Rotational Jumps of the Tyrosine Side Chain in Crystalline Enkephalin. ^2H NMR Line Shapes for Aromatic Ring Motion in Solids

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Abstract: Deuterium NMR spectra of polycrystalline [*tyrosine*-3,5-$^2H_2$][Leu^5]enkephalin show that the aromatic tyrosyl ring of this pentapeptide is executing 180° flips about the C^β–C^γ axis in the solid state. Specifically, the axially symmetric powder pattern observed at low temperature collapses to an axially asymmetric pattern with $\eta \simeq 0.6$ at high temperature. Computer simulations of the NMR line shapes, which account for spectral distortions induced by the quadrupole echo technique, indicate that at room temperature the flipping rate is approximately 5×10^4 s^{-1} and that it increases to about 10^6 s^{-1} at 101 °C.

In the past few years it has become apparent that solid-state NMR spectra can be employed to investigate the rate and mechanism of molecular reorientation processes in solids, and there are now several examples in the literature which demonstrate this point.[2-11] To date these studies have focussed on small crystalline molecules, but the techniques are equally applicable to large molecules such as peptides and proteins, where they can be used to obtain information on phenomena such as the motion of amino acid side chains. As part of a study of side-chain reorientation in peptides and proteins by solid-state NMR, we report here an investigation of the motion of the aromatic tyrosine ring in polycrystalline [Leu^5]enkephalin. Specifically, the ^2H NMR powder line shapes of [*tyrosine*-3,5-$^2H_2$][Leu^5]enkephalin show that the tyrosyl group is not rigid, but instead is executing jumps of 180° about the C^β–C^ζ axis of the aromatic ring. At room temperature the jump rate is approximately 10^4 s^{-1} and inceases to about 10^6 s^{-1} at 101 °C.

[Leu^5]enkephalin is a natural pentapeptide with the sequence Tyr-Gly-Gly-Phe-Leu which has been chemically labeled with [3,5-$^2H_2$]tyrosine for this study as is depicted in Figure 1. The peptide is of considerable interest because of its structural similarity to opiate agonists. Theoretical and experimental evidence from solution measurements[12] reveal that enkephalin may exist in a relatively rigid conformation in which the phenyl and tyrosyl rings are separated by a distance similar to that of opiates, so that these groups may participate in binding to the receptor. [Leu^5]enkephalin has been crystallized, and its structure determined;[13-15] the X-ray studies have suggested that the orientation of the tyrosyl side chain is not unique, the ring experiencing disorder of either a static or dynamic nature. Because of the possible existence of dynamic disorder, and because NMR can detect this type of disorder and reveal the details of its nature, a solid-state NMR investigation of [Leu^5]enkephalin appeared promising.

Experimental Section

DL-[3,5-$^2H_2$]Tyrosine was purchased from Merck, Sharpe, and Dohme (St. Louis, Mo.). The DL form was resolved by treatment of the *N*-trifluoroacetyl derivative with carboxypeptidase A to yield the L isomer of the free amino acid. The latter was used to synthesize [Leu^5]enkephalin by the procedure reported previously.[12] The ^2H labeled peptide was recrystallized from ethanol.

Samples for the NMR experiments typically consisted of approximately 50 mg of the ^2H-labeled peptide and were placed in vacuum-sealed glass tubes. The ^2H NMR spectra were obtained on a home-built spectrometer operating at 45.1 MHz for ^2H, using a quadrupole echo pulse sequence.[16,17] The $\pi/2$ pulse width was 2–2.5 μs and the τ value in the echo experiment was 30 μs. In order to ensure that the line shapes we obtain are accurate, we have found it necessary to employ quadrature, rather than single sided, phase detection. With the latter method gross asymmetries in the line shapes arising from misadjustment of the echo experiment can be masked by the spectral folding process. Methods for adjusting the experiment have been described elsewhere.[18]

Results and Discussion

Dynamic disorder or motion of an aromatic ring could manifest itself in rotation about two bonds (Figure 1), the C^α–C^β and the C^β–C^γ, as well as in motion of the peptide backbone. Of most importance, however, is motion about the C^β–C^γ bond, which results in rotation of the tyrosyl ring about the C^β–C^ζ axis which is parallel to the *Y* axis of the Cartesian coordinate system in Figure 1. If surrounding groups are densely packed and inflexible, this motion might be limited to small-angle torsional oscillations. However, if this is not the case, then the ring might execute continuous diffusion about the *Y* direction, or alternatively it could execute 180° jumps between the two symmetric positions of minimum energy. We now consider the ^2H NMR line shapes which result from these cases—rigid lattice, continuous diffusion, and 180° jumps—and we will see that they are quite different and offer a means to clearly distinguish among these possibilities.

