

## Effects of High Pressure on Isotope Effects and Hydrogen Tunneling

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**Abstract:** Kinetic isotope effects arising from transition state phenomena such as tunneling are sensitive to changes in pressure, whereas those arising from differences in zero point energies in the reactant state are not (Isaacs, N. S. *Isotope Effects in Organic Chemistry*; Buncl, E., Lee, C. C., Eds.; 1984; Vol. 6, pp 67–105). A new equation is derived which differentiates between the two origins as a function of pressure. Fitting published isotope effects on hydride transfer between chloranil and leucocystal violet (Isaacs, N. S.; Javid, K.; Rannala, E. *J. Chem. Soc., Perkin Trans. 2* 1978, 709–711) to this equation yields  $\Delta V_Q = 36.5 \pm 3.0$  mL/mol for the apparent volume difference between the transition states of hydride versus deuteride transfer,  $k_H/k_D = 7.8 \pm 0.1$  for the semiclassical isotope effect due to differences in zero point energies, and  $Q_H/Q_D = 1.44 \pm 0.02$  for the transition state effect. Thus, tunneling accounts for  $33 \pm 1\%$  of the observed deuterium isotope effect at atmospheric pressure. Incorporating the transition state effect into a pair of Bell tunneling correction equations (Bell, R. P. *The Tunnel Effect in Chemistry*; Chapman and Hall; London and New York, 1980) allows the precise estimation of the reaction frequency as  $\nu_H^\ddagger = 797 \pm 12$  cm<sup>-1</sup> with  $Q_H = 1.97 \pm 0.05$ . Quantifying hydrogen tunneling in this way provides a powerful new tool for probing transition state chemistry.

According to semiclassical kinetic theory, normal deuterium isotope effects arise solely from differences in zero point vibrational frequencies and should be  $k_H/k_D \approx 7$  or less. When much larger values are found, the excess is usually attributed to hydrogen tunneling. In a few cases this inference is supported by experimental data either in the form of anomalous Arrhenius plots or in the breakdown of the Swain–Schaad relationship.<sup>1</sup> However, there are problems and ambiguities with both experimental approaches in that anomalies and breakdowns may have other origins,<sup>2</sup> and both suffer from a lack of precision,<sup>2,3</sup> which nearly precludes quantifying the degree of hydrogen tunneling much beyond whether it is statistically significant.

Isaacs pioneered an alternative approach based on applying high hydrostatic pressure.<sup>4</sup> Because the origin of all normal isotope effects lies in changes in vibrational frequencies within the Bigeleisen equation,<sup>5</sup>

$$\frac{k_H}{k_D} = \frac{\nu_H^\ddagger}{\nu_D^\ddagger} \frac{\left( \prod (v_i^H/v_i^D) e^{-\Delta\mu_i/2} \frac{(1 - e^{\mu_i^D})}{(1 - e^{\mu_i^H})} \right)}{\left( \prod (v_j^H/v_j^D) e^{-\Delta\mu_j/2} \frac{(1 - e^{\mu_j^D})}{(1 - e^{\mu_j^H})} \right)} \quad (1)$$

where  $\nu$  represents a vibrational frequency and  $\mu$  a reduced mass of hydrogen or deuterium as indicated by the superscripts, Isaacs first examined whether the vibrational frequencies were sensitive to pressure. He concluded from infrared and Raman spectra at

(1) For reviews, see: Kohen, A.; Klinman, J. P. *Acc. Chem. Res.* 1998, 31, 397–404, and references therein.

(2) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; John Wiley & Sons: New York, 1980; p 15.

(3) Northrop, D. B.; Duggleby, R. G. *Bioorg. Chem.* 1990, 18, 435–439.

(4) Isaacs, N. S. In *Isotope Effects in Organic Chemistry, Volume 6*; Buncl, E., Lee, C. C., Eds.; 1984; pp 67–105.

(5) Bigeleisen, J.; Wolfsberg, M. *Adv. Phys. Chem.* 1958, 1, 15.

high pressure that the sensitivities are small and likely to be insignificant within the experimental range of pressure available to kinetic studies. This conclusion was verified experimentally using a variety of reactions displaying normal isotope effects. Isaacs then asked whether pressure might affect quantum mechanical tunneling, and concluded that since pressure influences solvation, it is likely to alter the tunnel correction,  $Q_H/Q_D$ , described by Bell.<sup>6</sup> He went on to examine experimentally eight chemical reactions suspected of tunneling, including hydrogen transfer, hydride transfer, and proton transfer, and found that in each case the observed  $k_H/k_D$  decreased with increasing pressure. These decreases were reported qualitatively, and have not been evaluated in any quantitative way.

