Collisionally Activated Decomposition of Poly(ethylene glycol)s: an Investigation of High-Mass Ion Abundances in the Collisional Activation Technique with Large Molecules

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The utility of the collisional activation technique in structure determination of ions is limited as parent ion mass increases. Optimum collisionally activated dissociation yield is often obtained at parent masses of 1000–2000 u, after which daughter ion yield decreases. The apparent decrease in the efficiency of the collisional activation process has been thought of as a degree-of-freedom effect: as new rotational-vibrational modes are added to the parent ion, its lifetime with respect to dissociation increases. We have investigated this effect using an easily characterized system of several poly(ethylene glycol) homologs from the 15-mer to the 35-mer. Observed trends in the collisional activation spectra as parent mass increases support the postulated 'degree-of-freedom' effect in general. The loss of C_2H_4O from the $[M - H]^-$ parents, a fragmentation which has a high activation barrier, however, actually becomes more favored as the parent ion becomes larger. This effect is explained in terms of statistical rate theory.

INTRODUCTION

Collisionally activated dissociation (CAD)¹ is a mainstay of mass spectrometrists in ion structure determination problems. It has found wide application in the study of large, biologically important molecules, such as oligosaccharides² and oligopeptides.³ A frustrating observation that has been made in structural studies of these biopolymers is that daughter ion yield, and hence structural information gained from a CAD experiment, decreases as parent ion mass increases above an optimum value.^{4,5} Two effects combine to produce this problem: first, parent ion beam intensity seems to decrease with increasing molecular mass with some ionization techniques (such as fast atom bombardment (FAB)),⁶ and the decrease must affect absolute daughter ion yield;⁷ and second, the efficiency of CAD processes, particularly those producing peaks diagnostic of sequence, may decrease as parent mass increases. The latter phenomenon⁸ has been attributed to a decrease in the rate constant for decomposition as the number of internal degrees of freedom increases.⁴

Here the decrease in CAD efficiency with increasing

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0030-493X/88/050342-08\$05.00 © 1988 by John Wiley & Sons, Ltd. mass has been studied to determine when it begins to be a problem in the spectra of certain related polymers.

We chose a simpler system than biopolymers, but one for which much of the chemistry can be well defined: poly(ethylene glycol) (PEG) homologs $HO(CH_2CH_2O)_nH$, where n = 15-35. The parent $[M + H]^+$ and $[M - H]^-$ ions of these molecules may be produced by FAB, as well as by other techniques.¹⁰ Their study by FAB is particularly useful because either ion has only a few characteristic fragmentations, for some of which the mechanisms and thermodynamics can be characterized. We correlated reaction energetics with fragment ion yield to understand more fully some aspects of the relation between ion mass and CAD efficiency.

EXPERIMENTAL

Experiments were carried out using a four-sector (*BEEB*) ZAB-4F mass spectrometer (VG Analytical, Manchester, UK), which has been described.¹¹ In addition to these, certain experiments were performed using an ion cyclotron resonance (ICR) mass spectrometer operating in rapid-scan data acquisition mode.¹² This instrument has also been previously described.¹³ Parent $[M + H]^+$ ions were produced with the FAB technique (8 kV) from commercial mixed PEG samples (Aldrich Chemical Co., Milwaukee, Wisconsin), from matrices of either tetra- or hexaethylene glycol (Aldrich). Of all the common FAB solvents, these gave the largest positive

ion yields for the PEG samples. Parent $[M - H]^-$ ions were produced similarly, either from the same matrices or from these matrices with a small amount of diethanolamine added to increase negative ion yield.¹⁴ The most intense isotope peak of each parent ion cluster was selected by the first magnetic and first electric sector of the instrument to serve as the primary or main beam (MB), for collision with helium in the collision cell between the two electric sectors; analysis of fragment ions was made with the second electric and second magnetic sectors of the instrument, at a pressure that gave a constant 20% reduction in parent ion current for all CAD experiments. This condition is different from those used in other polymer studies, but we assumed that constant reduction of the main beam would be related to constant energy deposition in a series of oligomers; some study of the relationship of the fraction of daughter ions collected to the fractional reduction of the main beam has been made.¹⁵

For the ICR experiments, the sample of tetraethylene glycol dimethyl ether (Aldrich) was introduced at the tip of an unheated probe located about 1 cm below the ICR cell. This sample was allowed to react with alkoxide bases generated from alkyl nitrites in a dissociative electron capture process. Generation of alkyl nitrites *in situ* for these experiments has been described.¹⁶

