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Review Article

Isotope effect on chemical shifts in hydrogen-bonded systems†

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Abstract: An overview of recent developments in the use of isotope effects in hydrogen-bonded systems with special emphasis on intramolecular hydrogen bonding is given. The systems investigated cover both resonance-assisted hydrogen bonded (RAHB) and non-RAHB systems. Deuterium isotope effects on ^{13}C chemical shifts are discussed extensively together with effects on ^{1}H, ^{19}F and ^{15}N chemical shifts. The compounds cover a large range from hydrogen-bonded esters over amides, thioamides, Schiff bases also covering proteins, nucleic acids and carbohydrates. Isotope effects are discussed both in static and tautomeric systems and in the liquid and solid states. Theoretical approaches to isotope effects on chemical shifts are likewise treated. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: deuterium isotope effects; chemical shifts; intramolecularly hydrogen-bonded systems; theoretical calculations; ^{13}C; ^{15}N; intrinsic isotope effects; equilibrium isotope effects

Introduction

Deuterium isotope effects on chemical shifts have been studied extensively in hydrogen-bonded systems. We must in this context distinguish between inter- and intramolecular hydrogen bonding. In the latter case between those that are resonance-assisted hydrogen bonded (RAHB) and those that are not. Furthermore, we need to distinguish between tautomeric and non-tautomeric systems. Isotope substitution will cause a change in the nuclear shielding (chemical shift) of the neighboring nuclei. For one-bond this can be explained by the change in the average bond length say upon deuteriation.[1] This shortening will cause a change in the nuclear shielding as seen in Figure 1. As the average bond length decreases upon deuteriation (at least for an asymmetric hydrogen bond potential) (see Figure 1), the average bond length decreases.

For isotope effects further away from the site of deuteriation the theory by Jameson can also be used.[1]

The nuclear shielding is expanded in a series as given in the following equation:

$$\langle\sigma\rangle = \sigma_e + \sum_i \left(\frac{\delta\sigma}{\delta r_i}\right)_e \langle\Delta r_i\rangle + \sum_{ij} \left(\frac{\delta^2\sigma}{\delta r_i \delta r_i}\right)_e \langle\Delta r_i \Delta r_j\rangle \sum_{ij} \left(\frac{\delta\sigma}{\delta\alpha_{ij}}\right)\langle\Delta\alpha_{ij}\rangle + \cdots \quad (1)$$

where r_i are the averaged equilibrium distances, Δr describes the change in the bond length due to the stretching vibration and $\Delta\alpha$ describes the bond angles distortion.

The isotope effect can be expressed as follows:

$$\langle\sigma\rangle - \langle\sigma^*\rangle = \sum_i \left(\frac{\delta\sigma}{\delta r_i}\right)_e [\langle\Delta r_i\rangle - \langle\Delta r_i\rangle^*] + \sum_{ij} \left(\frac{\delta^2\sigma}{\delta r_i \delta r_i}\right)_e [\langle\Delta r_i \Delta r_j\rangle - \langle\Delta r_i \Delta r_j\rangle^*] + \sum_{ij} \left(\frac{\delta\sigma}{\delta\alpha_{ij}}\right)[\langle\Delta\alpha_{ij}\rangle - \langle\Delta\alpha_{ij}\rangle^*] + \cdots \quad (2)$$

where * refers to the heavier isotope.

The deuterium isotope effect observed at the neighboring atom C can be approximated by the following:

$$\sigma - \sigma^* = \sum \left(\frac{\delta\sigma}{\delta r_{CH}}\right)_e [\langle\Delta r_{CH}\rangle - \langle\Delta r_{CD}\rangle] \quad (3)$$

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In both cases the theory is based on the Born–Oppenheimer approximation. In principle all types of isotope effects can be studied. However, the largest effects are of course found for H/D or even better H/T pairs and for nuclei with very large chemical shift ranges such as ^{15}N or ^{19}F to keep to those dealt with in the present review. As seen from the Jameson equation (Equation (3)) the long range isotope effects depend on the transmission of the isotope effects (the change in the nuclear shielding upon bond lengths changes) and upon the change in the bond length upon isotope substitution (in this case deuteriation). From a chemical point of view we therefore expect rather large isotope effects in conjugated systems. This is exactly what is found. Looking at deuterium isotope effects on chemical shifts it is obvious that large effects are often observed for RAHB systems. An important factor is bond order (or orbital overlap). Steric effects have also been found to increase isotope effects.[2] The isotope effects described by the Jameson equation are called intrinsic isotope effects. Intrinsic isotope effects will of course level off as the number of bonds increases. In aliphatic systems very quickly, in conjugated systems less rapidly.

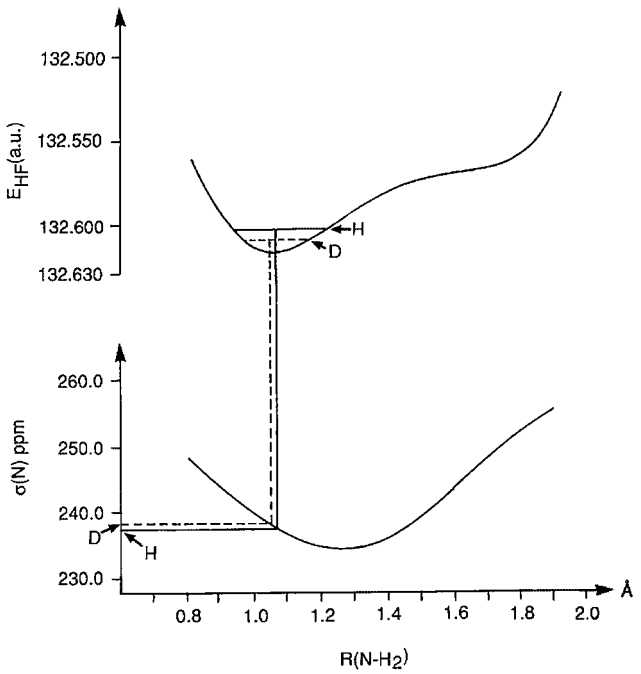
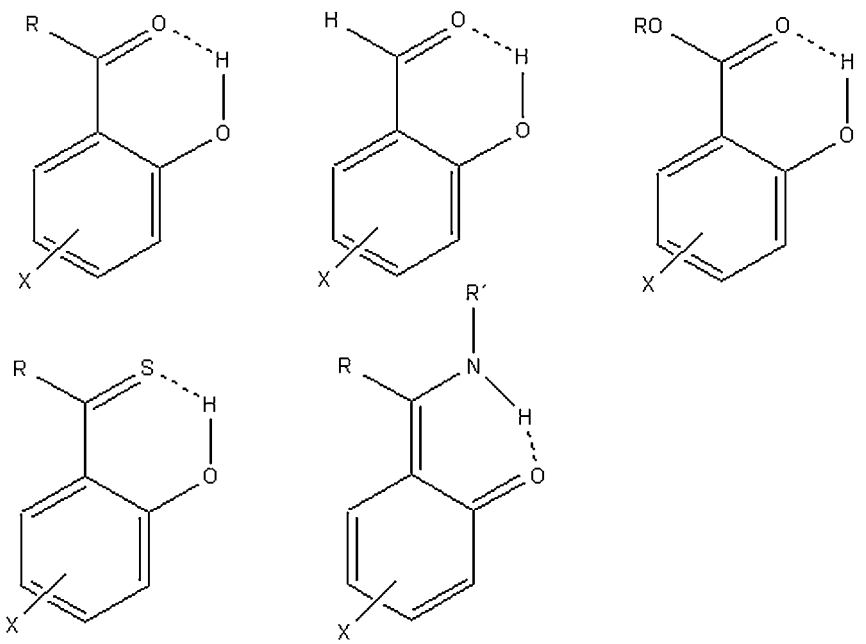


Figure 1 Hydrogen bond potential and nuclear shielding surface for a hydrogen bonded N–H system.

Isotope effects on chemical shifts are defined as $^{n}\Delta C - x(OD) = \delta C(OH) - \delta C(OD)$. As this definition is contrary to that used in some other papers, for all data using the opposite convention the sign is changed in this paper. Such data are marked with an asterisk.

Despite the expression isotope effects on chemical shifts we would also like to include primary isotope effects as they are closely related to secondary isotope effects: $^{p}\Delta H(OD) = \delta H - \delta D$. Isotope effects are in ppb if nothing else is mentioned.

The present review will not give an overview of all possible data and compounds as such reviews exist.[3–8] The intention is more to look into mechanisms and try to provide some general rules to be used especially for



Scheme 1

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intramolecularly hydrogen-bonded systems. The intermolecular cases have recently been covered by a review by Limbach.[8] A full presentation of intermolecular cases is outside the limits of the present review. The present review will give a condensed version of that interesting story based on very recent results.