Hydrostatic pressure affects chemical systems by shifting preexisting equilibria. As a result it is a particularly interesting system variable because nothing new is being added to the system itself,<sup>7</sup> yet it has only recently been exploited to a significant degree.<sup>8</sup> Chemical equilibria in solution vary as a function of pressure as shown in eq 2, where  $K_o$  is an equilibrium

$$K_p = K_o e^{-\Delta V_p/RT} \quad (2)$$

constant at either atmospheric or zero pressure (the difference is insignificant),  $\Delta V$  is the change in partial molar volumes of products and reagents in milliliters per mole ( $V_{\text{prod}} - V_{\text{react}}$ ),  $p$  is the pressure in bars (0.98692 standard atmosphere),  $R$  is the gas constant at 82.0578 mL·bar/mol·K, and  $T$  is the temperature

(6) Bell, R. P. *The Tunnel Effect in Chemistry*; Chapman and Hall: London and New York, 1980.

(7) Royer C. A. *Methods Enzymol.* 1995, 259, 357–77.

(8) (a) Markely, J. L.; Royer, C. A.; Northrop, D. B. *Effects of High Pressure on Molecular Biology and Enzymology*; Oxford University Press: New York, 1996. (b) Van Eldik, R.; Hubbard, C. D. *Chemistry Under Extreme or Non-Classical Conditions*; John Wiley & Sons: New York, 1997.

in Kelvin. In absolute rate theory,<sup>9</sup> this same treatment is applied to the quasi-equilibrium between a reactant state and a transition state, resulting in a pressure-dependent rate constant:

$$k_p = k_o e^{-\Delta V^\ddagger/RT} \quad (3)$$

where  $k_o$  is the rate constant and  $\Delta V^\ddagger$  represents the difference between the volume of the reactants and their volume in the transition state of the reaction. To extend this treatment to the effect of pressure upon an isotope effect requires consideration of a contribution from the transition state. Such a contribution was postulated for tunneling corrections as described by Bell:<sup>6</sup>

$$\left| \frac{k_H}{k_D} \right|_{\text{obsd}} = \frac{k_H Q_H}{k_D Q_D} \quad (4)$$

where  $k_H/k_D$  is the semiclassical isotope effect and  $Q_H/Q_D$  is the tunneling correction. The obvious approach to an expression for the effect of pressure on this ratio of kinetic terms would be to apply the pressure function of eq 3 to each component of eq 4:

$$\left| \frac{k_H}{k_D} \right|_{\text{obsd}} = \frac{k_H e^{-\Delta V_H^\ddagger p/RT} Q_H e^{-\Delta V_{QH}^\ddagger p/RT}}{k_D e^{-\Delta V_D^\ddagger p/RT} Q_D e^{-\Delta V_{QD}^\ddagger p/RT}} \quad (5)$$

Because of the lack of pressure effects on normal isotope effects as demonstrated by Isaacs,  $\Delta V_H^\ddagger$  and  $\Delta V_D^\ddagger$  should be equal and their exponential functions will cancel. When  $\Delta V_{QH}$  and  $\Delta V_{QD}$  are unequal and do not cancel, eq 5 becomes

$$\left| \frac{k_H}{k_D} \right|_{\text{obsd}} = \frac{k_H}{k_D} \frac{Q_H}{Q_D} e^{-(\Delta V_{QH} - \Delta V_{QD})p/RT} \quad (6)$$

However, eq 6 cannot be correct because it describes a decrease of an observed isotope effect to a value of zero at very high pressure when  $\Delta V_{QD}$  is more negative than  $\Delta V_{QH}$ , as was the case in all eight reactions examined by Isaacs and co-workers, and not to the finite values of isotope effects they observed experimentally.

