Chapter 6  Electron Ionization

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Conventions Used in This Chapter and Throughout the Book

This is a typical graphical representation of a molecule, \( n \)-propanol:

\[
\begin{array}{c}
\text{H} \quad \text{O} \quad \text{H} \\
\text{H} \quad \text{C} \quad \text{H} \\
\text{H} \quad \text{H} \quad \text{H} \\
\end{array}
\]

**Structure A**

The lines between the symbols for each of the elements represent pairs of electrons shared by the two adjacent atoms. The two pairs of nonbonding electrons on the oxygen atom are represented by the two pairs of dots above and below its symbol; similar dots will be used to represent nonbonding electrons on other heteroatoms.

The \( n \)-propanol molecule can also be represented by this type of structure:

\[
\begin{array}{c}
\text{H}_{3}\text{C} \quad \text{CH}_{2} \quad \text{H}_{2}\text{C} \quad \text{OH} \\
\end{array}
\]

**Structure B**

The \( n \)-propanol molecule can also be represented by this type of structure:

\[
\begin{array}{c}
\text{H}_{3}\text{C} \quad \text{CH}_{2} \quad \text{H}_{2}\text{C} \quad \text{OH} \\
\end{array}
\]

**Structure C**

In a given formula, ions are designated as odd electron (OE) using the sign of the charge (\( + \) or \( - \)) and a dot (\( \cdot \)) for the unpaired electron; or as even electron using only the sign of the charge (\( + \) or \( - \)). \( \text{M}^+ \) is the symbol for a molecular ion.

A single-barbed arrow is used to indicate the movement of a single electron:

\[
\begin{array}{c}
\text{A} \quad \text{C} \quad \text{H} \\
\end{array}
\]

A double-barbed arrow is used to indicate the movement of a pair of electrons:

\[
\begin{array}{c}
\text{C} \quad \text{H} \\
\end{array}
\]

Sigma-bond ionization is indicated using the following symbolism: \( \text{C}^+ \cdot \text{C} \) or \( \text{C} \cdot + \text{C} \).

The \( \Delta \) or \( \uparrow \) symbol on the abscissa of a mass spectrum indicates the position of the molecular ion peak. The \( \Delta \) indicates that a molecular ion peak is not present. The \( \Delta \) indicates that an intensity value is in the mass spectrum for the molecular ion (although it may be so low that the molecular ion peak may not be observable in the graphic display due to the display resolution).
I. Introduction

Still the most widely used technique in mass spectrometry, electron ionization (EI) produces molecular ions from gas-phase analytes. These molecular ions then fragment in a reproducible way, which results in a “fingerprint” of the analyte. Because of the uniqueness of these “chemical fingerprints”, commercially available libraries containing hundreds of thousands of standard EI mass spectra can be used to facilitate identification of unknown compounds.

At one time the abbreviation for EI meant “electron impact”. This name gives an incorrect impression of the fundamental processes involved in this important ionization technique. As was pointed out by Ken Busch [1], if an ionizing electron (70 eV) actually collided with the nucleus of one of the atoms that compose the analyte molecule, the gain in internal energy by the molecule would be infinitesimal. The maximal fraction of translational energy (70 eV, in this case) that can be converted into internal energy of the molecule during an inelastic collision with an electron is related to a ratio of the masses of the colliding bodies via the “center-of-mass” formula; because of the disparity in mass between an electron (~1/2000 the mass of a proton) and any common atom in a molecule, such a fraction would be vanishing small. Furthermore, from the perspective of providing cross-sectional area for a target, the molecule is composed of mostly free space between the nuclei of its atoms, meaning that the probability of a “head-on” collision between an ionizing electron and one of the molecule’s nuclei or one of its electrons is very small. Therefore, the analogy of a cue ball hitting a billiard ball (an elastic collision) is inappropriate for the excitation of a gas-phase molecule leading to ionization, and the term “electron impact” is no longer used.

Energy sufficient for ionizing and fragmenting gas-phase analyte molecules is acquired by interaction with 70 eV electrons produced from a hot filament and accelerated through a 70-V electric field. The ionization chamber is maintained at low pressure (~10^{-4} \text{ Pa}). The pressure in the ion volume (1 cm^3, high gas conductance chamber) of the EI source is ~10^{-1} \text{ Pa}, depending on the method of sample introduction (via GC, batch inlet, or direct insertion probe), to minimize ion/molecule collisions.

Some structural features of the analyte molecule can be deduced from the fragmentation pattern of the molecular ion (M^+). Knowledge gained from organic-solution chemistry concerning electron shifts together with a consideration of the relative stabilities of chemical bonds, odd- and even-electron ions, and radicals (neutral species that have an odd number of electrons) are used to suggest decomposition mechanisms of molecular ions in vacuo to rationalize observed fragmentation pathways.

II. Ionization Process

The source of electrons in nearly all EI mass spectrometers (regardless of the type of m/z analyzer) is a thin ribbon or filament of metal that is heated electrically to incandescence or to a temperature at which it emits free electrons (Figure 6-1). The emitted electrons are attracted to an anode (trap) situated on the opposite side of the ionization chamber from the filament or cathode. The electrons are forced by electric fields through the central space of the ionization chamber as a beam collimated by a slit near the filament. A superimposed magnetic field causes the electrons to move in a tight helical path, which increases the path length, and thus the probability of interaction with a molecule as they transverse the 15–20 mm gap between the slit and the anode.
There are three indicators of direct current that can be monitored during operation of the ion source as illustrated conceptually in Figure 6-2. These indicators are:

1. The electrical heating current through the filament, which results in thermal emission of electrons; this will vary depending on the composition and condition of the filament, but will usually be on the order of 3 to 4 amperes.

2. The total-emission current, which provides an index of the total number of electrons emitted by the filament.

3. The trap or target current, which is the portion of the emitted electron current that flows directly from the filament across the central space of the ionization chamber to the anode. The trap current (that available for ionization) is smaller than the total emission of free electrons because some collide with the filament slit and housing. The magnitude of the trap current is usually on the order of 100 µA.

The energy of the electrons is controlled by the potential difference between the filament and the source block.

Other conditions being constant, the efficiency of ionization of molecules by the electron beam increases rapidly with electron energy from 10 eV to approximately 20 eV for most organic compounds (see Figure 6-3), which brackets the range of the ionization energy of most organic compounds. The energy of the electron beam is reported in electron volts; 1 eV is the energy gained (equivalent to 23 kcal mole\(^{-1}\)) by an electron in traversing an electric field maintained by a potential difference of 1 V. Most reference
spectra are obtained at 70 eV because at that level the perturbations in electron energy have negligible effects on ion production (Figure 6-3) and because reproducible fragmentation patterns are obtained that can be compared with thousands of reference spectra that also were acquired at 70 eV. The linear part of the curve in Figure 6-3 can be extrapolated to the abscissa to obtain the appearance potential for ions [2].

The efficiency of EI is also related to the degree of interaction between sample molecules and energetic electrons. This interaction can be effectively increased by (a) increasing the trap current, (b) increasing the cross section of the electron beam, or (c) increasing the sample pressure in the ionization chamber. However, each of these reasonable suggestions has practical limitations. Most often, the sensitivity or efficiency of ionization is increased by increasing the trap current. The limitation in this case relates

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**Figure 6-2. Electrical circuit schematic of filament and trap in an El source.**

**Figure 6-3. Relationship between ion production and energy (electron volts) of ionizing electrons: A, threshold region, principally molecular ions produced; B, production of fragment ions becomes important; C, routine operation, mostly fragment ions.**
Electron Ionization

to the lifetime of the filament; usually, increasing the filament current above recommended levels to double the sensitivity will more than halve the filament lifetime. The other two suggestions for increasing ionization efficiency are limited by factors that may degrade the resolving power of the mass spectrometer. Some designs of ion optics are based on the formation of ions at a point source; in such designs, the dimensions of the ionizing medium (cross section of the electron beam) should be kept to a practical minimum. Finally, although an increase in sample pressure (short of that causing chemical ionization) in the ionization chamber would increase ionization efficiency, the accompanying increase in pressure in the analyzer would lead to a reduction in resolution due to band-broadening resulting from increased ion/molecule collisions and changes in fragmentation patterns due to collisional activation and subsequent dissociation of ions formed in the source. This approach is practical only with an instrument that has a powerful \((250 \text{ L sec}^{-1} \text{ or greater})\) pumping system or is differentially pumped using two high-vacuum pumps or a split-flow pump, as explained in Chapter 2 on Vacuum Systems.

The pressure in the ion-source housing is maintained at approximately \(10^{-4} \text{ Pa}\) so that the mean free path of the ions is approximately equal to the distance from the ion source to the detector. This relationship gives a satisfactory margin of protection against ion/molecule interactions in instruments that have lesser vacuum systems designed for low or unit resolution. If high-resolution data are required, the pressure in the analyzer must be lower than that reasonably achieved in the ion source; this can be accomplished most effectively with differential pumping of the ion source and analyzer (see Chapter 2). Alternatively, a differentially pumped instrument also provides the opportunity to increase sensitivity in special cases by increasing the sample pressure in the ion source by an order of magnitude over that permissible on a singly pumped instrument.

Regardless of the mode of sample introduction, the sample vapors should be channeled into the ion volume of the ionization chamber. This arrangement leads to maximum sensitivity because all of the sample must pass through the ionization chamber before it expands into the relatively large volume of the ion-source housing and escapes through the vacuum system.

EI might be considered something of an enigma. It is a process that, based upon close examination, appears not to work. Only about 0.01–0.001\% of the analyte molecules that enter the ion volume will be ionized. The remaining unionized molecules are pumped away; i.e., 99.99\% of the sample is wasted! However, even with this dismal ionization efficiency, a sample of 10 pg (some modern instruments claim even smaller amounts) of octafluoronaphthalene injected into a GC will yield a signal in a transmission quadrupole scanned from \(m/z\) 50 to \(m/z\) 300 at a rate of 3 scans sec\(^{-1}\) that can be quantitated using a mass chromatographic peak with a signal-to-background of >10:1 for the molecular ion. This quantity of material is sufficient to produce a full-scan mass spectrum that can be matched against the standard spectrum in the NIST/EPA/NIH Mass Spectral Database with a match factor of >90\%. For this and other reasons, it has long been said, “More information about the structure of an analyte can be obtained from less sample by using mass spectrometry than by any other technique.”
III. Strategy for Data Interpretation

1. Assumptions

When presented with a spectrum of peaks, the investigator assumes that all ions represented by the peaks are derived from a common source, namely the molecular ion of the compound of interest. This assumption may not be correct, as demonstrated by consideration of several criteria to be described shortly. Ions that may derive from contaminants should be identified and disregarded. Ions that result from traces of solvent are examples of those most easily recognized and disregarded. Elucidation of the structure of unknown compounds from their mass spectra should involve consideration of the structural significance of all ions of high mass or prominent abundance.

For those mass spectra that will be used for “fingerprinting” or comparison with reference spectra (mass spectral libraries), the interpreter will assume that the operator has obtained the mass spectra under conditions that reflect the true fragmentation pattern, not those that may distort peak intensities in the overall mass spectrum, such as scanning the m/z range of the mass spectrometer during GC elution when the sample concentration in the ion source may be changing rapidly during the scan (see Chapter 10).

During attempts to rationalize the losses observed from the molecular ion, it is generally assumed that the odd electron and positive charge are localized on a heteroatom or at the site of a double bond, if either of these is present. This assumption provides a rational starting point from which to consider various pathways of fragmentation of the molecular ion. The thermochemical basis for assuming that the odd electron in a molecular ion most likely resides on a heteroatom or at the site of a double bond is based on the fact that the ionization potential for an electron in a nonbonding orbital or a π bond is lower than for those in other orbitals (e.g., in a sigma bond), as explained in the following section.

2. The Ionization Process

A neutral molecule absorbs energy during the interaction of the ionizing electron with its electron cloud. As shown in Scheme 6-1, the analyte molecule interacts with an electron having 70 eV of kinetic energy, and through the interaction, absorbs approximately 14 eV as internal energy, which quickly causes the ejection of one of the electrons from the analyte (a thermal electron with <1 eV energy) and a residual energetic electron now having 56 eV of kinetic energy. Whereas some analyte molecules do absorb approximately 14 eV of energy from this interaction, there is actually a wide distribution of energies absorbed, such that a few molecular ions may absorb 30 eV, others only 9 eV of energy, etc. The greater the amount of energy absorbed from the ionizing electron, the greater the tendency for the nascent molecular ion to decompose into fragment ions. In principle, the 56-eV electron shown at the right in Scheme 6-1 can interact with yet another molecule of the analyte effecting ionization.

\[ M + e^- \rightarrow M^{+\bullet} + e^- + e^- \]

Scheme 6-1
Because the analyte molecule has many electrons, it is reasonable to ask, “Which electron leaves during the ionization process?” Whereas sufficient energy may have been absorbed by the analyte molecule to eject any one of its electrons, the most probable ejection will be of an electron that is least tightly bound. Therefore, it is predictable that the most likely site of ionization will be where electrons are loosely bound; e.g., in the nonbonding orbital of a heteroatom.

Consideration of the ionization potential and electronic configuration of a few simple molecules provides some useful insight into the phenomenon of ionization. Imagine that you have a very basic EI mass spectrometer in which you can control the energy of the bombarding electrons so that you can measure the minimum energy necessary to cause ion current to be generated in the ion source, pass through the m/z analyzer, and strike the detector. This basic instrument would be equipped with a reservoir inlet system into which you could place the gaseous analyte, allowing a small quantity to continuously leak into the ion source while you increase the energy of the electrons in an effort to prepare a graph similar to that in Figure 6-3 to determine the minimum energy necessary to ionize the compound. Now place ethane in the reservoir and increase the energy of the ionizing electrons until the ionization potential is reached. For ethane, this will require approximately 11.5 eV to produce molecular ions, which would then be accelerated through the mass spectrometer to the detector.

