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Application of Natural Abundance ^{2}H NMR. Simultaneous Measurement of Primary and Secondary Kinetic Deuterium Isotope Effects

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Internal and external reference methods are introduced for the study of kinetic deuterium isotope effects (KDIEs) by natural abundance ^{2}H NMR. These methods take account of both inter- and intra-molecular competitive reactions of fully protonated and naturally monodeuteriated molecules of the substrate in the reaction. Specifically deuteriated compounds are not necessary using these methods. These techniques have further advantages over other methods; for example, primary and secondary KDIEs of different types can be measured simultaneously. The methodology is summarized and examples of its application are presented.

KEY WORDS: Natural Abundance ^{2}H NMR Isotope effects

INTRODUCTION

Since Martin and Martin[1] discovered the existence of the important variations in the 'internal' distribution of deuterium in organic compounds by high-field ^{2}H NMR, the technique has been broadly applied to the identification of the origin of natural and synthetic products[2–6] and to fundamental studies of chemistry and biochemistry.[7–11] One of its most important applications in fundamental studies is in the investigation of deuterium isotope effects.[11–17] Using natural abundance (NA) ^{2}H NMR, the study of deuterium isotope effects can be carried out without the need for specific labelling. Since the synthesis of specifically deuteriated compounds is tedious, NA ^{2}H NMR is advantageous. In addition, it is a unique technique for the simultaneous measurement of the relative numbers of all molecules monodeuteriated at different sites in a sample. Based on this exclusive ability of NA ^{2}H NMR, we have been able to develop a set of new competitive methods for the study of kinetic deuterium isotope effects (KDIEs) that have advantages over other techniques, in particular the simultaneous measurement of primary and secondary KDIEs.[14–17] In contrast to conventional competitive methods, which generally lead to two (intra- and inter-molecular) primary KDIEs for a reaction,[18] by our methods only one 'pure' primary KDIE value is obtained for a given reaction while the α-secondary KDIE for the C—H bond breaking of —CH_2D and CHD can be measured separately. The direct measurement of this effect by conventional methods has not been reported.[19]

In this paper we present the methodology for the estimation of KDIEs by NA ^{2}H NMR based on both intra- and inter-molecular competitive reactions, using an internal or an external reference, and some examples of its application.

THEORETICAL

Internal reference method[14]

The internal reference method can be used when there is a hydrogen atom(s) site in the substrate molecule whose secondary KDIE can be neglected and the ^{2}H NMR signal is not perturbed by other signals. The side-chain halogenation of ethylbenzene is used here as an example to illustrate the method.[14] The natural existence of substrate molecules monodeuteriated at every H atom(s) site is the basis for the simultaneous measurement of primary and secondary KDIEs of different types. There are both fully protonated and monodeuteriated substrate molecules which must be considered. One protium or deuterium is replaced by a halogen atom during the reaction. In terms of deuteriated molecules, for example $C_6H_5CHDCH_3$, the cleavage of the C—D bond gives rise to a primary KDIE whereas in the reactions of $C_6H_5CH_2CH_2D$ and $C_6H_4DCH_2CH_3$, β and remote secondary effects should be observed.

The rate equation of ring monodeuteriated molecules (H) is

$$-\frac{d[H]}{dt} = 2k_H[H][X_2] \tag{1}$$

Although the deuterium atom in $C_6H_4DCH_2CH_3$ can be responsible for a remote secondary KDIE, it is reasonable to assume that the value of this effect is unity. Consequently, $2k_H$ is equal to the rate constant of the fully protonated molecules, $C_6H_5CH_2CH_3$.