Intramolecular hydrogen bonded systems

RAHB

A number of systems have now been studied in detail as seen in Scheme 1.

These include *o*-hydroxyacylaromatics, *o*-hydroxyesters and enol forms of β-ketoesters, *o*-hydroxythioketones and enamine forms of *o*-hydroxy Schiff bases and enaminones as well as *o*-hydroxynitro compounds[9], *o*-hydroxy Schiff base *N*-oxides[10] and acyltetronic acids.[11]

The *o*-hydroxyacyl aromatics show some interesting trends:

For XH systems the effects over two-bond are usually the largest. ROH $\sim$ RC = O $\sim$ RO...O $\sim {}^{2}\Delta C(OD)$.[2,9] Furthermore, ${}^{2}\Delta C(OD) \sim {}^{4}\Delta C(OD) \sim {}^{5}\Delta C{=}O(OD)$, but not with ${}^{4}\Delta C{=}O(OD)$ (Figure 2). 'Four-bond' isotope effects of the type ${}^{n}\Delta C{=}X(YD)$ (see Scheme 1) can in principle both be transmitted via the normal bonds (four bond) or via the hydrogen bond (two bonds). A number of factors will determine which pathway is the most favorable such as the X...Y distance and relation to the orbital overlap, the C = X bond order and the YH bond length, all parameters that describe the hydrogen-bonded system. An analysis of this situation has been carried out for nitro hydroxyacylaromatics.[9]

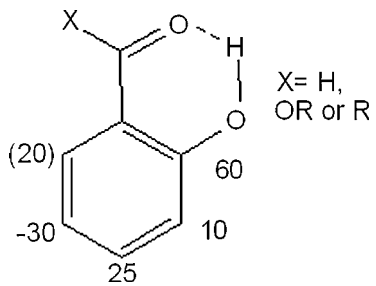

Figure 2 Proportionality factors of isotope effects.

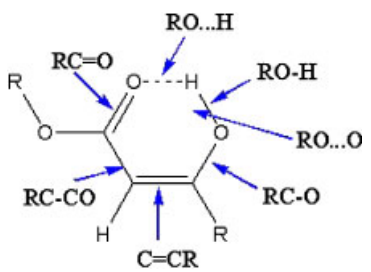

Figure 3 Distances and bond length around the hydrogen bond system.

An analysis of four-bond isotope effects of *o*-hydroxyesters and enolic forms of β-ketoesters, Meldrums acids, etc. based on distances around the six-membered hydrogen bond system (Figure 3) revealed that ${}^{4}\Delta C{=}O(OD)$ can be predicted rather well for compounds as different as Meldrums acids (Figure 4) and simple β-ketoesters.

Furthermore, by looking at the ratio ${}^{4}\Delta C{=}O(OD)/{}^{2}\Delta C(OD)$ one can tell about the transmission of isotope effects via the hydrogen bond versus transmission via the normal bonds.[12] From the results for *o*-hydroxyacylaromatics and *o*-hydroxyesters it can be seen that for ${}^{4}\Delta C{=}O(OD)$ transmission via the hydrogen bonds leads to a positive contribution to the isotope effect, whereas transmission via the normal bonds lead to a negative contribution. Furthermore, electron-withdrawing groups in conjugation with the hydrogen bond donor lead to large positive isotope effects partly because of the longer O–H bond but also because of the higher C = O bond order in such compounds (see Figure 5). ${}^{4}\Delta C{=}O(OD)$ isotope effects can be predicted using the following equation:

$$\begin{aligned} {}^{4}\Delta C{=}O(OD) &= -0.21 * RO\ldots O - 0.35 * ROH \\ &\quad + 5.40 * ROH - 1.92 * RC \\ &= O - 4.24 * RC - O + 0.71 * RC \\ &= C + 2.01 * RC - CO \end{aligned} \tag{4}$$

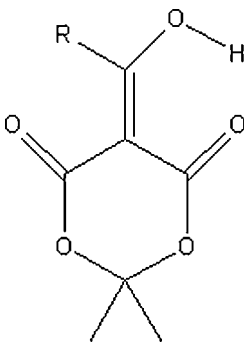

Figure 4 Meldrums acids.

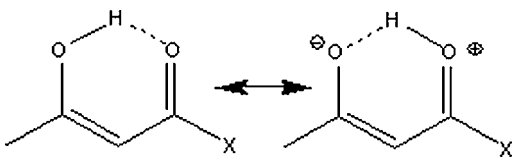

Figure 5 Resonance forms of enolic β-diketones.

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For *o*-hydroxythioketones, $^4\Delta C = S(OD)$ is found to be rather large and negative[13] and also in enolic forms of β-ketothioamides.[14] The latter may in special cases show tautomerism[15] (see section on Equilibrium). The negative four-bond isotope effect was ascribed to the longer distance between O and S as compared with the oxygen analogue. The longer distance results in less transmission via the hydrogen bond (Figure 6).

On the basis of these studies we can predict how the $^4\Delta C = O(OD)$ of *o*-hydroxyamides will behave. As amides and esters are rather similar we expect the $^4\Delta C = O(OD)$ to be positive. Electron-withdrawing groups will lead to more positive $^4\Delta C = O(OD)$. Very few compounds have been investigated[16,17] so far but the examples given in Figure 7 show that enol forms of β-ketoamides[14] show positive $^4\Delta C = O(OD)$ isotope effects.

For enolic forms of β-ketothioesters very few results exist, but they are not unexpectedly seen to behave much like the corresponding β-ketoesters.[14]

$^4\Delta C = O(ND)$ of *o*-hydroxy Schiff bases on the enamine form and enamines have also been studied. The observation of isotope effect of the NH-form of Schiff bases is relatively new.[18–22] $^4\Delta C = O(ND)$ is seen to vary from very large negative in derivatives of gossypol,[18] 2-acetyl-1,8-dihydroxy-3,6-dimethylnaphthalene[19] and 5,8-Dihydroxy-1,4-bis-phenylamino-2,3-dihydro-anthraquinone[20] (Figure 8), whereas for simple enaminones the values are usually positive.[23] The values can again be explained by the distances around the six-membered hydrogen bond ring. An early negative example was that of 1,4-diaminophenyl-9,10-anthraquinone[16] (see Figure 9). The finding that the $^4\Delta C = O(ND)$ is primarily negative can be ascribed to a finding that the NH form is primarily on the charged form (see Figure 10).

The above finding can be used to predict the magnitude and sign of amide NH involved in intramolecular hydrogen bonding. In that case one does not have to worry about the resonance form involving charges and we can therefore predict a positive $^4\Delta C = O(ND)$. A few examples are known[14] as seen in Figure 11. For thioamindes[14] involved in hydrogen bonding much the same pattern is seen (Figure 12) with respect to distances around the hydrogen bond ring.

Non-RAHB cases

Protonated DMAN's (Figure 13) is such a case and have been shown to have a very low barrier to interconversion. Isotopic perturbation of equilibrium has demonstrated that the system is a two-potential well system.[24,25] Protonated DMAN's is a well-known example showing usual effects. The two-bond isotope effects

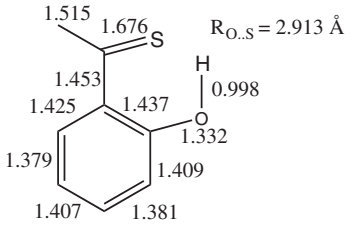

Figure 6 Distances and bond length of *o*-hydroxythioxoketones.

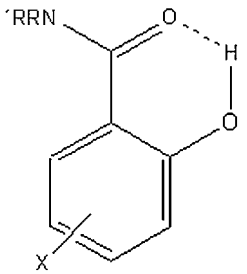

Figure 7 *o*-Hydroxybenzamides.

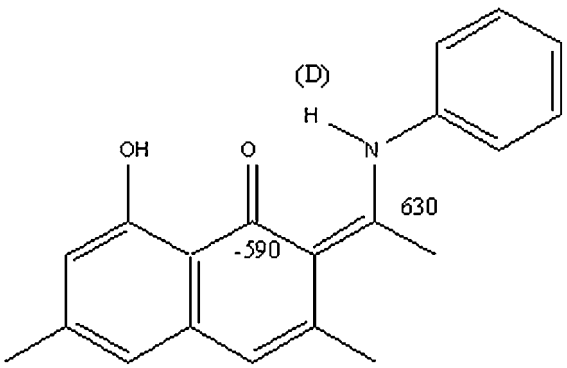

Figure 8 Two- and four-bond deuterium isotope effects on ^{13}C chemical shifts of Schiff base of 1,8-dihydroxy-3,6-dimethylacenaphthone deuteriated at OH-1.

