J. Am. Chem. Soc. **1988**, *110*, 7345-7349 7345

Registry No. $ClCH_2^{\bullet}$, 6806-86-6; $Cl_2CH^{\bullet}$, 3474-12-2; $Cl_3C^{\bullet}$, 3170-80-7; $BrCH_2^{\bullet}$, 16519-97-4; $Br_2CH^{\bullet}$, 14362-13-1; $Br_3C^{\bullet}$, 4471-18-5; $ICH_2^{\bullet}$, 16519-98-5; $ClCH_2CH_2^{\bullet}$, 16519-99-6; $ClCHCH_3^{\bullet}$, 16520-13-1; $BrCH_2CH_2^{\bullet}$, 16520-00-6; $BrCHCH_3^{\bullet}$, 16520-14-2; $ClCH_2CH_2OH$, 107-07-3; $ClCH_2C(CH_3)_3$, 753-89-9; Cl_2CHCH_2OH, 598-38-9; $Cl_2CHCOCH_3$, 513-88-2; Cl_3CCH_2OH, 115-20-8; $BrCH_2CH_2OH$, 540-51-2; $H_3CCH(OH)CH_2Br$, 19686-73-8; $BrCH_2COOH$, 79-08-3; Br_2CHCH_2OH, 83206-47-7; Br_3CCH_2OH, 75-80-9; ICH_2CH_2OH, 624-76-0; $ClCH_2CH_2COCH_3$, 6322-49-2; $Cl(CH_2)_3OH$, 627-30-5; $H_3CCH(Cl)COCH_3$, 4091-39-8; $H_3CCH(Cl)CH_2OH$, 78-89-7; $BrCH_2CH_2COOH$, 590-92-1; $Br(CH_2)_3OH$, 627-18-9; $H_3CCH(Br)COOH$, 598-72-1; $H_3CCH(Br)COCH_3$, 814-75-5.

Fragmentation of the Enolate Ions of Some Cycloalkanones. A Comparison of High-Energy and Low-Energy Collisional Activation

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Abstract: The collision-induced dissociation reactions of the enolate ions of cyclohexanone, the methylcyclohexanones, and cycloheptanone have been studied following activation by 8-keV collisions and as a function of collision energy over the range 5 to 100 eV (laboratory scale). Those ions which fragment to form detectable products have higher mean internal energies when activated by 50-eV collisions than when activated by 8-keV collisions. This difference is attributed to differences in the mechanism of activation. High-energy collisional activation involves electronic excitation, and those ions which receive large amounts of excitation energy suffer electron detachment before the excess energy can be redistributed as the vibrational energy necessary for bond rupture. On the other hand, low-energy collisional activation involves vibrational excitation of the ion; more energy can be accommodated in vibrational modes before electron detachment occurs. As a result low-energy collisional activation is likely to prove more useful than high-energy collisional activation in the structure elucidation of negative ions of low electron affinity by tandem mass spectrometry.

The analytical uses of negative ion mass spectrometry have undergone considerable development over the past 15 years, particularly in the areas of negative ion chemical ionization[1-3] and negative ion fast atom bombardment.[4,5] These soft ionization techniques usually yield ions characteristic of the molecular weight of the compound of interest but often do not produce the fragment ions which are necessary to derive structural information. Collision-induced dissociation of polyatomic ions has proved[6] to be a powerful method of providing structural information through formation of fragment ions from the "quasi-molecular" ions formed in the initial ionization process. As a consequence there is a continuing interest in the collision-induced fragmentation reactions of gaseous ions, particularly negative ions for which there was relatively little information until recently.[4,5] In addition to the analytical uses, such collisional activation studies provide fundamental information concerning the behavior of gaseous ions.[4,7]

