^{13}C, ^{2}H Coupling Constants and ^{2}H-Induced ^{13}C NMR Isotope Chemical Shifts in Deuteriated Cyclohexanes: Application for Measurements of Conformational Equilibrium Isotope Effects

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^{13}C, ^{2}H coupling constants and ^{2}H-induced ^{13}C isotope shifts have been measured in [^{2}H$_1$]-cyclohexane **1** and [1,1-^{2}H$_2$]cyclohexane **2**. We have observed differences in ^{13}C, ^{2}H coupling constants in **1** and **2** and also between the ^{13}C isotope shifts in **1** and **2** for each deuterium substitution. The observed differences in coupling constants for C^1, C^2 and C^3 are sums of intrinsic and thermodynamic effects and the intrinsic contribution predominates for C^1 while the thermodynamic contribution predominates for C^3. The differences in the ^{13}C isotope shifts are almost completely due to the non-additivity effects. Thus only the vicinal isotope effects for ^{13}C, ^{2}H or ^{13}C, H coupling constants can be expected to be used for the estimates of shifts of conformational equilibria in deuteriated cycloalkanes.

In recent years there have been many experimental and theoretical studies of the effects due to ^{2}H/^{1}H substitution on conformational equilibria in flexible molecules. Deuteriated cyclohexanes have proved to be the most interesting in this respect as they have been studied both experimentally and theoretically. While studying the low-temperature ^{2}H NMR spectra of [^{2}H$_1$]cyclohexane, Aydin and Günther[1] showed as long ago as 1981 that at 193 K the equilibrium (**1ax** $\rightleftharpoons$ **1eq**) is shifted towards the form with equatorial deuterium (**1eq**). The equilibrium constant K = [**1eq**]/[**1ax**] was found to be 1.060 ± 0.014. These data were criticised by Anet and Kopelevich[2] who estimated the equatorial preference for a deuterium using *trans*- and *cis*-1,4-diprotioperdeuteriocyclohexanes and the Saunders isotopic perturbation technique.[3] Later Anet and O'Leary[4] reported data from measurements of the low-temperature ^{2}H NMR spectra of [^{2}H$_1$]cyclohexane. They attempted to account for several substantial systematic errors and showed that K is equal to 1.024 ± 0.002 at 178 K corresponding to a free energy of equilibrium of −34.3 ± 3.8 J mol^{-1}.

The equatorial preference for heavier hydrogen isotopes in the cyclohexane ring has been also confirmed by the integration of the signals for the individual conformers in the low-temperature ^{3}H NMR spectra of [^{3}H$_1$]cyclohexane.[5] In addition Williams[6] has estimated the equatorial–axial equilibrium in monodeuteriocycloxane by calculating the free energies for two conformers using a scaled quantum-mechanical 3-21G force field approach and several known stretching frequencies in the IR spectrum of cyclohexane. He has also confirmed the equatorial preference of deuterium.

Although the conformational preference of cyclohexane was established by integration of the low-temperature NMR spectra, it is evident that for many conformationally flexible organic systems this technique is unlikely to be widely adopted as it needs temperatures as low as 120–130 K or even lower. That is why the Saunders isotopic perturbation method remains the only practical way to estimate isotope effects on the equilibrium constant. For the two-site exchange (**1ax** $\rightleftharpoons$ **1eq**) the equilibrium constant K can be found by using eqn. (1) where

$$K = (\theta_{obs} - \theta_{ax})/(\theta_{eq} - \theta_{obs}) \qquad (1)$$

θ_{obs} is the averaged (or observable) value of the parameter θ and θ_{ax} and θ_{eq} are the values for the limiting forms **1ax** and **1eq**.

