An Equilibrium Deuterium Isotope Effect of Surprising Magnitude

M. J. Goldstein* and E. J. Pressman[1]

Department of Chemistry, Cornell University
Ithaca, New York 14853

Received June 29, 1981

Any equilibrium isotope effect measures the difference between local force fields at two different sites. The heavier isotope always prefers the more constrained site. Deuterium equilibrium isotope effects can also be used to calibrate the more frequently measured secondary deuterium *kinetic* isotope effects. For example, K_{HD} for *tert*-butyl-d_9 cation exchange (2.36, 25 °C) is hardly distinguishable from k_H/k_D for hydrolysis[2] (2.39), i.e., from $K_{HD}^{\ddagger}$.[3] It follows that the structure of the hydrolytic transition state—$[C_4H_9CCl]^{\ddagger}$—closely resembles that of a *tert*-butyl cation.[4] The

$$(CH_3)_3CCl + (CD_3)_3C^+ \overset{K_{HD}}{\rightleftharpoons} (CH_3)_3C^+ + (CD_3)_3CCl$$

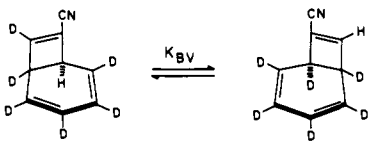
$$(CH_3)_3CCl + H_2O \xrightarrow{k_H} (CH_3)_3COH + HCl$$

$$(CD_3)_3CCl + H_2O \xrightarrow{k_D} (CD_3)_2COH + HCl$$

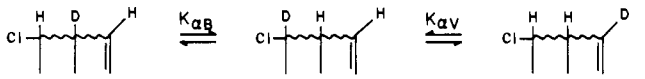
$$(CH_3)_3CCl + [C_4D_9CCl]^{\ddagger} \overset{K_{HD}^{\ddagger}}{\rightleftharpoons} [C_4H_9CCl]^{\ddagger} + (CD_3)_3CCl$$

differences between these two different kinds of isotope effects become larger and more variable in [3,3]-sigmatropic shifts; so also do the corresponding differences in transition-state structures.[5]

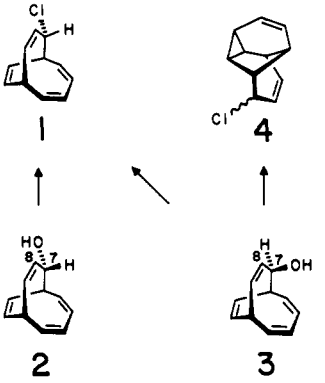
In the absence of such thermodynamic calibration, secondary deuterium kinetic isotope effects are occasionally difficult to distinguish from small primary isotope effects. For example, a solvolytic β-secondary deuterium isotope effect greater than ca. 1.2 (per deuterium atom) is occasionally taken to suggest the incursion of rate-determining deprotonation[6a] or hydrogen participation.[6b] Nevertheless, larger *equilibrium* isotope effects than this have been reported. In the cyanobicyclo[4.2.0]octatrienes-d_6, for example, deuterium prefers a bridgehead (B) to a vinyl (V) site by $K_{BV} = 1.55$ (6).[7]



We now report the preference of deuterium for an α-chloro site (α) to a bridgehead site (B) of $K_{\alpha B} = 1.48$ (4) and of this same α-chloro site to a vinyl site (V) of $K_{\alpha V} = 3.0$ (1). To the best of our knowledge, this latter isotope effect exceeds any previously reported value, at least for deuterium equilibration between two different carbon sites.[8]



Both isotope effects were obtained from samples of *anti*-7-chlorobicyclo[4.3.2]undecatetraene-d_1 (**1**-d_1). The vinyl site (V) is here an average over eight nonequivalent locations, and the bridgehead site (B) is an average over two. As we report elsewhere,[9] **1** is obtained as the exclusive product of anti alcohol (**2**) and as coproduct of syn alcohol (**3**), the second coproduct being an epimeric mixture of armilenyl chlorides (**4**). When these latter chlorides (**4**) were obtained from specifically deuterated **3**, their ^{1}H NMR spectrum revealed a deuterium distribution that was essentially random. The corresponding distribution in **1**-d_1, however, was subtly different.