In using the basic mass spectrometer to assess the ionization potential for each of the compounds listed in Table 6-1, it would be observed that only 10.5 eV is required to generate ion current from ethene rather than 11.5 eV for ethane [3]. Examination of the electronic configuration of these two compounds reveals that ethane consists of seven \( \sigma \) bonds, whereas ethene only has five \( \sigma \) bonds, but it also has two overlapping \( p \) orbitals to give a \( \pi \)-bond orbital. Because the principal difference between these two compounds is the presence of the \( \pi \)-bond orbital in ethene, it must be that an electron is easier to remove from a \( \pi \)-bond orbital than from a \( \sigma \)-bond orbital.

Carrying out the experiment further, it will be found that only \( \sim 10 \) eV are required to cause ionization of dimethyl ether. An examination of the electronic configuration of dimethyl ether shows that it consists of eight \( \sigma \) bonds and two nonbonding orbitals. The implication from the experimental results so far is that it is easier to remove an electron from one of the nonbonding orbitals of oxygen than from the \( \pi \)-bond orbital in ethene or from a \( \sigma \)-bond orbital of ethane.

Table 6-1. Ionization energy for selected compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ionization energy* in electron volts (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_3\text{C}–\text{NH}–\text{CH}_3 )</td>
<td>8.23</td>
</tr>
<tr>
<td>( \text{H}_3\text{C}–\text{CH}_2–\text{NH}_2 )</td>
<td>8.86</td>
</tr>
<tr>
<td>( \text{H}_3\text{C}–\text{O}–\text{CH}_3 )</td>
<td>10.03</td>
</tr>
<tr>
<td>( \text{H}_2\text{C}=\text{CH}_2 )</td>
<td>10.51</td>
</tr>
<tr>
<td>( \text{H}_2\text{C}–\text{CH}_3 )</td>
<td>11.52</td>
</tr>
</tbody>
</table>

Continuing further with the analysis of ethylamine, it will be noted that it requires only approximately 8.9 eV to generate detectable ion current. The electronic configuration of ethylamine consists of nine σ-bond orbitals and one nonbonding orbital. Once again, the implication is that it requires less energy to remove an electron from a nonbonding orbital than from a π-bond orbital or from a σ-bond orbital, but, in addition, there is a disparity between the nonbonding orbital of nitrogen and that of oxygen. This can be explained by their relative positions in the periodic table, where oxygen is farther to the right, indicating a greater electronegativity, which is consistent with its greater reluctance to give up an electron from its nonbonding orbital relative to nitrogen relinquishing one of its nonbonding electrons.

Although it is certainly possible to generate a $M^+$ from ethene, dimethyl ether, or ethylamine by removing an electron from one of its many σ-bond orbitals, this is a more expensive process in terms of the amount of energy required than is removing an electron from either a π-bond orbital or a nonbonding orbital. Therefore, the probability of the electronic configuration of the $M^{2+}$ of ethene, dimethyl ether, or ethylamine being in the form of a one-electron σ bond is much less than that for one in which the odd electron resides in a π-bond orbital or in a nonbonding orbital, respectively. It is important to remember that although there is a “most probable site” for the charge and for the odd electron in the $M^+$ of a specific analyte, in reality there can be a wide variety and distribution of molecular ions differing in charge-radical sites, especially in analytes containing a variety of heteroatoms, π-bond orbitals, and so forth.

When considering the fragmentation pattern of dimethyl ether or that of ethylamine, the fragmentation schemes in which the radical site or odd electron is on the heteroatom, namely oxygen or nitrogen, should dominate. In the case of the $M^{2+}$ for ethene, it should be assumed that the odd electron resides in between the two carbon atoms. In the case of ethane, consideration of the electron being removed from any one of the seven σ-bond orbitals would be a good starting point for the fragmentation process.

The location of the charge and radical sites in a $M^{2+}$ assumes that the molecule has a known structure. How can this knowledge of the charge-radical site be of benefit in determining the structure of an unknown? By understanding the way in which molecular ions of known compounds fragment, it is possible to deduce the structure of an unknown analyte from the presence of peaks in the mass spectrum and from the dark matter represented by the numerical difference between peaks representing fragment ions and molecular ions. Methods for interpreting a mass spectrum are provided in great detail in Chapter 5. This chapter describes the overall appearance of the EI mass spectra and mechanisms by which molecular ions fragment; these features assist in the deduction of what structural moieties are present in the corresponding analyte molecule.

As explained in Chapter 5, the first step in the interpretation of a mass spectrum is to determine whether a $M^+$ peak is present in the mass spectrum. In many cases, the elemental composition of the $M^+$ can be determined from the isotope peaks associated with it or from its accurately determined mass. The Nitrogen Rule, used in establishing the validity of a peak as representing the $M^+$, also provides some information about the elemental composition of the analyte. The elemental composition provides information as to whether heteroatoms are present and the possible number of π-bond orbitals that may be in the $M^+$ (as explained in Chapter 5). If no $M^+$ peak is present, it is still possible to identify the analyte. This can be done through an examination of other peaks in the mass spectrum or through the use of a different ionization technique such as
Electron Ionization

In some types of EI mass spectrometers, the M** peak candidate can be confirmed by low-electron-energy ionization. As the effective energy of the electron beam is reduced to a level near the ionization potential of the organic compound (typically 9–12 eV), fragmentation of the M** diminishes because less excess energy is absorbed, making the M** more stable and therefore more abundant relative to other ions in the spectrum. In this way, the validity of the choice for the M** peak can be tested by acquiring the mass spectrum at low electron energy, even though the detection limits are worse because the ionization efficiency also decreases with energy.

Consider the “unknown” mass spectrum in Figure 6-4A. Based on an initial examination, it appears that the peak at m/z 98 represents the nominal M**. However, if that were the case, the ions with m/z 84 and 85 would have to be generated by losses of 14 Da and 13 Da, respectively, from the M**. As such losses are not allowed, it is likely that the sample in the heated-inlet reservoir system, in this case, is impure. This means that Figure 6-4A is a mass spectrum of two or more analytes of different nominal masses. To prove this point, the electron energy is decreased to 16 eV for a second analysis (Figure 6-4B). Important changes in the relative intensities of several peaks are observed. Whereas in the original spectrum the base peak was m/z 84, the peak at m/z 85 is the base peak in this spectrum. The peak at m/z 98 now has a relative intensity equivalent to that of most of the peaks below m/z 84. Decreasing the electron energy still further to 12 eV for a third analysis gives the result in Figure 6-4C. Nearly all fragment ions represented by peaks below m/z 84 are insignificant; the peak at m/z 85 is not only the base peak, but it represents the majority of the ion abundance.

Figure 6-4. Three mass spectra illustrating the effect of ionization potential on the relative ion current of a M** and the fragment ions.
The peaks at m/z 85 and 98 in Figure 6-4C must represent the molecular ions of a two-component mixture, as these are the only two significant peaks remaining at low ionizing potential. Based on a logical-loss rational, it can be assumed that the peak at m/z 84 in all three mass spectra represents the loss of a hydrogen radical from the component that exhibits a M⁺ peak at m/z 85. It is also clear from the decreased abundance that the component that has a nominal mass of 98 Da has a higher ionization potential than the component that has a nominal mass of 85 Da. This makes sense because based on the Nitrogen Rule (Chapter 5) the component with a nominal mass of 85 Da must contain an odd number of nitrogen atoms.

There are two important features to appreciate about obtaining spectra at low ionizing energy. The first is that as the ionizing energy is reduced to near the ionization potential of most organic molecules (10–11 eV), there will be very little excess energy transferred to the nascent molecular ions, which means that fragmentation of these molecular ions will be minimal. This feature is illustrated in the sequence in Figure 6-4 A–C, which shows a profound diminution in fragmentation in going to a lower ionizing potential. The second feature is not so obvious from viewing the sequence in Figure 6-4; the efficiency of the ionizing process also drops off dramatically with the reduction in ionizing energy (see Figure 6-3). The absolute magnitude of the M⁺ current at m/z 85 and at m/z 98 in Figure 6-4C is approximately three orders of magnitude less than that in Figure 6.4A; however, the absolute intensity of the fragment ions diminishes by a factor of 4–5 orders of magnitude in going from 70 eV (Figure 6-4A) to 12 eV (Figure 6-4C). On a relative basis, the M⁺ peak appears to grow with decreasing ionizing potential. Of course, it is the discrimination against fragmentation that is useful in this technique.

Finally, to complete the explanation of the spectra in Figure 6-4, the peak at m/z 85 represents the M⁺ of piperidine, which contains one nitrogen atom; therefore, it has an odd nominal mass. Piperidine fragments readily by eliminating a hydrogen radical to form an ion of mass 84; because little energy is required to produce this fragment, a trace of it remains in the 12-eV spectrum (Figure 6-4C). The peak at m/z 98 represents the M⁺ of cyclohexanone, a compound containing only carbon, hydrogen, and oxygen.

The general appearance of the data also indicates the type of compound that generated the mass spectrum. One important aspect of the mass spectrum is the percent total abundance of the M⁺. This term “percent total abundance” expresses the abundance of a particular ion compared with the sum of the abundances of all ions in a specified m/z range. One convention that has been used to aid the determination of a total abundance scale is shown in Figure 6-5. In this convention, the scale for percentage total ionization is presented on the ordinate at the right or the high-mass end of the mass spectrum with a sigma (Σ) symbol, such as %Σ₁₂ in Figure 6-5, where %Σ₁₂ indicates that all of the ion current from m/z 12 up to and including the M⁺ (and its isotope peaks, as described in Chapter 5) was used as the basis for determining the contribution of any given ion to the total ionization in the specified m/z range. For example, in Figure 6.5 the peak at m/z 43 (the base peak) in the specified m/z range is responsible for 47% of the total ion current (TIC) represented by all the peaks in the range m/z 12 to m/z 60; the peak at m/z 58 has a relative intensity of about 25%, which represents approximately 12% (25% of 47%) of the total ion current as estimated from the bar graph. Most data systems no longer provide this convenient graphic presentation, which is unfortunate. Percentage of total ion current (%Σₙ) is the only expression of ion
Figure 6-5. Illustration of the concept of \( \Sigma \) to show the percentage of the total ion current represented by any peak; in this presentation, the base peak is not assigned 100%, but it still represents the largest fraction of the total ion current.

abundance that can be used in a valid comparison of the mass spectra of different types of compounds, such as those of isomers.

Depending on the type of compound, the M\(^{•}\) may represent only a small fraction of the total ion current. For example, compare the abundance of the molecular ions of the several compounds in Table 6-2 [4]. Each of the compounds in Table 6-2 contains ten carbons, but in combination with heteroatoms or structures ranging from aromatic to aliphatic, normal chain to highly branched. Note that the M\(^{•}\) of polyaromatic naphthalene resists fragmentation, and therefore the M\(^{•}\) peak represents a large fraction of TIC (36.7\%\( \Sigma \)). Although many aliphatic compounds have a M\(^{•}\) peak of only 1\%\( \Sigma \), this peak is still readily discernible in the spectrum; however, the M\(^{•}\) peak for long-chain alcohols or branched compounds may not be detectable (<0.01\%\( \Sigma \)), especially at the data acquisition rates associated with techniques such as GC/MS using capillary GC columns.

Figure 6-6. Mass spectrum of hexadecane showing the ion series incremented by m/z 14 corresponding to –CH\(_2\)– homologs.
Table 6-2. Abundance of molecular ions in mass spectra of selected compounds of various structures and elemental compositions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abundance (%Σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>44.3</td>
</tr>
<tr>
<td>Quinoline</td>
<td>39.6</td>
</tr>
<tr>
<td>n-Butylbenzene</td>
<td>8.26</td>
</tr>
<tr>
<td>trans-Decalin</td>
<td>8.22</td>
</tr>
<tr>
<td>tert-Butylbenzene</td>
<td>7.00</td>
</tr>
<tr>
<td>Alloocimene</td>
<td>6.40</td>
</tr>
<tr>
<td>Diamyl sulfide</td>
<td>3.70</td>
</tr>
<tr>
<td>n-Decane</td>
<td>1.41</td>
</tr>
<tr>
<td>n-Decylmercaptan</td>
<td>1.40</td>
</tr>
<tr>
<td>Diamylamine</td>
<td>1.14</td>
</tr>
<tr>
<td>Methyl nonanoate</td>
<td>1.10</td>
</tr>
<tr>
<td>Myrcene</td>
<td>1.00</td>
</tr>
<tr>
<td>Cycloododecane</td>
<td>0.88</td>
</tr>
<tr>
<td>3-Nonanone</td>
<td>0.50</td>
</tr>
<tr>
<td>n-Decylamine</td>
<td>0.50</td>
</tr>
<tr>
<td>Diamyl ether</td>
<td>0.33</td>
</tr>
<tr>
<td>cis-cis-2-Decalol</td>
<td>0.08</td>
</tr>
<tr>
<td>3-Nonanol</td>
<td>0.05</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.04</td>
</tr>
<tr>
<td>3,3,5-Trimethylheptane</td>
<td>0.007</td>
</tr>
<tr>
<td>n-Decanol</td>
<td>0.002</td>
</tr>
<tr>
<td>Tetrahydrolinalool</td>
<td>0.000</td>
</tr>
</tbody>
</table>


EI mass spectra that exhibit few peaks, several of which may have relatively high intensities, usually are of resonantly stabilized compounds such as aromatic compounds. Mass spectra that have a large number of peaks represent analytes that are more aliphatic in nature. One term that is used in the description of a mass spectrum is “ion series”. An ion series is represented by peaks separated by a specific number of m/z units. The spectrum of hexadecane (Figure 6-6) shows periodic clusters of peaks; in most cases, there is a spacing of 14 m/z units between a peak at the highest m/z value (that does not represent an isotopic ion) in one cluster and that of an adjacent cluster.
These clusters of peaks represent ions composed of sequential increments of a $-$CH$_2$—unit. That is not to say that a given ion is formed by the loss of a $-$CH$_2$—unit from the ion represented by a peak 14 m/z units higher in the mass spectrum. These ions are formed by the loss of homologous radicals (of successively larger numbers of $-$CH$_2$—units) from the molecular ion.