RESULTS AND DISCUSSION

The positive ion FAB spectra of poly(ethylene glycol)s are well documented.¹⁰ The spectra show a series of peaks with a spacing of 44 u which correspond to the homologous series of ions $[M + H]^+$. The ions in this series may either be parents or homologous daughters, differing from the parent ions by one or more $(-CH_2CH_2O-)$ units. Analysis of field desorption (FD) ion intensities with average relative molecular masses (RMM) indicates that the FD ions are almost entirely $[M + 1]^+$ ions;^{10b} comparison of FD and FAB spectra indicates that while in FAB there is a contribution from the daughters of oligomers with a few more $(-CH_2CH_2O-)$ units. We estimate this contribution to be less than 10% of the total intensity, based on comparison of the spectra.^{10a,b}

We first assume that the ions in the FAB spectrum of the mixture represent true parents to a very large extent, at least at the higher masses; and the validity of this assumption may in principle significantly affect the internal energy of the ion selected for study and therefore affect its fragmentation, since daughters formed by exothermic processes must in general contain lower internal energy than their parents. With the FAB method of ionization, selected RO⁻ ions from these compounds displayed identical unimolecular and collisionally activated daughter ion spectra whether selected as source-generated 'parent' ions, or as true daughters separated after decomposition of these parents in the third field-free region of the instrument. (In the ZAB-4F, analysis of the fragments produced by these daughter ions may be accomplished using the second electric and second magnetic sectors.) We therefore assume that most parent ions are generated with

internal energy distributions sufficiently close to thermal that the question of whether a particular ion is a true $[M + 1]^+$ or daughter becomes moot.

Positive ions

From the commercially available mixtures of PEG oligomers we have been able to obtain sufficiently high yields of these parent ions up to 1400 u to study their unimolecular and CAD with good daughter ion yields after accumulation of 20 scans. Representative positive ion unimolecular and collisional activation (CA) spectra in the mass region of interest in these experiments are shown in Figs 1 and 2. There are two fragmentation pathways represented here, shown in Eqns (1) and (2). In the ZAB-4F instrument, daughters formed in either process may be selected for a second CAD experiment. This technique was used to show that the paths are interlinked mechanistically; an $[M + H]^+$ daughter generated in (1) may lose water and move into pathway (2), or an $[M + H - H_2O]^+$ ion generated in (2) may lose acetylene and continue to follow pathway (1):

$$[H(OCH_2CH_2)_nOH_2]^+$$

$$\rightarrow [H(OCH_2CH_2)_{n-m}OH_2]^+ + (C_2H_4O)_m \qquad (1)$$

$$[H(OCH_2CH_2)_nOH_2]^+$$

$$\rightarrow [H(OCH_2CH_2)_n]^+ + H_2O$$
⁽²⁾

Figures 3 and 4 show the correlation between the mass of the parent ion and the yield of selected daughter ions formed in both unimolecular and CAD processes. Daughter ion yield is represented as the ratio of the intensity of the daughter peak to the intensity of the main beam (since the intensity of the main beam



Figure 1. Positive ion unimolecular dissociation spectrum of the PEG $[M + H]^+$ ion at m/z 1163. The upper scan shows the daughter ions on a scale 100 times larger than the main beam (MB); the lower scan magnifies the upper by a factor of 10. The width of peaks in all spectra is due to tracking problems in accumulation, not saturation.

× 10

835

Figure 2. Positive ion CAD spectrum of protonated PEG oligomer at m/z 1163. Scaling of the spectrum is as in Fig. 1.

m/2

1031

declines significantly with increasing mass under FAB ionization). Each point represents the average of 18–22 scans. The daughter ions selected are all of the series represented by Eqn (1), and are ions formed by losses of one, two and three units of 44 u from the main beam, respectively. These sequence ions were selected in order to minimize mechanistic complications in their formation; lower mass sequence ions may be formed by sequential fragmentations more commonly than daughters close to the parent mass. In addition, ions from the same fragmentation series were selected so that differences in thermochemistry might be examined within a single mechanism.

The principal route to these three ions is not sequential loss of C_2H_4O neutral fragments. The decrease in



Figure 3. Correlation of the relative intensities (fragment ion to main beam) of the first three sequence ions produced by unimolecular dissociation of an $[M + H]^+$ parent with parent ion mass.



