Simultaneous Determination of Intermolecular and Intramolecular ^{13}C and ^{2}H Kinetic Isotope Effects at Natural Abundance

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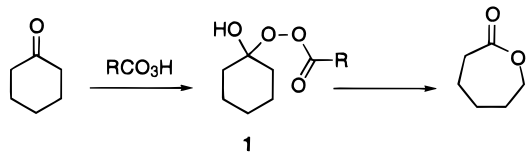
Kinetic isotope effects (KIEs) are a uniquely powerful probe of reaction mechanisms. Most measurements of KIEs provide *intermolecular* effects on the rate-limiting step for a reaction. This is true whenever the rates of labeled versus unlabeled materials are compared, whether by absolute kinetics or in competition reactions.[1] As such, most isotope effect determinations probe only the rate-limiting step and provide no direct information about the rest of a mechanism. The elegant use of *intramolecular* isotope effects to overcome this limitation was pioneered by Dolbier[2] and elaborated by Stevenson.[3] When a partially labeled molecule that is committed to react (having passed through the rate-limiting transition state) still has a stereochemical or regiochemical choice of reactive isotopes, the product distribution reflects the KIE for the product-determining step. Studies of these intramolecular KIEs have been used in numerous cases to obtain otherwise rare information about intermediates after the rate-limiting step.[4] In addition, comparisons of intermolecular and intramolecular KIEs are useful for distinguishing single-step from multi-step mechanisms.[5]

Such studies of intramolecular/intermolecular isotope effects face several difficulties and limitations. The intermolecular and intramolecular KIEs must be reliably distinguished as having different values, requiring either very precise KIE determinations or the presence of large KIEs. In practice, these studies are often limited to reactions involving large primary H/D KIEs, and comparisons of intermolecular and intramolecular ^{13}C KIEs have not been reported at all. In addition, the required regio- or stereospecific synthesis of partially labeled materials as well as fully labeled substrates can be arduous, often prohibitively so. We describe here simple methodology that overcomes these problems and allows the simultaneous measurement of the intramolecular and intermolecular ^{2}H and ^{13}C KIEs from a single experiment at natural abundance.

The test reaction for the methodology was the Baeyer−Villiger oxidation of cyclohexanone to ϵ-caprolactone. Considerable evidence supports the Criegee two-step mechanism for the Baeyer−Villiger reaction, involving hemiperacetals (e.g., **1**) as key intermediates.[6,7] Mechanistic study has generally focused on whether the first or second step is rate limiting and the nature of the alkyl migration step. Under many reaction conditions the second step has been established as rate limiting, and the full repertoire of physical organic probes, including kinetic isotope effects,[8] have been used to characterize the migration step. Such studies have supported a concerted loss of the leaving group and alkyl migration. Rate-limiting formation of the hemiperacetal has been suggested in some cases, though this mechanism is less well established. Necessarily, little is known about the alkyl migration step when the first step is rate limiting.



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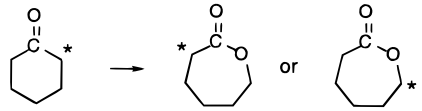
Intermolecular isotope effects for the oxidation of cyclohexanone with m-CPBA were determined by previously reported methodology for the multisite measurement of isotope effects at natural abundance.[9] Reactions of cyclohexanone on an ≈0.3 mol scale in methylene chloride were taken to 79 ± 3% and 63 ± 3% conversion by the slow addition of m-CPBA in portions, and the unreacted cyclohexanone was reisolated by an extractive workup followed by chromatography. The change in ^{2}H and ^{13}C isotopic composition in the recovered cyclohexanone compared to the original material was then analyzed by NMR using the C4 methylene group as an internal standard with the assumption that its isotope composition does not change. From the changes in isotope composition the ^{2}H and ^{13}C KIEs and errors were calculated as previously described.[9] The resulting intermolecular KIEs are summarized in Figure 1a.

The intramolecular isotope effects for this oxidation can be determined from the same reactions! Alkyl migration in **1** involves a choice between the migration of two enantiotopic α-methylene groups. When either α-methylene group contains a ^{13}C or ^{2}H, the rate of its migration compared to the other, unlabeled, α-methylene group will depend on the KIE for the migration step.[10] This will be reflected in the isotopic distribution in the product caprolactone. The intramolecular KIEs can thus be determined in principle from the natural-abundance ^{13}C or ^{2}H NMR integrations for the caprolactone.



In practice this requires an accurate and precise measurement of the relative integrations of two different peaks within an NMR spectrum.[11] This is more difficult than the process used to determine the intermolecular isotope effects, as that methodology involves the comparison of relative integrations versus a standard and allows the cancellation of many potential sources of systematic error. Systematic errors are a particular problem with ^{13}C spectra because of the great accuracy required in measuring small ^{13}C KIEs—there is less of a problem with ^{2}H KIEs in part

(1) An exception occurs when the material of interest is not involved in the rate law, as in some nitrations of aromatics. In such cases, a competition reaction could provide KIEs for a product-determining step after the rate-limiting step.

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(10) The observed intramolecular KIEs are *relative*, reflecting both the isotope effect on the rate of migration of a methylene group and any isotope effect on the rate of the migration when the label does not migrate. The latter effect is likely negligible for the ^{13}C KIE, as the nonmigrating carbon remains singly bonded to the same atoms throughout, but a possibly significant ^{2}H KIE in the nonmigrating methylene group must be considered in interpreting the intramolecular KIEs.

