MEASURING KINETIC ISOTOPE EFFECTS OF CARBON AT NATURAL ABUNDANCE

Reported by John D. Baird October 31, 2002

INTRODUCTION

Crucial to understanding the selectivity of a reaction is knowledge of its mechanism and rate-limiting step. Methods used to study reaction mechanisms include: linear free-energy relationships,[1] kinetic studies,[2] solvent effect studies,[3] and isotope effects.[4] The measurement of kinetic isotopic effects (KIE) is a well-established and powerful tool for probing reaction mechanisms, and this method has been used to elucidate events taking place in the rate-limiting step of a wide range of reactions. Isotope effects are usually measured by kinetic competition reactions using isotopically labeled reactants. A common example is the observation of primary ^{2}H KIEs to provide information regarding H transfer or secondary ^{2}H KIE to investigate changes in hybridization at an adjacent carbon center. Kinetic isotope effects are not limited to the use of ^{2}H, and KIEs of ^{13}C have been used to determine whether fundamental bonding changes of carbon atoms are occurring in the rate-limiting step of a reaction. While these experiments provide useful information leading to fundamental knowledge in the mechanistic pathway of reactions, the synthesis of isotopically labeled carbon reactants and experimental design required for each reaction can be cumbersome, and the cost of enriched starting materials can be high.

Since carbon exists as a mixture of isotopes, one alternative to the synthesis of ^{13}C-labeled compounds is the use of materials at natural abundance. For example, high precision isotope ratio mass spectrometry (MS) of materials labeled at natural abundance can be used, but this technique relies on selective degradations (without isotopic fractionation) of materials into small molecules suitable for (MS) analysis, such as CO_2.[5] The use of NMR would be an attractive alternative to such degradation experiments, as it would provide both quantitative and position-specific information for KIEs. In the past, techniques using NMR to measure isotope effects were unreliable because the uncertainty in the measurement either exceeded or equaled the size of the KIE due to the inherently low precision of ^{13}C NMR integrations.[6] Because the natural abundance of ^{13}C is 1.1%, measurements must be optimized for high precision when using NMR.[7] Recently, Singleton and coworkers have popularized the use of high field NMR to examine KIEs at natural abundance. Conditions have been developed so that NMR can be used to quantify small kinetic isotope effects at natural abundance in a multiplexed manner.[8] In principle, this allows one to obtain natural abundance KIEs for each carbon in the substrate from one experiment, without the use of degradation reactions or the arduous synthesis of site-specific labeled compounds.

Theory

The theory behind this experiment derives from the relative ratio of competitive reactivity of isotopically labeled substances in reactions. In the course of a reaction, the starting materials are anticipated to become fractionally enriched in the heavier (slower reacting) isotopic components. The observed natural abundance KIEs can then be determined by comparing the percent composition of the recovered (enriched) starting material to the isotopic composition of starting material from the same lot that was not subject to the reaction conditions. The relative ratio of a minor isotopic component in the residual enriched material from the reaction relative to the unreacted starting material can be defined as (R/R_0). This term is a function of the fractional conversion of reactants (F) and the KIE for the reaction (eqn. 2). As seen in Figure 1, as a reaction having a KIE greater than one approaches completion, R/R_0 approaches ∞, and KIEs will become greatly enhanced in the observable R/R_0.[9]

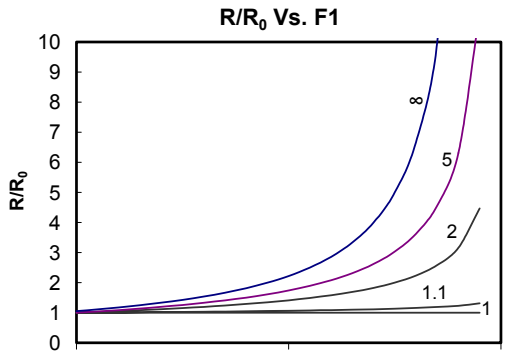


Figure 1. Graph of R/R_0 vs. F for eq. 2. A range of KIE values are shown above each line.

