induced disproportionation proceeds to yield C_8V. The latter product mediates the debromination process. The next reaction corresponds to the debromination of **1** by formate (eq 5). The turnover numbers of this process are also summarized in Table I.

$$HCO_2^- + PhBrCH\text{-}CHBrPh \rightarrow CO_2 + t\text{-}PhHC{=}CHPh + 2Br^- + H^+ \quad (5)$$

In conclusion, we have demonstrated the novel application of enzyme-catalyzed photochemical reactions in multiphase systems. The photosensitized electron transfer process provides a means to regenerate NAD^+ from NADH. The proper hydrophilic-hydrophobic balance of the redox couple $C_8V^{2+}/C_8V^{+\cdot}$ allows the formation of the two-electron charge relays in the organic phase and its subsequent utilization in chemical reactions. The stability of the photochemical system for the debromination of **1**, where ethanol is an ultimate electron donor, might find a synthetic merit.

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Deuterium/Protium Fractionation Factors for Polyfunctional Organic Molecules: Direct Determination by Carbon-13 NMR Spectroscopy†

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The isotope exchange processes between water and readily exchangeable groups (e.g., OH, NH, and SH) are of interest in studying reaction mechanisms and elucidating the structural dynamics of biological macromolecules.[1-3] A knowledge of the equilibrium partioning of deuterium and protium between the different types of exchangeable hydrogens is needed for a detailed interpretation of kinetic and equilibrium data and for the understanding of these processes.[1-3] Jarret and Saunders have eloquently summarized the limitations of existing methods for the determination of deuterium/protium fractionation factors.[4] They have outlined an NMR method for obtaining fractionation data at multiple sites in *rapidly exchanging* systems in an aqueous medium. Their approach is based on the isotope effects on carbon-13 chemical shifts[5] and involves the measurement of chemical shifts between solutions (contained in concentric tubes) in H_2O and D_2O and between solutions in H_2O and H_2O/D_2O mixtures.[4] This paper describes a method for the direct determination of fractionation factors from the integrated intensities of the carbon-13 resonances of deuterio and protio species under conditions of *slow chemical exchange*.

The substitution of deuterium for protium leads usually to an upfield shift in the carbon-13 resonances of atoms in the vicinity of the exchangeable site.[5] Under conditions of slow hydrogen exchange relative to the magnitude of this isotope effect, separate resonances are observed for the individual isotopomers. For amides[6,7] and ammonium derivatives[8] such isotopic multiplets can be observed in an aqueous medium. Non-hydroxylic organic solvents, e.g., Me_2SO, have been employed to achieve conditions of slow exchange for other functional groups.[9-12] The carbon-13 resonance of an atom in the vicinity of a partially deuterated group containing one exchangeable hydrogen atom is a doublet, the components of which correspond to the XH and XD species. Higher multiplets are observed when several equivalent exchangeable hydrogens are present.[7,8,11] The relative intensity of the mth component in a multiplet due to n equivalent isotope effects is given by

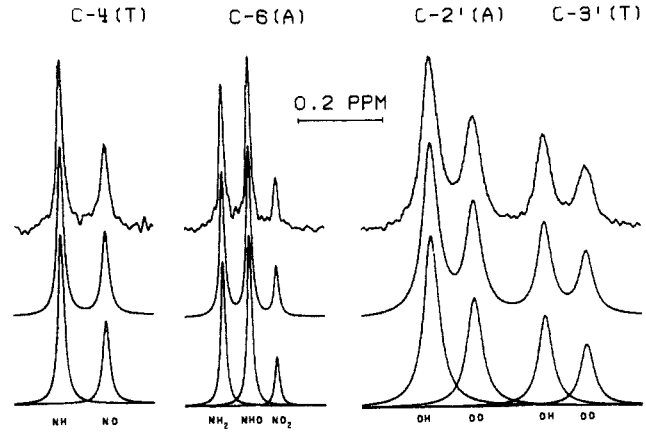

Figure 1. Isotopic multiplets for some of the carbon-13 resonances of adenosine and thymidine in their 2:1 mixture in Me_2SO-d_6: top, experimental; middle, calculated; bottom, individual components. The isotopic species are indicated under the peaks. Deuterium was introduced as CH_3OD. At equilibrium the deuterium/protium ratio as obtained from the relative areas of the CH_3OH and CH_3OD peaks was 0.71.