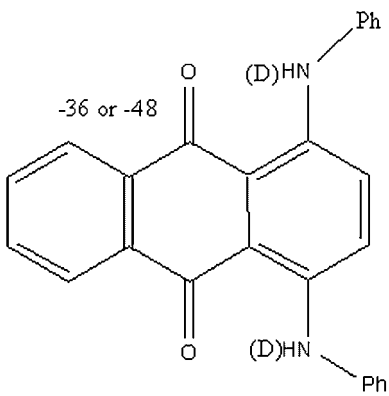

Figure 9 Four-bond deuterium isotope effects on ^{13}C chemical shifts.

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caused by deuteriation at the NH position are unusually small considering that we are dealing with a rather strong hydrogen bond.[26] However, we will see the same trend for acids (see below). This is in opposition to a two-bond isotope effect for RAHB cases.[4] For the non-symmetrical DMAN's the isotope effects are partly ascribed to equilibrium isotope effects (see later).[26]

Thioamides: A series of thioamides (Figure 14(A)) deuteriated at the NH position show very long-range deuterium isotope effect at both carbons and fluorines. In the latter case formally over nine bonds. The effects depend on hydrogen bonding as seen from Figure 14(B). The effects were described as electric field effects caused by the strong polarization of the NH bond. The electric field effects are seen both at C-2 (negative three-bond isotope effect), at C-1″, C-4″ as well as at the F-4[27] when this is present (Figure 14(C)). Effects via the hydrogen bond are probably also present and may contribute to the effects at C-1″, but they could neither explain the effect at C-4″ nor at the fluorine.

The two-bond effect, $^{2}\Delta C(ND)$, does also depend on the intrinsic isotope effect and is also dependent on the substituent at the nitrogen. Benzyl groups give smaller effects than aromatic ones.[28]

Long-range deuterium isotope effects on ^{19}F chemical shifts have also been observed in a number of *o*-hydroxyazom compounds and *o*-hydroxyhydrazo compounds.[29]

A couple of unusual thioamide-like compounds have been investigated (Figure 15). The question is how to interpret the isotope effects on ^{13}C chemical shifts when deuteriating the XH protons (NH and/OH). The effects at the CH_2 groups are quite normal (intrinsic effects). However, the isotope effects observed at the C = X carbons are more unusual. The systems are non-RAHB type. Nevertheless, an interesting feature does exist in the resonance forms as seen in Figure 16 provided that the multicharged resonance form contributes substantially to the total picture. Another factor not involving hydrogen bonding is electron-withdrawing properties. For c $^{2}\Delta C = O(OD)$ is clearly smaller than for amides in general. For b the effect of HNC = S seems to be larger than for HNC = O compared with c. This could also explain why the effect is close to zero in a.

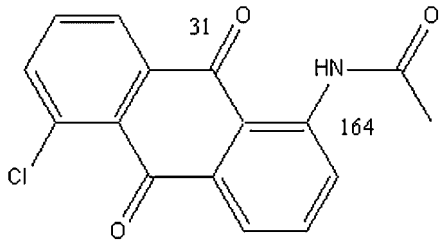

Figure 11 Deuterium isotope on ^{13}C chemical shifts of hydrogen-bonded amides deuteriated at the NH position.

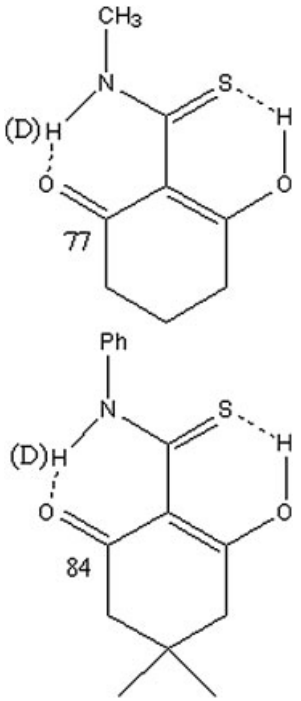

Figure 12 Deuterium isotope on ^{13}C chemical shifts of hydrogen-bonded thioamides deuteriated at the NH position.

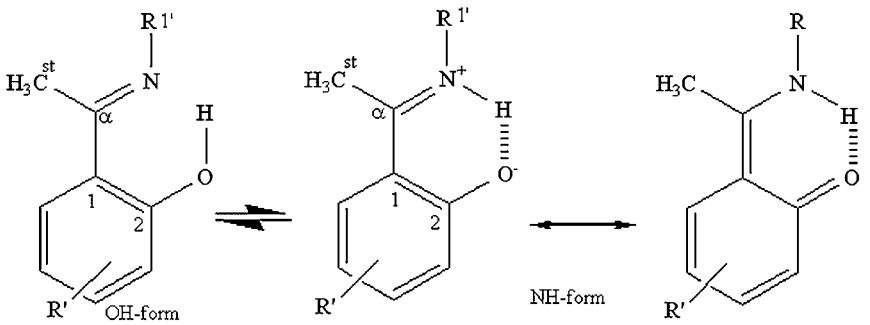

Figure 10 Equilibrium and resonance forms of *o*-hydroxy Schiff bases.

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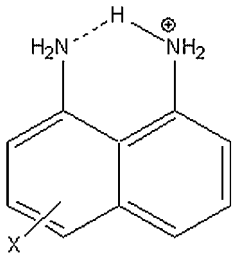

Figure 13 DMAN's.

Acids

Deuterium isotope effects on carboxylic acid carbons in carboxylic acid derivatives (OH position deuteriated) have been investigated only to a limited extent. They are found to be small in the reported cases even for strongly hydrogen-bonded ones like picolinic acid *N*-oxide (Figure 17) and quinaldinic acid *N*-oxide in line with values for other simple acids.[30] A very interesting variation is seen for picolinic acid by changing solvent from $CDCl_3$ to acetonitrile-d_3. The

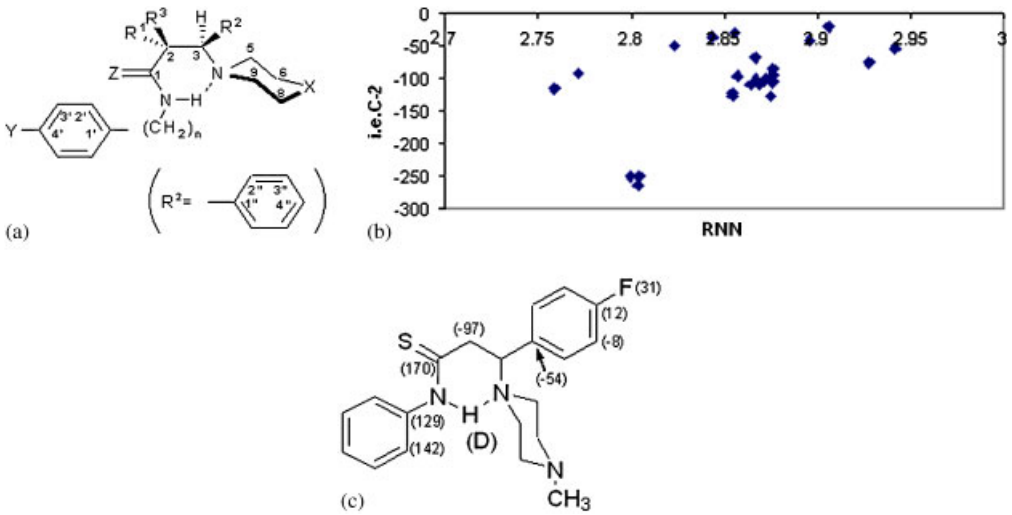

Figure 14 (A) *N*-substituted-3-(cycloamine)thiopropionamides or amides. (B) Long-range deuterium isotope effects at C-1" of N-substituted-3-(cycloamine)thiopropionamides or amides. (C) Deuterium isotope effects on ^{13}C and ^{19}F chemical shifts of *N*-substituted-3-(cycloamine)thiopropionamide.

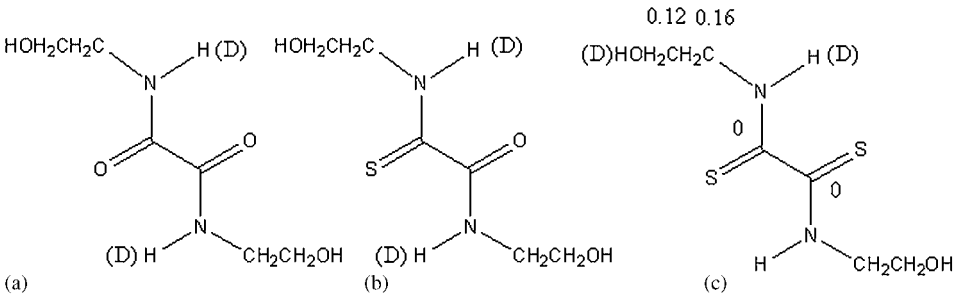

Figure 15 Deuterium isotope on ^{13}C chemical shifts of hydrogen-bonded amides and thioamides of deuteriated at the NH position.