Similarly, the rate equations for $C_6H_5CHDCH_3$ (D) and $C_6H_5CH_2CH_2D$ (D′) are

$$-\frac{d(D)}{dt} = k_D(D)(X_2) \tag{2}$$

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Table 1. Number of molecules of fully protonated and deuteriated species and molar fractions of deuteriated species in the halogenation of ethylbenzene

	Number of molecules (molar fraction of monodeuteriated species)				
Reactant	$C_6H_5CH_2CH_3$	$C_6H_4DCH_2CH_3$	$C_6H_5CHDCH_3$		$C_6H_5CH_2CH_2D$
Starting	1	r, (r)	d, (d)		d', (d')
Remaining	F	Fr, (r)	$dF^{(x+y)/2}$, $[dF^{(x+y)/2-1}]$		$d'F^z$, $(d'F^{z-1})$
Converted	$1-F$	$(1-F)r$	$d(1-F^{(x+y)/2})$		$d'(1-F^z)$
	$2k_H \downarrow$	$2k_H \downarrow$	$k_{D,I} \swarrow$	$\searrow k^{\alpha}_{D,II}$	$k^{\beta}_{D,II} \downarrow$
Product	$C_6H_5CHXCH_3$	$C_6H_4DCXHCH_3$	$C_6H_5CHXCH_3$	$C_6H_5CDXCH_3$	$C_6H_5CHXCH_2D$
	$1-F$	$(1-F)r$, (r)	$\frac{x}{x+y}d(1-F^{(x+y)/2})$	$\frac{y}{x+y}d(1-F^{(x+y)/2})$, $\left(\frac{y}{x+y}\frac{d}{1-F}[1-F^{(x+y)/2}]\right)$	$d'(1-F^z)$, $\left(d'\frac{1-F_z}{1-F}\right)$

and

$$-\frac{d(D')}{dt} = 2k^{\beta}_{D,II}(D')(X_2) \tag{3}$$

respectively. When Eqn (1) is divided by Eqn (2), integration of the resulting equation gives

$$\frac{2k_H}{k_D} = \frac{\ln([H]/[H]_0)}{\ln([D]/[D]_0)} \tag{4}$$

[H] and $[H_0]$ or [D] and $[D]_0$ are the concentrations of H or D at the time when the reaction was stopped and at the beginning of the reaction, respectively. Let $F = [H]/[H]_0$, where F refers to the molar fraction of the remaining fully protonated substrate in the reaction mixture after the reaction. Hence Eqn (4) can be written as

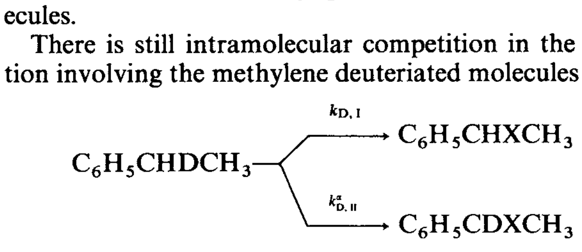
$$[D]/[D]_0 = F^{k_D/2k_H} \tag{5}$$

Similarly,

$$[D']/[D']_0 = F^{k^{\beta}_{D,II}/k_H} \tag{6}$$

[D′] and $[D']_0$ are concentrations of D′ at the time when the reaction was stopped and at the beginning, respectively. Equations (5) and (6) therefore represent quantitatively the intermolecular competition between the deuteriated and fully protonated substrate molecules.

There is still intramolecular competition in the reaction involving the methylene deuteriated molecules:



where $k_D = k_{D,I} + k^{\alpha}_{D,II}$. The rate equations of the two competitive reactions are

$$\frac{d[C_6H_5CHXCH_3]'}{dt} = k_{D,I}[D][X_2] \tag{7}$$

and

$$\frac{d[C_6H_5CDXCH_3]}{dt} = k^{\alpha}_{D,II}[D][X_2] \tag{8}$$

respectively. In Eqn (7), $[C_6H_5CHXCH_3]'$ refers to the concentration of the fully protonated halide derived from $C_6H_5CHDCH_3$. From Eqns (7) and (8) we have

$$k^{\alpha}_{D,II}/k_{D,I} = [C_6H_5CDXCH_3]/[C_6H_5CHXCH_3]' \tag{9}$$

and from Eqns (5) and (9) the following equations can be derived:

$$[C_6H_5CDXCH_3] = \frac{k^{\alpha}_{D,II}}{k_{D,I} + k^{\alpha}_{D,II}}[D]_0 \times [1 - F^{(k^{\alpha}{}_{D,II} + k_{D,I})/2k_H}] \tag{10}$$

and

$$[C_6H_5CHXCH_3]' = \frac{k_{D,I}}{k_{D,I} + k^{\alpha}_{D,I}}[D]_0 \times [1 - F^{(k^{\alpha}{}_{D,II} + k_{D,I})/2k_H}] \tag{11}$$

