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## Carbon Isotope Effects on Proton Transfers from Carbon, and the Question of Hydrogen Tunneling[1]

*Sir:*

We wish to report evidence that the tunnel effect makes an important contribution to hydrogen isotope effects in proton transfers but does not control the form of the dependence of the isotope effect on the strength of the attacking base.

The three-center model predicts that hydrogen isotope effects on proton transfers should be at a maximum when the proton is half transferred in the transition state.[2,3] Base and/or substrate variation have often been observed to lead to such maxima.[4-7] More recently, the significance of the observed maxima has been questioned by Bell, Sachs, and Tranter,[8] who suggest that the maxima arise from variation in the tunnel effect contribution rather than changes in stretching force constants of the carbon–hydrogen and base–hydrogen bonds. Model calculations show that the semiclassical (the isotope effect without tunneling) and the tunnel effect contributions to carbon isotope effects on proton transfers from carbon should vary in distinctly different ways with the extent of proton transfer.[9,10] Consequently, we undertook a study of β-carbon-13 isotope effects on E2 reactions of 2-phenylethyldimethylsulfonium (**1**) and -trimethylammonium (**2**) ions with hydroxide ion in mixtures of water and dimethyl sulfoxide. Both reactions show maxima in $k_H/k_D$ as the percentage of dimethyl sulfoxide is varied.[6,7]

The substrates **1** and **2** were oxidized quantitatively with potassium permanganate to benzoic acid, which in turn was treated with hydrazoic and sulfuric acids to give carbon dioxide. This carbon dioxide was compared in an isotope-ratio mass spectrometer[11] with carbon dioxide from substrate recovered after partial (usually 40–75%) reaction. The *m/e* 44/45 ratio for the original sample ($R^0$) and the recovered sample ($R$) were substituted into eq 1, along with the fraction of reactant remaining ($F$).[12] The resulting isotope effects, expressed as the percentage by which $k_{12}/k_{13}$ exceeds unity, are compared with $k_H/k_D$ values[6,7] in Figure 1 for **1**, and Figure 2 for **2**. Each point is the result of three–ten determinations, and standard deviations of the mean run 0.03–0.18%.

$$k_{12}/k_{13} = \log F/\log (RF/R^0) \quad (1)$$

The scatter at the higher water concentrations of the results on **1** (Figure 1) exceeds the combined standard deviations, and may result from error occasioned by the slowness of the reactions in these media. No such unusual scatter is observed in the results on **2** (Figure 2). In both cases, however, the most important point is clearly evident: the $^{12}C/^{13}C$ and H/D isotope effects depend on solvent composition in distinctively different ways.

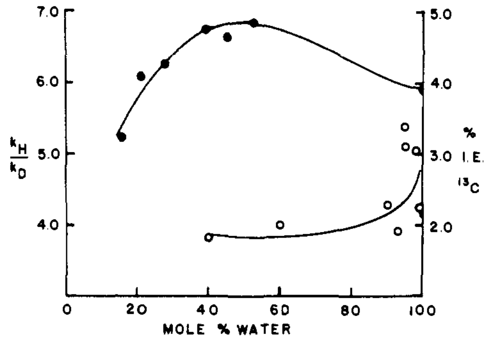

Figure 1. Observed $k_H/k_D$ values (left-hand ordinate and solid circle) and β-$^{13}C$ isotope effects, expressed as $(k_{12}/k_{13} - 1) \times 100$ (right-hand ordinate and open circles), for the elimination reaction of 2-phenylethyldimethylsulfonium ion with hydroxide ion in mixtures of water and dimethyl sulfoxide at 30°C.

