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^{13}C and ^{15}N Kinetic Isotope Effects on the Decarboxylation of 3-Carboxybenzisoxazole. Theory vs Experiment

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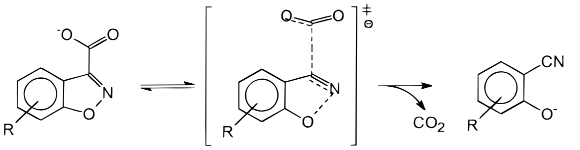
Nitrogen and carbon kinetic isotope effects were measured on the decarboxylation of 3-carboxybenzisoxazole at room temperature. The nitrogen isotope effect in acetone is 1.0312 ± 0.0006. The carbon isotope effect shows no dependence on the solvent polarity: 1.0448 ± 0.0007 in 1,4-dioxane, 1.0445 ± 0.0001 in acetonitrile, 1.0472 ± 0.0013 in DMF, and 1.0418 ± 0.0027 in water. These isotope effects were modeled theoretically at the semiempirical (AM1, PM3, SAM1) and ab initio (up to B3LYP/6-31++G**) levels. The comparison of the theoretical and experimental results leads to the conclusion that none of the theory levels employed is capable of quantitatively predicting these isotope effects.

Theoretical calculations of isotope effects are frequently used as a research tool to obtain a detailed picture of transition states. At the same time, experimental values of isotope effects are used to test theoretical protocols on which these calculations are based. Among recently published studies on theoretical predictions of isotope effects,[1−5] however, some recent reports question their predictive capabilities. Jensen et al.[4a,c] has shown theoretically the lack of the usually accepted correlation between the kinetic isotope effect and the geometry of the transition state in the case of E2 reactions as well as the dependence of calculated kinetic isotope effects on the basis set. We have also indicated that, in the case of the Menshutkin reaction,[1b,6] neither of the semiempirical NDDO methods was able to reproduce correctly all of the experimental isotope effects. To use information from isotope effects to analyze reaction mechanisms, these disturbing findings require more attention.

Simple chemical reactions, with well-established mechanisms, are attractive models for the evaluation of the newest methods of quantum chemistry. The so-called Kemp's reaction[7,8] is one of them:



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Its advantages are high sensitivity to the polarity and prototropicity of the solvent and simple, well-studied kinetics. Furthermore, Hilvert et al.[9] recently engineered catalytic antibodies which catalyzed the above reaction, and carbon kinetic isotope effects were measured.[10] Since then a number of contributions was published on modeling the reaction pathway,[11] isotope effects,[1c,d,2k] and solvent effects.[12−14]

In our approach to the problem of the reliability of the calculations of isotope effects, we are using comparison of experimental data with theoretical calculations to find a dependable and cost-effective method that correctly predicts the values of isotope effects. Differences in the interpretation of the carbon isotope effects on the above

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reaction and discrepancies between values predicted by different theoretical models have prompted us to make additional measurements which promised to resolve our doubts. Herein, we present results of nitrogen and carbon kinetic isotope effects measured for the unsubstituted benzisoxazole (R = H) together with their theoretical evaluations.

Experimental Section

Reagents. 3-Carboxybenzisoxazole was synthesized as described by Kemp et al.[7] using *o*-nitrophenylacetic acid (Aldrich) as the starting material. Triethylamine (Aldrich) was distilled and stored over KOH. All solvents (diethyl ether, acetone, acetonitrile, 1,4-dioxane, and DMF) were analytical grade and were stored over molecular sieves. Organic solvents and deionized water for carbon isotope effect determinations were freed of traces of CO_2 by 2 h purging with N_2 passed through an Ascarite trap, as described earlier.[10]

Nitrogen Isotope Effect. Samples for ^{15}N isotope effect measurement were prepared in the following way: to a vigorously stirred solution of 3.1 mmol of 3-carboxybenzisoxazole in 50 mL of acetone maintained at 21 °C was added 28.7 mmol of triethylamine. The reaction was quenched by the addition of 5 mL of 70% H_2SO_4. The reaction mixture was then diluted with cold water and extracted with ether. The extract was dried and evaporated, and a small sample was used to determine the conversion ratio (by means of ^1H NMR). Salicylonitrile was then separated by means of preparative thin-layer chromatography (Merck, Kieselgel 60, without fluorescent indicator). Quenching after 60 and 80 s yielded conversions of 15.9 and 19.7%, respectively.