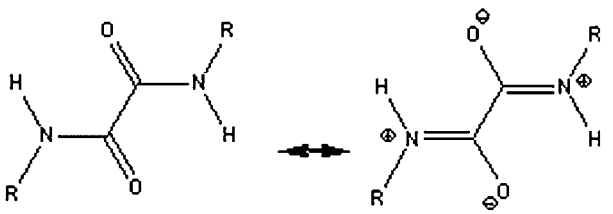

Figure 16 Resonance forms of *N*-substituted oxdiamide, oxthioamide and oxdithioamide.

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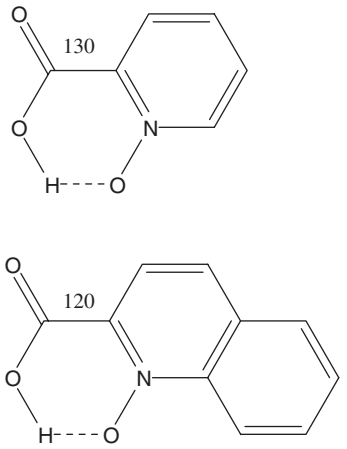

Figure 17 Deuterium isotope on ^{13}C chemical shifts of picolinic acid *N*-oxide and quinaldinic *N*-oxide.

isotope effect decreases from 130 to 20 ppb. This is followed by a change in the OH chemical shift from 18.9 to 19.1 ppm.[31] For the 6-methylpicolinic acid with a change in temperature from 300 to 260 K a change in OH chemical shifts is seen from 18.53 to 18.89 ppm.[32] For citrinin values of 261–296 ppb were reported (in this case also the C = O group is hydrogen bonded).[33] The highest value was found at low temperature. The small value of deuterium isotope on ^{13}C chemical shifts of carboxylic acid carbons can probably be related to the rather small change in the C = O nuclear shielding upon stretching of the OH bond (see Figure 18).

A small isotope effect of 180 ± 40 ppb was found in HIV-1 protease. In this case two aspartic acid side chains are involved in hydrogen bonding. Smith *et al.*[34] assumed as only one isotope effect was found that only one of the two carboxylic acids was protonated. Yamasaki *et al.*[35] reported a similar result but also found a small negative isotope effect for the other carboxylic acid group. More recently Piani *et al.*[36] have calculated the deuterium isotope effect at the carboxylic acid carbon in this case and they suggest that both carboxylic acids are protonated based on energy considerations. They explained the lack of an isotope effect by a weak hydrogen bond in a non-polar region of the protein. This suggestion is very interesting. Recently, Limbach and Co-workers[37] have investigated acetic acid and acetate complexes. They found at very low pH an isotope effect at the carboxylic acid carbon. They suggested that a zero effect of carboxylic acid can also be obtained if the carboxylic acid is involved in a low-barrier hydrogen bond.

Nucleic acids

Use of deuterium isotope effects on ^{13}C chemical shifts is very interesting in nucleic acids through which bond

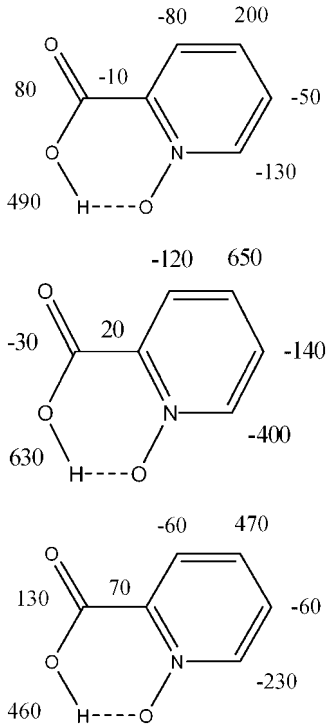

Figure 18 (A) Changes in ^{13}C chemical shifts as a function of OH bond stretch. Values refer at chance in the OH bond length of 0.01 Å. (B) Changes calculated using a 2D potential surface. (C) Experimental values.

isotope effects can be observed. Deuteriation of H-3 of uracil or of thymidine leads to isotope effects at C-2 of adenine in A:U and A:T base-pairs (Figure 19).[38–40] These isotope effects are transmitted through the hydrogen bond. Vakonakis and LiWang[38] found that the isotope effects are smaller for A:T than for A:U base pairs. This experimental observation led to a theoretical study showing that the difference was simply due to the methyl group.[41]

However, this has been questioned by DFT calculations by both LiWang and Co-workers[40] and Carloni and Co-workers.[42] The latter pointed to the crucial importance of the conformation of the base pair as well as magnetic and electrostatic interactions with the surroundings. A correlation is also found between ^{1}JNH and hydrogen bond isotope effect.[43] It has been demonstrated most recently that hydrogen bonding and pi–pi stacking are coupled in DNA using isotope effects.[44]

Deuterium isotope effects on ^{15}N chemical shifts

Deuterium isotope effects on ^{15}N chemical shifts are typically 0.6 ppm in amides and amines, but may be larger in RAHB systems.[45] It is natural to investigate this type of isotope effects in proteins and peptides in

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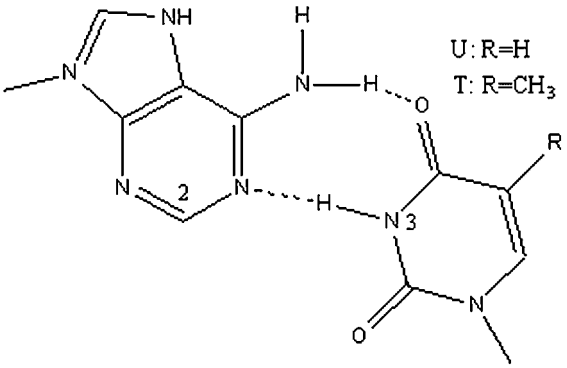

Figure 19 A:U and A:T base pairs.

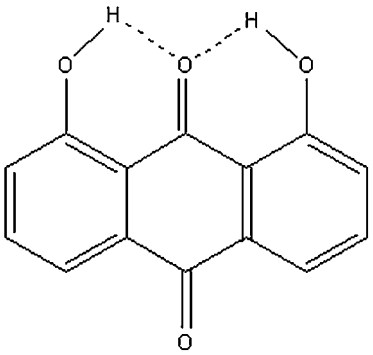

Figure 20 1,8-Dihydroxy-9,10-anthraquinone.

which intramolecular hydrogen bonding can occur.[46] Grzesiek and Co-workers[47] correlated $^{1}\Delta N(D)$ with ^{15}N chemical shifts (poor correlation). They also found a small decrease of $^{h3}JNC'$ of 0.03 ± 0.03 Hz upon deuteriation. They concluded that the effect is consistent with the Ubbelohde effect[48] and with a shortening of the H...O distance.

A different approach has been taken in which $^{1}\Delta N(D)$ are related to the H...O distance (calculated by DFT calculations) and the directional angles.[49] A good correlation is found for amino acids without charged side chains. In cases with charged side chains electric field effects may cause problems.[50] Deuterium isotope effects on ^{15}N chemical shifts have also been studied in the solid state (see later).

Deuterium isotope effects on ^{1}H chemical shifts

These are usually not large, but for hydrogen-bonded systems they can be quite useful. The effects can be of both inter- and intramolecular type. The latter are typically described in Figure 20 in which deuteriation at one OH-position will lead to a change in the isotope effect of the other OH-chemical shift. An overview is given in Reference [4]. The effect can usually be ascribed to a change in one hydrogen bond strength upon deuteriation leading to a change in the hydrogen bond strength of another. The effects can become large if one part is involved in a tautomeric equilibrium as in Figure 21(B).

Deuteriation at the position with the weak hydrogen bond (OH-8) of ADH has only a small effect at the other proton (0.0295 ppm), whereas deuteriation at OH-1 has an effect at OH-8 of 0.161 ppm.[51] For the symmetrical compound mono deuteriation leads to an effect of 0.66 ppm[19] very indicative of an equilibrium between two positions with vastly different chemical shifts.

