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Measurements of Heavy-Atom Isotope Effects Using ^{1}H NMR Spectroscopy

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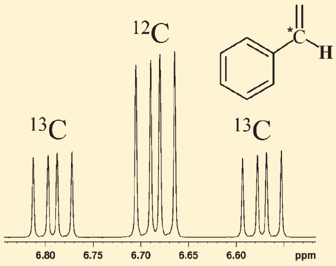
S Supporting Information

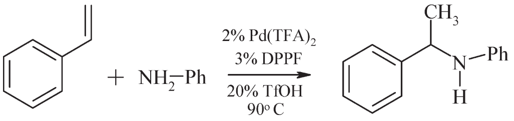
ABSTRACT: A novel method for measuring heavy-atom KIEs for magnetically active isotopes using ^{1}H NMR is presented. It takes advantage of the resonance split of the protons coupled with the heavy atom in the ^{1}H spectrum. The method is validated by the example of the ^{13}C-KIE on the hydroamination of styrene with aniline, catalyzed by phosphine-ligated palladium triflates.



Kinetic isotope effects (KIEs, ratios of rate constants of light-to-heavy isotopologues) are very subtle tools for studying mechanisms of organic reactions that allow the deduction of the structure and properties of transition states, entities not yet amenable to direct experimental scrutiny.[1] Their use is, however, hampered by the high cost of isotope-ratio mass spectrometers and the tedious procedure required frequently for conversion of reactants into gaseous form suitable for analysis. Therefore, other experimental techniques of measuring KIEs are being sought. One of these emerging tools is nuclear magnetic resonance (NMR) spectroscopy.[2] Singleton has introduced ^{13}C NMR using samples of natural isotopic abundance.[3] Measuring deuterium isotope effects by NMR using natural abundance was also proposed in the literature.[4] The main limitation of this approach is its low sensitivity and the necessity of using large amounts of material. It also suffers from the fact that only relative isotope effects can be measured; thus, the isotope effect of a chosen reference atom has to be assumed. Bennet improved this method to gain higher sensitivity and to avoid dependence on the reference KIE at the expense of isotopic synthesis by using ^{13}C-labeled material.[5] Both of these methods suffer from the low sensitivity of ^{13}C NMR compared to ^{1}H NMR. Recently, Bennet used the splitting of ^{19}F NMR signals for the purpose of measuring kinetic isotope effects.[6]

Here we present a novel method for measuring KIEs for magnetically active heavy-atom isotopes (e.g., ^{15}N, ^{13}C), which is based on the isotopic change of the signal shape observed in the ^{1}H NMR spectra. In this method we use a combination of a compound at natural abundance and its heavy-atom isotopologue, labeled with ^{13}C at a position for which the isotopic fractionation is measured. The resonance split of the protons coupled with the heavy atom in the ^{1}H spectrum compared to the unlabeled compound allows for direct measurement of the ratio of concentrations of the two isotopologues (isotopic ratio, R). As an example of this, we have chosen the ^{13}C-KIE for the hydroamination of styrene with aniline, catalyzed by phosphine-ligated palladium triflates (Scheme 1), which has been shown to exhibit a substantial ^{13}C-KIE at the benzylic carbon.[7]

Scheme 1



In the proposed methodology, we have used a combination of two isotopologues, styrene of natural abundance and (α-^{13}C)-styrene, in order to obtain the ^{13}C/^{12}C ratio from ^{1}H NMR signals of benzylic hydrogen of the unreacted substrate. One of these signals in the unlabeled isotopologue is a doublet of doublets at 6.685 ppm, while it is additionally split with a coupling constant of 153.3 Hz in the ^{13}C-labeled reactant, as illustrated in Figure 1.

The obtained value of ^{13}C-KIE, 1.036, is in excellent agreement with the value of 1.037 determined theoretically and is slightly higher than 1.030 ± 0.003 (95% confidence level) determined experimentally.[7] The difference between the two experimental KIE values may be due to the better sensitivity of the method proposed herein or a small secondary isotope effect of the reference carbon atom. This latter case would indicate that the model in which aniline is truncated to ammonia does not yield correct results. In order to find out if the theoretical model is in fact the source of the discrepancy between theoretically predicted and experimentally observed KIE, we have performed theoretical calculations extending the model to include the whole aniline molecule. Three transition states, **1a**−**c**, illustrated in Figure 2 (detailed geometries, energies, and frequencies are given

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in the Supporting Information), have been found differing in the C6−C9−N12−C30 dihedral angle (atom numbering and values of the torsional angle given in Figure 2). The most stable structure **1a**, given on the left, is more stable by 0.9 and 1.6 kcal/mol than structures shown in the center (**1b**) and on the right (**1c**) of Figure 2, respectively. The obtained ^{13}C-KIEs for α and *para* positions are 1.0372 and 1.0007, respectively, in agreement with the results reported previously by Singleton. Thus, it seems that the method presented herein yields results slightly more accurate that those based on ^{13}C NMR.