Equation 1. $R/R_0 = (1 - F)^{(1/KIE)-1}$

Equation 2. $KIE_{obs} = \dfrac{\ln(1- F)}{\ln[(1-F)R/R_0]}$

In order for KIE data to be of value, the uncertainty present in the observed KIEs must be low. Therefore, the precision of the analytical technique ($\Delta R/R_0$) and the uncertainty present in the measured F (ΔF) must be sufficiently low to permit analysis by NMR. Because of the inherently low precision of NMR integrations, the uncertainty present in the KIE is dominated by the uncertainty in $\Delta R/R_0$.[8] However, the impact of this uncertainty decreases rapidly as F increases and ΔF becomes insignificant. For example, if a reaction with a KIE of 1.02 is taken to 98.0% conversion, and 2.0% ± 0.1 of unreacted starting material is recovered (1-F = 0.02 ± 0.001), then a 1.5% uncertainty in NMR integrations results in an uncertainty of only 0.003 in the observed KIE. If, however, a reaction having a KIE of 1.02, were taken to only 50% completion (0.50 ± 0.001), then the uncertainty in the KIE would be 0.02 and the KIE would not be significant.

While this technique offers the potential for probing reaction mechanisms, it has several limitations including the requirement that relatively large quantities of material be used. Unreacted starting material must be recovered in sufficient quantity for NMR spectroscopy; reactions chosen for study must be irreversible, and the reaction mechanism must not change as the reaction proceeds. The reaction of interest must be clean with no side product formation.

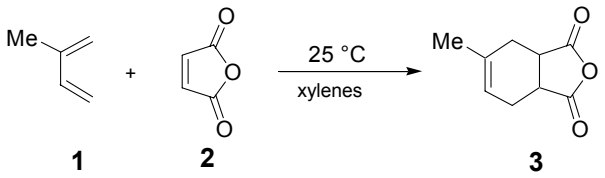
APPLICATION

The determination of natural abundance ^{13}C KIEs by NMR has been applied to a range of reactions including: Diels-Alder cycloadditions,[10] the epoxidation of olefins,[11] Baeyer-Villiger oxidation,[12] organocuprate additions to enones,[13] ene-reactions,[14] the dihydroxylation of olefins,[15] the Claisen rearrangement,[16] palladium-catalyzed trimethylenemethane cycloadditions,[17] cycloadditions of dichlorocarbenes to alkenes,[18] ester aminolysis,[19] the decarboxylation of orotic acid,[20] β–lactone formation,[21] and the polymerization of 1-hexene.[22] Determination of ^{13}C KIEs for these reactions has been particularly useful for understanding changes in bond organization in the rate-limiting step. Furthermore, this method has been extended to the study of intramolecular KIEs, where mechanistic insight arises from competition in the product-determining step.[13] To highlight KIEs measured at natural abundance, several representative examples are presented.

THE DIELS-ALDER REACTION

To determine the KIEs in a diene of a Diels-Alder reaction, Singleton and coworkers carried out the reaction of isoprene **1** with maleic anhydride **2** in xylenes at 25 °C and measured the observed KIEs for the diels-alder adduct shown **3** at natural abundance (Scheme 1).[8] They found that this reaction proceeded via a concerted, slightly asynchronous mechanism. The reaction was carried out to 98.9% completion, and the unreacted starting material was analyzed for isotopic enrichment. The isoprene methyl group was used as an "internal standard" with the assumption that its isotopic composition would not change during the course of the reaction since it is not involved in bond formation or breaking. The results shown in Figure 2 illustrate the isotopic enrichment of the C_1 and C_2 carbons of the isoprene **1**. Using equation 2, the KIEs for carbons 1-4 were calculated (Figure 2b). The KIE values at C_1 and C_4 of 1.022 and 1.017, respectively, suggest a slightly asynchronous process (Figure 2b). Theoretical calculations using Gaussian 94 and Becke3LYP/6-31G* (B3LYP) suggest a value for $\Delta\Delta G^{\ddagger}$ of 1.1kcal/mol favoring the endo pathway.[11] The calculated Becke3LYP/6-31G* (B3LYP) transition state for this reaction suggests that the methyl group introduces a small amount

Scheme 1.