Entries 3–12 of Table I show that the vinyl proton areas of **1**-d_1 are consistently greater than anticipated for a completely random distribution. The α-proton and bridgehead proton areas are correspondingly lower, albeit to different extents. These discrepancies persist despite differences in the stereochemistry of the alcohol precursor in the original locations of the deuterium label, in the reaction conditions, or in the purification of the product. They cannot be due to partial dedeuteration by extraneous protons, because concordant results were obtained in the presence of both HCl (oxalyl chloride conditions) and excess triethylamine (mesyl chloride conditions). They vanish when a ^{13}C label is used[9] or in the absence of any label at all (entries 1 and 2). These discrepancies must therefore be measuring a deuterium isotope effect.

Is this also an *equilibrium* deuterium isotope effect? It need not be if chloride ion capture of equilibrating carbocations were irreversible. The small secondary *kinetic* isotope effect, to be expected of such capture, might then be amplified by incomplete carbocation equilibration.[10] Even larger effects might then also be "induced" by deprotonating side reactions.[11] Fortunately, no such reactions could be detected in the ^{1}H NMR spectra, even of "crude" product. To guarantee the reversibility of chloride ion capture is more difficult, at least in the absence of specifically deuterated **1**-d_1.

That equilibration does extend to the covalent chlorides was first suggested by related experiments with specifically deuterated armilenyl chlorides (**4**-d_1). These equilibrate rapidly by a carbocation mechanism, even in chloroform solution at ambient temperatures.[12] Because the bicyclic chloride (**1**) hydrolyzes more rapidly than do the armilenyl chlorides (**4**),[9] one expects the isotopic equilibration of **1** also to be more rapid. Confirmatory evidence was provided by entry 13 of Table I. The otherwise slow

(1) Taken in part from the Ph.D. Thesis of E. J. Pressman, Cornell University, 1981.
(2) Evans, J. C.; Lo, B. Y.-S. *J. Am. Chem. Soc.* **1966**, *88*, 2118-2122.
(3) Gold, V.; Satchell, D. P. N. *Q. Rev. Chem. Soc.* **1955**, *9*, 51-72.
(4) For more recent data and critical evaluation, see: Burton, G. W.; Sims, L. B.; Wilson, J. C.; Fry, A. *J. Am. Chem. Soc.* **1977**, *99*, 3371-3379 and references cited.
(5) (a) McMichael, K. D.; Korver, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 2746-2747. (b) Gajewski, J. J.; Conrad, N. D. *Ibid.* **1979**, *101*, 2748-2750, 6693-6704. (c) Hartshorn, S. R.; Shiner, V. J., Jr. *Ibid.* **1972**, *94*, 9002-9012.
(6) (a) Shiner, V. J., Jr.; Dowd, W.; Fisher, R. D.; Hartshorn, S. R.; Kessick, M. A.; Milakofsky, L.; Rapp, M. W. *J. Am. Chem. Soc.* **1969**, *91*, 4838-4843. (b) Shiner, V. J., Jr.; Jewett, J. G. *Ibid.* **1965**, *87*, 1382-1383, 1383-1384.
(7) (a) Paquette, L. A.; Kitching, W.; Heyd, W. E.; Meisinger, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 7371-7372. (b) The authors explicitly recognize this to be a maximum value, the product of K_{BV} with a secondary *kinetic* deuterium isotope effect of unknown magnitude. The latter can be estimated as ca. 1.08, whence $K_{BV} \approx 1.44$.
(8) The autoprotolysis constant of H_2O exceeds that of D_2O by a factor of 6.88. See: Kingerly, R. W.; LaMer, V. K. *J. Am. Chem. Soc.* **1941**, *63*, 3256-3261.
(9) Goldstein, M. J.; Tomoda, S.; Pressman, E. J.; Dodd, J. A. *J. Am. Chem. Soc.*, preceding paper in this issue.
(10) For an example of amplification during carbanionic rearrangement, see: Thibblin, A.; Bengtsson, S.; Ahlberg, P. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1569-1572. Thibblin A.; Ahlberg, P. *J. Am. Chem. Soc.* **1979**, *101*, 7311-7318.
(11) Samuelson, A. G.; Carpenter, B. K. *J. Chem. Soc., Chem. Commun.* **1981**, 354-356.
(12) Goldstein, M. J.; Pressman, E. J., manuscript in preparation.