Such collisional activation studies may be carried out by examining ion/neutral collisions in the keV collision energy range using either B/E linked scans in a conventional-geometry mass spectrometer[8] or electric sector scans (the MIKES technique[9]) in a reversed-geometry instrument to detect the product ions. Alternatively, the ion/neutral collisions may occur at low collision energies (5–100 eV) using quadrupole collision cells and quadrupole mass analyzers,[10,11] multi-Fourier transform mass spectrometric (FTMS) techniques,[12,13] or an ion trap mass spectrometer.[14] This low-energy approach has the advantage that the collision energy can be varied readily, the energy-resolved mass spectrometric (ERMS) technique,[10,11,15,16] thus providing qualitative information on the energy dependence of the fragmentation reactions. There is ample evidence, from positive ion studies, that, at these low collision energies, the kinetic energy transformed into internal energy in the ion/neutral collision increases with increasing collision energy,[17-22] although the energy deposited reaches a

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maximum at higher collision energies.[20,21]

In positive ion studies there is substantial evidence[20-22] that the mean energy of the fragmenting ions does not differ greatly for ions activated by single collisions in the keV range and ions activated by single collisions in the 30–100-eV range. Consequently, similar CID spectra are observed for simple organic cations in both high-energy and low-energy CID.[20,21] By contrast, two recent communications from this laboratory have compared the 8-keV CID mass spectra of ester enolate ions[23] and alkoxide ions[24] with the 50-eV CID mass spectra and have concluded that, in these negative ion systems, the mean internal energy of those ions fragmenting to form detectable products is greater for ions activated by low-energy collisions. The present work extends this comparison of high-energy and low-energy collisional activation to the enolate ions of cyclohexanone, the methylcyclohexanones, and cycloheptanone. Hunt and co-workers[25,26] first reported that the low-energy collisional activation of the enolates of cyclohexanone and 2-methylcyclohexanone resulted in H_2 and C_2H_4 elimination. Very recently, Raftery and Bowie[27] have reported a detailed study of the 8-keV collision-induced dissociation mass spectra of a large number of cyclohexanone enolate ions. Using deuterium labeling they have elucidated the details of the major reactions occurring. For the enolate ions of cyclohexanone and the methylcyclohexanones, the major fragmentation reaction was found to be H_2 elimination. However, other minor reactions involving CH_4 and alkene elimination provided useful structural information. It will be shown in the present paper that these structurally characteristic reactions have higher critical reaction energies than elimination of H_2 and are much more important following activation by 50-eV collisions than following activation by 8-keV collisions. This difference is attributed to differences in the mean internal energy of the ions fragmenting to form detectable products and arises from the different mechanisms of activation in high-energy and low-energy collisional activation. Cooks and co-workers[22] have shown that the mean energy deposited in an ion in a collisional experiment can increase significantly if one is working under multiple collision conditions. Thus, in a comparison of high-energy and low-energy collisional activation, it is necessary that both experiments be carried out under single collision conditions. This aspect is addressed in detail in the following section.

Experimental Section

All experiments were carried out using a VG Analytical ZAB-2FQ mass spectrometer which has been described previously.[28] Briefly, the instrument is a reversed-geometry (BE) double-focusing mass spectrometer with a third stage consisting of a deceleration lens system, an rf-only quadrupole collision cell, and a quadrupole mass analyzer. This assembly permits collisional studies to be carried out in the low-energy range. In addition, the instrument is equipped with a collision cell at the focal point between the magnetic (B) and electric (E) sectors which provides the capability of studying collision processes in the keV energy range.

In the low-energy collisional experiments the ions of interest were mass-selected by the double-focusing BE instrument at 8-keV ion energy, decelerated to the appropriate collision energy (5 to 100 eV, laboratory scale), and introduced into the quadrupole cell where they underwent collision with N_2. The ionic products were mass analyzed using the final quadrupole. The pressure of N_2 was varied from 5×10^{-8} to 4×10^{-7} torr, as indicated by an ionization gauge attached to the pumping line from the quadrupole stage. Over this pressure range there was no significant variation in the CID spectra observed; the data shown in the following were obtained at an N_2 pressure of 1.5×10^{-7} torr. Studies of the charge exchange reactions of $^{36}Ar^+$ with ^{40}Ar in the quadrupole cell[29] indicated that this pressure corresponded to an effective target gas thickness of 0.6×10^{14} cm^2, clearly in the single collision regime.[30]

