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Communications to the Editor

High-Precision Simultaneous Determination of Multiple Small Kinetic Isotope Effects at Natural Abundance

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Small heavy-atom and secondary hydrogen kinetic isotope effects (KIEs) can provide detailed information about the mechanism of an exceptional range of chemical reactions.[1,2] However, there are significant general limitations in methods for the determination of these KIEs. Because absolute rate measurements are rarely sufficiently precise, small KIEs are usually determined in competition reactions of isotopically labeled and unlabeled materials. This is possible only in systems carefully chosen to allow the precise measurement of isotopomer ratios with appropriate analytical techniques, such as scintillation counting for ^{3}H and ^{14}C KIEs. The synthesis of isotopically labeled materials can be arduous, often prohibitively so, and a new synthesis, competition reaction, and analysis are required for each KIE of interest. A broadly useful alternative, particularly for ^{13}C KIEs, is to employ the high precision of isotope ratio mass spectrometry to study KIEs in materials labeled only at natural abundance.[3] A major restriction is that each site of interest must be selectively degradable without isotopic fractionation into an analyzable small molecule, most often CO_2.

The isotope- and position-specific information inherent in NMR techniques seems ideally suited to measuring KIEs at natural abundance. The utility of ^{2}H NMR for determining large ^{2}H KIEs at natural abundance has been established,[4] and in theory, all of the individual KIEs in reactions of complex natural abundance materials can be determined simultaneously![5] In practice, however, NMR quantitation has not been sufficiently precise to be useful with small KIEs, the uncertainty in the few cases tried generally rivaling or exceeding the size of the isotope effects.[4,5] We report here a simple general method for attaining chemically significant precision while simultaneously measuring all of the KIEs for reactions at natural abundance.

As any reaction proceeds, the starting materials are fractionatively enriched in isotopically slower-reacting components. The proportion of a minor isotopic component in recovered material compared to the original starting material (R/R_0) is related to the fractional conversion of reactants (F) and the KIE (relative rate for major/minor isotopic components) by eq 1.[6] As a reaction approaches completion (F→1), R/R_0 approaches ∞, and KIEs become greatly magnified in the observable R/R_0. For example, a KIE of 1.05 results in ∼25% enrichment of a slower-reacting isotopic component at 99% conversion. It has long been recognized that this ordinary effect of kinetic fractionation can improve the precision of KIE determinations,[7] but the degree of improvement possible depends critically on the magnitude of the KIE, the precision of the analytical technique ($\Delta R/R_0$), and the uncertainty in F (ΔF).

$$R/R_0 = (1 - F)^{(1/\mathrm{KIE})-1} \tag{1}$$

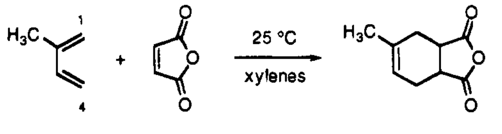
$$\mathrm{KIE}_{\mathrm{calcd}} = \frac{\ln(1 - F)}{\ln[(1 - F)R/R_0]} \tag{2}$$

$$\Delta\mathrm{KIE}_F = \frac{\partial \mathrm{KIE}}{\partial F}\Delta F = \frac{-\ln(R/R_0)}{(1 - F)\ln^2[(1 - F)R/R_0]}\Delta F \tag{3}$$

$$\Delta\mathrm{KIE}_R = \frac{\partial \mathrm{KIE}}{\partial (R/R_0)}\Delta(R/R_0) = \frac{-\ln(1 - F)}{(R/R_0)\ln^2[(1 - F)R/R_0]}\Delta(R/R_0) \tag{4}$$

The uncertainties in calculated KIEs (eq 2[6]) due to $\Delta R/R_0$ ($\Delta\mathrm{KIE}_R$) and ΔF ($\Delta\mathrm{KIE}_F$) are shown in eqs 3 and 4, respectively.[8] Most commonly, either the KIE is large or the measurement of R/R_0 is highly precise ($\Delta R/R_0$ small). In these cases, the uncertainty in the KIE is dominated by $\Delta\mathrm{KIE}_F$ (not counting any systematic error), and no advantage is gained at high conversion. However, for the relatively low precision of NMR integrations, the uncertainty in the KIE is dominated by $\Delta\mathrm{KIE}_R$, which decreases greatly as F increases, and $\Delta\mathrm{KIE}_F$ is nearly negligible. For example, with a KIE of 1.02 and a 1.0% ± 0.1 measurement of unreacted starting material ($1 - F = 0.01 \pm 0.001$), $\Delta\mathrm{KIE}_F$ is only 0.0004, and a 1.5% uncertainty in NMR integrations results in an uncertainty ($\approx\Delta\mathrm{KIE}_R$) of only 0.003 in the KIE.