0002-7863/81/1503-6533$01.25/0

Table I. Fractional ^{1}H NMR Areas and Equilibrium Constants (35 °C) of *anti*-7-Chlorobicyclo[4.3.2]undecatetraene-d_1 (**1**-d_1)

structural assignment:		vinyl	α	bridgehead			
δ_H, ppm[a]		6.10–5.45	4.80–4.70	3.65–3.40			
areas calcd for random distribution:		0.727	0.091	0.182			
precursor	reaction conditions	fractional areas obsd[b]			Isolated yield[c]	$K_{\alpha B}$[b]	$K_{\alpha V}$[b]
1. *syn*-**3**-d_0	$SOCl_2$, pyr/ether	0.725 (3)	0.092 (2)	0.184 (1)			
2. *anti*-**2**-d_0	$SOCl_2$, pyr/ether	0.730 (3)	0.091 (1)	0.179 (2)			
3. *syn*-**3**-**8**-d_1	$SOCl_2$, pyr/ether	0.749 (6)	0.083 (4)	0.168 (4)	98%	1.1 (3)	2.7 (6)
4. *syn*-**3**-**8**-d_1	MesCl Et_4NCl, Et_3N	0.748 (3)	0.079 (2)	0.173 (2)	97%	1.6 (2)	3.3 (3)
5. *syn*-**3**-**7**-d_1	$SOCl_2$, pyr/ether	0.763 (5)	0.073 (5)	0.167 (2)	100%	1.4 (2)	6 (2)
6. *anti*-**2**-**8**-d_1	$SOCl_2$, pyr/ether	0.746 (6)	0.085 (2)	0.169 (6)	98%	1.00 (6)	2.2 (2)
7. *anti*-**2**-**8**-d_1	MesCl, Et_4NCl, Et_3N	0.743 (4)	0.086 (2)	0.171 (3)	94%	1.0 (3)	2.0 (4)
8. *anti*-**2**-**7**-d_1	$SOCl_2$/pentane	0.754 (3)	0.073 (5)	0.172 (5)	94%	1.7 (3)	3.8 (6)
9. *anti*-**2**-**7**-d_1	$SOCl_2$/pyr, ether	0.757 (7)	0.076 (2)	0.167 (2)	100%	1.6 (1)	4.3 (5)
10. *anti*-**2**-**7**-d_1	$(ClCO)_2$/PhH(vac)[d]	0.747 (4)	0.080 (3)	0.172 (1)	*e*	1.8 (3)	3.6 (7)
11. *anti*-**2**-**7**-d_1	$(ClCO)_2$/PhH	0.750 (1)	0.076 (2)	0.174 (1)	*e*	1.5 (2)	3.0 (7)
12. *anti*-**2**-**7**-d_1	$(ClCO)_2$/PhH	0.744 (3)	0.079 (3)	0.176 (2)	*f*	1.83 (9)	2.9 (3)
13. *anti*-**1** (from 12)	$SnCl_4$, CH_2Cl_2	0.743 (3)	0.077 (3)	0.181 (3)	*f*	2.2 (1)	3.1 (3)
	weighted mean[g]	0.749 (1)	0.079 (1)	0.172 (1)		1.48 (4)	3.0 (1)

[a] Continuous wave spectra in $CDCl_3$ at 90 MHz except where otherwise specified. [b] Mean and standard deviations in the last digit as obtained from 3–5 scans. Except where otherwise specified, all samples were purified via HPLC. [c] Prior to HPLC purification. [d] Vacuum line techniques were used. Inert atmosphere syringe techniques were used elsewhere. [e] Analysis without prior HPLC purification. [f] FT spectra at 80 MHz. [g] $\bar{x} = \Sigma(x_i/s_i)/\Sigma(1/s_i)$ and $s = 1/(\Sigma 1/s_i^2)^{1/2}$.

structural isomerization of **1**-d_1 to **4**-d_1 was catalyzed by stannic chloride, and the process was halted after 60% completion. Recovered unreacted **1**-d_1 should then have had ample opportunity for reversible chloride dissociation to be complete. Because the resulting isotopic distribution remained unchanged, it seems reasonable to identify its persistence with chemical equilibrium.