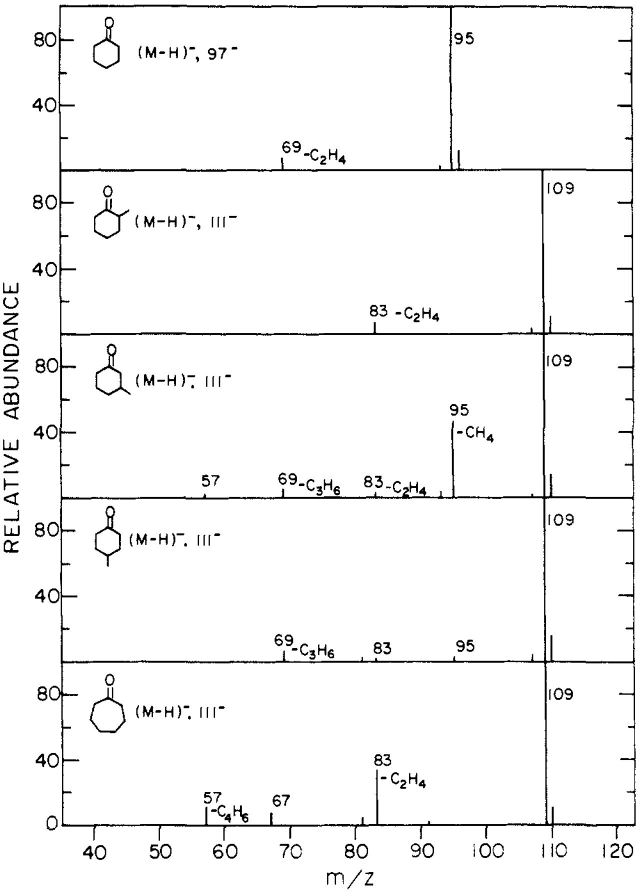

Figure 1. 8-keV CID mass spectra of cycloalkanone enolates.

For the high-energy collisional experiments, the ions of interest were mass-selected by the magnetic sector and underwent collision, at 8-keV energy, with He in the collision cell located between the magnetic and electric sectors. The ionic fragmentation products were analyzed according to their kinetic energy by scanning the voltage applied to the electric sector, the so-called MIKES technique.[9] The He pressure was varied from 7.0×10^{-8} to 4×10^{-7} torr as measured by the ionization gauge on the pumping line from the region, with no change in the spectra observed. The results reported in the following were obtained at a He pressure of 1.5×10^{-7} torr; experiments with positive ions showed this pressure leads to ~20% attenuation of the main beam, an attenuation which corresponds predominantly to single collisions.[31] Unimolecular metastable ion fragmentation reactions occurring in the drift region between the magnetic and electric sectors were studied by the MIKES technique in the absence of He gas in the collision cell.

The enolate ions were prepared by reaction of the appropriate ketone with OH^- in a chemical ionization source operating at ~200 °C and 50-eV ionizing electron energy. The OH^- was prepared by electron impact on a 10% N_2O/90% CH_4 mixture. The cycloalkanones were obtained from the Aldrich Chemical Co. and were used as received.

Results and Discussion

The 8-keV collision-induced dissociation (CID) mass spectra of the enolate ions of cyclohexanone, the isomeric methylcyclohexanones, and cycloheptanone are presented in bar graph form in Figure 1. The results obtained for the first four compounds are in essential agreement with the results reported by Raftery and Bowie.[27] Clearly the dominant fragmentation reaction for all the enolate ions is elimination of H_2; Raftery and Bowie[27] have shown that loss of H_2 occurs from the 3,4- and 3,6-positions with the former being more pronounced. For the cyclohexanone enolate ions the only other fragment ion of more than 10% relative