To test this idea, the Diels−Alder reaction of isoprene with maleic anhydride (eq 5) was studied. The relative proportion of ^{2}H and ^{13}C at the various positions of isoprene, compared to the original starting material, could be readily determined by ^{2}H and ^{13}C NMR[9] using the methyl group as an "internal standard" on the assumption that its isotopic composition does



(5)

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(1) For some varied recent applications of heavy-atom isotope effects, see: Burger, R. M.; Tian, G.; Drlica, K. *J. Am. Chem. Soc.* **1995**, *117*, 1167. Jankowski, S.; Quin, L. D.; Paneth, P.; O'Leary, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 11675. Holm, T. *J. Am. Chem. Soc.* **1994**, *116*, 8803. Zhang, Y.; Bommuswamy, J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1994**, *116*, 7557. Kupczyk-Subotkowska, L.; Saunders, W. H., Jr.; Shine, H. J.; Subotkowski, W. *J. Am. Chem. Soc.* **1993**, *115*, 5957. Yamataka, H.; Nagareda, K.; Takatsuka, T.; Ando, K.; Hanafusa, T.; Nagase, S. *J. Am. Chem. Soc.* **1993**, *115*, 8570. Rao, M.; Barlow, P. N.; Pryor, A. N.; Paneth, P.; O'Leary, M. H.; Quinn, D. M.; Huskey, W. P. *J. Am. Chem. Soc.* **1993**, *115*, 11676.

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(4) Pascal, R. A., Jr.; Baum, M. W.; Wagner, C. K.; Rodgers, L. R.; Huang, D.-S. *J. Am. Chem. Soc.* **1986**, *108*, 6477 (see especially footnote 9b).

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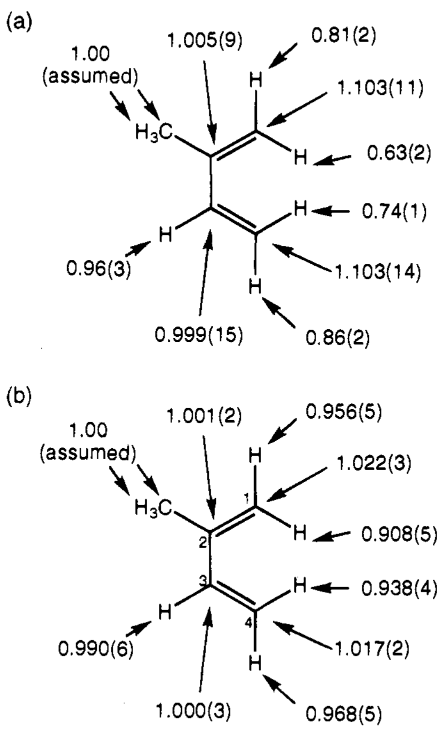

Figure 1. (a) ^{2}H and ^{13}C isotopic composition of isoprene recovered from a reaction taken to 98.9% completion, relative to starting isoprene, with standard deviations in parentheses (n =11 (3 samples) for ^{13}C data; n = 5 (2 samples) for ^{2}H data). (b) ^{2}H and ^{13}C KIEs (k_H/k_D and $k_{^{12}C}/k_{^{13}C}$) calculated from the results in (a) and eq 2.

not change.[10] As the reaction proceeds, the relative proportion of ^{13}C at positions 1 and 4 of isoprene increases, and the proportion of deuterium in these positions decreases. Isoprene recovered by distillation[11] from a reaction taken to 98.9(1)% completion[12] was analyzed in detail, and the results are shown in Figure 1a. The ^{2}H and ^{13}C KIEs (k_H/k_D and $k_{^{12}C}/k_{^{13}C}$) could be calculated from eq 2 and are shown in Figure 1b.

The KIEs determined in this manner are consistent with KIEs estimated at lower conversion (with concomitantly decreased precision).[13] Within experimental error, the calculated KIEs are independent of F. They are also in line with general expectations, prior experimental results, and theoretical calculations. The KIEs for C_2, C_3, and H_3 are very small, as would be expected for nonreacting centers. The individual ^{2}H KIEs for the 1 and 4 positions of isoprene may be compared with aggregate KIEs from previous studies of the reactions of [1,1,4,4-D_4]butadiene or [1,1,4,4-D_4]isoprene with symmetrical dienophiles.[14,15] At 25 °C, previous studies found KIEs per deuterium (=KIE$^{0.25}$) of 0.935,[14] 0.943,[15] 0.948,[15] and 0.938,[15] compared to a geometric mean of 0.942 in the current study. It is notable that there have been no previous studies of either ^{13}C or ^{14}C KIEs for the diene in a Diels–Alder reaction.[16] However, the ^{13}C KIEs for C_1 and C_4 are in line with recent predictions of ^{14}C KIEs for the concerted Diels–Alder reaction of butadiene with ethylene based on theoretical calculations.[17] The same calculations predicted that significantly more pronounced ^{2}H isotope effects would be observed for a diene's "inside" hydrogens (H_{1Z} and H_{4Z}) than for the "outside" hydrogens (H_{1E} and H_{4E}). The results experimentally verify that prediction.