$$\frac{n!R^{m-1}}{(m-1)!(n-m+1)!} \quad (1)$$

where R is the deuterium/protium ratio and the first component is the protio form.[11] Thus, from the integrated intensities of the multiplet components one can obtain the deuterium/protium ratio at a given molecular site. If R_{water} is known, e.g., in dilute solutions in D_2O/H_2O mixtures of known composition, the fractionation factor

$$\alpha = R_{substance}/R_{water} \quad (2)$$

can be readily determined. In nonaqueous solvents methanol can be used as a reference compound and $R_{methanol}(R_{ref})$ measured from the components of the methanol doublet in the carbon-13 NMR spectrum. The fractionation factor for methanol has been accurately determined (α = 1.12 at 25 °C)[1] and can be used to obtain the fractionation factor of interest:

$$\alpha = \alpha_{ref}R_{substance}/R_{ref} \quad (3)$$

In this work a Nicolet 360 WB spectrometer was employed operating at 90.56 MHz for carbon-13. Accurate integrated intensities were obtained by curve-fitting of the spectral bands using the NMCCAP routine supplied by the instrument manufacturer. The rms deviation between experimental and calculated curves was usually less than 1%. An example is presented in Figure 1, where some of the resonance of thymidine and adenosine (in their mixture in Me_2SO-d_6) are shown. Deuterium was introduced as CH_3OD.

In aqueous solutions, as well as in the presence of acids or bases, the hydrogen exchange of methanol is fast. This limitation on the general applicability of the proposed method can be obviated by using *N*-methylacetamide ($CH_3CONHCH_3$) as the reference compound. This material is soluble in a variety of solvents and has the advantage of slow hydrogen exchange under most conditions, except for very high pH values. All three carbon-13

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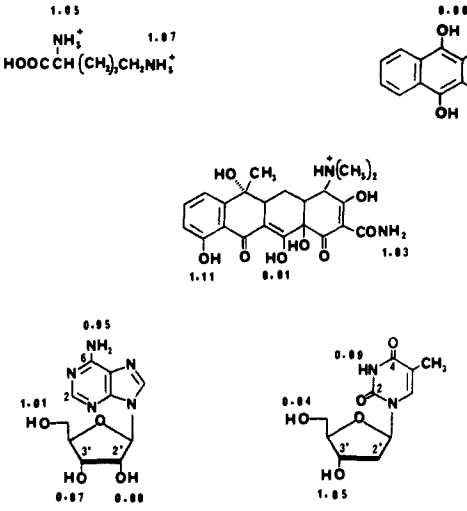
Table I. Deuterium Isotope Effects on Carbon-13 Shifts, Deuterium/Protium Ratios, and Fractionation Factors in Different Solvents

	D_2O/H_2O	$(CD_3)_2SO$	$(CD_3)_2CO$	$CDCl_3$	C_6D_6
methanol					
Δ^a	189[b]	127	141	155	141
R		0.527	2.622	0.677	0.495
N-methylacetamide					
$\Delta(CH_3N)^a$	140	127	142	145	143
$\Delta(C{=}O)^a$	94	88	85	87	74
$\Delta(CH_3CO)^a$	50	46	42	49	42
R		0.603	2.638	0.662	0.585
α^c	1.16[d]	1.18[e]	1.13[e]	1.10[e]	1.32[e]

[a] Upfield isotope shift in ppb ± 3. [b] This is the shift between H_2O and D_2O solutions under conditions of fast exchange, from ref 4. [c] Estimated uncertainty ±0.03. [d] Calculated with eq 2. [e] Calculated with eq 3 using $\alpha_{methanol} = 1.12$.

Chart I. Deuterium/Protium Fractionation Factors



resonances of *N*-methylacetamide in solutions of D_2O/H_2O mixtures appear as doublets. The fractionation factor was determined in a series of such mixtures of known composition. The fractionation factor in organic solvents was determined by using methanol as the reference and CH_3OD as the deuterium source. The results along with the isotope effects on the chemical shifts are summarized in Table I. The variation of the fractionation factor with the solvent is within experimental error, except for the C_6D_6 solution. Extensive solute–solute interactions in the nonpolar solvent may be responsible for the anomaly. The solvent dependence of the isotope shifts, which results from isotope perturbations on bond lengths and bond angles,[13] demonstrates that solute–solvent and solute–solute interactions are important in governing the isotope effects in this system.