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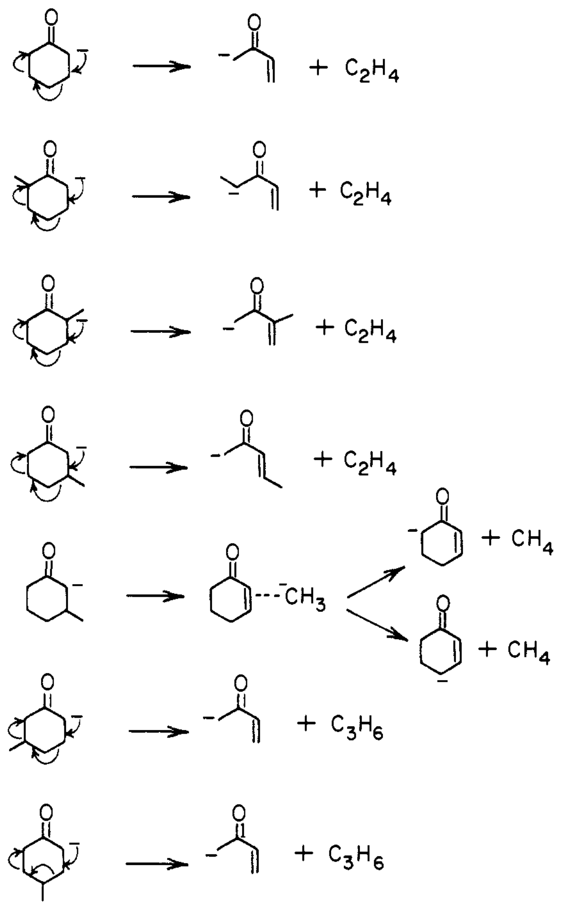
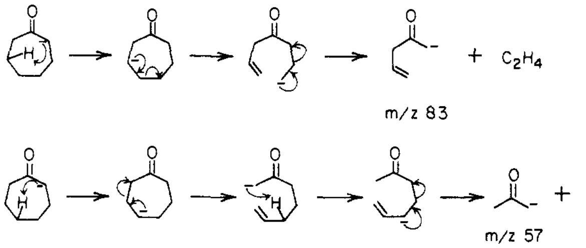
Scheme I



Scheme II



abundance is *m*/*z* 95 corresponding to elimination of CH_4 from the enolate of 3-methylcyclohexanone. Other relatively minor ions, which serve to distinguish the methylcyclohexanones, are observed corresponding to elimination of C_2H_4 and/or C_3H_6 from the enolate ions. The mechanisms for elimination of alkene molecules and CH_4, as elucidated by Raftery and Bowie,[27] are shown in Scheme I. In addition to elimination of H_2, the enolate ion of cycloheptanone shows mainly elimination of C_2H_4 and elimination of C_4H_6; possible mechanisms for these two reactions are outlined in Scheme II.

The 50-keV (laboratory scale) CID mass spectra of the same enolate ions are presented in bar graph form in Figure 2. Clearly, the alkene elimination products, which characterize the structures of the cycloalkanones, are much more abundant, relative to the H_2 loss peak, in the 50-eV CID mass spectra than they are in the 8-keV CID mass spectra of Figure 1. Some light is thrown on the origin of this difference by examining the breakdown graphs (i.e., the fractional fragment ion intensities versus collision energy) for the enolate ions. These are shown in Figures 3 to 7 for the five systems studied. Note that the abscissas in these figures are the center-of-mass collision energy; the data of Figure 2 correspond to 11.2-eV center-of-mass collision energy for the cyclohexanone enolate and to 10.0-eV center-of-mass collision energy for the remaining enolates. Accepting that, at least at low collision energies, the internal energy of the fragmenting ions increases with increasing collision energy,[17-22] one notes that for the enolates of cyclohexanone, 2-methylcyclohexanone, and 4-methylcyclohexanone elimination of H_2 is the dominant fragmentation reaction at the lowest internal energies attainable with elimination of alkene increasing rapidly in importance with increasing collision energy and, hence, internal energy. For the enolates of 3-methylcyclohexanone elimination of both H_2 and CH_4 are observed at the lowest collision (internal) energies attainable while for the enolate

