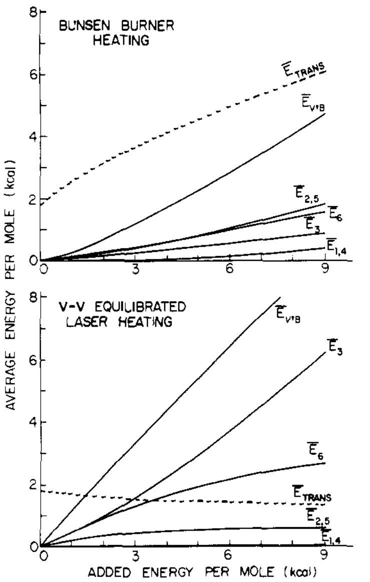

Figure 1. (Upper) Plot of the average energy in the translational, rotational, and vibrational degrees of freedom of CH_3F vs. added energy. A Boltzmann equilibrium at a single temperature and harmonic oscillator states are assumed. $\overline{E}_{TRANS}$ is the sum of the translational and rotational energies, $\overline{E}_{VIB}$ is the total average vibrational energy, and $\overline{E}_i$ is the mean vibrational energy of mode i. (Lower) Plot of the average energy distribution in CH_3F vs. laser input energy for a system at vibration–vibration equilibrium obeying the energy transfer mechanism of eq 1–5; 3 kcal per mole is equivalent to 1 laser photon absorbed per molecule. The calculated curves neglect vibration–translation/rotation relaxation.

anharmonic effects and radiative cooling via spontaneous emission.[17]

Figure 1 presents a comparison of the Boltzmann equilibrium energy distribution in CH_3F with the laser induced steady-state distribution for the input energy range equivalent to 0–9 kcal/mol (0–3 photons absorbed per molecule). The upper plot represents the case where the added energy is divided among the various degrees of freedom according to the equilibrium equipartition, Boltzmann description. Compared to any particular vibrational mode the translational and rotational degrees of freedom receive the majority of the added energy. Yet the energies of the vibrational degrees of freedom do not change greatly their absolute or relative values compared to ambient conditions. The lower plot representing the pulsed laser heated, V–V equilibrated case differs substantially from the Bunsen burner-like expectations. Here the translational/rotational energy is slowly decreasing with increasing input energy reflecting the fact that the relaxation pathway is translationally endothermic. The rate of increase of vibrational energy, on the other hand, is markedly different for each mode. The C–H stretches ν_1,ν_4 receive practically no excitation while the C–F stretch ν_3 is overwhelmingly enhanced compared to the remaining vibrational modes, particularly at excitation levels greater than 4.5 kcal/mol (1.5 photons per molecule). Therefore CH_3F in this high exctation regime will trap most of the input laser energy in the ν_3 mode for a period lasting for thousands of gas kinetic collisions. The overall features of the distinctive laser induced energy distribution of Figure 1 have also been observed experimentally.[18] Similar results have been obtained independently for laser pumped CCl_3F[19] using the CH_3F model[18] and an assumed energy transfer path with constant translational temperature.

The assumption of a vibrational steady state in which the molecular modes are equilibrated with each other but not with the translational and rotational degrees of freedom is not the only model nor the only effect which can lead to vibrational energy localization.[20] Nevertheless, the effects which are described here for CH_3F are quite general. Any laser pumped polyatomic molecule which undergoes nonresonant mode-to-mode equilibration processes much faster than the overall V–T/R relaxation can exhibit highly localized metastable vibrational energy distributions.

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Kinetic Energy Release as a Transition State Probe

Sir:

A central problem in chemistry is that of increasing our knowledge of transition states. There are clear advantages in using a molecular beam in studies of this problem, so that information, which might otherwise be lost due to collisions following the collapse of a transition state (as in solution experiments), can be conserved. The molecular beam approach can be employed using conventional magnetic sector mass spectrometers. In appropriate experiments, the unimolecular dissociations of ions are observed some 10^8 vibrations after their formation; reactions taking place so long after the ion formation ensure that there is little excess energy present in the transition state. Thus, in such experiments primary deuterium isotope effects are often large, and span the range found in solution experiments.[1] The most important advantage of the method is that if a dissociation involves a reverse activation energy, then the fraction of the reverse activation energy which appears as mutual repulsion of the products (the kinetic energy release) can be directly measured.[2]