Carbon Isotope Effects Samples. ^{13}C isotope effects were determined by means of a general procedure described by O'Leary.[15] All experiments were carried out at 21 °C. For each sample, to a 4 mL solution (under nitrogen) containing 0.31 mmol of 3-carboxybenzisoxazole was added 1 mL (0.57 mmol) of a solution of triethylamine dissolved in the same solvent through a stopper. The reaction was stopped by anaerobic addition of 1.5 mL of 70% phosphoric acid. Then the CO_2 formed during the reaction was purified by cryodistillation through two dry ice/2-propanol traps and a liquid nitrogen-cooled trap. The amount of the carbon dioxide was measured manometrically and then it was distilled into a sample tube. Extents of the reaction were of about 5–20% (lower in the case of reactions carried out in water). Samples of CO_2 for isotopic composition measurements after full conversion were prepared in the similar manner. Desired full conversion was achieved by leaving the reaction overnight (in DMF) and confirmed manometrically.

Isotope Effects Measurements. The samples for nitrogen isotope effects were combusted, then the products were passed through a catalyzer system in order to convert the nitrogen compounds to N_2. The procedure was automatic in a Heareus system coupled on-line with the mass spectrometer. Then the nitrogen was introduced into a Finnigan Delta S isotope ratio mass spectrometer. Carbon dioxide samples were introduced directly through a direct inlet system. Isotope effects were calculated according to the following formula:[16]

$$\frac{k_L}{k_H} = \frac{\ln(1 - f)}{\ln(1 - fR_f/R_0)} \qquad (2)$$

where f is fraction of reaction, R_0 is the isotopic ratio (ratio of heavy to light isotope concentration) of the reactant before the reaction, and R_f is the isotopic ratio of the product after the fraction of reaction f. From the mass balance, R_0 equals R_∞, the isotopic ratio of the product after full conversion. We have used this latter ratio in determinations of the carbon kinetic isotope effects. The equality of R_0 and R_∞ was tested experimentally in the case of nitrogen samples. The ratio of the isotope ratios can be obtained from "deltas"—results of mass spectroscopic measurements—from the following equation:

$$R_f/R_0 = (1000 + \delta_f)/(1000 + \delta_0) \qquad (3)$$

where δ relates the isotopic ratio of a measured sample to the same ratio of home standard, e.g.,

$$\delta^{13}C = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 = \left(\frac{[^{13}C]/[^{12}C]_{\text{sample}}}{[^{13}C]/[^{12}C]_{\text{standard}}} - 1\right) \times 1000 \qquad (4)$$

Calculations. MOPAC 93, Amsol 5.4, and Ampac 5.0 packages[17−19] running on DEC alpha workstations under OSF/1; Gaussian 94[20] running on a Cray J916 under UNICOS 8.0.4; HyperChem 5[21] running on a dual processor Pentium Pro 200 MHz PC under Windows NT 4.0; and GAMESS[22] running on a SGI Power Challange L under Irix 6.1 were employed. Transition states were obtained by a coordinate driving method from optimized structures of substrates, followed by final reoptimization of the structures of maximum potential energy. Then force field analysis was performed for both substrate and transition state structures, and finally, kinetic isotope effects were calculated by means of ISOEFF version 6[23] according to the Bigeleisen equation.[16] In the case of semiempirical Hamiltonians, two continuum solvent models were also employed: COSMO[24] (with two values of environment dielectric constant, 2.0 and 80.0) and SM4[25] (with the parametrization for water). All the ab initio calculations were performed in vacuum.