Equations 1 and 2 were then used to define and evaluate the two equilibrium constants.[13] Each n_i represents the mole fraction

$$K_{\alpha B} \equiv \frac{2n_\alpha}{n_B} = \frac{a_V - 9a_\alpha + a_B}{a_V + a_\alpha - 4a_B} \quad (1)$$

$$K_{\alpha V} \equiv \frac{8n_\alpha}{n_V} = \frac{4a_V - 36a_\alpha + 4a_B}{-a_V + 4a_\alpha + 4a_B} \quad (2)$$

of one isotopic isomer with its deuterium atom at site i; the a_i are the corresponding observed areas. Because the algebraic form of these equations attenuates experimental precision, nonrandom error propagation was avoided by evaluating $K_{\alpha B}$ and $K_{\alpha V}$ separately for each NMR scan. To the extent that precursors were incompletely deuterated, 1.48 and 3.0 should be regarded as *lower limits* of the true equilibrium constants. One recalls too that $K_{\alpha V}$ is an average over 8 nonequivalent sites; some of them might well require larger equilibrium isotope effects than the rest.[14]

Why are these isotope effects so large? Molecular models reveal no apparent steric constraints about the α hydrogen, but constraints might well be present nonetheless. Nucleophiles attack the corresponding ketone almost exclusively from the anti direction.[15] Perhaps the preference for bridgehead to vinyl deuteration ($K_{BV} = K_{\alpha V}/K_{\alpha B} = 2.0$) is greater than that in the cyanobicyclo[4.2.0]octatrienes, because there the CH bond is also a less constrained cyclobutyl CH bond.[16]

A more interesting possibility is that these isotope effects only appear to be large for want of adequate comparison. If so, they might profitably encourage discovery of still larger effects in molecules that provide correspondingly greater contrasts in local force fields. Empirical calibration of secondary deuterium *kinetic* isotope effects could then rest more securely upon a wider range of structurally well-defined equilibrium isotope effect data.

Acknowledgment. We are grateful to E. S. Lewis and V. J. Shiner, Jr., for critical comments and to the National Science Foundation for its contributions to research (CHE77-26482) and to the purchase of an NMR spectrometer (CHE76-05884). Spectra were also obtained at the Northeast Regional NSF-NMR Facility (270 MHz) and, through the courtesy of E. R. Stimson, at Carnegie-Mellon University (600 MHz).

Supplementary Material Available: A brief derivation of eq 1 and 2 (1 page). Ordering information is given on any current masthead page.

(13) See supplementary material. A more general derivation will be presented elsewhere. The critical reader can verify eq 1 and 2 at the extremes; e.g., $K_{\alpha B} = K_{\alpha V} = 1$ when $a_V = 8$, $a_\alpha = 1$, and $a_B = 2$.

(14) 270-MHz NMR spectra resolved the two bridgehead protons and showed them to be of equal area. The vinyl protons remained incompletely resolved, even at 600 MHz.

(15) (a) Goldstein, M. J.; Kline, S. A. *Tetrahedron Lett.* **1973**, 1089–1092. (b) *J. Am. Chem. Soc.* **1973**, *95*, 935–936. (c) Groves, J. T.; Ma, K. W. *Ibid.*, **1977**, *99*, 4076–4082.

(16) See: Sunko, D. E., Borčić, S. In "Isotope Effects in Chemical Reactions"; Collins, C. J., Bowman, N. S., Eds.; Van Nostrand Reinhold: New York, 1970; pp 188–189.

Formation of *N*-Phenylheme in the Hemolytic Reaction of Phenylhydrazine with Hemoglobin

Paul R. Ortiz de Montellano* and Kent L. Kunze

Department of Pharmaceutical Chemistry
School of Pharmacy and Liver Center
University of California
San Francisco, California 94143

Received April 17, 1981

The formation of a green pigment in erythrocytes of animals treated with phenylhydrazine was described by Hoppe-Seyler in 1885.[1] Pigment formation has subsequently been found to be intimately associated with the precipitation of hemoglobin in the form of Heinz bodies and with the ensuing hemolytic anemia.[2] Despite a century of continuous scrutiny, however, the nature of the green chromophore and the mechanism by which it is formed remain unknown. The green substance was actually isolated in

(1) Hoppe-Seyler, G. *Z. Physiol. Chem.* **1885**, *9*, 34–39.

(2) (a) Beutler, E. *Pharmacol. Rev.* **1969**, *21*, 73–103. (b) Webster, S. H. *Blood* **1949**, *4*, 479–497.

0002-7863/81/1503-6534$01.25/0