If $x = k_{D,I}/k_H$, $y = k^{\alpha}_{D,II}/k_H$ and $z = k^{\beta}_{D,II}/k_H$, then x^{-1}, y^{-1} and z^{-1} will refer to primary, α- and β-secondary KDIEs, respectively, for the reaction. If it is assumed that the reaction begins with 1 mol of fully protonated ethylbenzene, and if r, d and d' refer to the number of moles of ring-, methylene- and methyl-monodeuteriated molecules, respectively, then from Eqns (5), (6), (10), (11) and $k_D = k^{\alpha}_{D,II} + k_{D,I}$, the number of moles of each species involved in the reaction can be easily obtained (Table 1). The molar fraction of the deuteriated species in the sample is given in parentheses in Table 1. For example, in the remaining reactant, the number of methylene-deuteriated ethylbenzene molecules is $dF^{(x+y)/2}$ and the total number of molecules (approximately that of fully protonated molecules) is F, and therefore the molar fraction of $C_6H_5CHDCH_3$ in the remaining reactant will be $dF^{(x+y)/2-1}$.

The value of the KDIE induced by the deuterium in the benzene ring can be considered to be unity. Consequently, the number of converted molecules of this species is directly proportional to that of the fully protonated substrate. Therefore, the molar fraction of ring-deuteriated species in the starting and remaining reactant, and also in the product, should be a constant (r). Hence the ring-deuteriated molecules can be employed as the internal reference for quantitative ^2H NMR analysis.

Since in NA ^2H NMR the intensity of the signal of a monodeuteriated molecule is directly proportional to its number, the ratio of the signal intensity of site i-deuteriated molecules to that of the reference represents the ratio of the molar fraction of the deuteriated species to that of the reference in the sample. Hence from the ^{2}H NMR experimental data we can establish the following equations, in which S_i is the peak area of i. From the ^{2}H NMR spectrum of the starting reactant:

$$A = S_{C_6H_5CHDCH_3}/S_{ref} = d/r \qquad (12)$$

$$M = S_{C_6H_5CH_2CH_2D}/S_{ref} = d'/r \qquad (13)$$

From the spectrum of the remaining reactant, where the sample is isolated from the reaction medium:

$$B = S_{C_6H_5CHDCH_3}/S_{ref} = \mathrm{d}F^{(x+y)/2-1}/r \qquad (14)$$

$$N = S_{C_6H_5CH_2CH_2D}/S_{ref} = d'F^{z-1}/r \qquad (15)$$

From the spectrum of α-haloethylbenzene, where the sample is also isolated from the reaction medium:

$$C = S_{C_6H_5CDXCH_3}/S_{ref} = \frac{y}{x+y}\cdot\frac{d}{r}\cdot\frac{1-F^{(x+y)/2}}{1-F} \qquad (16)$$

$$P = S_{C_6H_5CHXCH_2D}/S_{ref} = \frac{d'}{r}\cdot\frac{1-F^{z}}{1-F} \qquad (17)$$

Solving Eqns (12)–(17) for x, y and z leads to the following results:

$$(k_H/k_{D,I})^{-1} = 2\left(\ln\frac{B}{A}\Big/\ln F + 1\right) - (k_H/k^{\alpha}_{D,II})^{-1} \qquad (18)$$

$$(k_H/k^{\alpha}_{D,II})^{-1} = 2C(1-F)\ln\frac{BF}{A}\Big/[(A-BF)\ln F] \qquad (19)$$

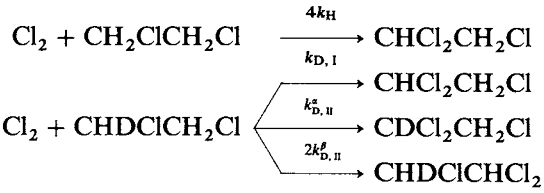
and

$$(k_H/k^{\beta}_{D,II})^{-1} = \ln\frac{N}{M}\Big/\ln F + 1$$
$$= \ln\left[1-\frac{P}{M}(1-F)\right]\Big/\ln F \qquad (20)$$