The fractionation factors determined for a series of small molecules of biological interest are summarized in Chart I. *N*-Methylacetamide was used as the reference for L-lysine (in Me_2SO-d_6 containing D_2O and concentrated HCl), leucoquinizarin (in Me_2SO-d_6), and tetracycline hydrochloride (in Me_2SO-d_6), while methanol was the reference in a 2:1 adenosine/thymidine mixture in Me_2SO-d_6. Conditions of fast exchange prevailed for functional groups other than those indicated. The results confirm eariler observations[4] that hydroxyl groups engaged in intramolecular hydrogen bonds exhibit a preference for protium over deuterium. However, a direct correlation with the hydrogen bond energy (as measured by the hydroxyl proton chemical shift[14]) was not observed.

Acknowledgment. The excellent technical assistance of David S. Rice is gratefully acknowledged.

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Note Added in Proof. The fractionation factor for the SH group in 2-mercaptoethanol and cysteine derivatives was determined by the present method and found to be in the range 0.44 ± 0.01.[12]

Registry No. L-Lysine, 56-87-1; leucoquinizarin, 17648-03-2; tetracycline monohydrochloride, 64-75-5; adenosine, 58-61-7; thymidine, 50-89-5.

Kinetics of the Thermal Isomerization of 7,7-Dimethylbicyclo[4.1.1]octa-2,4-diene and of 8,8-Dimethylbicyclo[5.1.0]octa-2,4-diene. Evidence for a Nonconcerted 1,5-Carbon Sigmatropic Shift

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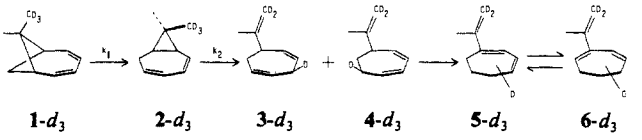
The previously reported flow system thermolysis of 7,7-dimethylbicyclo[4.1.1]octa-2,4-diene (**1**) to 8,8-dimethylbicyclo[5.1.0]octa-2,4-diene (**2**) and to the hydrogen shift products from



1-d_3 **2**-d_3 **3**-d_3 **4**-d_3 **5**-d_3 **6**-d_3

2 is remarkable.[1] To the extent that **3** and **4** come exclusively from **2**, the 1,5-sigmatropic shift of **1** occurs primarily (~80%) via Woodward–Hoffmann[2a] orbital symmetry "forbidden" but Berson–Salem[2b] "allowed" suprafacial-inversion pathway; this follows from the deuterium positions shown above. Moreover, **4** is the result of a homo-1,7-deuterium shift process from **2**, which here competes favorably with the generally observed, orbital symmetry allowed, homo-1,5-deuterium shift process that converts **2** to **3**. While previous work indicated that **2** was an intermediate in the thermolysis of **1**, it seemed appropriate to determine the kinetics of reaction of **1** and **2** to measure the relative values of k_1 and k_2. Further, activation parameters for loss of **1** are useful to gauge the extent of concert in this rearrangement.

The kinetics for loss of **1** were determined in a well-conditioned 2-L static reactor from 173.0 to 209.0 °C over one half-life using GC to follow the reaction; this gave $\log k_1(s^{-1}) = (15.1 \pm 0.4) - (41\,900 \pm 900)/2.3RT$ (E_a in cal/mol). Since **2** rearranged rapidly at 125 °C, the rates were determined in degassed benzene-d_6 in sealed NMR tubes from 100 to 139 °C; $\log k_2(s^{-1}) = 11.8 - (29\,400 \pm 800)/2.3RT$ (E_a in cal/mol).[3]

The initial product distribution from both **1** and **2** appeared to be roughly independent of temperature but the homo-1,5-shift product, **3**, is converted slowly to the homo-1,7-shift products **4**–**6** in the pyrolysis of **1**. There may be reversibility in the homo-1,5-shift, giving back **2**, which ultimately affords the more stable trienes. The initial distribution of homo-1,5- to homo-1,7-hydrogen shift products from **1** at 191 °C is 1:1.59 with the ratio of the major 1,7-shift products **5** and **6** being 2.2:1. The ratio 1,5- to

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