The quadrupole coupling constant (e^2qQ/h) for a deuteron on an aromatic ring is about 176 kHz and η, the asymmetry parameter, is nearly zero.[19] Thus, if the ring is rigid an axially

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(2) Griffin, R. G.; Ellett, J. D.; Mehring, M.; Bullett, J. G.; Waugh, J. S. *J. Chem. Phys.* **1972**, *57*, 2147–2155.
(3) Ellett, J. D.; Griffin, R. G.; Waugh, J. S. *J. Am. Chem. Soc.* **1974**, *96*, 345–347.
(4) Spiess, H. W. *Chem. Phys.* **1974**, *6*, 217–225.
(5) Spiess, H. W., Grosescu, R., Haeberlen, U. *Chem. Phys.* **1974**, *6*, 226–234.
(6) Alexander, S.; Baram, A.; Luz, Z. *Mol. Phys.* **1974**, *27*, 441–455.
(7) Campbell, R. F.; Meirovitch, E.; Freed, J. H. *J. Phys.* Chem. **1979**, *83*, 525–533.
(8) Meirovitch, E.; Freed, J. H. *Chem. Phys. Lett.* **1979**, *64*, 311–316.
(9) Huang, T. H.; Skarjune, R. P.; Wittebort, R. J.; Griffin, R. G.; Oldfield, E. *J. Am. Chem. Soc.* **1980**, *102*, 7377–7379.
(10) Pschorn, O.; Spiess, H. W. *J. Magn. Reson.*, **1980**, *39*, 217–228.
(11) Wemmer, D. E.; Ruben, D. J.; Pines, A. *J. Am. Chem. Soc.* **1981**, *103*, 28–33.
(12) Stimson, E. R.; Meinwald, Y. C.; Scheraga, H. A. *Biochemistry* **1979**, *18* 1661–1671 and references cited therein.
(13) Smith, G. D.; Griffin, J. F. *Science* **1978**, *199*, 1214–1216.
(14) Blundell et al.[15] suggest that the structure deduced previously[13] can "be described as an average of four nearly identical molecules". They account for the disorder of the tyrosine side chain in terms of two crystallographically independent conformations.
(15) Blundell, T. L.; Hearn, L.; Tickle, I. J.; Plamer, R. A.; Morgan, B. A.; Smith, G. D.; Griffin, J. F. *Science* **1979**, *205*, 220.
(16) Solomon, I. *Phys. Rev.* **1958**, *110*, 61.
(17) Davis, J. H.; Jeffrey, K. R.; Bloom, M.; Valic, M. I.; Higgs, T. P. *Chem. Phys. Lett.* **1976**, *42*, 390–394.
(18) Griffin, R. G. *Methods Enzymol.*, in press.

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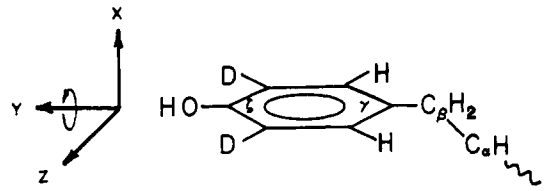

Figure 1. The tyrosyl side chain of [*tyrosine*-3,5-$^2H_2$][Leu^5]enkephalin illustrating the two CD bond vectors and a Cartesian coordinate system where X is perpendicular to the plane of the ring, Y is parallel to the C_β–C_ζ axis (the ring axis), and Z lies in the plane of the ring, perpendicular to these directions. The unique component of the rigid-lattice tensor is along the CD direction. For continuous diffusion about C_β–C_γ the Y direction corresponds to the unique axis of the axially symmetric motionally averaged tensor, while for discrete 180° jumps about C_β–C_γ the principal values of the axially asymmetric $\eta = 0.6$ tensor appear along the X,Y,Z directions.