Figure 4. Correlation of the relative intensities of the first three sequence ions produced by CAD from $[M + H]^+$ parents with parent mass. Spectra are corrected by subtraction of the intensities of unimolecular products.

product yield would have been steeper for each sequential loss if that were the case. Loss of neutrals of mass 44n seems to occur for many values of n, although losses of 44 and 88 u dominate both unimolecular and CAD spectra. It is unwise to speculate on the nature of the neutral products of these processes. The structure of the parent $[M + H]^+$ ion is uncertain. The charge site probably resembles a protonated crown ether or small PEG dialkyl ether (glyme) molecule, with the proton hydrogen bonded to an ether oxygen, hydrogen-bonded to a second, and further solvated by a number of ether dipoles.¹⁷ Further disposition of the rest of the chain is unknown. Fragmentation may even occur remote from the site of the charge in the ion,¹⁸ as has been demon-strated for negative ions. With the structure of the reactant and the site of the reaction undetermined, it is hazardous to propose detailed mechanisms for the processes, and the choice of daughter ions to select for study is necessarily arbitrary.

The general trend illustrated in Figs 3 and 4 decreases, as expected; the scatter of data is so great that we have not pretended to fit the data to the logarithmic mass scale theory demands for a degree-offreedom effect.¹⁹ As parent mass increases, there is an obvious fall-off in daughter ion yield for the high-yield daughters. For the ion $[MB - 132]^+$ the trend is not dramatic, but the trend may be impossible to observe with a low-intensity product ion. But if the loss of $C_6H_{12}O_3$ is indeed independent of the mass of the precursor ion, the independence would be compatible with the suggestion that randomization of energy within large ions is incomplete before fragmentation,²⁰ contrary to the assumptions of quasi-equilibrium theory:²¹ if the number of vibrational states among which the energy is distributed reaches a limit, then ions with a greater number of states will have only the limiting number of states participating, and the rate constant will be invariant. However, there cannot be effective randomization for one fragmentation (loss of C₂H₄O) but not for another (loss of $C_6H_{12}O_3$). The lack of

100

50

0 10

5

n

1180

Relative intensity

1163 (MB)

100

1119

1075

dependence on mass must have some other explanation associated only with the one reaction. An accidental proportional increase in the number of states in the activated complex and the density of states in the molecule would also produce the rate independence, but so far as we know has no precedent.

The observed trend in Figs 3 and 4 may be rationalized in a general way by using statistical rate theory. Increasing parent mass increases the sum and density of rovibrational states for the reactant parent ion; if the corresponding change in the sum of states for the activated complex is less than that for the activated ion, the rate of the decomposition must decrease. Without confident knowledge of the structure of the reactant or products, it is impossible to guess the structure of the transition state, and therefore to quantify the effect of increasing parent mass on the activated complex. In general, as the number of degrees of freedom in the reactant increases, the rate constant for the decomposition k(E) rises more slowly as the internal energy E^* of the ion is increased, and the effective threshold for the collisionally activated reaction increases.9 Given nearthermal energy distributions for each of the parent ions, the number of fragment ions produced should decrease as mass increases. The CAD and unimolecular spectra both show this effect; because the approximate dependences are very nearly the same in the two plots, the increase in E^* for the parent on collision must be small relative to the zero-point energy of the molecule.

There would also be a reduction in intensity of these fragments if new competitive pathways are introduced with increasing MB mass. In this range of masses no new kind of processes were noted, although the same kind of process may occur once more each time the chain is lengthened, so that competition generally increases.

Negative ions

The spectra of the $[M - 1]^-$ ions produced by FAB from poly(ethylene glycol) samples present far fewer problems in interpretation than do the $[M + H]^+$ spectra. As with the positive ions, the negative ion spectrum shows a series of homologous peaks, differing in mass by 44 u. Again, these may be assumed to have energy distributions close to thermal whether they represent true parents or fragments. Ions up to 1200 u were selected for further fragmentation studies with good daughter yields, and representative unimolecular and CAD spectra are shown as Figs 5 and 6.