Results

The results of experiments and calculations are shown in the Tables 1−3. The ^{15}N kinetic isotope effect (Table 1) is an average of nine individual values from two independent series: five samples of the series of 16% reaction completion and four samples of 20% conversion. The ^{13}C kinetic isotope effects are averages of five or six independent samples for each solvent. The large standard deviation in the case of the reaction carried out in water was caused by measurements of very small amounts of CO_2, due to the very slow decarboxylation rate in this solvent. For a comparison, Table 1 contains also a set of

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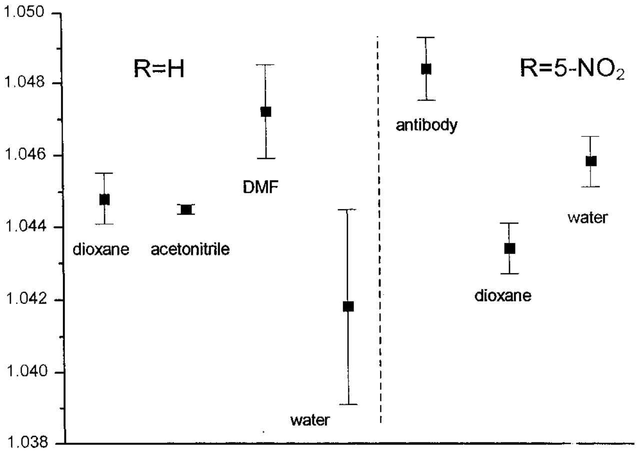

Figure 1. Carbon kinetic isotope effects on decarboxylation of 3-carboxybenzisoxazole and 3-carboxy-5-nitrobenzisoxazole.

Table 1. Measured Nitrogen and Carboxylic Carbon Isotope Effects on Decarboxylation of 3-Carboxybenzisoxazole and 3-Carboxy-5-nitrobenzisoxazole at 21 °C.

solvent	kinetic isotope effect R = H	kinetic isotope effect R = 5-NO_2[a]
	nitrogen	
acetone	1.0312 ± 0.0006	
	carbon	
1,4-dioxane	1.0448 ± 0.0007	1.0434 ± 0.0007
acetonitrile	1.0445 ± 0.0001	
DMF	1.0472 ± 0.0013	
water	1.0418 ± 0.0027	1.0458 ± 0.0007

[a] From ref 10.

carbon kinetic isotope effect values measured previously for the decarboxylation of 5-nitrobenzisoxazole.[10]

Discussion

We start our discussion with the problem of transition state location on the reaction pathway. Kemp postulated that, despite the tremendous rate change with solvent polarity, the transition state for reaction 1 remains unchanged. This was recently questioned on the basis of theoretical modeling of carbon kinetic isotope effects.[2k] We have argued earlier[10] that experimental results obtained for 5-nitro-substituted substrate (see Figure 1) support Kemp's postulate. Also QM/MM calculations performed at the AM1/TIP3P level led to similar conclusions.[11]

It should be noticed that the experimental data was obtained for the 5-nitro derivative while the calculations were performed on the unsubstituted substrate (R = H). One might expect that the transition states for these two reactions (R = H and R = NO_2) may differ since the nitro group in the para position to the phenolate oxygen of the product should strongly stabilize the transition state, the phenomenon which manifests itself experimentally by the activation barrier being over 5 kcal/mol lower in the case of the nitro derivative. We have addressed this question experimentally by measuring carbon kinetic isotope effects for the unsubstituted substrate in a variety of solvents, as reported in the Results.

A few observations are worth noting. First, isotope effects in the presence and absence of the 5-nitro substituent are the same within the experimental error (note that standard deviations and not statistical errors are reported), as illustrated in Figure 1.

