According to transition state theory,

$$k_r = \frac{\kappa kT}{h} e^{-\Delta G^{\ddagger}/RT} \qquad \text{and} \qquad K_c = e^{-(-\Delta G_c)/RT}$$

$$\text{product ratio} = \frac{(\kappa kT/h)\, e^{-\Delta G_a^{\ddagger}/RT}\, e^{+\Delta G_c/RT}}{(\kappa kT/h)\, e^{-\Delta G_b^{\ddagger}/RT}}$$

$$= e^{(-\Delta G_a^{\ddagger}+\Delta G_b^{\ddagger}+\Delta G_c)/RT}$$

But from Fig. 4.8,

$$\Delta G_b^{\ddagger} - \Delta G_a^{\ddagger} + \Delta G_c = G_b^{\ddagger} - G_a^{\ddagger}$$

The product ratio is therefore not determined by ΔG_c but instead primarily on the relative energy of the two transition states leading to A and B.

The conclusion that the ratio of products formed from conformational isomers is not determined by the conformation population ratio is known as the *Curtin–Hammett principle.*[32] While the rate of the formation of the products is dependent upon the relative concentration of the two conformers, since $\Delta G_b^{\ddagger}$ is decreased relative to $\Delta G_a^{\ddagger}$ to the extent of the difference in the two conformational energies, the conformational preequilibrium is established rapidly, relative to the two competing product-forming steps.[33] The position of the conformational equilibrium cannot control the product ratio. The reaction may proceed through a minor conformation if it is the one which provides access to the lowest-energy transition state.

The same arguments can be applied to other energetically facile interconversions of two potential reactants. For example, many organic molecules undergo rapid proton shifts (tautomerism) and the chemical reactivity of the two isomers may be quite different. It is not valid, however, to deduce the ratio of two tautomers on the basis of subsequent reactions which have activation energies greater than that of the tautomerism. Just as in the case of conformational isomerism, the ratio of products formed in subsequent reactions will not primarily be controlled by the position of the facile equilibrium.

4.5. Isotope Effects

A special type of substituent effect that has proved very valuable in the study of reaction mechanisms is the replacement of an atom by one of its isotopes. Isotopic substitution has most often involved replacing protium by deuterium (or tritium),

32. D. Y. Curtin, *Rec. Chem. Prog.* **15**, 111 (1954); E. L. Eliel, *Stereochemistry of Carbon Compounds*, Mc-Graw-Hill, New York, 1962, pp. 151–152, 237–238.
33. For a more complete discussion of the relationship between conformational equilibria and reactivity, see J. I. Seeman, *Chem. Rev.* **83**, 83 (1983).

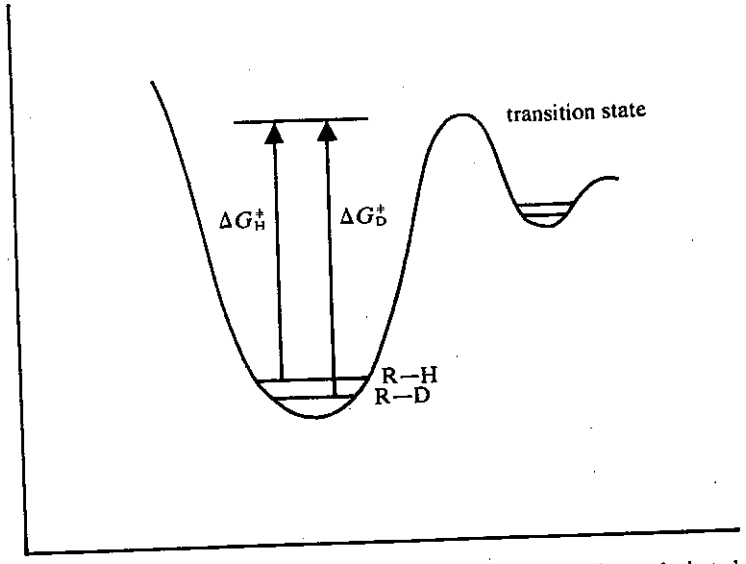


Fig. 4.9. Differing zero-point energies of protium- and deuterium-substituted molecules as the cause of primary kinetic isotope effects.

but the principle is applicable to nuclei other than hydrogen. The quantitative differences are largest, however, for hydrogen. Isotopic substitution has no effect on the qualitative chemical reactivity of the substrate, but it often has an easily measured effect on the rate at which reaction occurs. Let us consider how this modification of the rate arises. Initially, the discussion will concern *primary kinetic isotope effects*, those in which a bond to the isotopically substituted atom is broken in the rate-determining step. We will use C—H bonds as the specific topic of discussion, but the same concepts apply for other elements.