The more pronounced KIEs for ^{2}H substitution on C_1 over C_4 indicate some asynchronicity in bond formation to C_1 versus C_4 at the transition state. In Gajewski's elegant study of the reaction of [4,4-D_2]- and [1,1,4,4-D_4]isoprene with symmetrical dienophiles, some of the KIE results had also suggested asynchronicity but were always consistent, within 2–5% error limits, with a perfectly synchronous mechanism.[15] The indication of asynchronicity in the current results is well outside of experimental error.

The major limitation on the determination of KIEs by this method is that the reaction must be sufficiently scalable to allow recovery of adequate starting material for the ^{2}H NMR analysis. The method also requires that the starting material be isolable from a large amount of product. The reaction of interest must be irreversible, and the reaction mechanism must not change over the course of the reaction. However, the KIEs should be unaffected by secondary conversions of the products. This method should also be relatively insensitive to impurities in the analyzed material. Indeed, it is completely insensitive to impurities unless they overlap in the NMR, and a 2% natural abundance impurity causes only ~0.004 error in a KIE measured at 99% conversion. It should be noted that such contamination in the ^{2}H NMR can be detected from a careful ^{1}H NMR integration.

By this method, a level of detailed KIE information often unattainable in conventional ways can be quickly obtained with routine instrumentation, and the determination of KIEs can become a routine part of the characterization of chemical reactions. We are currently applying this technique to a wide variety of classical and topical reactions.

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Supporting Information Available: Procedures for reactions, NMR measurements, and calculations (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(9) As described in the supporting information, a number of precautions were taken to minimize both random and systematic errors in the NMR analysis. See: Rabenstein, D. L.; Keire, D. A. In *Modern NMR Techniques and Their Application in Chemistry*; Popov, A. I., Hallenga, K., Eds.; Marcel Dekker: New York, 1991; pp 323–369. Samples were prepared identically, a T_1 determination was carried out for each sample, and the results from a single sample that exhibited significantly different T_1's were excluded. ^{13}C spectra were obtained with gated ^{1}H decoupling and a 63 s delay between calibrated $2\pi/9$ pulses. Variations in the data workup (integration regions, baseline correction methods, phasing), when applied consistently for both original and recovered material, did not significantly affect the calculated KIEs. The major source of variability in integrations appeared to be random effects from signal-to-noise limitations and baseline variations.

(10) Because of this assumption, the resulting KIEs are "relative", erring systematically to whatever extent the assumed ^{2}H and ^{13}C KIEs for the methyl group are incorrect. A small systematic error would not affect the interpretation of the current results. In cases where a reasonable internal standard is absent, the isotopic composition can be referenced to an external standard (see ref 5) or, better, groups introduced by chemical derivatization.

(11) Because the NMR analysis compares relative amounts of monodeuterated or mono-^{13}C materials, the effect of isotopic fractionation in the reisolation should be negligible.

(12) Based on a determination of 1.1(1)% remaining isoprene by GC analysis versus heptane as an internal standard.

(13) The calculated ^{13}C KIEs for reactions taken to 72(2), 81(2), 90(1), 95(1), and 96.0(0.4)% conversions were, respectively: for C_1, 1.022(7), 1.025(8), 1.019(4), 1.020(5), and 1.023(4); for C_2, 0.999(9), 1.000(6), 1.001(5), 1.000(3), and 1.000(4); for C_3, 1.007(8), 1.004(4), 0.999(4), 1.004(3), and 1.001(4); for C_4, 1.020(8), 1.017(8), 1.014(5), 1.017(5), and 1.019(3), respectively. The ^{2}H KIEs for reactions taken to 81(2) and 90(1)% conversions were, respectively: for H_{1E}, 0.961(9), 0.966(13); for H_{1Z}, 0.909(13), 0.899(10); for H_3, 0.982(19), 0.994(9); H_{4E}, 0.968(12), 0.959(9); H_{4Z}, 0.940(11), 0.947(10).

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(16) For a study of ^{14}C KIEs in the *dienophile*, see: Kupczyk-Subotkowska, L.; Shine, H. J. *J. Am. Chem. Soc.* **1993**, *115*, 5296.

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