External reference method

The external reference method can be useful when an appropriate internal reference cannot be found in the substrate molecule. For example, while 1,2-dichloroethane has only one chemically equivalent site for H atoms, an external reference method must be used in the study of the KDIE of the chlorination of this compound. The kinetic analysis for the external reference method is analogous to that for the internal reference method. For the calculation the ^{2}H NMR data for the starting and remaining reactant (1,2-dichloroethane) and that of the product (1,1,2-trichloroethane) are required. The value of F must also be determined precisely. The external reference compound is contained in a sealed capillary, and has only one ^{2}H NMR signal which cannot perturb that of the samples. In the case of the chlorination of 1,2-dichloroethane we used $CHCl_3$ ($\delta = 7.29$ ppm) which had been mixed with a small amount of $CDCl_3$ so that its signal intensity is comparable to that of the samples. The different samples for the NMR experiments are measured with the capillary situated coaxially in the cell, and its ^{2}H NMR signal intensity can therefore be used as a reference for the quantitative analysis of the samples.

During the chlorination of 1,2-dichloroethane there are both inter- and intra-molecular competitive reactions, and the following reaction scheme is valid:



$$Cl_2 + CH_2ClCH_2Cl \xrightarrow{4k_H} CHCl_2CH_2Cl$$

$$Cl_2 + CHDClCH_2Cl \begin{cases} \xrightarrow{k_{D,I}} CHCl_2CH_2Cl \\ \xrightarrow{k^{\alpha}_{D,II}} CDCl_2CH_2Cl \\ \xrightarrow{2k^{\beta}_{D,II}} CHDClCHCl_2 \end{cases}$$

$4k_H$, $k_{D,I}$, $k^{\alpha}_{D,II}$ and $2k^{\beta}_{D,II}$ are the rate constants of the reactions. The following equation can be derived for the intermolecular competition between the fully protonated molecules (H) and the deuteriated molecules (D):

$$\frac{4k_H}{k_{D,I} + k^{\alpha}_{D,II} + 2k^{\beta}_{D,II}} = \frac{\ln([H]/[H]_0)}{\ln([D]/[D]_0)} \qquad (21)$$

Let $x = k_{D,I}/k_H$, $y = k^{\alpha}_{D,II}/k_H$, $z = k^{\beta}_{D,II}/k$ and $F = [H]/[H]_0$. The number of fully protonated and monodeuteriated molecules in the starting reactant are assumed to be 1 and d, respectively. The number of deuteriated molecules in the remaining reactant will be $\mathrm{d}F^{(x+y+2z)/4}$, and the number of $CDCl_2CH_2Cl$ and $CHCl_2CHDCl$ molecules in the product should be

$$\frac{y}{x+y+2z}\,d(1 - F^{(x+y+2z)/4})$$

and

$$\frac{2z}{x+y+2z}\,d[1 - F^{(x+y+2z)/4}],$$

respectively. The number of deuteriated molecules of the reference in the probe is denoted by r. Table 2, which is analogous to Table 1, can then be constructed.

The molar fraction of the deuteriated species of interest in different samples can be compared using the external reference in ^{2}H NMR experiments. The spectrum of the starting reactant and the external references gives

$$A = S_{CH_2ClCHDCl}/S_{ref} = d/r \qquad (22)$$

The spectrum of the remaining reactant with the reference gives

$$B = S_{CH_2ClCHDCl}/S_{ref} = \mathrm{d}F^{(x+y+2z)/4-1}/r \qquad (23)$$

The spectrum of the product with the reference gives

$$C = JS_{CDCl_2CH_2Cl}/S_{ref}$$
$$= \frac{y}{x+y+2z}\cdot\frac{d}{r}\cdot\frac{1-F^{(x+y+2z)/4}}{1-F} \qquad (24a)$$

and

$$C' = JS_{CHDClCHCl_2}/S_{ref}$$
$$= \frac{2z}{x+y+2z}\cdot\frac{d}{r}\cdot\frac{1-F^{(x+y+2z)/4}}{1-F} \qquad (24b)$$