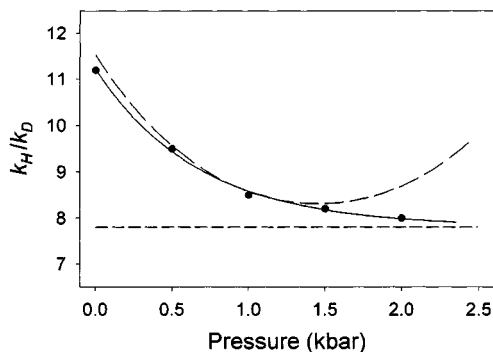
One needs to recall that before mathematical manipulations are performed on rate equations with isotope effects, it is necessary to subtract "1" from the effect, because the absence of an isotope effect has a value of 1, not 0.<sup>10</sup> In this case, subtracting  $k_H/k_D$  from both sides of eq 4 followed by rearrangement yields

$$\left| \frac{k_H}{k_D} \right|_p = \frac{k_H}{k_D} \left( \frac{Q_H}{Q_D} - 1 \right) + \frac{k_H}{k_D} \quad (7)$$

Application of the exponential pressure function of eq 6 to the parenthetical expression containing the tunneling correction minus 1 yields

$$\left| \frac{k_H}{k_D} \right|_p = \frac{k_H}{k_D} \left( \frac{Q_H}{Q_D} - 1 \right) e^{-\Delta V_Q p/RT} + \frac{k_H}{k_D} \quad (8)$$

where  $\Delta V_Q$  is the apparent volume difference between a hydride and deuteride ion accompanying the tunneling correction. Because of the algebraic form of eq 8 and because pressure can also affect reaction rates indirectly by changing the solvent



**Figure 1.** Deuterium isotope effects on the hydride transfer from chloranil to leucocrystal violet as a function of hydrostatic pressure.<sup>11</sup> The solid line is a fit to eq 8. The short-dashed line is the asymptote to the fitted curve and represents the semiclassical isotope effect. The long-dashed curve was calculated from the published quadratic parameters obtained by fitting  $k_H$  and  $k_D$  separately to eq 9.

**Table 1.** Pressure Parameters of Hydrogen Tunneling<sup>a</sup>

$k_H/k_D$	$7.8 \pm 0.1$
$Q_H/Q_D$	$1.44 \pm 0.02$
$\Delta V_Q$	$36.5 \pm 3.0$ mL/mol

<sup>a</sup> Data points<sup>11</sup> shown in Figure 1 were fitted to eq 8.

viscosity and dielectric constant,  $\Delta V_Q$  need not be taken literally as a real difference in volume of hydride and deuteride in the transition state. Equation 8 successfully divides the observed isotope effect into a transition state function that is dependent upon pressure, and a reactant state function that is not dependent. While framed in the symbols of Bell for hydrogen tunneling, it is not specific to this particular quantum mechanical phenomenon but rather is a general expression that may be applicable to other processes which contribute unequally to the two states, such as coupled motion.

The new equation has been tested using the experimental data of Isaacs, Javaid, and Rannala<sup>11</sup> who examined the effects of pressure on the oxidation of leucocrystal violet by chloranil (tetrachloro-*p*-benzoquinone), because Lewis and co-workers<sup>12</sup> had shown earlier that the deuterium isotope effect for this reaction is abnormally large, i.e.,  $k_H/k_D > 12$ , and that it has an anomalous temperature dependence. Their results, obtained at 29 °C in acetonitrile at pressures up to 2 kbar, are reproduced graphically in Figure 1. The data were fitted to eq 8 using the nonlinear regression routine of Duggleby<sup>13</sup> to obtain the results shown in Table 1, from which the solid line was drawn in Figure 1. The fit shows a remarkably good agreement between point and line, with a difference between the vertical intercept and the horizontal asymptote consistent with the pressure-sensitive component contributing  $33 \pm 1\%$  to the observed isotope effect at atmospheric pressure. This exercise in analytical geometry shows that the effects of pressure on an isotope effect can be used, not only to detect transition state phenomena as was demonstrated by Isaacs, but also to quantify such phenomena with considerable precision.

In contrast, Isaacs and co-workers fitted their primary data to the general quadratic function

$$\ln k = A + Bp + Cp^2 \quad (9)$$

which is standard procedure when logarithmic plots of reaction

(9) Glastone, S.; Laidler, K. J.; Eyring, H. *The Theory of Rate Processes*; McGraw-Hill: New York, 1941.

(10) Northrop, D. B. In *Isotope Effects on Enzyme-Catalyzed Reactions*; Cleland, W. W., O'Leary, M. H., Northrop, D. B., Eds.; University Park Press: Baltimore, 1977; pp 122–152.