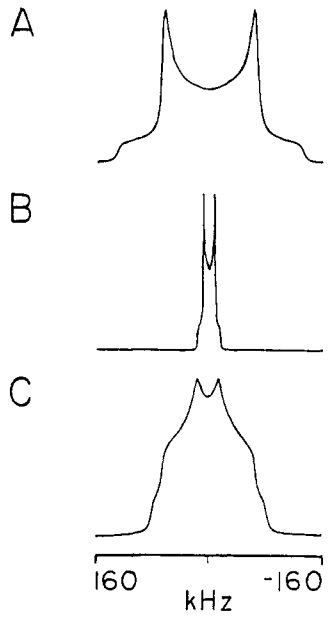

Figure 2. Theoretical ^2H NMR line shapes for [3,5-$^2H_2$]tyrosine. (A) Rigid lattice spectrum exhibiting a splitting between perpendicular edges of $D = 135$ kHz. (B) Spectrum resulting from fast continuous diffusion about the Y axis with a splitting between perpendicular edges of $D/8 = 17$ kHz. (C) Axially asymmetric spectrum arising from fast 180° jump diffusion about C_β–C_γ. The principal values of this spectrum are $V_{ZZ} = 5D/4$, $V_{XX} = -D$, and $V_{YY} = -D/4$ yielding $\eta = |V_{XX} - V_{YY}|/V_{ZZ} = 0.6$.

symmetric powder pattern with a splitting between the perpendicular edges, $\Delta\nu_{Q\perp}$, of $D \equiv (3e^2qQ/4h) = 135$ kHz would be observed. This is shown in Figure 2A. In the limit of fast continuous diffusion or of fast but small angle jump diffusion[7,20] about the ring axis the spectrum is narrowed substantially. The unique axis of the rigid lattice electric field gradient tensor, $V_\parallel$, is along the CD bond and, for the 3,5 positions of the tyrosyl ring, makes an angle of 60° with respect to the diffusion (Y) axis. Fast continuous diffusion always results in an axially symmetric, motionally averaged tensor with the unique axis coincident with the diffusion axis.[21] The separation between the parallel edges of the motionally averaged pattern is

$$\Delta\nu_{Q\parallel}{}^R = \left(\frac{3eQ}{4h}\right)(3\cos^2\theta - 1)V_\parallel = D(3\cos^2\theta - 1) \quad (1)$$

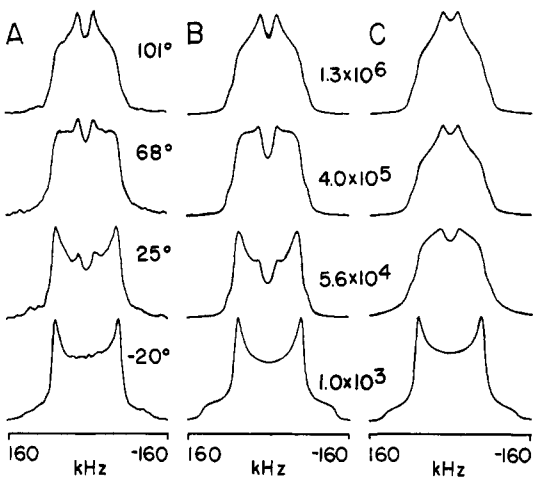

Figure 3. (A) Experimental 45.1 MHz ^2H quadrupole echo spectra of polycrystalline [*tyrosine*-3,5-$^2H_2$][Leu^5]enkephalin as a function of temperature. A 55-mg sample required 30000–50000 scans, the $\pi/2$ pulse width was 2–2.5 μs and τ in the echo experiment was 30 μs. The recycle delay at −20 °C was 4 s and for the other spectra 1 s. Quadrature phase detection was employed. (B) Simulations of the spectra using a 180° rotational jump model including corrections for power roll off and for intensity distortions produced by the quadrupole echo. The jump rate at each temperature is indicated in the figure. (C) Line shapes at the same jump rate but without the echo distortion corrections. Note the dramatic differences between the corrected and uncorrected spectra in the intermediate exchange region (25 and 68 °C).