Examination of the unimolecular decomposition spectra of these negative ions allows some important generalizations about the fragmentation pathways to be made. (We did not compare the yields of fragments to the yield of electron detachment products.) The loss of a single C_2H_4O unit is not favored; the loss of $C_4H_8O_2$ is. In the CAD spectrum, loss of 44 becomes possible because of the energy deposited on collision. Since the charge site for these $[M - H]^-$ ions must be located at the end of the polymer chain, an alkoxide anion, there are two possible mechanisms involving the charge site that would allow losses of one or more chain units in a single step. The products of these are shown in Eqns (3) and (4) for generalized cases; the first is an internal displacement, while the second involves an internal 'bimolecular' elimination transition state. Both possibilities are consistent with our observation that the $[M - H]^-$ parent and the daughter ions which have the same putative structure produce the same CAD and unimolecular spectra, but this result does not distinguish between them. However, when it can occur, the elimination reaction should be strongly favored over displacement.²²⁻²⁵ The most reasonable hypothesis explaining this effect is that the transition state for a displacement is highly ordered (tight); while that for the corresponding elimination

$$[H(OCH_2CH_2)_nO]^- \rightarrow [H(OCH_2CH_2)_{n-m}O]^-$$

$$+ CH_2CH_2(OCH_2CH_2)_mO \quad (3)$$

$$[H(OCH_2CH_2)_nO]^- \rightarrow [H(OCH_2CH_2)_{n-m}O]^-$$

$$+ H_2C=CH(OCH_2CH_2)_{m-1}OH$$

$$(4)$$

is looser, more closely resembling that for a proton transfer.²⁶ We have been unable to find any example in the literature in which an alkoxide leaving group is produced in a gas-phase displacement reaction when elimination is a competing channel; elimination is always more facile, even when displacement is thermodynamically favored.²³ Loss of a single chain unit should take place via displacement, but higher-order losses should proceed via Eqn (4).

A third mechanism that might be used to explain the inconsistency in behavior between the losses of C_2H_4O and higher-mass neutrals would be a thermal reaction remote from the charge site,¹⁸ as illustrated in Eqn (5). If this process were

$$\begin{bmatrix} HO-CH-CH_{2} \\ I \\ H \\ O-CH_{2}CH_{2}(OCH_{2}CH_{2})_{n}O]^{-} \\ \rightarrow [HOCH_{2}CH_{2}(OCH_{2}CH_{2})_{n}O]^{-} + HOCH=CH_{2} \\ \end{bmatrix}$$
(5)

favored for the loss of C_2H_4O but not for the higherorder losses, the difference in behavior could be explained by this mechanism. Given similar thermochemistry, the four-centered transition state required in this mechanism for the loss of vinyl alcohol would make this reaction less likely than the analogous process producing $C_4H_8O_2$, because of entropic considerations. In that case the dominant mechanisms for the negative ion fragmentations would be charge-initiated.

In order to verify that displacement does not compete with elimination at parent ion internal energies close to thermal, ion cyclotron resonance (ICR) spectrometry was used to examine the reaction of methoxide ion with tetraethylene glycol dimethyl ether, a system in which both the displacement and the elimination reactions can occur and produce different ionic products. In an ICR spectrometer, rate constants for reactions are measurable, and could be determined for both processes should they occur. A comparison of rate constants would allow a more informed comparison of transition state structure and energetics for the two channels.

Tetraethylene glycol dimethyl ether may react with methoxide to produce the ionic products illustrated in



Figure 5. Negative ion unimolecular dissociation spectrum of the PEG $[M - H]^-$ ion at m/z 1029. The daughter ions are shown on a scale 100 times larger than the main beam.

Eqn (6) through either the elimination or displacement mechanism. The reaction shown in Eqn (7), however, can take place only through a displacement mechanism.

$$[CH_{3}O]^{-} + CH_{3}O(CH_{2}CH_{2}O)_{4}CH_{3}$$

$$\rightarrow CH_{3}OH + CH_{3}O - CH = CH_{2}$$

$$+ [O(CH_{2}CH_{2}O)_{3}CH_{3}]^{-}$$

$$\rightarrow CH_{3}OH + CH_{3}OCH_{2}CH_{2}OCH = CH_{2}$$

$$+ [O(CH_{2}CH_{2}O)_{2}CH_{3}]^{-}$$

$$\rightarrow CH_{3}OH + CH_{3}O(CH_{2}CH_{2}O)_{2}CH = CH_{2}$$

$$+ [OCH_{2}CH_{2}OCH_{3}]^{-}$$

$$\rightarrow CH_{3}OH + CH_{3}O(CH_{2}CH_{2}O)_{3}CH = CH_{2}$$

$$+ [OCH_{3}]^{-}$$
(6)

$$[CH_{3}O]^{-} + CH_{3}O(CH_{2}CH_{2}O)_{4}CH_{3} \rightarrow CH_{3}OCH_{3}$$
$$+ [O(CH_{2}CH_{2}O)_{4}CH_{3}]^{-}$$
(7)

