Tritium NMR in Conformational Analysis: Isotopic Perturbation of the Ring Inversion Equilibrium in [^{3}H]Cyclohexane

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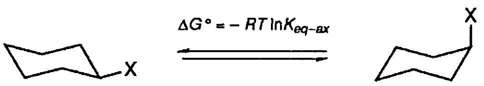
Integration of the axial and equatorial tritium signals in the ^{3}H NMR spectrum of [^{3}H]cyclohexane at −88 °C shows that tritium prefers the equatorial site by 11.2 ± 0.5 cal mol^{-1} (1 cal = 4.184 J), in agreement with expectations based on previous NMR work with [^{2}H]cyclohexane.

The perturbation of conformational equilibria by isotopic substitution has recently received considerable attention, especially in six-membered rings.[1–9] The parent molecule for these studies is cyclohexane, and thus the isotopic perturbation of the ring inversion equilibrium in the chair form of this molecule is of importance, both experimentally[7–9] and theoretically.[8,10] Previous work on cyclohexane has involved deuterium substitution.[7–9] A direct way to determine the conformational preference of deuterium in [^{2}H]cyclohexane **1** is to integrate the separate axial and equatorial ^{2}H resonances at low temperatures, where ring inversion is slow on the NMR chemical shift timescale. Because the difference in the integrals is small (*ca.* 2%), this experiment must be executed with care, especially with respect to minimizing integration artifacts arising from baseline distortions and improper phasing. Using this approach, we have reported[9] that ^{2}H prefers the equatorial position in **1** by 8.2 ± 0.9 cal mol^{-1} (1 cal = 4.184 J), in contrast to the results of earlier work,[7] but in satisfactory agreement with ^{1}H NMR data on a mixture of *cis*- and *trans*-[$^2H_{10}$]1,4-cyclohexane. In the latter study[8] it was found that ^{2}H preferred the equatorial site by 6.3 ± 1.5 cal mol^{-1}.

To our knowledge, ^{3}H NMR has not yet been used in any equilibrium isotope effect studies. As an illustration of the usefulness of tritium in isotopic perturbation studies, we present results obtained in a low temperature integration study of [^{3}H]cyclohexane **2**. This work was performed to obtain the first experimental ΔG° value (*i.e.*, Winstein's *A* value) for the conformational preference of tritium in **2**. With suitable precautions[11] one can work safely with compounds



1 X = D
2 X = T

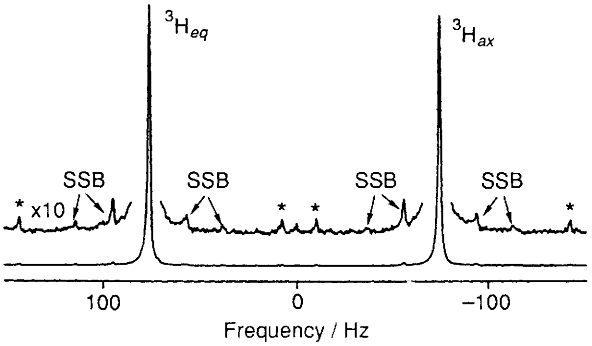


Fig. 1 320 MHz $^3H\{^1H\}$ NMR spectrum of 10% cyclohexane (0.25% ^3H, 33.4 mCi) in CS_2–CD_2Cl_2 (7:2) at −88 °C. The signals shown are the tritium resonances ($^3H_{ax}$ and $^3H_{eq}$) of [^3H]cyclohexane, associated ^{13}C satellites (*) and spinning side bands (SSB), and a small spike at the transmitter frequency (0 Hz)

Table 1 Ratios of integrals for the axial and equatorial tritium peaks in **2**

Data set no.	Integral ratios (K_{eq-ax}) *a*	*b*	*c*	Mean	Mean $\Delta G°$ /cal mol^{-1}
1	0.968	0.975	0.972	0.972	10.5
2	0.969	0.969	0.975	0.971	10.8
3	0.964	0.972	0.973	0.970	11.2
4	0.971	0.971	0.973	0.972	10.5
5	0.969	0.968	0.975	0.971	10.8
6	0.960	0.968	0.966	0.965	13.1
			Total mean:[d]	0.970 ± 0.001	11.2 ± 0.5

[a] 16K time domain points zero filled to 32K with exponential multiplication (EM) with a line broadening (LB) of 0.5 Hz. [b] Same digital resolution as in *a* but with a combined window function [Lorentzian–Gaussian with Bruker parameters, Gaussian broadening (GB) = 0.4 and LB = −0.5, followed by an EM with LB = 0.5 Hz]. [c] 16K time domain points zero filled to 64K with identical window functions as in *b*. [d] Errors are standard deviations in the total means.

containing enough tritium to guarantee ^3H NMR spectra with excellent signal-to-noise ratios. The axial–equatorial peak separation in the $^3H\{^1H\}$ NMR spectrum of **2** is calculated to be 150 Hz at a ^3H resonance frequency of 320 MHz (^1H frequency of 300 MHz) from data on other cyclohexane isotopomers.[9] In our analogous ^2H experiment,[9] the peak separation was only 36 Hz, even though the field strength, which corresponded to a ^1H frequency of 500 MHz, was higher. Furthermore, ^3H has a spin of 1/2 and gives sharp lines at low temperatures, unlike ^2H, which has a spin of 1 and therefore can relax by the quadrupolar mechanism. These factors are important because the accuracy of integration improves when the spectral lines are both sharp and well separated.

