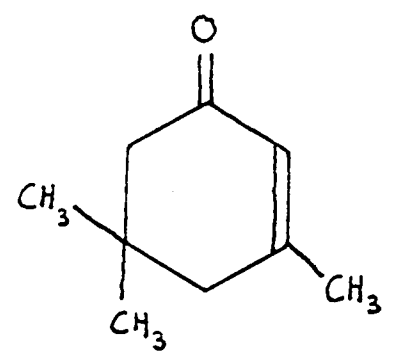


IXL

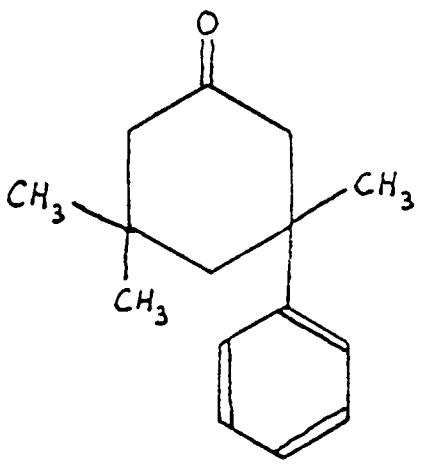
trans



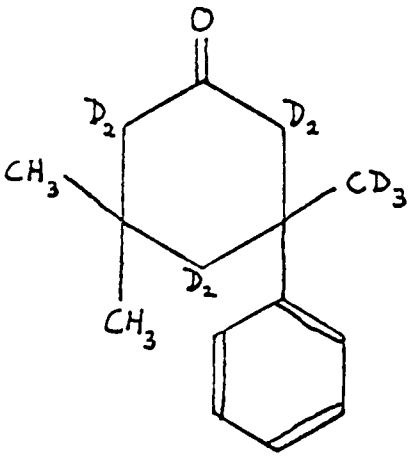
XLI



XLII



XXXVI



XXXVII

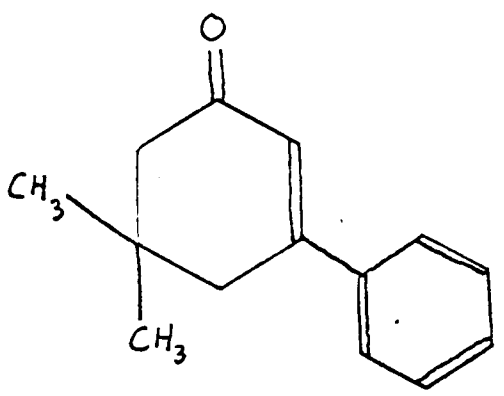
bonded interactions with the axial 2-substituent and the axial 6-hydrogen which an axial 4-methyl group would encounter. Hence the 2-ethoxycarbonylmethyl group is equatorial in XL, the cis-isomer, and axial in IXL, the trans-isomer. The 2-methylene protons are much closer to the C-4 chiral centre in the trans-isomer leading to a greater degree of magnetic non-equivalence (an AB quartet in the NMR spectrum). In the cis-isomer the 2-methylene protons are far enough away from the chiral centre to be approximately isochronous (a singlet in the NMR spectrum). Therefore XL and IXL are present in the reaction product mixture in the ratio 2:3.

As observed in the GLC chromatograms obtained with analytical columns using various stationary phases, the less easily eluted, cis-isomer, XL, is more strongly retained as the polarity of the stationary phase increases to a greater extent than the more easily eluted, trans-isomer, IXL. This corresponds to a relatively greater increase of solvation of XL over IXL with an increase in the polarity of a solvent medium. Therefore a change from a less polar to a more polar solvent will push the equilibrium XL $\rightleftharpoons$ IXL to the left under suitable conditions for equilibration (for example, in the presence of a trace of dry acid catalyst).

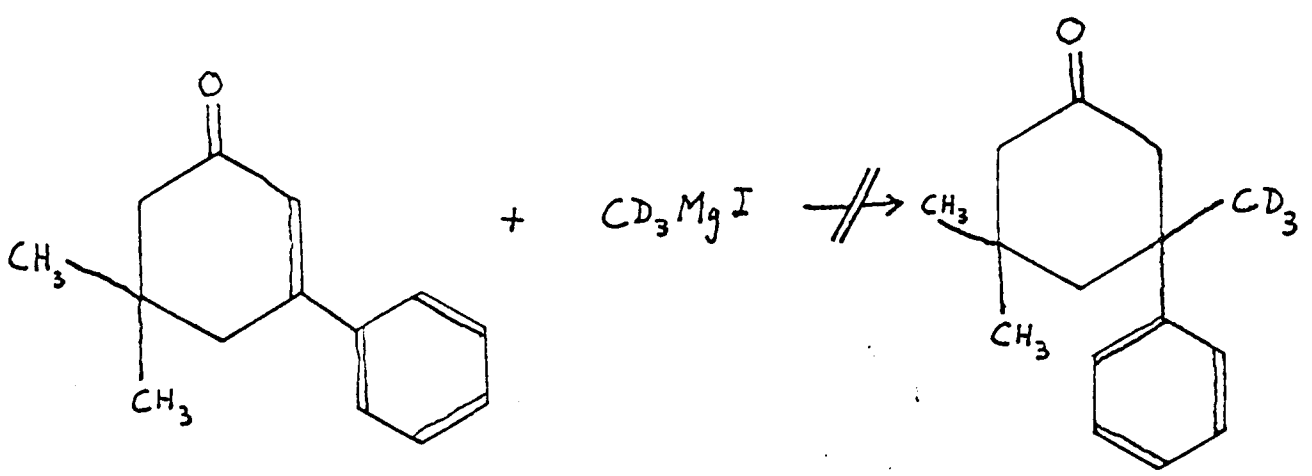
The Preparation of Cyclohexanone Derivatives

5,5-Dimethyl-3-trideuteriomethyl-2,4,4,6,6-pentadeuteriocyclohex-2-en-1-one, XLI, was prepared in 64% yield by the repeated deuteriation of 3,5,5-trimethylcyclohex-2-en-1-one, XLII, with an acidic mixture of heavy water and ethanol-OD, generated by the hydrolysis of triethyl orthoformate with a trace of phosphorus oxychloride in heavy water.

The preparations of 3-phenyl-3,5,5-trimethylcyclohexanone, XXXVI, and 3-phenyl-5,5-dimethyl-3-trideuteriomethyl-2,2,4,4,6,6-hexadeuteriocyclohexanone,



XLIII



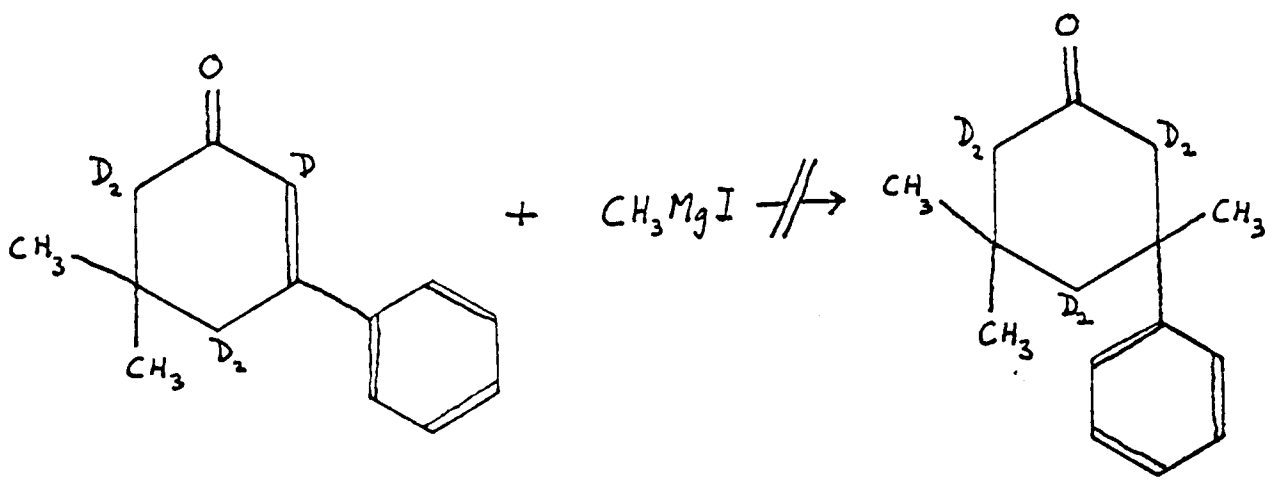


Figure 9

XXXVII, were achieved by the reaction with phenyl magnesium bromide of XLII and XLI respectively. Copper II acetate dissolved in tetrahydrofuran, as suggested by Birch[30] was used as the catalyst. The yields obtained (32 and 35%) were an improvement upon the yield (24%) reported by Balasubramanian and Souza[12] who used copper I chloride as the catalyst for the preparation of XXXVI from XLII.

Attempts to prepare XXXVI by the reaction of methyl magnesium iodide on 5,5-dimethyl-3-phenylcyclohex-2-en-1-one, XLIII, under similar conditions, a model reaction for those indicated in Fig.9, failed. The NMR spectra of the crude reaction residues indicated the absence of the desired product and were compatible with 1,2-addition.

The relative success of the addition of phenyl magnesium bromide to XLII and the failure of the 1,4-addition of methyl magnesium iodide to XLIII may be ascribed to steric control of the relative kinetics of 1,2- and 1,4-addition. From investigation of the attack by Grignard reagents on α,β-unsaturated ketones, House[31] has shown that an increase in the size of the Grignard reagent favours 1,4-attack over 1,2-attack.

There was insufficient time to attempt the preparation of XXXVI from XLIII using methylcopper reagents recently investigated by House[32] and by Piers and Keziere.[33]

EXPERIMENTAL

GENERAL PROCEDURES (applying to preparative work in all three chapters)

All starting materials were purified using standard procedures[34] until a suitable physical property, usually melting point, boiling point or refractive index, was either in agreement with a value quoted in the literature or would not improve upon further treatment. Often in the latter case an NMR, UV or IR spectrum would be used as an additional criterion of purity.

Melting points were recorded on a Kohfler block using uncorrected thermometers. The refractive indices of liquids were measured on an Abbé refractometer.

Microanalyses were carried out by Dr. F. B. Strauss and his staff.

PREPARATION OF KETONES AND A DIOL FOR THE SYNTHESIS OF 1,3-DIOXANES

Methyl 4,4-dimethyl-3-oxopentanoate

Sodium hydride (60% sodium hydride in an oil dispersion, 50 g, 1.25 mole) was washed by decanting three portions (80 ml each) of petroleum ether (b.p. 30-40°) from it, and then suspended, by stirring, in dimethyl carbonate (220 ml, 2.6 mole) with methanol (2 ml). The suspension was heated to boiling and then pinacolone (78 g, 0.63 mole) was added at such a rate as to maintain brisk boiling under reflux unaided. More dimethyl carbonate (100 ml) was added to keep the mixture mobile towards the end of the pinacolone addition.

After one more hour of boiling under reflux, the mixture was cooled in an ice bath and poured into a mixture of concentrated hydrochloric acid (108 ml, 1.25 mole) and ice (200 g). Enough tartaric acid was dissolved in the resulting solution to neutralise it. Ethereal extracts (350 ml) of the solution were washed with brine and dried over magnesium sulphate. The residue from evaporation was

distilled at 13 mm pressure and the fraction b.p. 74-80° was collected and redistilled at the same pressure. Yield 75 g, 76%, b.p. 74-77°/13 mm, n_D^{20} 1.4329. Literature[35] b.p. 91-93°/20 mm.

Methyl 2-methyl-3-oxopentanoate

Sodium hydride (60% sodium hydride as a dispersion in oil, 50 g, 1.25 mole) was washed as above and then a solution of methanol (2 ml) in tetrahydrofuran (200 ml) which had been dried with lithium aluminium hydride, was added. The stirred suspension was brought to the boil and methyl propionate (133 g, 1.5 mole) was added dropwise in an hour.

With continuous stirring the mixture was boiled under reflux until, after 16 hours, no gas was evolved from the cooled mixture when a few drops of methanol were added. Ether (200 ml) was poured into the mixture, followed by an ice-cold solution of glacial acetic acid (80 ml, 1.3 mole) in water (200 ml). The two resulting liquid phases were separated, ethereal washings of the aqueous one being combined with the organic one, which was then washed and dried in the usual way. The residue from evaporation was distilled and the fraction b.p. 65-69°/11 mm was then distilled through a 15" Vigreux column. Yield 30.5 g, 39%, b.p. 78.5-78.8°/16 mm, n_D^{20} 1.4230. Literature[36] b.p. 74-76°/15 mm, n_D^{20} 1.4211.

4-t-Butyl-2-methoxycarbonyl-6-methylcyclohexanone

Excess dimethyl carbonate (110 ml, 1.3 mole) as solvent was condensed with 4-t-butyl-2-methylcyclohexanone (52.5 g, 0.31 mole) using sodium hydride (0.63 mole) as base by following the same procedure as for condensation with pinacolone in the preparation of methyl 4,4-dimethyl-3-oxopentanoate above, except that the reaction mixture was heated at about 80° for 16 hours before work-up.

The crude product was distilled at 0.6 mm pressure, and the fraction b.p. 107.5-108.0° was collected. Yield 36.5 g, 52%. n_D^{20} 1.4790. $C_{13}H_{22}O_3$ requires 68.99% C, 9.80% H. Found 69.1% C, 9.7% H.

The NMR spectrum of the product (10% w/v in carbon tetrachloride) had signals at -2.1 τ and -2.3 τ, indicating the presence of equal quantities of c-4-t-butyl-r-2-methoxycarbonyl-c-6-methylcyclohex-1-enol and c-4-t-butyl-r-2-methoxycarbonyl-t-6-methylcyclohex-1-enol.

2,2-Dibenzylpropane-1,3-diol

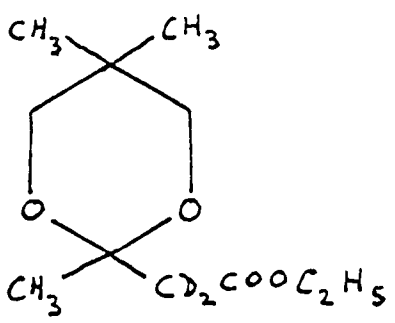
Using the methods described earlier by the author,[29] diethyl dibenzylmalonate, prepared by the double benzylation of diethylmalonate with sodium ethoxide and benzyl chloride, was reduced with a suspension of a slight excess of lithium aluminium hydride in boiling dioxane in 58% yield, m.p. 81-83°. Literature[37] m.p. 82-83°.

PREPARATION OF 1,3-DIOXANES BY THE CONDENSATION OF DIOLS WITH KETONES

Listed below are the various compounds made in this way with, in each case, the quantities of reactants and solvent, the reaction time, properties of the product and any modifications of the following general procedure.

Diol and ketone were mixed, with whichever was more easily removed in the work-up (a water soluble diol or relatively volatile ketone) in slight excess. The required quantity of benzene or, rarely, toluene was added and the mixture was boiled under reflux through a Dean-Stark apparatus to remove water azeotropically. If necessary, portions of heavy water or water were added with azeotropic removal of water/heavy water mixtures after each addition to effect any exchange required for the reactants. The acid catalyst (toluene-p-sulphonic acid, in quantities

XXI



XXII

between 4 and 25 g/mole ketone) was then added and the reaction mixture was refluxed as briskly as possible to facilitate removal of the water formed, for a specified reaction time.

With protection from moisture the solution was cooled, and then washed twice with its own volume of ice-cold aqueous sodium hydroxide solution (0.5 M) and once with half its own volume of brine followed by drying over anhydrous potassium carbonate. The residue from evaporation, together with a drop of triethylamine, was distilled at oil pump pressure.

All products were stored in a desiccator over potassium hydroxide pellets.

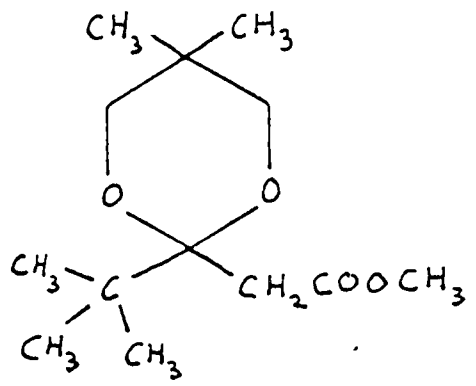
XXI 2-Ethoxycarbonylmethyl-2,5,5-trimethyl-1,3-dioxane

2,2-Dimethylpropane-1,3-diol (10 g, 0.10 mole) and ethyl acetoacetate (13.8 g, 0.11 mole) were condensed in the standard way with toluene-p-sulphonic acid (1.0 g, 10 g/mole ketone) in benzene (500 ml) with a reaction time of 45 minutes. The product was collected as the fraction b.p. 75.7-76.0°/0.7 mm, n_D^{20} 1.4407. Yield 11.5 g, 55%. $C_{11}H_{20}O_4$ requires 61.09% C, 9.32% H. Found 61.2% C, 9.4% H.

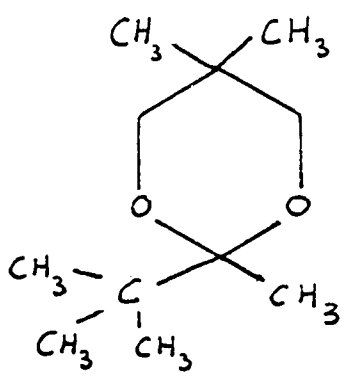
XXII 2-Ethoxycarbonyldideuteriomethyl-2,5,5-trimethyl-1,3-dioxane

Five portions of heavy water (5 x 4 ml, 5 x 0.2 mole) were used for exchange with 2,2-dimethylpropane-1,3-diol (9.5 g, 0.09 mole) and ethyl acetoacetate (13.0 g, 0.10 mole) in benzene (250 ml). Afterwards toluene-p-sulphonic acid-OD (prepared by evaporating a solution of the H-acid in heavy water several times, 0.4 g, 4 g/mole ketone) was added and 10 minutes reaction time allowed. The product had b.p. 96-98°/3 mm, n_D^{20} 1.4408. Yield 11.8 g, 59%.

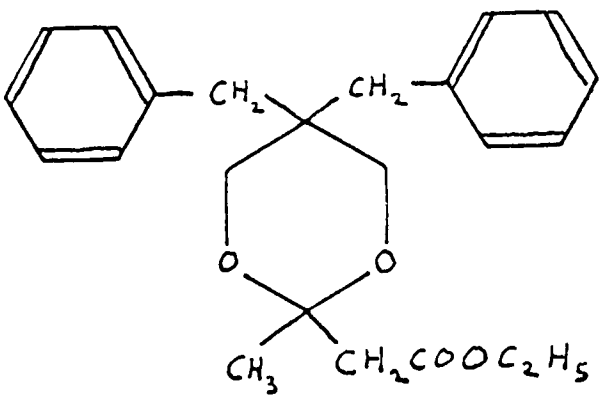
The low resolution mass spectrum, compared with that of XXI recorded under



XLIV



XXIII



XXIV

the same conditions, indicated, from the intensities of the M^+, $(M-CH_3)^+$ and $(M-CH_2COOC_2H_5)^+$ groups of lines, the equivalent of 93% dideuteriation at the 2-methylene position and 16% monodeuteriation at the 2-methyl position.

Attempted Preparation of XLIV 2-t-Butyl-5,5-dimethyl-2-methoxycarbonylmethyl-1,3-dioxane

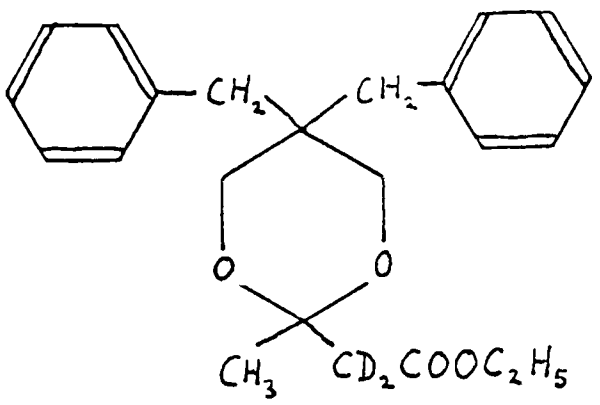
The most productive of several attempts used equimolar quantities of 2,2-dimethylpropane-1,3-diol and methyl 4,4-dimethyl-3-oxopentanoate in toluene (1.6 l/mole ketone) with toluene-p-sulphonic acid (10 g/mole ketone) and 70 minutes reaction time. Material of b.p. 98-100°/0.6 mm corresponding to a yield of about 4% was obtained. The NMR spectrum of a solution in carbon tetrachloride indicated the presence of a high proportion of the desired product. However, decomposition, with the formation of a water soluble solid, took place on standing before, and during, a further distillation.

XXIII 2-t-Butyl-2,5,5-trimethyl-1,3-dioxane

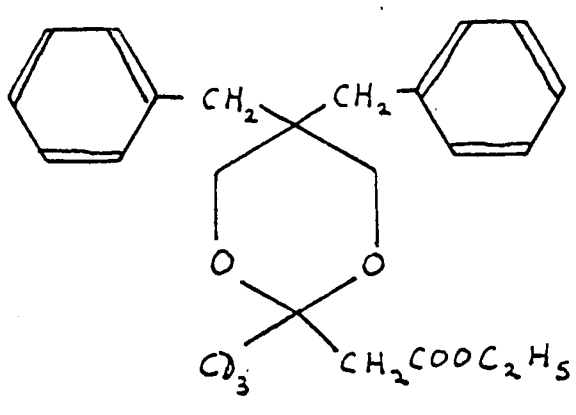
A sample, prepared in the standard way using toluene as solvent, was provided by Dr. M.J.T. Robinson.

XXIV 5,5-Dibenzyl-2-ethoxycarbonylmethyl-2-methyl-1,3-dioxane

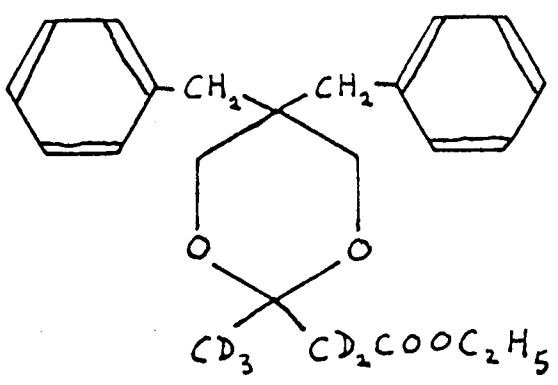
2,2-Dibenzylpropane-1,3-diol (2.9 g, 0.011 mole) was condensed with ethyl acetoacetate (1.7 g, 0.013 mole) using toluene-p-sulphonic acid (0.24 g, 20 g/mole ketone) in benzene (120 ml) over 20 minutes in the usual way. The product had b.p. 175-178°/0.2 mm, n_D^{20} 1.5434. Yield 2.75 g, 68%. $C_{23}H_{28}O_4$ requires 74.97% C, 7.66% H. Found 74.7% C, 7.8% H.



XXV



XXVI



XXVII

Attempted Preparation of XXV 5,5-Dibenzyl-2-ethoxycarbonyldideuteriomethyl-2-methyl-1,3-dioxane

The preparation of XXIV above was repeated with deuterium exchange with the reactants preceding the addition of the (deuteriated) acid catalyst. The M^+, $(M-CH_3)^+$ and $(M-CH_2COOC_2H_5)^+$ line groups in the mass spectrum of the product and of XXIV indicated scrambling of the substituted deuterium between the 2-methyl and 2-methylene groups. Accordingly XXV was prepared by direct deuteriation of XXIV described below.

XXVI 5,5-Dibenzyl-2-ethoxycarbonylmethyl-2-trideuteriomethyl-1,3-dioxane

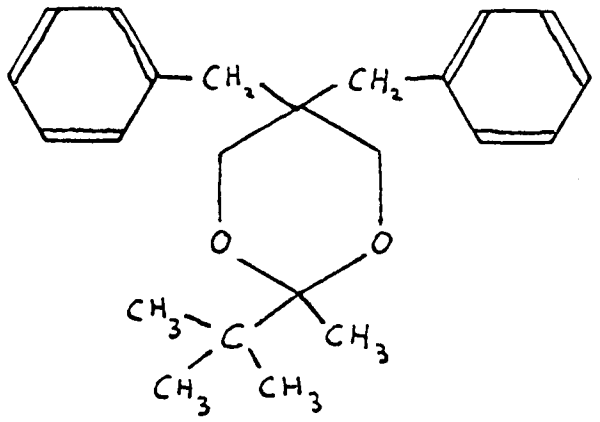
In earlier work[29] a sample of ethyl pentadeuterioacetoacetate was prepared in low yield (9%) by base-catalysed deuterium exchange. Four portions of water (4 x 0.18 ml, 4 x 0.01 mole) were used for exchange with this ketone (0.92 g, 0.0068 mole) and 2,2-dibenzylpropane-1,3-diol (1.45 g, 0.0055 mole) in benzene (70 ml) before the acid catalyst (0.12 g, 20 g/mole ketone) was added and 20 minutes reaction time was allowed.

The product was distilled from bulb to bulb at 0.2 mm, the temperature of the oil bath being 185° during distillation. Crude yield 1.97 g, 95%, n_D^{20} 1.5424. The mass spectrum of the product, in comparison with that of XXIV indicated the equivalent of 65% trideuteriation at the 2-methyl position and 13% monodeuteriation at the 2-methylene position.

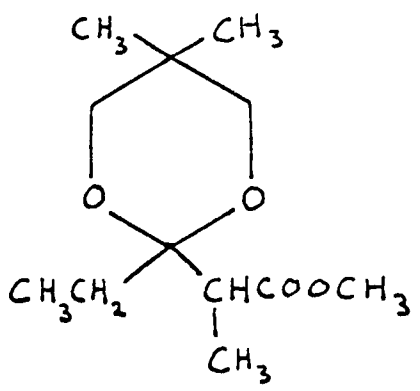
XXVII 5,5-Dibenzyl-2-ethoxycarbonyldideuteriomethyl-2-trideuteriomethyl-1,3-dioxane

In one flask ethyl acetoacetate (3.9 g, 0.03 mole) and toluene-p-sulphonic acid (0.3 g, 10 g/mole ketone) in refluxing benzene (60 ml) were treated with seven

64



XXVIII



XXIX

portions of heavy water (7 x 1.55 ml, 7 x 0.0755 mole) with azeotropic removal of heavy water/water mixtures after each addition through a Dean Stark apparatus, and then the contents of the flask was allowed to cool with protection from atmospheric moisture.

In a second flask 2,2-dibenzylpropane-1,3-diol (2.56 g, 0.01 mole) in benzene (60 ml) was treated with seven portions of heavy water (7 x 0.3 ml, 7 x 0.015 mole) in the same way. Then the contents of the first flask was added to that of the second at such a rate as would not stop the boiling and 30 minutes reaction time was allowed.

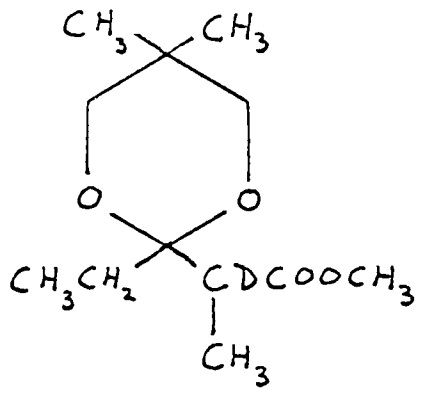
The product had b.p. 172-175°/0.1 mm, n_D^{20} 1.5431. Yield 1.55 g, 41%. The mass spectrum indicated the equivalent of 78% trideuteriation at the 2-methyl positions and 84% dideuteriation at the 2-methylene positions.