^{2}H/^{1}H induced isotope shifts for ^{13}C nuclei and ^{13}C, H coupling constants for two limiting forms have been obtained in the low-temperature ^{13}C NMR spectrum of [^{2}H$_{11}$]cyclohexane.[7] ^{2}H/^{1}H substitution in **1** (*vs.* cyclohexane C_6H_{12}) is considered here to be antisymmetrical to ^{1}H/^{2}H substitution in [^{2}H$_{11}$]cyclohexane (*vs.* C_6D_{12}). The averaged isotope shifts for ^{13}C nuclei and ^{13}C, H coupling constants have been reported also.[7] It is evident from the general point of view (see, *e.g.*, ref. 8) that the accuracy in applying eqn. (1) depends critically on 'the sensitivity factor', S_f, of the parameter used (where S_f is proportional to the difference of the values for the axial and equatorial forms, $|\theta_{eq} - \theta_{ax}|$). Thus the spectral parameters for C^1 and C^3 nuclei seem to be more promising as they reveal the largest differences $|\theta_{eq} - \theta_{ax}|$. However, application of the Saunders isotopic perturbation technique to the values given in ref. 7 produce unreasonable values of K ranging from 0.9 to 1.2. The main cause of the large scatter of these estimates is the temperature dependence of spectral parameters. Hence the parameters established at low temperatures should be extrapolated to ambient temperatures. In order to avoid difficulties connected with the temperature-dependent isotope shifts, Aydin and Günther[1] compared the isotope shifts in **1** with those in [1,1-^{2}H$_2$]cyclohexane **2** where the equilibrium is automatically equalised. However to be valid the comparison assumes strict additivity of isotope shifts upon deuterium substitution. As Anet and O'Leary noted[4] it is valid only for measurements with moderate accuracy as noticeable deviations are observed in very accurate studies (see, *e.g.*, ref. 9). Indeed, if the additivity of the isotope shifts of C^1 is assumed the raw experimental data for **1** and **2** lead to even more erroneous results (K = 1.15) than those obtained from the low-temperature data on isotope shifts of C^1 in **1**, *viz.* K = 1.10 if the correction for temperature dependence is included and K = 1.037 if it is not.

It is worth noting that Aydin and Günther[1] limited themselves only to isotope shifts of C^1 presumably due to the insufficient resolution in the low-temperature ^{13}C NMR spectra of a mixture of **1** and **2**. In this paper we shall demonstrate the possibility of applying the Saunders technique to vicinal ^{13}C, ^{2}H coupling constants.

Thus our intention was to measure the isotope effect for ^{13}C, ^{2}H coupling constants, $\Delta^nJ(^{13}C, ^2H)$, defined as shown in eqn. (2), where $^nJ(^{13}C, ^2H)$ are the ^{13}C, ^{2}H coupling constants

$$\Delta^nJ(^{13}C, ^2H) = {}^nJ(^{13}C, ^2H)(\mathbf{2}) - {}^nJ(^{13}C, ^2H)(\mathbf{1}) \qquad (2)$$

through n bonds. When there is no substantial secondary

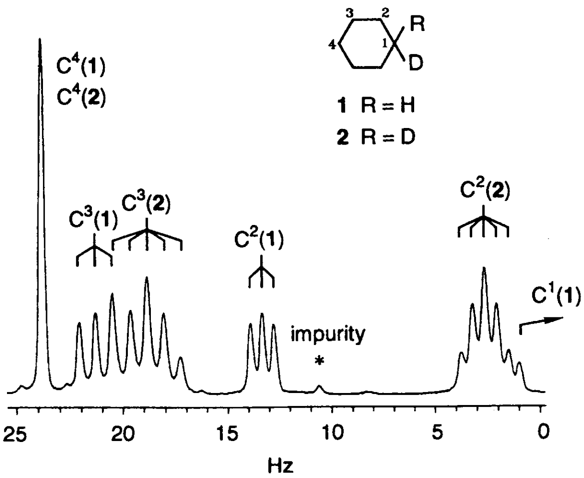

Fig. 1 A portion of the ^{13}C NMR spectrum of the mixture of **1** and **2** (sample A) with multiplets for C^2, C^3 and C^4 carbons. The high-field portion of the C^2 (**1**) signal also reveals the left component of the 1 : 1 : 1 triplet of C^1 (**1**).

isotope effect[10] on the coupling constants one can determine the isotope effect on conformational equilibria in **1**. It should be noted here that small but noticeable isotope effects for the one-bond coupling constant $^1J(^{13}C, H)$ were found recently for methane by Bennett and Raynes[11] and for toluene by Leshcheva *et al.*[12] Thus a more detailed analysis of the separation of thermodynamic and intrinsic isotope effects is needed.

Furthermore we expected to obtain more accurate data for ^{13}C isotope shifts in the mixture of **1** and **2** than those reported previously, see refs. 1, 13 and 14.