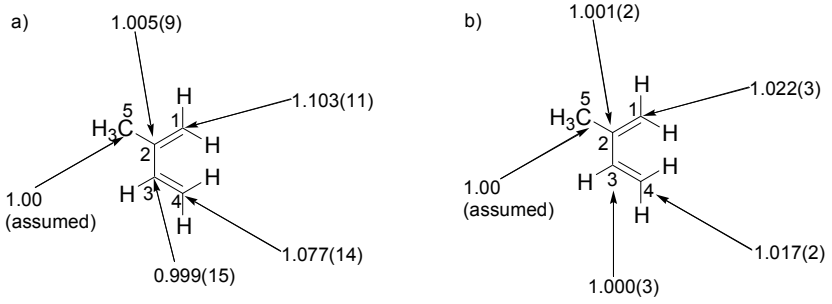


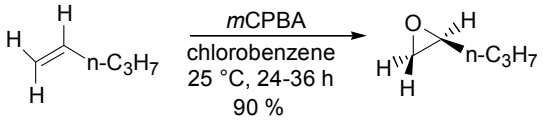
Figure 2. (a) 13C isotopic composition of isoprene recovered from a reaction taken to 98.9% conversion. Standard deviations are listed in parentheses. (b) 13C KIEs (k12C/k13C) calculated using the data in (a) and equation 2.

of asynchronicity in the transition state, with bond formation being more advanced at C_1 of the isoprene unit which is consistent with the experimentally observed KIEs.

EPOXIDATION OF OLEFINS

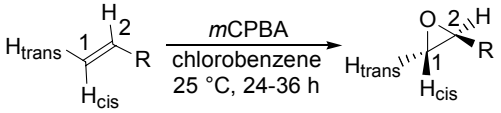
In the epoxidation of olefins, conventional kinetic studies have suggested that the two CO bonds are formed in a nearly synchronous manner.[23] However, MP2/6-31G* calculations suggested that the transition state is highly unsymmetrical and the bond breaking/formation event is asynchronous.[12] To address this issue, Singleton and coworkers chose to study the epoxidation of 1-pentene **4** with meta-chloroperbenzoic acid (mCPBA) to afford oxirane **5** by observing KIEs at natural abundance (Scheme 2).[12] The residual unreacted starting material was recovered, purified, and analyzed by NMR for ^{13}C enrichment, employing the methyl group on 1-pentene as an internal standard. The ^{13}C enrichment in the starting material was compared to material from the same lot analyzed in an identical manner. The KIE values shown in Table 1 for both C_1 and C_2 suggest that the reaction proceeds via a concerted, nearly synchronous formation of both C-O bonds as the observed ^{13}C KIEs are almost identical within experimental uncertainty. To substantiate this observation, theoretical calculations were conducted for the epoxidation of 1-propene with performic acid using both MP2/6-31G* (MP2) and Becke3-LYP calculations. Subsequently, excellent agreement was found between predicted values using (B3LYP) and the experimental results. The MP2 calculations, however, did not correlate well with the experimentally observed values. In several other cases studying concerted cycloadditions, the (B3LYP) calculations have been more successful in predicting KIEs.

Scheme 2.



4 **5**

Table 1.



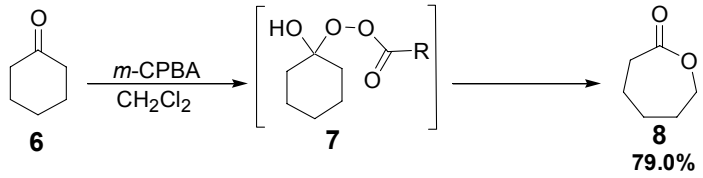
	MP2 [a]	B3LYP [a]	exp 1 [b]	exp 2 [b]	exp 3 [b]
C_1	1.015	1.012	1.013(4)	1.012(3)	1.010(3)
C_2	1.023	1.009	1.009(4)	1.009(4)	1.006(3)
R	CH_3	CH_3	n-C_3H_7	n-C_3H_7	n-C_3H_7

Calculated and experimental ^{13}C KIEs. [a] experiments 1, 2, and 3 were carried out to 90.0(8), 89.4(8), and 81(1.4)% completion, respectively. Standard deviations are listed in parentheses.