where the superscript R denotes the rotationally averaged splitting. $V_\parallel = eq$ is the unique component of the rigid lattice axially symmetric electric field gradient tensor, and θ is the angle between the diffusion axis and $V_\parallel$. For the case of interest here ($\theta = 60°$) $\Delta\nu_{Q\parallel} = -D/4$, which amounts to 34 kHz. Correspondingly the perpendicular edges of the pattern, which are easier to observe experimentally, are separated by $D/8 = 17$ kHz. The approximate order of magnitude narrowing for this case, which is illustrated in Figure 2B, is due to the fact that the CD bond vector, and thus $\nu_\parallel$ of the rigid lattice tensor, is near the "magic angle" with respect to the diffusion (Y) axis.[20,21]

In contrast, fast discrete reorientation by 180° flips results in a dramatically different spectrum whose shape and breadth are easily understood. Since the two ring orientations are equally probable, the motionally averaged tensor will have components along the X,Y,Z coordinate system shown in Figure 1. The splitting perpendicular to the plane of the ring (X) is unaffected by the motion and remains $\Delta\nu_{Q_{XX}} = -D$ whereas the splitting along the jump (Y) axis is that calculated above, $\Delta\nu_{Q_{YY}} = -D/4$. Since the tensor must remain traceless, the third component (Z) must be $\Delta\nu_{Q_{ZZ}} = 5D/4$.[21] Thus, fast discrete reorientation yields an axially asymmetric spectrum ($\eta = 0.6$) with a breadth about half that of the rigid lattice pattern and the central portion of the spectrum yields peaks which are 34 kHz rather than 17 kHz apart as in the case of continuous diffusion. A theoretical line shape for fast 180° discrete reorientation is shown in Figure 2C and is easily distinguishable from the rigid limit and continuous diffusion spectra.

Note that a 180° flip of an aromatic ring results in a 120° change of the ^2H tensor orientation and yields the spectrum of Figure 2C. However, because magnetic resonance tensors are of rank two, an equivalent spectrum would result from a mechanism which produced a 60° change in the ^2H tensor orientation. In general in the type of experiments described here, it is not possible to distinguish jumps by θ from those by 180 - θ. However, since the Tyr side chain has approximate C_2 symmetry, a 60° jump does not appear physically plausible.

In Figure 3A are shown a set of ^2H spectra obtained with a quadrupole echo pulse sequence[16] from a sample of [*tyrosine*-3,5-$^2H_2$][Leu^5]enkephalin at temperatures between −20 and 101 °C. The recycle delays and other relevant parameters needed to obtain the spectra are given in the caption for Figure 3. It should

(19) Barnes, R. G. *Adv. Quadrupole Reson.* **1974**, *4*, 335–355.
(20) For a complete discussion of the effects of continuous diffusion on magnetic resonance line shapes see ref 7 and: Freed, J. H.; Bruno, G. V.; Polnazek, C. F. *J. Chem. Phys.* **1971**, *55*, 5270–5281; *J. Phys. Chem.* **1971**, *75*, 3385–3399. Mason, R. P.; Polnazek, C. F.; Freed, J. H. *Ibid.* **1974**, *78*, 1324–1329.
(21) Mehring, M.; Griffin, R. G.; Waugh, J. S. *J. Chem. Phys.* **1971**, *55*, 746–755.

be pointed out that in a truly rigid solid T_1 can be very long (minutes or even hours) and it would be easy to saturate signals from any rigid component, particularly in the −20 and 25 °C spectra, if it is present. Accordingly we have checked that the experimental line shapes shown in Figure 3A do not change when longer recycle delays are employed. The fact that the −20 °C spectrum could be obtained with a 4 s recycle delay is itself an indication that there is some motion present. In addition, experiments on other flipping aromatic rings suggest that T_1's for spectra like those shown in Figure 3A range from approximately 1 s to 0.1 s.[22]