We may estimate²⁷ the enthalpy changes for Eqns (6) and (7), using group additivity rules²⁸ and estimates based on thermochemical cycles. Equation (6) should have $\Delta H_r^{\circ} = -4.6 \ (\pm 25) \ \text{kJ} \ \text{mol}^{-1}$ whatever the ionic products, since loss of a $-\text{CH}_2\text{CH}_2\text{O}-$ unit from the reactant should be cancelled by the gain of the same unit in the neutral product; while ΔH_r° for Eqn (7) is calculated to be 19.7 (± 25) kJ mol⁻¹. The relative error for these values should be much smaller than the absolute error indicated. The reaction did not produce an ion at mass 207 [CH₃(OCH₂CH₂)₄O]⁻ attributable to displacement across the terminal methyl group. Because of the endothermicity of the displacement, it is unlikely that this reaction would be observable in the near-



Figure 6. Negative ion CAD spectrum of the PEG $[M - H]^-$ ion at m/z 1029. Scale is as in Fig. 5.

thermal conditions of the ICR, but the products shown in Eqn (6) are observed at long detection times.³⁰ These are therefore products of the elimination mechanism exclusively, and this observation provides evidence that this pathway is the preferred one. (It must of course also be preferred on collisional activation.)

With CA, the additional energy necessary for the loss of C₂H₄O is available. Losses of larger fragments undoubtedly take place through the more favorable elimination pathway, but the displacement mechanism is necessary to explain the loss of a single chain unit. We can again estimate thermochemistry for these pathways using group additivity rules.²⁸ The loss of each chain unit is calculated to decrease ΔH_f° of the ion by 165 kJ mol⁻¹. The $\Delta H_{\rm f}^{\circ}$ for each of the neutral products either can be calculated²⁶ or is available through the literature.³¹ Thus we estimate relative heats of reaction for the losses of C_2H_4O , $C_4H_8O_2$, and $C_6H_{12}O_3$ to be approximately 0, 15 and 51 kJ mol⁻¹, respectively. These values will adequately show the relative enthalpies of the fragmentations, but should not be interpreted as absolute because of uncertainties in the conformations (and hence heats of formation) of the parent and daughter ions. The neutral fragment C₂H₄O is taken to be acetaldehyde, rather than the neutral that would be produced if the activated complex broke up immediately after the displacement. The requirement that the transition state for the reaction is a cyclic form does not specify the way in which the activated complex finally breaks up, even though it does control the reaction kinetics. Acetaldehyde as the neutral fragment is more accessible thermodynamically than ethylene oxide by 113 kJ mol⁻¹ and vinyl alcohol³² by 41 kJ mol⁻¹. Further reaction of the ionic and neutral product within the activated complex (the base-catalyzed rearrangement illustrated in Eqn (8)) is well supported in the literature.³³ Vinyl alcohol might be the kinetic product of the last proton transfer, but there is no reason to exclude further tautomerization within the complex, increasing the exothermicity of the reaction.

All of these reactions, even with their kinetic shifts, are thermodynamically accessible for an ion produced by FAB. Differences in the behavior of different

$$H(OCH_{2}CH_{2})_{n}O^{-} \qquad O \\ \rightarrow [H(OCH_{2}CH_{2})_{n-1}O - H - CH - CH_{2}]^{-} \rightarrow O \\ Or [H(OCH_{2}CH_{2})_{n-1}O - H - OCH = CH_{2}]^{-} \rightarrow O \\ \rightarrow [H(OCH_{2}CH_{2})_{n-1}O - H - H_{2}C - CHO]^{-} \\ \rightarrow [H(OCH_{2}CH_{2})_{n-1}O]^{-} + CH_{3}CHO$$
(8)

ions with respect to each loss must then be discussed in terms of kinetic barriers. This information proves helpful in attempting to correlate the yield of each of these processes with parent ion mass.