For intermolecular hydrogen bonds this kind of reasoning has been inferred to explain the complicated isotope effects observed at low temperature.[52] A recent example is that of isotope effects in dimethylphosphoric acid. This compound is shown to exist as dimers and trimers at low temperature (140 K) in CDF_3+CDF_2Cl (1:3) (Figure 22).[53]

A coupled (cooperative) change in bond strength leads to the isotope effects. The main difference between intra- and intermolecular cases is that the intramolecular ones represent usually only medium–strong hydrogen bonds with heavy atom distances (X...X) of intermediate length, whereas for the intermolecular ones very short X...X distances may occur. For the very short X...X distances the trend is opposite to normal ones.[8]

Equilibrium cases

These can be of different kinds, rotameric ones, acid–base equilibria or tautomeric ones. In recent years much emphasis has been laid on systems with low barriers to interconversion (Figure 23). This is true for β-diketones,[54] malondialdehyde,[55,56] β-thioxoketones,[57] for monoprotonated dicarboxylic acids and for a number of enzyme systems.[58,59] For these systems it has so far not been possible to cool down enough for the single tautomers to be studied. The barrier to interconversion can be calculated in vacuum and the calculations predict very low barriers. For the mentioned systems this means that the barrier is below the zero-point energy level and the potential should be a single-well type (Figure 23). For *o*-hydroxy Schiff bases the calculated barrier is higher[7,60] but the observed isotope effect patterns are exactly the same as for the above-mentioned compounds. The low barrier has been discussed extensively by Frey and Co-workers[60] and has recently been reviewed by Perrin and Lau[56] and Lau and Perrin[61]. It has been shown from isotopic perturbation of equilibrium studies of phthalate anions, malondialdehyde and nitromalondialdehyde, etc. that these compounds are in an equilibrium between two enol forms. This is proved in malondialdehyde by introducing a deuterium at one

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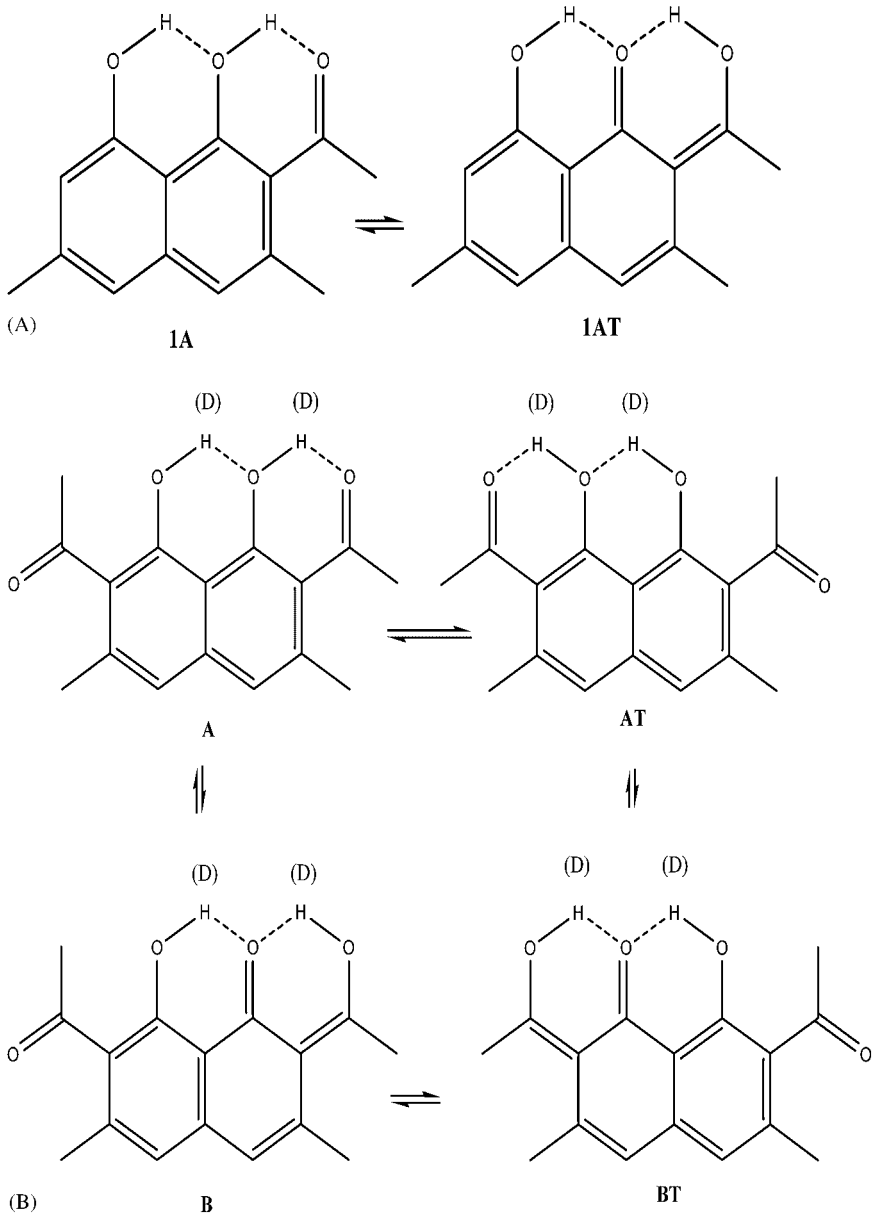

Figure 21 (A) 1,8-Dihydroxy-3,6-dimethyl-2-acetylnaphthalene (ADHDMN) and (B) 1,8-dihydroxy-3,6-dimethyl-2,7diacetylnaphthalene.

carbon[56,62] (Figure 24). According to Perrin and Lay[56] this does not imply a symmetrical double-potential well but can be a solvent-influenced asymmetric single-potential well as seen in Figure 25. However, as the form of the potential is independent on isotopes it is difficult to understand how the isotopes can perturb the equilibrium.

Non-symmetrical cases

The best studied system is that of enolic forms of β-diketones. For a review of these see Reference 4. It has been shown that the isotope effects in equilibrium systems will show a dependence on the mole fraction as seen in Figure 25.[54] In this analysis the isotope effects are divided into intrinsic and equilibrium isotope effects. Strictly speaking, it is the equilibrium part that shows dependence and an S-shape (Figure 26).

One system studied earlier was β-thioxoketones (Figure 27). They show very large isotope effects. These can in a simple-minded fashion be divided into intrinsic and equilibrium contributions. Recently, it has been realized that the intrinsic effect $^{24}\Delta C = S(OD)$ can be rather large and negative.[13] This explains why some discrepancies were found for β-thioxoketones.[63]

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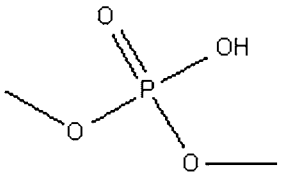

Figure 22 Dimethylphosporic acid (figure slightly different from original).

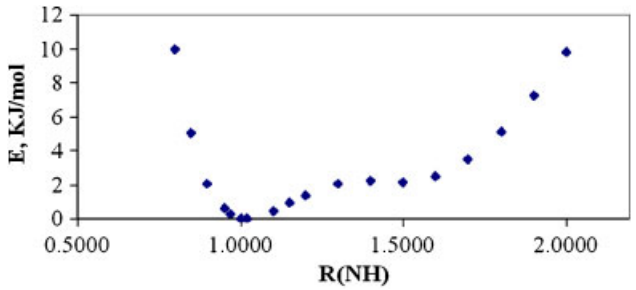

Figure 23 Low-barrier hydrogen bond.

Using the new values it is found as also seems logical that the $^{2}\Delta C=O(XD)$ is very much the same in all systems and the hydrogen bond strength is rather similar.

o-Hydroxy Schiff bases have been studied in detail using isotope effects.[21,22,60,64] It was shown earlier that $^{1}\Delta N(D)$ depended on the mole fraction.[65] The idea of dividing isotopes effects into intrinsic and equilibrium isotopes has been tested in *o*-hydroxy Schiff bases.[60] As seen from Equation (5) one is expected to find a linear relationship between isotope effects at different carbons if the intrinsic contribution is small

$$^{n}\Delta X(D)_{obs} = {}^{n}X(D)_{int} + {}^{n}\Delta X(D)_{eq} \quad (5)$$

$$^{n}X(D)_{int} - (1-\chi_D)^{n}X(D)_{OH} + \chi_D\,{}^{n}X(D)_{NH} \quad (6)$$

$$^{n}X(D)_{eq} = (\delta X_{NH} - \delta X_{OH})(\chi - \chi_D) \quad (7)$$

This is clearly the case for carbons far from the center of deuteriation.[60] A linear dependence can therefore be expected and actually found as shown in Figure 28. For Schiff bases the deuterium isotope effects on carbon chemical shifts can also be calculated to a very good extent following the Jameson Scheme.[1] Deuterium isotope effects on carbon chemical shifts have also been analyzed using principal component analysis again supporting the importance of equilibrium contributions.[66,67]

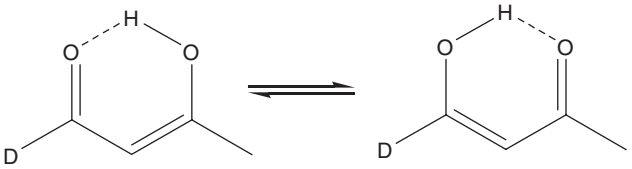

Figure 24 Example of isotopic perturbation of equilibrium.