In Eqns (24a) and (24b) $J = d_R M_P/d_P M_R$,[20] where d and M are the density and molecular weight of the sample, respectively, and while the subscripts R and P

Table 2. Numbers of molecules of fully protonated and deuteriated species and molar fractions of deuteriated species in the chlorination of 1,2-dichloroethane

		Number of moleculares (molar fraction of monodeuteriated species)	Reference
Reactant	CH_2ClCH_2Cl	$CHDClCH_2Cl$	
Starting	1	d, (d)	r
Remaining	F	$dF^{(x+y+2z)/4}$, $(dF^{(x+y+2)/4-1})$	r
Converted	$1-F$	$d(1-F^{x+y+2z/4})$	
	$k_H \downarrow$	$k_{D,I} \swarrow$ $k^{\alpha}_{D,II} \downarrow$ $\searrow k^{\beta}_{D,II}$	
Product	$CHCl_2CH_2Cl$	$CHCl_2CH_2Cl$ $CDCl_2CH_2Cl$ $CHCl_2CHDCl$	
	$1-F$	$\frac{x}{x+y+2z}d(1-F^{(x+y+2z)/4})$, $\frac{y}{x+y+2z}d(1-F^{(x+y+2z)/4})$, $\frac{2z}{x+y+2z}d(1-F^{(x+y+2z)/4})$,	
		$\left[\frac{y}{x+y+2z}\cdot\frac{d}{1-F}(1-F^{(x+y+2z)/4})\right]$, $\left[\frac{2z}{x+y+2z}\cdot\frac{d}{1-F}(1-F^{(x+y+2z)/4})\right]$	r

denote reactant and product, respectively. The aim of the introduction of this factor is to correct the number of product molecules so that it is equal to that of the reactant molecules in the probe. The values of the primary and secondary KDIEs can be obtained on solving the following equations:

$$(k_H/k_{D,I})^{-1} = 4\,[\ln(B/A)/\ln F + 1] - (k_H/k^{\alpha}_{D,II})^{-1} - 2(k_H/k^{\beta}_{D,II})^{-1} \tag{25}$$

$$(k_H/k^{\alpha}_{D,II})^{-1} = 4C\ln(BF/A)(1-F)/[(A-BF)\ln F] \tag{26}$$

$$(k_H/k^{\beta}_{D,II})^{-1} = 2C'\ln(BF/A)(1-F)/[(A-BF)\ln F] \tag{27}$$

RESULTS AND DISCUSSION

The results of the application of the methods to some examples are shown in Table 3. Most are cited from our previous work, except for the chlorination of 1,2-dichloroethane. The results show that the primary and α-secondary KDIEs are normal for the reaction, while the β-secondary KDIE ≈ 1. The interpretation of these results is not discussed since the aim of this paper is to deal with the methodology by means of which they are obtained.

Although theoretically, on using the methods described above, in most cases primary and all secondary KDIEs can be investigated simultaneously in a one-pot experiment, in practice the capability of the methods is limited by two major difficulties, viz. the resolution and the sensitivity of the NMR spectrometer. Because of the low resolution in ^2H NMR (only 1/6.5 of that of ^1H NMR) there will be many cases in which some signals are not well resolved, so that sufficient data are not available for the calculation of all KDIE values. For example, for the halogenation of benzene and of ethylbenzene and the oxidation of benzyl alcohol, only average $k_H/k_{D,II}$ $[=5k_H/(k^{p}_{D,II} + 2k^{o}_{D,II} + 2k^{m}_{D,II})]$ values of phenyl-deuteriated substrates can be obtained, since the signals of *o*-, *m*- and *p*-deuteriated species cannot be completely resolved. The deviations of the KDIE values in Table 3 were calculated using partial derivatives. Generally, $k_H/k_{D,I}$ is much more sen-

Table 3. Results of ^2H NMR measurements and calculation of KDIE values of some reactions (standard deviations are in parentheses)