Another important feature of EI mass spectra is the presence of certain peaks that represent “diagnostic” ions in the mass spectrum. The $m/z$ values for these peaks should indicate the possibility of a structural moiety in the analyte or a possible contaminant in the sample. The $m/z$ values (or loss from the M$^{+•}$) and structural formula for some common diagnostic ions are listed in Table 6-3.

Table 6-3. List of significant peaks in EI mass spectra representing diagnostic ions of importance.

<table>
<thead>
<tr>
<th>Compound Type</th>
<th>Structure of Ions</th>
<th>$m/z$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic amines</td>
<td>R$_2$C=NH$_2$ or H$_2$N=CR$_2$</td>
<td>30, 44,58</td>
</tr>
<tr>
<td>Aliphatic alcohols</td>
<td>R$_2$C=OH</td>
<td>31, 45, 59</td>
</tr>
<tr>
<td>Aliphatic ketones</td>
<td>(CH$_3$)$_2$C=OH</td>
<td>58</td>
</tr>
<tr>
<td>Aliphatic acids</td>
<td>H$_2$C(C=O)OH</td>
<td>60</td>
</tr>
<tr>
<td>Methyl esters</td>
<td>H$_3$C(C=O)OCH$_3$ and °OCH$_3$ loss</td>
<td>74, M – 31</td>
</tr>
<tr>
<td>Aromatics</td>
<td>C$_6$H$_5$, C$_7$H$_7$, H$_3$C–C$_8$H$_4$, ring fragments</td>
<td>77, 91, 105, and 39, 51, 65</td>
</tr>
<tr>
<td>Phthalate</td>
<td>(C$_6$H$_4$)(C=O)$_2$OH</td>
<td>149</td>
</tr>
<tr>
<td>Column bleed</td>
<td>[(CH$_3$)$_2$SiO]$_n$ – CH$_3$ where n ≥ 3</td>
<td>207, 281</td>
</tr>
<tr>
<td>Trimethylsilyl derivative</td>
<td>Si(CH$_3$)$_3$</td>
<td>73</td>
</tr>
</tbody>
</table>

IV. Types of Fragmentation Pathways

Once an $m/z$ value for the M$^{+•}$ and its probable elemental composition has been established, it is possible to see which peaks may represent the various possible fragment ions resulting from cleavage of certain bonds in the M$^{+•}$. The odd-electron (OE) site formed in the M$^{+•}$ during EI can initiate cleavage of chemical bonds to form an even-electron (EE) species or another OE species. These species will be ions of the same charge sign or will be neutrals. When a M$^{+•}$ fragments, it produces an ion with the same charge sign (positive in EI) and a lesser mass along with a neutral, which can be a molecule with a smaller mass (an even-electron species) or a radical with lesser mass than the molecular ion (an odd-electron species). EI fragmentation does not result in an ion pair (two ions of opposite charge sign).

After identifying the M$^{+•}$ peak and ascertaining as much information as possible from this peak and using the general appearance of the mass spectrum to formulate some idea as to the type of compound that may have produced it, the next step is to identify any peaks that may represent odd-electron fragment ions (for more detail, see Chapter 5). Odd-electron fragment ions formed from molecular ions are very important because they require the presence of specific structural features. Odd-electron fragment ions result from rearrangements. Unlike the other three mechanisms for the M$^{+•}$
Types of Fragmentation Pathways

fragmentation listed below, rearrangements involve breaking more than one chemical bond and the formation of new chemical bonds. The other three mechanisms involve only single-bond cleavage. Peaks representing OE ions can be recognized from the mass spectra according to whether they occur at even or at odd m/z values coupled with a knowledge of the elemental composition of the M$^+$ from which they derive. Remembering from the Nitrogen Rule that a molecule that contains an even number of nitrogen atoms (e.g., a molecule containing zero or two atoms of nitrogen) has an even nominal mass, a M$^+$ peak for such a species, an OE ion, would occur at an even m/z value in the spectrum. On the other hand, a fragment ion that contains an even number of nitrogen atoms, formed by loss of a radical from a M$^+$, would have an even number of electrons, but would be represented by a peak in the mass spectrum occurring at an odd m/z value. As a simple example, a molecule that does not contain any atoms of nitrogen will be represented by a M$^+$ peak at an even m/z value in the mass spectrum. When the odd-electron molecular ion (an odd-electron species) containing no nitrogen undergoes single-bond fragmentation through the loss of a radical, the resulting even-electron ion will have an odd m/z value (see Scheme 6-2). When a M$^{++}$ of the same compound fragments through a rearrangement, a molecule (an even-electron species) is the neutral loss (dark matter), and the resulting odd-electron ion will have an even mass.

Scheme 6-2

Now look at a more complicated case of a M$^{++}$ that contains two atoms of nitrogen represented by a peak at an even m/z value. This M$^{++}$ (an odd-electron ion) fragments with the loss of a radical (an odd-electron species) that retains one of the two nitrogen atoms. The resulting fragment ion will have an odd number of nitrogen atoms; because it was formed by the loss of an odd-electron radical from an odd-electron ion, the fragment ion will have an even number of electrons, and because it contains an odd-number of nitrogen atoms, it is represented by a peak at an even m/z value (Scheme 6-3). There is an important structural significance of odd-electron fragment ions (in signaling the existence of a rearrangement i.e., the elimination of a molecule) that cannot be overemphasized.

Scheme 6-3
Fragmentation of the \( \text{M}^{++} \) following formation by EI can occur by a variety of processes, most of which can be rationalized by one of the following four mechanisms:

A. Sigma-Bond Cleavage
B. Homolytic or Radical-Site-Driven Cleavage
C. Heterolytic or Charge-Site-Driven Cleavage
D. Rearrangements

1. Sigma-Bond Cleavage

The homolytic and sigma-bond cleavage pathways are initiated by the odd electron in OE ions, whereas the heterolytic cleavage pathways can be initiated by an electron pair in either OE ions or EE ions. Rearrangements are usually initiated by a radical site in an OE ion or by an electron pair in an EE ion. A given type of ion, whether it is an OE or an EE species, has the possibility of participating in more than one of the four different fragmentation processes.

In general, the driving force for fragmentation of an ion is somewhat dependent on the bond strength in the original ion, the stability of the resulting ion, and the stability of the neutral loss (radical or molecule) relative to the energetics of the original ionic species. That is to say, if the sum of the internal energies of ion \( B \) and radical \( C \) are less than the internal energy of \( \text{M}^{++} \) in Scheme 6-4, then fragmentation will proceed spontaneously as it is an overall exothermic process. Following this concept, “paper mechanisms” can be drawn to explain certain fragmentations if the site of electron deficiency and/or positive charge is presumed to be known. The paper mechanisms that are defined, described, and given as examples on the next several pages can be used to anticipate the kinds of data to expect during analysis of a given molecule by EI, or used to rationalize the data observed during analysis of a given compound by EI.

\[
\text{M}^{++} \rightarrow ^{+} B \quad \text{and} \quad ^{+} C
\]

\textit{Scheme 6-4}

A rigorous treatment of the thermodynamics and spectroscopic considerations associated with the fragmentation of ions during the electron ionization process can be found in \textit{Mass Spectrometry: A Textbook} by Jürgen H. Gross (Springer-Verlag, Berlin, 2004).

It is important to realize that these paper mechanisms are generally attempts to extend the rationale of organic chemistry (in which solvents are usually involved) to an understanding of unimolecular decompositions \textit{in vacuo}. These mechanisms should not be taken as an indication of detailed knowledge of actual electron movement within the ion. However, the general concepts described below have been used remarkably well in studies of the mechanisms of decomposition of OE ions [5–7]. The art and science of assigning structures to gas-phase ions have been reviewed [8].
Most of the bonds broken in a mass spectral fragmentation process are sigma bonds. The term “sigma bond cleavage” is reserved for fragmentation that occurs as a result of the loss of a $\sigma$-bond electron during the ionization process (sigma-bond ionization) that forms the $M^{+}$. As shown in Scheme 6-5, the site of sigma-bond ionization is represented by a single-electron bond; this is the weak link in the $M^{+}$ in which all other bonds consist of two electrons. As shown in species A of Scheme 6-5, the $M^{+}$ of $n$-butane consists of four carbon atoms held together by three $\sigma$ bonds; two of the bonds consist of two electrons and one of the bonds consists of a single electron. The $M^{+}$, species A in Scheme 6-5, is represented by a peak at $m/z$ 58 (an even value) because it does not contain an odd number of nitrogen atoms. Those molecular ions of $n$-butane illustrated in Scheme 6-5 will fragment at the bond between the methyl carbon and the other three carbons giving rise to a methyl radical (species C) that will not be detected (because it does not carry a charge) and a charged species (the propyl ion) that will be detected at $m/z$ 43.

In other words, the charge is retained by the propyl portion of the $M^{+}$ and the radical is retained by the methyl portion (the neutral loss). If the other electron in this bond had been removed during ionization, sigma-bond cleavage of that one-electron bond would have left the odd electron on the propyl portion and the charge on the methyl group (a propyl radical and a methyl ion). A sigma-bond electron could have been lost from any of the carbon–carbon bonds or from a carbon–hydrogen bond in butane. In many cases (but not all), when sigma-bond cleavage occurs, ions of both possible $m/z$ values will be represented in the mass spectrum, but rarely by peaks of equal intensity.

Unlike charge sites resulting from the loss of an electron associated with heteroatoms and $\pi$ bonds, charge sites resulting from the loss of sigma-bond electrons can migrate throughout the $M^{+}$, resulting in cleavage at any point along the carbon–carbon skeleton. There are a number of guidelines that can be applied to the species produced by the fragmentation of a $M^{+}$. As with many sets of guidelines, there are numerous exceptions:

- The charge site will be associated with the more stable of two possible ions.
- The radical site will be associated with the portion that has the highest ionization potential.
- There is a tendency to lose the largest possible neutral species.
- Fragmentation will occur at the weakest bond.

These guidelines not only apply to fragmentation resulting from sigma-bond cleavage but also to that resulting from the three other driving forces listed above.
Examination of the mass spectrum of \( \text{n-butane} \) (Figure 6-7) shows that the most stable ion is the propyl ion, which is represented by the base peak; the intensity of this peak might also suggest that the methyl radical is the most stable radical. However, methyl radicals are considerably less stable than larger radicals. The disparity in the intensities of the peaks representing the methyl and propyl ions is also very noticeable, indicating that the propyl ion is formed in preference to the methyl ion even through both ions could be formed by cleavage of the carbon–carbon bond between the number 1 carbon and the number 2 carbon. Although ethyl ions could be formed by retention of the charge by either the number 2 or the number 3 carbon atom, the intensity of the peak at \( m/z \) 29 representing this ion is only about half that of the base peak. There is another important observation that can be made from this mass spectrum. Acylium ions of the form \( \text{C}_n\text{H}_{(2n - 1)}\text{O}^+ \) have the same nominal mass as aliphatic ions of the form \( \text{C}_2\text{H}_{(4n + 1)} \), i.e., both \( \text{H}_3\text{C}--\text{C}'\text{H}_2 \) and \( \text{HC}=\text{O}^+ \) have a nominal mass of 29 Da. In a mass spectrum where all \( m/z \) values are reported to the nearest integer, these two ions cannot be distinguished from one another; however, an examination of the mass spectrum of 3-pentanone (Figure 6-8) shows two fragment ion peaks, one at \( m/z \) 29 and the other at \( m/z \) 57, representing an ethyl ion and a methyl acylium ion, respectively. There are peaks of significant intensities at \( m/z \) 28, 27,
and 26 preceding the peak at $m/z$ 29, indicating secondary fragmentation of the aliphatic ion through the losses of hydrogen radicals and molecules, whereas such peaks representing secondary fragmentation ions are not associated with the peak at $m/z$ 57, which represents the acylium ion. This tendency for aliphatic ions to undergo secondary fragmentation allows these types of ions to be differentiated from acylium ions of the same nominal mass in many cases. A number of factors determine the extent to which this secondary fragmentation of these aliphatic ions occurs. These factors include the pressure in the ion source, whether the aliphatic ion results from a fragmentation of the $M^+$ and whether the aliphatic ion was formed by the expulsion of a molecule from a larger aliphatic ion produced from a $M^+$.

2. Homolytic or Radical-Site-Driven Cleavage

Homolytic or radical-site-driven cleavage results from the tendency of the odd electron associated with a heteroatom or a $\pi$ bond in the $M^+$ to pair with another electron for better stability; like cops and snakes, electrons travel in pairs. Typically, an initial consideration of the fragmentation mechanism assumes that the OE species has the “plus/dot” (the charge/the odd electron) at a single location, possibly in a nonbonding orbital of a heteroatom such as that of oxygen as illustrated in Scheme 6-6. The movement of single electrons in the paper mechanism (Scheme 6-6) is indicated by a single “fishhook” or single-barbed arrow [5]. The overall electron movement in homolytic cleavage will commonly be indicated with fishhooks a and b in Scheme 6-6; fishhook c is shown here for the sake of completeness. Fishhooks a and b indicate movement of two single electrons that form an additional bond between carbon and oxygen in this case.

![Scheme 6-6](image)

The radical site on the OE molecular ion (A) stimulates transfer of a single electron from an adjacent orbital in an effort to cause a pairing of electrons on the oxygen to achieve a lower-energy state. In Scheme 6-6 it should be noted that the electron pairing with the radical-site electron is associated with a bond between the atom attached to the atom that bears the radical and charge sites and an adjacent atom. The “pairing” of the original odd electron on the heteroatom in Scheme 6-6 causes an OE site to occur elsewhere in the $M^+$. It may appear that no advantage is gained overall because an OE species still exists. However, depending on the element and its electronegativity, some radical species are more stable than other radical species, and if the fragmentation process proceeds, obviously a more favorable energetic state has been achieved. As illustrated in Scheme 6-6, the initial OE ion undergoes homolytic cleavage of the adjacent carbon–carbon bond by migration of a single electron (via fishhook b) to pair with the odd electron (via fishhook a) that was originally on the oxygen. This causes the formation of an EE oxonium ion (B) and the formation of an alkyl radical (via fishhook c). The sum of the internal energies of the oxonium ion and the alkyl radical are less than the energy of the original OE species and, therefore, the fragmentation process proceeds as shown.
During the homolytic cleavage process, the positive charge does not move; note that the positive charge is on the oxygen atom in both species A and species B in Scheme 6-6. There is no longer an odd electron on species B because the odd electron originally on oxygen has now been paired up with an electron that was taken from the bond between the two carbons. As the total number of electrons in the system is conserved, an odd electron now appears on another species, namely the methyl radical (species C) in this scheme. Species C is a neutral species, that is to say it has no net charge and is not detected by the mass spectrometer. In summary, species A in Scheme 6-6 is an OE ion and species B is an EE ion, whereas species C is an OE neutral (a radical). The oxonium ion can also be written in a resonance form with the charge on the carbon atom connected to the oxygen atom and the double bond moves to a position between the carbon atom and the R₁ group; this is another rationalization of stability borrowed from classical organic chemistry.