XXVIII 2-t-Butyl-5,5-dibenzyl-2-methyl-1,3-dioxane

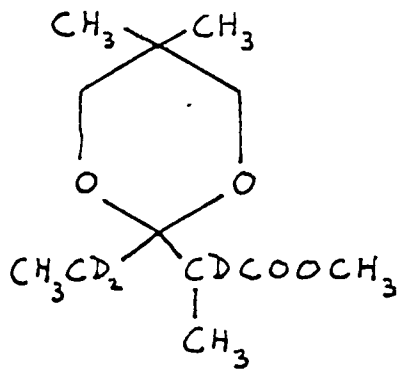
This compound was prepared in earlier work[29] from the condensation of pinacolone and 2,2-dibenzylpropane-1,3-diol using the standard method with toluene as solvent.

XXIX 5,5-Dimethyl-2-ethyl-2-(1-methoxycarbonylethyl)-1,3-dioxane

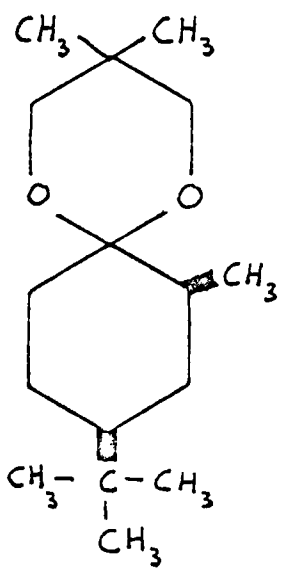
In the usual way 2,2-dimethylpropane-1,3-diol (9 g, 0.09 mole) was condensed with methyl 2-methyl-3-oxopentanoate (7.2 g, 0.05 mole) in benzene (200 ml) with toluene-p-sulphonic acid (1.0 g, 20 g/mole ketone) over 90 minutes. The product had b.p. 84-85°/0.7 mm, n_D^{20} 1.4498. Yield 5.7 g, 50%. $C_{12}H_{22}O_4$ requires 62.58% C, 9.63% H. Found 63.0% C, 9.6% H.



XXX



XXXII



XXXIII

Attempted Preparation of XXX 5,5-Dimethyl-2-ethyl-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane

Attempts to prepare this compound following the method above for XXIX with deuteriation of the reactants before addition of the (deuteriated) acid catalyst led to a product whose mass spectrum, in comparison with that of XXIX , indicated scrambling of the substituted deuterium between the 2-methylene and 2-methine positions. Accordingly XXX was prepared by direct deuteriation of XXIX as described below.

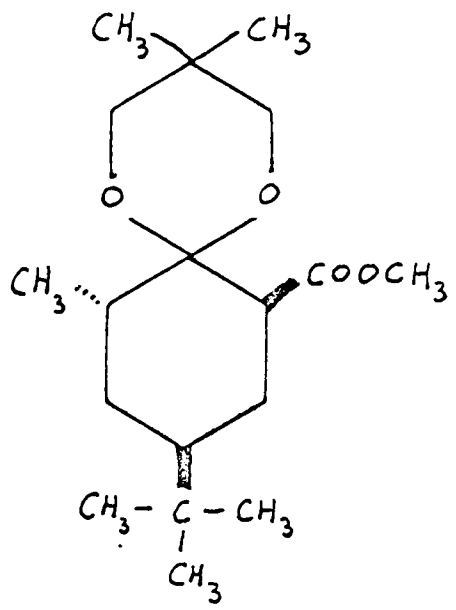
XXXII 5,5-Dimethyl-2-(1,1-dideuterioethyl)-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane

Following the "two flask" method used in the preparation of XXVII, in one flask methyl 2-methyl-3-oxopentanoate (7.2 g, 0.05 mole) and toluene-p-sulphonic acid (1.0 g) in benzene (80 ml) were deuteriated with seven portions of heavy water (7 x 1.7 ml, 7 x 0.085 mole). Seven portions of heavy water (7 x 2.7 ml, 7 x 0.135 mole) were also used to deuteriate 2,2-dimethylpropane-1,3-diol (8 g, 0.08 mole) and toluene-p-sulphonic acid (1.5 g) in benzene (80 ml) in a second flask. Afterwards the contents of the first flask was added to that of the second and 105 minutes reaction time allowed.

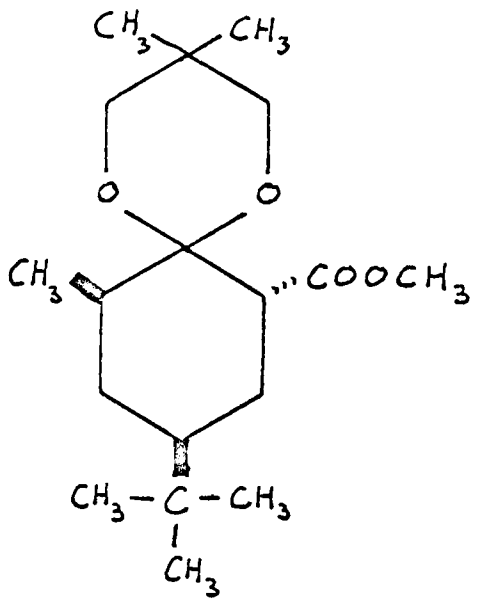
The product had b.p. 69-70°/0.15 mm, n_D^{20} 1.4496. Yield 3.13 g, 27%. The mass spectrum indicated the equivalent of 97% dideuteriation at the 2-methylene position and 93% monodeuteriation at the 2-methine position.

XXXIII r-9-t-Butyl-3,3,c-7-trimethyl-1,5-dioxaspiro[5.5]undecane

The standard procedure was used for the condensation of 2,2-dimethylpropane-1,3-diol (5.5 g, 0.055 mole) with 4-t-butyl-2-methylcyclohexanone (n_D^{20} 1.4625.



XXXV



XXXIV

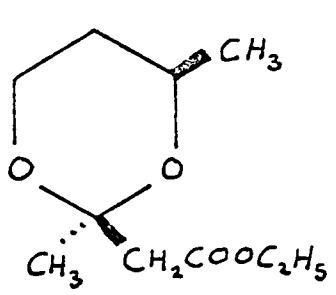
Literature[38] n_D^{20} 1.4604 for a sample 93% cis and 7% trans, 8.4 g, 0.05 mole) in benzene (250 ml) using toluene-p-sulphonic acid (0.5 g, 20 g/mole ketone) with a reaction time of 135 minutes.

The fraction b.p. 98-100°/0.2 mm solidified and was recrystallised from petroleum ether (b.p. 30-40°) at -78°. M.p. 57-61°. Yield 10.71 g, 91%. $C_{16}H_{30}O_2$ requires 75.53% C, 11.89% H. Found 75.8% C, 12.0% H.

XXXV c-9-t-Butyl-r-7-methoxycarbonyl-3,3,t-11-trimethyl-1,5-dioxaspiro[5.5]-undecane and XXXIV t-9-t-Butyl-r-7-methoxycarbonyl-3,3,t-11-trimethyl-1,5-dioxaspiro[5.5]undecane

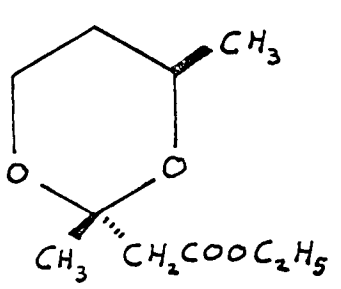
In the usual way 2,2-dimethylpropane-1,3-diol (5.5 g, 0.055 mole) and 4-t-butyl-2-methoxycarbonyl-6-methylcyclohexanone (5.65 g, 0.025 mole) were condensed in toluene (100 ml) using toluene-p-sulphonic acid (0.5 g, 20 g/mole ketone) over 180 minutes. The NMR spectrum of the crude product (10% w/v in carbon tetrachloride) had two small signals around -2 τ, indicating the presence of cis and trans enolic forms of the keto ester starting material.

The crude product (8 g) was dissolved in ethanol (10 ml) and stirred as a solution of sodium hydroxide (0.6 g, 0.015 mole) in aqueous ethanol (1:1, 12 ml) was added followed by lanthanum nitrate hexahydrate (0.22 g, 0.005 mole) in aqueous ethanol (1:1, 12 ml). The mixture was stirred under water-pump pressure for 90 minutes and then water (200 ml) was added. The aqueous suspension was extracted with petroleum ether (b.p. 30-40°, 300 ml), which was then filtered, washed with brine and dried over anhydrous potassium carbonate. The residue from evaporation was distilled to give the product b.p. 123-125°/0.2 mm, n_D^{20} 1.4734. Yield 4.2 g, 53%. $C_{18}H_{32}O_4$ requires 69.19% C, 10.32% H. Found 69.5% C, 10.2% H.



cis

XL



trans

IXL

The two isomers XXXV and XXXIV were separated by elution of a sample of the product on Kieselgel coated plates with an ether petroleum ether (b.p. 30-40°) mixture (1:19). The NMR spectra of the two chromatographic fractions (10% w/v in benzene) in comparison with that of the original mixture (recorded on a 270 MHz instrument) indicated that the first-eluted isomer was XXXIV (a doublet of doublets attributed to a 7-equatorial proton centred at 6.23 τ) and the second-eluted isomer was XXXV (a doublet of doublets attributed to a 7-axial proton centred at 7.06 τ). The spectrum of the product mixture indicated that XXXV and XXXIV were present in quantities of 1:4 parts.

XL 2,c-4-Dimethyl-r-2-ethoxycarbonylmethyl-1,3-dioxane and IXL 2,t-4-Dimethyl-r-2-ethoxycarbonylmethyl-1,3-dioxane

In the usual way butane-1,3-diol (9 g, 0.1 mole) and ethyl acetoacetate (13.75 g, 0.11 mole) were condensed in benzene (400 ml) using toluene-p-sulphonic acid (1 g, 10 g/mole ketone) over a reaction time of 100 minutes. The product had b.p. 82°/0.3 mm, n_D^{20} 1.4414. Yield 11.9 g, 59%. $C_{10}H_{18}O_4$ requires 59.38% C, 8.97% H. Found 59.8% C, 9.2% H.

The NMR spectrum of the product (20% w/v in carbon tetrachloride) showed for the 2-methylene protons an AB quartet for the trans isomer (J_{AB} = 12.5 Hz, τ_A = 7.19 ppm, τ_B = 7.33 ppm), relative intensity 0.61, and a singlet (τ = 7.53 ppm), relative intensity 0.39, for the cis isomer, the combined intensities corresponding to two protons.

GLC of the product at 75° using 5 ft columns and a flow rate of 40 ml nitrogen/minute gave traces as shown in the following table.

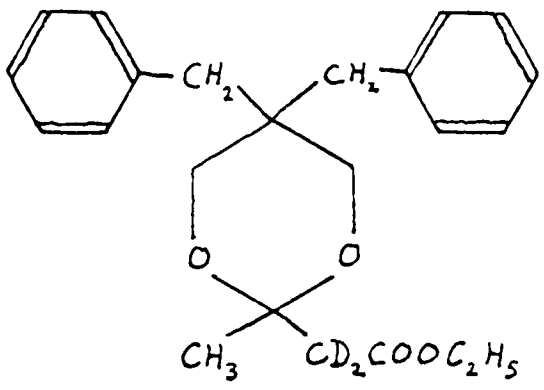
Support	Stationary Phase	trans peak: Retention Time (min)	trans peak: Relative Intensity	cis peak: Retention Time (min)	cis peak: Relative Intensity	Ratio of Retention Times
Chromosorb G	3% Silicone Oil	97	0.60	128	0.40	0.76
Diatomite C	5% Polyethylene Glycol "600"	118	0.60	158	0.40	0.75
Diatomite C	5% Tris-Cyanoethoxypropane	168	0.61	249	0.39	0.67

DIRECT DEUTERIATION OR PROTIATION OF 1,3-DIOXANES

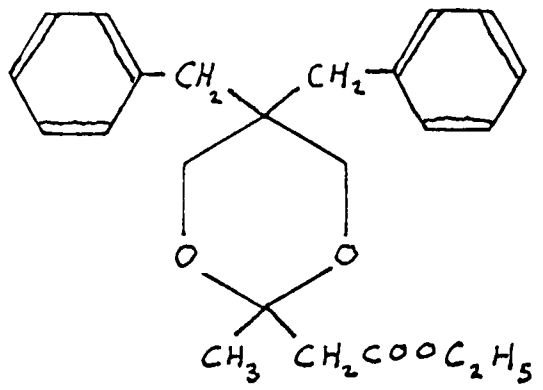
Ethanol-OD and methanol-OD were prepared using the method of Streitwieser [39] et al.

The following procedure was used for the preparation of the three compounds listed below with, in each case, the quantities of reactants, reaction time and properties of the product.

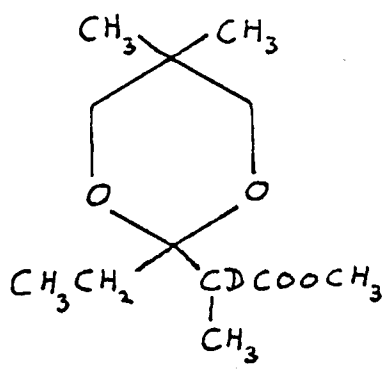
Freshly drawn sodium wire was dissolved in the required dry alcohol under dry nitrogen. With suitable protection from moisture the alcoholic base was added to the substrate and either dimethyl or diethyl carbonate and the resulting solution was heated at 35° in a sealed tube for the required time. The contents of the tube were washed into a conical flask with a little dry ether and the headspace above the stirred solution was evacuated with a water-pump through a solid carbon dioxide trap. When the volume of the solution had been reduced by about three quarters, ether and water (both in equal quantities to the volume used in the sealed tube) were added and the



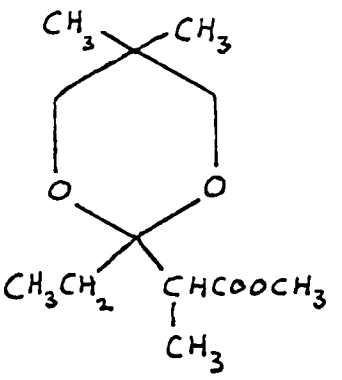
XXV



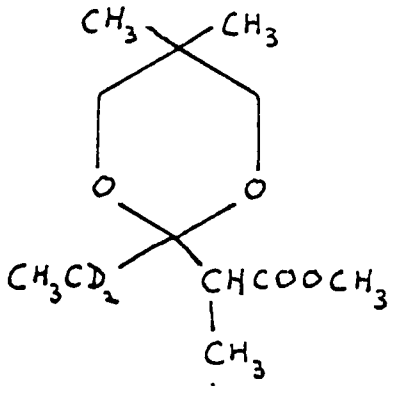
XXIV



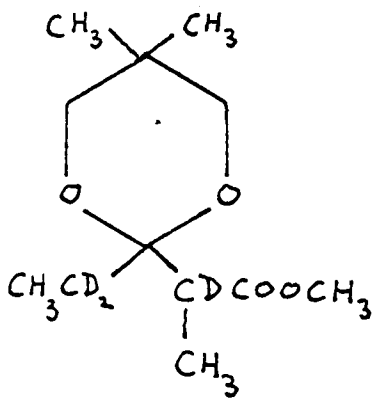
XXX



XXIX



XXXI



XXXII

two phases were separated. The ethereal solution was washed with brine and dried over potassium carbonate. The product was collected as a fraction from the distillation of the residue after evaporation.

The products were stored above potassium hydroxide pellets in a desiccator.

XXV 5,5-Dibenzyl-2-ethoxycarbonyldideuteriomethyl-2-methyl-1,3-dioxane

In three batches the undeuteriated compound (*XXIV*, total 3 g, 0.009 mole) and dry diethyl carbonate (3 ml, 0.025 mole) were treated with sodium (2.1 g, 0.09 g atom) in ethanol-OD (90 ml, 1.5 mole) for 5 days. The product had b.p. 187-189°/0.1 mm, n_D^{20} 1.5427. Yield 1.58 g, 52%. Comparison of the mass spectra of the starting material and product indicated no deuteriation at the 2-methyl position and the equivalent of 85% dideuteriation at the 2-methylene position.

XXX 5,5-Dimethyl-2-ethyl-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane

The undeuteriated compound (*XXIX*, 0.50 g, 0.022 mole) and dimethyl carbonate (0.2 g, 0.002 mole) were treated with sodium (0.23 g, 0.010 g atom) in methanol-OD (10 ml, 0.24 mole) for 24 hours. The product had b.p. 67°/0.4 mm, n_D^{20} 1.4498. Yield 0.40 g, 80%. The mass spectrum of the product, in comparison with that of the starting material, indicated no deuteriation at the 2-methylene position and 96% monodeuteriation at the 2-methine position.

XXXI 5,5-Dimethyl-2-(1,1-dideuterioethyl)-2-(1-methoxycarbonylethyl)-1,3-dioxane

The trideuteriated starting material, 5,5-dimethyl-2-(1,1-dideuterioethyl)-2-(1-methoxycarbonyl-1-deuterioethyl)-1,3-dioxane (*XXXII*, 0.50 g, 0.0021 mole) and dimethyl carbonate (0.2 g, 0.002 mole) were treated with sodium (0.23 g, 0.010 g atom) in methanol-OH (10 ml, 0.24 mole) for 5 days. The product had

b.p. 60.5°/0.1 mm, n_D^{20} 1.4498. Yield 0.43 g, 85%. The mass spectrum indicated no deuteriation at the 2-methine position and the equivalent of 96% dideuteriation at the 2-methylene position.

PREPARATION OF CYCLOHEXANONE DERIVATIVES

5,5-Dimethyl-3-trideuteriomethyl-2,4,4,6,6-pentadeuteriocyclohex-2-en-1-one

A partially deuteriated sample of 3,5,5-trimethylcyclohex-2-en-1-one was provided by Dr. M.J.T. Robinson. The NMR spectrum of the sample (10% w/v in carbon tetrachloride) indicated approximately 50% deuteriation at each of the required positions.

Heavy water (11.3 ml, 0.62 mole) was added to partially deuteriated 3,5,5-trimethylcyclohex-2-en-1-one (10 g, 0.07 mole) followed by phosphorus oxychloride (1 ml, 0.01 mole) and enough triethyl orthoformate (11 ml, 0.067 mole) to produce a clear solution. This mixture was sealed in a tube and heated in boiling water for 24 hours. The contents of the cooled tube were extracted with dichloromethane (50 ml) and the extract was washed twice with its own volume of aqueous dipotassium hydrogen orthophosphate solution (1 M), once with brine (25 ml) and then dried over anhydrous sodium sulphate. The residue from evaporation was used for a further exchange process. This cycle of operations was performed five times in all.

The final residue was distilled to give the product, b.p. 86-89°/17 mm, n_D^{20} 1.4765 (Literature[40] n_D^{20} 1.4775 for the undeuteriated compound). Yield 6.5 g, 64%. The NMR spectrum of the product (20% w/v in carbon tetrachloride) consisted of a singlet (τ = 9.0 ppm) of an intensity at least twenty times the combined intensities of all other signals between 0 and 10 τ.

3-Phenyl-3,5,5-trimethylcyclohexanone

To phenyl magnesium bromide in ether (40 ml), prepared from magnesium (3.6 g, 0.15 g atom) and bromobenzene (23.6 g, 0.15 mole), was added a solution of 3,5,5-trimethylcyclohex-2-en-1-one (6.9 g, 0.05 mole) in tetrahydrofuran (30 ml) saturated with copper II acetate monohydrate (0.8 g, 0.004 mole) over one hour, as the chilled mixture was stirred. The surrounding ice bath was allowed to warm to room temperature. After 20 hours' stirring, the reaction mixture was boiled under reflux for an hour and then cooled in an ice bath as a mixture of water (5.4 ml, 0.3 mole) with an equal volume of tetrahydrofuran was added followed by an excess of saturated aqueous ammonium chloride solution.

Ethereal extracts of the aqueous phase were added to the organic one and then washed firstly with dipotassium hydrogen orthophosphate solution and then with brine. The solution was dried over anhydrous magnesium sulphate and evaporated.

The crude residue (16.5 g), dissolved in ethanol (16 ml), was added to a stirred solution of semicarbazide hydrochloride (6.5 g, 0.05 mole) and pyridine (4.1 g, 0.05 mole) in aqueous ethanol (1:1, 40 ml). After two hours of stirring, petroleum ether (b.p. 30-40°, 10 ml) was added to break up the sticky precipitate which had formed and the mixture was stirred for another hour. The precipitated semicarbazone was filtered off and washed with copious amounts of aqueous ethanol (1:1) and petroleum ether (b.p. 30-40°) and dried at 100° before recrystallisation from ethanol dioxane (1:1, 110 ml). Yield 5.2 g, 38%. M.p. 193-195°. Literature[12] m p. 198-199°.

Triethyl orthoformate (10 ml, 0.06 mole) with phosphorus oxychloride (1 drop) was added to the semicarbazone which had been moistened with water (2.0 ml,

0.11 mole). This mixture was stirred with petroleum ether (b.p. 40-60°, 100 ml) as a solution made by the reaction of phosphorus oxychloride (3 g, 0.02 mole) with water (10 ml, 0.55 mole) was added. When the mixture had been stirred for 20 hours the petroleum solution was separated, washed with aqueous dipotassium hydrogen orthophosphate solution and brine, and dried over magnesium sulphate. The residue from evaporation was distilled to give the product, b.p. 154-156°/12 mm, n_D^{20} 1.5292. Literature[12] b.p. 149-150°/11 mm, n_D^{30} 1.5254. Yield 3.5 g, 32% overall.

The reaction of methyl magnesium iodide with 5,5-dimethyl-3-phenylcyclohex-2-en-1-one (prepared by the reaction of phenyl magnesium bromide with the ethyl ester of 5,5-dimethylcyclohexane-1,3-dione by the method of Woods[41]) was investigated using, separately, copper I chloride and copper II acetate as catalysts. NMR spectra of solutions of the crude residues from work-up indicated the presence of little, if any, of the desired product.

5,5-Dimethyl-3-phenyl-3-trideuteriomethyl-2,2,4,4,6,6-hexadeuteriocyclohexanone

Using the sample of 5,5-dimethyl-3-trideuteriomethyl-2,4,4,6,6-pentadeuteriocyclohex-2-en-1-one, the preparation of which is described above, as starting material, the same procedure was followed on the same scale as for the preparation of 3-phenyl-3,5,5-trimethylcyclohexanone above. The only change was the use of heavy water in place of water where molar quantities are specified.

The yield of semicarbazone was 5.8 g, 42%, m.p. 197-201°. That of the product was 3.9 g, 35% overall, b.p. 99-100°/0.3 mm, n_D^{20} 1.5275.

The mass spectrum of the product in comparison with that of the undeuteriated compound indicated the equivalent of 94% nonadeuteriation, and an NMR spectrum

of the product (10% w/v in carbon tetrachloride) indicated the presence of aromatic and two sets of methyl protons with intensity ratio 5:3:3. The relative intensity of the residual signal of the 3-methyl group indicated 97% trideuteriation.

3,3-Dimethyl-5-phenylcyclohexanone

A sample of 3,3-dimethyl-t-5-phenylcyclohexanol, supplied by Dr. M.J.T. Robinson, was oxidised according to the method of Jones[42] to give the product in 84% yield, m.p. 54-57°. Literature[43] m.p. 53.5°.

THE MEASUREMENT OF NMR CHEMICAL SHIFT DIFFERENCES

The following general method was used for the preparation of 1,3-dioxane and cyclohexanone derivatives as solution samples for NMR spectrometry. About 0.8 ml of solution was made up in a test-tube and filtered through a small amount of alumina over a cotton wool plug into an NMR tube with two constrictions. The alumina was omitted for trideuterioacetonitrile solutions. These operations were carried out in a cold-room in the cases when the solvent was fluorotrichloromethane.

The solution was then subjected to three freeze-pump-thaw cycles[44] to remove air, using liquid nitrogen as refrigerant. For mixed solvent solutions, the pumping was started several minutes after immersion in liquid nitrogen on each occasion as an extra precaution against the selective loss of one of the solvent components. On the third cycle, the tube, immersed in liquid nitrogen and evacuated, was sealed with a hand-torch at the upper constriction. The second constriction was then sealed as symmetrically as possible (to ensure smooth spinning of the tube), and the tube between the two seals was discarded.

It was essential to ensure that seed crystals formed at the bottom of the tube before full immersion of the solution in liquid nitrogen in order to avoid the mechanical

strain from sudden crystallisation of large portions of solution. Strain from uneven melting during warming-up processes was avoided by ensuring that the upper portions of the solution melted first.

For samples run in a J.E.O.L. JNM-4H-100 spectrometer, spectra were calibrated using markers whose positions relative to tetramethylsilane were measured with a frequency counter. The calibration scales, derived from a linear least-squares fit, were used to convert distances measured between lines which bisected the areas of the signals into chemical shift differences. Thermometry was based upon a linear relation between the chemical shift of the hydroxylic protons in methanol and temperature.[45]

For samples run in a Perkin Elmer R14 (100 MHz) instrument, the distance between the tetramethylsilane signal and a 200 Hz oscillator-induced side-band was used for calibration. The probe temperature, measured by the direct insertion of a mercury thermometer, varied by less than 0.1 degree throughout the measurements.

For solutions of 5,5-dibenzyl-2-ethoxycarbonylmethyl-2-methyl-1,3-dioxane and its deuteriated analogues in trideuterioacetonitrile, direct measurement of the distance between the area bisectors of the two benzyl methylene signals was not possible since this distance was only slightly greater than the signal width at half-height. The distance measured was that between the two observed maxima. The distances between the two maxima of the sum of two Lorenzian curves with various centroid separations were computed and expressed, in an exponential least-squares fit, as a function of the ratio of centroid separation to width at half-height. This function was used to obtain "true" separation values from the experimental maxima separations.

CORRECTIONS FOR IMPERFECT DEUTERIATION

Observed isotope effects on chemical shift differences required correction for imperfect deuteriation. These corrections were based upon the assumption that the effects of individual deuterium atoms substituted for protium on the same carbon atom were simply additive. That is, that the changes in conformational free energy differences ($\Delta\Delta G$) between, for example, the species with $-CD_3$ and $-CH_3$, $-CHD_2$ and $-CH_3$ and between $-CH_2D$ and $-CH_3$ were in the ratio 3:2:1. As the observed isotope effects on conformational equilibria were small (changes of less than $2\frac{1}{2}\%$ of the equilibrium constants), additivity of free energy changes corresponds, within 0.1% of the change in chemical shift difference for one substituted deuterium atom, to additivity of changes between chemical shift differences for individual isotopic species. The validity of the assumption of additivity depends upon a lack of rotational preference for the deuterium over protium (or vice versa) of a partially deuteriated substituent in the crowded molecular environments studied. Even the largest isotope effect upon the conformational equilibria studied (16 cal/mole) was small in comparison with RT (612 cal/mole at 35°). Therefore the largest change in rotameric populations resulting from an isotopic preference in partially deuteriated substituents was $1:e^{-16/612}$, that is, $2\frac{1}{2}\%$. The largest of this type of contribution to an isotope effect from partially deuteriated molecules in any of the compounds studied was calculated to correspond to an error of $\frac{1}{2}\%$ in the isotope effect.