THE BAEYER-VILLIGER OXIDATION: PROBING THE PRODUCT DETERMINING STEP

Singleton and coworkers sought to investigate the Criegee intermediate of the Baeyer-Villiger oxidation.[24] Previous work establishes substantial evidence for a two-step reaction mechanism (Scheme 3). However, some debate exists as to which step is rate-limiting, as well as the nature of the migration step. To determine both the inter-, as well as the intramolecular KIEs for this reaction, the authors studied the oxidation of cyclohexanone **6** with *m*-CPBA at natural abundance.

Scheme 3.



Residual starting material was recovered, and intermolecular KIEs were calculated[13] using eqn. 2 (Figure 3a). The significant intermolecular KIE present on the carbonyl carbon suggests rate-limiting formation of the hemi-peracetal intermediate **7,** whereas the very small KIE at the α-methylene carbon suggests that there is very little bonding change at this carbon in the rate-limiting step (Figure 3a). Intramolecular KIEs for this reaction were determined by recovery and analysis of the product lactone **8**. Experimental techniques have been developed to examine a partially labeled molecule that is committed to react (after passing through the rate-limiting step) and has a choice of reactive isotopes, where the product distribution will reflect the KIE for the product-determining step.[25] Because alkyl migration will involve a choice between one of two enantiotopic methylene groups in the hemiperacetal intermediate, the rate of migration of alkyl group bearing a ^{13}C or ^{2}H will be slower compared to the other unlabeled isotope. The difference in rate of migration will depend on the KIE. This is seen in Figure 3b where the relative depletion of ^{13}C in the ε-position, is indicative of a slower migration of a ^{13}C nucleus in the product determining step.[12] The relative intramolecular KIE is calculated by taking the reciprocal of the ratio of $^{13}C/^{12}C$ (Figure 3b).

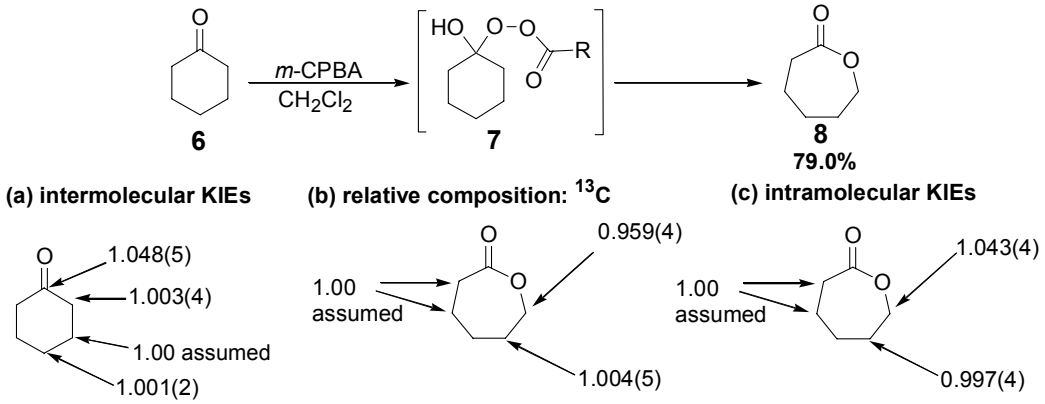
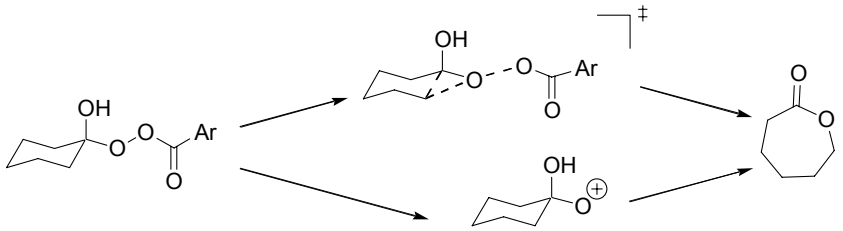


Figure 3. (a) Intermolecular KIEs (k12C/k13C) for the Baeyer-Villiger oxidation of cyclohexanone. (b) relative isotopic composition in product. (c) intramolecular KIEs calculated using equation 2 and the results in (b). Standard deviations are listed in parentheses.