In conclusion, we present a new NMR approach to measurements of heavy-atom isotope effects that takes advantage of the high sensitivity of ^{1}H NMR spectra. It should be noted, however, that it can be applied only in the advantageous cases when the proton signal of interest is not overlapping with any other signals, a much less frequent case than in ^{13}C NMR spectra, although there is some flexibility here; only one satellite signal may be used for kinetic studies if the other one overlaps with other peaks. In such fortunate cases it can be applied to both chemical as well as enzymatic reactions; we are currently working on the application of this method to the studies of binding isotope effects on binding inhibitors to lactic dehydrogenases that should allow us to distinguish isoforms of the enzyme.[8] On the other hand, measurements of ^{15}N KIEs using this method would be challenging in systems that have exchangeable N−H protons in the reactant and product.

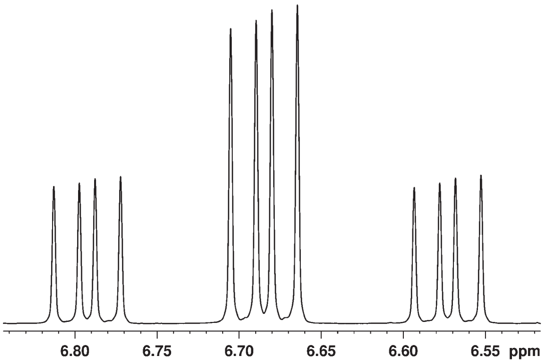

Figure 1. ^{1}H NMR signals of benzylic hydrogen of a mixture of styrene at natural abundance and (α-^{13}C)styrene.

EXPERIMENTAL SECTION

The reaction protocol followed that described in the literature[7] with a small modification that was made because we did not observe full separation of the reactants until the reactant mixture was saturated with gaseous HCl and amine reactants were converted into their respective ammonium salts. Additionally, we used anhydrous deuterated benzene (distilled from Na/benzophenone, 99.5% isotopic purity, The Radioisotope Production and Distribution Centre, Swierk, Poland) as the reaction medium, which simplified the preparation of NMR samples, and the scale was reduced to the 0.63 mmol level. The reaction was carried out at 90 °C. Integrations of vinylic peaks of styrene (5.709 and 5.176 ppm) and the sum of quartet (4.323 ppm) and satellite quartets (4.418 and 4.223 ppm) corresponding to benzylic protons of the reaction product were used in the determination of the fraction of reaction, *f*. The isotopic composition of the starting material at the α-^{13}C position was kept close to unity (see the Supporting Information). Styrene samples with a natural abundance of isotopes (ReagentPlus grade, $\geq$99%) and α-^{13}C labeled (99% isotopic purity) were obtained from Sigma-Aldrich and used without further purification.

All samples for NMR measurements were prepared using the same amount of styrene (20 mg) in 0.7 mL of C_6D_6 in a 5 mm NMR tube. The ^{1}H spectra were recorded at 700.21 MHz at 300 K, using 85.5 s delays between 45° pulses, an 11.010 s acquisition time, and 131 072 points. Because of the relatively high time constant T_1 = 10.5 s of the proton attached to the α-^{12}C carbon atom, the $8T_1$ repetition time and a pulse of $\pi/4$ instead of $\pi/2$ were applied to ensure the complete magnetization recovery. A zeroth order baseline correction was applied, and integrations of each doublet of doublets of 6.685 ppm signals were performed using an integration region equal to the width of doublet of doublets expanded by 10 half-widths of singlet lines. Spectra were recorded for 64 or more scans to achieve a signal-to-noise ratio of at least 1000. Isotopic ratios were calculated from the ratio of sum of integrations of satellite doublets of doublets and central doublet of doublets (Figure 1).