The example shown in Scheme 6-6 is called alpha cleavage because the bond that is cleaved is the bond between the carbon of a functional group and an adjacent carbon atom. A–H₂C–O– of an alcohol, ether, or ester and a –CO– of a ketone, aldehyde, acid, or ester are considered to be functional groups. The terminology describing this special case of homolytic cleavage was first suggested by Budzikiewicz, Djerassi, and Williams in 1964 [5]. McLafferty and Tureček [6] define "α-cleavage" as "cleavage of a bond adjacent to the atom alpha to that possessing the original radical site", which clearly indicates limitation of the use of the term "α-cleavage" to homolytic cleavages. Replacement of the oxygen with other heteroatoms (S, N, P, or a halogen) constitutes a similar functionality. Homolysis can also result in a beta cleavage, as will be seen later in this chapter in the discussion of the fragmentation of aliphatic amines. This alpha, beta, and gamma nomenclature convention in organic chemistry is illustrated in Scheme 6-7.

Another example of homolytic cleavage is illustrated in Scheme 6-8, which shows the M⁺⁺ of diethyl ether with the "plus/dot" on the oxygen atom in a nonbonding orbital. The odd electron on the oxygen stimulates the migration of a single electron from the carbon–carbon bond alpha to the carbon–oxygen bond as illustrated in Scheme 6-8. Homolytic cleavage at the carbon–carbon bond forms an EE oxonium ion and expels a methyl radical as indicated in this scheme. Those molecular ions of diethyl ether that do not fragment would be detected at m/z 74, an even m/z value as the molecule contains an even number (namely 0) of nitrogen atoms, and the fragment resulting from homolytic fission of the carbon–carbon bond would be detected at m/z 59, an odd m/z value, which is consistent with an EE ion containing no atoms of nitrogen. The methyl radical would not be detected.
3. Heterolytic or Charge-Site-Driven Cleavage

The positive charge on an ion, rather than the odd electron of an OE species, can stimulate fragmentation by inducing heterolytic capture of both electrons out of an adjacent bond as illustrated in Scheme 6-9. The driving force in heterolytic cleavage (so-called because the electron pair constituting the original chemical bond stays with one atom of the two originally connected) is the induction effect of the positive charge causing the electron pair to migrate toward the positive charge resulting in neutralization at the original site. The movement of both electrons from a given bond causes an electron deficiency on the other species, the first carbon of the propyl group in Scheme 6-9, which is reflected by the positive charge developing on that carbon atom as indicated on species B. Of course, the original bond from which both electrons migrated to the positive charge is broken as is shown with the carbon–chlorine bond of species A in Scheme 6-9; this movement of electrons is coupled with the formation of a radical species indicated by C.

The movement of two electrons is shown by a double-barbed arrow in the heterolytic fragmentation scheme. In inductive cleavage (synonymous with heterolytic cleavage) as illustrated in Scheme 6-9, the charge appears to migrate from its original locus in the M^+•. In Scheme 6-9, the M^+•, an OE species, that would be detected with an m/z 78 (an even value) has the positive charge on the chlorine. The fragment ion, species B, has the positive charge on the carbon of an EE ion that would be detected at m/z 43. The chlorine radical (an atom of chlorine) is quite stable, and because it is neutral will not be detected. In this scheme, the charge migrated from its original position on the chlorine atom to the carbon atom of the ethyl moiety.

Another example of charge-driven or heterolytic cleavage is illustrated in Scheme 6-10 for the M^+• of diethylether. Once again, note that the “+•” is located on the oxygen atom as this is the most likely locus from which to lose an electron during the ionization process. In this case, as opposed to that in Scheme 6-9, the charge site induces the movement of both electrons from an adjacent carbon–oxygen bond to neutralize the original charge on the oxygen atom to give rise to the radical species C shown in Scheme 6-10, a species with no charge, which therefore will not be detected. In addition to the radical species, the charged species B is formed with the positive charge now on the methylene carbon of the ethyl ion, which is detected at m/z 29, an odd m/z value. This is consistent with the ethyl ion being an EE species.
It is interesting to compare the fragmentation processes of the diethylether M\(^{+}\) illustrated in Schemes 6-8 and 6-10 that produce ions of different elemental composition and different mass. Even though the ions formed from diethylether via Schemes 6-8 and 6-10 have different \(m/z\) values, namely \(m/z\) 59 and \(m/z\) 29, both of these \(m/z\) values are odd because they both represent EE ions that do not contain an odd number of nitrogen atoms.

It is important to remember that heterolysis involves the movement of a pair of electrons from the bond that connects the atom with the charge and the adjacent atom. In some cases, this may not be clear. A good example involves heterolytic cleavage that takes place when the charge and the radical are associated with the carbonyl oxygen atom of a ketone (Scheme 6-11). The actual cleavage in this case occurs at the alpha carbon. However, this bond cleavage only occurs after a two-step process as illustrated in Scheme 6-11. Even though an alpha-carbon bond is broken in this process, it would be inappropriate to refer to this as alpha cleavage. The term alpha cleavage (as stated above) is reserved for the special case of homolytic cleavage that occurs at an alpha carbon.

Unlike homolytic cleavage, which always results in the formation of an ion and a radical, heterolytic cleavage can result in the formation of an ion and a molecule or an ion-radical pair. The ion and molecule formation associated with heterolytic cleavage usually occurs when the heterolytic cleavage proceeds as a secondary reaction in an ion formation. Secondary reactions involved with rearrangements of charges and/or radical sites followed by either homolytic or heterolytic cleavage are discussed in more detail below. A good example of heterolytic cleavage resulting in the formation of an ion–molecule pair is the common propensity for acylium ions (formed by homolytic cleavage of molecular ions) to lose a molecule of carbon monoxide as illustrated in Scheme 6-12. The propensity for this heterolytic cleavage to occur is based on the stability of the resulting ion versus that of the ion that undergoes the secondary fragmentation.
The tendency for homolytic cleavage or heterolytic cleavage to occur is influenced by the chemical nature of the original electron removed during ionization. Homolytic cleavage will occur in preference to heterolytic cleavage when the charge and the radical site result from the loss of a nonbonding electron from a nitrogen or phosphorus atom. If the charge and radical site are associated with a nitrogen or phosphorus atom, it is very unlikely that ions resulting from heterolytic cleavages will be observed. If the charge and radical site are due to the loss of an electron from a nonbonding orbital of a halogen (F, Cl, Br, or I), the probability of heterolytic cleavage is more likely; however, ions resulting from both types of cleavage will be observed in the mass spectrum, though the abundances of ions produced by heterolytic cleavage will be significantly greater. When the charge and the radical sites are due to the loss of an electron from a π-bond orbital or a nonbonding orbital of an oxygen or sulfur atom, homolytic cleavage can be promoted by adjacent electron-withdrawing groups. Peaks due to both homolytic and heterolytic cleavages will be observed in the mass spectra of compounds that have π-bond orbitals or atoms of oxygen or sulfur, and these ions sometimes can have nearly equal abundances.

4. Rearrangements

Fragmentation as a result of a rearrangement involves multiple-bond cleavages and new bond formations. The neutral loss in a fragmentation involving a rearrangement is always a molecule, an EE species. Unlike the previously described mechanisms associated with single-bond cleavages, which result in EE ions being formed from OE ions and OE ions being formed from EE ions, rearrangement fragmentation processes produce the same type of ions as the precursor ion; i.e., EE ions produce EE fragment ions and OE ions produce OE fragment ions. In an EI mass spectrum, peaks representing OE ions are important because they signal the involvement of rearrangement fragmentations of OE species, which can provide insight about the location of heteroatoms and/or double/triple bonds in the analyte molecule, and/or to the overall structure of the analyte molecule.

In EI, rearrangement fragmentation of EE ions is always associated with a secondary fragmentation of the M⁺⁺, a process that significantly affects the appearance of the spectrum as exemplified by the mass spectra of aliphatic alcohols and amines. Rearrangements involve a shift of a hydrogen atom or a hydride, and in some cases a small group of atoms in the form of a radical, which is analogous to a hydrogen atom. After a hydrogen or radical shift occurs, which involves the breaking of one bond and the forming of another, a distonic ion [9, 10] is formed with a new radical site, but with the charge remaining in its original position in the case of a rearrangement fragmentation of an OE ion; in the case of rearrangement fragmentations of an EE ion, the nascent ion has a different charge location after the shift occurs. Species C with m/z 58 in Scheme 6-13 is a distonic ion. The next step in the rearrangement fragmentation involves the movement of a single
electron (a homolytic cleavage) in response to the new radical site or the movement of a pair of electrons (a heterolytic cleavage) in response to a new or the original charge site, both of which usually involve cleavage of a second bond and the formation of a second new bond, as illustrated in Schemes 6-13 and 6-14.

The charge and radical sites in an odd-electron ion are usually associated with the same atom (e.g., as is the case when an electron is lost from a nonbonding orbital of an oxygen atom in the formation of a molecular ion of a ketone) or from a bond between two connected adjacent atoms (e.g., as is the case when a π-bond electron is lost in the formation of the molecular ion of an aromatic compound) in a nascent molecular ion. When the charge and radical sites are not associated with the same or two connected adjacent atoms, the ion is referred to as a distonic ion.

The formation of a primary oxonium ion, illustrated in Scheme 6-14, is a good example of a rearrangement mechanism involving the secondary fragmentation of an EE ion produced by the primary fragmentation of a M$^+$ ion. The same process of multiple-bond cleavages and new bond formations described above for the rearrangement fragmentation of a M$^+$ (an OE ion) also occurs in these hydride-shift rearrangements of EE ions.

Scheme 6-14

A. Hydrogen-Shift Rearrangements

A commonly observed rearrangement occurs in the case of a M$^+$ containing a double bond and a γ hydrogen relative to the double bond as shown generally in Scheme 6-13. In species A, the γ hydrogen is positioned in such a way as to participate in a virtual six-membered ring involving the carbonyl oxygen, which provides the double bond and the radical site. This overall rearrangement process involves the shift of a hydrogen atom (a proton and an electron) from its original position on a carbon atom that is in the gamma position relative to the carbonyl group. This γ-hydrogen shift occurs because of the need for the radical electron on the oxygen atom to pair with another electron. The resulting odd-electron ion has the same mass (and elemental composition) and the same number of electrons as the original ion, but with a new geometry and a new radical site. The odd electron constituting the new radical site on the γ carbon initiates the subsequent cleavage of the β bond. This mechanism was originally described by Nicholson [11], who compared photolytic and EI decomposition products of aliphatic ketones; however,
because of extensive studies by Fred W. McLafferty over the last five decades [12–15],
this general rearrangement has affectionately taken on the name **McLafferty rearrangement**.

According to studies by McLafferty [13–15] and others [16, 17], the overall
rearrangement proceeds according to the two discrete steps described above in
Scheme 6-13. The first step in Scheme 6-13, involving the transfer of the γ hydrogen to the
radical site on oxygen in species A, forms a new bond in a stable enol ion [18]. This
exothermic step leads to generation of the distonic species B, which has the radical site on
the γ carbon, and which is still detectable at m/z 100 because all of the atoms originally
present are still connected to one another, albeit in a different order. If sufficient internal
energy is available, the radical site on the γ carbon in some of species B stimulates
homolytic fission of the bond between the α- and β-carbon atoms to form the OE species C
appearing at m/z 58, together with elimination of the EE species D as a molecule of ethene.

As seen in Scheme 6-15, the γ-hydrogen shift can also be followed by a heterolytic
cleavage. In this particular example, the driving force for the heterolytic cleavage is the
formation of the resonantly stabilized substituted ketene OE ion.

![Scheme 6-15](image)

In Scheme 6-15, the charge and radical sites resulting from the loss of one of the
π electrons on the aromatic ring is shown. As described earlier in this chapter, the most
probable electron to be lost during the formation of a M⁺ for this compound is one of the
nonbonding electrons associated with carbonyl oxygen. Although this is true, there may be
some molecular ions formed with the charge and the radical associated with a π bond in the
aromatic ring (as shown in Scheme 6-15) as opposed to those formed with the charge and the
radical associated with the carbonyl oxygen. The ions with the charge and the radical
associated with the ring will be far less abundant than those with the charge and radical
sites associated with the carbonyl oxygen; none the less, there will be some molecular ions
with the charge and radical sites associated with the ring.

It is always important to keep in mind that even though there is a most probable position for the charge and radical sites in a molecular ion, some molecular ions with localized charge and radical sites in other positions might also exist; such a variety of possible positions for ionization provides a distribution of isomeric molecular ions.
An even more significant example of a hydrogen-shift rearrangement followed by a heterolytic cleavage is illustrated in the loss of water from an aliphatic alcohol as shown in Scheme 6-16. Again, a virtual six-membered ring plays a role in promoting the hydrogen shift from a $\gamma$ carbon in response to the radical site created by the expulsion of an electron from a nonbonding orbital of the oxygen atom. The $\gamma$-hydrogen shift induces a heterolytic cleavage that comes about when the pair of electrons that constitute the bond between the carbon and oxygen atoms moves to neutralize the charge that is still on the oxygen atom (see Scheme 6-16). This heterolytic movement of electrons causes the charge to move to the carbon atom that was attached to the oxygen atom and releases a neutral molecule of water. As will be seen later on in this chapter during discussion of the fragmentation of aliphatic alcohols, the resulting distonic OE ion in Scheme 6-16 can undergo a series of secondary fragmentations, which account for the large number of peaks in the mass spectra of such compounds.