If the assumption of additivity is accepted, the position of a signal for an imperfectly deuteriated compound will be given by the maximum (strictly, the area bisector) of the sum of strongly overlapping signals corresponding to the individual isotopic species present. The strong overlap is a consequence of the small size of

the isotopic shifts relative to the band widths observed in this work.

For a sample consisting of a fraction A of monodeuteriated molecules and 1 - A of undeuteriated molecules the signal intensity, y, as a function of the NMR sweep, x, for Lorenzian lines, will be

$$y = \frac{A}{1 + \frac{4}{w^2}(x - \delta)^2} + \frac{1 - A}{1 + \frac{4x^2}{w^2}} \qquad (1)$$

where w is the signal width at half-height and δ is the shift between the signals for the two isotopic species. For such a system the observed isotopic shift, δ_{obs}, will be the position of the maximum value of y. For the compounds studied, signal widths at half-height were in the range 1.4 to 1.6 Hz and δ values were between 0.01 and 0.15 Hz (for spectra recorded on a 100 MHz instrument). Accordingly, values of δ_{obs} were computed for various degrees of deuteriation, A, using w = 1.5 Hz with δ varying from 1 to 10% of the value of w.

For the relation:

$$\delta_{obs} = CA\delta$$

C is a function of A and the ratio $\frac{\delta}{w}$. As $\frac{\delta}{w}$ tends to zero, C approaches unity, at which point δ_{obs} and A are simply proportional. The departure of C from unity is a measure of the error resulting from the assumption of a linear relation between δ_{obs} and A. For any particular value of $\frac{\delta}{w}$ in the range $0 < \frac{\delta}{w} < 1$ (that is, relatively strong overlap), C is unity at A = 0, 0.5 or 1 and is furthest from unity at A = 0.75 (or 0.25). Therefore, to estimate the maximum error resulting from the use of the linear relation as an approximation for the dependence of δ_{obs} on δ, values of C

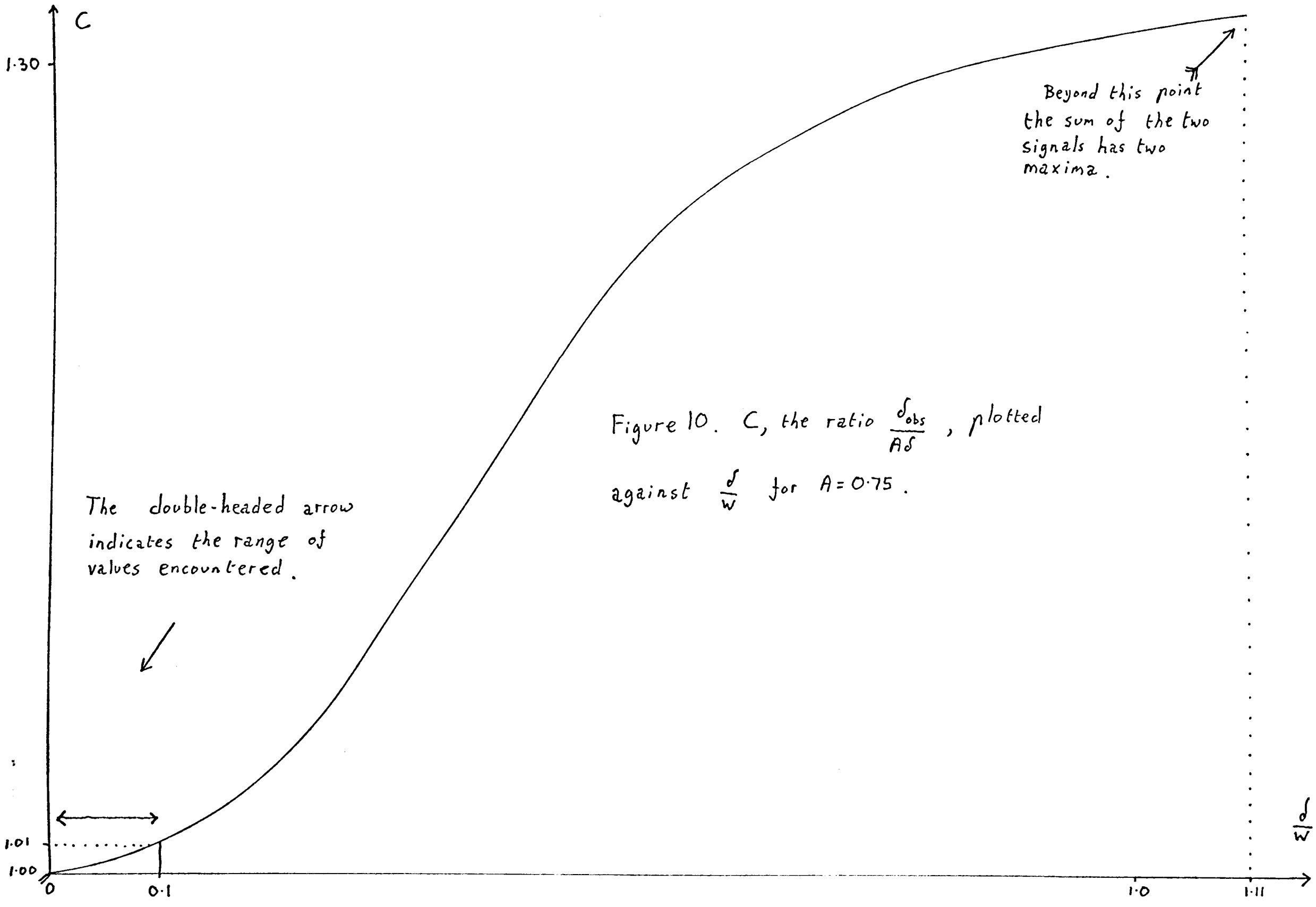


Figure 10. C, the ratio $\frac{\delta_{obs}}{A\delta}$, plotted against $\frac{\delta}{w}$ for $A = 0{\cdot}75$.

were calculated for a range of $\frac{\delta}{w}$ for A = 0.75. Figure 10 is a plot of C against $\frac{\delta}{w}$ and the double headed arrow in the lower left hand corner shows the limits of the range of $\frac{\delta}{w}$ values encountered experimentally. It is apparent that in the worst case the error resulting from assuming a simple linear relationship between δ_{obs} and A of an imperfectly deuteriated sample will be not greater than 1% of the isotope effect for A = 0.75. This error is reduced to $\frac{1}{3}$% if a Gaussian shape for signal components is assumed. For the "two species" samples that were studied, A was greater than 0.85.

For values of $\frac{\delta}{w}$ greater than 1.11 (A = 0.75), the sum of the two Lorenzian signals has two maxima, and, if the position of the greater maximum is chosen for δ_{obs}, C approaches a value of $\frac{4}{3}$ $(= \frac{1}{A})$ asymptotically as $\frac{\delta}{w}$ tends to infinity. This region of relative isotope effects describes spectra for which the signals of individual isotopic species are separately discernible. In the limiting situation where the signal width is negligible in comparison with the isotope effect there are two signals of relative intensities A and 1 - A and of chemical shift difference δ.

The model used above to establish the validity of linear corrections to isotopic signal shifts is applicable to a mixture of monodeuteriated and undeuteriated species, or to a system with one species having N equivalent deuterium atoms and the other having N-1 deuterium atoms. The low resolution mass spectra of the deuteriated compounds which were prepared indicated that all but three of the compounds were such "two-component" systems with no significant population of N - 2 deuteriated species. In the worst case of the three compounds which did have a significant number of N - 2 deuteriated molecules (8% of $-CH_2COOC_2H_5$ in $-CD_2COOC_2H_5$), the error in using a linear correction as opposed to a correction directly based upon

the position of the maximum of a sum of three Lorenzian signals was calculated to be less than 2% of the isotope effect.

Corrections for imperfect deuteriation in more than one substituent of a compound were calculated by treating the effect of deuteriation in one substituent as independent of the effect of deuteriation in another. This independence was observed experimentally for those groups of 1,3-dioxanes with deuteriation distributed in four different ways between two substituents.

The linear correction for imperfect deuteriation of 3-phenyl-5,5-dimethyl-3-trideuteriomethyl-2,2,4,4,6,6-hexadeuteriocyclohexanone was made for deuterium deficiency in the 3-trideuteriomethyl group alone. The assignment of the isotope effect on the chemical shift difference of the 5-methyl proton signals solely to deuterium substitution in the 3-methyl group has been explained in the discussion on conformational equilibrium isotope effects.

MEASUREMENTS FROM NMR SPECTRA

Below are listed the various chemical shift differences, D (expressed in Hz for spectra recorded on 100 MHz instruments), between indicator groups ($-CH_2Z$, Z = H or C_6H_5) measured for the conformationally equilibrating and "locked" compounds. All measurements were at 35.2° unless otherwise stated. The concentrations of the solutions have been specified in the discussion. The key to the compound numbers faces page 28.

The D values are quoted as measured. That is, they are not corrected for overlap (trideuterioacetonitrile solutions), nor are they corrected for imperfect deuteriation.

The value for XXVI as a solution in trichlorofluoromethane was not measured.

For comparison with other compounds in the same isotopic series a ΔG value from earlier work[29] with lower concentrations was used, with the assumption that the effect of a change in concentration on ΔG was the same for XXVI as for the undeuteriated compound, XXIV.

Compound Number		Solvent	D (Hz)
XXI	(41.4°)	CCl_4	6.87 ± 0.09
XXI	(40.3°)	CCl_3F	7.88 ± 0.03
XXII	(41.4°)	CCl_4	7.26 ± 0.08
XXII	(40.3°)	CCl_3F	8.27 ± 0.03
XXIII	(41.4°)	CCl_4	42.20 ± 0.05
XXIII	(40.3°)	CCl_3F	41.80 ± 0.05
XXIV		CCl_3F	3.761 ± 0.066
XXIV		CD_3CN	1.082 ± 0.053
XXV		CCl_3F	4.375 ± 0.107
XXV		CD_3CN	1.464 ± 0.072
XXVI		CD_3CN	1.256 ± 0.088
XXVII		CCl_3F	4.444 ± 0.064
XXVII		CD_3CN	1.612 ± 0.075
XXVIII		CCl_3F	64.59 ± 0.15
XXVIII		CD_3CN	58.454 ± 0.089
XXIX		CH_3OH/C_6H_6	10.054 ± 0.028
XXX		"	10.212 ± 0.040
XXXI		"	10.131 ± 0.049
XXXII		"	10.293 ± 0.041
XXIII		"	45.387 ± 0.055
XXXIII		"	47.517 ± 0.057
XXXIV	(1 M)	"	44.557 ± 0.043
XXXIV	(0.5 M)	"	44.683 ± 0.024
XXXV		"	35.875 ± 0.016
XXXVI	(between 5-methyl groups)	CCl_3F	65.402 ± 0.220
XXXVII	"	CCl_3F	64.733 ± 0.157

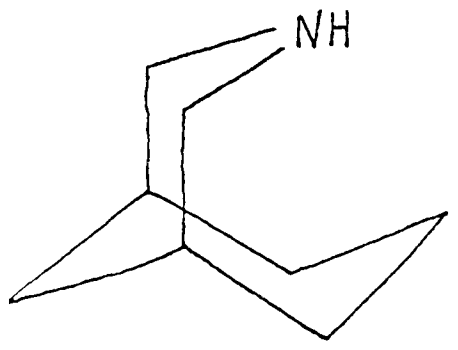
Compound XXXVIII had an equatorial 5-methyl proton signal at 8.904 ± 0.001 τ (CCl_3F solution).

Robinson[10] extrapolated the chemical shift of the 5-methyl group protons cis- to the phenyl ring in XXXVI to 10.0 τ as the temperature tended to absolute zero, and observed that the chemical shift of the 5-methyl proton group trans- to the phenyl ring in XXXVI, at 8.873 τ, changed by less than 0.01 ppm over the temperature range -90 to +40°, corresponding to a fourfold change of equilibrium constant.

CHAPTER TWO

ISOTOPE EFFECTS ON INTRAMOLECULAR AND INTERMOLECULAR EXCHANGE

DISCUSSION



L

THE ORIGIN OF THE INVESTIGATION

An isotope effect on the exchange of hydrogen between two different kinds of molecules may be expected to lead to an isotopic fractionation, that is, a non-statistical distribution of protium and deuterium between the two exchange sites, upon partial deuteriation of the system. The measurement of such a fractionation for exchange between, for example the N-hydrogen atoms in a variety of secondary amines and a standard "partner", an enol, would provide a probe with which steric hindrance in such amines as 3-azabicyclo[3.3.1]nonane, L, derivatives might be studied. However, experimental difficulties over the required accurate control of relative concentrations, and over exchange with such impurities as traces of water adsorbed from the atmosphere, prevented investigation in this direction and restricted quantitative measurements to intramolecular systems.

THE CHOICE OF SYSTEMS FOR INVESTIGATION OF ISOTOPE EFFECTS ON INTRAMOLECULAR AND INTERMOLECULAR EXCHANGE

Equilibrating systems with a bound hydrogen atom, which may be in one of two different steric and/or electrostatic environments, have been shown, in Chapter One, to provide a basis for studying the change in preference between the two environments when deuterium is substituted for protium. The accuracy with which such effects may be measured is greatest when there is little preference between the two environments (K_H and $K_D \simeq 1$).

Systems with hydrogen atoms bonded in <u>two</u> different, but rapidly exchanging, environments may provide examples of this as illustrated in the equilibrium,

$$H'A + BH'' \rightleftharpoons H''A + BH',$$

where A and B represent sites at which hydrogen is bonded either on the same molecule (HABH), or an equal number of two different molecules (AH and BH), then "K_H" = 1. It is apparent that measurements on a single compound system will not be subject to any concentration requirements for control of the stoichiometry.

For the "two compound" equilibrium, the value of a suitable property, for example the position of the time averaged exchanging hydrogen NMR signal, will depend simply on the two concentrations [AH] and [BH], and will lie between the two "extreme" values which may be measured for AH and BH individually. Any isotopic preference between bonding at site A and site B will lead to a shift of the time-averaged signal on partial deuteriation of the equilibrating substances. Exchangeable hydrogens with widely different chemical shifts and "uncluttered" spectra are therefore requirements for the substances AH and BH.

t-Butylmethylamine has a simple NMR spectrum and a readily exchangeable hydrogen with an NMR signal at high field (9.4 τ for a solution 10% w/v in carbon tetrachloride). Compounds with intramolecularly hydrogen-bonded exchangeable hydrogens will be suitable "partners" for t-butylmethylamine, provided that they are not too acidic and that exchange is fast on an NMR time scale, since the chemical shift of an intramolecularly hydrogen-bonded proton is at low field and less sensitive to concentration changes than shift values for "free" hydroxyl protons.

For a "one-compound" equilibrium, partial deuteriation of molecules of the type HA-BH gives interconverting molecules with three different empirical formulae: ABH_2, ABHD and ABD_2. The equilibrium,

$$HA\text{-}BD \rightleftharpoons DA\text{-}BH ,$$

for the ABHD molecules represents a balance between the free energy difference of

protium in the two environments and the free energy difference of deuterium, and any isotope effect on these free energy differences will lead to a departure of the equilibrium constant, K_{HD}, from unity.

K_{HD} may be determined if a property for measurement is chosen which is sufficiently different in value for the two types of molecule HA-BD and DA-BH. The time-averaged NMR chemical shift of the exchanging hydrogen atoms will be different for the undeuteriated (ABH_2) and partially deuteriated (ABHD) system if $K_{HD} \neq 1$. If the chemical shifts of hydrogen bound to site A and hydrogen bound to site B are far enough apart, either separate proton signals for the species ABHD and ABH_2 would be observed for systems with intermolecular exchange NMR-slow, or a time-averaged signal between those of ABHD and ABH_2 (the position depending on the relative concentrations of the two species) would be observed for systems with NMR-fast intermolecular exchange. Model compounds may be used to estimate the chemical shifts of protons bound at one or both of the two sites A- and B-.

Compounds of the type $RCH_2NR"_2$, RCHO, $RCOCH_3$ and $RCHNOCH_3$, where R represents 3,5-di-t-butyl-2,6-dihydroxyphenyl are readily prepared from resorcinol and soluble in low polarity NMR solvents which are easily dried and which have no signals (or very few) in the range to be examined. These resorcinols provide sites for bonding to hydrogen in the two hydroxyl groups which are rapidly exchanging environments. One hydroxyl group has a hydrogen which is hydrogen-bonded to the nitrogen atom of an amino group, the oxygen atom of a carbonyl group or the nitrogen atom of an oxime group and which is consequently deshielded by its environment. The other hydroxyl hydrogen is not deshielded by any such intramolecular effect, nor, if the solution is sufficiently dilute, by significant intermolecular association.

The compounds of the type $RCH_2NR"_2$ which were prepared and examined, 3,5-di-t-butyl-2,6-dihydroxy-N,N-dimethylaminomethylbenzene and 3,5-di-t-butyl-2,6-dihydroxypiperidinomethylbenzene, had NMR hydroxyl proton signals with chemical shifts which were significantly dependent upon concentration, at the concentrations required for reliable integration of spectra, and were therefore rejected as unsuitable compounds for further investigation.

PRELIMINARY INVESTIGATION OF EXCHANGE FOR "TWO COMPOUND" SYSTEMS

To investigate the exchange

$$t\text{-}BuCH_3NH + BD \rightleftharpoons t\text{-}BuCH_3ND + BH$$

for any departure from a statistical distribution of the exchanging protium and deuterium between the available sites by NMR spectroscopy, the compound BH must have an exchangeable hydrogen with a low-field chemical shift value which is relatively insensitive to concentration. A series of compounds was tested for their suitability for the role of BH.

Each compound was tested for the sensitivity of the chemical shift of the exchangeable proton signal to concentration changes. 8-Quinolinol and pyrazole, being significantly intermolecularly associated at the concentrations required for NMR spectroscopy,[46] were rejected since the exchanging proton signals in their NMR spectra shift by about 0.5 ppm for a change from 5 to 2% concentration.

The exchange studied must be NMR-fast for the observation of a single time-averaged NMR signal for exchanging protons. The NMR spectrum of 8-quinolinol-1-oxide has a sharp hydroxylic proton signal (at -5.8 τ for solutions 1.25 and 2.5% w/v in benzene) for solutions in benzene, deuteriochloroform, pyridine and nitrobenzene which is broadened by the addition of small quantities of t-butylmethylamine. The extent of the broadening increased as more t-butylmethylamine was added until, at about one tenth of a molar equivalent of the 8-quinolinol-1-

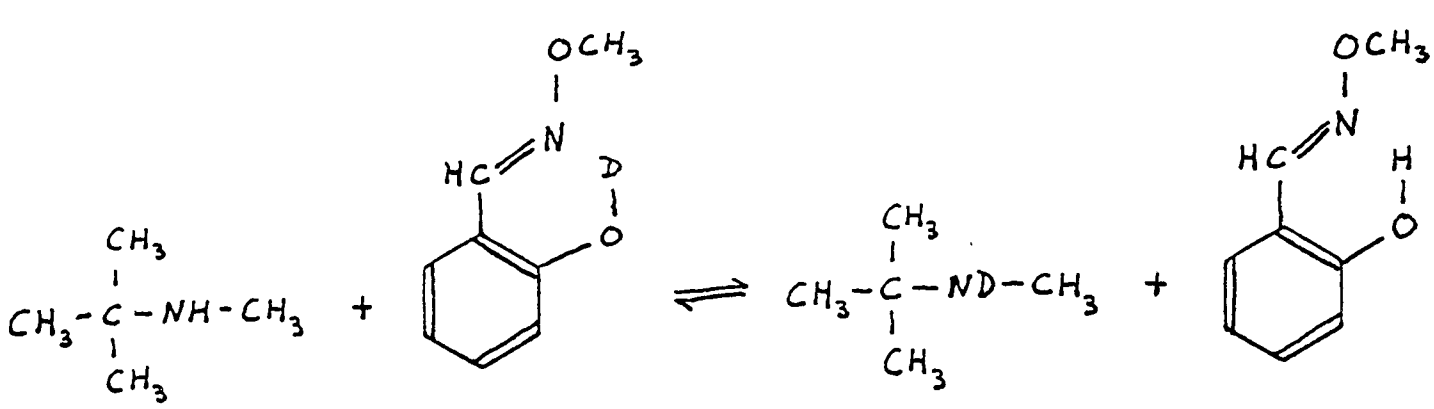


Figure X

oxide, the signal was so "broad" that it could not be located. Therefore 8-quinolinol-1-oxide was rejected as a candidate for BH since proton exchange with t-butylmethylamine, in a variety of solvents, was too slow on an NMR time scale.

Salicylaldehyde was found to be unsatisfactory since the time-averaged exchanging proton signal in the NMR spectra of mixtures with t-butylmethylamine, although sharp, changed in chemical shift value with time (shifts of 0.1 ppm in 20 hours). This was attributed to an association between salicylaldehyde and t-butylmethylamine. After 2 days, yellow crystals formed in an equimolar mixture of these two substances. The crystals were separated from the remaining liquid and turned to a black oil either on standing for an hour at 15 mm pressure or on standing for a week at 760 mm pressure, indicating the formation of a complex when salicyl-aldehyde and t-butylmethylamine are mixed.

The O-methyloxime of salicylaldehyde suffered from none of the disadvantages discussed above for the other "candidates" which were tested. The chemical shift of the hydroxylic proton signal of the O-methyloxime (2 and 10% w/v in benzene) was -0.06 τ. The signal moved upfield, with retention of sharpness, on addition of small quantities of t-butylmethylamine to the sample solution.

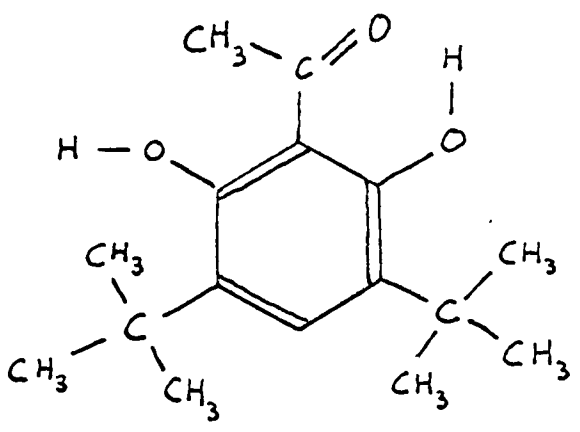
Apparently the most practicable way to investigate the exchange shown in Figure X is to study the change in NMR chemical shift values of the time-averaged hydroxylic proton signal as accurately measured quantities of salicylaldehyde O-methyloxime-OD (the less volatile of the two compounds in the system) are added, with efficient mixing, to a standard solution of t-butylmethylamine. Atmospheric (or any other) moisture must be excluded throughout these operations, and any loss of material by evaporation during the additions must be minimised. No experimental system which satisfactorily met these demands was devised.

CALCULATION OF ISOTOPE EFFECTS ON EXCHANGE IN "ONE-COMPOUND" SYSTEMS

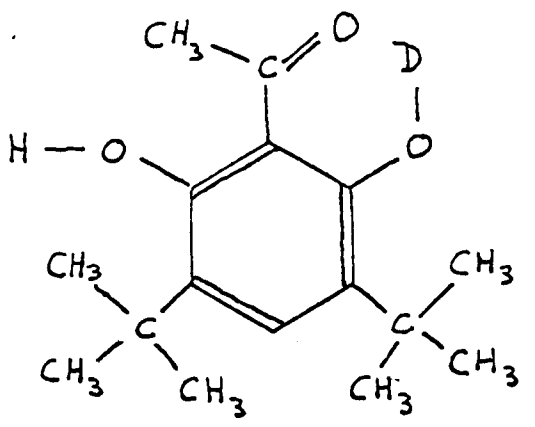
The NMR spectrum of a solution containing the three species ABH_2, ABHD and ABD_2 will have two hydroxylic proton signals, corresponding to ABH_2 and ABHD (provided that the chemical shift difference between these two signals is large compared with the signal widths) for systems with NMR-slow intermolecular exchange, as for a solution of partially deuteriated 3,5-di-t-butyl-2,6-dihydroxyacetophenone in carbon tetrachloride. In this case τ_{HH} and τ_{HD}, the chemical shift values corresponding to the signals for ABH_2 and ABHD respectively, may be measured directly.

For those systems where intermolecular exchange is NMR-fast, only one hydroxylic proton signal is observed on partial deuteriation. The shift of this signal from τ_{HH} will be directly proportional to the ratio of the number of exchanging deuterium atoms to the total number of exchanging atoms for a statistical distribution of deuterium between ABH_2, ABHD and ABD_2. Therefore a value for τ_{HD} may be obtained from extrapolation to 100% deuteriation using the slope of a plot of the isotopic shift against extent of deuteriation. This determination of τ_{HD} was used for solutions of partially deuteriated 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde O-methyloxime. The determination of τ_{HD} by this method renders the value of the difference $\tau_{HD} - \tau_{HH}$ less accurate (standard deviation ~ 10% of $\tau_{HD} - \tau_{HH}$) than values of $\tau_{HD} - \tau_{HH}$ measured directly (standard deviation ~ 1% of $\tau_{HD} - \tau_{HH}$) for systems with NMR-slow intermolecular exchange.

For the compound ABH_2, τ_{HH} will be half-way between the chemical shift of a proton bound at site A, τ_A, and the chemical shift of a proton bound at site B, τ_B.



LI



LII

The values τ_A and τ_B may not be measured directly at room temperature and compounds with single bonding sites for exchangeable hydrogen atoms which are models of A- and B- must be used to determine τ_A' and τ_B', approximations for τ_A and τ_B. The relation

$$\tau_A - \tau_{HH} = \tau_{HH} - \tau_B$$

provides either a test of the applicability of a pair of model compounds or the means by which the value of τ_A may be calculated if a particularly appropriate model for site B- can be found.