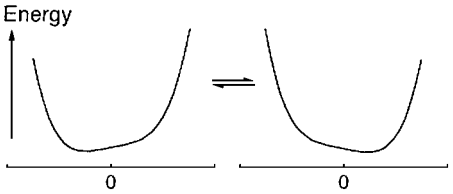

Figure 25 Equilibrium between asymmetric single-potential wells.

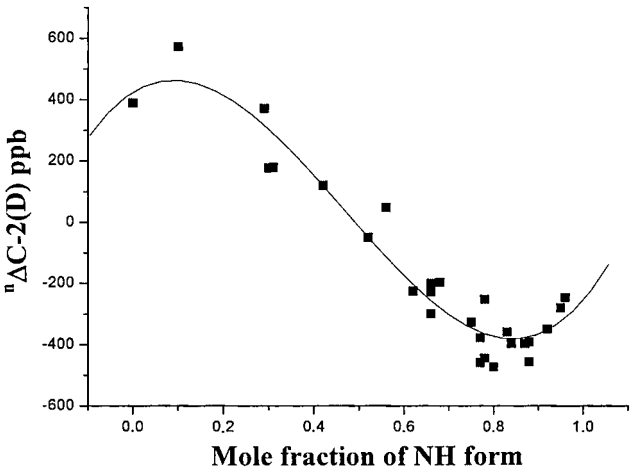

Figure 26 Plot of $^{1}\Delta N(D)$ versus NH mole fraction.

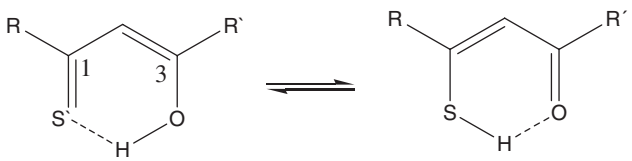

Figure 27 Substituent effects of β-diketones.

Rotational cases

Symmetrical systems such as 2,6-dihydroxyacetophenones and esters of 2,6-dihydroxybenzoic acids have been investigated. In all cases it was found that the deuterium preferred the non-hydrogen-bonded position (Figure 29). Similar studies have been done involving non-RAHB systems like cage diols. The diols are particularly interesting as they may be considered as models for carbohydrates. For the diols it was found

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that the deuterium preferred the intramolecular hydrogen bond (Figure 30). O' Leary and Co-workers[68] have used the cage diol of Figure 30 together with derivatives to determine the equilibrium constant for mono deuteriation in DMSO-d_6 to 1.018. However, the isotope effect observed depends on the nature of the solvent: −12.0 ppb in DMSO-d_6, −1.0 ppm in acetone-d_6, −12.3 ppb in THF-d_8 and −46.0 ppb in CD_2Cl_2. For the latter solvent the equilibrium constant is determined using both isotope effects and coupling constant is found to be very close to 1.045.[69] Solvents such as pyridine-d_5 and benzene-d_6 are found to amplify the isotope effects.[70] The studies of the diols have helped to decide the question whether the isotope effects observed in carbohydrates are due to equilibrium isotope effects or not (for a recent review see Reference (5)). Diols show that for suitable carbohydrates equilibrium isotope effects were found (see later).

Very recently attempts were made to calculate the preferred position of deuteriation in the above-mentioned compounds. A conspicuous result of these calculations is the importance of entropy. For hydrogen-bonded systems entropy is normally considered to be rather unimportant when comparing H and D compounds. However, for the present compounds the energy differences are very small. Furthermore, it is absolutely crucial to do the calculations in the anharmonic mode.[69]

Conformational effects have also been studied in CH_2D groups.[71]

An account of carbohydrates is given in Reference 5. Use of both deuterium isotope effects on ^{13}C and ^{1}H chemical shifts is complementary. It has been suggested that deuterium isotope effects caused by OD groups on ^{13}C chemical shifts can tell whether the OH group is a donor or an acceptor. The solvent is shown to play an important role both in determining the hydrogen bond pattern and in explaining the variations in the isotope effects. A better understanding of solvent effects is crucial for the use of isotope effects in structural studies of carbohydrates.

Solid state NMR

Deuterium isotope effects on chemical shifts in the solid state have not been reported very often. The reason is undoubtedly the large line width in solid state spectra that prohibits the observation of normal intrinsic isotope effects. Some of the isotope effects presented therefore are found in tautomeric systems. Isotope effects measured in the solid state can be very useful as they may lead to values for one of the tautomers of systems that are tautomeric in the liquid state but occurs as a single species in the solid state (for an example see β-thioxoketones later). A couple of things can give rise to mistakes. It is in the solid state like in the liquid state essential to measure the H- and D-compounds in the same sample. Even doing this, deuteriation may lead to a different crystal form than

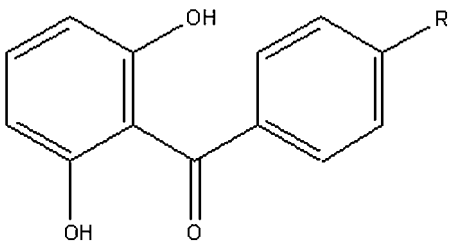

Figure 29 2,6-Dihydroxyacylaromatics.

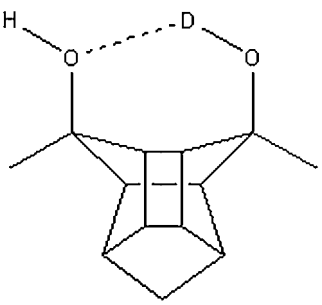

Figure 30 Symmetrical cage diol.

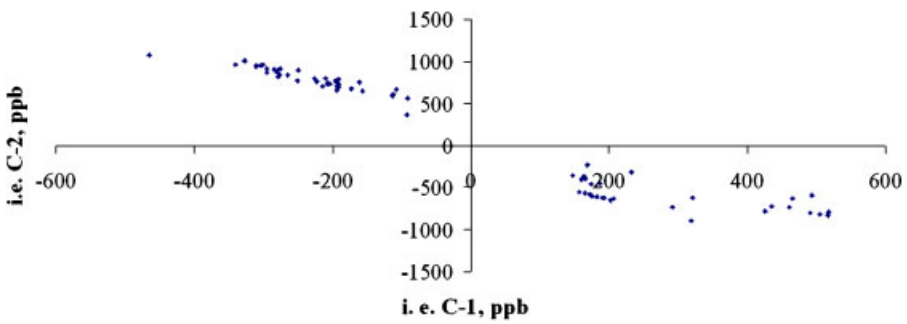

Figure 28 Linear correlation between isotope effects for a series of *o*-hydroxy Schiff bases.

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for the H-compound. How this will influence the chemical shifts is unknown.

In the solid state hydrogen bonding is the case for OH- and NH-containing compounds. For charged compounds one also gets a chance to study the effect of counter ions. A simple example is ammonium ions.[72]

Potentially tautomeric systems have been studied. $^{1}\Delta N(D)$ isotope effects of 1.65* and −2.41* ppm were reported for di-Schiff bases between *trans*-1,2-diaminocyclohexane and 2-hydroxynaphthaldehyde.[73] Using the correlation between mole fraction and $^{1}\Delta N(D)$[65] one compound is primarily at the OH-form and the other primarily at the NH-form. For Schiff bases of 2-hydroxynaphthaldehyde even larger $^{1}\Delta N(D)$ was observed.[74] In both cases tautomerism in the solid state could be established.

Deuterium isotope effects on ^{13}C chemical shifts are studied in ADHDMN (Figure 31). The $^{2}\Delta C\text{-}1(OD)$ of 1.5 ppm together with the finding of similar ^{13}C chemical shifts in the liquid and solid state suggested that ADHDMN show tautomerism in the solid state.[19] For diphenylthioacetylacetone, which is found to be fully on the enol form in the solid state, a $^{4}\Delta C = S(OD)$ isotope effect of −1.2 ppm was found in the solid state. This value helped us to explain the deuterium isotope effects seen in β-thixoketones in the liquid state.[5,63] Deuterium isotope effects on ^{13}C chemical shifts can also be observed in β-diketones in the solid state.[75]

Primary isotope effects

Primary isotope effects are important for at least two reasons, the observation of tritium resonance results in lower zero-point energies than can be obtained with deuterium (Figures 32–34) and the resonances are usually much sharper and the primary isotope effects on either tritium or deuterium are related to ^{1}H chemical shifts which are different from those of ^{13}C or ^{15}N chemical shifts.