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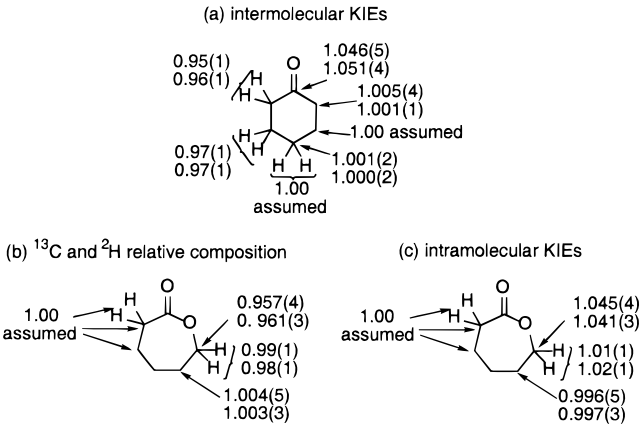
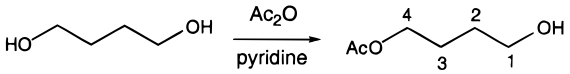


Figure 1. Intermolecular and intramolecular KIEs (k_H/k_D or $k^{12}C/k^{13}C$) for the Baeyer−Villiger oxidation of cyclohexanone, and relative isotopic compositions in the product caprolactone. Standard deviations are shown in parentheses.

because they are usually bigger and in part because the lower signal-to-noise attainable at natural abundance causes random error to predominate. To define the methodology necessary for accurate ^{13}C relative integrations within a single spectrum, we first investigated the effect of various NMR parameters and conditions with a sample of 4-acetoxy-1-butanol (**2**) obtained from acetylation of 1,4-butanediol. Owing to the symmetry of the initial diol and the negligible expected isotope effects at C1/C4 for acetylation, the amounts of ^{13}C at C1 and C4 of **2** should be equal. The same should be true of C2 and C3.



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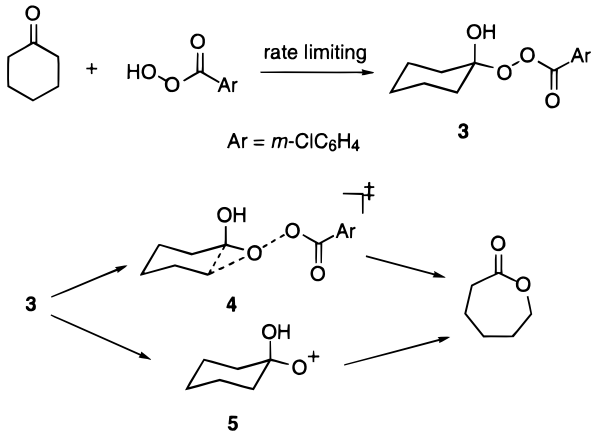
Optimal conditions for obtaining equal integrations at C1 versus C4 and C2 versus C3 of **2** included (1) centering the transmitter frequency between the peaks of interest, (2) a high spectral width but high digital resolution (collecting 225000 points and zero-filling to 512 K with a 400 ppm spectral width at 100 MHz), (3) long delays between pulses ($>8 \times T_1$), (4) a calibrated 90° pulse width, and (5) integration ranges that are a constant 10× multiple of the peak width at half-height. Under these conditions the integrations at C1 versus C4 and C2 versus C3 were consistently equal within the reproducibility of their relative integrations (±0.5%).

Applying this methodology to the product caprolactone from the Baeyer−Villiger oxidation gave the relative ^{13}C and ^{2}H compositions shown in Figure 1b, assigning 1.00 to the nonmigrating side of the lactone. The relative depletion of ^{13}C in the migrating α-methylene group is a measure of the slower rate of migration of a ^{13}C nucleus. The intramolecular KIE in normal form (k_{12C}/k_{13C}) may then be calculated from the reciprocal of the relative compositions in Figure 1b.[10] The results are shown in Figure 1c.

The most notable result is that the oxidation exhibits a large intramolecular ^{13}C KIE and a negligible intermolecular KIE for the α-methylene group. The differing intramolecular and intermolecular KIEs are strong evidence that the migration step occurs after the rate-limiting step. The very large ^{13}C intermolecular KIE at the carbonyl carbon is indicative of rate-limiting addition to the carbonyl,[12] and the slightly inverse α-methylene ^{2}H KIE is consistent with formation of a tetrahedral intermediate.[13] The very small α-methylene ^{13}C KIE indicates that this carbon is not undergoing any substantial change in bonding at the stage of the rate-limiting step.



The intramolecular α-methylene ^{13}C KIE is large and is consistent with a concerted migration and departure of the leaving group.[10] An intramolecular ^{13}C KIE might also be expected if the formation of caprolactone from **3** involved the oxylium ion **5** as an intermediate, as **5** still has a choice of alkyl groups to migrate. However, the highly exothermic alkyl-group migration in **5** would be expected to exhibit a relatively small ^{13}C KIE as it should involve a very early transition state. In support of this idea, calculations (Becke3LYP/6-31G*) only locate **5** and its ring-flipped conformational isomer as saddle points on the potential energy surface when C_s symmetry is enforced, and removal of the symmetry constraint results in barrierless alkyl migration. We therefore consider the large observed intramolecular ^{13}C KIE as inconsistent with **5** and supporting the concerted migration mechanism. The β-methylene ^{13}C KIE of 0.996(5)−0.997(3) provides an internal check of the accuracy of the NMR measurements, as a negligible β KIE would be expected for any mechanism.

Comparison of intermolecular and intramolecular isotope effects is applicable in principle whenever there is a selection between homotopic or enantiotopic groups in a reaction.[14] This first example with ^{13}C KIEs demonstrates the power of this mechanistic probe. The ability to execute this probe at natural abundance should make it conveniently useful in a variety of systems.

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(12) The formation of **2** must also involve proton-transfer step(s), undefined by the results here.

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