Such a correlation is illustrated in Figs 7 and 8, for the unimolecular and CAD spectra, respectively. Again, for the low-intensity daughters a significant change in yield is not observable. The yield of $[MB - 88]^-$, where MB denotes the main beam $[M - H]^-$, decreases with increasing parent mass, as found for the positive ions. The increasing yield of $[MB - 44]^-$ with increasing parent mass after CA has only a few observed parallels, mostly when the mass of the neutral is larger.¹⁸



Figure 7. Correlation of relative fragment ion intensity (fragment ion over main beam) for the first three sequence ions produced in unimolecular dissociation of $[M - H]^-$ parents with parent ion mass. Spectra are corrected by subtraction of intensities of unimolecular products.



Figure 8. Correlation of relative fragment ion intensity with parent ion mass for sequence ions produced after CA of parent $[M - H]^-$ ions.

This observed increase might be due to a difference in the collection efficiency of the third (electric) sector of the instrument caused by radically different kinetic energy releases in the losses of 44 and 88 u fragments. This effect is well documented,³⁴ and may be compensated for by measurement of the kinetic energy release of a fragmentation and taking into account the spatial spreading of the beam possible without loss of ions in a particular instrument. With this correction in ZAB geometry instruments,³⁵ measured kinetic energy releases for the losses of 44 u and 88 u fragments from the $[M - H]^-$ ion of the model compound hexaethylene glycol after CA are 30 and 68 meV, respectively, so that in the ZAB-4F, the ionic fragments left by these processes should be collected by the third (electric) sector at 91% and 80% efficiency, respectively, for a parent of mass 600, and at 94% and 84% efficiency for a parent of mass 1200. Since the observed variation in kinetic energy release with mass of separating fragments is not large enough to alter collection efficiency sufficiently here, this discrimination cannot be responsible for the observed effect. The difference in fragment ion yield must be related to the differences in the kinetics for the two processes.

If the displacement reaction transition state is indeed tight, the sum of states in the transition state manifold for this reaction is lower than that for the elimination reaction, and this reduction must decrease the rate. The fundamental equation for RRKM theory³⁶ (Eqn (9)) illustrates the effect on k(E) for an ion of energy E^* of the sum of vibrational states in the transition state, $G^+(E^+ - E_0)$. $N^*(E)$ is the density of vibrational states for the activated ion, and L^+ is a degeneracy factor. Q^* and Q^+ are rotational partition functions for the activated ion and the transition state. The increase in E^* provided by collision with a

$$k(E) = \frac{L^+ Q^+ G^+ (E^+ - E_0)}{h Q^* N^*(E)}$$
(9)

helium atom brings both reaction rate constants above threshold, but only the elimination occurs without CA. When parent ion mass increases, the number of degrees of freedom increases for the activated ion and for the transition state, and the number and density of states increases for both manifolds. The rotational partition functions Q^* and Q^+ for the activated ion and the transition state also increase, but the increase is not the same in both manifolds. For a loose transition state, some free rotations are retained in the transition state, and the loose, newly forming bond and the breaking bond across the proton transfer adds a large number of vibrational states to the transition state manifold. $G^+(E^+ - E_0)$ and Q^+ for this transition state are both high, and the increase in them caused by lengthening the parent chain is not significant compared to the activated parent ion, where a corresponding increase occurs. In the tight displacement transition state, however, both $G^+(E^+ - E_0)$ and Q^+ are low, and any increase in these is dramatic with respect to their original values. Lengthening the parent chain causes an increase in k(E) because the increased ease with which the complex moves over the activation barrier is not offset by the increased lifetime of the activated ion.9 For our high-barrier reaction, the displacement of C₂H₄O, the fragment yield increases as parent mass increases because of the competing effects on the rate constant.

CONCLUSIONS

There are natural limits to the utility of the CA technique in structural studies of large molecules: difficulty in producing large yields of parent ions and difficulty in interpreting the complex spectra that are often produced by complex molecules. (Of course they are not always found in practice, so the technique is generally useful.) This investigation points to additional difficulties. First, from comparison of the unimolecular and the CAD spectra, the increase in the energy for a very large ion by a single collision may be small relative to the zero-point energy; the increase in fragmentation produced by the relatively small increase in energy may not yield much more information. Second, for the cases in which the small increase in energy does increase the intensities of certain daughter peaks, these ultimately are likely to be daughters from processes with tight transition states. In other words, as parent mass increases within a homologous series, the CAD spectra change. Large peaks from the lower-mass parents often important in interpretation become smaller. The less useful peaks increase in intensity, and may dominate the spectra of higher homologs.⁵ In general, we conclude that CAD spectra do not simply decrease in intensity as mass increases, but they may change in appearance as well. A peptide of RMM 2400 has been sequenced successfully,³⁷ and we do not imply that this difficulty has a single universal RMM value for onset irrespective of molecular structure. Eventually, though, for any class of compound, there must be an RMM at which rearrangements are the only peaks measurable, in any large ion that can rearrange. Some sequencing difficulties of this kind have already been noted in FAB/CAD spectra of peptides as a class above RMM 2000,⁵ and the breadth of such observations-some 50 examples-suggests that successful sequencing far above this limit could be difficult, at least without further refinement of the technique. This onset RMM must be different for other polymer classes.