Tritium as seen in Figure 1 experiences a more symmetrical potential than ^{1}H or D. A large study of non-tautomeric compounds did not reveal any conspicuous differences between $^{n}\Delta D(H)$ and $^{n}\Delta T(H)$.[76] For symmetrical tautomeric systems the picture is much the same despite the very low barriers for some of the systems like β-diketones. Primary isotope effects are to a large extent proportional to those of $^{2}\Delta C(OD)+^{4}\Delta C(OD)$.[76] Primary isotope effects have also been correlated with OH chemical shifts.[77–79] Looking in more detail it was also found that hydroxyl *N*-oxides and β-diketones had plots with different slopes.[79] More extensive plots are shown in Figure 32 based on primary data from References 72–74 and from Reference 4.[80]

For non-symmetrical tautomeric systems rather large positive as well as negative isotope effects can be found due to the contribution of equilibrium isotope effects. Very clear cut examples are those of β-thioxoketones and *o*-hydroxy Schiff bases. Schilf *et al.*[81] have recently measured primary deuterium isotope effects in *o*-hydroxy Schiff bases. Their values have been used in a larger context to demonstrate that both the primary and secondary isotope effects show a dependence of the mole fraction and have a maximum around $X = 0.8$.[82]

Frey and Co-workers[60] reported a $^{p}\Delta H(D)$ 1.1 ± 0.5 ppm for the XH proton of the transition state a transition state analogue complex of chymotrypsin. This combined with other evidence is taken as a sign of a low-barrier hydrogen bond. Primary isotope effects

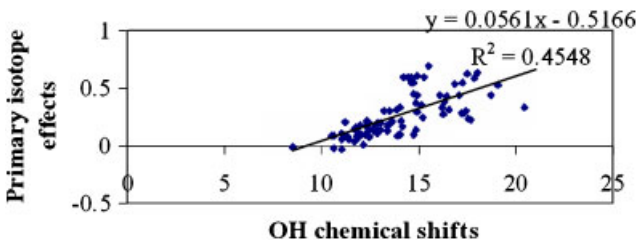

Figure 32 Plot of primary deuterium isotope effects versus δOH. Static cases.

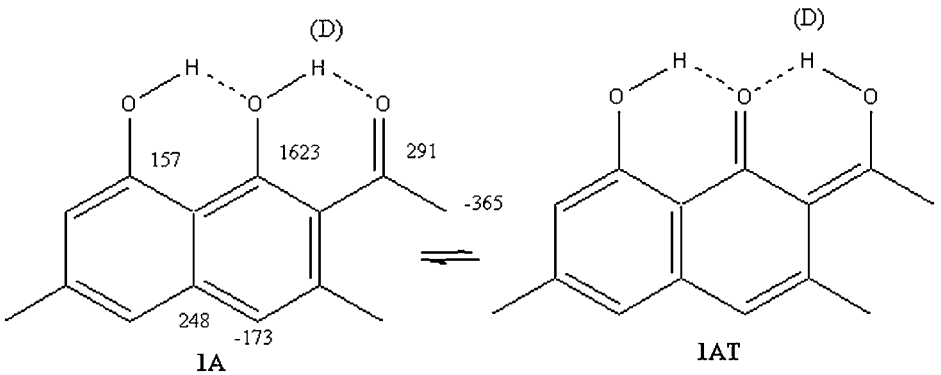

Figure 31 Deuterium isotope effects at ADHDMN.

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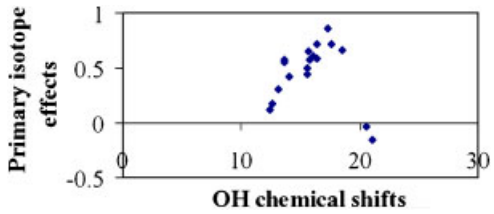

Figure 33 Symmetrical tautomeric equilibria.

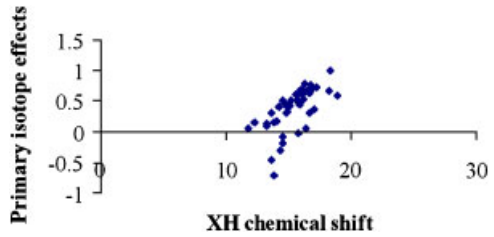

Figure 34 Plot of primary deutrium isotope effects vs. δOH Non-symmetrical tautomeric equilibria.

have also been plotted versus O...O distances and a very clear distinction is obtained between static and tautomeric systems.[83]

Theoretical calculations

Isotope effects on nuclear shieldings can be calculated based on the Jameson approach. Two factors are needed in the simple approach, the change in the nuclear shielding upon stretching of the isotope substituted bond and the change in that bond length upon isotope substitution. The change in the nuclear shielding can easily be calculated using the DFT-GIAO approach. An important requirement is that the shielding surface is linear around the equilibrium geometry (see Figure 35). The change in the bond length can be estimated from a scan of the potential surface as demonstrated for *o*-hydroxyacetophenones. This approach has since been used for *o*-hydroxy Schiff bases[59] and in DNA and RNA base pairs[70] as well as for proteins.[36]

For tautomeric systems calculation of nuclear shieldings is especially important because the chemical shifts for the two tautomers are normally not known and these are necessary to estimate the equilibrium contribution (see Equation (7)). The nuclear shieldings can be calculated for all carbons. A rather good agreement with experimental values is obtained for Schiff bases.[59]

A more advanced approach has been taken by Mavri and Co-workers[31] predicting isotope effects of picolinic acid *N*-oxide. In this approach the change was calculated on two-dimensional chemical shift surfaces. However, the fit was in no way better than using a one-dimensional approach (see Figure 18).

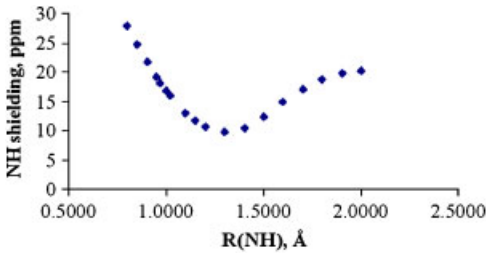

Figure 35 Calculated nuclear shielding surface (equilibrium distance 1.03 Å).

A different approach has been taken by Limbach and Co-workers. They used the formulas developed for intermolecular hydrogen-bonded systems. They also divided the isotope effects into geometric and equilibrium isotope effects but in a different fashion from the simple way done in Equations (2)–(4).

They found a very large variation with the mole fraction and ascribed this to a change in the intrinsic isotope effect. They found the largest variations for the mole fraction to be close to 0.5. From DFT calculations it is seen that neither the OH nor the NH bond lengths become very long. Furthermore, the ^{15}N chemical shifts are rather similar for all Schiff bases derived from 2-hydroxyacetophenone. According to the Jameson theory the geometric isotope effects should therefore not change dramatically.

REFERENCES

1. Jameson C. In *Isotopes in the Physical and Biomedical Sciences*, vol. 2, Buncel E, Jones JR (eds). Elsevier Science: Amsterdam, 1991, 1.
2. Abildgaard J, Bolvig S, Hansen PE. *J Am Chem Soc* 1998; **120**: 9063–9069.
3. Hansen PE. *Isotope Effects on Chemical Shifts as Tools in Structural Studies*. Roskilde University Press: Frederiksberg, 1996.
4. Hansen PE, Bolvig S. *Curr Org Chem* 2000; **4**: 19–54.
5. Hansen PE, Rozwadoski Z, Dziembowska T. *Prog Nucl Magn Reson* 2004; **45**: 1–29.
6. Hansen PE. In *Isotopes in Chemistry and Biology*, Kohen A, Limbach H-H (eds). CRC Press: Boca Raton, FL, 2006.
7. Filarowski A. *J Phys Org Chem* 2005; **18**: 686–698.
8. Limbach H-H. In *Isotopes in Chemistry and Biology*, Kohen A, Limbach H-H (eds). CRC Press: Boca Raton, FL, 2006.
9. West-Nielsen M, Dominiak P, Wozniak K, Hansen PE. *J Mol Struct* 2006; **789**: 81–91.
10. Dziembowska T, Rozwadowski Z, Majewski E, Ambroziak K. *Mag Reson Chem* 2001; **39**: 484–488.