Tritiated water (18 µl, *ca.* 5% ^3H) was prepared by reacting PtO_2 with a $^3H_2/^1H_2$ mixture in a closed system.[12] This was transferred under a vacuum to a flask cooled in liquid nitrogen and the stoichiometric amount of cyclohexylmagnesium chloride in ether (1.5 mol dm^{-3}, Aldrich Chemical Co.) was introduced *via* a syringe. After warming, cyclohexane (750 µl) was added and the product was isolated by distillation as the third fraction (300 µl, b.p. 80 °C) following diethyl ether and a diethyl ether/cyclohexane azeotrope (b.p. 45–50 °C). The NMR sample (5 mm Wilmad 528 pp tube) contained 10% cyclohexane (0.25% ^3H, 33.4 mCi),[13] 70% freshly distilled CS_2 and 20% CD_2Cl_2 by volume, and was not degassed.

The NMR spectra were acquired at −88 °C on an AF-300 NMR spectrometer (IBM Instruments, Inc.) operating at 300 and 320 MHz for ^1H and ^3H respectively and equipped with a 5 mm $^3H/^1H$ probe. The protons were decoupled by broadband irradiation. The resolution was adjusted on the tritium free induction decay of **2** so as to obtain narrow peaks with symmetrical line shapes and an acquisition microprogram, which is described elsewhere,[9] was used to avoid baseline distortions (Fig. 1).[14] A variety of transmitter offsets were employed as a precaution against unequal excitation of the two peaks. The $^3H\{^1H\}$ spin-lattice relaxation times for the axial and equatorial peaks were determined by an inversion-recovery pulse sequence and found to be virtually the same (2.53 and 2.51 s, respectively). This near equality allows the integration to be safely performed under conditions of partial saturation, and the spectra have a better signal-to-noise ratio than when obtained under conditions that prevent saturation. Integration data were collected in six sets of 512 scans each with an acquisition time of 2 s, a 75° flip angle, and a total recycle time of 3 s. Each data set was processed with three different procedures as shown in Table 1; the integrals covered a region of ±60 Hz about each peak.

The measured integrals in Table 1 are not randomly distributed about the mean. One integral lies considerably outside the range of the others, but because we have no reason to distrust the particular data set from which this arises, the mean and errors given in Table 1 cover all the available data. The conformational preference ($\Delta G°$) of ^3H in **2** at −88 °C is 11.2 ± 0.5 cal mol^{-1}. This value can be compared to the corresponding deuterium value by consideration of zero point energy (ZPE) differences for vibrations involving hydrogen isotopomers. Assuming that the vibrations are harmonic and that the mass of carbon is infinitely greater than that of any hydrogen isotope, we have $\Delta_{ZPE}(^1H_{ax-eq}) = x$, $\Delta_{ZPE}(^2H_{ax-eq}) = x/\sqrt{2}$, and $\Delta_{ZPE}(^3H_{ax-eq}) = x/\sqrt{3}$, where x is the ZPE difference for C–H stretching in the axial and equatorial positions. The ratio in eqn. (1) is thus obtained.

$$\frac{\Delta_{ZPE}(^1H_{ax-eq}) - \Delta_{ZPE}(^3H_{ax-eq})}{\Delta_{ZPE}(^1H_{ax-eq}) - \Delta_{ZPE}(^2H_{ax-eq})} = \frac{x - x/\sqrt{3}}{x - x/\sqrt{2}} = 1.44 \qquad (1)$$

Molecular mechanics calculations of the conformational equilibrium in **1** with the Delphi program and a modified Boyd force field[15] gives a $\Delta G°$ of 6.2 cal mol^{-1}.[8] Similar calculations for **2** give a value of 9.0 cal mol^{-1} and thus a factor of 1.45 for the ratios of $\Delta G°$ s for the tritium and deuterium equilibria, in agreement with the expectation that the ZPE contributions largely control the positions of these equilibria. Therefore, the measured $\Delta G°$ of 11.2 cal mol^{-1} for tritium corresponds to 7.7 cal mol^{-1} for deuterium, in good agreement with our previously published values (6.3 and 8.2 cal mol^{-1}).[8,9] The equatorial preferences of the heavy hydrogen isotopes are the result of the larger stretching force constant for the equatorial as compared to the axial CH bond in cyclohexane, although this is partly compensated by bending contributions, which operate in the opposite direction.[8]

The ^3H nucleus offers several advantages for NMR when compared to ^2H, namely, inherently higher sensitivity, larger chemical shifts (in frequency units), and narrower lines.

Although simple spectra can be obtained by means of $^1H\{^2H\}$ NMR on highly deuteriated molecules,[16] the use of $^3H\{^1H\}$ NMR at low tritium abundance obviates the need for deuterium substitution, and is therefore synthetically easier. These advantages should lead to a wider use of ^3H NMR in conformational analysis despite the radioactivity of tritium.

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