Because of the breadth of ^2H spectra ($\sim 10^5$ Hz), an echo technique is required to avoid effects such as receiver overload and acoustic probe ringing which would otherwise prevent observation of ^2H powder line shapes.[17] However, in a chemically exchanging system an echo pulse pulse sequence can introduce another type of spectral distortion[23] and this is illustrated in the computer simulations of Figures 3B and 3C where we compare theoretical spectra obtained with a Bloch decay (Figure 3C) and with a quadrupole echo (Figure 3B). The physical origin of this spectral distortion and the differences between these sets of theoretical spectra will be discussed below. The theoretical spectra shown in Figure 3 have also been corrected for power roll off due to the finite radiofrequency pulse widths used in the experiments.[24]

At −20 °C the experimental spectrum is axially symmetric with a splitting of 135 kHz between perpendicular edges, in agreement with the rigid lattice value of the quadrupole coupling of [3,5-$^2H_2$]tyrosine. Simulation of this spectrum indicates that any ring flips occur with a rate of less than 10^3 s^{-1}. Moreover, the spectrum at 101 °C resembles the fast exchange spectrum of Figure 2C (or 3C), indicating that at this temperature the ring is undergoing jumps at a rate of approximately 10^6 s^{-1}. Note that in this experimental spectrum the $-D/4$ = 34 kHz splitting is evident, as well as the shoulders yielding splittings of $-D$ = 135 kHz and $5D/4$ = 169 kHz. The observation of these distinct spectral features provides convincing evidence that the tyrosyl ring is undergoing discrete 180° flips rather than continuous (or small angle jump) diffusion about the C^β–C^γ axis.

At intermediate temperatures, 25 and 68 °C, which correspond to the intermediate exchange region, the spectra contain features of both the high- and low-temperature spectra. The 34-kHz splitting is apparent as well as the 135-kHz component, and the 34-kHz component grows in intensity with increasing temperature. Simulation of these experimental line shapes, without accounting for the fact that they were obtained with a two-pulse quadrupole echo, yields line shapes such as those shown in Figure 3C, which are clearly different from those observed experimentally. The physical origin of this spectral distortion effect, observed at 25 and 68 °C, is the fact that the motional correlation times are of the same order as the reciprocal of the anisotropic spin interactions for some crystallite orientations, and as a consequence the effective T_2 is anisotropic.[25] In particular, for crystal orientations which correspond to the magnetic field being perpendicular to the ring plane or along the ring axis (the X or Y directions, respectively), T_2 is relatively long, since the flipping motion does not modulate the quadrupole coupling. Thus, for these orientations the spins are always in the fast exchange limit. Conversely, for crystal orientations corresponding to H_0 being aproximately parallel or perpendicular to the CD direction, T_2 can be very short, because a ring flip results in the splitting changing from $V_\parallel = -2V_\perp$ to $\sim V_\perp$ or vice versa. Now, if the exchange rate is in the intermediate regime, and an echo pulse is applied after a time τ, then magnetization from those regions where $T_2 < \tau$ will not be completely refocussed. Fourier transformation starting at the echo peak will then lead to spectra with apparent intensity anomalies, such as are observed at 25 and 68 °C in Figure 3A.

In Figure 3B we show simulations of the experimental spectra resulting from a computer program[26] based on the calculations of Spiess and Sillescu[23] which accounts for these echo distortions, and a comparison of these simulations with the experimental results shows good agreement between the two. Note that intensity loss in the intermediate exchange region is most pronounced in the spectral region between $D/2$ and $D/8$ and $-D/8$ and $-D/2$ and to a lesser but nevertheless perceptible extent between $\pm D/8$. This is the expected behavior since these regions of the powder spectrum correspond to those where dephasing of the spins due to the exchange process is maximal. Note also that even at 101 °C the exchange rate does not completely satisfy the fast limit condition, since there remains some difference between the Bloch decay and quadrupole echo simulation. In fact, fast limit spectra are not observed until the hopping rate exceeds 10^7 s^{-1} because of the echo distortion.