Scheme 6-16

The importance of the virtual six-membered ring in a rearrangement fragmentation cannot be overemphasized; however, eight- and ten-membered ring transitions are also observed, as will be seen later on in this chapter in the discussion of the fragmentation of methyl stearate. Tighter shifts (meaning shifts from carbons closer than five atoms away from the site of the charge or radical) can also take place, as seen with hydride and hydrogen shifts in aliphatic amines and alcohols. This process is illustrated in Scheme 6-17, which shows a hydrogen shift from the $\alpha$ carbon of ethyl amine in response to the radical site created by the expulsion of an electron from a nonbonding orbital of nitrogen during the formation of the M$^+$.$^*$. Some of these distonic ions then undergo heterolytic cleavage with the loss of a molecule of ammonia and the formation of an OE ion of ethene.

Scheme 6-17

Another important factor associated with the occurrence of a hydrogen shift and the associated virtual six-membered ring is the type of radical that is produced. If the $\gamma$ carbon has three hydrogen atoms attached, the result of a hydrogen shift will be a primary radical (the radical-ion product in Scheme 6-17 is an example of a primary radical), which is not as stable as a secondary radical. The intensity of the peak representing the OE fragment ion formed by a $\gamma$-hydrogen shift involving intermediate formation of a primary radical in the mass spectrum of 2-pentanone (Figure 6-9) has a lower relative intensity than does the peak with the same $m/z$ value ($m/z$ 58) in the mass spectrum of 2-hexanone.
Figure 6-9. EI mass spectrum of 2-pentanone.

(Figure 6-10) where the \( \gamma \)-hydrogen shift results in the intermediate formation of a secondary radical (like that shown with the second structure in Scheme 6-18). Another example of the influence of the intermediate formation of a primary vs secondary radical during a \( \gamma \)-hydrogen-shift rearrangement can be found later in Section V in the comparison of the mass spectra of \( n \)-propylbenzene (Figure 6-23) and \( n \)-pentylbenzene (Figure 6-25); for propylbenzene, formation of the OE ion of \( m/z \) 92 is virtually nonexistent in the mass spectrum (Figure 6-23), whereas, for \( n \)-pentylbenzene, formation of the same ion involves an intermediate secondary radical, and the peak at \( m/z \) 92 is 80% of the base peak (Figure 6-25).

The one exception to suppression of the \( \gamma \)-hydrogen-shift rearrangement by the intermediate formation of a primary radical is when the ion that is undergoing the rearrangement has just been produced by a preceding \( \gamma \)-hydrogen-shift rearrangement. The products of two successive \( \gamma \)-hydrogen shifts are represented by peaks at \( m/z \) 86 and \( m/z \) 58 in the mass spectrum of 4-decanone (Figure 6-11 and Scheme 6-18). The peak at \( m/z \) 86 represents the OE fragment ion formed by the loss of a molecule of 1-pentene, as shown early in Scheme 6-18. This OE ion will then undergo a \( \gamma \)-hydrogen shift that involves the intermediate formation of a primary radical, as shown in the second line of Scheme 6-18; the subsequent \( \beta \) cleavage of the distonic ion in the form of a primary radical
produces an OE ion represented by the peak at m/z 58. The intensity of the peak at m/z 58 indicates that there is a strong tendency for this secondary reaction (involving the intermediate formation of a primary radical) to take place.

Another factor that cannot be ignored in the formation of these OE ions resulting from γ-hydrogen shifts is the electronic configuration of the α, β, and γ carbons. If these carbons have an electronic configuration other than that of an sp³ hybrid, the ion geometry and configuration may preclude formation of the virtual six-membered ring making the reaction less likely to occur, resulting in lower abundances of these corresponding OE fragment ions.

B. Hydride-Shift Rearrangements

Fragmentation occurring as a result of a hydride-shift rearrangement is mostly restricted to secondary fragmentation of EE ions formed by single-bond cleavage in a M⁺. An example of a hydride-shift rearrangement fragmentation of a secondary oxonium ion produced by the alpha cleavage of a M⁺ of a secondary alcohol was shown earlier in Scheme 6-14. Such rearrangements observed in an even-electron system have also been studied and explained by McLafferty [19]. As stated earlier, these hydride-shift rearrangements play a key role in the EI-induced fragmentation of aliphatic amines.
An example of a hydride-shift rearrangement in the secondary fragmentation of an amine is shown in Scheme 6-19 as indicated by the double-barbed arrows in the species having an $m/z$ value of 58. As shown in this scheme, the elimination of ethylene, an EE species of even mass, from an EE ion (containing one nitrogen) with $m/z$ 58, produces another EE species (also containing the nitrogen) with $m/z$ 30, another even number. The $m/z$ values of these ions in Scheme 6-19 provide an example of a corollary to the Nitrogen Rule; namely that an EE fragment ion retaining an odd number of nitrogen atoms will have an even $m/z$ value. In this case, the species at $m/z$ 58 is a fragment ion (an EE species) from a larger OE species that contains at least one atom of nitrogen.

Another example of the significance of a hydride-shift rearrangement is seen in the mass spectrum of diethyl ether (Figure 6-12). This mass spectrum exhibits a $M^+$ peak at $m/z$ 74 of significant intensity. There are major peaks at $m/z$ 59, 45, 31, and 29. All the peaks in the mass spectrum appear to be consistent with the Nitrogen Rule and the formation of EE-fragment ions through single-bond cleavage.

The $M^+$ peak in the mass spectrum of diethyl ether (Figure 6-12) is at an even $m/z$ value which is consistent with the analyte not containing an odd number of nitrogen atoms. Because there are no atoms of nitrogen in the analyte, none of the fragment ions formed will contain an atom of nitrogen. If the fragment ions are EE ions, then according to the Nitrogen Rule they must have an odd $m/z$ value. The peak at $m/z$ 59 represents an oxonium ion produced by alpha cleavage in the $M^+$ of diethyl ether with the charge and radical sites on the oxygen atom as shown earlier in Scheme 6-8. Some of the molecular ions with the charge and radical sites on the oxygen atom will undergo heterolytic cleavage to produce aliphatic ions with $m/z$ 29, as was illustrated in Scheme 6-10. The peak at...
m/z 29 obviously represents the aliphatic ethyl ion because of the presence of peaks at m/z 28, 27, and 26, which represent ions formed by the secondary fragmentation of the ethyl ion. The peak at m/z 59 probably does not represent an alkyl ion because it is not accompanied by peaks at m/z 58, 57, and 56 that would correspond to secondary fragmentation of such an ion. Both peaks at m/z 29 and m/z 59 represent ions formed by single-bond cleavage. It is also possible to rationalize the peak at m/z 45 as representing an ion formed through a single-bond cleavage mechanism resulting in the loss of an ethyl radical from the M⁺.

The origin of the ion represented by the peak at m/z 31 in Figure 6-12 cannot be rationalized by any single-bond cleavage mechanism relating to the fragmentation of diethyl ether. Ions with m/z 31 are listed in Table 6-3 as being a primary oxonium ion that is diagnostic for aliphatic alcohols. Therefore, in Figure 6-12, the only rationale for the formation of the ion represented by the peak at m/z 31 is a fragmentation of diethyl ether induced by a hydride-shift rearrangement as shown in Scheme 6-20. A hydride from the terminal carbon of the ethyl moiety of the oxonium ion with m/z 59 shifts in response to the charge on the oxygen atom in concert with the movement of the pair of electrons between the methylene carbon of the ethyl moiety and the oxygen in response to the new electron deficiency created by the hydride shift. As shown in Scheme 6-20, the charge remains on the oxygen atom, but now the oxonium ion with m/z 31 is composed of a hydrogen attached to the oxygen rather than to the ethyl group. The hydride-shift rearrangement, illustrated in Scheme 6-20, results in the formation of a molecule of ethene. Examination of a series of spectra of dialkyl ethers reveals that although an alkyl-substituted primary oxonium ion is formed regardless of the size of the chain on either side of the ether oxygen, the peak for the ion with m/z 31, while present, has a significant intensity only when one of the alkyl chains is an ethyl group. This fact supports the supposition that some rearrangement fragmentations most likely involve the expulsion of “small” molecules.

Another important feature that distinguishes the mass spectra of ethers and aliphatic alcohols is that those of most aliphatic ethers exhibit a M⁺ peak, whereas those of aliphatic alcohols containing more than four carbons do not. The intensity of the peak at m/z 31 in the mass spectra of all primary aliphatic alcohols always has a relative intensity of about 25%. The fragmentation of aliphatic alcohols and aliphatic ethers is discussed in more detail later in this chapter.

V. Representative Fragmentations (Spectra) of Classes of Compounds

This section compares the so-called “paper mechanisms” with the peaks observed in the mass spectra of a series of different types of compounds. By proposing paper mechanisms based on structures and observed peaks, it becomes easier to deduce a structure from an EI mass spectrum. Frequent practice with this strategy or procedure will
make it easier to recognize the relationship between a structural moiety or feature in the molecule and observed peaks of specific \( m/z \) values or to specific neutral losses in the mass spectrum. Gaining familiarity with representative mass spectra of many different classes of compounds is another important aspect of learning to deduce a structure from an unknown mass spectrum. When the quality of a mass spectrum is being determined by the reviewers at NIST, they always rely on spectra of similar compounds where possible.

1. Hydrocarbons

There are four types of hydrocarbons: alkanes, alkenes, alkynes, and aromatics. Alkanes, alkenes, and alkynes can be broken down further into straight-chain, branched, and cyclic compounds. Aromatic hydrocarbons usually involve rings; however, a conjugated double-bond system such as found in butadiene also constitutes an aromatic compound. From a mass spectrometry standpoint, hydrocarbons can be defined a little differently. There are hydrocarbons that have \( \pi \) bonds (alkenes, alkynes, and aromatic) and there are hydrocarbons that do not have \( \pi \) bonds (saturated hydrocarbons: alkanes). There are linear hydrocarbons (those that have only two terminal carbon atoms and no carbons attached to more than two other carbon atoms) and there are cyclic hydrocarbons in which all the carbon atoms (no terminal carbons) are attached to two or more other carbon atoms. Branched hydrocarbons have more than two terminal carbon atoms, and contain some carbons that are attached to more than two other carbon atoms. An aromatic compound can have alkyl substituents. The fragmentation of saturated hydrocarbons is principally through \( \sigma \)-bond cleavage. Fragmentation of unsaturated hydrocarbons is initiated by the loss of an electron from a \( \pi \)-orbital electron in the formation of the \( \text{M}^+ \). Fragmentation of saturated cyclic compounds involves a two-step process. Because the driving force for the fragmentation of the various types of compound is different, the interpretation of their mass spectra will be discussed separately.

A. Saturated Hydrocarbons

1) Straight-Chain Hydrocarbons

The fragmentation of a saturated hydrocarbon results in the formation of carbenium ions. For straight-chain hydrocarbons, the mass spectrum is dominated by peaks that represent primary carbenium ions separated by 14 \( m/z \) units, as seen in Figure 6-13. Unlike molecular ions involved in charge or radical-site-driven cleavages, the location of the charge cannot be easily assigned in the \( \text{M}^+ \) of a saturated hydrocarbon. Even if an electron is ejected between two specific carbon atoms, the charge can migrate throughout the nascent \( \text{M}^+ \) because of the lack of highly localized electrons as exist in \( \pi \) orbitals of a double bond. It should also be mentioned that an electron in a carbon–carbon bond is more likely to be lost than an electron from a carbon–hydrogen bond. This is probably because the carbon–carbon bond consists of the overlay of two hybridized orbitals and a carbon–hydrogen bond is the overlay of a hybridized orbital and an \( s \) orbital; therefore, it is likely that the more highly hybridized bond can better accommodate the electron deficiency.

The abundance of the \( \text{M}^+ \) produced by straight-chain saturated hydrocarbons is about 2–5% of the total ion current. Sometimes, the rate of dissociation of molecular ions is high enough that the residence time of ions in the mass spectrometer can appreciably affect the intensity of the molecular ion peak. A long residence time, the interval between ion formation and detection, might be long enough for all sufficiently unstable molecular ions to decompose depending on the kinetics of the process. The residence time in a magnetic sector, TOF, or transmission quadrupole instrument is microseconds, whereas in a quadrupole ion trap it can be as much as 25 msec. The molecular ions of some
saturated hydrocarbons are stable enough to be detected even when the data are acquired using a quadrupole ion trap mass spectrometer.

There are several other important factors that should be noted about the mass spectrum of hexacosane (Figure 6-13). Looking at the spectrum as a whole, a characteristic pattern is observed. This pattern is sometimes referred to as the “ski-slope” pattern. This ski-slope pattern is the underlying pattern exhibited by the mass spectrum of compounds that have a straight-chain hydrocarbon backbone; it derives from a series of peaks that represent $C_2$, $C_3$, $C_4$, $C_5$, $C_6$ primary carbenium ions. These ions dominate because of their stability and the fact that they come from at least two different sources.

To some extent, the ski-slope pattern can be explained by the half-$C_n$ rule (where $C_n$ represents the number of C atoms in the molecular ion); this rule states that the majority (~80%) of the ions that contain half or more of the carbons comprising the complete chain result from direct fragmentation of the $M^+$. Only about 20% of the ions containing fewer than half of the carbon atoms result from direct fragmentation of the $M^+ [20]$; that is, most of the ions containing fewer than half the carbon atoms in the original molecule result from two or more stages of fragmentation. The source of most of the fragment ions in the mass spectrum of a straight-chain hydrocarbon (certainly down to the half-$n$ position where $n = \text{total number of carbon atoms in the analyte molecule}$), are molecular ions; however, as described above, below the half-$n$ position, the precursor is most likely a high-mass fragment ion. For example, the peak at $m/z$ 281 in Figure 6-13 represents a fragment ion containing $C_{20}$ (as $C_{20}H_{41}$), which most likely was formed by the expulsion of $C_6$ (as the radical $C_6H_{13}$) from the $C_{26}$ molecular ion. Furthermore, the peak at $m/z$ 113 represents a fragment ion containing $C_9$ (as $C_9H_{17}$), which was likely formed by loss of $C_{10}$ (as the olefin $C_{10}H_{20}$) from the $C_{18}$ ion (as $C_{18}H_{37}$) or the loss of $C_{11}$ (as an olefin) from the fragment ion comprising $C_{10}$ or the loss of $C_9$ from the $C_{17}$ ion, etc., as well as the loss of the radical $C_{18}$ from the $C_{26}$ molecular ion.