Kinetic energy release profiles must contain useful information about transition state structures and geometries. For example, we have previously shown that symmetry-forbidden 1,2-eliminations of H_2 occur with relatively large releases of

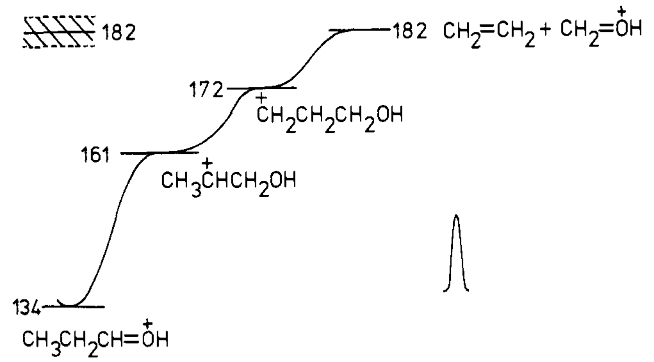


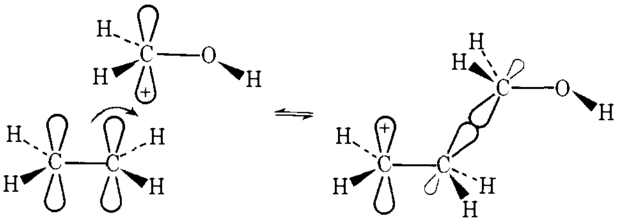
Figure 1. Potential energy surface for a dissociation of $CH_3CH_2CH{=}OH^+$; the hatched area represents the experimentally determined energy required for dissociation, and the kinetic energy release profile is schematically shown.

kinetic energy,[3] but in general (with a few exceptions[4-6]) it has been difficult to interpret existing data. We now report the inference of some transition state structures from transition state energy and kinetic energy release measurements, and show that the approach has led to correct predictions.

One of two slow unimolecular dissociations undergone by protonated propionaldehyde is that shown in eq 1,[7]

$$CH_3CH_2CH{=}OH^+ \rightarrow CH_2{=}CH_2 + CH_2{=}OH^+ \quad (1)$$

The heats of formation of reactant[8] and products[8,9] allow us to fix the starting and final points of the reaction surface (Figure 1). Using the conventional approach to the mechanism of organic reactions, the dissociation may proceed via the primary carbonium ion $^+CH_2CH_2CH_2OH$ (Figure 1), which can smoothly pass to products. The estimated heat of formation[10] of $^+CH_2CH_2CH_2OH$ shows that internal energy of this species will be required to stretch and break its central C—C bond, and the products may then drift apart without mutual repulsion. Looking at the reaction in reverse, $CH_2{=}CH_2$ and $CH_2{=}OH^+$ would apparently not have to be pushed together since the new C—C bond could be formed by initial π-bond overlap and a continuous decrease in electronic energy, **1** ⇌ **2**.



1 2

The essential features of the proposed reaction surface are supported by two experimental measurements: (i) the appearance potential for the production of $CH_2{=}OH^+$ corresponds, within experimental error, to the sum of the heats of formation of C_2H_4 (12 kcal mol^{-1}) and $CH_2{=}OH^+$ (170 kcal mol^{-1}); thus, as surmized from mechanistic and energetic considerations **1** → **2** does not involve a significant activation energy (ii) the energy release profile (metastable peak) for eq 1 establishes the release of only a very small amount of kinetic energy (an average[11] of 0.8 kcal mol^{-1})[7b] as the dissociation **2** → **1** proceeds. This observation is in accord with the conclusion from (i).

We had long been intrigued that the dissociation of the corresponding nitrogen analogue (eq 2) proceeds with a large and relatively specific kinetic energy release (~9 kcal mol^{-1}).[12]