The ^{13}C-KIE has been evaluated from the logarythmic dependence correlating the isotopic ratios of the remaining reactant with the reaction progress:

$$\ln R_f = \left(\frac{1}{\mathrm{KIE}} - 1\right)\ln(1-f) + \ln R_0 \qquad (1)$$

where *f* is the extent of reaction, and R_f and R_0 are the isotopic ratios of styrene (ratios of concentrations of heavy to light species) determined after fraction of reaction *f* and for the starting material, respectively. The obtained dependence is illustrated in Figure 3. Triplicates of such

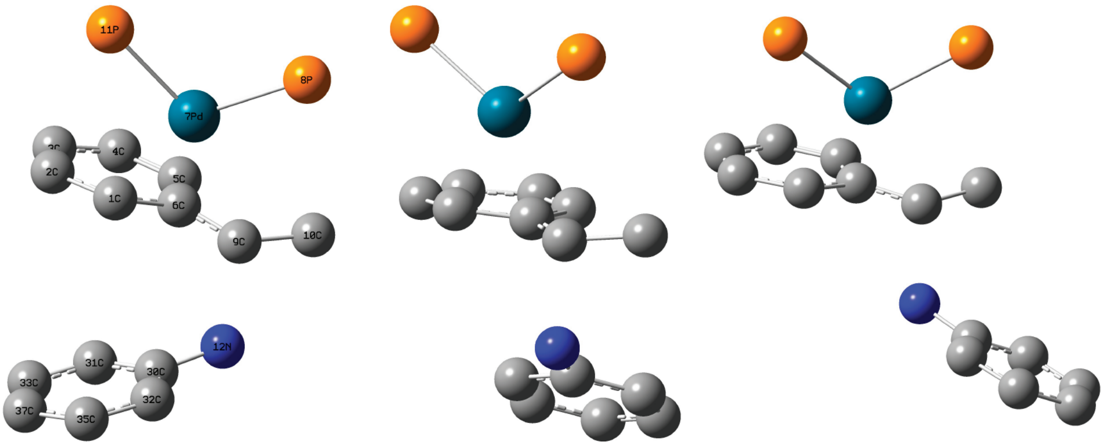

Figure 2. Transition state structures characterized by the C6−C9−N12−C30 dihedral angle of −58.7 (**1a**), 40.4 (**1b**), and −162.9° (**1c**) (left to right). Hydrogen atoms are omitted for clarity.

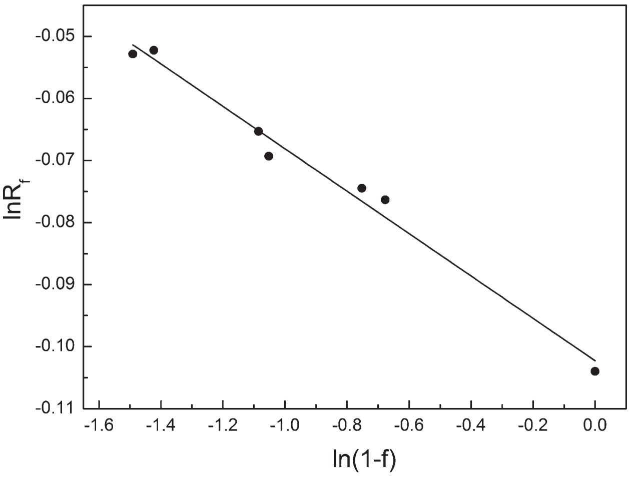

Figure 3. Evaluation of the ^{13}C-KIE from the ^{1}H NMR determined isotopic ratios.

measurements of the KIE (each based on six or seven points) have yielded the ^{13}C-KIE value of 1.0360 ± 0.0015 (95% confidence level).

Theoretical calculations were carried out at the DFT level using the M05-2X functional[9] expressed in the SDD with an effective core potential[10] (for Pd) and 6-31+G**[11] (on remaining atoms) basis set with the inclusion of the PCM solvent continuum model,[12] as implemented in Gaussian09.[13] We have shown previously that this theory level performs well in calculations of KIEs.[14] Geometry optimizations were carried out using default convergence criteria. Vibrational analysis was performed to confirm that the obtained structures are stationary points on the potential energy surface and correspond to either a local minimum ($3n - 6$ real normal modes of vibrations) or a transition state (exactly one imaginary frequency). KIEs were calculated using the complete Bigeleisen equation[15] as implemented in the ISOEFF program.[16]

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information.** Tables giving individual experimental points used in the determination of the ^{13}C-KIE with linear regression parameters, geometries of the transition state structures, Gibbs free energies, and imaginary frequencies. This material is available free of charge via the Internet at http://pubs.acs.org.

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