Another feature of note in the mass spectrum of hexacosane (Figure 6-13) is the pattern of peak clusters at intervals of 14 $m/z$ units. Usually, the most intense peak in these clusters represents fragment ions with the elemental composition $C_nH_{2n+1}$. The serial clusters of peaks differing by 14 $m/z$ units, up to the 1/2 $C_n$ position in the spectrum, represent the integral effect of a large population of isomeric molecular ions losing a
particular member of a homologous series of radical species, i.e., those containing different numbers of methylene (–CH\(_2\)–) groups; it does not represent the successive loss of methylene groups (which would suggest the loss of a diradical) from a particular fragment ion!

It should also be noted that in the mass spectra of straight-chain hydrocarbons there is the lack of a peak representing the \([M – \text{CH}_3]^+\) ion; this feature relates to the low stability of the methyl radical, especially as compared to radicals containing higher numbers of carbon atoms. The intensity of peaks representing ions formed from losses of successively larger radicals from the \(M^+\) increases as the number of carbon atoms comprising the radical increases down to the half-n value of the molecular ion. After the half-n value is reached, the intensity of the peaks representing ions with successfully smaller numbers of carbon atoms increases at a much higher rate because these ions are also due to secondary fragmentation of larger fragment ions.

Although the loss of a methyl radical is used extensively in the explanation of some fragmentation mechanisms, it should always be remembered than the \([M – 15]^+\) peak indicates the presence of a *special* methyl (i.e., the methyl group is in a special location) in the \(M^+\).

As seen in Scheme 6-21, σ-bond cleavage can result in the formation of either an ethyl ion or the \([M – 29]^+\) ion. Formation of the ethyl ion is favored due to the increased stability accompanying the \(\text{C}_{29}\text{H}_{49}\) radical compared to that of the ethyl radical that accompanies the \([M – 29]^+\) ion. This is the basis for the Stevenson rule [21], which states (paraphrased from McLafferty [6]):

Cleavage of a carbon–carbon bond in an odd-electron ion due to the loss of a σ-bond electron can lead to two sets of ion-radical products; i.e., the \(M^+\) \(\text{ABCD}^+\) can produce \(\text{A}^+\) + \(\text{BCD}^+\) or \(\text{A}^+\) + \(\text{BCD}^+\). The fragment with the higher ionization energy (IE) should be the fragment with the greater tendency to retain the unpaired electron. This means that there should be a higher probability for the formation of the ion corresponding to the species that has the lower ionization energy. The ions with the lower ionization energy are usually more stable and, therefore, will account for the more abundant of the two ions.

The Stevenson rule also corresponds to the *loss of the largest alkyl* radical statement often used in the description of EI mass spectra. Looking at mass spectra of compounds that form aliphatic ions through single-bond cleavage, it will be seen that these spectra are dominated by peaks that represent ions formed by the loss of the largest radical moiety. This *loss of the largest alkyl* will dominate over the *greatest stability of the ion formed* principle. It is a well-established fact that a tertiary carbenium ion is more stable than a secondary carbenium ion. Looking at the partial mass spectrum of 3-methyl-\(n\)-heptane (Figure 6-14), it is clear that the three secondary carbenium ions...
Electron Ionization

Figure 6-14. Abbreviated EI mass spectrum of 3-methyl heptane.

formed by the respective loss of a butyl (m/z 57), ethyl (m/z 85), or methyl radical (m/z 99) from the M** are represented by peaks of decreasing intensity. This spectrum supports the loss of the largest alkyl principle and the Stevenson rule. The ion that should be the most stable (the tertiary carbenium ion formed by the loss of a hydrogen radical) is the least abundant; this observation is consistent the loss of the largest group and the instability of the hydrogen radical. Although there are three independent factors in sigma-bond cleavage (stability of the ion formed, stability of the neutral loss, and tendency to lose the largest moiety), all three must be considered as a whole, rather than from just their individual contributions.

The series of peaks that starts with an m/z value of x and proceeds to about m/z x – 4 represents ions formed by successive losses of hydrogen radicals and hydrogen molecules from the ion with m/z x. These patterns are characteristic of aliphatic ions. There are oxonium and acylium ions that have m/z values corresponding to those of alkyl ions, but the mass spectrum representing these ions does not exhibit the secondary fragmentation pattern associated with the presence of alkyl ions. The presence and absence (or minimization) of these secondary fragmentation patterns can be used to distinguish between alkyl ions and ions containing oxygen atoms that have the same nominal m/z values.

Not restricted to the mass spectra of hydrocarbons, alkyl ions formed in the mass spectra of many compounds can undergo secondary fragmentation through the loss of a molecule of methane, ethene, or larger olefins. These losses result from rearrangements that take place in the even-electron fragment ions to produce lighter even-electron product ions. A good example is seen in the spectrum of n-butyrbromide (Figure 6-26 later in this chapter), which has peaks at m/z 41 and m/z 29 representing ions formed by the respective loss of methane (16 Da) and ethene (28 Da) from an even-electron butyl ion with m/z 57.

2) Branched Hydrocarbons

The mass spectrum of a branched hydrocarbon is very similar to that of the straight chain saturated hydrocarbon:

1. Low intensity, but discernible M** peak.
2. Peaks every 14 m/z units beginning with [M – 29]⁺.
3. A recognizable “ski-slope” pattern with the spectrum dominated by peaks representing C₂, C₃, C₄, C₅, and C₆ ions.
4. A moderate secondary fragmentation pattern associated with alkyl ions.
The one significant difference in the mass spectra of a branched hydrocarbon, as seen in the mass spectrum of 9-butyldocosane in Figure 6-15, is the presence of what could be called extruding “bushes” along the “ski slope”, peaks with far greater than expected intensities for a normal chain. These peaks represent secondary carbenium ions that are formed by fragmentation at the branch points in the backbone chain. The intensity of these peaks is greater than that of those representing the primary carbenium ions because secondary carbenium ions are more stable. Scheme 6-22 shows the formation of a secondary carbenium ion with the loss of one branch from the hydrocarbon backbone. Scheme 6-23 shows the formation of another secondary carbenium ion due to the loss of a different branch from the hydrocarbon backbone. These two ions are represented by bushes sticking up above the ski-slope at m/z 182 and 253, respectively, in Figure 6-15.

Processing the mass spectra with Boolean logic allows determination of the location of the branch point on the hydrocarbon backbone. The branched hydrocarbon is schematically represented by Scheme 6-24. Let box A contain all the carbon atoms that make up the secondary carbenium ion in Scheme 6-22. Let box B contain all the carbon atoms that make up the secondary carbenium ion shown in Scheme 6-23. As illustrated in...
Scheme 6-24, the total number of carbon atoms in the molecule subtracted from the sum of the carbon atoms in boxes A and B is equal to the number of carbon atoms in the branch plus one (the branch point carbon).

To determine the number of carbon atoms in a saturated hydrocarbon (branched or not), subtract two from the nominal mass \(366 - 2 = 364\) in this example) and divide by 14, which is the mass of a \(-\text{CH}_2-\) unit. In this example (Figure 6-15), the total number of carbon atoms in the hydrocarbon that has a single branch point is 26 \((= 364 / 14)\). To determine the number of carbon atoms in either box A or box B, subtract 1 from the mass of the carbenium ion represented by the particular box and then divide the resulting number by 14. For the current example, the result of this arithmetic process indicates that the number of carbon atoms in Box A is 13 \((= [183 - 1] / 14)\) and the number of carbon atoms in Box B is 18. Substituting these numbers into the Boolean equation shown in Scheme 6-24 yields the number 5 \((= 13 + 18 - 26)\), which is the sum of the carbon atoms in the branch plus the branch point carbon; this means that the branch consists of four carbon atoms, a butyl group. The next step is to determine the location of the branch point. By convention, the branch point is designated numerically from the nearest terminus of the main carbon chain; for this reason, box A was chosen for computation because it contained fewer carbons than box B. In this case, box A contains 13 carbon atoms; because 4 of these 13 carbon atoms are in the form of a butyl side chain, the other 9 carbon atoms must be in the main chain. Therefore, the butyl group is attached to carbon 9.

The above computational example based on Boolean logic was contrived so that the number of carbon atoms in box A would be consistent with the correct name of the compound, namely 9-butyldocosane. Any two of the three high-intensity peaks representing secondary carbenium ions could have been assigned to box A and box B, and the resulting structure would be the same although the numbering would not be consistent with IUPAC nomenclature; e.g., 5-octyloctadecane. Whenever the \(m/z\) values for peaks representing secondary carbenium ions can be identified, the location of this secondary carbon atom can be established. Another example of the use of this Boolean logic is illustrated later (Figure 6-89) in this chapter in the context of using mass spectral data to establish the location of a double bond in the hydrocarbon backbone of an aliphatic ester.
Although the intensity of the $M^+$ peak is easily discernible in the mass spectrum of 9-butyldocosane, as the number of branches in a hydrocarbon increases, identification of a $M^+$ peak can be difficult. This is especially true in data acquired by rapid scanning (problem relates to poor ion-counting statistics) GC/MS instruments or in a quadrupole ion trap where the time between ion formation and ion detection can be quite long (the problem relates to a relatively short half-life of the $M^+$). Using chemical ionization in an internal ionization quadrupole ion trap mass spectrometer with acetonitrile as the reagent gas can be a good way to determine the nominal mass of these highly branched compounds [22].

3) Cyclic Hydrocarbons

The $M^+$ peak of a cyclic hydrocarbon is more intense than the $M^+$ peak for either straight-chain or branched saturated hydrocarbons (each of these latter compound types can be referred to as linear hydrocarbons to differentiate them from cyclic hydrocarbons). The reason for the greater intensity of the $M^+$ peak for the cyclic compound is due to the fact that cleavage of a single bond does not result in the production of a fragment ion. Breaking of a carbon–carbon bond in the cyclohexane $M^+$ results in an ion with the charge and radical sites on nonadjacent carbon atoms (see Scheme 6-25), but all of the atoms originally in the analyte are still connected to one another.

The distonic ion of cyclohexane produced by $\sigma$-bond cleavage (see Scheme 6-25) undergoes various reactions involving hydrogen-shift rearrangements, followed by homolytic or heterolytic cleavages resulting in single-bond fragmentations. Like the mass spectra of linear hydrocarbons, the mass spectra of cyclic hydrocarbons will exhibit peaks every 14 $m/z$ units; however, the peak pattern and the appearance of the peak clusters will be more erratic than observed in the spectra of the linear hydrocarbons. A good example is the mass spectrum of cyclohexane shown in Figure 6-16.

As can be seen from the fragmentation pathways illustrated in Scheme 6-25, many of the ions in the cyclohexane spectrum can result from fragmentation of the $M^+$; however, these fragmentations may be induced by hydrogen shifts; e.g., the peaks at $m/z$ 69 ($[M - CH_3]^+$), $m/z$ 55 ($[M - C_2H_5]^+$), and $m/z$ 41 ($[M - C_3H_7]^+$) represent ions resulting from homolytic cleavages induced by the respective shifts of a hydrogen atom from the number 4, 3, and 2 carbon atoms (the number 1 carbon atom is the site of the charge) to the radical site on the number 6 carbon. It is possible to have competing pathways for ion formation. Not only does the ion with $m/z$ 41 result from a homolytic cleavage of $M^+$ induced by a hydrogen shift, this fragment ion can also be the result of a heterolytic cleavage of the ion.

Figure 6-16. EI mass spectrum of cyclohexane, a cyclic saturated hydrocarbon.
with \( m/z \) 69. Fragmentations of fragment ions contribute more to the appearance of the mass spectrum of cyclic saturated hydrocarbons than they do to the appearance of the spectra of aliphatic saturated hydrocarbons. The peaks at \( m/z \) 43 and \( m/z \) 29 represent ions formed by hydride-shift rearrangements, also shown in Scheme 6-25. It is clear from the spectrum in Figure 6-16 and the mechanism proposed in Scheme 6-25 that a number of different simultaneous reactions take place.

As the ring becomes increasingly substituted by alkyl groups, the relative intensity of the \( M^{+*} \) peak decreases; this is especially true when a given carbon has more than one substituent. Compare the spectra of 1-ethyl-1-methyl cyclohexane and 1-ethyl-2-methyl cyclohexane in Figure 6-17. The \( M^{+*} \) peak in the mass spectrum of 1-ethyl-2-methyl cyclohexane is obvious at \( m/z \) 126, whereas in the mass spectrum of 1-ethyl-1-methyl cyclohexane, for all practical purposes, there is no \( M^{+*} \) peak.

![Scheme 6-25](image)

**Scheme 6-25**

Substitution of heteroatoms into the ring or attachment of functional groups to the ring will influence the fragmentation patterns. The principles associated with location of the charge and radical sites on heteroatoms, discussed for specific types of aliphatic compounds later in this chapter, provides a rationale for the formation of these patterns. It
is important to remember that an initial bond cleavage in a cyclic molecular ion usually does not result in the formation of a fragment ion.

When the EI mass spectra of a given class of compounds becomes of interest, study spectra of as many compounds in that class as possible. Use the NIST Mass Spectral Database for this purpose.

**B. Unsaturated Hydrocarbons**

Unsaturated hydrocarbons can be straight-chain, branched, or cyclic hydrocarbons. These compounds are called alkenes because they have one or more double bonds. They do not have the resonance structure that simulates alternating double and single bonds as do aromatic compounds. They do have one or more pairs of $\pi$-bond electrons. The most probable site of the charge and radical sites in the $M^+$ of an alkene is between the two carbon atoms originally connected by the double bond. The $M^+$ is formed by the loss of a
Figure 6-18. EI mass spectra of 3-dodecene (bottom), a linear hydrocarbon with a single site of unsaturation, and n-dodecane (top).