11. Hofmann JP, Hansen PE, Bond AD, Duus F. *J Mol Struct* 2006; **790**: 80–88.
12. Hansen PE, Kamounah FS, Ullah S. *J Mol Struct*, submitted.
13. Nguyen TT, Le TN, Hansen BVK, Duus F, Hansen PE. *Magn Reson Chem* 2007; **45**: 245–252.
14. Hansen PE, Duus F, Bolvig S, Jagodzinski TS. *J Mol Struct* 1996; **378**: 45–59.
15. Wesowowska A, Jagodzinski TS, Sósnicki JG, Hansen PE. *Pol J Chem* 2001; **75**: 387–400.
16. Hansen PE, Kolonicny A, Lycka A, *Mag Reson Chem* 1992; **30**: 786–795.
17. Hansen P, Duus F, Bolvig S, Jagodziński TS. *J Mol Struct* 1996; **378**: 45–59.
18. That QT, Nguyen KPP, Hansen PE. *Magn Reson Chem* 205; **43**: 302–308.
19. Hansen PE, Kamounah FS, Hansen BVK, Spanget-Larsen J. *Magn Reson Chem* 2007; **45**: 106–117.
20. Hansen PE, Kamounah FS. Submitted.
21. Rozwadowski Z. *J Mol Struct* 2005; **753**: 127–131.
22. Rozwadowski Z. *Magn Reson Chem* 2006; **44**: 881–886.
23. Zheglova DKh, Genov DG, Bolvig S, Hansen PE. *Acta Chem Scand* 1997; **51**: 1016–1023.
24. Perrin CL, Ohta BK. *J Am Chem Soc* 2001; **123**: 6520–6526.
25. Perrin CL, Ohta BK. *J Mol Struct* 2003; **644**: 1–12.
26. Grech E, Klimkiewicz J, Nowicka-Scheibe J, Pietrczak M, Schilf W, Pozharksi AF, Ozeryanskii VA, Bolvig S, Abildgaard J, Hansen PE. *J Mol Struct* 2002; **615**: 121–140.
27. Sosnicki JC, Hansen PE. *Tetrahedron Lett* 2005; **46**: 839–842.
28. Sosnicki JC, Langaard M, Hansen PE. *J Org Chem* 2007; **72**: 4108–4116.
29. Hansen PE, Bovlig S, Buvari-Barcza A, Lycka A. *Acta Chem Scand* 1997; **51**: 881–888.
30. Ladner HK, Led JJ, Grant DM. *J Magn Reson* 1975; **20**: 530–534.
31. Stare J, Jerzierska A, Ambrozic G, Kosir IJ, Kidric J, Koll A, Mavri J, Hadzi J. *J Am Chem Soc* 2004; **126**: 4437–4443.
32. Hansen PE. Private communication.
33. Hansen PE, Langaard M, Bolvig S. *Pol J Chem* 1998; **72**: 269–276.
34. Smith R, Brereton IM, Chai RY, Kent SBH. *Nature Struct Biol* 1996; **3**: 946–950.
35. Yamazaki T, Kiso Y, Wang Y-X, Freedberg DI, Torchia DA, Wingfield P, Stahl SJ, Kaufman JD. *Peptide Chemistry*, Kitada C (ed.), Protein Research Foundation: Osaka, 1997; 337–340.
36. Piani S, Sebatiani D, Carloni P, Parinello M. *J Am Chem Soc* 2001; **123**: 8730–8737.
37. Tolstoy PM, Schah-Mohammedi P, Smirnov SN, Golubev NS, Denisov GS, Limbach H-H. *J Am Chem Soc* 2004; **126**: 6521–6534.
38. Vakonakis I, LiWang AC. *J Am Chem Soc* 2004; **126**: 5688–5689.
39. Vakonakis I, LiWang AC. *J Biomol NMR* 2004; **29**: 65–72.
40. Kim Y-E, Manolo MN, Peréz L, LiWang A. *J Biomol NMR* 2006; **34**: 229–236.
41. Swart M, Guerra CF, Bicelhaupt FJ. *J Am Chem Soc* 2004; **126**: 16718–16719.
42. Vidossich P, Piani S, Miani A, Carloni P. *J Am Chem Soc* 2006; **128**: 7215–7221.
43. Manolo MN, Kong X, LiWang A. *J Am Chem Soc* 2005; **127**: 17974–17995.
44. LiWang A. Personal communication.
45. Hansen PE, Kawecki R, Krowczynski A, Kozerski L. *Acta Chem Scand* 1990; **44**: 826–832.
46. Hansen PE. *Magn Reson Chem* 2000; **38**: 1–10.
47. Jaravine VA, Cordier F, Grzesiek S. *J Biomol NMR* 2004; **29**: 309–318.
48. Ubbelohde AR, Gallagher KJ. *Acta Cryst* 1955; **8**: 71–83.
49. Abildgaard J, Manolo, LiWang A, Bax A, Hansen PE. *J Am Chem Soc*, submitted.
50. Munch M, Hansen AE, Bowman T, Hansen PE. *Acta Chem Scand* 1992; **46**: 1065–1071.
51. Hansen PE, Bolvig S. *Magn Reson Chem* 1997; **35**: 520–528.
52. Benedict H, Limbach H-H, Wehlan M, Fehlhammer W-P, Golubev NS, Janoschek R. *J Am Chem Soc* 1998; **120**: 2939.
53. Detering C, Tolstoy PM, Golubev NS, Denisov GS, Limbach H-H. *Dokl Phys Chem* 2001; **379**: 191–193.
54. Bolvig S, Hansen PE. *Magn Reson Chem* 1996; **34**: 467–478.
55. Perrin CL, Nielson JB. *Annu Rev Phys Chem* 1997; **48**: 511–544.
56. Perrin CL, Lau JS. *J Am Chem Soc* 2006; **128**: 11820–11824.
57. Hansen PE, Duus F, Schmitt P. *Org Magn Reson* 1982; **18**: 58–61.
58. Cassidy C, Lin J, Frey PA. *Biochem Biophys Res Commun* 2000; **273**: 789–792.
59. Perrin CL, Nielson JB. *J Am Chem Soc* 1997; **119**: 12734–12741.
60. Filarowski A, Koll A, Rospenk M, Krol-Starzomska I, Hansen PE. *J Phys Chem A* 2005; **109**: 4464–4473.

61. Lau JS, Perrin CL. In *Isotope Effects in Chemistry and Biology*. Kohen A, H-H Limbach (eds). CRC Press: Boca Raton, FL, 2006.
62. Perrin CL, Kim YJ. *J Am Chem Soc* 1998; **120**: 12641–12645.
63. Andresen B, Duus F, Bolvig S, Hansen PE. *J Mol Struct* 2000; **552**: 45.
64. Dziembowska T, Rozwadowski Z, Filarowski A, Hansen PE. *Magn Reson Chem* 2001; **39**: S67.
65. Hansen PE, Sitkowski J, Stefaniak L, Rozwadowski Z, Dziembowska T. *Ber Buns Phys Chem* 1998; **102**: 410–413.
66. Dominiak PM, Filarowski A, Hansen PE, Woźniak K. *Chem Eur J* 2005; **11**: 4758–4766.
67. Hansen PE, Dziembowska T, Rozwadowski Z. *Curr Org Chem*, submitted.
68. Vasquez Jr T, Bergset JM, Firman MB, Nelson A, Roth J, Khan SI, O'Leary DJ. *J Am Chem Soc* 2002; **124**: 2931–2938.
69. Hansen PE, Hansen BKV, O'Leary DJ. To appear.
70. Craig BN, Janssen MU, Wickersham BM, Rabb DM, Chang P, O'Leary DJ. *J Org Chem* 1996; **61**: 9610–9613.
71. Allen BD, O'Leary DJ. *J Am Chem Soc* 2003; **125**: 9018–9019.
72. Hansen PE. Private communication.
73. Dziembowska T, Ambroziak K, Rozwadowski Z, Shilf W, Kamienski B. *Magn Reson Chem* 2003; **41**: 135.
74. Rozwadowski Z, Schilf W, Kamienski B. *Magn Reson Chem* 2005; **43**: 573–577.
75. Hansen PE, Lindon J, Borisov EV, Liu M-L. Private communication.
76. Bolvig S, Hansen PE, Morimoto H, Wemmer D, Williams P. *Magn Reson Chem* 2000; **38**: 525–535.
77. Hibbert F, Elmsley J. *Adv Phys Org Chem* 1990; **26**: 255–379.
78. Mazzini S, Merlini L, Mondelli R, Nasini G, Ragg E, Scaglioni L. *JCS Perkin Trans* 1997; **2**: 2013–2021.
79. Dziembowska T, Rozwadowski Z, Hansen PE. *J Mol Struct* 1997; **436–437**: 189–199.
80. Hansen PE, Borisov EV. Unpublished results.
81. Schilf W, Bloxsidge JP, Jones JR, Lu S-Y. *Magn Reson Chem* 2004; **43**: 556–560.
82. Hansen PE, Filarowski A. *Magn Reson Chem*, submitted.
83. Bolvig S, Hansen PE, Wemmer D, Williams P. *J Mol Struct* 1999; **509**: 171–181.
84. Sharif S, Denisov GS, Toney MD, Limbach H-H. *J Am Chem Soc* 2006; **128**: 3375–3387.

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