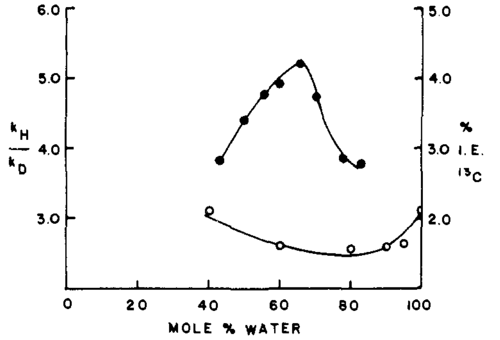

Figure 2. Observed $k_H/k_D$ values (left-hand ordinate and solid circles) and β-$^{13}C$ isotope effects, expressed as $(k_{12}/k_{13} - 1) \times 100$ (right-hand ordinate and open circles) for the elimination reaction of 2-phenylethyltrimethylammonium ion with hydroxide ion in mixtures of water and dimethyl sulfoxide at 60°C.

This point is important because the Bell, Sachs, and Tranter[8] model achieves variation in the tunnel effect primarily by changes in the activation energy (strictly speaking, the barrier height in whichever direction the proton transfer is exothermic, but our E2 reactions are all exothermic in the forward direction). It follows that variations in the carbon and deuterium isotope effects cannot both be controlled by tunnel-effect changes, for the activation energies, and their changes with solvent composition, should be nearly the same for all of the isotopic species. Actually, the activation energies for the reactions of **1** decrease monotonically as the water content of the medium decreases,[6] a pattern which is qualitatively consistent with the carbon isotope effects but quite inconsistent with the maximum in $k_H/k_D$. There is, however, no reason to believe that the variation in the carbon isotope effect would be controlled by the tunnel effect when the variation in $k_H/k_D$ is not,[13] so we conclude that variations with solvent composition in both the $^{12}C/^{13}C$ and H/D isotope effects reflect primarily force-constant changes.

Comparison of calculated[9,10] and observed isotope effects will be considered in more detail in a full paper. Here we will simply present briefly the evidence bearing on the role of the tunnel effect. Calculations based on purely semiclassical models of the E2 transition state predict slightly *inverse* $^{12}C/^{13}C$ isotope effects in the vicinity where $k_H/k_D$ is at a maximum.[9,14] This is clearly not the case in Figures 1 and 2, so one is forced to the conclusion that there must be a substantial tunnel effect superimposed on the semiclassical effect. A $^{12}C/^{13}C$ tunnel effect in the vicinity of 1.5–2.5% would fit the observed results. Such a tunnel effect for

$^{12}C/^{13}C$ would correspond in our models to a tunneling contribution to $k_H/k_D$ of ca. 1.5–2.0.[15]

Our results indicate that the Bell, Sachs, and Tranter[8] model is right in predicting substantial tunnel corrections. It does not seem to be right, however, in making the tunnel effect primarily or wholly responsible for variations in the isotope effect.

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(13) It seems very likely that any tunnel effects in both cases would be of the same origin, for the $^{12}C/^{13}C$ tunnel effect probably reflects the effect of carbon mass on the tendency of *hydrogen* to tunnel.
(14) The form of the predicted dependence of the semiclassical $^{12}C/^{13}C$ effect on extent of hydrogen transfer is qualitatively similar to the observed dependencies on mole percent water in Figures 1 and 2.
(15) A referee has asked us to comment on the relation of our data to available $A_H/A_D$ values. Values calculated from data in ref 6 show considerable scatter, but with one exception are 0.5 or below. Other elimination reactions of 2-phenylethyl derivatives give $A_H/A_D$ near 0.5.[16,17] Model calculations[10] giving a $^{12}C/^{13}C$ tunnel correction of 1.5% predict an $A_H/A_D$ of about 0.5, so our carbon isotope effects are qualitatively consistent with available data on the temperature dependence of $k_H/k_D$.[18]
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## Optically Detected Zero-Field Magnetic Resonance Studies of the Photoexcited Triplet State of the Photosynthetic Bacterium *Rhodospirillum rubrum*

*Sir:*

There has been much interest in the triplet states which are observed in fully reduced reaction center preparations and intact cells of photosynthetic bacteria.[1] Optical[2] and high field EPR studies[3–5] have led to renewed speculation concerning the role of a triplet state in the mechanism of bacterial photosynthesis and the structure of the reaction center which gives rise to the observed optical and triplet EPR spectra.