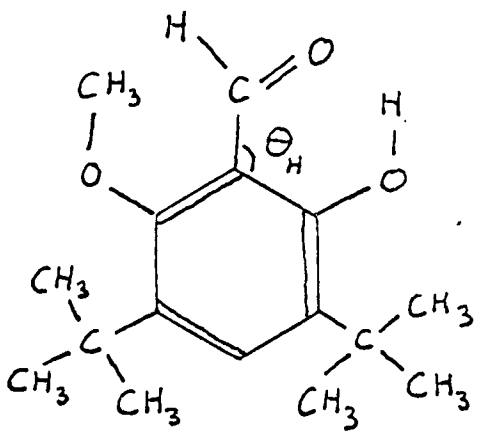
If the chemical shift difference between τ_{HH} and τ_{HD}, $\Delta\tau = \tau_{HD} - \tau_{HH}$, may be solely attributed to an isotopic fractionation resulting from a non-statistical distribution of protium and deuterium between sites A- and B-, then K_{HD}, the equilibrium constant for the equilibrium:

$$HABD \rightleftharpoons DABH$$

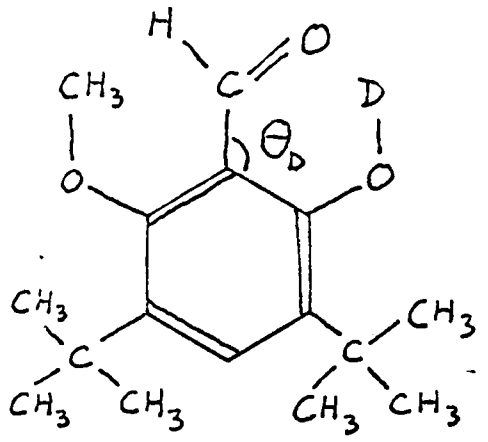
is given by

$$K_{HD} = \frac{[DABH]}{[HABD]} = \frac{\tau_A - \tau_{HH} - \Delta\tau}{\tau_A - \tau_{HH} + \Delta\tau} = \frac{\tau_{HH} - \tau_B - \Delta\tau}{\tau_{HH} - \tau_B + \Delta\tau} \qquad (3)$$

The assumption upon which the applicability of equation (3) is based is invalid if there be any significant difference between, for example, the chemical shift of a proton bound at site A in HABD and the chemical shift of a proton bound at site A in HABH. For example, substitution of deuterium for intramolecularly hydrogen-bonded protium in conformation LI to give conformation LII may lead to a change in the geometry of the molecule resulting in a difference in value between the chemical shift of the "free" hydroxyl proton in LI and LII. Rapid interconversion of the two


H
C
O
CH3
θH
H
O
O
CH3
C
CH3
CH3
CH3
C
CH3
CH3
CH3

LIII


H
C
O
CH3
θD
D
O
O
CH3
C
CH3
CH3
CH3
C
CH3
CH3
CH3

LIV

kinds of hydroxylic group prevents any direct attempt to detect such an effect at room temperature. However, the compounds LIII and LIV provide a model system for such an investigation: any change in, for example, the orientation of the aldehydic group with respect to the plane of the benzene ring, or any difference between the angles Θ_H and Θ_D, will be expected to lead to a difference in value of the chemical shifts of the methoxy methyl signals in LIII and LIV. Examples of an isotopic change in molecular geometry are changes in the lengths of hydrogen bonds in crystals when deuterium is substituted for protium in the hydrogen bonds.[47]

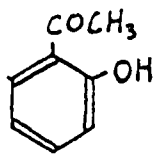
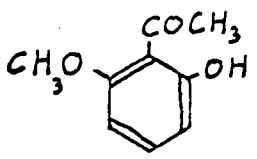
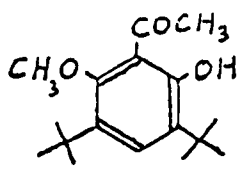
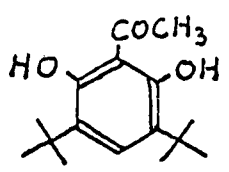
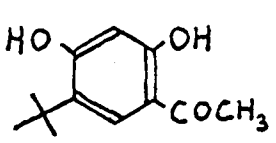
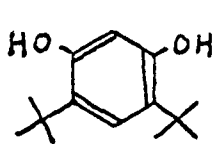
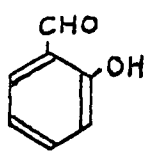
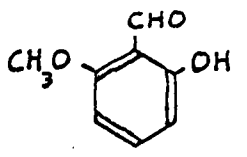
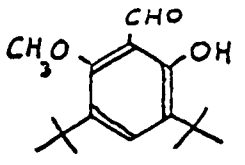
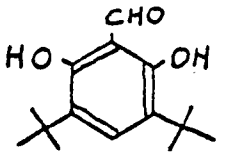
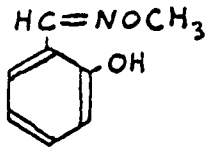
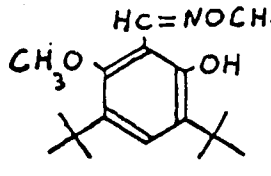
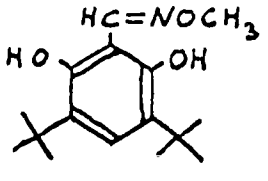
Using p-dimethoxybenzene as an internal standard, the NMR chemical shifts of the methoxy proton signals of LIII and LIV (as 0.36 M solutions in carbon tetrachloride) were measured 32 times each. The change in chemical shift from the protio to the deuterio compound was -0.0002 ± 0.0002 ppm. This chemical shift difference is less than one per cent of the smaller of the two isotopic shifts of time-averaged signals which were measured.

THE PROBLEM OF MODEL COMPOUNDS

Calculation of K_{HD}, and hence an isotopic change in free energy difference, from equation (3) requires values for τ_A or τ_B, the chemical shifts of protons bound to the sites A- or B-. Model compounds will be used with hydrogen bound at sites corresponding, as closely as possible, to the environments at A- and B- in ABH_2. The NMR spectra of these models will provide approximations for τ_A and τ_B, designated as "model" values with a dash: τ_A' and τ_B'.

Table 3 shows a series of compounds with the corresponding chemical shifts of hydroxylic protons in intramolecularly hydrogen bonded and "free" environments, τ_B' and τ_A', together with the time-averaged hydroxylic proton chemical shifts for

Table 3. Hydroxylic proton NMR chemical shifts. (All compounds except Nos. LIX and LXII as 5% w/v solutions in carbon tetrachloride at 35.2°)

Compound		Chemical Shift (ppm on τ scale)		
Number	Structure	Intramolecularly H-bonded (τ_B')	Exchanging (τ_{HH})	"Free" (τ_A')
LV		-2.07		
LVI		-2.95		
LVII		-1.80		
LVIII			0.49	
LIX*		-2.16		4.81
LX				5.57
LXI		-1.32		
LXII+		-1.84		
LXIII		-2.29		
LXIV				1.0 (broad signal)
LXV		0.48		
LXVI		-0.30		
LXVII				2.33

* From an accumulated spectrum (131 scans) at ½% concentration.

\+ Value at 33°, between 3 and 6% concentration, measured by Forsen and Åkermark.[48]

compounds LVIII, LXIV and LXVII with rapid exchange between the two environments, τ_{HH}. Of these three compounds, τ_{HD} values were measured for LVIII and LXVII after partial deuteriation (LXIV was not amenable to such investigation as the hydroxylic signal was too broad) and therefore models for the environments of the two hydroxylic protons in both LVIII and LXVII are required.

In Table 3, the sensitivity of τ_B' values to effects induced by the variation of substituents on the benzene ring is illustrated by the compounds LV to LXIII, LXV and LXVI. A superficial initial choice of LIX as a model for both hydroxylic proton sites in the exchanging compound LVIII gives a model "exchanging environment" chemical shift (that is, the arithmetic mean of $\tau_A' = 4.81$ ppm and $\tau_B' = -2.16$ ppm) which is 0.84 ppm greater than τ_{HH} for LVIII; hence the model values τ_A' and τ_B' which are to be used must be derived from molecules which more closely reproduce the structure of the "exchange" molecules.

In compound LVIII, for example, the "free" hydroxyl substituent will be expected to contribute towards the chemical shift of the intramolecularly hydrogen-bonded proton. In the model compounds studied, a methoxy group meta- to the hydrogen-bonded hydroxyl group is used to provide a similar contribution. Forsen and Åkernark[48] have measured the hydroxylic proton chemical shifts of 2-hydroxy-x-methoxybenzaldehydes and 2-hydroxy-x-methoxyacetophenones (x = 3,4,5 and 6). Good linear correlations were found between these chemical shifts and both the stretching frequency of the chelated carbonyl group and the stretching frequency of the phenolic hydroxyl group. This suggests that the major factor responsible for variations in the hydroxyl proton chemical shift among the methoxy derivatives is the variation in the electron distribution in the atoms participating in the intramolecular

hydrogen bond. Therefore the effect of a 6-methoxy group on the 2-hydroxyl proton chemical shift in model compounds LVI and LXII appears to be primarily an inductive one and may be expected to be a close reproduction of the effect of a 6-hydroxyl group.

Comparison of the τ_B' values for the two pairs of compounds, LVI and LVII, LXII and LXIII indicates that the 3- and 5-t-butyl groups influence the chemical shift of the hydroxylic protons, and therefore any model compounds for the intramolecularly hydrogen-bonded hydroxyl group in LVIII and LXVII must incorporate such effects. On these grounds, LVII would be expected to be the best model for LVIII in a "fixed" configuration, and LXVI the best for a fixed configuration of LXVII. However, using the τ_B' value of LVII and τ_{HH} for LVIII, a model value of 2.76 ppm for τ_A' is given by the relation

$$\tau_A' - \tau_{HH} = \tau_{HH} - \tau_B',$$

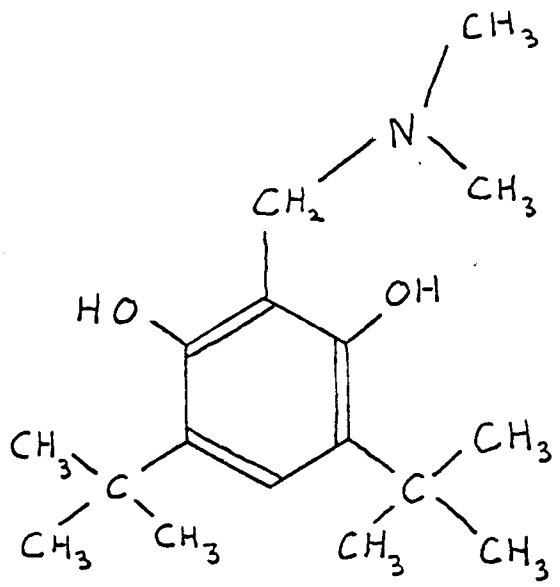
which suitable values of τ_A' and τ_B' will be expected to fulfil. A value of 2.76 ppm is exceptionally low for the chemical shift of a "free" phenolic hydroxyl group proton and the implication is that LVII is a bad model.

Examination of a Dreiding model of LVII shows severe strain resulting from steric hindrance between the protons of the 6-methoxy group and the protons of either the acetyl methyl group or the 5-t-butyl group. The expected distortion of bond angles from "normal" values resulting from such strain may influence the chemical shift of the hydroxylic proton, and the great difference between the change in τ_B' value from LVI to LVII (+1.15 ppm) and the change in τ_B' from LXII to LXIII (-0.45 ppm) illustrates this.

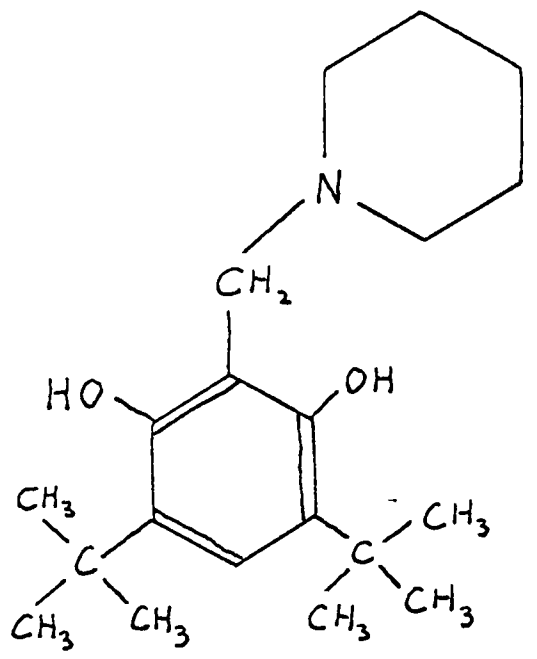
The steric crowding in LXIII and LXVI is small compared with that in LVII,

since LXIII and LXVI have no acetyl methyl groups to interact with the 6-methoxy group protons. Hence LXVI may be expected to be a suitable model for a fixed configuration of LXVII. This gives a reasonable τ_A' value of 4.93 ppm for the free hydroxylic proton in the exchanging system.

As has been discussed, the change in τ_B' values from LXII to LXIII (-0.45 ppm) is a better indication of the "non-steric" effect of the 3- and 5-t-butyl groups on the hydroxylic proton chemical shift than the change from LVI to LVII. Further support for the validity of LXIII as a model for a fixed configuration of LXIV is provided by Forsen and Åkermark's linear correlation of chelated carbonyl stretching frequencies and intramolecularly hydrogen bonded hydroxyl proton chemical shifts. The carbonyl stretching frequency of LXIV (as a 2.5% w/v solution in carbon tetrachloride) was 1643 cm^{-1}, corresponding to a chemical shift for the intramolecularly hydrogen bonded hydroxylic proton, τ_B, of -2.3 ppm using Forsen and Åkermark's correlation. The τ_B' value of LXIII was -2.29 ppm. Since LVII has been shown to be a poor model for a fixed configuration of LVIII, LVI will be used as the model with its τ_B' value modified for the di-t-butyl contribution as measured by the change in τ_B' from LXII to LXIII (-0.45 ppm). Therefore the chosen model value for the chemical shift of the intramolecularly hydrogen-bonded hydroxyl proton, τ_B, in LVIII is -2.95 - 0.45 = -3.40 ppm. The carbonyl stretching frequency of LVIII (as a 2.5% w/v solution in carbonyl tetrachloride) was 1625 cm^{-1} which corresponds to τ_B = -3.3 ppm, using Forsen and Åkermark's correlation, in good agreement with the selected τ_B' value derived from model compounds.


CH3
N
CH3
CH2
HO
OH
CH3
C
CH3
CH3
CH3
C
CH3
CH3

LXVIII


N
CH2
HO
OH
CH3
C
CH3
CH3
CH3
C
CH3
CH3

LXIX

PREPARATION AND PROPERTIES OF THE COMPOUNDS

3,5-Di-t-butyl-2,6-dihydroxy-1-dialkylaminomethylbenzenes

Compounds LXVIII and LXIX were prepared by the Mannich condensation[49] of 4,6-di-t-butylresorcinol with aqueous formaldehyde and a secondary amine, aqueous dimethylamine for LXVIII and piperidine for LXIX, in 85% yield.

As solutions in ethanol-free deuteriochloroform, LXVIII and LXIX gave NMR spectra with hydroxylic proton signals whose chemical shifts showed a strong dependence upon concentration over the range of concentration around the minimum required for reliable integration of the spectra (~ 0.1 M). For a change from 5 to 10% w/v solutions the hydroxyl proton signal of LXVIII moved downfield by 0.36 ppm whilst the same signal for LXIX moved downfield by 0.47 ppm.

3,5-Di-t-butyl-2,6-dihydroxybenzaldehyde and its O-methyloxime

Using a version of the Vilsmeier-Haark formylation reaction, reviewed by de Maheas,[50] 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde was prepared by the alkaline hydrolysis of the addition product made by treating dry 4,6-di-t-butyl-resorcinol with an equimolar mixture of phosphorus oxychloride and dimethylformamide, in 62% yield.

The NMR spectrum of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde (5 and 10% w/v in carbon tetrachloride) had a hydroxylic proton "signal" of 1 ppm width at half height with a centre of gravity at 1.0 τ which did not shift with the change in concentration. Any change in the position of the centroid for the spectrum of a partially deuteriated sample was less than 0.05 ppm and hence could not be detected by any integration method since the wide signals spanned the spectral noise associated with a 2 ppm section of the spectrum. The lack of facilities for variable temperature

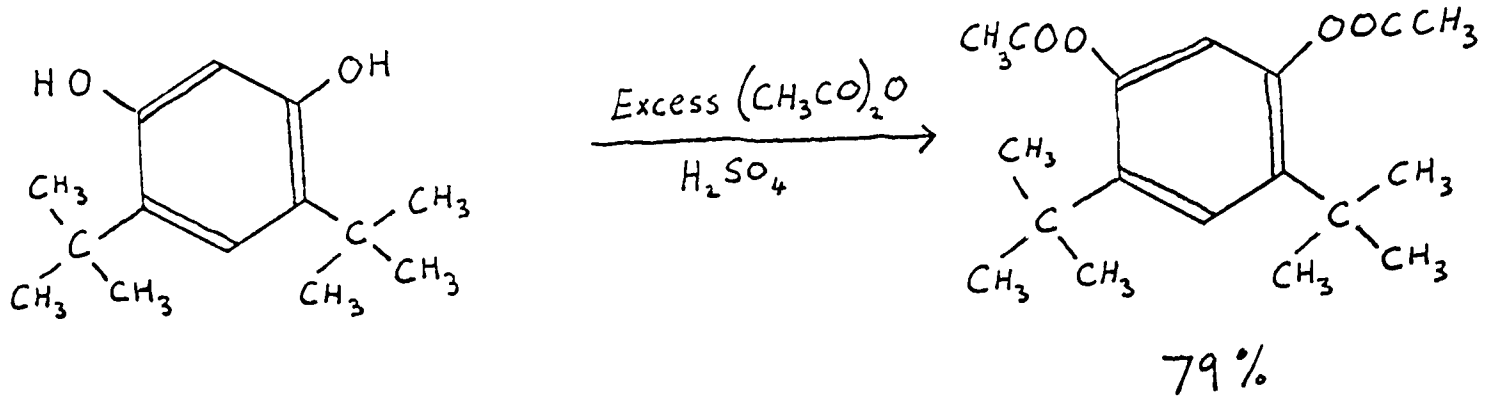


Figure 11

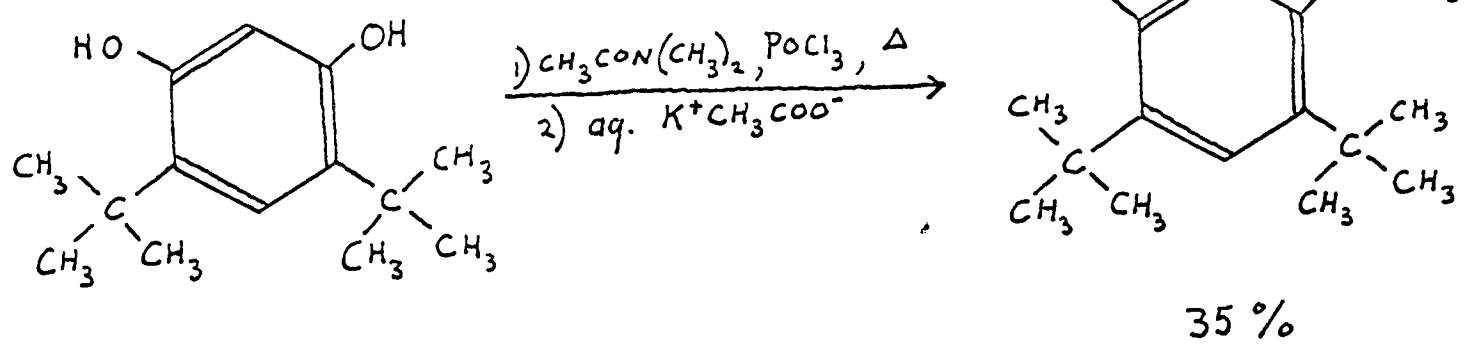


Figure 12

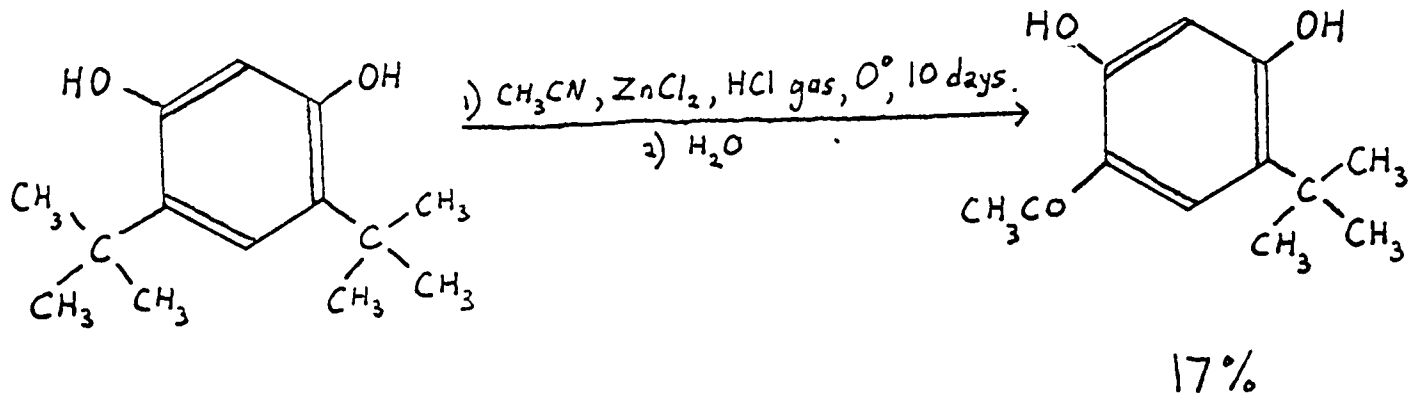


Figure 13

studies prevented investigation of the spectrum at high or low temperatures, which would be expected to correspond to the NMR-fast and NMR-slow intermolecular exchange situations.

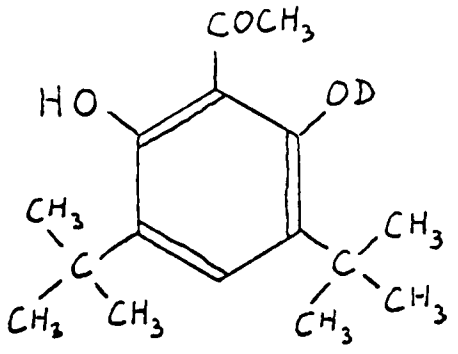
The NMR spectrum (4 and 8% w/v in carbon tetrachloride, dried over potassium hydroxide pellets) of the O-methyloxime of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde had a hydroxyl proton signal which shifted by only 0.01 ppm for the change in concentration. The spectra of a series of partially deuteriated samples (all 5% w/v in dry carbon tetrachloride) had hydroxylic signals whose chemical shift values followed a linear relation with the extent of deuteriation with a correlation coefficient of -0.98 for a least-squares fit.

3,5-Di-t-butyl-2,6-dihydroxyacetophenone

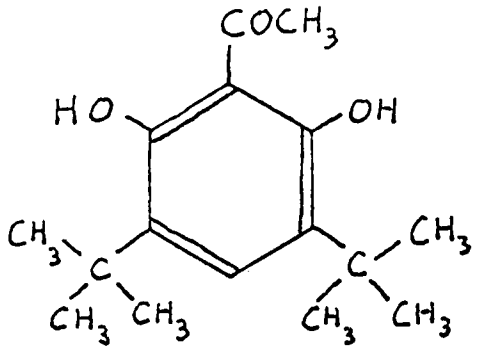
Treatment of 2,6-dihydroxyacetophenone (commercially available) with an excess of t-butyl alcohol mixed with orthosphosphoric acid gave a 50% yield of 3-t-butyl-2,6-dihydroxyacetophenone and 17% 3,5-di-t-butyl-2,6-dihydroxyacetophenone.

Attempts to acetylate 4,6-di-t-butylresorcinol, readily available and activated towards electrophilic attack, with a variety of acetylating reagents did not give 3,5-di-t-butyl-2,6-dihydroxyacetophenone, but in several cases specific products, characterised by their NMR spectra and elemental analyses, were obtained. With excess acetic anhydride and concentrated sulphuric acid, 4,6-di-t-butylresorcinol gave the diacetate (Fig.11). A Vilsmeier acetylation reaction[51] produced the monoacetate (Fig.12), and a Hoesch acetylation[52] gave 5-t-butyl-2,4-dihydroxyacetophenone (Fig.13).

NMR spectra of 3,5-di-t-butyl-2,6-dihydroxyacetophenone (4, 5 and 6% w/v



LXX



LXXI

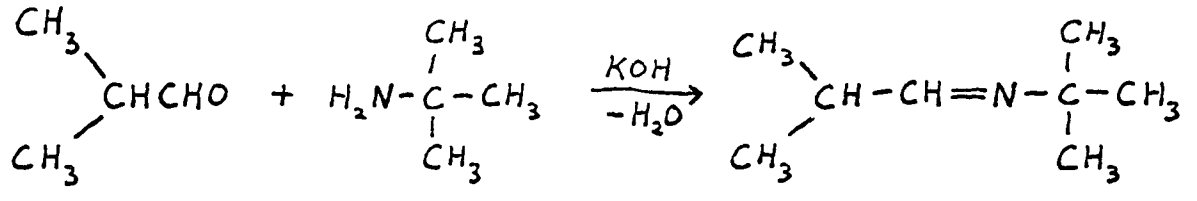


Figure 14

in carbon tetrachloride) had a hydroxylic proton signal at 0.49 τ. NMR spectra of partially deuteriated samples (5% w/v in dry carbon tetrachloride) showed two hydroxylic proton signals: one at 0.33 τ and one at 0.49 τ. From the increase of the relative intensity of the former to that of the latter with an increase in the extent of deuteriation of the sample, and from the correspondence of the chemical shift of the signal at higher field with the hydroxylic proton signal value for the undeuteriated compound, the signal at 0.33 τ was attributed to the species LXX and the signal at 0.49 τ was attributed to the species LXXI.

Monomethylation of Resorcinols

The method described by Nakazawa[53] was used to prepare 3,5-di-t-butyl-2-hydroxy-6-methoxybenzene derivatives in yields of 30 to 90%.

t-Butylmethylamine

t-Butylmethylamine was prepared using the reaction scheme shown in Fig.14. Hydrolysis of the dimethyl sulphate adduct of N-isobutylidene t-butylamine, followed by addition of excess potassium hydroxide gave a solution which was fractionally distilled. The NMR spectra of the distillate fractions, which had been dried with solid potassium hydroxide, showed the presence of t-butylamine as well as the desired secondary amine. The presence of this starting material was attributed to incomplete reaction between N-isobutylidene t-butylamine and dimethyl sulphate with the result that N-isobutylidene t-butylamine was hydrolysed as well as its dimethyl sulphate adduct. This incomplete reaction, together with the operations necessary for the removal of t-butylamine from the crude product (the addition of excess salicylaldehyde to "trap" the primary amine as the relatively involatile salicylidene imine whilst t-butylmethylamine was removed under reduced pressure), accounted for the low overall yield (6%).