Experimental

[1,1-$^2H_2$]Cyclohexane was synthesised by UV-irradiation of the mixture of 1,1-dichlorocyclohexane (4.2 mmol) with tributyltin deuteride (10 mmol) and AIBN (AIBN = azoisobutyronitrile; 1.2 mmol), degassed and sealed in a glass tube. The volatile product was isolated by vacuum transport, washed with $HClO_4$, and distilled to give 0.31 cm^3 of the product. [$^2H_1$]Cyclohexane was obtained by interaction of the Grignard reagent from bromocyclohexane with deuterium oxide.[1]

The NMR measurements were performed on a Varian VXR-400 spectrometer at 303 K using two samples A and B. Sample A contained a solution of 5% of **1** (v/v), 9% of **2** and 10% of [$^2H_{12}$]tetramethylsilane in CS_2. Sample B contained a solution of 5% of cyclohexane C_6H_{12}, 5% of **1**, 5% of **2** and 10% of [$^2H_{12}$]tetramethylsilane in CS_2. The samples were degassed and sealed off in 5 mm o.d. NMR tubes. The ^{13}C NMR spectra were recorded at a resonance frequency of 100 MHz with proton decoupling using WALTZ modulation. In ^{13}C NMR measurements the acquisition time was about 20 s and the number of scans varied from 200 to 2500. The exponential weighting function with the line broadening factor of 0.01 Hz to improve somewhat the signal : noise ratio and Fourier transformation with double precision were used. Zero-filling was used to minimise the digital Fourier transformation round-off errors, so that the digital resolution was about 0.01 Hz per point. The phased spectra were sent *via* a local network to an IBM PC for further processing. Part of the ^{13}C NMR spectrum of the sample A including the signals of C^2, C^3 and C^4 nuclei is reproduced in Fig 1. The approximate values of ^{13}C, ^2H coupling constants can be obtained from the experimental spectra directly. However, the spectra obviously contain additional broadening due to the scalar contributions *via* deuterium relaxation. Thus, in order to obtain the accurate values of the coupling constants we applied the lineshape fitting program QUADR. This software was already successfully used by us while analysing the ^{13}C, ^2H splittings in [$^2H_1$]benzaldehyde,[16] in toluenes deuteriated in the methyl group[12] and in [$^2H_3$]nitromethane.[15] A full description of the QUADR program may be found in refs. 12 and 16.

The deuteron spin–lattice relaxation times T_1 were measured for both **1** and **2** using the inversion recovery technique. $^2H\{^1H\}$ NMR spectra were accumulated with spectral width of 200 Hz, acquisition time of 10 s, while the line broadening factor of 0.2 Hz was applied. The interleave accumulation mode with 64 scans was used normally. The data for 16 delays were fitted with the three-parameter model and found to be 2.05(6) and 2.05(2) s for **1** and **2**, respectively. The measured T_1 for **1** is quite close to the data of Aydin and Günther.[13] During QUADR iterations the values of the $J(^{13}C, ^2H)$ coupling constants, the line widths and the isotope shifts $\Delta^{13}C(^2H/^1H)$ were fitted while the deuterium relaxation time T_1 was set constant.

Results and Discussion

^{13}C, ^2H *Coupling Constants*.—The results of the analysis of ^{13}C, ^2H splittings are presented in Table 1. High accuracy in the data for ^{13}C, ^2H coupling constants was achieved: 0.4–0.5 mHz for geminal and vicinal coupling constants and 2–6 mHz for one-bond coupling constants. These errors correspond to purely random statistical noise in the difference spectra and do not include possible systematic errors due to magnetic field inhomogeneity, solvent and temperature effects, and the data processing settings. However we may consider most of the systematic errors as the same for both isotopomeric forms **1** and **2**, thus neglecting any systematic corrections for the differences given by eqn. (2).

The observable isotope effect ΔJ (Table 1, column 4) can be presented as shown in eqn. (3), which can also be rewritten as

$$\Delta J = (1/2)[{}^nJ_{eq}(\mathbf{2}) + {}^nJ_{ax}(\mathbf{2})] - \{[K/(1 + K)]{}^nJ_{eq}(\mathbf{1}) + [1/(1 + K)]{}^nJ_{ax}(\mathbf{1})\} \quad (3)$$

shown in eqn. (4),

$$\Delta J = \Delta J_{therm} + \Delta J_{intr} \quad (4)$$

where

$$\Delta J_{therm} = (1/2)[(1 - K)/(1 + K)][{}^nJ_{eq}(\mathbf{1}) - {}^nJ_{ax}(\mathbf{1})] \quad (5)$$

and

$$\Delta J_{intr} = (1/2)\{[{}^nJ_{eq}(\mathbf{2}) - {}^nJ_{eq}(\mathbf{1})] + [{}^nJ_{ax}(\mathbf{2}) - {}^nJ_{ax}(\mathbf{1})]\} \quad (6)$$

ΔJ_{therm} and ΔJ_{intr} are the thermodynamic and intrinsic parts of the observed isotope effect ΔJ, respectively.