We have studied triplet state species of the photosynthetic bacterium *Rhodospirillum rubrum* in chemically reduced preparations[6] by zero-field optical detection of magnetic resonance at 2 K. An approximately $3 \times 10^{-2}$ *M* solution of sodium dithionite in 50% (by volume) glycerol and 0.1 *M* Tris-$Cl^-$ buffer, pH 7.4 (deoxygenated with nitrogen), was used to volume dilute packed whole cells of *R. rubrum*[7] by approximately six times forming a stock solution of dithionite treated whole cells. In practice a further dilution without dithionite of this stock solution by a factor 50 appeared to provide the best samples for optical detection experiments. Using CW dye laser excitation at 590 nm (Spectra-Physics Model 375 dye laser pumped by a Spectra-Physics Model 164 argon-ion laser) strong, structureless fluorescence with a maximum at 920 nm is observed from the cell preparations at 2 K. Using fluorescence-microwave techniques previously described,[8] two sharp zero field EPR transitions at 467 and at 668 MHz are observed, both as a microwave-induced decrease in fluorescence intensity monitored at 920 nm. The 467-MHz transition, shown in Figure 1, is approximately five times as intense as the transition at 668 MHz and corresponds to a change of <1% of the fluorescence intensity; the third transition (2E transition) could not be observed after extensive signal averaging. The line widths of the 467 and 668 MHz transitions are remarkably sharp (width at half-height of 7 and 15 MHz, respectively) and reproducibly symmetrical. The zero-field splitting parameters calculated from our optical detection measurements are in excellent agreement with the zero-field splitting parameters, $|D| = 0.0187\ \text{cm}^{-1}$, $|E| = 0.0034\ \text{cm}^{-1}$, reported for *R. rubrum* in X-band high field EPR experiments.[9] From the $|D|$ and $|E|$ values we conclude that we are observing optically detected triplet state transitions originating in the reaction center.

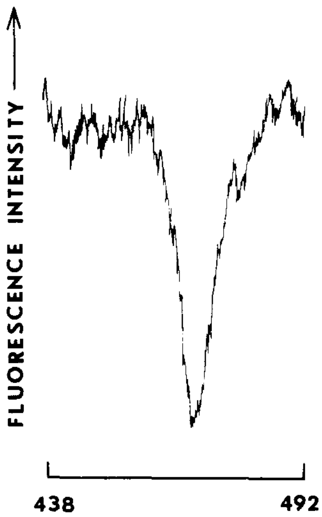

**Figure 1.** Fluorescence-detected zero field EPR transition in *R. rubrum* cell suspensions at 2 K. Excitation of fluorescence is at 590 nm with dye laser, and detection wavelength for optical-microwave double resonance experiment is 920 nm. Frequency scale is in megahertz.

The time dependence of the population changes among the zero field triplet spin sublevels was determined by monitoring the fluorescence response to square-wave microwave modulation, as described previously.[8] These experiments measure the rates at which each of the triplet spin sublevels undergo intersystem crossing into the ground state. Using the arbitrary designation (including the assumption that the sign of $D$ is positive) of x for the top energy spin sublevel, y for the middle, and z for the lowest energy spin sublevel, we find the decay rate constants, $k_i$, for the triplet spin levels to be $k_x = 2105 \pm 182\ \text{sec}^{-1}$, $k_y = 2885 \pm 400\ \text{sec}^{-1}$, and $k_z = 1335 \pm 58\ \text{sec}^{-1}$. From the observed intensity ratios of the zero field transitions, and relative populating-rates of the spin sublevels must also be of the same relative magnitude as the decay rates.

It is of interest to compare the depopulating rates measured at zero field for *R. rubrum* preparations with those measured in high field EPR and flash photolysis experiments. Dutton, Leigh et al. report triplet EPR signals at 10 K with transient times on the order of 6 μsec in *R. spheroides* preparations.[5] Parson et al. have measured transient optical absorbance changes in *R. spheroides* at 15 K with