Any C—H bond has characteristic vibrations which impart some energy to the molecule in its normal state. This energy is called the *zero-point* energy. The energy associated with these vibrations is related to the mass of the vibrating atoms. Because of the greater mass of deuterium, the vibrations associated with a C—D bond contribute less to the zero-point energy than do those of the corresponding C—H bond. For this reason, substitution of protium by deuterium lowers the zero-point energy of a molecule. For a reaction involving cleavage of a bond to hydrogen (or deuterium), a vibrational degree of freedom in the normal molecule is converted to a translational degree of freedom on passing through the transition state. The energy difference due to this vibration disappears at the transition state. The transition state has the same energy for the protonated and deuterated species. Since the deuterated molecule had the lower zero-point energy, it necessarily has a higher activation energy to reach the transition state. This is illustrated in Fig. 4.9.

Just how large the rate difference is depends on the nature of the transition state. The maximum effect occurs when the hydrogen being transferred is bound about equally to two other atoms at the transition state. The calculated maximum

for the isotope effect k_H/k_D involving C—H bonds is about 7 at room temperature.[34] When bond breaking is more or less than half complete at the transition state, the value is less and can be close to 1 if the transition state is very reactant-like or very product-like. Primary isotope effects can provide two very useful pieces of information about a reaction mechanism. First, the existence of a substantial isotope effect—that is, if k_H/k_D is 2 or more—is strong evidence that the bond to the isotopically substituted hydrogen atom is being broken in the rate-determining step. Second, the magnitude of the isotope effect provides a qualitative indication of where the transition state lies with respect to product and reactant. A relatively low primary isotope effect implies that the bond to hydrogen is either only slightly or nearly completely broken at the transition state. That is, the transition state must occur quite close to reactant or to product. An isotope effect near the theoretical maximum is good evidence that the transition state involves strong bonding of the hydrogen to both its new and old bonding partner.

Isotope effects may be observed even when the substituted hydrogen atom is not directly involved in the reaction. Such effects are called *secondary kinetic isotope effects.* Secondary isotope effects are smaller than primary ones and are usually in the range of $k_H/k_D = 0.7$–1.5. Secondary isotope effects may be normal ($k_H/k_D > 1$) or inverse ($k_H/k_D < 1$). They are also classified as α, β, etc., depending on whether the isotopic substitution is on the reacting carbon or farther away. Secondary isotope effects result from a tightening or loosening of the C—H bond at the transition state. The strength of the bond may change because of a hybridization change or a change in the extent of hyperconjugation, for example. If sp^3-hybridized carbon is converted to sp^2 as reaction occurs, a hydrogen bound to the carbon will experience decreased resistance to C—H bending. The freeing of the vibration for a C—H bond is greater than that for a C—D bond because the C—H bond is slightly longer and the vibration therefore has a larger amplitude. This will result in a normal isotope effect. Entry 5 in Scheme 4.2 is an example of such a reaction since it proceeds through a carbocation intermediate.

An inverse isotope effect will occur if coordination at the reaction center increases in the transition state. The bending will become more restricted. Entry 4 in Scheme 4.2 exemplifies such a case involving conversion of a tricoordinate carbonyl group to a tetravelent cyanohydrin. In this case the secondary isotope effect is 0.73.

Secondary isotope effects at the β position have been especially thoroughly studied in nucleophilic substitution reactions. When carbocations are involved as intermediates, substantial isotope effects are observed. This is because the hyperconjugative stabilization by the β-hydrogens weakens the C—H bond.[35] The observed

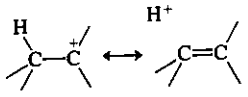
34. K. B. Wiberg, *Chem. Rev.* **55**, 713 (1955); F. H. Westheimer, *Chem. Rev.* **61**, 265 (1961).
35. V. J. Shiner, W. E. Buddenbaum, B. L. Murr, and G. Lamaty, *J. Am. Chem. Soc.* **90**, 809 (1968); A. J. Kresge and R. J. Preto, *J. Am. Chem. Soc.* **89**, 5510 (1967); G. J. Karabatsos, G. C. Sonnichsen, C. G. Papaioannou, S. E. Scheppele, and R. L. Shone, *J. Am. Chem. Soc.* **89**, 463 (1967); D. D. Sunko and W. J. Hehre, *Prog. Phys. Org. Chem.* **14**, 205 (1983).