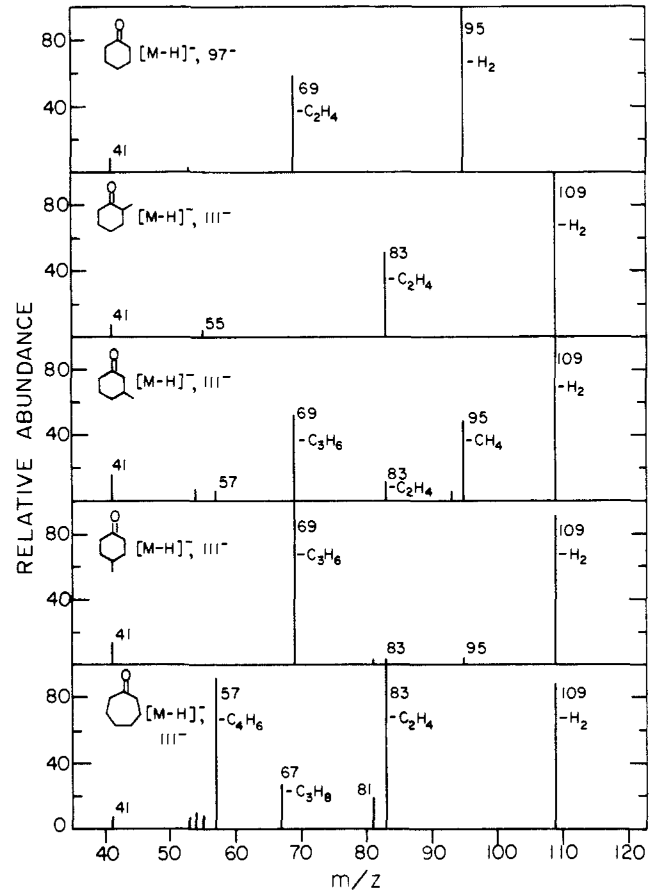


Figure 2. 50-eV CID mass spectra of cycloalkanone enolates.

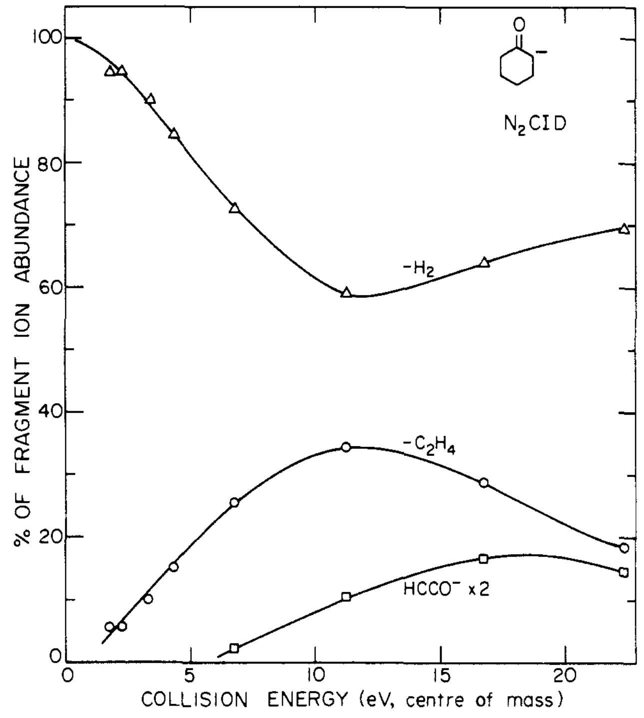


Figure 3. Breakdown graph for cyclohexanone enolate.