To use eqns. (4)–(6) we need additional information concerning the equilibrium constant K, the differences $\Delta J_D = {}^nJ_{eq}(\mathbf{1}) - {}^nJ_{ax}(\mathbf{1})$ and the values of the secondary isotope effects $\Delta J_{eq} = {}^nJ_{eq}(\mathbf{2}) - {}^nJ_{eq}(\mathbf{1})$ and $\Delta J_{ax} = {}^nJ_{ax}(\mathbf{2}) - {}^nJ_{ax}(\mathbf{1})$. In particular, we can estimate ΔJ_{intr} if only two other parameters (*i.e.* the equilibrium constant K, and the differences ΔJ_D) are known. Indeed, we can estimate the equilibrium constant K at 303 K from the accurate value of K at 178 K obtained by Anet and O'Leary[4] with the assumption that the entropy contribution to the conformational free energy is negligible. This assumption is indirectly confirmed by an estimate of the K value in **1** obtained by Anet and Kopelevich[2] for *trans*-1,4-diprotioperdeuteriocyclohexane [$\Delta G°(298\ K) = 26\ J\ mol^{-1}$] which leads to the $\Delta S°$

Table 1 ^{13}C, ^{2}H Coupling constants in **1** and **2**, observed isotope effect $\Delta^{n}J$, the differences $\Delta J_D = J_{eq}(\mathbf{1}) - J_{ax}(\mathbf{1})$ and the thermodynamic and intrinsic parts ΔJ_{therm} and ΔJ_{intr} (all values in Hz)

C	$J(^{13}C, ^{2}H)$					
	1	**2**	ΔJ[a]	ΔJ_D[b]	ΔJ_{therm}[c]	ΔJ_{intr}[d]
C^1	19.197(2)	19.161(6)	−0.036(6)	0.61	−0.0021(4)	−0.034(6)
C^2	−0.5804(5)	−0.5759(5)	0.0045(7)	0.04	−0.0001(1)	0.0046(7)
C^3	0.7874(5)	0.7831(4)	−0.0043(7)	0.92	−0.0032(6)	−0.0011(9)

[a] $\Delta J = J(\mathbf{2}) - J(\mathbf{1})$. [b] $\Delta J_D = (\gamma_D/\gamma_H)[J(^{13}C, H)_{eq} - J(^{13}C, H)_{ax}]$, data on ^{13}C, H coupling constants were taken from ref. 7. [c] ΔJ_{therm} is determined from eqn. (5) with $K = 1.014$ calculated from data of Anet and O'Leary[4] with the assumption $\Delta S^{\circ} = 0$, see also text. [d] $\Delta J_{intr} = \Delta J - \Delta J_{therm}$.

Table 2 ^{13}C Isotope shifts in **1** and **2** for C^1–C^3 carbons, the observed 'total' non-additivity Δ_{total}, the differences in isotope shifts for equatorial and axial deuterium in cyclohexane, $\delta_{eq} - \delta_{ax}$, thermodynamic (*C*) and intrinsic (*I*) parts of the non-additivity (all values in ppb)

C	Isotope shifts[a]					
	$\Delta^{13}C(\mathbf{1})$	$\Delta^{13}C(\mathbf{2})$	Δ_{total}[b]	$(\delta_{eq} - \delta_{ax})$[c]	C[d]	I[e]
C^1	−419.2(3)	−841.3(3)	−1.5	47.3	−0.16	−1.3
C^2	−105.4(3)	−212.4(3)	−0.8	−12.8	0.04	−0.8
C^3	−25.7(3)	−50.5(3)	0.5	−27.4	0.10	0.4

[a] Present paper, relative to C_6H_{12}. [b] $\Delta_{total} = (1/2)\,\delta(\mathbf{2}) - \delta(\mathbf{1})$ (with the accuracy of ±0.4 ppb). [c] From the data in the low-temperature ^{13}C NMR spectrum of $[^{2}H_{11}]$cyclohexane.[7] [d] *C* values were calculated by eqn. (9). [e] $I = \Delta_{total} - C$ (with the accuracy ±0.4 ppb).

value of the order of -0.06 J mol^{-1} K^{-1}. Then as a good approximation for $(^{n}J_{eq} - {}^{n}J_{ax})$ values, we may use the data for the ^{13}C, H coupling constants in the low-temperature ^{13}C NMR spectrum of $[^{2}H_{11}]$cyclohexane.[7] The calculated values of ΔJ_{therm} are given in Table 1 for one-bond, geminal and vicinal couplings. They all are negative and those for ^{1}J and ^{3}J are the largest in magnitude as expected.