Scheme 4.2. Some Representative Kinetic Isotope Effects

	Reaction	k_H/k_D(°C)[a]
A. Primary kinetic isotope effects		
1[b]	$PhCH_2{-}H^* + Br\cdot \longrightarrow Ph{-}CH_2\cdot + H^*{-}Br$	4.6 (77)
2[c]	$(CH_3)_2C(H^*){-}C(=O){-}C(H^*)(CH_3)_2 + OH^- \longrightarrow (CH_3)_2C(H^*){-}C(O^-)=C(CH_3)_2$	6.1 (25)
3[d]	H^*-cyclopentyl-$N^+(CH_3)_3 + OH^- \longrightarrow$ cyclopentene $+ (CH_3)_3N$	4.0 (191)
B. Secondary kinetic isotope effects		
4[e]	CH_3O-C_6H_4-$CH{=}O^* + HCN \longrightarrow CH_3O$-$C_6H_4$-$C(H^*)(OH)(C{\equiv}N)$	0.73 (25)
5[f]	H_3C-C_6H_4-$C(H^*)_2Cl \xrightarrow[CF_3CH_2OH]{H_2O} H_3C$-$C_6H_4$-$C(H^*)_2OH$	1.30 (25)
6[g]	CH_2^*, CH_2^* bridged bicyclic $\longrightarrow$ naphthalene $+ CH_2^*{=}CH_2^*$	1.37 (50)

a. Temperature of measurement is indicated in parentheses.
b. K. B. Wiberg and L. H. Slaugh, *J. Am. Chem. Soc.* **80**, 3033 (1958).
c. R. A. Lynch, S. P. Vincenti, Y. T. Lin, L. D. Smucker, and S. C. Subba Rao, *J. Am. Chem. Soc.* **94**, 8351 (1972).
d. W. H. Saunders, Jr., and T. A. Ashe, *J. Am. Chem. Soc.* **91**, 4473 (1969).
e. L. do Amaral, H. G. Bull, and E. H. Cordes, *J. Am. Chem. Soc.* **94**, 7579 (1972).
f. V. J. Shiner, Jr., M. W. Rapp, and H. R. Pinnick, Jr., *J. Am. Chem. Soc.* **92**, 232 (1970).
g. M. Taagepera and E. R. Thornton, *J. Am. Chem. Soc.* **94**, 1168 (1972).

secondary isotope effects are normal as would be predicted since the bond is weakened.



Detailed analysis of isotope effects reveals that there are many other factors which can contribute to the overall effect in addition to the dominant change in bond vibrations. For that reason, it is not possible to quantitatively predict the magnitude of either primary or secondary isotope effects for a given reaction.

Furthermore, there is not a sharp numerical division between primary and secondary effects, especially in the range between 1 and 2. For these reasons, isotope effects are usually used in conjugation with other criteria in the description of reaction mechanisms.[36]

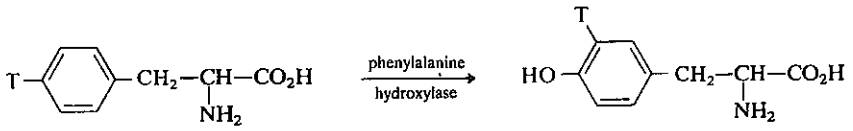
4.6. Isotopes in Labeling Experiments

A quite different use of isotopes in mechanistic studies involves their use as labels for ascertaining the location of a given atom involved in a reaction. As in kinetic experiments, the isotopic substitution will not qualitatively affect the course of the reaction. The nuclei most commonly used for isotopic tracer experiments in organic chemistry are deuterium, tritium, and the ^{13}C and ^{14}C isotopes of carbon. There are several means of locating isotopic labels. Deuterium can frequently be located by analysis of NMR spectra. In contrast to the normal ^{1}H isotope, deuterium does not show an NMR signal under the normal operating circumstances. The absence of a specific signal can therefore be used to locate deuterium. Both mass spectrometry and infrared spectroscopy also can be used to locate deuterium. Tritium and ^{14}C and other radioactive isotopes are usually located on the basis of the radioactivity. This is a very sensitive method. It should be borne in mind that, in most experiments in which radioactive labels are used, only a small fraction of the atoms at the site of substitution are the radioactive nuclide. The high sensitivity of radioactive atoms to detection makes the method practical. Usually, the location of ^{14}C requires a degradation process of some sort to separate the atoms that might conceivably be labeled. Carbon-13 has become an important isotope for tracer experiments relatively recently. Unlike normal ^{12}C, ^{13}C has a nuclear magnetic moment and can be detected in NMR spectrometers of the proper frequency. This avoids the necessity of developing a degradation scheme to separate specific carbon atoms.