One could try to put some meaning to the difference of averaged values of the carbon isotope effects in the aqueous solution for unsubstituted and 5-nitro-substituted compounds (0.4%) disregarding the precision. However, if this difference was meaningful, the increase of the isotope effect in aqueous solution compared to the isotope effect on the reaction in dioxane, which is observed for the 5-nitro derivative, should be paralleled by a similar tendency for the unsubstituted compound, which is not the case. Second, we have measured the carbon kinetic isotope effect for the unsubstituted 3-carboxybenzisoxazole in a number of solvents of different polarity and properties. Two solvents, dioxane and water, were chosen in order to make the results comparable to those previously obtained for the 5-nitro derivative and because of the large difference in their polarity. Since the hydrogen-bonding network present in water dramatically affects the rate constant of the reaction, we have also used two other, considerably polar solvents: acetonitrile and DMF. These two solvents constitute an interesting pair since while their apparent polarity is similar, the rate constants are different by an order of magnitude. Comparison of the results obtained in dioxane, acetonitrile, and DMF indicates that a relative increase of the rate constant of 2−3 orders of magnitude does not change the carbon isotope effects. Thus, upon inspection of all of the experimental values together with their statistical precision, we conclude that the carbon kinetic isotope effect is unperturbed by substituents or solvents, supporting the original assumption of Kemp that the transition state structure is very similar under all conditions. Consequently, we assume that the carbon kinetic isotope effect is about 1.045, regardless of the environment.

We now turn our discussion to the results of theoretical calculations. If what we have concluded thus far is correct, then the criterion for the evaluation of a theoretical protocol can be formulated: "a theory level properly modeling the carbon kinetic isotope effect should yield the value of about 4.5%". Following this line of argument it seems that desolvation equilibrium isotope effects deduced by Zipse et al. result from the erroneous value of the carbon isotope effect obtained at the 6-31G* level in the gas phase (1.0320).

Furthermore, the calculated values of the oxygen isotope effect seem to be erroneous, too. Intuitively one would expect that this isotope effect should be very small or even inverse (less than unity) since bonding to carboxylic oxygens increases along the reaction coordinate. Apart from one semiempirical result (AM1 Hamiltonian), all our calculations yielded inverse values, as listed in Tables 2 and 3. And even in this exception the value of the oxygen isotope effect is only 1.0007. We have also an experimental indication that this isotope effect is in fact inverse. It can be deduced from the $\delta^{18}O$ values obtained during isotopic ratio measurements (peaks 46/44 in the CO_2 isotopic analysis). The experimental procedure, however, may be the source of systematic error since the reaction is quenched by acidification with phosphoric acid. This promotes ^{18}O exchange between carbon dioxide and water and causes a wash-out of the isotope label. We have demonstrated earlier that under experimental conditions only a few percent of oxygen is being exchanged,[28] but since no tests were carried out

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Table 2. Calculated Nitrogen, Carboxylic Carbon, and Oxygen Kinetic Isotope Effects on Decarboxylation of 3-Carboxybenzisoxazole—Results of Semiempirical Calculations

method	AM1				PM3				
		COSMO		SM4		COSMO		SM4	
solvent model		$\epsilon = 2.0$	$\epsilon = 80.0$	H_2OSRP		$\epsilon = 2.0$	$\epsilon = 80.0$	H_2OSRP	SAM1
k_{14}/k_{15}	1.0540	1.0560	1.0604	1.0617 (1.0558)[a]	1.0146	1.0148	1.0200	1.0114 (1.0110)	1.0754
k_{12}/k_{13}	1.0300	1.0332	1.0380	1.0391 (1.0368)	1.0369	1.0458	1.0619	1.0503 (1.0490)	1.0199
$k_{16,16}/k_{18,18}$	1.0007	0.9995			0.9898	0.9866	0.9881		

[a] Values given in parentheses were obtained from scaled frequencies.