The intrinsic parts (Table 1, column 7) taken as the differences $(\Delta J - \Delta J_{therm})$ may be considered as the averaged values for two equally populated states with equatorial and axial ^{13}C, ^{2}H coupling constants.

It is clearly seen from Table 1 that for observable isotope effects, ΔJ, ranging from 4–5 mHz to 36 mHz, the intrinsic contributions may even predominate. On the other hand, in the case of the vicinal ^{13}C, ^{2}H coupling constant the observed isotope effect is almost completely thermodynamic and therefore it can be used to estimate the conformational isotope equilibrium effect.

It is worth noting that by multiplying the intrinsic isotope effect for ^{13}C, ^{2}H coupling constants by the factor (γ_H/γ_D) (= 6.514 40) we obtain the isotope effect for the corresponding ^{13}C, H coupling constant. Unfortunately data on secondary isotope effects for ^{13}C, H couplings are scarce (see *e.g.* data in ref. 10) and only the data for one-bond ^{13}C, H coupling constants seem to be unambiguous. The present data give the value of -0.22 Hz (±0.04 Hz) for the secondary isotope effect on the ^{13}C, H coupling constant which is similar to the values reported for methane (-0.30 Hz[11,17]) and for toluene deuteriated at the methyl group (-0.30 Hz[12]). The values of secondary isotope effects for ^{13}C, H coupling constants from -0.25 to -0.30 Hz were found recently in the halomethane series.[18]

^{2}H *Induced* ^{13}C *Isotope Shifts*.—Now we may return to the problem of estimating conformational equilibrium isotope effects by using ^{13}C isotope shifts. The isotope shifts in **1** and **2** measured relative to the signal of C_6H_{12} in sample B are given in Table 2. Comparing isotope shifts in **1** and **2** one can introduce the total non-additivity parameter defined by eqn. (7),

$$\Delta_{total} = (1/2)\Delta^{13}C(\mathbf{2}) - \Delta^{13}C(\mathbf{1}) \qquad (7)$$

which can be presented as the sum of two parts

$$\Delta_{total} = C + I \qquad (8)$$

C corresponds to the thermodynamic contribution and can be written in the form of eqn. (9), where δ_{eq} and δ_{ax} are the ^{13}C

$$C = (1/2)[(1 - K)/(1 + K)](\delta_{eq} - \delta_{ax}) \qquad (9)$$

isotope shifts for the corresponding conformational states, *I* is the purely non-additive contribution.

In order to estimate the thermodynamic part, *C*, we can again use the best *K* values obtained by Anet and O'Leary.[4] For the difference $(\delta_{eq} - \delta_{ax})$ we can take data from the low-temperature ^{13}C NMR spectra of $[^{2}H_{11}]$cyclohexane[7] (Table 2, column 5) thus ignoring possible temperature dependence of the difference of isotope shifts. The final values of thermodynamic and non-additive contributions are given in Table 2 (columns 6 and 7, respectively).

The Δ_{total} values seem to be extremely small but significant compared with the experimental error of ±0.4 ppb (Table 2). The contributions from thermodynamic effects do not exceed 0.2 ppb and in all cases the non-additivity contribution predominates. This explains why the use of ^{13}C isotope shifts can lead to the ambiguous results mentioned above.

Very recently data on non-additivity for the ^{13}C isotope shifts through one bond, $^{1}\Delta^{13}C(^{2}H/^{1}H)$, for successive $^{2}H/^{1}H$ replacements have been obtained[19] for several halomethanes with the same sign and close magnitudes to that obtained in the present study.

The approach developed here allows us to consider the measurements of vicinal ^{13}C, ^{2}H coupling constants as a useful technique to obtain data on small isotopic perturbations of conformational equilibria in other deuteriated cycloalkanes. We are currently performing measurements of the ^{13}C NMR of deuteriated cyclobutane, cyclopentane and cyclooctane. The results will be published shortly.

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