A comparison of the mass spectra of n-dodecane and 3-dodecane in Figure 6-18 shows the similarities and differences in the mass spectra of the two types of compounds. Both compounds are straight chain with a total of 12 carbon atoms. Both mass spectra show a M⁺ abundance of 2–5% of the total ion current. Both mass spectra exhibit a ski-slope pattern with peaks representing C₂, C₃, C₄, and C₅ ions dominating the spectrum and distinct clusters of peaks every 14 m/z units as they approach the M⁺ peak. A significant difference in these two spectra is the [M – 29]⁺ peak for n-dodecane vs the [M – 28]⁺ peak for 3-dodecene. The [M – 28]⁺ peak for 3-dodecene represents the loss of a molecule of ethene to form an odd-electron fragment ion. Another important difference in the two spectra is the increased abundance of the alkenyl and alkynyl ions for 1-dodecene that increment by two and four m/z units lower than the peak for the alkyl ions; e.g., m/z 41 and m/z 39 vs m/z 43. There are more peaks for OE ions in the mass spectrum of dodecene (e.g., m/z 42, 56, 70, etc.) than in that of dodecane; these represent odd-electron ions resulting from various olefin expulsions from the molecular ions of the alkene. The loss of ethene from an olefin is always observed when there is a terminal double bond; this loss is also sometimes observed when the double bond is deeper in the carbon chain.
C. Aromatic Hydrocarbons

Aromatic hydrocarbons are compounds composed of a ring structure that have a specific number of delocalized electrons so that the structure is consistent with alternating double and single bonds. The simplest such structure is a benzene ring. Aromatic compounds can be monocyclic or polycyclic. The most common monocyclic aromatic compounds are substituted benzenes. Some of the most studied polycyclic aromatic compounds are polycyclic aromatic hydrocarbons (PAHs), sometimes referred to as polynuclear aromatic hydrocarbons (PNAs) because of their health and environmental significance. The PAHs are composed of multiple fused aromatic rings. One of the most common PAHs is naphthalene, a compound used for control of moth infestation in clothing storage areas and as a disinfectant in urinals in public toilets.

The mass spectra of aromatic hydrocarbons (mainly those containing only carbon and hydrogen with all the carbon atoms arranged with conjugated double bonds and without substitutions) are very different from the mass spectra of aliphatic hydrocarbons. When molecular ions are formed from these aromatic molecules, it is due to the loss of a π-bond electron. This loss does not result in the cleavage of any bond. The resonance structure delocalizes the energy of the ionizing electron. This stabilization means that the mass spectra of these compounds are dominated by the M\(^+\) peak, which is usually the base peak in their mass spectra. The mass spectrum of chrysene (a polycyclic aromatic hydrocarbon that has no commercial use and is only a laboratory curiosity) shown in Figure 6-19 is a good example of the spectra produced from aromatic hydrocarbons. The M\(^+\) peak at m/z 228 is the base peak. There is a peak at m/z 226 that suggests that some of the M\(^+\) ions expel a molecule of hydrogen. The peak at m/z 227 is too intense to be exclusively the \(^{13}\)C-isotope peak related to the m/z 226 peak; it is likely that some of the ion current at m/z 227 represents the loss of a hydrogen radical from the M\(^+\). There are peaks at m/z 114 and m/z 113 that represent ions having two charges [23], i.e., the nominal mass of chrysene is 228 Da; the ion with a mass of 228 Da and two charges will have an m/z value of 114, the mass divided by the number of charges. The one observation that may be somewhat confusing is the intensity ratio of the peaks at m/z 113 and 114, which is about the same as that of the peaks at m/z 226 and 228. The abundance ratio of [M – H\(^2\)]\(^+\) and M\(^+\) is about 1:4; the fact that the abundance ratio of the double-charge ions is nearly the same is not readily explained.

![Figure 6-19. El mass spectrum of chrysene, a polynuclear aromatic hydrocarbon.](image-url)
A philosophical question. A molecular ion is formed by the addition or removal of an electron. Is a double-charge ion that retains all the atoms of the original molecule a molecular ion?

More likely to be encountered than the PAHs are compounds that contain a phenyl group. The mass spectra of compounds containing a phenyl group have the following characteristics:

1. $M^{++}$ peaks of significant intensity.
2. A dearth of peaks representing fragment ions.
3. Low-intensity peaks at $m/z$ 39 and 65 representing secondary fragmentation of compounds with a benzyl moiety.
4. A low-intensity peak at $m/z$ 51 for all phenyl-containing compounds (this includes compounds with a benzyl moiety).
5. Peaks at $m/z$ 77 (of wildly varying intensity) for all phenyl-containing compounds.
6. Peaks of significant intensity at $m/z$ 91 representing a tropylium ion for compounds containing a benzyl moiety.
7. An examination of the mass spectra of benzene and toluene shown in Figure 6-20 illustrates the difference a substituent on the phenyl ring makes in the appearance of the spectrum. The base peak in the mass spectrum of benzene is the $M^{++}$ peak, whereas in the toluene mass spectrum the $M^{++}$ peak is only about 20% of the intensity of the base peak at $m/z$ 91 representing the tropylium ion.

Scheme 6-26
Scheme 6-26 illustrates a fragmentation mechanism that results in the formation of the ion with $m/z$ 51 from a phenyl ion. Scheme 6-27 illustrates the two-step fragmentation of the tropylium ion with successive losses of acetylene in the formation of ions with $m/z$ values of 65 and 39. There is a peak at $m/z$ 39 in the mass spectrum of benzene. However, benzene does not have a benzyl moiety, which, if present, could participate in the formation of a tropylium ion (see Scheme 6-28), which is the precursor of the ion with $m/z$ 65. According to Scheme 6-27, the ion with $m/z$ 65 is the precursor of the ion with $m/z$ 39. However, there is no peak at $m/z$ 65 in the mass spectrum of benzene. So where does the ion with $m/z$ 39 come from in the mass spectrum of benzene? What is the number 39, besides the age of my coauthor’s girlfriend? Thirty-nine is half of seventy-eight! The peak at $m/z$ 39 in the mass spectrum of benzene represents a double-charge ion of the intact benzene molecule.

Scheme 6-27
Scheme 6-28 illustrates the formation of the tropylium ion [24] from the M'' of toluene. This cleavage of a hydrogen–carbon bond on the carbon attached to the ring with the charge and radical on the ring is called benzylic cleavage. Although the radical and charge sites are shown localized between carbons number 1 and number 2, it should be understood that in the actual M'', the charge and radical are part of the ring structure and are not localized. The localization of the charge and radical sites is shown in Scheme 6-28 for didactic purposes only.

Scheme 6-28

Figure 6-21. EI mass spectrum of ethylbenzene.

Figure 6-21 is the mass spectrum of ethylbenzene and Figure 6-22 shows the spectra of the three regioisomers of xylene; the four compounds have the elemental composition C₈H₁₀ configured on a single benzene ring. For all intents and purposes, these spectra are essentially identical, with the exception that the intensity of the peak at m/z 105 in the mass spectrum of ethylbenzene is far less than that in the mass spectra of the three regioisomers of xylene. As seen from the displayed structures on each of the spectra, molecular ions of all four compounds have structural features that can participate in benzylic cleavage. However, the three regioisomers of xylene can only lose a hydrogen radical in the formation of the tropylium ion, which in this case becomes a methyl-substituted tropylium ion as represented by the peak at m/z 105 of rather significant intensity. Ethylbenzene can lose either a hydrogen radical or a methyl radical. From a statistical standpoint, it would seem more likely that a hydrogen radical would be lost because there are two hydrogens and only one methyl group on the benzylic carbon. However, the low intensity of the peak at m/z 105 in Figure 6-21 indicates that this is not
Figure 6-22. EI mass spectra of o-xylene (top), m-xylene (middle), p-xylene (bottom).

the case. The favored loss of a methyl radical over a hydrogen radical during benzylic cleavage of the ethylbenzene $M^+$ relates to the greater stability of a methyl radical than a hydrogen radical. There is a peak at $m/z$ 105 in the mass spectrum of ethylbenzene, albeit very small; therefore, it cannot be said that the $M^+$ will not lose a hydrogen radical. It is just that the loss of the hydrogen radical is much less likely than the loss of a methyl radical. It
should also be noted that all four spectra are dominated by a peak at m/z 91. In the case of ethylbenzene, the tropylium ion is formed directly from M••. In the case of the xylene isomers, the peak at m/z 91 represents a tolyl ion [24, 25] due to the loss of a methyl radical from the xylene M••.

As would be expected, the base peak in the mass spectrum of n-propylbenzene, shown in Figure 6-23, is m/z 91, representing a tropylium ion. The mass spectrum in Figure 6-23 is typical of the mass spectra of many aromatic compounds:

1. Few peaks representing fragment ions.
2. An intense M•• peak.
3. A dominant base peak representing a phenyl or tropylium ion, or a substituted phenyl or tropylium ion.
4. Minor peaks at m/z 39, 51, and 65.

A structural isomer with the same elemental composition as propyl benzene (C₉H₁₂), 1-ethyl-4-methylbenzene (4-ethyltoluene) produces a mass spectrum (Figure 6-24) very different from that of propylbenzene. Benzylic cleavage involving the ethyl moiety results in the loss of a methyl radical and the formation of a methyl-substituted tropylium ion with m/z 105 (the base peak). The methyl radical will be lost in preference to the loss of a hydrogen radical from either of the two alkyl groups attached to the ring because of the methyl radical’s greater stability.

Careful examination of the spectra in Figures 6-21 and 6-22 can lead to a rather uncomfortable conclusion, which is that regioisomers of aromatic hydrocarbons cannot be distinguished by their mass spectra. It is clear that whereas the mass spectra of positional isomers such as propylbenzene and 4-ethyltoluene have very different appearances, the mass spectra of the three xylene regioisomers cannot be distinguished. However, because mass spectrometry for these types of compounds is often performed in conjunction with gas chromatography, these xylene isomers (with a unique mass spectrum as a group) can be distinguished from one another by their differences in retention times. As will be seen later in this chapter, when substitutions to the ring involve heteroatoms, the ortho effect can, in some cases, allow the ortho isomer to be distinguished from the meta and para isomers by the appearance of their mass spectra. The meta and para isomers can only be distinguished from one another by their retention time differences.

All of the mass spectra of aromatic hydrocarbons containing a phenyl ring exhibit an easily discernible M•• peak at an even m/z value and peaks representing fragment ions at odd m/z values. These spectra are consistent with the Nitrogen Rule, which was discussed in Chapter 5.

The mass spectrum of n-pentylbenzene (Figure 6-25) is characterized by the unusual feature of a significant peak in the fragmentation region at an even m/z value, m/z 92. The peak intensity at m/z 92 is much greater than can be explained from the stable isotope contribution from the species represented by the peak at m/z 91. When such a situation as this is observed in the mass spectrum of an unknown there should be concern that this m/z 92 peak might indicate the presence of superimposed spectra of two compounds, where the peak at m/z 92 might represent the M•• of a contaminant. As was the case for all the spectra viewed so far in this class of aromatic hydrocarbons (compounds that do not contain an odd number of nitrogen atoms), it would be expected that all fragment ions be represented by peaks at odd m/z values (consistent with the Nitrogen Rule).
Figure 6-23. EI mass spectrum of n-propylbenzene.

Figure 6-24. EI mass spectrum of 1-ethyl-4-methylbenzene (4-ethyltoluene).

Figure 6-25. EI mass spectrum of n-pentylbenzene.
One way to eliminate the possibility of superimposed spectra would be to examine each of several consecutively recorded mass spectra, assuming the sample was analyzed by GC/MS, to determine whether the peak intensity at \( m/z \) 92 is consistently 40% of that at \( m/z \) 91 in all spectra. Another way would be to prepare mass chromatograms (Chapter 1) for \( m/z \) 91 and \( m/z \) 92 to determine whether the chromatographic peaks rise and fall together (which would suggest a pure sample). Having ruled out the possibility of superimposed spectra associated with a contaminant or coeluting analyte, observing a peak at an even \( m/z \) value in the fragmentation region of the mass spectrum is an indicator that a rearrangement has occurred during fragmentation of the \( M^+ \).

Recalling Scheme 6-13 earlier in this chapter, a rearrangement can occur when there are hydrogen atoms on a moiety that is gamma in position to the sites of the charge and the radical is an odd-electron ion. These hydrogen atoms are called \( \gamma \) hydrogens. The peak at \( m/z \) 92 in the mass spectrum of \( n \)-pentybenzene can be explained as a rearrangement of a \( \gamma \) hydrogen, which subsequently promotes a homolytic cleavage. The nascent radical site on the \( \gamma \) carbon formed by the \( \gamma \)-hydrogen shift in response to the radical site in the \( M^+ \) stimulates cleavage at the \( \beta \) carbon (the bond between the \( \beta \) - and \( \alpha \)-carbon atoms) of the penty side chain as illustrated in Scheme 6-29. Observing the peak representing a rearrangement in the mass spectrum of \( n \)-pentybenzene is good evidence that most of the carbons in the side chain are in a normal chain. It is clear, for example, that there is no branch at the \( \alpha \) carbon because if there were, the branch would be carried along with the \( \alpha \) carbon in the rearrangement fragmentation product, and the peak at \( m/z \) 92 would be shifted to \( m/z \) 106 if a methyl group were on the \( \alpha \) carbon or to \( m/z \) 120 if an ethyl group were on the \( \alpha \) carbon. No evidence would be given in the mass spectrum for branching at the \( \beta \) carbon in the side chain as no fragment ions containing the \( \beta \) carbon and its substituents carry a positive charge. It is unlikely that any branching occurs at the \( \gamma \) carbon, although the rearrangement would still be possible if at least one \( \gamma \) hydrogen remained.