The presence of differing inter- and intramolecular KIEs of the migrating methylene suggests the formation of an intermediate **7.** The substantial α–methylene intramolecular ^{13}C KIE is indicative of migration of this group in the product-determining step, and the magnitude of this value is also consistent with a concerted migration and expulsion of the acid leaving group which would be expected to be large. (Scheme 4, upper path).

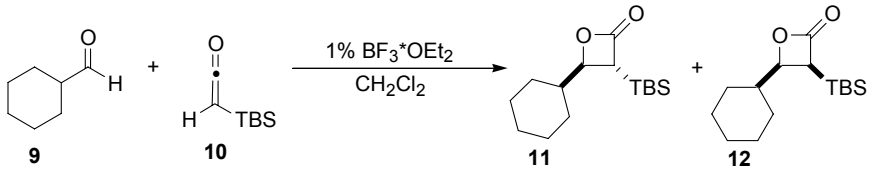
Scheme 4.



LEWIS-ACID CATALYZED FORMATION OF β-LACTONES: A COMPLEX MECHANISM

The synthesis of β-lactones is of great interest due to there usefulness as synthetic intermediates. While theoretical calculations have suggested a concerted process for their formation in some cases, other work has suggested a stepwise process. Recently, Singleton and coworkers have investigated the Lewis-acid catalyzed formation of these heterocyclic compounds to illuminate the mechanistic pathway of their formation.[21] A Lewis-acid catalyzed [2+2] cycloaddition between cyclohexane carboxaldehyde **9** and *tert*butyldimethylsilyl (TBS) ketene **10** afforded the trans-(**11)** and cis-(**12)** β–lactones (Scheme 5). The difficulty involved in recovering the highly reactive ketene required that this experiment be conducted by analyzing the product. In separate experiments taken to low conversion, the product was analyzed by determining the KIEs on the carbons and comparing these to prepared NMR standards of isotopically assured reference products.

Scheme 5.



The observed KIEs shown in Figure 4 suggest a stepwise mechanism for the formation of the *trans* product where the rate-limiting step is the formation of the C_α-C_β bond. However, the KIEs present on all carbons of the ring in the *cis* product suggests either an asynchronous concerted [2+2] cycloaddition or, rate limiting formation of the lactone bond where the KIEs present at the α-carbon and β-carbon arise from unusually large secondary ^{13}C KIEs observed in the formation of charged

intermediates.[21] These results suggest that two very different mechanisms are operating to account for the diastereomeric products. Theoretical calculations suggest a stepwise process with two differing rate-limiting steps where the first step is rate-limiting in the formation of the trans diastereomer and the second step is rate-limiting for the formation of the cis diastereomer. Despite the possible mechanistic explanations for the formation of the *cis* isomer, the differing observed KIEs for these products suggests two different mechanisms are operating in the formation of these products.