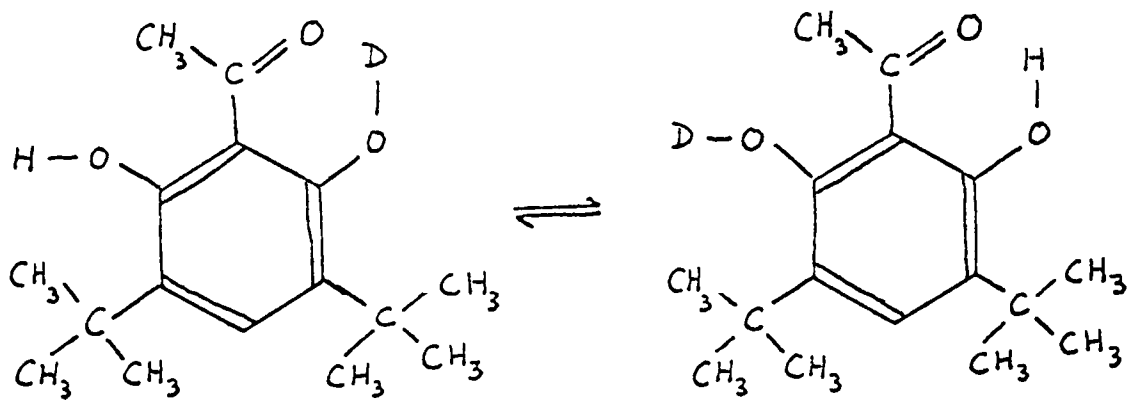


Figure 15

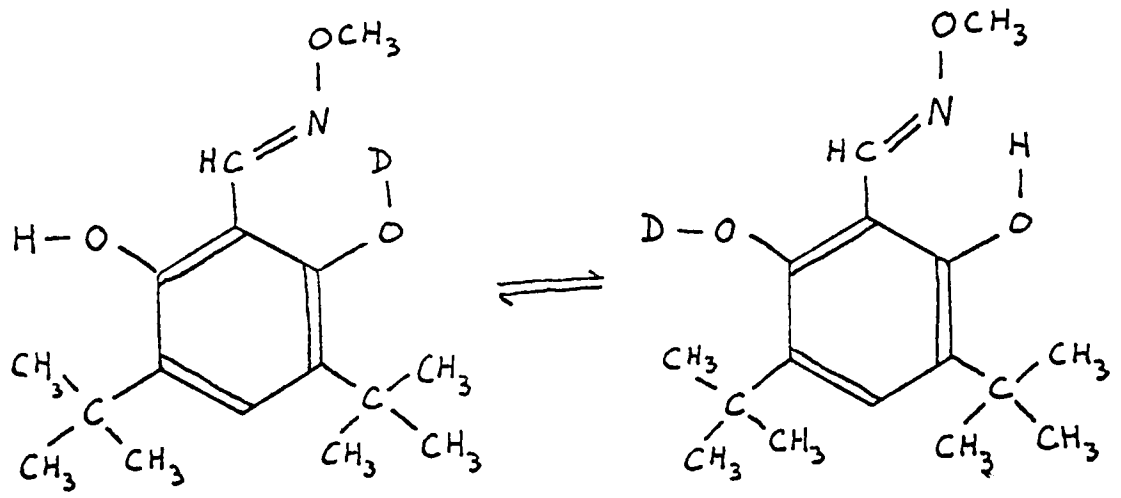


Figure 16

ISOTOPE EFFECTS IN COMPOUNDS WITH HYDROXYL GROUPS IN TWO DIFFERENT ENVIRONMENTS

As described above, $\Delta\tau$ values were measured for carbon tetrachloride solutions of partially deuteriated samples of 3,5-di-t-butyl-2,6-dihydroxyacetophenone and 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde *O*-methyloxime. The strong dependence of the time-averaged hydroxyl proton chemical shifts of 3,5-di-t-butyl-2,6-dihydroxy-1-dimethylaminomethylbenzene and 3,5-di-t-butyl-2,6-dihydroxy-1-piperidinomethylbenzene on concentration rendered these compounds unsuitable for the measurement of isotope effects. Also investigation of an isotope effect on partial deuteriation of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde was prevented by the broadness of the hydroxyl proton signal.

Using the measured $\Delta\tau$ values in conjunction with estimates of τ_A and τ_B derived from model compounds in the manner described above, the free energy changes, ΔG_{15} and ΔG_{16} for the equilibria shown in Figure 15 and Figure 16 respectively were calculated from the values of K_{HD} obtained from equation (3).

The free energy differences at 35.2° are:

ΔG_{15}	-51.5 ± 0.6 cal/mole
ΔG_{16}	-16 ± 1 cal/mole

Strictly each of these isotope effects is made up of two components: the change in free energy on substituting deuterium for protium in the "free" hydroxyl group and the change in free energy on substituting protium for deuterium in the intramolecularly hydrogen-bonded hydroxyl group. The first component is a change in the free energy of solvation of the "free" hydroxyl group in carbon tetrachloride, since the concentration dependence of the hydroxyl proton signals is so small for the

compounds studied, indicating that intermolecular association effects are negligible. The second component is a change in the free energy between an intramolecular protium bond and an intramolecular deuterium bond.

Bellamy and Williams have measured the hydroxyl stretching frequencies of phenol and 2-isopropylphenol as 0.07 M solutions in various solvents,[54] as a measure of the degree of association between solvent molecules and the phenolic hydroxyl group. For comparison, hydroxyl stretching frequencies of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde as 0.01 M solutions are shown in the table below:

Solvent	Hydroxyl Stretching Frequency (cm^{-1})		
	Phenol[54]	2-Isopropylphenol[54]	3,5-Di-t-butyl-2,6-dihydroxybenzaldehyde
Hexane	3622 ± 1.5	3625 ± 1.5	3622 ± 2
Carbon tetrachloride	3611 ± 1.5	3615 ± 1.5	3622 ± 2
Benzene	3559 ± 1.5	3560 ± 1.5	3610 ± 2
Ethyl acetate	3432 ± 1.5	3444 ± 1.5	3422 ± 10

If, as expected, there is no solvent-phenol association in hexane solutions, the change in hydroxyl stretching frequency from hexane to any other solvent is indicative of the degree of association in that solvent. The low "basicity" of carbon tetrachloride together with the steric hindrance provided by the 1- and 3-substituents of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde reduce solvent association with the free hydroxyl group to negligible proportions for the type of compounds studied in carbon tetrachloride solutions. Therefore the ΔG values which have been measured may be primarily interpreted as changes in free energy between intramolecular protium and deuterium bonds.

ISOTOPE EFFECTS ON HYDROGEN BONDS

Most investigations of the effect of deuteriation upon the properties of hydrogen bonded substances reported in the literature may not be directly interpreted in terms of the change in enthalpy or free energy between a protium bonded system and the analogous deuterium bonded system. For example McDougall and Long[55] have measured the change of pK_a values between acid-OH compounds in water and analogous acid-OD compounds in heavy water. This measurement corresponds to changes in the relative solvation of acid and anion between the undeuteriated and deuteriated system as well as any change in ionisation energies which may be sought as a measure of an isotope effect upon, for example, intramolecular hydrogen bonding in the bimaleate anion.[56]

Kolbe[57] reports data for isotopic effects on the association of alcohols with terriary amines, using spectroscopic measurements of -OH and -OD stretching intensities for solutions in toluene. Plourde[58] has used the same technique to investigate isotope effects on association between phenol and weak "bases" (benzene, ethyl acetate, tetrahydrofuran and di-n-butyl ether) for carbon tetrachloride solutions. The largest isotopic change in the enthalpy of association measured by Plourde was for phenol-benzene association. Therefore solute-solvent association effects will be expected to complicate any interpretation of Kolbe's results. Plourde introduces systematic errors by deriving her isotopic changes of the enthalpies and entropies of association from a comparison of her own measurements on phenol-OD with the results of another worker's investigations of phenol-OH.[59] Plourde's relatively large isotopic changes in the enthalpy and entropy of association values are based upon the temperature changes in free energy differences close to zero at 25°, the temperature at which

systematic errors are at a minimum. Systematic errors will be greater as the temperature of measurement is changed from room temperature and may well be different for experiments carried out by two different workers, possibly using two different instruments.

Bonner[60] has made IR spectroscopic measurements of the quotient of the concentration ratio of monomeric methanol-OH to polymeric methanol-OH and of the concentration ratio of monomeric methanol-OD to polymeric methanol-OD for solutions in carbon tetrachloride. Unfortunately the measurements are over a range of concentrations (0.125 to 2.5 mole/dm^3) at which the concentrations of monomers may only be measured with very low precision. However Bonner's conclusion that association is greater for methanol-OH is qualitatively in agreement with that of Grimison[61] who measured the freezing points of solutions of imidazole and 1-deuterioimidazole in naphthalene and observed an isotope effect of 80 calories per mole on the free energy of association favouring association in the undeuteriated system. This last isotope effect may, of course, include a contribution from an isotopic change in the free energy of association between imidazole and naphthalene, as suggested by Plourde's results!

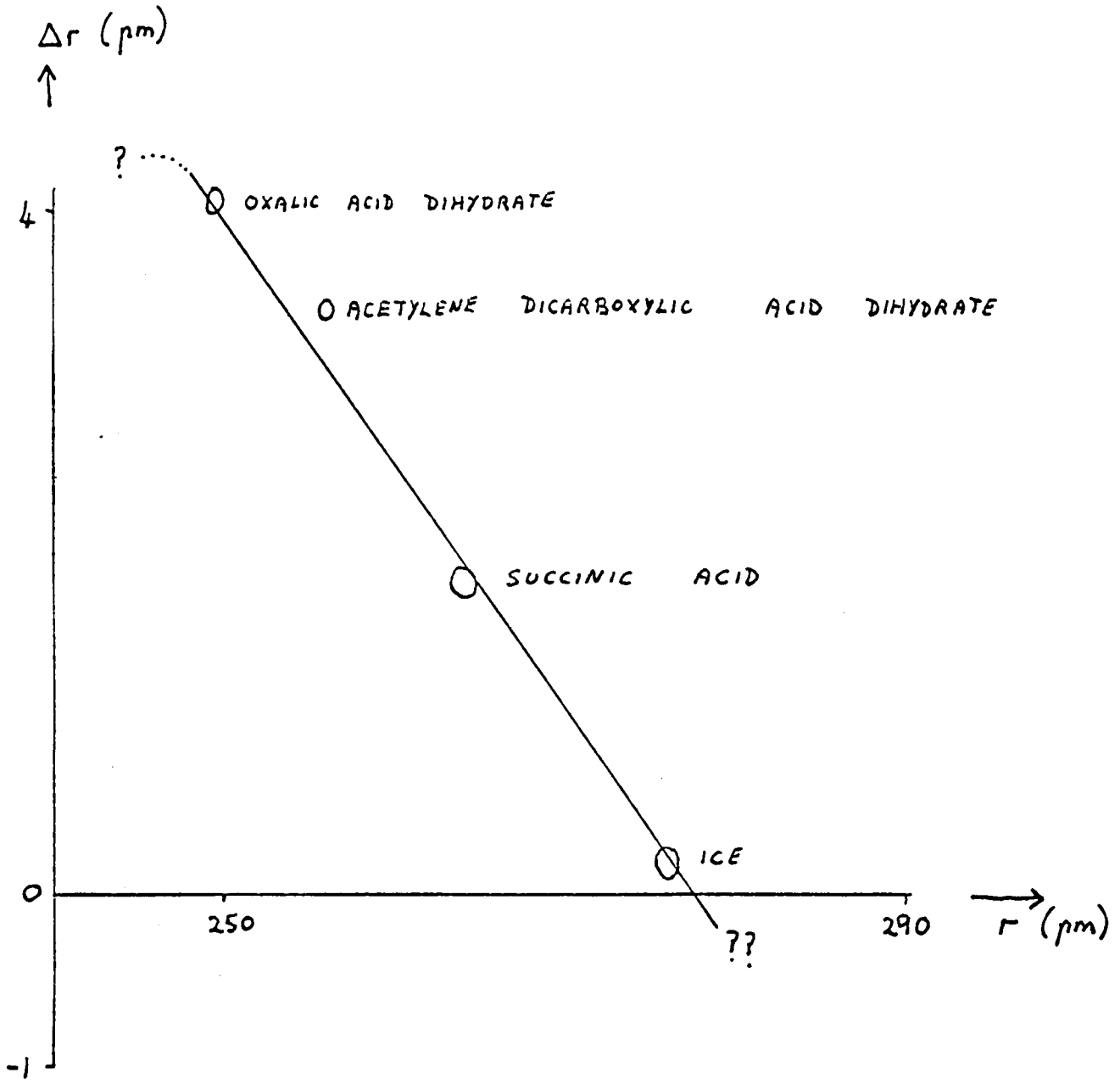
Studies of the change in the extent of enolisation between acetylacetone and 3,3-dideuterioacetylacetone have been made by Thompson and Allred,[62] using integration of the NMR signal intensities of diketone and enol-ketone methyl proton signals to measure enolisation equilibrium constants. A Varian A-60 instrument was used. Some of the measurements are reported for solutions in cyclohexane, a solvent which is strongly absorbing in the methyl proton chemical shift region. The isotopic changes reported in the enthalpy and entropy of enolisation, based upon measurements

at only three temperatures, are derived from the temperature variation of enolisation free energies which are the same, within experimental error, for the undeuteriated and deuteriated compounds, at the temperature (37°) for which measurements may be made most accurately. Thompson and Allred describe the large isotopic changes in the enthalpy difference as "difficult to explain", being too large for a simple analysis of zero-point energy contributions to apply.

The investigations mentioned above present a confusing picture of several isotope effects which may be interpreted in terms of isotopic weakening or strengthening of hydrogen bonds and of other effects which are subject to systematic errors.

Wolff has measured isotopic effects on the vapour pressure of ice and several hydrogen bonded liquids.[63,64] At all temperatures of measurement the vapour pressure of "light" ice is greater than that of "heavy" ice. Wolff refers to this as a "normal" isotope effect. The isotope effect on the vapour pressure of water and heavy water is normal at temperatures below 220° and the reverse (an "inverse" isotope effect) above this temperature. This reversal of a normal vapour pressure isotope effect with an increase of temperature is also observed for measurements on dimethylamine and heptadeuteriodimethylamine, methanol-OH and methanol-OD, and ammonia and trideuterioammonia. A normal isotope effect at one particular temperature is diminished and eventually inverted by increasing dilution with an inert solvent: for example hexane added to dimethylamine and heptadeuteriodimethylamine, or propane added to ammonia and trideuterioammonia. Since an increase in temperature or dilution decreases the concentration of hydrogen bonded polymers in the gas phase, Wolff's results for high temperatures or high dilution provide a measure of the change in free energy between protium bonds and deuterium bonds in the condensed phase, and

Figure 17. The change in hydrogen bond length on deuteriation, Δr., plotted against r, the hydrogen bond length. [From Gallagher][47]



? The short symmetrical FHF^- bond ($r = 226$ pm) shows a small contraction (Δr negative) and so a similar effect may be expected for very short OHO bonds.

?? The long bonds in copper sulphate pentahydrate ($r > 280$ pm) and urea ($r = 299$ pm) show small contractions.

indicate that protium bonding is stronger than deuterium bonding for the compounds studied.

Gallagher [47] has reported the effect of deuterium substitution on hydrogen bond length (that is the distance A----B, r, for a hydrogen bond A----H----B) in crystals. Figure 17 shows how Δr, the change in bond length from a protium bond to a deuterium bond, varies with r.

A correlation between an increase of the O----O distance in O----H----O bonds and a decrease in hydrogen bonding energy has been calculated by Lippincott and Schroeder, which compares well with experimental data for ice, alcohols, carboxylic acid dimers and oxalic acid.[65] Therefore a decrease in r may be used as an indicator of an increase in strength from one hydrogen bond to another, and, except for the very strongly bonded, probably symmetric, bifluoride anion, the value of Δr observed in crystals is positive for stronger bonds, decreasing as the strength of the bond diminishes. Comparison of Wolff's results with those of Gallagher for compounds with hydrogen bonding enthalpies in the range from ice through methanol to oxalic acid hydrate indicates that the series of positive Δr values corresponds to a weakening of the hydrogen bonds on deuterium substitutions.

If δG is the change in free energy between a protium bond and an analogous deuterium bond, then the general pattern of the results reported by Wolff and Gallagher is that δG is positive (an "inverse" isotope effect) and decreases as the hydrogen bond strength diminishes, accompanying an expansion of the hydrogen bond length, Δr, which also decreases with the hydrogen bond strength for the hydrogen bonds studied between oxygen and oxygen and between nitrogen and nitrogen. The very short FHF^- bond and the very long hydrogen bonds in copper sulphate pentahydrate and urea show contractions on deuteriation.

Both δG values measured in this work were positive (ΔG_{15} and ΔG_{16} correspond to a change from a deuterium bond to a protium bond) indicating that the hydrogen bonds studied conform to the general pattern described directly above for hydrogen bonds of intermediate length and enthalpy of formation. The effect for 3,5-di-t-butyl-2,6-dihydroxyacetophenone, LVIII, (δG = 52 cal/mole) is larger than that for 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde O-methyloxime, LXVII (δG = 16 cal/mole). This is consistent with a stronger intramolecular hydrogen bond in LVIII than in LXVII.

Wolff has observed that normal isotope effects on vapour pressures are strongly dependent upon temperature and concentration, which may be ascribed to association in both the gas and condensed phases. Inverse effects, under conditions without significant association in the gas phase, show a small temperature dependence. Therefore the origin of the inverse isotope effects observed in this work and in the literature may be initially ascribed to an isotope effect, δH, on the enthalpies of formation of hydrogen bonds. This enthalpy effect is given by the expression

$$\delta H = [\Sigma E_{associated} - \Sigma E_{free}]_D - [\Sigma E_{associated} - \Sigma E_{free}]_H ,$$

where the E terms are the energies of vibrations affected by isotopic substitution and the subscripts "associated" and "free" refer to the vibrational energies of the system with and without hydrogen bonding.

A rough estimate of δH for the isotopic change of the difference in vibrational energies between an unassociated phenol and an intramolecularly hydrogen bonded phenol (for example salicylaldehyde or 2-hydroxyacetophenone) may be made. The vibrations most significantly affected by isotopic substitution are the hydroxylic stretching (ν) and the out-of-plane torsional deformation (τ). A relatively smaller

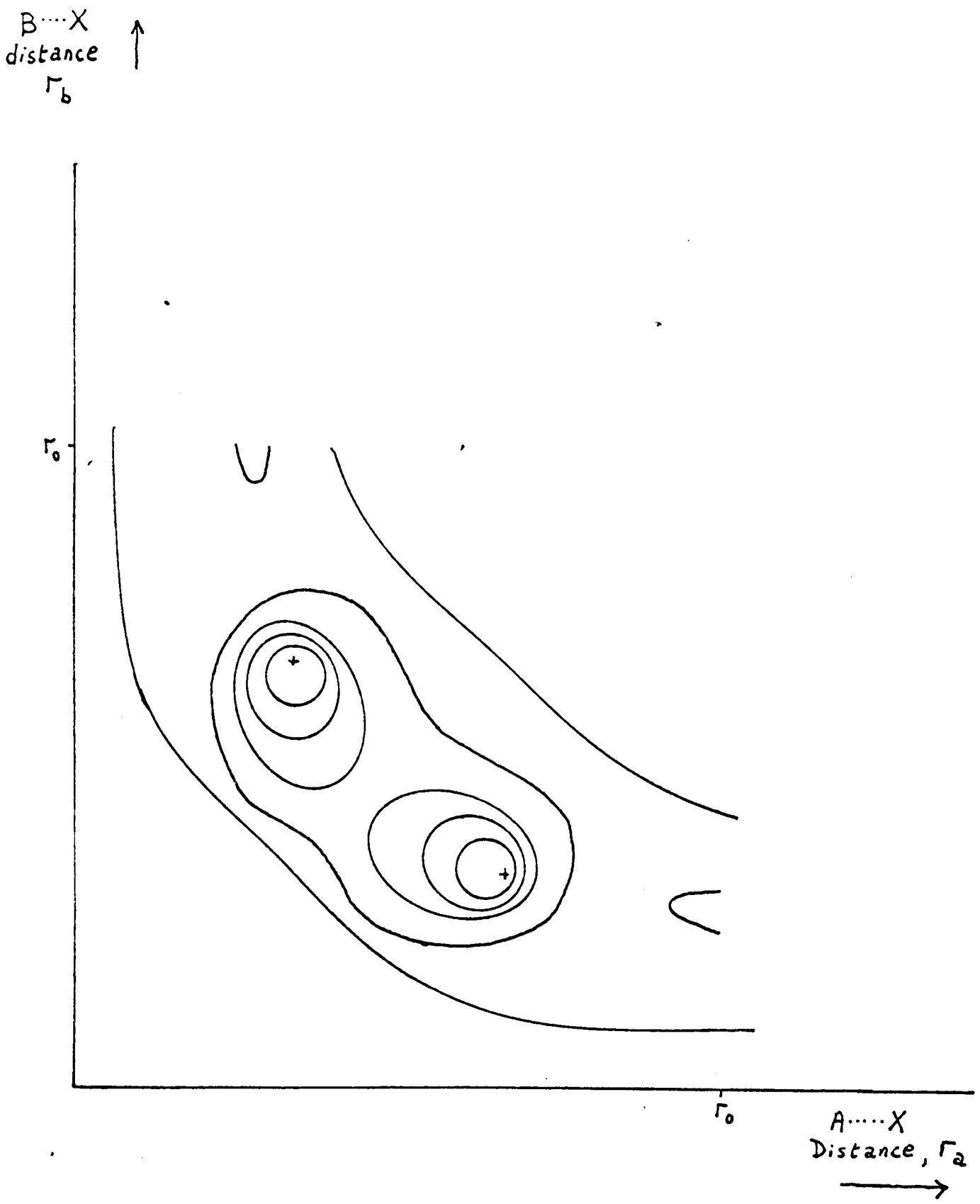


Figure 18. A potential energy contour map for an A····X····B (X = H or D) hydrogen bond of the type which expands upon deuteriation. [Only contours in the region of the potential energy minimum valley are shown].

contribution will be expected from the hydroxylic in-plane bending (δ). Literature values for the frequencies of these three vibrations in phenol-OH and phenol-OD[66,67] and in salicylaldehyde and 2-hydroxyacetophenone derivatives (for these compounds the relation $\delta_{OH} = 1.35\,\delta_{OD}$ was assumed. Nyquist[68] has observed this proportionality between ν_{OH} and ν_{OD})[68,69,70] were used to compute E values. As the frequencies of torsional vibrations are low (~ RT), the E values were computed by summation over all vibrational states with 1% or more of the total molecular population at the temperature of the measurements in this work (35°). The simple harmonic approximation of energetically equally spaced excited states was used. The calculated value of δH was 300 ± 100 calories per mole.

The enthalpy effect, then, favours the formation of protium bonds over the formation of deuterium bonds. Any entropy effect will probably favour the reverse; the ordering of the more widely vibrating -OH groups on hydrogen bond formation is greater than the equivalent ordering of -OD groups.

The somewhat surprising expansion of hydrogen bond length (Δr positive) on deuteriation for hydrogen bond lengths of a particular range (254 pm < r < 277 pm) remains to be considered. The expansion is explicable only if the potential surface for a particular bond of this type A----X----B (X = H or D) is of the form indicated by the potential contour map in Figure 18. For simplicity of discussion the map shown is for a bond with a symmetric double minimum, although similar considerations will apply to potentials with an asymmetric double minimum provided that the same general pattern for the potential gradients is exhibited.

In Figure 18 only contours in the region of the potential minimum valley are shown. The two minima are marked with crosses and lie on the line

$$r_a + r_b = r_o$$

where r_a is the A----X distance, r_b is the B----X distance and r_o is the A----B distance at minimum potential energy. Since X is a much lighter atom than either A or B, the vibration of X in the bond may be considered for fixed values of r (the distance from A to B) to a fair degree of approximation, and the zero-point vibration of X will be along lines parallel to that joining the two minima. The characteristics of the potential surface which determine the isotope effect on r are the relative potential gradients for small displacements of lines parallel with $r_a + r_b = r_o$ towards or away from the origin. For the system whose potential is represented in Figure 18, the presence of one minimum perturbs the potential surface around the other minimum (and vice versa) so that there is a shallower potential gradient for displacement from the line $r_a + r_b = r_o$ towards the origin (a contraction of the hydrogen bond) than for displacement away from the origin (an expansion). Hence the zero-point energy of the X atom's vibration will position the vibration along a line parallel with $r_a + r_b = r_o$ and displaced towards the origin. The greater the zero-point energy the greater will be this displacement towards the origin and hence the shorter will be the hydrogen bond. Therefore substitution of deuterium, with its lower zero-point energy, for protium as the atom X will lead to an expansion of the hydrogen bond (Δr positive).

For hydrogen bonds which are longer than the type discussed above (as in copper sulphate pentahydrate and urea), the two minima are far enough apart for a "normal" potential energy surface with a steeper potential gradient for contraction of the hydrogen bond than for its expansion, and deuteriation has the opposite effect on the hydrogen bond length (Δr negative). Very short bonds approach a single

minimum situation as for the FHF^- bond where the decreased amplitude of vibration on changing from protium to deuterium will be expected to lead to a contraction of the bond, as Gallagher reports for potassium hydrogen fluoride.

Although the isotope effects considered in this chapter are upon hydrogen atoms in environments whose relative energies are more "electrostatic" in origin than those examined in the first chapter, the observed isotopic changes may still be accounted for in terms of the decreased vibrational amplitude resulting from a change from a bond to protium to a bond to deuterium.

EXPERIMENTAL

DI-t-BUTYLRESORCINOLS

4,6-Di-t-butylresorcinol dihydrate, prepared by the acid-catalysed reaction of t-butyl alcohol with resorcinol, was supplied by Dr. M.J.T. Robinson.

3,5-Di-t-butyl-2,6-dihydroxy-1-dimethylaminomethylbenzene

Dimethylamine (as an aqueous 25% solution, 27 ml, 0.15 mole) was added to 4,6-di-t-butylresorcinol dihydrate (12 9 g, 0 05 mole) followed by formaldehyde (as an aqueous 37% solution, 13 ml, 0.15 mole) and enough methanol (70 ml) to form a clear solution at 40°. The mixture was boiled under reflux for 2½ hours and, when cool, poured into an equal volume of water. The precipitate which formed was filtered off, dried and recrystallised from aqueous methanol (1:4). Yield 11.7 g, 85%. M.p. 120-122° (decomposition). $C_{17}H_{29}NO_2$ requires 73.07% C, 10.46% H, 5.01% N. Found 73.3% C, 10.4% H, 4.9% N.

In the NMR spectrum of the product (in deuteriochloroform), the chemical shift of the hydroxylic proton varied with concentration: at 5%, τ_{OH} = 2.49 ppm, whilst at 10%, τ_{OH} = 2.13 ppm.

3,5-Di-t-butyl-2,6-dihydroxy-1-piperidinomethylbenzene

The above preparation was carried out on half the scale using piperidine (7.5 ml, 0.075 mole) in the place of dimethylamine solution. Yield 6.5 g, 84%. M.p. 137-141° (decomposition). $C_{20}H_{33}NO_2$ requires 75.19% C, 10.41% H, 4.38% N. Found 75.3% C, 10.4% H, 4.4% N.

In the NMR spectrum of a 5% solution in deuteriochloroform τ_{OH} = 2.37 ppm and for 10% concentration τ_{OH} = 1.90 ppm.

3,5-Di-t-butyl-2,6-dihydroxybenzaldehyde

A solution of 4,6-di-t-butylresorcinol dihydrate (12.9 g, 0.05 mole) in

1,2-dichloroethane (100 ml) was dried by azeotropic distillation as more 1,2-dichloroethane was added at such a rate as to maintain the volume of the solution. The cooled solution was added dropwise, with stirring, to an ice cold mixture of phosphorus oxychloride (16.8 g, 0.11 mole) and dimethylformamide (8 g, 0.11 mole) which had been allowed to stand at room temperature for 20 minutes. The reaction mixture was boiled under reflux until evolution of gas ceased (about 20 minutes) and then cooled.

With vigorous stirring a solution of potassium acetate (54 g, 0.55 mole) in water (100 ml) was added as rapidly as possible and the resulting mixture was boiled under reflux for half an hour. Ethereal extracts (2 x 50 ml) of the cooled aqueous phase were added to the organic one to give a combined solution which was washed twice with its own volume of aqueous dipotassium hydrogen orthophosphate (2M), once with brine and dried over anhydrous potassium carbonate. The residue from evaporation was recrystallised from petroleum ether (b.p. 40-60°) to give the product as yellow crystals m.p. 119-121°. Yield 7.8 g, 62%. $C_{15}H_{22}O_3$ requires 71.97% C, 8.86% H. Found 72.4% C, 8.5% H.