$$CH_3CH_2CH{=}NH_2^+ \rightarrow CH_2{=}CH_2 + CH_2{=}NH_2^+ \quad (2)$$

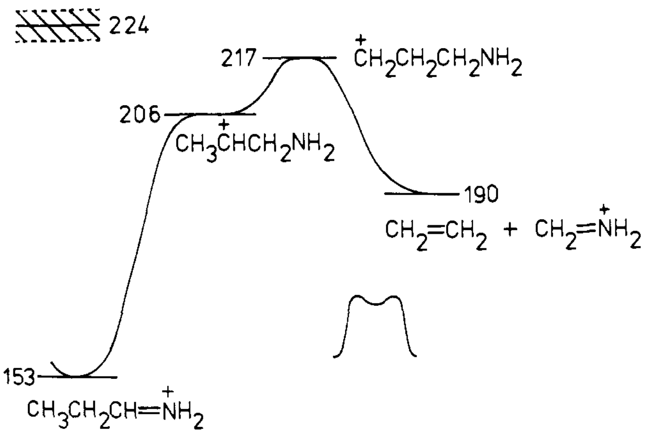


Figure 2. Potential energy surface for a dissociation of $CH_3CH_2CH{=}NH_2^+$; the hatched area represents the experimentally determined energy required for dissociation, and the kinetic energy release profile is schematically shown.

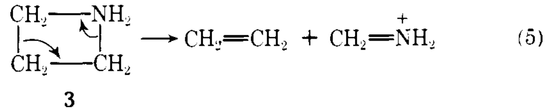
We have therefore constructed the analogous reaction surface for the protonated imine (Figure 2). Interestingly, it transpires that the postulated reacting configuration, $^+CH_2CH_2CH_2NH_2$, should now approximate to a transition state[13] and dissociate exothermically to C_2H_4 and $CH_2{=}NH_2^+$ (ΔH_f = 178 kcal mol^{-1}). Apprximately one-third of the reverse activation energy (27 kcal mol^{-1}) is released as kinetic energy. The fundamental difference between the surfaces given in Figures 1 and 2 is a consequence of the greater efficiency of N (relative to O) to stabilize an adjacent carbonium ion. This difference can be quantitatively assessed for a specific case by comparing the isodesmic reactions 3 and 4; the endothermicities are 56 and 21 kcal mol^{-1}, respectively.

$$\begin{array}{lcccc} & H_2NCH_2^+ & + \; CH_3CH_3 \rightarrow & H_2NCH_3 & + \; CH_3CH_2^+ \\ \Delta H_f & 178 & -20 & -5 & 219 \end{array} \quad (3)$$

$$\begin{array}{lcccc} & HOCH_2^+ & + \; CH_3CH_3 \rightarrow & HOCH_3 & + \; CH_3CH_2^+ \\ \Delta H_f & 170 & -20 & -48 & 219 \end{array} \quad (4)$$

Experimental evidence that the reacting configuration, $^+CH_2CH_2CH_2NH_2$, has been correctly chosen for reaction 2 is available from appearance potential measurements for $CH_2{=}NH_2^+$ production. This gives a transition state energy of ~224 kcal mol^{-1} (i.e., an activation energy of ~71 kcal mol^{-1} starting from $CH_3CH_2CH{=}NH_2^+$), in close agreement with the estimated ΔH_f value (217 kcal mol^{-1}) of $^+CH_2CH_2CH_2NH_2$.

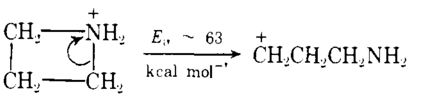
It is noteworthy that the products of eq 2 would also be available from a concerted dissociation of protonated azetidine (ΔH_f = 154 kcal mol^{-1})[14] (eq 5). Occurring from a planar, or



(5)

3

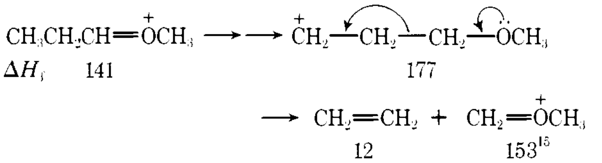
near planar, conformation, this reaction would be isoelectronic with the classic case of symmetry-forbidden dissociation of cyclobutane to two molecules of ethylene, and as such, would occur with kinetic energy release.[3] However, the measured transition state energy for the dissociation establishes that even if the configuration **3** is sampled prior to dissociation (as appears likely, since it is in a deep potential well), the internal energies available are sufficient to cause heterocyclic opening of **3** (eq 6). Thus, dissociation from **3** would be stepwise rather than concerted.



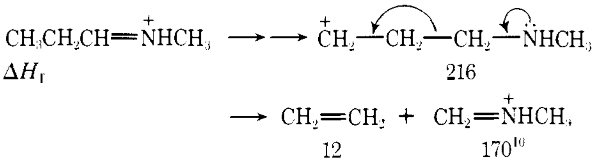
(6)

3

If the above descriptions of the reaction surfaces are appropriate, then it should be possible to predict (in a qualitative sense) the kinetic energy releases that will occur in similar reactions. Therefore we have examined reactions 7 and 8 in which a heteroatom X–H group of the earlier examined reactants is replaced by a X–CH_3 group.