(11) Isaacs, N. S.; Javaid, K.; Rannala, E. *J. Chem. Soc., Perkin Trans. 2* **1978**, 709–711.

(12) (a) Lewis, E. S.; Robinson, *J. Am. Chem. Soc.* **1968**, *90*, 4337. (b) Lewis, E. M.; Perry, J. M.; Grinstein, *J. Am. Chem. Soc.* **1970**, *92*, 709.

(13) Duggleby, R. G. *Comput. Biol. Med.* **1984**, *14*, 447–455.

rates versus pressure are nonlinear. However, values for  $k_H/k_D$  calculated from their quadratic parameters generate the dashed curve shown in Figure 1, which does not approach a finite asymptote but curves upward at pressures above 1.5 kbar. This fitting procedure is obviously incorrect: a pressure-sensitive phenomenon should not decrease as a function of low pressure and then at some point reverse directions and start increasing at higher pressure, which in turn suggests that the widespread use of eq 9 in pressure kinetics is misguided. An expanded version of eq 3 is needed, to replace the arbitrary but ill-suited quadratic expression.

With a precise experimental value of  $Q_H/Q_D$  at hand, it becomes possible for the first time to apply the Bell tunneling correction equation to a real chemical reaction suspected of tunneling, and not just a computer model of one. Bell assumed a truncated parabolic energy barrier and derived the following series function:

$$Q_t = \frac{1/2 u_t}{\sin(1/2 u_t)} - \sum_{n=1}^{\infty} (-1)^n \frac{\exp\left(\frac{u_t - 2n\pi}{u_t} \alpha\right)}{u_t - 2n\pi} \quad (10)$$

where  $u_t = h\nu^\ddagger c/k_B T$  and  $\alpha = E/k_B T N$ .<sup>14</sup> Fortunately, Isaacs, Javaid, and Rannala<sup>11</sup> also determined the temperature dependence of hydride and deuteride transfer and obtained activation energies of  $E_H = 8.7 \pm 0.5$  kcal/mol and  $E_D = 10.3 \pm 0.5$  kcal/mol. According to Hook's law, the imaginary frequencies of hydride and deuteride transfer are related by the ratios  $\nu_H^\ddagger/\nu_D^\ddagger = 1^{-1/2}/2^{-1/2} = 1.414$ . These parameters, constants, and relationships were combined with protium and deuterium versions of eq 10 in a computer program written in BASIC.<sup>15</sup> Only the deuterium activation energy was used, under the assumption that deuterium tunnels either very little or not at all. When a finite value was obtained for deuterium tunneling, i.e.,  $Q_D = 1.36$ , the activation energy was corrected upward accordingly, but the small correction did not alter the fit significantly. The program iterated through a series of reaction frequencies until the ratio of  $Q_H$ , calculated from the protium version, and  $Q_D$ , calculated from the deuterium version, reached the preset value reported in Table 1. The process was then repeated at plus and minus the standard error of the ratio. The frequency at convergence for the pressure data on the oxidation of leucocrystal violet was thereby estimated to be  $\nu_H^\ddagger = 797 \pm 12$  cm<sup>-1</sup> at  $Q_H = 1.97 \pm 0.05$ .

(14) Equation 10 was taken from Melander and Saunders,<sup>2</sup> where Plank's constant  $h = 1.592 \times 10^{-37}$  kcal/s, the speed of light  $c = 2.9979 \times 10^{10}$  cm/s, Boltzman's constant  $k_B = 3.3 \times 10^{-27}$  kcal/K, and Avogadro's number  $N = 6.023 \times 10^{23}$ /mol. The expression of Bell evolved through several forms, and unfortunately his final version of 1980 has an error;  $\beta$  is defined as  $2\pi/h\nu^\ddagger$ , as it was in an earlier version, but it should have been identified as  $\beta = E/h\nu^\ddagger$  (see Bell's Appendix C). K. L. Grant and J. P. Klinman (*Bioorg. Chem.* **1992**, *20*, 1-7) encountered the error and inserted the activation energy,  $E$ , into the series and removed it from Bell's  $\alpha$  term while retaining the earlier definition of  $\beta$ . They were able to calculate accurate corrections, but their published expression is missing some parentheses, which can be identified by comparison to a parallel version of the equation using a typesetting that did not require these parentheses (Klinman, J. P. In *Enzyme Mechanism from Isotope Effects*, Cook, P. F., Ed.; CRC Press: Boca Raton, FL, 1991; pp 127-148). The clearest discussion and most reliable documentation of the Bell expression can be found in Melander and Saunders.<sup>2</sup> This version nevertheless fails to include the speed of light (which is discussed later) and Avogadro's number (which is not), both of which are necessary for proper units conversion, and are here included in the component terms of eq 10,  $u_t$  and  $\alpha$ , respectively.