NMR spectra of the product (5 and 10% w/v in carbon tetrachloride) showed broad (width at half-height 1 ppm) signals with centroids at 1.0 τ for the hydroxylic protons.

3,5-Di-t-butyl-2,6-dihydroxybenzaldehyde O-methyloxime

A solution of methoxyamine hydrochloride (0.8 g, 0.01 mole) in water (1 ml) was mixed with one of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde (2 g, 0.008 mole) in methanol (5 ml). Pyridine (0.9 ml, 0.01 mole) was added to the mixture which was kept clear by heating on a water bath. Enough methanol (5 ml) was added to

the cooled mixture to clarify it at room temperature, and the solution was left to stand for three hours. The precipitate which had formed was filtered off and washed with cold aqueous methanol (1:5) before recrystallisation from a more polar mixture (9 parts water, 1 part methanol). Yield 1.68 g, 76%. M.p. 137-140°. $C_{16}H_{25}NO_3$ requires 68.78% C, 9.02% H, 5.01% N. Found 69.3% C, 8.8% H, 5.0% N.

The product discoloured after several weeks on standing with protection from light or after a few days without such protection.

NMR spectra of the product (4 and 8% w/v in carbon tetrachloride) had $\tau_{OH} = 2.33$ ppm.

Samples of the product were dissolved in 1,2-dimethoxyethane and varying quantities of heavy water (to give up to 90% deuteriation at the hydroxylic positions) were added to each sample. The solutions were dried in vacuo and the NMR spectra of the residues (5% w/v in carbon tetrachloride) were recorded. The extent of deuteriation of a sample was measured from the relative intensities of the iminic, aryl and hydroxylic proton signals.

3,5-Di-t-butyl-2,6-dihydroxyacetophenone

2,6-Dihydroxyacetophenone (5 g, 0.033 mole) was dissolved in t-butyl alcohol (45 g, 0.61 mole) and then, with swirling, orthophosphoric acid (88% in water, 40 g, 0.4 mole) was added. The mixture was heated at 70° for 30 hours, cooled and poured into water (100 ml). The resulting suspension was stirred with petroleum ether (b.p. 30-40°, 100 ml) and the undissolved solid was collected and recrystallised from aqueous methanol to give 3-t-butyl-2,6-dihydroxyacetophenone as yellow crystals, m.p. 176-179°. Yield 3.0 g, 50%. $C_{12}H_{16}O_3$ requires 69.21% C, 7.74% H. Found 69.5% C, 7.8% H. The NMR spectrum (1% w/v in

carbon tetrachloride) showed an AB quartet (τ_A = 2.83 ppm, τ_B = 3.93 ppm, J_{AB} = 9 Hz) for the two aryl protons.

The two phases of the filtrate obtained in the work-up were separated and the organic one was washed with brine and dried over anhydrous magnesium sulphate. The residue from evaporation was heated at 100° under 0.5 mm pressure for half an hour, cooled and then recrystallised twice from petroleum ether (b.p. 30-40°) at -78° to give 3,5-di-t-butyl-2,6-dihydroxyacetophenone as yellow crystals m p. 77-79°. Yield 1.5 g, 17%. $C_{16}H_{24}O_3$ requires 72.69% C, 9.15% H. Found 72.9% C, 9.3% H. NMR spectra (4, 5 and 6% w/v in carbon tetrachloride) had τ_{OH} = 0.49 ppm.

Two partially deuteriated samples were prepared by the evaporation of the product in 1,2-dimethoxyethane/heavy water solutions.

Some of the many attempts that were made to prepare 3,5-di-t-butyl-2,6-dihydroxyacetophenone using anhydrous 4,6-di-t-butylresorcinol as starting material yielded characterised products of interest.

Treatment of anhydrous 4,6-di-t-butylresorcinol with acetic anhydride (an eightfold excess) and concentrated sulphuric acid (equimolar to the resorcinol) for 15 hours at room temperature gave 4,6-di-t-butylresorcinol diacetate, m.p. 133-137° after recrystallisation from aqueous ethanol, in 79% yield. $C_{18}H_{26}O_4$ requires 70.56% C, 8.55% H. Found 70.8% C, 8.4% H. The NMR spectrum (10% w/v in carbon tetrachloride) showed two aryl proton singlets at 2.77 τ and 3.41 τ, both of relative intensity 1, and two methyl proton singlets at 7.80 τ, relative intensity 6, and at 8.67 τ, relative intensity 18.

The method described above for the preparation of 3,5-di-t-butyl-2,6-

dihydroxybenzaldehyde was followed using N,N-diethylacetamide in the place of dimethylformamide. The product m.p. 169-173° had an NMR spectrum (10% w/v in deuteriochloroform) with two aryl proton singlets at 2.81 τ and 3.63 τ, both of relative intensity 1, a broad hydroxylic proton signal at 4.09 τ, of relative inetnsity 1 and two methyl proton signals, one at 7.69 τ of relative intensity 3 and the other at 8.67 τ of relative intensity 18. This NMR spectrum was compatible with the structure of 4,6-di-t-butylresorcinol monoacetate. Yield 35%. $C_{16}H_{24}O_3$ requires 72.69% C, 9.15% H. Found 72.9% C, 9.0% H.

Treatment of anhydrous 4,6-di-t-butylresorcinol in dry ether (as a 25% solution) with acetonitrile (a twofold excess), saturated with hydrogen chloride, over zinc chloride (0.25 molar equivalent) at 0° for 10 days gave a precipitate which was filtered off and dissolved in water. The residue from ethereal extracts of this solution was recrystallised from toluene to give a product m.p. 199-203°. The NMR spectrum (2% w/v in deuteriochloroform) was compatible with the structure of 5-t-butyl-2,4-dihydroxyacetophenone: two aryl proton singlets at 2.43 τ and 3.80 τ, both of relative intensity 1, a hydroxylic proton signal at 4.30 τ of relative intensity 2, and two methyl proton signals, one at 7.46 τ of relative intensity 3, and the other at 8.62 τ of relative intensity 9. Yield 17%. $C_{12}H_{16}O_3$ requires 69.21% C, 7.74% H. Found 69.4% C, 7.7% H.

PREPARATION OF PHENOLS USED AS MODEL COMPOUNDS FOR HYDROXYL GROUPS

5-t-Butyl-2,4-dihydroxyacetophenone

The Hoesch acetylation of 4,6-di-t-butylresorcinol described above gave the product in 17% yield.

Salicylaldehyde O-methyloxime

The procedure described by Feigl and Bondi[71] was used to prepare the product in 66% yield. M.p. 24-26°. Literature[71] m.p. 28°.

3,5-Di-t-butyl-2-hydroxy-6-methoxyacetophenone

3,5-Di-t-butyl-2,6-dihydroxyacetophenone (0.5 g, 0.002 mole) was dissolved in a mixture of acetone (AnalaR, 2.5 ml) and dry 1,2-dimethoxyethane (2.5 ml). Iodomethane (3 g, 0.02 mole) was added and the mixture was boiled under reflux over anhydrous potassium carbonate (0.3 g, 0.002 mole) for five hours. The suspension was cooled and filtered. The residue from evaporation of the filtrate was twice recrystallised from methanol to give the product, m.p. 94.5-96.5°. Yield 0.15 g, 29%. $C_{17}H_{26}O_3$ requires 73.34% C, 9.41% H. Found 73.1% C, 9.3% H.

3,5-Di-t-butyl-2-hydroxy-6-methoxybenzaldehyde

3,5-Di-t-butyl-2,6-dihydroxybenzaldehyde was monomethylated using the procedure described directly above on a five-fold scale, except that the crude product was once recrystallised from petroleum ether (b.p. 30-40°) at -78°. M.p. 73-75°. Yield 1.8 g, 68%. $C_{16}H_{24}O_3$ requires 72.69% C, 9.15% H. Found 72.6% C, 9.1% H.

2-Hydroxy-6-methoxyacetophenone

2,6-Dihydroxyacetophenone was monomethylated in the usual way to give a crude reaction product which was recrystallised from aqueous methanol. Yield 50%. M.p. 54-56°. Literature[48] m.p. 57-58.5°.

3,5-Di-t-butyl-2-hydroxy-6-methoxybenzaldehyde O-methyloxime

A solution of methoxyamine hydrochloride (0.4 g, 0.005 mole) in water

(0.5 ml) was added to one of the parent aldehyde (1.0 g, 0.004 mole) in methanol (16 ml). The mixture was heated to 50° to keep it clear as pyridine (0.45 ml, 0.005 mole) was added. The solution was left to cool in the dark overnight and then chilled to 0°. The crystals which separated were filtered off and recrystallised from methanol. Yield 1.04 g, 91%. M.p. 92.5-95.5°. $C_{17}H_{27}NO_3$ requires 69.59% C, 9.28% H, 4.77% N. Found 69.4% C, 9.3% H, 4.9% N.

PREPARATION OF SUBSTANCES FOR PRELIMINARY INVESTIGATIONS OF INTERMOLECULAR EXCHANGE

Salicylaldehyde O-methyloxime was prepared as described above.

t-Butylmethylamine

A solution of isobutyraldehyde (144 g, 2 mole) in ether (250 ml) was added over a period of 3½ hours to a solution of t-butylamine (146 g, 2 mole) in ether (250 ml) cooled in an ice-salt bath, with continuous stirring. The mixture was warmed to room temperature by the addition of potassium hydroxide pellets (16 g, 0.3 mole). After half an hour the aqueous phase which had formed was removed and more potassium hydroxide (20 g, 0.3 mole) was added to the ethereal solution. The aqueous syrup which formed overnight was removed and the organic phase was dried over more potassium hydroxide. The solution was then distilled and the fraction b.p. 114-119°/768 mm was collected as N-isobutylidene t-butylamine. Literature[72] b.p. 115°. Yield 149 g, 60%.

This product (149 g, 1.17 mole) was added to a solution of dimethyl sulphate (148 g, 1.17 mole) in dry benzene (300 ml) and left to stand overnight with suitable protection from atmospheric moisture. The lower of the two layers which formed was poured into a mixture of water (250 ml) and ethanol (150 ml) and the resulting mixture

was boiled under reflux for two hours before evaporation under reduced pressure (15 mm) in a boiling water bath. The residue was cooled, with stirring, in an ice bath as a cold solution of potassium hydroxide (100 g, 1.6 mole) in water (180 ml) was slowly added. The resulting sludge was filtered and the filtrate was distilled, the fraction with b.p. less than $90^{\circ}/760$ mm being stored over potassium hydroxide (10 g). After a day the aqueous phase which had formed in this distillate was removed and the organic one was dried over more potassium hydroxide before fractional distillation through a 7" Dixon ring column. The distribution of fractions in the boiling range $59-72^{\circ}$ indicated the presence of t-butylamine.

Accordingly the fractions were recombined and mixed with an excess of salicylaldehyde to "trap" the primary amine as its involatile salicylidene imine whilst t-butylmethylamine was collected in a solid carbon dioxide trap at reduced pressure. The product obtained in this way was distilled and the fraction b.p. $70-94^{\circ}$ was recycled through the same purification procedure to give a crude product b.p. $69-81^{\circ}$ with an NMR spectrum (20% w/v in carbon tetrachloride) indicating the absence of t-butylamine.

Distillation through a 9" Dixon ring column gave the product as two fractions b.p. $69.5-71.3^{\circ}$ and $71.3-71.6^{\circ}/768$ mm, both with n_D^{20} 1.3962. Yield 10 g, 6% overall. Literature[73] b.p. $73-74^{\circ}$, n_D^{25} 1.3952.

8-Quinolinol-1-oxide

8-Quinolinol (oxine) was oxidised in glacial acetic acid with hydrogen peroxide solution (100 volumes) according to the method described by Ramiah and Srinivasan[74] in 33% yield. M.p. of the sublimed product $131.5-134.0^{\circ}$. Literature crude yield 37%, m.p. 139°.

MEASUREMENT OF THE HYDROXYLIC PROTON NMR CHEMICAL SHIFTS OF PARTIALLY DEUTERIATED DI-t-BUTYLRESORCINOLS

NMR spectra of samples of 3,5-di-t-butyl-2,6-dihydroxybenzaldehyde O-methyloxime, partially deuteriated at the hydroxylic positions (5% w/v in carbon tetrachloride which had been dried over potassium hydroxide for a week) were recorded. Calibration of a chemical shift scale was based upon the distance between the iminic and aryl proton signals which had a standard deviation of 0.2%. The distances of the hydroxylic proton signal from the two standard signals were measured for each sample and the extent of deuteriation was estimated from the intensities of the three signals. A linear least-squares fit was made between the chemical shift of the hydroxylic proton signal and the extent of deuteriation of the sample corresponding to a shift of $-(3.4 \pm 0.2) \times 10^{-4}$ ppm for every per cent of deuteriation.

The NMR spectra of samples of 3,5-di-t-butyl-2,6-dihydroxyacetophenone, partially deuteriated at the hydroxylic positions (5% w/v in dry carbon tetrachloride) showed two hydroxylic proton signals. The chemical shift difference between these two signals was measured, using the signal of tetramethylsilane and a 200 Hz oscillator-induced side band for calibration, as -0.1634 ± 0.0020 ppm, for 50 and 65% deuteriation (measured from the intensity of the signals). The intensity of the lower field signal relative to that of the other increased with the extent of deuteriation.

CHAPTER THREE

ISOTOPE EFFECTS FROM UV SPECTROSCOPY

DISCUSSION

CHANGES IN UV SPECTRA ASSOCIATED WITH DEUTERIATION

The two principal changes in UV absorption bands between the spectra of an undeuteriated and of a deuteriated compound in a condensed phase that are possible are changes of intensity and changes in the frequency of the maxima or centroids.

An isotopic change in intensity may be the result of a change in the oscillator strength of absorption by a single species, or the result of a change in an equilibrium between species with different absorptions. Earlier work[29] on the spectra of highly enolised β-diketone compounds and p-nitroaniline indicated that any change in the oscillator strength, f, of absorptions upon deuteriation was less than 1% of the value of f and was consequently of the same order of magnitude as the expected errors in the preparation of solutions. Therefore the more intense absorption in the spectrum of acetylacetone (as a 10^{-5} molar solution in cyclohexane) compared with the absorption in the spectrum of 3,3-dideuterioacetylacetone, recorded under the same conditions by Thompson and Allred[62] may be ascribed to a greater preponderance of the more strongly absorbing conjugated enol form in the undeuteriated acetylacetone than in the deuteriated compound.

Thompson and Allred also report that "Substitution of deuterium into the 3-positions does not alter noticeably either the position or the shape of the enol band." For the same deuteriation the author has observed a shift in the maximum of this enol absorption (for spectra of solutions in water or heavy water with 1,2-dimethoxyethane) from 274.5 nm to 272.0 nm.[29] Isotopic shifts were also observed for all the highly enolised compounds studied as well as p-nitroaniline. Since deuteriation is primarily a perturbation of the σ electronic configuration of a

molecule, many of these shifts corresponded to surprisingly large changes in excitation energy (for example ~ 1 kcal/mole for dibenzoylmethane) for a $\pi \rightarrow \pi^*$ transition, and may only be interpreted in terms of significant geometric changes between the ground and excited states (for example the flattening of amino groups in anilines, discussed on Page 158). Schubert et al.[75] have measured smaller isotopic shifts of the K absorption of nitrobenzenes, anilines and anisoles on deuteriation either at the 4-position or at an alkyl substituent at the 4-position. It has been shown that, within experimental error, these isotopic shifts are independent of the nature of the solvent used for the measurements.[76]

Thompson and Allred's anomalous observation may be erroneous, since, for a 10^{-5} molar solution of 3,3-dideuterioacetylacetone in cyclohexane, only 0.2 mg per litre of water in the solution (less than 1 μg in a typical UV cell) would be required for a 50% distribution of deuterium between the diketone and water. Unless special precautions, not mentioned by Thompson and Allred, are taken, such quantities of water are readily dissolved from the walls of the apparatus and from the air. Therefore the spectrum recorded may be that of the undeuteriated or partially deuteriated compound. This casts doubt upon the reliability of the value of the isotope effect on the keto-enol equilibrium composition of acetylacetone measured by Thompson and Allred.

In this work, intensity changes, as well as frequency shifts, have been measured in the long wavelength $\pi \rightarrow \pi^*$ UV absorption bands of acetylacetone and cis- and trans-4,6-di-t-butylcyclohexane-1,3-diones in order to investigate isotope effects on the keto-enol equilibria of the three compounds. The presence of conjugation in the enol forms gives rise to the allowed $\pi \rightarrow \pi^*$ absorption which was

studied. The diketo forms can only absorb in a similar frequency region through low intensity n $\rightarrow \pi^*$ carbonyl absorptions leading to bands of oscillator strengths negligible in comparison with those of enol absorptions.

Acetylacetone was chosen as an example of a compound with an intramolecularly hydrogen bonded enol, for which any isotopic change in the relative stabilisation of the enolic and diketone tautomers may be interpreted in terms of a change in the strength of the intramolecular hydrogen bond on deuteriation. The two cyclohexanediones have no intramolecular hydrogen bond in the enol form since the six-membered ring holds the two carbon oxygen bonds in a trans-configuration. Therefore any isotope effect upon the relative stabilisation of the two tautomers for the cyclohexanediones by hydrogen bonding will be the result of changes in hydrogen bonding with the solvent.

The spectra of 4-aminopyridine and several of its derivatives are examined for isotopic frequency shifts on substitution of deuterium in the amino group for basic and acidic solutions. The extent of such shifts may depend upon the varying hybridisation of the N-H bond in these compounds. Accordingly, the shift values are compared with those measured for p-toluidine, 4,4'dinitrodiphenylamine and p-phenylenediamine in this study and with those measured for other benzenoid compounds by White[77] to assess the influence of a nitrogen hetero atom in the aromatic ring as well as the effects of ring substituents. Also the ^{15}N-^{1}H NMR coupling constants of 4-^{15}N-aminopyridine and several of its derivatives are measured, as another quantity which depends upon the hybridisation of the N-H bond, to give values for comparison with the measurements of Axenrod et al.[78] on a series of ^{15}N-aniline derivatives.

PREPARATION OF COMPOUNDS FOR UV SPECTROMETRY AND THE MEASUREMENT OF ^{15}N-^{1}H NMR COUPLING CONSTANTS

Most of the substances studied were either purified samples of commercially available materials or prepared using the straightforward procedures described in the experimental section. Discussed below are those preparations which involved experimental difficulties or unusual reactions.

4-Aminopyridine-1-oxide

Pentimalli[79] reports the preparation of 2-aminopyridine-1-oxide hydrochloride in 76% yield by the acid extraction of the crude residue from the oxidation of 2-aminopyridine with perbenzoic acid. Using the same reaction conditions the direct oxidation of 4-aminopyridine was attempted, producing 4-aminopyridine-1-oxide hydrochloride in 19% yield. The hydrochloride was readily converted to the free 4-aminopyridine-1-oxide in 93% yield using aqueous silver oxide. The colourless N-oxide product was extremely sensitive to light, turning yellow after a few minutes exposure to daylight, a property not mentioned by Katritsky et al.[80] who had prepared 4-aminopyridine-1-oxide from the reduction of 4-nitropyridine-1-oxide.

Therefore the low yield of 4-aminopyridine-1-oxide hydrochloride may be attributed to the exposure of the 4-aminopyridine/perbenzoic acid reaction mixture to light, and an improvement in the yield is to be expected for reactions carried out in the dark.

4,4'-Dinitrodiphenylamine

Equimolar quantities of p-bromonitrobenzene and p-nitroaniline with an equivalent of anhydrous potassium carbonate and a catalytic amount of copper I iodide were heated at about 200° in nitrobenzene for a day. The reaction mixture

was then steam distilled to get rid of the nitrobenzene and washed with concentrated hydrochloric acid. Kloetzel et al.[81] report a 40% yield of 4,4'-dinitrodiphenylamine of unspecified melting point from the extraction of the dried insoluble matter from the reaction procedure described above, with large quantities of boiling nitrobenzene. Owing to the obvious practical difficulties of extractions with boiling nitrobenzene (the boiling point of nitrobenzene is 211° at 760 mm[82]) this relatively efficient method was abandoned in favour of continuous extraction with ethanol for isolation of the product, as only enough 4,4'-dinitrodiphenylamine for UV spectroscopic measurements was required. This accounts for the very low yield of the product (1%).

4-^{15}N-Aminopyridine

Vompe et al.[83] report the preparation of 4-aminopyridine in 90% yield from heating 4-phenoxypyridine with ammonium chloride at 300° "in an open vessel" for an hour. This reaction was repeated several times on a small scale, for which it was found that a shorter reaction time (35 minutes) and an atmosphere of dry nitrogen above the reaction mixture optimised the yields of sublimed 4-aminopyridine (~ 60%). The use of ammonium sulphate in the place of ammonium chloride gave no detectable yield of the desired product.

Accordingly 4-^{15}N-aminopyridine was prepared by heating together 4-phenoxypyridine and ^{15}N-ammonium chloride for 35 minutes at 300° under a slow stream of dry nitrogen in 53% yield. The ^{15}N-ammonium chloride was prepared by allowing ^{15}N-ammonia, generated from a mixture of ^{15}N-ammonium sulphate and potassium hydroxide solution, to diffuse into a slight excess of dilute hydrochloric acid. 4-Phenoxypyridine was prepared by the condensation of phenol with 4-chloropyridine using the method reported by Hill and McGraw for the preparation of 2-aryloxypyridines.[84]

^{15}N-Enriched 4-aminopyridine derivatives

Derivatives of 4-aminopyridine with ^{15}N-enrichment were prepared using reaction conditions which had been established by experiments on 4-^{14}N-amino-pyridine as described in the experimental section.

The low yield (20%) of 4-amino-p-nitrophenylpyridinium chloride from heating p-chloronitrobenzene and 4-aminopyridine in xylene at 130° (the highest temperature for an experiment which could safely be left unattended for long periods) for three days is a result of the very low rate of reaction. Crude samples of 4-amino-p-nitrophenylpyridinium fluoride, which is formed from the much faster reaction of p-fluoronitrobenzene and 4-aminopyridine, reacted with dimethylsulphoxide, the solvent to be used for the determination of the NMR ^{15}N-^{1}H coupling constant of a 4-^{15}N-amino-p-nitrophenylpyridinium compound, to give a deep red precipitate.

THE SELECTION OF CONDITIONS FOR THE RECORDING OF SPECTRA

In order to study the spectrum of a selected compound and the spectrum of the same compound with deuterium substituted in the place of all readily replaceable hydrogens, compounds were dissolved in an aprotic solvent mixed with a suitable quantity of water or acid in water for the former, and mixed with an equivalent quantity of heavy water or deuterio-acid in heavy water for the latter. 1,2-Dimethoxyethane ("glyme"), dried with lithium aluminium hydride and stored over sodium wire, was selected as the most suitable aprotic solvent since it combined the properties of miscibility with water and solvation of the appropriate compounds, with transparency to ultraviolet light with wavelength longer than 255 nm. Any absorptions at shorter wavelengths (details are in the experimental section) remained constant in intensity over a period of six weeks' standing, unlike the absorption of, for

example, dry tetrahydrofuran even with suitable protection from light. The higher boiling point of glyme (84-85°) compared with that of tetrahydrofuran (65°) lessens the problem of solvent evaporation during the running of the spectra, although, of course, cells with tight stoppers were used at all times.

Compounds whose spectra were examined were acetylacetone, 4-amino-pyridine, 4-aminopyridine-1-oxide, 4-amino-1-methylpyridinium methanesulphonate, p-toluidine, p-phenylenediamine, 4,4'-dinitrodiphenylamine, and cis- and trans-4,6-di-t-butylcyclohexane-1,3-dione. Acidic solutions were used for p-toluidine, to observe an isotope effect upon p-toluidinium ion absorption and for the two cyclohexanediones to suppress enolate anion formation. 4-Aminopyridine and 4-amino-pyridine-1-oxide spectra were recorded for both neutral and acidic solutions in order to observe isotope effects upon absorptions of the free bases and conjugate acids respectively.

The proportions of water and glyme in the solvent were such that the water (or heavy water) was always vastly in excess of the absorbing compound. In the worst case (p-toluidinium salt solution) the molecular ratio was greater than 100:1. The concentrations were chosen to give optical densities of about 1 at 0.1 or 0.2 cm path length at the wavelengths with maximum absorption. However the relatively low extinction coefficient at maximum absorption for p-toluidinium ion combined with the need to keep the molar ratio of water (or heavy water) to absorbing species above 100:1 led to the use of 1 cm path length for p-toluidinium salt solutions.

The solvent composition for acetylacetone was chosen so that the extinction coefficient at the maximum of the absorption band around 274 nm was about half that quoted for the absorption of the pure enol by Belford, Martell and Calvin[85]

incorporating the bromometric measurements of Meyer.[86] The selection of a solvent composition with the keto-enol equilibrium constant near unity was based upon the optimisation of the sensitivity of the spectra, with maximum optical density about one, to any possible isotope effects upon the equilibrium. For a given isotopic change in the free energy difference between the keto and enol tautomers, the change in the absorbance increases with a decrease in the enol content for the equilibrium studied. However, with too small an enol content the forbidden diketone absorption would become significant.

For acidic aqueous glyme solutions of cis- and trans-4,6-di-t-butylcyclohexane-1,3-dione, the intensity of the absorption band near 250 nm increased with an increase in the proportion of water in the solvent from 1 to 20% by volume indicating an increase in the more strongly absorbing enol content for both compounds. The use of greater proportions of water led to no significant further increase of absorption intensity with water/acetonitrile mixtures as solvent.[10] Spectra of these cyclohexanediones were run in solvents using both ~ 1 and ~ 20% by volume aqueous acid in glyme as examples of relatively low enol content equilibria and "highly enolised" equilibria respectively. The aqueous acid used for the former was ten times more dilute than the acid for the latter, since, for low proportions of water (~ 1%), time-dependent spectra were observed for solutions made up with more concentrated acid, presumably as the result of some acid-catalysed decomposition of the cyclohexanediones.

The spectra of both "protio" and "deuterio" solutions were recorded in the usual way ("direct" spectra) together with recordings of the base-line using the solvent in cells placed in sample and reference beams, and with the cells exchanged

in position. Any divergence between the two base-lines indicated the presence of dirt in the cells and so base-line recording was continued until such divergence was minimised.

For more accurate measurement of any isotope effects on the spectra, "difference" spectra were recorded. Two "base-lines" were run with protio solution in both cells, at first with cell A in the sample beam and cell B in the reference beam, and then the reverse. The mean absorbance of these two "base-lines" gave a base-line which was not subject to any path length difference between the two cells, a significant source of error if solutions of optical densities near, or greater than, unity are in both beams of the spectrometer. Two optical difference spectra were then run with deuterio solution in the sample beam and protio solution in the reference beam, again with cells first one way and then the other. Arithmetic averaging of these two spectra eliminated the effects of a difference in path length, and subtraction of the averaged base-line provided an optical difference spectrum (deuterio-protio) which depended only upon differences in absorption properties between the two solutions.