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REFERENCES

- (a) F. W. McLafferty, P. F. Bente III, R. Kornfeld, S.-C. Tsai and I. Howe, J. Am. Chem. Soc. 95, 2120 (1973); (b) F. W. McLafferty, R. Kornfeld, W. F. Haddon, K. Levsen, I. Sakai, P. F. Bente III, S.-C. Tsai and H. D. R. Schuddemage, J. Am. Chem. Soc. 95, 3886 (1973).
- A. Dell, H. R. Morris, H. Egge, H. von Nicolai and O. Stiecker, Carbohyd. Res. 115, 41 (1983).
- 3. H. R. Morris, M. Panico, M. Barber, R. S. Bordoli, R. D.

Sedgurde and A. N. Tyler, *Biochem. Biophys. Res. Commun.* 101, 623 (1981).

- 4. G. M. Neumann, M. M. Sheil and P. J. Derrick, *Z. Naturforsch.* **39a**, 584 (1984).
- 5. D. J. Harvan, personal communication (1984).
- 6. M. Barber, R. S. Bordoli, R. D. Sedgwick and A. N. Tyler, J. Chem. Soc. Chem. Commun. 325 (1981).
- 7. B. N. Green, private communication (1985).

- A. G. Craig and P. J. Derrick, J. Am. Chem. Soc. 107, 6707 (1985).
- (a) W. Forst, *Theory of Unimolecular Reactions*, Academic Press, New York (1973); (b) P. F. Bente III, F. W. McLafferty, D. J. McAdoo and C. Lifshitz, *J. Phys. Chem.* **79**, 713 (1975).
- (a) R. P. Lattimer, Int. J. Mass Spectrom. Ion Proc. 55, 221 (1983); (b) R. P. Lattimer and G. E. Hansen, Macromolecules 14, 776 (1981); (c) J. Saito, S. Toda and S. Tanaka, Shitsuryo Bunseki 28, 175 (1980); (d) S.-T. F. Lai, K. W. Chan and K. D. Cook, Macromolecules 13, 953 (1980).
- R. B. Cole, C. R. Guenat and S. J. Gaskell, Anal. Chem. 59, 1139 (1987).
- (a) R. L. Hunter, Ph.D. thesis, University of California at Irvine (1972); (b) R. L. Hunter and R. T. McIver Jr, *Amer. Lab.* 9, 13 (1977); (c) R. L. Hunter and R. T. McIver Jr, *Chem. Phys. Lett.* 49, 577 (1977).
- (a) R. T. McIver Jr, *Rev. Sci. Instrum.* 41, 555 (1970); (b) R. T. McIver Jr, *Rev. Sci. Instrum.* 49, 111 (1978); (c) R. T. McIver Jr, R. L. Hunter, E. B. Ledford Jr, M. J. Locke and T. J. Francl, *Int. J. Mass Spectrom. Ion Phys.* 39, 65 (1981); (d) J. E. Bartmess and G. Caldwell, *Int. J. Mass Spectrom. Ion Phys.* 42, 125 (1981).
- (a) J. F. Gower, *Biomed. Mass Spectrom.* 12, 191 (1985);
 (b) B. N. Pramanik, A. K. Mallams, P. L. Bartner, R. R. Rossman, J. B. Morton and J. H. McGlotten, *J. Antibiot.* 37, 818 (1984);
 (c) K. I. Harada, M. Suzuki and H. Kambara, *Org. Mass Spectrom.* 17, 386 (1982).
- M. L. Gross, K. B. Tomer, R. L. Cerny and D. E. Giblin, *Mass Spectrometry in the Analysis of Large Molecules*, ed. by C. J. McNeal, p. 171. Wiley, Chichester (1986).
- G. Caldwell and J. E. Bartmess, Org. Mass Spectrom. 17, 456 (1982).
- (a) R. B. Sharma and P. Kebarle, J. Am. Chem. Soc. 106, 3913 (1984); (b) Y. C. Lee, A. I. Popov and J. Allison, Int. J. Mass Spectrom. Ion Phys. 51, 267 (1983); (c) G. Poidda, L. Corda, A. M. Maccioni and P. Traldi, Org. Mass Spectrom. 21, 395 (1986).
- N. J. Jensen, K. B. Tomer and M. L. Gross, J. Am. Chem. Soc. 107, 1863 (1985).
- P. F. Bente III, F. W. McLafferty, D. J. McAdoo and C. Lifshitz, J. Phys. Chem. 79, 713 (1975).
- 20. P. J. Derrick, Fresenius Z. Anal. Chem. 324, 486 (1986).
- (a) H. M. Rosenstock, M. B. Wallenstein, A. L. Wahrhaftig and H. Eyring, *Proc. Natl. Acad. Sci. USA* 38, 667 (1952); (b) F. W. McLafferty, *Interpretation of Mass Spectra*, 3rd edn, University Science Books, Mill Valley, California (1980).