One of the questions Isaacs, Javaid, and Rannala<sup>11</sup> asked was whether solvent had any effect on hydrogen tunneling, because their experience suggested that tunneling only occurred when solvent was largely excluded from a reactive center. They proposed that when solvent is not excluded, then coupled motion between hydride transfer and the solvent could raise the effective mass in the transition state and thereby shorten the de Broglie wavelength to a degree that would make tunneling impossible. To find out, they repeated their experiments in isobutyronitrile, a solvent of similar polarity but larger bulk than acetonitrile, and reported the following: "It was found that the reduction of isotope effect with pressure was slightly less rapid than with acetonitrile"—an answer that is not very satisfying. Now, with the new pressure function available, their question can be asked again in a more quantitative way. Fitting their isobutyronitrile data to eq 8 gives  $Q_H/Q_D = 1.72 \pm 0.24$ , and this value gives  $Q_H = 2.66 \pm 0.62$  in eq 10. A comparison of these data to the acetonitrile data above indicates that significantly more tunneling does indeed occur in the bulkier solvent, just as had been postulated.

**Conclusion.** The effect of pressure on a large deuterium isotope effect suspected of arising in part from hydrogen tunneling must be a continuous function. Such a function is now described, and fitting experimental data to the new function generates precise estimates of the contribution of tunneling and of the Bell tunneling correction factor,  $Q_H/Q_D$ . These in turn allow precise estimates of  $Q_H$  and the imaginary frequency  $\nu^\ddagger$ , which provide for a new type of structure/activity relationship: one can now examine the effects of changes in reactant structures and in reactant conditions not only on rates of reactions but also on the degree and nature of hydrogen tunneling. This is an important advance because even if hydrogen tunneling is not present, the results from such a relationship when posited in terms of tunneling may yield nonsensical results and thereby discredit a false postulate. Should that occur, parameters from this analysis will in all likelihood be instrumental in formulating new transition state theory to replace hydrogen tunneling. The effect of pressure on an isotope effect provides a direct probe into the physical properties of a transition state that is unprecedented and holds great potential.

(15) The following is the BASIC program used to calculate tunneling corrections:

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100 Rem          BASIC PROGRAM TO CALCULATE TUNNELING CORRECTIONS
110 Print "Enter value for Bell's tunneling correction:",
120   Input Q
130 Print "Enter value for the energy of activation in kcal/mol:",
140   Input E
150 Print "Enter temperature in degrees Celcius:",
160   Input T
170 H=1.583695e-37;          Rem Plank's Constant, kcal/s
180 K=3.3300669e-27;        Rem Boltzman's Constant, kcal/K
190 G=6.022e23;             Rem Avogadro's number, molecules/mol
200 C=2.9979e10;           Rem Speed of light, cm/second
210 T=T+273.15;            Rem Temperature, Kelvin
220 For F=500 to 3000 by 1; Rem Iteration of H frequency
230   A=E/(K*T*G);         Rem Bell's alpha term
240   For I=1 to 3;         Rem I is the particle mass
250     V(I)=F*(I)^(-.5)
260     UT=H*V(I)*C/(K*T)
270     For N=1 to 7
280       X(N)=(-1)^N*(exp(A*(UT-2*N*pi)/UT))/((UT-2*N*pi)/UT)
290     Next N
300     S=X(1)+X(2)+X(3)+X(4)+X(5)+X(6)+X(7)
310     Y=0.5*UT/(sin(0.5*UT))-S
320     On I goto 330,340,350;   Rem Y is Q of I
330     QH=Y; Goto 360
340     QD=Y; Goto 360
350     QT=Y
360   Next I
370   If QH/QD<Q then 380 Else 390
380 Next F
390 Print "Qh =",QH
400 Print "Qd =",QD
410 Print "Qt =",QT
420 Print "Qh/Qd =", QH/QD
430 Print "Qh/Qt =", QH/QT
440 Print "v = ",F
450 Stop

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