Table 3. Calculated Nitrogen, Carboxylic Carbon, and Oxygen Kinetic Isotope Effects on Decarboxylation of 3-Carboxybenzisoxazole—Results of ab Initio Calculations[a]

theory level	k_{14}/k_{15}	k_{12}/k_{13}	$k_{16,16}/k_{18,18}$
STO-3G	1.0667	1.0153	0.9808
3-21G*	1.0370	1.0322 1.0307[b]	0.9850
UHF/3-21G*	1.0370 (1.0416)[c]	1.0322 (1.0367)	0.9850
B3LYP/3-21+G*	1.0184	1.0292	0.9808
6-31G*		1.0320[b]	1.0115[b]
UHF/6-31G*	1.0448	1.0421	0.9850
B3LYP/UHF/6-31G*	1.0259	1.0238	0.9915
B3LYP/UHF/6-31+G*	1.0275	1.0276	0.9893
B3LYP/6-31++G**	1.0275	1.0276	0.9894

[a] Scaling factors appropriate for each basis set were taken from ref 32. [b] From ref 2k. [c] Values given in parentheses were obtained using unscaled frequencies.

for the oxygen exchange in the present studies, we are reluctant to report quantitative values of the carboxylic oxygens isotope effect. Nevertheless, we can safely say qualitatively that this isotope effect is inverse. This conclusion agrees with the experimental data of Headley and O'Leary, who have found an inverse oxygen isotope effect of 0.5% per oxygen atom for the decarboxylation of 4-pyridylacetic acid in aqueous solution.[29] Thus we formulate the second criterion of the correctness of theoretical calculations: the predicted oxygen isotope effect should be inverse.

Initially we restricted our theoretical modeling to the semiempirical level, which is most cost-effective among quantum chemical theory levels. We have found a huge difference between the nitrogen kinetic isotope effect obtained by the AM1 and PM3[30] methods (see Table 2). Thus, we thought measuring this value experimentally was "the unequivocal experiment". As can be seen from Tables 1 and 2, measuring this value raised more questions than it answered (a typical outcome of unequivocal experiments). On the basis of this experiment we have formulated the third condition a good theoretical method should fulfill: the modeled nitrogen kinetic isotope effect should be around 3.1%.

Armed with criteria derived from the analysis of carbon, oxygen, and nitrogen isotope effects, we started on our quest for the "best theoretical approach" to modeling of isotope effects on the Kemp's reaction. Since we have argued that the environment has little to no effect on isotope effects, the logical choice was to study the gas-phase reaction. This choice was also supported by the results of simulations using combined QM/MM calculations carried out by Gao[11] and studies of the solvent effect by Hilvert et al.[12] It should be noted here that perturbations of the reacting system upon changing solvents may be different from those caused by going from gas phase to a solvent. Our previous calculations of isotope effects using the COSMO model indicate that the value obtained for the gas-phase coincides with the one extrapolated from the dependence of the isotope effect on the solvent polarity.[1d] This supports the assumption that the gas-phase results can be used in the present case. However, caution is necessary since COSMO was developed for polar systems. Furthermore, contributions to isotope effects from specific interactions, e.g. hydrogen bonding, are lost by using continuum solvent models (COSMO and SM4 in our case).

The goal was to find methods which properly model all three isotope effects and, if more then one can be identified, to pinpoint the most cost-effective one. We have started with the cheapest semiempirical methods and moved through ab initio to DFT methods. Unfortunately, as can be seen from results collected in Tables 2 and 3, there is no level among those studied that correctly reproduces the isotope effects of carbon and nitrogen simultaneously. Thus it is not possible to argue the details of the transition state structure, such as bond lengths and timing of bond breaking, and extent of bond breakage, based on our calculations. Nevertheless, a few general observations are worth mentioning. The transition state structures obtained using the 3-21G basis sets are planar, while those obtained in other calculations have the carboxyl rotated about 90°. Occasionally we were able to identify a transition state for this rotation for which the imaginary frequency was at the level of $50/i\ \mathrm{cm}^{-1}$, the same value as reported by Houk's group.[2k]

A large k_{14}/k_{15} isotope effect of over 3% suggests that the N—O bond is significantly cleaved in the transition state and that its structure is probably closer to the salicylonitrile anion than to the substrate. This leads to the conclusion that the "transition state with delocalized charge" is in the present case misleading: the net negative charge in the transition state is probably mostly localized on the oxygen atom of the former isoxazole ring and similarly delocalized as in the substrate anion. This supports, on the molecular level, the explanation of the influence of the solvent properties on the reaction rate given by Hilvert,[12] who suggested that the reaction is running slower in dipolar, nonprototropic solvents, in agreement with the Ingold rule, and the net acceleration observed is due to the ion pairing effect.