ANALYSIS OF THE SPECTRA

The analysis of the recorded spectra had two goals: the determination of isotope effects on integrated intensities of the absorption bands and on the frequency position of the centroids of the bands. The positions of centroids of the bands were chosen as representatives of the frequency position of the most probable electronic excitation rather than the position of maxima, since a centroid is a property of an absorption band as a whole and far less susceptible to random uncertainties in its frequency position than a maximum which is a "single reading" property. Therefore

the precision with which the frequency position of a centroid may be measured is greater than the precision of measurement of a maximum. The high precision with which centroid positions can be measured is illustrated by a study of IR carbonyl stretching bands discussed below. A further disadvantage of a maximum is that it is by definition in the frequency region with the largest absolute uncertainty in intensity for spectra recorded on an instrument like the Cary 14 with spectral noise increasing as the transmitted light in the sample and reference beams becomes more unbalanced.

The accuracy with which the centroid of a band may be located depends upon how much of the band may be measured without interference from other, overlapping bands. The long wavelength $\pi \rightarrow \pi^*$ absorptions examined in this work were selected for their relative freedom from this overlap which set the limits over which the bands could be scanned ("truncation").

Provided that the systematic errors in assigning the frequency position of the centroid of a band to the position of the most probable electronic excitation are the same for the spectrum of an undeuteriated and deuteriated compound, the difference in the frequency positions of the centroids will be a good approximation for the difference between the frequencies of the most probable excitations. This condition is met if the band shape is the same for the undeuteriated and deuteriated species.

Calculated direct spectra for the protio and deuterio species were computed from the sum of the two observed direct spectra combined with the difference spectrum as explained in the experimental section. This use of the difference spectrum leads to calculated intensities of the direct spectra which are less subject to calibration errors, since the calibration of the difference spectrum is "automatic". It also leads

to a reduction in the uncertainty of the ratio of the two direct spectrum absorption intensities, since the noise level of the difference spectrum is less on an absolute scale than that of the two direct spectra which are recorded with more unbalanced transmitted beams of light.

The positions of the centroids and the intensities of both the calculated direct spectra were computed by numerical integration of the bands. Care was taken to ensure that both bands were integrated between limits which were at the same absorbance on either side of the maxima and at the same fractional absorbance (the extinction coefficient expressed as a fraction of the extinction coefficient at the maximum) for the undeuteriated and deuteriated compounds. This ensured that, as far as possible, the errors resulting from the integration of truncated bands were the same for the protio and deuterio spectra, reducing the sensitivity of the values of isotopic changes in the position of the centroid or the integrated intensity (expressed as a ratio of intensities) to these errors.

A test of the precision of measurements derived from the analysis described directly above was devised. The IR spectra of the carbonyl stretching vibrations of a series of cyclohexanones (as ~ 0.05 molar solutions in carbon tetrachloride made up by weight) were recorded using a Perkin-Elmer 257 instrument with an expanded frequency scale. "Difference" spectra were also recorded between pairs of the compounds in all possible combinations, and the spectra were analysed, with integration at 2 cm^{-1} intervals, in the manner described to determine the centroid shifts between the various bands. The isolated nature of the bands eliminated truncation problems in the integrations. In the table below, the centroid positions of the bands relative to the centroid of the band of cyclohexanone itself (arbitrarily

chosen as being at 0 cm^{-1}) are shown for the various derivatives. The underlined values are for shifts measured straightforwardly from cyclohexanone. The other values are for shifts calculated from the difference between such straightforwardly measured values. That is, the shift from cyclohexanone to compound A is taken as the shift from cyclohexanone to compound B added to the shift from compound B to compound A.

Cyclohexanones (Indicated by the substituents)	Frequency shift of the centroid of the carbonyl stretching band from the centroid of that of cyclohexanone (cm^{-1})
4-t-Butyl	<u>2.70</u>, 2.48, 2.69, 2.71
4-Methyl	<u>1.72</u>, 1.74, 1.75, 1.71
r-3,c-5-Dimethyl	<u>-0.16</u>, -0.18, -0.18, 0.06
r-3,c-4,c-5-Trimethyl	<u>0.02</u>, 0.04, -0.01, 0.05

For the difference in centroid positions between 4-t-butylcyclohexanone and 4-methylcyclohexanone, measured straightforwardly as -0.99 cm^{-1}, the calculation was repeated with the calibration of the direct spectrum of 4-t-butylcyclohexanone shifted by 1 cm^{-1}. The recalculated centroid shift was -1.02 cm^{-1}, illustrating the relative insensitivity of centroid shifts to calibration errors between the two direct spectra.

The high reproducibility of centroid shifts from independent determinations shown in the table indicates the suitability of this approach for the measurement of isotopic shifts of UV bands.

MEASURED ISOTOPE EFFECTS ON UV ABSORPTIONS

Table 4 below shows the details of the measured effects.

"M acid", in the column listing the composition of solvents is a solution prepared by the reaction of 1.33 moles of phosphorus oxychloride with one litre of water (or heavy water, as necessary). Therefore, of course, "M/20 acid" refers to the same solution diluted twentyfold with water (or heavy water). Although the composition of solvents is indicated on a volume per cent basis, it should be noted that this only provides a guide to the composition of solutions which were in fact made up by weight. A table showing the correspondence between the volume compositions and weight fractions is provided in the experimental section.

All integrations were made using readings taken at 400 cm^{-1} intervals (with suitable end corrections) except for acetylacetone spectra, for which 250 cm^{-1} intervals were used, and for p-toluidinium salt spectra, for which 100 cm^{-1} intervals were necessary to allow for the fine structure of the band. The integration limits in the table are expressed as a fraction (%) of the extinction coefficient at the frequency of maximum absorption in the calculated direct spectra. For p-toluidinium ion, the absorption band was so structured that a mean extinction coefficient in the region of maximum absorption was used as a "maximum" and the wavelength at "maximum" absorption in the protio calculated direct spectrum is simply an indication of the position of this region.

The last two columns in Table 4 show the ratio of the integrated intensity of the calculated deuterio band to that of the protio band, and the isotopic change in the electronic excitation energy (deuterio-protio) as measured by the shift in the frequency position of the centroids. Uncertainties in the isotope effects upon the

TABLE 4 MEASURED ISOTOPE EFFECTS ON UV ABSORPTIONS

	Compound	Solvent Composition (By Volume)	Integration Limits as % of extinction coefficient at maximum absorption
1)	Acetylacetone	60% water 40% glyme	1
2)	4-Aminopyridine	1% water 99% glyme	23
3)	"	1% "M acid" 99% glyme	5
4)	4-Aminopyridine-1-oxide	1% water 99% glyme	10
5)	"	1% "M acid" 99% glyme	5
6)	4-Amino-1-methylpyridinium methanesulphonate	1% water 99% glyme	10
7)	p-Toluidine	1% "M acid" 99% glyme	10
8)	4,4'-Dinitrodiphenylamine	1% water 99% glyme	5
9)	p-Phenylenediamine (Primary Band)	1% water 99% glyme	35
10)	" (Secondary Band)	"	33
11)	cis-4,6-Di-t-butylcyclohexane-1,3-dione	20% "M/20 acid" 80% glyme	15
12)	"	1% "M/200 acid" 99% glyme	33
13)	trans-4,6-Di-t-butylcyclohexane-1,3-dione	20% "M/20 acid" 80% glyme	5
14)	"	1% "M/200 acid" 99% glyme	10

TABLE 4 (continued)

	Wavelength of Maximum Absorption of undeuteriated compound (nm)	Ratio of Integrated Intensities (D/H)	Change in excitation energy (D - H) (cal/mole), ΔE
1)	275	0.995	711
2)	243	0.998	239
3)	264	1.001	215
4)	292	0.995	99, 116
5)	272	0.972	292
6)	272	0.992	167
7)	260 to 265	0.975	143
8)	402	1.006	28
9)	252	1.005	397
10)	324	0.996	240
11)	257	1.033	307
12)	252	1.195	194
13)	258	0.998	357
14)	253	1.024	150

electronic excitation energies are difficult to assess. There is a table in the experimental section showing how the centroid shift for acetylacetone varies with the degree of truncation in the choice of integration limits. However, this only provides a semi-quantitative guide to these uncertainties for other compounds. Also in the experimental section are details of the calculation of a "minimum detectable isotope effect", corresponding to a change in excitation energy of ~30 cal/mole for a band with a maximum around 263 nm wavelength.

A repeat of experiment number (4) in Table 4 (the solutions for (4) were the most difficult to prepare) led to a 16% change in the value of the centroid shift. White[77] has performed experiments numbers (2) and (3) and obtained centroid shifts corresponding to 303 and 192 cal/mole respectively.

Isotope Effects upon the Integrated Intensities of Absorption

The lack of integrated intensity changes on deuteriation for all the compounds examined which were not mixtures of equilibrating species with different absorptions is indicative of the same degree of "allowedness" (in terms of selection rules) for protio and deuterio absorptions, since the size of the oscillator strength for an absorption is a measure of how strongly allowed the absorption is. All the absorptions studied in this work are strongly allowed $\pi \rightarrow \pi^*$ transitions. Since the primary effect of isotopic substitution is a change in the amplitude of anharmonic vibrations, any isotopic change in oscillator strengths will be the result of a change in vibronic contributions to the allowance of an electronic excitation, which will be most important for forbidden transitions, but of decreasing significance as the allowance of the transition studied increases. Worden[87] has reported reductions of about 10% in the value of oscillator strengths on deuteriation for the vibronically induced

forbidden n $\rightarrow \pi^*$ transitions of acetone, acetaldehyde and propionaldehyde.

The observation of negligible changes of intensity on deuteriation for strongly absorbing species suggests that, in the event of conditions being found for which there is an isotope effect on absorption intensity for a keto-enol equilibrium mixture with a significant population of both tautomers, the change in inetnsity may be used to calculate the change in the free energy difference of the equilibrium caused by deuteriation. For such calculations, "100% enol" values of oscillator strengths are required. These may be obtained from an NMR spectroscopic[88] or bromometric[86] determination of equilibrium composition combined with UV spectroscopy or, if possible, from the examination of the spectra of isolated samples of the pure enol form.[89] Another approach, used in this work, to the oscillator strength of the enolic absorption is to change the solvent composition so that an absorption intensity for the highly enolised system may be measured.

NMR spectroscopy is only accurate enough to provide equilibrium composition values leading to rough values of oscillator strengths for the purely enolic system, and is not sufficiently sensitive for the study of isotope effects on equilibrium concentrations. Solvent composition effects on the "allowedness" of electronic transitions may lead to uncertainties in enolic absorption oscillator strengths derived from measurements at one solvent composition in order to calculate isotope effects on equilibria at another solvent composition. However, rough values of the oscillator strengths for the purely enolic absorptions are all that would be required for UV spectroscopic investigation of these changes, since the results have shown that the pure enol absorption intensity is not significantly affected by deuteriation. The calculation of isotope effects, therefore, is not critically dependent on absolute

values of the tautomeric equilibrium constant, K_T, calculated from the equation

$$K_T = \frac{[\text{enol}]}{[\text{keto}]} = \frac{I_{eq}}{I_{enol} - I_{eq}} \qquad (4)$$

where I_{eq} is the integrated intensity of the absorption observed for the equilibrium mixture and I_{enol} is the integrated intensity (between the same "fractional absorbance" limits) for the enol, derived from the UV absorption spectrum and some independent estimate of the equilibrium. The difference between $\ln K_T$ values for deuteriated and undeuteriated systems will be proportional to the isotope effect on the change in free energy associated with the tautomeric equilibrium.

No isotope effect was observed, within experimental error, upon the integrated intensity of the acetylacetone absorption. Therefore deuteriation did not affect the position of the acetylacetone keto-enol equilibrium in the solvent used, and so, under such conditions, any change in the difference in sums of zero-point vibrational energies of normal modes in the keto and enol forms between the undeuteriated and deuteriated systems is either negligibly small or compensated by an isotope effect on the difference in entropy between the two forms.

For solutions in a solvent which was by volume 20% aqueous acid and 80% glyme, the integrated intensities of the absorptions of cis- and trans-4,6-di-t-butylcyclohexane-1,3-diones were the same for the undeuteriated and deuteriated species. As for acetylacetone, this equivalence could be a coincidence. However, since the absorption intensity of these compounds increases sharply with the size of small fractions of water in water/glyme solvent mixtures and then levels off for mixtures with larger proportions of water, it appears that the compounds are fully

(or almost fully) enolised in solutions with about 20% water (by volume) and the absence of any significant isotope effect upon intensities may be ascribed to the strongly allowed character of the enolic absorption.

Assuming that the solutions of cyclohexanediones with 20% aqueous acid are "purely enolic", the per cent enol contents, and hence rough K_T values from equation (4) were calculated for the protio solutions with 1% aqueous acid using intensities integrated between the same limits of fractional absorbance for the two solvent compositions, 10% of the maximum for trans-4,6-di-t-butylcyclohexane-1,3-dione, and 33% of the maximum for cis-4,6-di-t-butylcyclohexane-1,3-dione. In a mixture of 1% "M/200 acid" and 99% glyme, the trans-compound was 75% in the enolic form and the cis-compound was 34% in the enolic form. Using the ratio of intensities of the deuteriated and undeuteriated species' absorptions shown in Tabie 4, the isotope effect on the free energy change from the keto tautomer to the enol tautomer, $\Delta\Delta G$, was calculated for each compound.

At 20°C for solutions in 1% "M/200 acid" and 99% glyme,

$\Delta\Delta G = -60 \pm 40$ cal/mole for trans-4,6-di-t-butylcyclohexane-1,3-dione,

$\Delta\Delta G = -144 \pm 13$ cal/mole for cis-4,6-di-t-butylcyclohexane-1,3-dione.

The quoted uncertainties in these isotope effects are based upon the standard deviation of the measured ratio of integrated intensities between deuterio and protio spectra for the compounds with no equilibrium between species with very different absorptions.

Both the isotope effects upon cyclohexanedione equilibria are of a sign which indicates a greater preference for the enol tautomer in the deuteriated compound than in the undeuteriated compound. As the proportion of enol rises as water is added to a glyme solution for both compounds, the enol form is stabilised relative to the

keto form by hydrogen bonding to the solvent. Therefore the $\Delta\Delta G$ values indicate stronger hydrogen bonds to the solvent (the geometry of the cyclohexanediones does not permit the formation of intramolecular hydrogen bonds) in the deuteriated enols than in the undeuteriated enols.

These effects for cyclohexanediones are in the opposite direction to the isotope effects upon the free energies of formation of hydrogen bonds measured and discussed in Chapter Two. The entropy effect favouring deuterium-substituted bonds (deuterium bonds) over ordinary hydrogen bonds (protium bonds), mentioned on page 120, is thus enhanced by, or less impeded by, any isotopic enthalpy change, $\Delta\Delta H$, from changes in zero-point energy contributions. For acetylacetone and the two cyclohexanediones, the change from the diketone tautomer to the ketoenol tautomer involves replacement of C-X (X = H or D) bonds by hydrogen-bonded O-X bonds. The isotopic change in the difference in stretching frequency between these two bonds, probably the dominant contribution to $\Delta\Delta H$, may be positive or negative since ν_{C-X} and $\nu_{O-X\,bonded}$ lie in the same region of the IR spectrum (X = H, 3000 cm^{-1} and X = D, 2200 cm^{-1}). Thus $\Delta\Delta H$ will be arithmetically smaller than δH in Chapter Two (page 118) which was dominated by the larger isotopic change in the decrease of stretching frequency from a free O-X group going to a hydrogen bonded O-X----Z (Z = O or N) group. This accounts for the preference for deuterium bonds over protium bonds in the two cyclohexanediones, a preference which will be expected to vary with the hydrogen bonding strength of the solvent, and perhaps change sign for solvents with extremely strong hydrogen bonding to the enol, and also accounts for the lack of an isotopic preference in acetylacetone, an "indifference" which is not likely to change with the solvent since the hydrogen bonding is primarily intramolecular.

The isotope effects measured on the enthalpy and entropy changes in the keto-enol equilibrium of acetylacetone by Thompson and Allred using NMR spectra[62] have been criticised as being subject to systematic errors. However, inasmuch as the results are valid, they apply to "neat" acetylacetone and a 50:50 weight fraction solution in cyclohexane, conditions under which intermolecular hydrogen bonding will be significant.

Isotope Effects upon the Excitation Energies of Electronic Transitions

Isotopic changes in the most probable excitation energies, ΔE, are shown in the last column of Table 4 . Isotopic differences in the changes of sums of energies for vibrational modes on excitation are not enough to account for many of the observed ΔE values unless there is a significant change of geometry between the ground state and electronically excited state.

The nature of the individual excitations may be used as a basis for analysis of the signs and sizes of the ΔE values. For the enols of acetylacetone and the two cyclohexanediones, the absorption of the enolate anions at longer wavelengths indicates that the enol is a stronger acid and therefore probably a weaker base (at the carbonyl group), in the excited state than in the ground state. These two conflicting effects on an intramolecular hydrogen bond (the enol of acetylacetone) may be expected to result in a weakening of the hydrogen bond on excitation by analogy with fluorine substitution on the strength of the bond in acetylacetone.[70] (The $\pi \rightarrow \pi^*$ absorption moves π electrons away from the plane of the molecule, resulting in an increased electronegativity of the enol towards σ electrons). Therefore excitation of the enol of acetylacetone involves a transition from a potential well for vibration of the hydrogen atom which approximates more closely

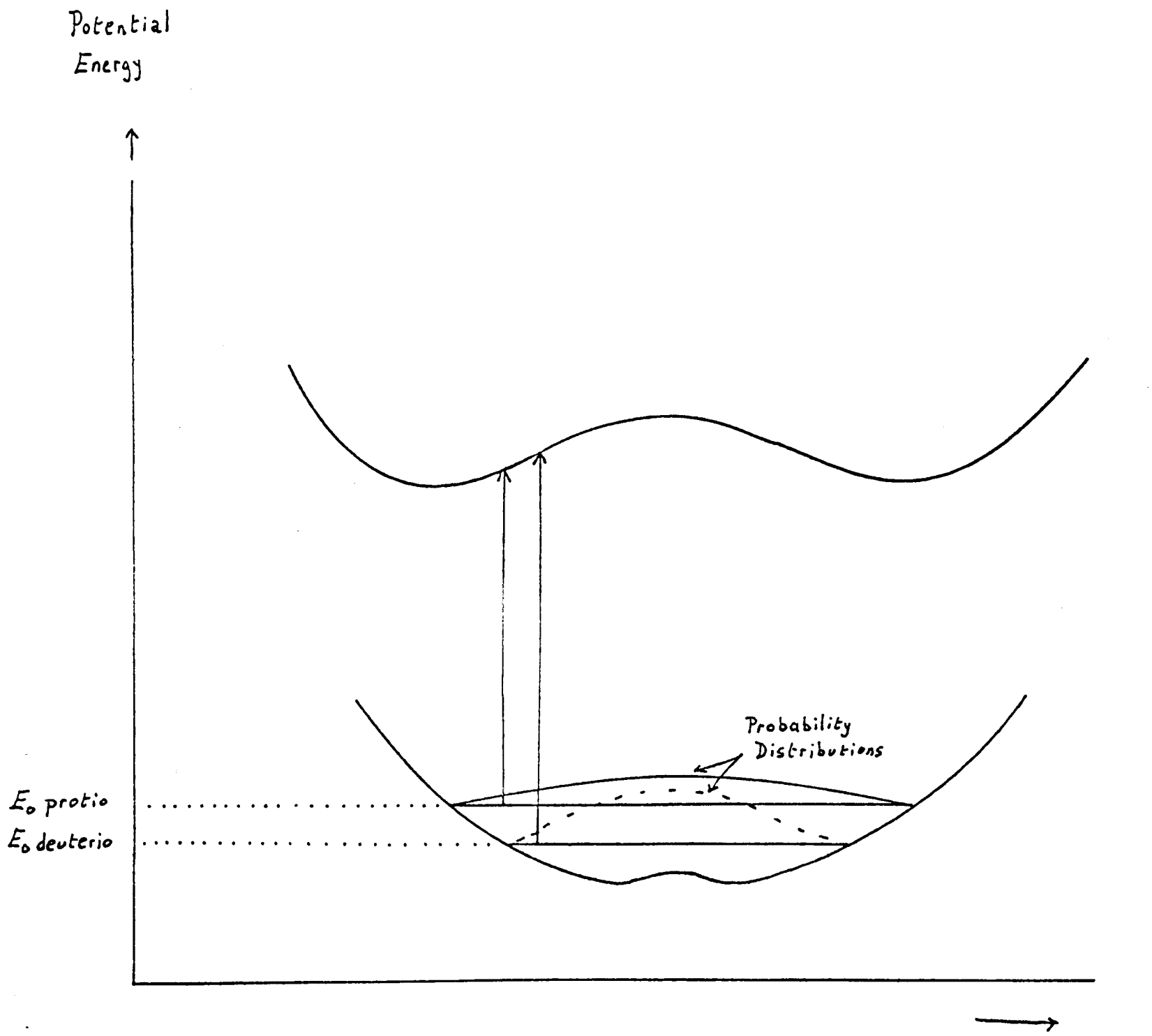


Figure 19.

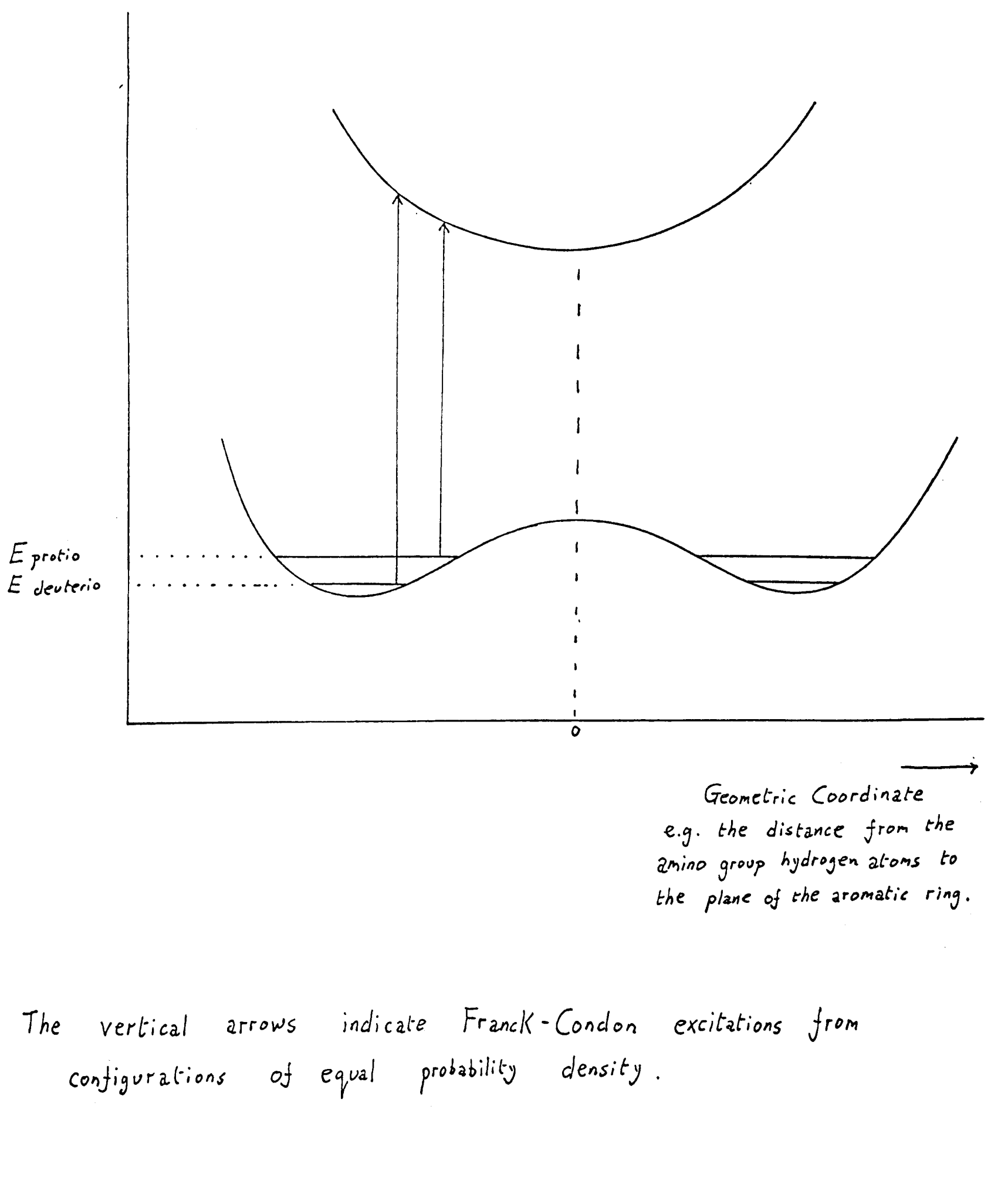
The vertical arrows indicate Franck-Condon excitations from configurations of equal probability density.

to a single minimum symmetric well in the ground state to a potential well in the excited state with more pronounced double minimum character, as illustrated in Figure 19. The probability distributions of protium and deuterium (a dashed line for the latter) are shown for the zero point vibrations in the ground state. The greater amplitude of vibration of protium leads to a more widely spread probability distribution with the result that Franck-Condon excitation to the lower vibrational levels of the excited state is more favoured for protium than for deuterium, leading to a positive value for ΔE (711 cal/mole). This is consistent with the observation that the UV absorption band of the deuterio spectrum is slightly narrower than that of the protio spectrum,[29] indicating transitions from a wider range of vibrational displacements for the protio system.

The electronic absorption of the cyclohexanediones is of the same character, but no intramolecular hydrogen bonding is possible in their fixed trans-configurations. The increased acidity of the molecules in the excited state will result in stronger hydrogen bonding to the solvent whose basicity remains independent of the excitation. As was seen in Chapter Two, a strengthening of the hydrogen bond is accompanied by a contraction of the hydrogen bond length, r (the distance between the two oxygen atoms in the hydrogen bond). If the strengths of the hydrogen bonds to the solvent of the ground state cyclohexanediones are of the approximately same size as the strengths of carboxylic acid dimer hydrogen bonds or alcohol polymer hydrogen bonds, then $r_{protio} < r_{deuterio}$ (Chapter Two) and the excitation will be energetically more favourable for the undeuteriated cyclohexanediones than for the deuteriated cyclohexanediones, leading to the positive ΔE values observed.

The increases in the ΔE values for the cyclohexanediones with a change from

Figure 20 Potential wells for an aromatic amine with inversion of the amino group in the ground state.



The vertical arrows indicate Franck-Condon excitations from configurations of equal probability density.

1 to 20% aqueous acid in the solvent may be ascribed to the more extensive hydrogen bonding of water and/or hydrated protons (or their deuteriated equivalents) to the carbonyl oxygen of the enols for the solutions with the larger proportion of aqueous acid. (The acidic properties of glyme are negligible). Any difference between the acidities of the protio and deuterio acids will remain the same for the ground and excited states and so a contribution to ΔE must be sought in the change of the basicity of the carbonyl oxygen. If, as suggested above, this site becomes less basic on excitation, the strengths of hydrogen bonds to it will decrease on excitation, bringing energies of vibrations associated with such bonds closer together in the excited state than in the ground state. Thus the contribution to ΔE will be positive.

The ΔE values for anilines, 4-aminopyridine and its derivatives as free bases may be interpreted in terms of the difference in the geometries of the ground and excited states. White[77] measured $\Delta E = 0$ for N, N-dimethylaniline and $\Delta E = 50$ cal/mole for pyridine. Thus isotope effects on hydrogen bonding to the solvent are probably too small to account for the ΔE values measured for aromatic amines in this work.