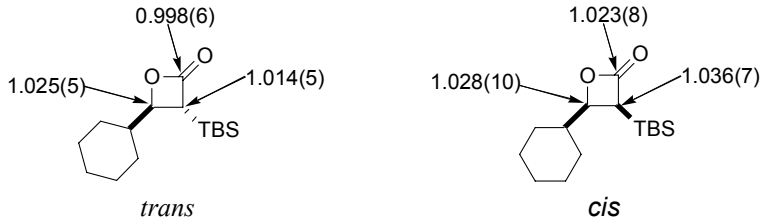


Figure 4. KIEs for cis and trans ß-lactones

CONCLUSION

The use of NMR spectroscopy to measure KIEs at natural abundance is a powerful tool in the probing of reaction mechanisms. The ability to establish mechanistic understanding in reactions can lead to the development of new variations and improve the ability to predict reactivity. This technique is particulary useful because it allows for the rapid analysis of KIEs within a sample in a multiplexed fashion, utilizing the naturally occurring isotopes of nuclei. In contrast to classical KIE experiments requiring sometimes complex site-specific isotopic labeling, this method allows in theory, for the survey of KIEs at every carbon in a substrate. Singleton and coworkers have demonstrated the use and applicability of this new method in a wide scope of reactions. This relatively new technique should find a broad range of application in mechanistic studies in the near future. Furthermore, with respect to advances in NMR technology, this method should become easier and more precise in the immediate future.

REFERENCES

(1) Topsom, R. *Prog. Phys. Org. Chem*. **1976**, *12*, 1.
(2) Wiberg, K. *Physical Organic Chemistry*; Wiley; New York, **1964**; pp 305-351.
(3) Amis, E.; Hinton, J. *Solvent Effects on Chemical Phenomena*; Academic Press; New York, **1973**; pp 15-35.
(4) Westheimer, F. *Chem. Rev.* **1961**, *61*, 265.
(5) O'Leary, M. *Methods Enzymol.* **1980**, *64*, 83.
(6) Zhang, B. *Magn. Reson. Chem.* **1988**, *26*, 955.
(7) Canet, D. *Nuclear Magnetic Resonance*; Wiley: New York, **1996**, pp 3-21.
(8) Singleton, D.; Thomas, A. *J. Am. Chem. Soc.* **1995**, *117*, 9357.
(9) Melander, L.; Saunder, W. *Reactions Rates of Isotopic Molecules*; Wiley; New York, **1980**; pp 91-102.
(10) Beno, B.; Houk, K.; Singleton, D. *J. Am. Chem. Soc.* **1996,** *118*, 9984.

(11) Singleton, D.; Merrigan, S.; Liu, J.; Houk, K. *J. Am. Chem. Soc.* **1997**, *119*, 3385.
(12) Singleton, D.; Szymanski, M. *J. Am. Chem. Soc.* **1999**, *121*, 9455.
(13) Frantz, D.; Singleton, D.; Snyder, J. *J. Am. Chem. Soc.* **1997**, *119*, 3383.
(14) Singleton, D.; Hang, C. *J. Org. Chem.* **2000**, *65*, 895.
(15) Delmonte, A.; Haller, J.; Houk, K.; Sharpless, K.; Singleton, D.; Strassner, T.; Thomas, A. *J. Am. Chem. Soc.* **1997**, *119*, 9907.
(16) Meyer, M.; Delmonte, A.; Singleton, D. *J. Am. Chem. Soc.* **1999**, *121*, 10865.
(17) Singleton, D.; Schulmeier, B. *J. Am. Chem. Soc.* **1999**, *121*, 9313.
(18) Keating, A.; Merrigan, S.; Singleton, D.; Houk, K. *J. Am. Chem. Soc.* **1999**, *121*, 3933.
(19) Singleton, D.; Merrigan, S. *J. Am. Chem. Soc.* **1996**, *118*, 9984.
(20) Singleton, D.; Merrigan, S.; Kim, B.; Beak, P.; Phillips, L.; Lee, J. *J. Am. Chem. Soc.* **2000**, *122*, 3296.
(21) Singleton, D.; Wang, Y.; Yang, H.; Romo, D. *Angew. Chem. Int. Ed.* **2002**, *41*, 1572.
(22) Landis, C.; Rosaaen, K.; Uddin, J. *J. Am. Chem. Soc.* **2002**, *124*, 12062.
(23) Lynch, B.; Pausacker, K. *J. Chem. Soc.* **1955**, 1525.
(24) Criegee, R. *Justus Liebigs Ann. Chem.* **1948**, *560*, 127.
(25) Dai, S.; Dolbier, W. *J. Am. Chem. Soc.* **1972**, *94*, 3946.