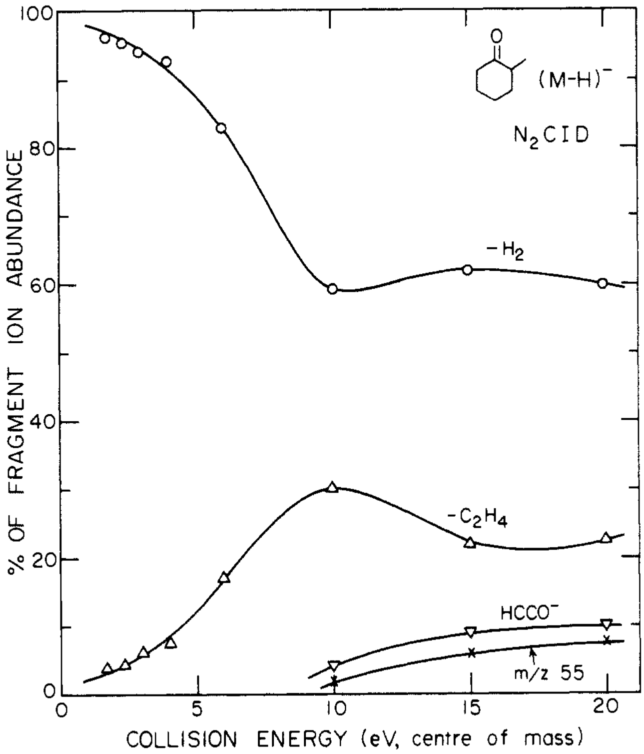


Figure 4. Breakdown graph for enolates of 2-methylcyclohexanone.

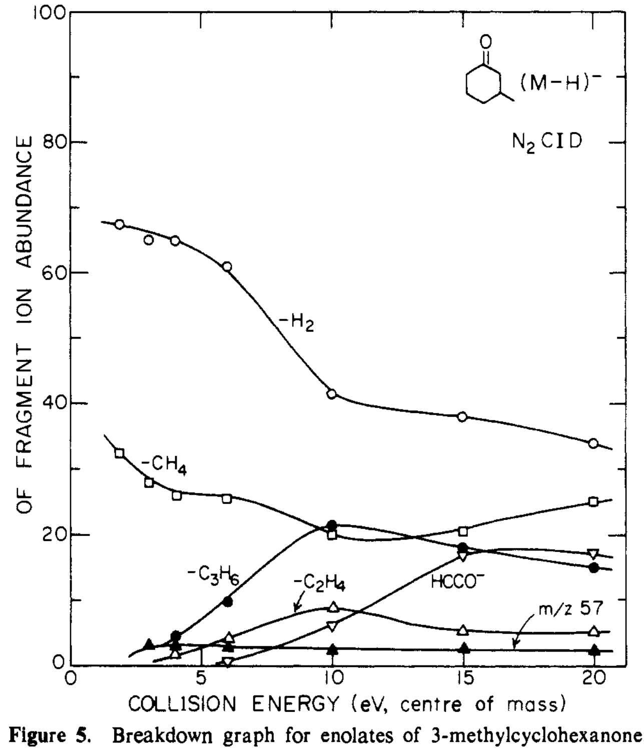


Figure 5. Breakdown graph for enolates of 3-methylcyclohexanone.