There are many excellent examples of isotopic labeling experiments in both organic chemistry and biochemistry.[37] An interesting example is the case of hydroxylation of the amino acid phenylalanine which is carried out by the enzyme phenyl-

36. For more complete discussion of isotope effects, see W. H. Saunders, in *Investigation of Rates and Mechanisms of Reactions*, E. S. Lewis (ed.), *Techniques of Chemistry*, Third Edition, Vol. VI, Part 1, Wiley-Interscience, New York, 1974, pp. 211–255; L. Melander and W. H. Saunders, Jr., *Reaction Rates of Isotopic Molecules*, Wiley, New York, 1980; W. H. Saunders, in *Investigation of Rates and Mechanisms of Reactions*, C. F. Bernasconi (ed.), *Techniques of Chemistry*, Fourth Edition, Vol. VI, Part 1, Wiley-Interscience, New York, 1986, Chapter VIII.
37. For examples of use of isotopic labels in mechanistic studies, see V. F. Raaen, in *Investigation of Rates and Mechanisms of Reactions*, E. S. Lewis (ed.), *Techniques of Chemistry*, Vol VI, Part 1, Wiley-Interscience, New York, 1974, pp. 257–284, and *Isotopes in Organic Chemistry*, Vols. 1–4, E. Buncel and C. C. Lee, (eds.), Elsevier, New York, 1975–1978; C. Wentrup, in *Investigation of Rates and Mechanisms of Reactions*, C. F. Bernasconi (ed.), *Techniques of Chemistry*, Fourth Edition, Vol. VI, Part 1, Wiley-Interscience, New York, 1986, Chapter IX.

alanine hydroxylase.



When this reaction was studied by use of tritium, the phenylaline was labeled with tritium at the 4-position of the phenyl ring. When the product, tyrosine, was isolated, it retained much of the original radioactivity, even though the 4-position was now substituted by a hydroxyl group. When this was studied in detail, it was found that the ^3H originally at the 4-position had rearranged to the 3-position in the course of oxidation. This hydrogen shift, called the *NIH shift*,[38] has subsequently been found to occur in many biological oxidations of aromatic compounds.

4.7. Characterization of Reaction Intermediates

Identification of the intermediates in a multistep reaction is a major objective of studies of reaction mechanisms. When the nature of each intermediate is fairly well understood, a great deal is known about the reaction mechanism. The amount of an intermediate present in a reacting system at any instant of time will depend on the rates of the steps by which it is formed and the rate of its subsequent reaction. A qualitative indication of the relationship between intermediate concentration and the kinetics of the reaction can be gained by considering a simple two-step reaction mechanism:

$$\text{reactants} \xrightarrow{k_1} \text{intermediate} \xrightarrow{k_2} \text{products}$$

In some reactions, the situation $k_1 > k_2$ exists. Under these conditions, the concentration of the intermediate will build up as it goes on more slowly to product. The possibility of isolating, or at least observing, the intermediate then exists. If both k_1 and k_2 are large, the reaction may proceed too rapidly to permit isolation of the intermediate, but spectroscopic studies, for example, should reveal the existence of two distinct stages for the overall reaction. It should be possible to analyze such a system and determine the two rate constants.

If the two steps are of about equal rates, only a small concentration of the intermediate will exist at any time. It is sometimes possible to interrupt such a reaction by lowering the temperature rapidly or adding a reagent that stops the reaction and isolate the intermediate. Intermediates can also be "trapped." In this approach, a compound that is expected to react specifically with the intermediate is added to the reaction system. If trapping occurs, the intermediate is diverted from

38. From its discovery at the National Institutes of Health (NIH); for an account of this discovery, see G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, and S. Udenfriend, *Science* **157**, 1524 (1967).