While the calculated values of the nitrogen isotope effect tend to approach the experimental one with the increase of the basis set, the same is not true in the case of the carbon isotope effect. Most of the calculations yielded this kinetic isotope effect at the level of 3%. We have argued earlier that the rotation around the C—C bond has a surprisingly large influence on the isotope effect.[1d] This may also be the reason the calculated value

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is different from the observed isotope effect. For example, for the reaction going from the flat substrate, the carbon isotope effect changes from 1.0286 for the rotated transition state to 1.0414 for the flat transition state.

Another possible explanation of this situation could be that the calculated isotope effect for the reaction in question is correct and it is the experimental value that is more complex and represents an apparent isotope effect. Hilvert argued that the reaction is actually stepwise with preequilibrium tight ion pair separation and subsequent decarboxylation. In such a case, the observed isotope effect would be a product of the equilibrium isotope effect on ion pair separation and the kinetic isotope effect on decarboxylation. However, the equilibrium carboxylic carbon isotope effect on the tight ion pair separation has to be very small. By far the upper limit of its value is the equilibrium isotope effect on the deprotonation of an carboxylic acid. Bron[31] reported the secondary carbon equilibrium isotope effect on deprotonation of benzoic acid to be 1.001 at 30 °C. The preequilibrium mechanism is an extreme case. Other stepwise mechanisms would introduce isotopically insensitive steps to the apparent rate, causing the observed carbon isotope effect to be even smaller. Thus we can discard this explanation.

A dogma in isotope effect calculations states that the absolute values of normal modes are not important since the true source of isotope effects lies in isotopic differences. While this is basically true, it is not always appreciated how large these “not important” effects could be. We have used our results to shed some light on this problem. We illustrate this issue with the 3-21G* results. In their excellent study of over a thousand frequencies Scott and Radom[32] evaluated optimal scaling factors relating theoretical harmonic frequencies calculated at different levels of theory to the observed fundamentals. The values given in Table 3 in parentheses show the dependence of the calculated kinetic isotope effects on the scaling factor. It is apparent that an incorrectly chosen scaling factor can considerably change the value of the calculated isotope effect. The problem is even more severe with most of the post-Hartree−Fock methods, where the scaling factor for low-frequencies is larger than unity while the standard scaling factor is fractional. Because of the problems with scaling frequencies, one should expect about 0.5% uncertainty in the accuracy of the calculated isotope effects. The problem of scaling is even more serious with semiempirical methods. Traditionally frequencies obtained with these methods were used directly without scaling. We have shown a small scaling effect previously.[1a] Radom et al., suggest scaling factors of 0.9532 and 0.9761 for frequencies obtained by means of AM1 and PM3, respectively. This can lead to changes in isotope effects of about 0.5%, as illustrated by the results given in Table 2.

The highest theory level used here is B3LYP/6-31++G*. This level may still not be sufficient for the qualitative calculations of isotope effects. Glad and Jensen have shown[4a] considerable variation of isotope effects, calculated by means of DFT method, upon the change of basis set. Results of Melissas and Truhlar indicate[3e] that higher correlation corrections and bigger basis sets are necessary to quantitatively model isotope effects.

Conclusions

The carbon isotope effect on the Kemp’s reaction shows no dependence on the solvent polarity despite tremendous dependence of the rate constant. The comparison of the theoretical and experimental results of kinetic isotope effects leads to the conclusion that none of the theory levels employed, neither semiempirical nor ab initio, are capable of predicting isotope effects. While it is possible that by using higher theory levels one can correctly model these isotope effects, our results warn against drawing mechanistic conclusions from the low-to-medium calculation levels. Clearly more research is needed to establish acceptable computational framework.

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