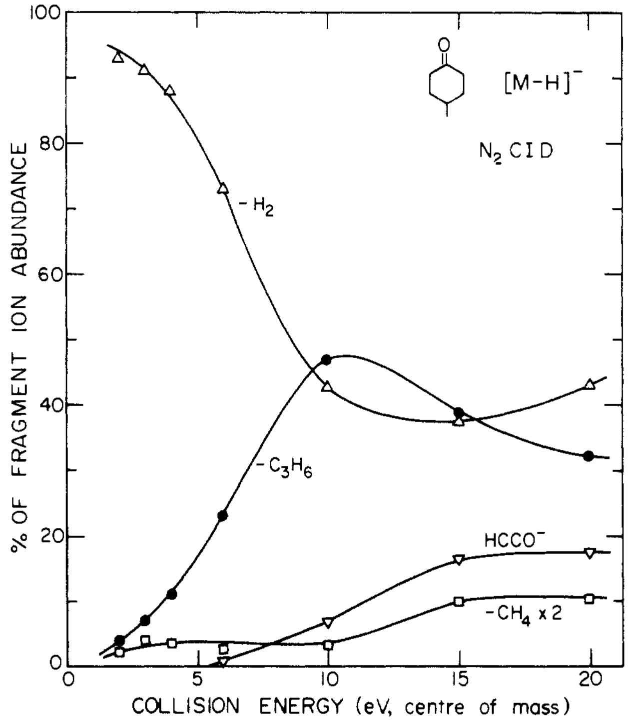


Figure 6. Breakdown graph for 4-methylcyclohexanone enolate.

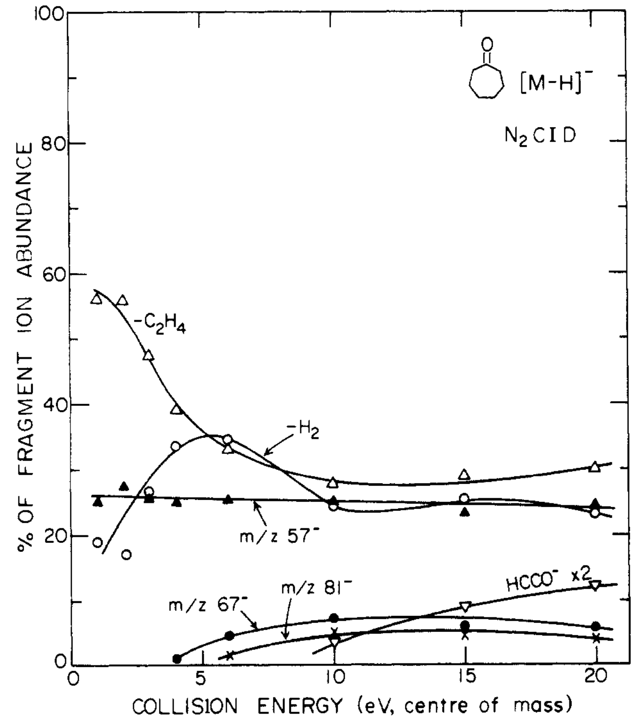


Figure 7. Breakdown graph for cycloheptanone enolate.

Table I. Unimolecular Fragmentation of Cycloalkanone Enolates

cycloalkanone	$(M - H)^-$ *m/z*	% of total ion signal -H	$-H_2$	$-CH_4$	$-C_2H_4$	$-C_4H_6$
cyclohexanone	97	10.3	88.8		0.9	
2-methylcyclohexanone	111	13.9	85.3		0.9	
3-methylcyclohexanone	111		79.1	20.9		
4-methylcyclohexanone	111	12.0	88.0			
cycloheptanone	111	4.8	49.2		32.5	13.5

of cycloheptanone elimination of H_2, C_2H_4, and C_4H_6 are all observed at the lowest energies attainable. These characterizations of the lowest energy fragmentation reactions are in essential agreement with the results obtained from studying the unimolecular fragmentation reactions of the enolate ions occurring in the field-free region between the magnetic and electric sectors, presented in Table I.

Comparison of the high-energy CID mass spectra of Figure 1 with the breakdown graphs (Figures 3–7) clearly indicates that the high-energy collisional data match the low-energy collisional data for center-of-mass collision energies of 2 to 4 eV. By contrast, as noted earlier, the results of Figure 2 correspond to 10.0–11.2-eV center-of-mass collision energy. Accepting, as has been clearly established in positive ion studies,[17-22] that the mean internal energy of the activated ions increases with increasing collision energy over this range, it is clear that the ions fragmenting to yield identifiable products have a lower mean internal energy after activation by 8-keV collisions than they do after 50-eV collisions.

This conclusion is in agreement with the conclusions reached in earlier studies,[23,24] but the present systems represent more dramatic examples.