The character of the absorption of anilines, for example, p-nitroaniline,[90] has been analysed in terms of a shift of π electrons from the amino nitrogen into the ring and, where applicable, from the ring to an accepting substituent. Such absorption results in a flattening of the amino group with a change from tetrahedral or near tetrahedral[91] to trigonal bonding between nitrogen and hydrogen.

The potential well for inversion of the ground state amino group is shown in Figure 20 with the well for the same out-of-plane distortion of the flattened amino group in the excited state. The zero-point energies for ground state vibrations of

the protio and deuterio molecules are shown, the N-H bonds having the greater vibrational amplitude. The outer walls of the potential well of the ground state are steeper than the sides of the inversion hump, so that the average configuration of the undeuteriated molecules is closer to a flattened form than is the average configuration of the deuteriated molecules, providing a smaller excitation to the trigonal excited state in the protio system. Hence positive values of ΔE are observed.*

The closer to trigonal (and further from tetrahedral) is the amino group the less will be this "inversion" contribution to ΔE, which explains the decrease in the value of ΔE in the series:

	ΔE (cal/mole)
p-Phenylenediamine (Secondary Band)	240
Aniline[77]	158
p-Nitroaniline[77]	101
4,4'-Dinitrodiphenylamine	28

since the four compounds are in the order of increasing π electron withdrawal from the amino group and hence increasing trigonal character of N-H bonds. Such a

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* Although electronic transitions from double minimum to single minimum wells (aromatic amines) and from single minimum to double minimum wells (acetylacetone) have been shown to give rise to $\Delta E > 0$, positive ΔE values are not necessarily general. Transition from a symmetric single minimum potential well to a steeper single minimum well with little accompanying displacement of the minimum, for example, may lead to a negative value of ΔE.

decreasing inversion contribution may also explain the decrease of ΔE values in the series:

	ΔE (cal/mole)
4-Aminopyridine	239
4-Amino-1-methylpyridinium methanesulphonate	167
4-Aminopyridine-1-oxide	108 (mean value of two measurements)

Axenrod et al. have measured the J (^{15}N-^{1}H) coupling constants and proton chemical shifts of ^{15}N-amino groups in aniline and a series of its derivatives.[78] Solutions (1 M or saturated for less soluble compounds) in hexadeuteriodimethylsulphoxide and trideuteriochloroform were used and coupling constants were quoted with uncertainties of 0.2 Hz. Several of these measurements are shown in the table below, together with coupling constants and chemical shifts for 4-^{15}N-aminopyridine and two of its derivatives measured (for 1% w/v solutions) in this work (listed on the right side of the table).

^{15}N-Aniline[78]	J (^{15}N-^{1}H)(Hz)		τ_{NH}	4-^{15}N-Aminopyridinium
Substituents	in DMSO	in $CDCl_3$	(ppm)	"Substituent"
	93.5 ± 0.3		1.1	1-p-Nitrophenyl
2,4-Dinitro	92.6		1.68	
	91.7 ± 0.1		2.03	1-Methyl
4-Nitro	89.4	86.4	3.37	———
	87.7 ± 0.1	85.7 ± 0.1	4.15	4-^{15}N-Aminopyridine
3,5-Dimethyl-4-nitro	87.0	83.2	4.16	
2,4-Dibromo	86.0	82.7	4.58	
4-Bromo	84.0	79.6	4.85	
none	82.6	78.6	5.10	
4-Methyl	81.4	76.5	5.30	
4-Methoxy	79.4	75.6	5.52	
4-Amino		74.9		

Axenrod et al.'s results indicate an increase of J (^{15}N-^{1}H) and a decrease of τ_{NH_2} as the substituents in the anilines become less π-electron donating and more electron withdrawing, which may be correlated with a trend towards a trigonal amino group in the ground state. This trend is followed by J (^{15}N-^{1}H) and τ_{NH_2} for 4-^{15}N-aminopyridine, 4-^{15}N-amino-1-methylpyridinium methanesulphonate and 4-^{15}N-amino-1-p-nitrophenylpyridinium chloride, correlating with a larger ΔE value for 4-aminopyridine than for 4-amino-1-methylpyridinium methanesulphonate.

The increase in value of J (^{15}N-^{1}H) and the decrease in τ_{NH_2} on changing from ^{15}N-aniline to 4-^{15}N-aminopyridine suggests that the effect of aza substitution in the benzene ring of aniline is electron withdrawal from the amino group. Binsch et al.[92] have measured J (^{15}N-^{1}H) for ^{15}N-acetanilide (as a solution in acetone of unspecified concentration) as 90.9 ± 0.2 Hz. In this work J (^{15}N-^{1}H) for 4-^{15}N-acetamidopyridine (5% w/v in acetone) was measured as 91.1 ± 0.2 Hz. The negligible change in J (^{15}N-^{1}H) between these two compounds with trigonal amido N-H bonds suggests that electron withdrawal by the nitrogen in the aromatic ring of 4-aminopyridine is of π-withdrawal character rather than σ-withdrawal character. (However the ab initio calculations of Clementi[93] suggest the reverse for electron withdrawal by the nitrogen atom in pyridine). Thus the ΔE values for 4-aminopyridine and its derivatives are expected to be smaller than those for aniline and corresponding derivatives from an analysis of NMR data.

Experimentally (aniline ΔE = 158 cal/mole,[77] 4-aminopyridine ΔE = 239 cal/mole) the opposite change of ΔE values is found, and so some significant contribution to ΔE, other than the inversion contribution, must be sought which is greater for 4-amino-pyridine than for aniline. Possibly hydrogen bonding to water or heavy water at the

ring nitrogen atom is more significant for 4-aminopyridine than for pyridine owing to the greater basicity of the ring nitrogen atom in the former. If excitation involves a weakening of these hydrogen bonds, with the result that protio and deuterio vibrational energy levels are closer together in the excited state than in the ground state, such a contribution to ΔE would be greater for 4-aminopyridine than for pyridine (ΔE = 50 cal/mole[77]), and is, of course, absent for aniline.

Wolff[94] has observed that the absorption maxima in the spectrum of p-toluidine as a free base are at longer wavelengths than the equivalent maxima for p-toluidinium chloride. Therefore p-toluidinium ion is a stronger acid in the excited state than in the ground state. The correlation between an increase of acid strength and a decrease of the O-H stretching frequency has been well established for alcohols, phenols and carboxylic acids,[1] and probably, therefore, the N-X (X = H or D) bonds in the p-toluidinium ion will be weakened on excitation. Thus the vibrational energies of the protio and deuterio compounds will be closer in the excited state than the ground state, giving a positive ΔE value, as observed experimentally (143 cal/mole). Phenols would be expected to give positive ΔE values for similar reasons, although only one bond to protium or deuterium is involved. White has observed ΔE values ~ 30 cal/mole for several simple phenols.[77]

The pK_a measurements of Gardner and Katritzky on aminopyridine-1-oxides indicate that 4-aminopyridine-1-oxide protonates on the oxygen atom in acidic solutions,[80] therefore the "inversion" constribution to ΔE will be preserved on changing from 4-aminopyridine-1-oxide to the conjugate acid. The shift of the absorption maximum for this change indicates that the conjugate acid is a stronger acid in the excited state than in the ground state. Hence the O-X bond is weakened

on excitation to give a further, positive, contribution to ΔE with a consequent increase in the value on changing from 4-aminopyridine-1-oxide (mean ΔE = 108 cal/mole) to the conjugate acid (ΔE = 292 cal/mole).

From an exmination of the IR spectra of aminopyridines, aminopyridinium chlorides and pyridones, Spinner concludes that protonation on the ring nitrogen of 4-aminopyridine leads to π-electron withdrawal from the amino group, with a resulting change of the N-H bonds to more trigonal character.[95] This change on protonation combined with the weaker acidity of the conjugate acid in the excited state, and consequent strengthening of N-H bonds on excitation, would be expected to reduce the value of ΔE significantly for the change from 4-aminopyridine to its conjugate acid. The small experimentally observed reduction (4-aminopyridine ΔE = 239 cal/mole, 4-aminopyridinium chloride ΔE = 215 cal/mole) is probably an indication of inadequacies in this interpretation rather than the sum of these reducing effects on ΔE with protonation!

These examples show how ΔE values may be interpreted, albeit speculatively and with the benefit of hindsight, in terms of the decrease in vibrational energies on deuteriation.

It is apparent that isotope effects on excitation energies may have some application in the investigation of hydrogen bonding. Another possible use may be in the characterisation of UV absorption bands of, for example, natural products. Only those bands with electronic changes around groups with readily exchangeable hydrogen atoms or hydrogen bonding to the solvent will be expected to show an isotope effect. Hence the isotopic optical difference spectrum will only have sigmoid "bands" owing to those chromophores influenced by deuteriation or by hydrogen bonding.

EXPERIMENTAL

SUBSTANCES FOR UV AND VISIBLE SPECTROMETRY

Acetylacetone

Acetylacetone (B.D.H. Chemicals Laboratory Reagent) was distilled and the fraction b.p. 136-138°/610 mm was twice recrystallised from three times its own volume of petroleum ether (b.p. 30-40°) at -78°. The recrystallised material was fractionally distilled to give pure acetylacetone, b.p. 140-141°/732 mm, n_D^{20} 1.4536. Literature[96] n_D^{20} 1.4529.

4-Aminopyridine

A sample supplied by R. White was resublimed to give crystals m.p. 152.5-154.5°. Literature[97] m.p. 157-158°.

4-Aminopyridine-1-oxide

A solution of perbenzoic acid in benzene was prepared according to the method of Swern et al.[98]

4-Aminopyridine (5 g, 0.053 mole) was dissolved in 1,2-dimethoxyethane (60 ml) and a solution of perbenzoic acid (8.1 g, 0.059 mole) in benzene (46 ml) which had been cooled to 8° was added with swirling in an ice-bath. The mixture was allowed to warm to 10° and kept at that temperature for three hours after which it was left to stand at room temperature for nineteen hours. The residue from evaporation at reduced pressure was taken up with concentrated hydrochloric acid (30 ml) and the resulting suspension was filtered. The residue from evaporation of the filtrate was twice recrystallised from ethanol to give crystals of the product hydrochloride m.p. 169-173°. Yield 1.5 g, 19%. A further portion of crude hydrochloride (2.0 g) was obtained from aqueous extracts of the precipitate. Literature[99] m.p. 181-183° (decomposition). Calculated for $C_5H_7N_2OCl$,

40.97% C, 4.81% H, 19.11% N, 24.19% Cl. Found 40.7% C, 5.0% H, 18.8% N, 24.3% Cl.

4-Aminopyridine-1-oxide hydrochloride (0.74 g, 0.005 mole) dissolved in water (10 ml), was stirred with silver oxide (0.7 g, 0.003 mole) for two hours in the dark. After this time, a drop of the suspension gave no precipitate when added to a solution of silver nitrate in dilute nitric acid. The suspended material was removed centrifugally and the resulting solution was evaporated and heated at 80°/0.5 mm pressure in the dark for four hours to give the crude product (0.50 g, 93%).

On exposure to light, the colourless product crystals became bright yellow in a few minutes.* For the determination of m.p., microanalysis and the preparation of solutions for UV spectrometry, samples of the crude product were recrystallised from ethanolic ethyl acetate and dried in the dark just before use. M.p. 219-223° (decomposition). Literature[80] m.p. 235-236°. Calculated for $C_5H_6N_2O$, 54.54% C, 5.49% H, 25.44% N. Found 53.6% C, 5.5% H, 25.1% N.

4-Amino-1-methylpyridinium methanesulphonate

A solution of dimethyl sulphate (2.52 g, 0.02 mole) in ethanol (6 ml) was added dropwise to 4-aminopyridine (1.9 g, 0.02 mole) in ethanol (6 ml) with swirling at such a rate as to bring about gentle boiling of the mixed solutions. As the mixture cooled to room temperature a precipitate was deposited. This product was filtered off and recrystallised from ethanol. Yield 3.57 g, 81%. M.p. 124-127°. $C_7H_{12}N_2O_4S$

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* The low yield of 4-aminopyridine-1-oxide hydrochloride may be attributed to the exposure of the reaction mixture to light.

requires 38.17% C, 5.49% H, 12.72% N, 14.56% S. Found 38.2% C, 5.3% H, 12.8% N, 14.6% S.

p-Toluidine

A commercially supplied sample was sublimed to give crystals, m.p. 42-45°. Literature[100] m.p. 43.7°.

p-Phenylenediamine

A commercially supplied sample was recrystallised from ethanol and then was sublimed to give crystals m.p. 135-139°. Literature[101] m.p. 142°.

4,4'-Dinitrodiphenylamine

p-Bromonitrobenzene (11.2 g, 0.055 mole), p-nitroaniline (8 g, 0.058 mole), anhydrous potassium carbonate (4 g, 0.03 mole), copper I iodide (0.16 g, 0.0008 mole) and nitrobenzene (56 ml) were heated together at 205° for 26 hours. The cooled reaction mixture was steam distilled until no more nitrobenzene was collected in the distillate. Concentrated hydrochloric acid (60 ml) was added to the residue and the resulting sludge was filtered. The insoluble material was dried and then extracted with ethanol (150 ml) in a Soxhlet apparatus for 18 hours. The ethanolic solution was left to stand at 0° overnight and the precipitate which formed was filtered off and recrystallised from ethanol to give the product as dark violet crystals m.p. 206-210°. Literature[102] m.p. 216-216.5° for "amber-coloured needles with a steel-blue reflection". Yield 0.15 g, 1%. Calculated for $C_{12}H_9N_3O_4$, 55.60% C, 3.50% H, 16.21% N. Found 55.1% C, 3.7% H, 16.1% N and, on repetition 55.6% C, 3.5% H, 16.0% N.

trans-4,6-Di-t-butylcyclohexane-1,3-dione

A sample supplied by J. D. Schofield was twice recrystallised from aqueous ethanol to give crystals m.p. 134-136°. Literature[103] m.p. 137°.

cis-4,6-Di-t-butylcyclohexane-1,3-dione

A sample supplied by A. P. Foster was twice sublimed to give crystals m.p. 49-51°. Literature[103] m.p. 50-51°.

SUBSTANCES FOR THE MEASUREMENT OF ^{15}N-^{1}H NMR SPIN-SPIN COUPLINGS

^{15}N-Ammonium Chloride

^{15}N-Ammonium sulphate (95.4% enriched, 0.25 g, 0.0018 mole) was weighed into a conical Quickfit flask. A test-tube, with a piece of universal indicator paper held on to the outside of its neck with a rubber band, containing hydrochloric acid (2 M, 3 ml) was placed in the flask and then potassium hydroxide solution (3 M, 2.5 ml) was rapidly pipetted onto the floor of the flask. As soon as possible after this the flask was closed with a greased stopper. The indicator paper turned from orange to green in a few minutes. After 3 days the colour of the paper had changed back to orange. The flask was opened and the contents of the test-tube was evaporated to give the product. Yield 0.19 g, 97%.

4-Phenoxypyridine

4-Chloropyridine hydrochloride (4.6 g, 0.04 mole), phenol (7.5 g, 0.08 mole), anhydrous potassium carbonate (8.3 g, 0.06 mole) and copper powder (0.16 g, 0.0025 g atom) were heated together, with stirring, under an air condenser for six hours in an oil bath at 155°. The cooled mixture was stirred with ice cold aqueous sodium hydroxide solution (2 M, 32 ml) to give a suspension which was extracted with ether (75 ml). The extracts were washed with brine and dried over anhydrous potassium carbonate. The residue from evaporation was distilled to give the product b.p. 82.0-82.5°/0.3 mm. Yield 2.7 g, 42%. Literature[104] b.p. 141-142°/14 mm.

The product solidified to crystals m.p. 42.5-45.5°. Literature[105] m.p. 44-46°.

4-^{15}N-Aminopyridine

^{15}N-Ammonium chloride (0.175 g, 0.00321 mole), mixed with 4-phenoxy-pyridine (0.549 g, 0.00321 mole), was heated in a metal bath at 300° under a slow stream of nitrogen for 35 minutes. Aqueous sodium hydroxide solution (2 M, 5 ml) was added to the cooled reaction mixture and the resulting solution was extracted with petroleum ether (b.p. 30-40°, 5 ml) and then with n-butanol (15 ml). The n-butanol solution was washed with brine (2 ml) and dried over anhydrous potassium carbonate. The residue from evaporation was washed with ether (2 ml) and then with petroleum ether (b.p. 30-40°, 2 ml) before sublimation to give the product m.p. 149-152°. Yield 0.162 g, 53%.

NMR spectra of the product (1% w/v solutions) had $J_{^{15}N^{1}H} = 87.7 \pm 0.1$ Hz for dimethylsulphoxide solution and $J_{^{15}N^{1}H} = 85.7 \pm 0.1$ Hz for deuteriochloroform solution.

The success of the above preparation eliminated the need for an investigation of the action of ^{15}N-ammonia on the N-hydroxysuccinimide ester of isonicotinic acid, which had been prepared by the method of Sachdev and Fruton.[106]

4-^{15}N-Amino-1-methylpyridinium methanesulphonate

Following the method described above for the preparation of the unlabelled compound by direct mixing of the reactants, 4-^{15}N-aminopyridine (10.8 mg, 0.114 mmole) dissolved in dimethyl sulphoxide (0.25 ml) was mixed with dimethyl sulphate (14.3 mg, 0.114 mmole) dissolved in an equal volume of the same solvent. In this way a solution (5% w/v) of the product in dimethyl sulphoxide, ready for the recording of an NMR spectrum, was prepared. The spectrum had $J_{^{15}N^{1}H} = 91.7 \pm 0.1$ Hz.

4-^{15}N-Amino-p-nitrophenylpyridinium chloride

The following preparation of the unlabelled compound was carried out in order to establish the reaction conditions required and the identity of the product.

p-Chloronitrobenzene (0.40 g, 0.0025 mole) and 4-aminopyridine (0.20 g, 0.0021 mole) in dry xylene (5 ml) were heated, with stirring, under an air condenser for 3 days in an oil bath at 130°. The hot liquid was poured away leaving the product which had precipitated on the walls of the vessel. When these crystals had cooled they were washed with acetone and dried at 100°. Yield 0.12 g, 20%.

A sample of the product was recrystallised from ethanolic ethyl acetate to give crystals m.p. 279-283° (decomposition). $C_{11}H_{10}ClN_3O_2$ requires 52.50% C, 4.01% H, 16.70% N, 14.09% Cl. Found 52.0% C, 4.2% H, 16.6% N, 14.3% Cl, and, on repetition 52.0% C, 4.2% H, 16.3% N.

The preparation was repeated on one tenth the scale using 4-aminopyridine which was 80% unlabelled and 20% enriched material, the preparation of which is described above. The washed product was dissolved in dimethyl sulphoxide (0.5 ml) to give a solution (1% w/v) ready for the recording of an NMR spectrum, which had $J_{^{15}NH}$ 93.5 ± 0.3 Hz.

4-^{15}N-Acetamidopyridine

The following preparation of the unlabelled compound was carried out in order to establish the reaction conditions required and the identity of the product.

4-Aminopyridine (0.94 g, 0.01 mole) dissolved in 1,2-dimethoxyethane (10 ml) was added to a solution of acetic anhydride (1.02 g, 0.01 mole) in 1,2-dimethoxyethane (10 ml). The mixed solution was evaporated at reduced pressure and the residue was heated at 100° under 0.1 mm pressure for half an hour before

recrystallisation from water. The recrystallised product was heated at 100°/0.1 mm for a further two hours to remove water of crystallisation. Yield 0.99 g, 73%. M.p. 145-148°. Literature[107] m.p. 148°.

A labelled sample of 4-aminopyridine (10% 4-^{15}N-aminopyridine, prepared as described above, 0.19 g, 0.002 mole) dissolved in 1,2-dimethoxyethane (1 ml) was added to a solution of acetic anhydride (0.22 g, 0.0022 mole) in 1,2-dimethoxyethane (2 ml) and the mixture was evaporated. The residue was heated at 100°/0.15 mm for half an hour and then used for the preparation of solutions for NMR spectra. For a solution 5% w/v in acetone $J_{^{15}NH}$ = 91.05 ± 0.1 Hz and for a solution 5% w/v in dimethylsulphoxide $J_{^{15}NH}$ = 90.9 ± 0.1 Hz.

UV AND VISIBLE SPECTROMETRY

1,2-Dimethoxyethane ("glyme") was dried by refluxing over lithium aluminium hydride (1 g/100 ml glyme) and then distilled, b.p. 83-84°/746 mm, n_D^{20} 1.3797. Literature[108] b.p. 84.7-84.8°/760 mm, n_D^{20} 1.3797. The UV spectrum of the glyme compared with distilled water consisted only of absorption at wavelengths less than 260 nm (optical density 0.17 at 250 nm, 0.30 at 240 nm, 0.48 at 230 nm, 0.63 at 220 nm and 1.03 at 210 nm for a 10 mm path length). The spectrum of a sample which was left to stand over sodium wire for six weeks was the same.

The substance whose spectrum was to be examined was made up as a solution in glyme by weight to give a "master" solution. Two equal volumes of this master solution were pipetted into separate flasks and made up to give solutions of optical density near to unity as follows. In one case the required quantity of water or aqueous acid (prepared from the reaction of a measured amount of phosphorus oxychloride with water) would be added followed by glyme to make the solution up to

the necessary volume. For the second sample, heavy water or acid prepared from the reaction of phosphorus oxychloride with heavy water was substituted for water or aqueous acid. In all cases the components of a solution were weighed as each one was added to the flask to obtain accurate weight-fraction values which were converted to molarities using specific volumes of water-glyme mixtures measured by Wallace and Matthews.[109]

In addition to the "protio" and "deuterio" solutions, a "solvent" mixture was prepared from water or aqueous acid and glyme to duplicate, as accurately as possible, the weight fractions of these components in the "protio" solution.

The table below shows the corresponding weight fraction of glyme for each solution composition described in terms of volume. "M acid" refers to a solution made by the reaction of 1⅓ moles of phosphorus oxychloride with water (or heavy water) and hence "M/20 acid" is the same solution diluted twentyfold with water (or heavy water).

Volume Composition	Weight Fraction Glyme	
	Protio Solution	Deuterio Solution
1% water 99% glyme	0.988	0.987
1% "M acid" 99% glyme	0.987	0.986
1% "M/200 acid" 99% glyme	0.988	0.987
20% "M/20 acid" 80% glyme	0.776	0.757

For 4-aminopyridine-1-oxide and 4-amino-1-methylpyridinium methane-sulphonate it was necessary to use separate portions of the substances dissolved in water or aqueous acid to prepare the protio and deuterio solutions owing to very low

solubilities in dry glyme.

For the three solutions associated with any compound: "protio solution", "deuterio solution" and "solvent", spectra were recorded on a Cary 14 instrument, using 1 mm, 2 mm or, rarely, 10 mm path length stoppered cells as follows:

	In sample beam	In reference beam
No.1	Solvent in cell A	Solvent in cell B
No.2	Solvent in cell B	Solvent in cell A
No.3	Protio solution in cell B	Solvent in cell A
No.4	Deuterio solution in cell B	Solvent in cell A
No.5	Deuterio solution in cell B	Protio solution in cell A
No.6	Protio solution in cell B	Protio solution in cell A
No.7	Protio solution in cell A	Protio solution in cell B
No.8	Deuterio solution in cell A	Protio solution in cell B

With cell cleaning between each run, spectra Nos.1 and 2 were rerun until they diverged by no more than 0.01 absorbance in any part of the spectrum at longer wavelength than 220 nm before the rest of the spectra were recorded.

Analysis of UV and Visible Spectra

Absorbance values obtained by subtracting spectrum No.2 from each of Nos.3 and 4 were taken to be the "observed direct" spectra of the protio and deuterio solutions respectively. The mean absorbance of spectra Nos.6 and 7 substracted from that of Nos.5 and 8 gave the "difference" spectrum. Both the "observed direct" and the "difference" spectra were computed in terms of extinction coefficients and then calculated direct spectra were computed as follows:

Calculated protio spectrum, series $\epsilon_i^H = \dfrac{\text{Sum of observed direct spectra} - \text{difference spectrum}}{2}$

Calculated deuterio spectrum, series $\epsilon_i^D = \dfrac{\text{Sum of observed direct spectra} + \text{difference spectrum}}{2}$

The centroid of each spectrum, $\bar{\nu}$, defined as

$$\bar{\nu}^H \equiv \frac{\int \epsilon_i^H \nu_i \, dv}{\int \epsilon_i^H \, dv} \quad \text{and} \quad \bar{\nu}^D \equiv \frac{\int \epsilon_i^D \nu_i \, dv}{\int \epsilon_i^D \, dv}$$

where ϵ_i is an extinction coefficient value at frequency ν_i, was computed by numerical integration of the calculated direct spectra over suitable frequency increments using a Newton-Cotes closed formula with degree of precision 2 (The Trapezium Rule).

The maximum permissible limits for integration were set by overlap with an absorption band at higher, or less often, lower, frequency. The limits were chosen so that the two calculated direct absorption bands associated with a compound were integrated between frequencies at which the extinction coefficients were the same fraction of the extinction coefficient at maximum absorption for both bands. The effect of varying this "truncation" of the bands was examined in the analysis of the spectrum of acetylacetone in a solution three parts water and two parts glyme by volume. The following table shows the variation of the centroid difference between the deuterio and protio bands with the extent of truncation used to set the integration limits:

Limiting extinction coefficient values, expressed as a percentage of the extinction coefficient at maximum absorption	$\delta\bar{\nu} = \bar{\nu}^D - \bar{\nu}^H$ (cm^{-1})
1%	249
5%	247
10%	259
20%	271
30%	278
40%	309

A change in concentration of one of the two solutions relative to the other was shown, within experimental error, to have no effect upon the value of $\delta\bar{\nu}$ in the case of 4-aminopyridine-1-oxide solutions which offered the most scope for error in their preparation, since the compound was hygroscopic, sensitive to light and of such a low solubility in dry glyme as to require the use of separate samples for the protio and deuterio solutions.

Computation of the smallest detectable isotopic shift was made using a theoretical direct spectrum of the same frequency position and dimensions as that of 4-aminopyridine in a solution 1% (by volume) aqueous hydrochloric acid (4 M) and orthophosphoric acid (1.3 M) and 99% glyme, of maximum absorbance 1.25 combined with an assumed difference spectrum with the smallest detectable change in amplitude (from -0.005 to +0.005 absorbance units). This minimum isotopic shift was 9 cm^{-1} for a band with absorption maximum at 38,000 cm^{-1}.

IR SPECTRA

A series of cyclohexanone derivative samples was supplied by Dr. J. D. Schofield. Each compound was purified by distillation or sublimation and made up by weight as a solution in dry carbon tetrachloride. Using cells whose path length had been measured by the interference method and a Perkin-Elmer 257 IR spectrometer, the "direct" spectra of the C=O stretch bands were recorded. "Difference" spectra were recorded using solutions of two different compounds (in all possible combinations) in the sample and reference beams of the instrument following a pattern analogous to that used to obtain "deuterio-protio" UV difference spectra.

Centroid shifts of the bands from one compound to another were computed in the same way as UV isotopic shifts. The isolated nature of the C=O stretch bands eliminated the need for "truncation" of the integration limits.

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