- (a) D. P. Ridge and J. L. Beauchamp, J. Am. Chem. Soc. 96, 3595 (1974); (b) S. A. Sullivan and J. L. Beauchamp, J. Am. Chem. Soc. 98, 1160 (1976); (c) S. A. Sullivan and J. L. Beauchamp, J. Am. Chem. Soc. 99, 5017 (1977).
- (a) C. H. Depuy and V. M. Bierbaum, J. Am. Chem. Soc. 103, 5034 (1981); (b) C. H. Depuy, E. C. Beedle and V. M. Bierbaum, J. Am. Chem. Soc. 104, 6483 (1982).
- 24. J. C. Kleingold and N. M. M. Nibbering, *Tetrahedron Lett.* 21, 1687 (1980).
- J. P. Kiplinger, PhD thesis, Indiana University, Bloomington, Indiana (1984).
- M. J. Pellerite and J. I. Brauman, J. Am. Chem. Soc. 102, 5993 (1980).
- 27. Heats of formation: $[CH_3Q]^- = -145.2 \text{ kJ mol}^{-1}$, $CH_3OH = -201.7 \text{ kJ mol}^{-1}$. Alkoxide ions: Heat of formation = gas phase acidity $-\Delta H_t^{\circ}(H^+) + \Delta H_t^{\circ}(\text{conjugate acid})$; gas-phase acidity estimated 1544 kJ mol}^1 for all ROH, based on comparison with known values (see Ref. 24); $\Delta H_t^{\circ}(H^+) = 1530$ kJ mol}^1 (Ref. 29). Other neutrals estimated using group additivity rules (Ref. 28).
- S. W. Benson, *Thermochemical Kinetics*, Wiley, New York (1976).
- 29. J. E. Bartmess, J. Phys. Chem. Ref. Data, to be published.
- T. A. Lehman and M. M. Bursey, *Ion Cyclotron Resonance Spectrometry*, Wiley, New York (1976).
- K. Bystrom and M. Mansson, J. Chem. Soc., Perkin Trans. 2 567 (1987).
- J. L. Holmes and F. P. Lossing, J. Am. Chem. Soc. 104, 2648 (1982).
- (a) P. Longevialle, *Spectrosc. Int. J.* 3, 139 (1984); (b) J. H. Stewart, R. H. Shapiro, C. H. Depuy and V. M. Bierbaum, *J. Am. Chem. Soc.* 99, 7650 (1977); (c) C. H. Depuy and V. M. Bierbaum, *Acc. Chem. Res.* 14, 146 (1981).
- 34. (a) P. J. Todd, R. J. Warmack and E. H. McBay, Int. J. Mass Spectrom. Ion Phys. 50, 299 (1983); (b) R. K. Boyd, F. M. Harris and J. H. Beynon, Int. J. Mass Spectrom. Ion Proc. 66, 185 (1985).
- B. A. Rumpf, C. E. Alison and P. J. Derrick, Org. Mass Spectrom. 21, 295 (1986).
- P. J. Robinson and K. A. Holbrook, Unimolecular Reactions, Wiley, New York (1972).
- K. Biemann, S. A. Martin, H. A. Scoble, R. S. Johnson, I. A. Papayannapoulos, J. E. Biller and C. E. Costello, *Mass Spectrometry in the Analysis of Large Molecules*, ed. by C. McNeal, p. 131. Wiley, Chicheşter (1986).