Reaction	A	B	C	$M(C_{\beta}{}^{e})$	N	P	J	F	$k_H/k_{D,I}$	$k_H/k^{\alpha}_{D,II}$	$k_H/k^{\beta}_{D,II}$ [f]
Bromination of	0.324	0.358	0.201	0.348	0.348	0.346		0.6048	2.39	0.84	1.00
ethylbenzene[a]	(0.006)	(0.012)	(0.000)	(0.003)	(0.001)	(0.001)			(>1)	(0.03)	(0.02)
Chlorination of	3.117	3.094	2.561				1.141[g]	0.517[k]	0.89		1.01
benzene[b]	(0.017)	(0.016)	(0.022)					(0.002)	(0.19)		(0.02)
Oxidation of	0.328[i]	0.476[i]	0.162[i]	0.815[j]	0.812[j]			0.487[k]	7.63	1.20	1.00
benzyl alcohol[c]	(0.024)	(0.006)	(0.007)	(0.022)	(0.021)			(0.004)	(>1)	(0.21)	(0.08)
Chlorination of	1.588	1.787	0.354	0.863			1.156[h]	0.430[k]	1.89	1.18	0.97
1,2-dichloroethane[d]	(0.013)	(0.021)	(0.009)	(0.022)				(0.002)	(>1)	(0.07)	(0.08)

[a] Ref. 14; using an internal reference, ^2H NMR data are the average of only two spectra.
[b] Ref. 16; using an external reference, ^2H NMR data are the average of six spectra.
[c] Ref. 16; both internal and external references were used; ^2H NMR data are the average of six spectra.
[d] This work using an external reference; A and B are the averages of six spectra and C and C_{β} are the averages of twelve spectra.
[e] For the reaction of 1,2-dichloroethane.
[f] Average $k_H/k_{D,II}$ of phenyl-deuteriated molecules for the chlorination of benzene, the bromination of ethylbenzene and the oxidation of benzyl alcohol.
[g] $d_R = 0.876$, $d_P = 1.106$.
[h] $d_R = 1.235$, $d_P = 1.440$.
[i] Using an internal reference.
[j] Using an external reference.
[k] Results of GC analysis; average of 5–7 experiments.

sitive to experimental errors than is $k_H/k_{D,II}$. For most examples in Table 3 the error in the $k_H/k_{D,I}$ value is >1, since in these cases the A and B values are too small and their relative deviations are large. The error in $k_H/k_{D,I}$ could be decreased by choosing correctly the (internal or external) reference so that A, B, M and N are as large as possible, and by minimizing their standard deviations. Although the deviation of $k_H/k_{D,II}$ can be small, in practice many remote secondary KDIEs have to be neglected, i.e. their values are assumed to be unity, since the difference between their real values and 1 should be within experimental error. The use of a large number of spectral data for the statistical analysis will increase the reliability of the final results, and the precision of the measurement will be related to the progress of the NMR technique. Nevertheless, in spite of these difficulties, it is important to demonstrate the potential of NA ^{2}H NMR spectroscopy in the study of KDIEs and to establish a theoretical basis for the simultaneous measurement of primary and secondary KDIEs of different types.

EXPERIMENTAL

NMR experiments

The ^{2}H NMR spectra were recorded using a Varian XL 400 spectrometer operating at 61.37 MHz, without field-frequency locking, in the ^{1}H broad band decoupling mode; 10 mm cells were used. The spectra were measured with 500 accumulations obtained with an acquisition time of 7 s (90° pulse) over a range of 1000 Hz; $T = 22 \pm 0.5\,^\circ$C. Each FID was treated by exponential multiplication before Fourier transformation ($LB = 3$ and 4). Integrations are used for the calculation.

Chlorination of 1,2-dichloroethane

Chlorine gas was bubbled into 20 ml of purified 1,2-dichloroethane contained in a flask under irradiation from a 100 W lamp. The temperature was maintained at 83–84 °C. The progress of the reaction was monitored with a Shimadzu GC-9A gas chromatograph (2 m OV-17 column). When nearly half the substrate had been converted, the chlorine gas stream was stopped and nitrogen was bubbled into the reaction mixture to drive off the residual chlorine and hydrogen chloride. The F value was then determined with the gas chromatograph. After washing successively with dilute sodium carbonate solution and water, the remaining reactant and the product (1,2,2-trichloroethane) were separated by fractional distillation and were then ready for NMR analysis.

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