(7)



(8)

In contrast to eq 1, the intermediate primary carbonium ion in eq 7 should now correspond to a transition state, since the methoxymethyl cation (ΔH_f = 153 kcal mol^{-1}) is considerably more stable than the hydroxymethyl cation (ΔH_f = 170 kcal mol^{-1}). Thus, the addition of C_2H_4 to $CH_2{=}OCH_3^+$ involves an activation energy of ~12 kcal mol^{-1}, and on the basis that approximately one-third of this should pass into kinetic energy (vide supra) in the reverse step (dissociation), the kinetic energy release should be ca. 4 kcal mol^{-1}. The kinetic energy release profile for reaction 7 is indeed much broader than that for reaction 1 and corresponds to an average kinetic energy release (computed from the width at half-height[11]) of 3 kcal mol^{-1}. Analogous arguments for eq 8 show that the reverse activation energy should be ca. 34 kcal mol^{-1}, and ca. 11 kcal mol^{-1} should be released as kinetic energy. The energy release profile is similar to that given in Figure 2, but the computed kinetic energy release (11 kcal mol^{-1}) is indeed larger than for eq 2, and in good agreement with the predicted value.

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Carbon-13 Nuclear Magnetic Relaxation Study on Cobalt Carbonic Anhydrase: Evidence on the Location of Enzyme Bound CO_2 and HCO_3^-

Sir:

Zinc metalloenzymes constitute a class of enzymes that can generally be substituted with Co(II) at the active site. This introduces a paramagnetic probe that allows NMR measurements that determine distances from individual atoms of bound ligands to the metal. In earlier studies, the relaxation times (T_1 and T_2) of ^{13}C-enriched HCO_3^- bound to bovine cobalt or zinc carbonic anhydrase (EC 4.2.1.1) were measured.[1] This communication reports work on the low activity human B form of the enzyme that gives information on the location of both bound substrate and product on the actively functioning enzyme.

It is well known that paramagnetic ions can decrease the longitudinal relaxation time, T_1, of nearby ^{13}C nuclei. With carbonic anhydrase, the paramagnetic contribution to the ^{13}C relaxation rate of the substrate gives the distance between the paramagnetic metal and the carbon nucleus of the bound substrate.[1] Much of the methodology required for studies of this kind has been developed over a decade ago in studies of inorganic complexes, and then extended to protein systems.[2-4]

The paramagnetic contribution to the longitudinal relaxation rate was calculated from

$$\frac{1}{T_{1_p}} = \frac{1}{T_1} - \frac{1}{T_1{}^0} \tag{1}$$

where $1/T_1$ is the measured [^{13}C]substrate relaxation rate in cobalt carbonic anhydrase solutions and $1/T_1{}^0$ is the corresponding rate in zinc carbonic anhydrase solutions.[5] Longitudinal relaxation was measured with a Bruker HFX-10 or a JEOL FX-60 using a conventional inversion recovery pulse sequence, with an equilibrium mixture of substrate and product (which is the substrate in the reverse direction), 90% carbon-13 enriched. The paramagnetic contribution was normalized by the factor f, $[\text{enzyme}]_0/[\text{substrate}]_0$, to yield $1/fT_{1_p}$.

At binding saturation, with fast exchange

$$\frac{1}{fT_{1_p}} = \frac{1}{T_{1_m}} \tag{2}$$

The distance r was calculated from the dipolar term of the Solomon–Bloembergen equation[4]

$$r = C[T_{1_m} f(\tau_c)]^{1/6} \tag{3}$$

where C is a product of physical constants, equal to 460 Å s$^{-1/3}$ in the present work,[6] and $f(\tau_c)$ is the well-known function of the correlation time for dipolar interaction, τ_c.[4] We have used a τ_c value of 1.2×10^{-11} s.[1,7]

Carbon-13 longitudinal relaxation times of CO_2 and HCO_3^- in solutions of cobalt(II) carbonic anhydrase at several pH values are given in Table I. In the experiments, the equilibrium between substrate and product varies from about 97% HCO_3^- at pH 7.8 to about 87% CO_2 at pH 5.5. In all cases, the ^{13}C longitudinal relaxation times are substantially decreased by