The experimental spectra in Figure 3 were obtained on heating the sample from −20 to 101 °C, and equilibration to a particular line shape occurs well within the time necessary to acquire the spectrum. However, when the sample is subsequently cooled, for example from 100 to 40 °C, a spectrum resembling a fast exchange spectrum is observed, which over an extended period (>24 h) converts to the original spectrum observed at that temperature. This spectral hysteresis suggests that [Leu^5]enkephalin crystals exist in a different form at high temperatures which may be supercooled. The temperature dependence of the tyrosine jump rate, together with studies of the motion of the Phe and Leu side chains and the peptide backbone, could reveal details of the changes in structure of the enkephalin crystal with temperature. Nevertheless, the rate constants obtained from the simulations of Figure 3 can be used to estimate an effective activation energy for the tyrosyl ring flips for the two forms of enkephalin, and we find E_a = 9.8 kcal/mol. This is lower than that determined for tyrosine ring flips in solution from ^1H spectra of proteins. For example, in ferrocytochrome *c* and in bovine pancreatic trypsin inhibitor (BPTI) activation energies of ~20 kcal/mol and ~15 kcal/mol, respectively, were measured.[27] But we note that in BPTI there are other aromatic rings which exhibit quite different behavior. For example, one phenylalanine ring is immobile up to 80 °C and four other tyrosine and phenylalanine rings are in fast exchange over the entire temperature range (4–80 °C) accessible to solution ^1H experiments. These rapidly moving rings likely exhibit lower activation energies.

Conclusions

In summary, the ^2H NMR spectrum of [*tyrosine*-3,5-$^2H_2$]-[Leu^5]enkephalin transforms from an axially symmetric rigid lattice spectrum at −20 °C to an axially asymmetric spectrum at 101 °C with $\eta \simeq 0.6$. At intermediate temperatures, where the exchange rate is comparable to the reciprocal of anisotropic spin interactions, the spectra exhibit intensity distortions which arise from the fact that they were obtained with a two-pulse quadrupole echo. The shape and breadth of these spectra clearly exclude the presence of rigid tyrosyl aromatic rings or the possibility that the rings are diffusing continously or in small-angle jumps about the ring axis. Spectral simulations assuming a model of 180° jumps about the ring axis agree well with the experimental results and provide convincing evidence that this is in fact the primary mode of motion of the tyrosyl ring.

Note Added in Proof. Since submission of this paper two other investigations of aromatic ring motion in amino acids and proteins have appeared.[28,29] These reports differ from the present study

(22) Rice, D. M.; Herzfeld, J.; Griffin, R. G., to be published.
(23) Spiess, H. W.; Sillescu, H. *J. Magn. Reson.* **1981**, *42*, 381–387.
(24) Bloom, M.; Davis, J. H.; Valic, M. I. *Can. J. Phys.* **1980**, *58*, 1510–1517.
(25) Strongly anisotropic line widths have been observed for HOD molecules executing 180° flips in single-crystal ^2H NMR investigations. See for instance: Van Willigen, H.; Haberkorn, R. A.; Griffin, R. G. *J. Chem. Phys.* **1977**, *67*, 917–924 and references therein.
(26) Wittebort, R. J.; Griffin, R. G., to be published.
(27) Campbell, I. D.; Dobson, C. M.; Moore, G. R.; Perkins, S. J.; Williams, R. J. P. *FEBS Lett.* **1976**, *70*, 91–100. Wagner, G.; DeMarco, A.; Wuthrich, K. *Biophys. Struct. Mech.* **1976**, *2*, 139–158.
(28) Gall, C. M.; DiVerdi, A.; Opella, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 5039–5043.
(29) Kinsey, R. A.; Agustin, K.; Oldfield, E. *J. Biol. Chem.* **1981**, *256*, 9028–9036.

in that the spectral distortions induced by the quadrupole echo were not considered. In both of these papers it was stated that fast limit spectra are observed when the hopping rate exceeds $\sim 10^5$ s^{-1}. The spectra in Figure 3 above illustrate that because of echo distortions rates $\geq 10^7$ s^{-1} are required to obtain such spectra. It is also clear from Figure 3 that echo distortions lead to intermediate exchange spectra with "pseudo"-rigid-lattice features—e.g., both strong perpendicular edges as well as a fast limit-$D/4$ splitting—and the distorted line shapes appear to be (but are not) a superposition of mobile and immobile rings. It is possible to obtain reasonable simulations of such spectra by superimposing rigid lattice (Figure 2A) and fast limit (Figure 2C) line shapes in varying proportions, implying that both mobile and immobile rings are present. This procedure was recently employed to analyze the ^2H spectra of phenylalanine side chain labeled bacteriorhodopsin[29] and is incorrect. We find that the ^2H spectra for both crystalline phenylalanine-d_5·HCl and phenylalanine-d_5 labeled bacteriorhodopsin can best be simulated by assuming a single jump rate for all rings, and that the "pseudo"-rigid-lattice features result from echo distortions.[22]