It is probable that this difference in internal energies arises from differences in the mechanisms of excitation in low- and high-energy collisions. High-energy collisional activation of low mass ions is thought[32] to involve electronic excitation and it is highly probable that those ions which receive a large amount of electronic excitation energy will undergo electron detachment before the excitation energy can be redistributed as vibrational energy, thus leading to bond cleavage. In contrast to high-energy collisional activation, low-energy collisional activation is considered[33,34] to involve translational to vibrational energy transfer, and it appears that a greater amount of energy can be deposited in vibrational modes without leading to electron detachment; i.e., the rate of vibrational to electronic energy redistribution is relatively slow. This difference in mean internal energies attainable is likely to be a common phenomena for organic negative ions of low electron affinity. For such species low-energy collisional activation is likely to be more useful than high-energy collisional activation in the determination of the structures of negative ions by negative ion tandem mass spectrometry. It should be noted that the enolate ions studied in the present work have electron affinities of about 1.5 eV;[35] as the electron affinity of the anion under study increases, one would anticipate that the differences between high-energy and low-energy collisional activation should decrease. We also note that negative ions on high-energy collisions undergo charge inversion reactions[36,37] involving loss of two electrons, so clearly large amounts of energy can be transferred to the anion in a single collision; these charge inversion reactions can be used to identify ion structures.[38]

Finally, in contrast with the results of table I, Raftery and Bowie[27] have concluded that all fragmentation reactions of the enolate ions have a unimolecular component. This conclusion was reached as a result of applying a voltage to the collision cell under CID conditions and observing that all peaks split into two components, one displaced in electric sector voltage by an amount determined by the cell voltage and the other not displaced by the applied cell voltage. The latter component was assigned to "unimolecular" reactions occurring outside the cell while the displaced component was assigned to collision-induced reactions occurring within the cell. We are concerned that collision gas leaking from the cell may result in collision-induced reactions occurring outside the cell leading to an undetermined "collision-induced" component to the undisplaced and apparently "unimolecular" reaction. Accordingly, the results in Table I have been obtained without admitting collision gas to the cell (background pressure $\sim 2 \times 10^{-9}$ torr), and we believe that they represent more truly the unimolecular fragmentation reactions of the enolate ions.

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Electronic Spectroscopy of Small Tryptophan Peptides in Supersonic Molecular Beams

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Abstract: Laser desorption provides an attractive approach to vaporizing thermally sensitive nonvolatile molecules. By coupling this vaporization technique with supersonic molecular beam methods, the high resolution electronic spectra of several tryptophan containing di- and tripeptides have been observed. The detailed spectra of these systems reveal the existence of a large number of stable conformations in the ground electronic state. In the majority of the peptides, strong vibronic activity is found in the spectrum of the conformer possessing the lowest energy electronic transition, suggesting that the excited-state potential energy surface is substantially displaced from the ground-state surface in this particular conformer. Since the electronic excitation in these peptides is localized on the indole ring system of tryptophan, differences in the electronic spectra reflect different perturbations to this chromophore arising from the various peptide environments. Comparison of the electronic spectra in a series of peptides having either an N-terminal or a C-terminal tryptophan residue suggests that the dominant perturbation to the indole chromophore is more closely associated with the location of the tryptophan residue in the chain than with the identity of the other amino acids of which the peptide is composed.

The use of seeded supersonic molecular beams as a means of simplifying complex electronic spectra is well documented.[1] By cooling the internal vibrational and rotational degrees of freedom and providing an isolated environment free from inhomogeneous site broadening, electronic spectra which show extensive congestion at room temperature may often be interpreted in great detail. One requirement of molecular beam studies is that the molecule of interest have sufficient vapor pressure to seed a supersonic expansion. For many nonvolatile, biologically relevant molecules this requirement presents a sufficient obstacle to limit spectroscopic studies to the condensed phase. The use of direct heating as a means of increasing vapor pressure is often not possible due to the instability of these compounds at elevated temperatures.

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