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Noncompeting Metastable Losses of Methyl and Ethylene from Gaseous Butanoic Acid Ions due to Isomerization Prior to Methyl Loss

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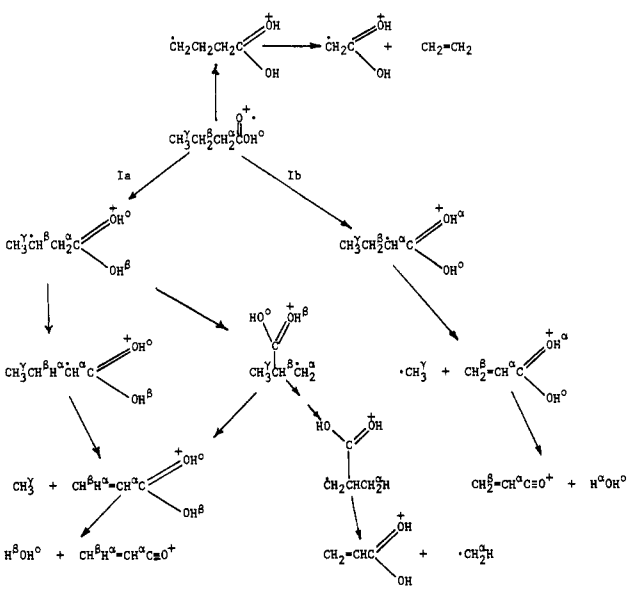
Abstract: Metastable $C_4H_8O^{+\cdot}$ ions obtained from butanoic acid and ethyl butanoate undergo considerable γ-hydrogen exchange prior to losing ethylene, but little exchange prior to losing methyl. Therefore the two fragmentations are not directly competing, contrary to the general assumption that all reactions of an ion in the gas phase are competitive. It is concluded that metastable butanoic acid ions which lose methyl isomerize essentially irreversibly to $CH_3CH_2\dot{C}HC(OH)_2^+$ and/or $CH_3CH(\dot{C}H_2)C(OH)_2^+$ before the γ-methyl becomes exchanged. This accounts for the difference between γ-hydrogen exchange prior to the loss of methyl and ethylene without invoking isolated electronic states, as previously proposed. Butanoic acid ions generated by the McLafferty rearrangement of butanoate esters have a much weaker metastable loss of ethylene than directly ionized butanoic acid. Collisional activation experiments demonstrate that this results from more of the butanoic acid ions derived from ethyl butanoate than from butanoic acid isomerizing prior to collision. Variation in internal energy probably causes this difference in degree of isomerization with the source of the ion.

Metastable butanoic acid ions in the mass spectrometer lose the γ-methyl with very little hydrogen exchange, while ethylene lost containing the same carbon is highly exchanged.[1] Therefore, the two decompositions must occur from different populations of ions. This is surprising, since both fragmentations take place from ions with the same initial structure, and all metastable decompositions occur in approximately the same time frame. The apparent lack of competition between the losses of methyl and ethylene is of interest because it is generally assumed that the fragmentations of a given ion at a given internal energy are directly competing.[2] For this reason, decomposition from different electronic states has been proposed to account for the difference in the degree of exchange accompanying the two metastable decompositions.[1a]

It has previously been concluded from the decomposition patterns of deuterium-labeled ions that the metastable butanoic acid ion loses ethylene by the McLafferty rearrangement, the γ-methyl following what appeared to be a 1,3-hydrogen shift to form $CH_3CH_2\dot{C}HC(OH)_2^+$, and methyl containing the α-hydrogens (Scheme I).[1,3] Symmetry-forbidden 1,3-hydrogen shifts are generally unfavored processes in gaseous ions,[4] making path Ib unusual. At higher energies, methyl loss occurs following transfer of a β-hydrogen to oxygen and transfer of an α-hydrogen to the β-carbon (Scheme I, path Ia).[1]

Scheme I



If ionized butanoic acid can exist in more than one isolated electronic state, then those states should be populated to different

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