![Scheme 6-29](image-url)

Scheme 6-29

Indirect evidence for all five carbons being in a straight chain is given by the fact that \( m/z \) 92 is quite intense in the mass spectrum. Compare the intensity of the peak at \( m/z \) 92 in the spectrum of \( n \)-propylbenzene in Figure 6-24 (top) with the intensity at \( m/z \) 92 in the mass spectrum of \( n \)-pentybenzene (Figure 6-25). Propylbenzene has three \( \gamma \) hydrogens; therefore, the rearrangement is possible. However, notice that the intensity at \( m/z \) 92 in the mass spectrum of \( n \)-propylbenzene is only slightly higher than would be expected from the isotopic composition of the ion with \( m/z \) 91. This low intensity means that the rearrangement process is not highly favored in the case of \( n \)-propylbenzene because the intermediate radical produced by the \( \gamma \)-hydrogen shift is a primary radical as opposed to a secondary radical in the rearrangement of \( n \)-pentybenzene, as shown in the
first step in Scheme 6-28. It can be concluded that when the γ hydrogens are on a terminal carbon (i.e., an ω carbon), the rearrangement process is frequently suppressed. Alternatively, as will be seen in the discussion of rearrangement fragmentations associated with aliphatic ketones later in this chapter where there are two sets of γ hydrogens and one set is on an ω carbon, this suppression effect is not a factor.

2. Alkyl Halides

Alkyl halides (a.k.a. haloalkanes) are composed of a saturated hydrocarbon (straight-chain or branched) and one or more halogen (fluorine, chlorine, bromine, or iodine) atoms. One-, two-, three-, and four-carbon alkyl halides are important environmental compounds that are found as chlorine disinfection by-products in drinking water; they are also used as solvents (CCl₄), aerosol propellants (CCl₃F – Freon 11), fire extinguishers (CF₃Br – Halogen 1301), anesthetics (CF₃CHClBr – Halothane or Fluothane), and in the production of plastics such as polyvinyl chloride (PVC) and polytetrafluorethene (PTFE – Teflon®).¹ Larger carbon chain alkyl halides are used in the chemical industry; e.g., lauryl chloride (1-chlorododecane) is used in the manufacture of photographic chemicals, pharmaceuticals, and surfactants. These higher nominal mass compounds, as a class, pose less of a health hazard than short-chain alkyl chlorides.

The most probable location of the charge and the radical sites in the M⁺• of an alkyl halide is associated with the halogen moiety because of the three pairs of nonbonding electrons of the halogen. As was pointed out earlier in this chapter, there is a greater tendency for heterolytic cleavage to occur than homolytic cleavage when the charge and the radical sites are on a halogen atom.

![Figure 6-26. EI mass spectrum of n-butylbromide with peak intensities above m/z 85 magnified by a factor of four.](image)

As seen in Figure 6-26, the base peak in the mass spectrum of n-butylbromide is at m/z 57, which represents the loss of a bromine radical from the M⁺•. The peak at m/z 41 represents a secondary fragment ion resulting in the loss of a molecule of methane (16 Da) from the ion with m/z 57. The peak at m/z 29 represents another secondary fragmentation product of the butyl ion resulting from the loss of a molecule of ethene (28 Da). Therefore,

¹ Vinyl chloride and polytetrafluorethene are not really alkyl halides because they contain a double bond; however, most references include these compounds in discussions about alkyl halides. Halogenated aromatic compounds will be discussed later in this chapter in the section on Multiple Heteroatoms or Heteroatoms and a Double Bond.
it can be concluded that the major fragmentation of the n-butylbromide M* occurs via heterolytic cleavage following the initial loss of a bromine radical.

The fragmentation pathways for the n-butylbromide M* are shown in Scheme 6-30. When viewing the mass spectrum of n-butylbromide in Figure 6-26, it should be noted that the intensities of all peaks above m/z 86 have been magnified by a factor of four.

Scheme 6-30

When the mass spectra of the four possible n-halobutanes (Figures 6-26 and 6-27) are compared, one feature stands out. The mass spectra of the fluoro- and chloro-analogs show a base peak at m/z 56 in contrast to one at m/z 57 in the spectra of the bromo- and iodo-analogs. There are peaks of significant intensity at m/z 43 in both the spectra of the fluoro- and chloro-analogs. There is essentially no peak at m/z 43 in the mass spectra of the bromo- and iodo-analogs. The peaks at m/z 56 represent odd-electron ions produced by the loss of H–X from the M*+. The peaks at m/z 43 represent the alternative loss of a H2C–X radical from the M*+ as a result of a heterolytic cleavage brought about by the movement of the pair of electrons between the number 1 and the number 2 carbon atoms in response to the charge site on the halogen atom.

The hydrohalogen loss to form the odd-electron ion with m/z 56 (which must involve a hydrogen shift in response to the radical site on the halogen) decreases as the size of the halogen increases. It should be noted that there is a peak of measurable intensity at m/z 56 in the mass spectrum of n-butylbromide, but there is no m/z 56 peak in the mass spectrum of n-butyliodide. These differences in the mass spectra of halocarbons where the only variable is the halogen are probably due to both the electronegativity and the steric factor associated with the size of the halogen.

In many cases, alkyl halides do not exhibit a M*+ peak. The lack of a M*+ peak is probably due to the high propensity for the loss of H–X, which will produce a distonic odd-electron fragment ion. If the hydrogen shift resulting in this distonic ion were from the number 4 carbon, a subsequent heterolytic cleavage involving the loss of a molecule of ethene would result in an odd-electron terminal olefin M*+, as was the case involving the loss of water from aliphatic alcohols. This means that the first possible peak in the mass spectrum of an alkyl chloride could correspond to [M – 64] (the loss of HCl followed by the loss of H2C=CH2). This process is especially prominent as the number of carbon atoms increases. Both chloromethane and chloroethane produce mass spectra with an obvious and intense M*+ peak, which is the base peak. The next member in this homologous series,
Figure 6-27. Mass spectra of fluoro- (top), chloro- (middle), and iodo-n-butane (bottom).
chloropropane, produces a mass spectrum with the base peak representing \([M - HCl]^+\). The \(M^{+}\) peak in chloropropane is vanishingly small. This trend of a very intense \([M - HCl]^+\) peak continues among alkylchlorides up to \(C_6\), at which point a peak at \(m/z\) 91 representing an ion that contains an atom of chlorine (obvious because of the isotope peak at \(m/z\) 93, which has an intensity about one-third that of the \(m/z\) 91 peak) becomes a prominent peak in the mass spectrum. A good example of a prominent \(m/z\) 91 peak is seen in the mass spectrum of 1-chlorodecane in Figure 6-28. There is also a pair of peaks with a 3:1 intensity ratio at \(m/z\) 105 and \(m/z\) 107, which represents the six-membered ring analog of the ion with a nominal \(m/z\) value of 91 (Scheme 6-31). Examination of the mass spectrum of 1-bromodecane shows pairs of peaks with almost equal intensity at \(m/z\) 135 and \(m/z\) 137 and at \(m/z\) 149 and \(m/z\) 151, representing the bromine analogs of the \(C_4H_8Cl^+\) and \(C_5H_{10}Cl^+\) ions formed by alkyl chlorides. In the mass spectrum of 1-iododecane there are peaks at \(m/z\) 155 and 169, which could possibly represent three- and four-membered ring analogs with the iodine being one of the ring members.

Scheme 6-31
These two competing reactions, the formation of the halogen-containing rings and the loss of H–X followed by the loss of H₂C=CH₂ involving the formation of a ring containing the halogen, both of which have very high tendencies to occur, diminishes the possibility of a M⁺ peak in the mass spectrum of an alkyl halide.

At first inspection of the mass spectrum of 1-chlorodecane there is no obvious pattern; however, if the spectrum is closely examined, excluding the peaks at m/z 91 and 93 and at m/z 105 and 107, representing the chlorine-containing ions, the ski-slope pattern of straight-chain hydrocarbons is apparent (see Figure 6-29).

In cases where the halogen (or multiple halogens) is (are) chlorine or bromine, the characteristic X+2 isotope peak patterns are obvious indicators of the number of atoms of such elements. The spectra will often have clusters of peaks separated by 14 m/z units in the C₂–C₆ region, as seen in the mass spectrum of 1-chlorododecane (Figures 6-27 and 6-28). Because of the lack of a M⁺ peak and the similarity between the mass spectra of many species of a homologous series of 1-chloroalkanes, an identification, even using a library search, can be difficult. The same is true for other compounds composed of different halogens in this class, and retention time becomes very important for an unambiguous identification.

A library search was conducted using the NIST Main Library mass spectrum of 1-chlorododecane against the NIST05 Database. Nine of the first 20 hits were for seven different members of a CₙH₂ₙ₊₁Cl homologous series. There were four hits in this group of twenty for α-ω-dichloro-ₙ-alkanes; all of these hits had Match Factors that were in the range of 884–720.

![Figure 6-29. EI mass spectrum of 1-chlorodecane with the peaks representing the chlorine-containing ions at m/z 91 and 93 and at m/z 105 and 107 removed. Notice that the spectrum’s pattern is characteristic of a straight-chain hydrocarbon.](image-url)
3. Oxygen-Containing Compounds
   A. Aliphatic Alcohols

The EI mass spectra of aliphatic alcohols are some of the most widely studied of all classes of compounds in mass spectrometry. This is because these compounds are volatile over a large molecular-mass range and they are used in a wide range of applications from disinfectants to ingredients of perfumes. The most probable location for the charge and the radical sites in the M$^+$ will be on the oxygen atom due to a loss of an electron from one of the two pairs of nonbonding electrons. The fragmentation of aliphatic alcohols is somewhat analogous to the fragmentation of alkyl halides in that the M$^+$ of alkyl halides of a certain minimum size have a tendency to lose H–X; the M$^+$ of alkyl alcohols of a certain minimum size (larger than the corresponding alkyl halide) have a tendency to lose water. The distonic ions formed by the loss of a molecule of water will then undergo one or more heterolytic cleavages to expel one or more molecules of H₂C=CH₂ to produce a terminal olefin M$^+$, which accounts for the unsaturated-hydrocarbon appearance of the mass spectrum. Because of the lack of a M$^+$ peak, it is difficult to use the mass spectra of aliphatic alcohols for identification without the concomitant use of GC retention times.

There is a peak at m/z 31 in the mass spectra of all 1°, 2°, and 3° alcohols that can be considered diagnostic for aliphatic alcohols. This peak at m/z 31 represents a primary oxonium ion (H₂C=O$^+$H) formed by homolytic cleavage of the M$^+$ at the α carbon (the number 2 carbon atom) as illustrated in Scheme 6-32.

The mass spectrum of 1-decanol, shown in Figure 6-30, is a good representation of the spectra produced by primary alcohols. There is no M$^+$ peak. The peak representing the ion formed by the loss of a molecule of water from the M$^+$ is vanishingly small. The first...
Representative Fragmentations (Spectra) of Classes of Compounds

A readily discernible peak, at m/z 112, represents the ion formed by the loss of H₂C=CH₂ from the [M − H₂O]⁺ ion. The peak at m/z 31, representing the primary oxonium ion, is readily discernible in this mass spectrum. The remainder of the mass spectrum looks very much like the spectrum of 1-decene with the characteristic pattern seen in the spectra of terminal olefins.

Formation of the primary oxonium ion with an m/z value of 31 from 2° and 3° alcohols is explained in Scheme 6-33. The intensity of the peak at m/z 31 decreases as the chain length increases. The intensity of the m/z 31 peak in the mass spectrum of n-butanol is ~98% of the base peak, which represents the [M − H₂O]⁺ ion, whereas the m/z 31 peak in the mass spectrum of n-decanol is ~20% of the base peak at m/z 43, which represents a propyl ion. There is also a decrease in the intensity of the m/z 31 peak in a series of mass spectra ranging from primary to tertiary alcohols. The m/z 31 peak in the mass spectrum of tert-butanol is only ~30% of the intensity of base peak at m/z 59, which represents the dimethyl-substituted oxonium ion. The ratio of the relative intensities of the m/z 31 peaks remains constant for primary and tertiary alcohols regardless of the main chain length.

The relative intensity of peaks in the mass spectrum is important; however, it is also important to be aware that as the abundance of an ion of a specific m/z value increases while that of another remains constant, the relative intensity of the second ion may appear to decrease if the first is represented by the base peak.

Scheme 6-33
Figure 6-31. EI mass spectrum of 2-methyl-2-decanol. The peak at m/z 31 is barely distinguishable.

Fred McLafferty and František Tureček, on page 241 of the 4th Edition of *Interpretation of Mass Spectra* [26], describes the formation of primary oxonium ions from mono- and disubstituted oxonium ions using a series of mechanisms involving the pairing of single electrons with no apparent driving force. The mechanisms in Scheme 6-33 all have a driving force related to a charge site attracting a pair of electrons. Such cause–effect mechanisms appear more logical than the hand-waving associated with odd-electron pairing for no apparent reason. However, it should be remembered that all of these schemes illustrate so-called paper mechanisms, and a degree of faith is required to accept them as “truth”.

Peaks representing mono- and disubstituted oxonium ions will be present in the mass spectra of 2° and 3° alcohols. The principle of the loss of the largest moiety will always be the driving force for the initial homolytic cleavage that results in formation of the oxonium ion. This is illustrated in Scheme 6-33 and corroborated by the fact that the base peak in the mass spectrum of 2-methyl-2-decanol (Figure 6-31) and 3-decanol (Figure 6-32) is at m/z 59.

Figure 6-32. EI mass spectrum of 3-decanol.
Scheme 6-34 illustrates the loss of water from the $M^+$ of decanol to produce a distonic fragment ion, some of which then undergo heterolytic cleavage to expel a molecule of ethene. The first step involves a hydrogen shift from the $\gamma$ carbon (in aliphatic alcohols, the number 4 carbon is the $\gamma$ carbon because the number 1 carbon is a part of the carbonyl functional group) as facilitated by the putative existence of a virtual six-membered ring involving the oxygen atom that carries both the radical and charge sites. The second step in Scheme 6-34 involves the actual loss of water via heterolytic cleavage of the distonic molecular ion.

As illustrated in Scheme 6-35, the ion with $m/z$ 112 produced by the 1,2-elimination of water, and then ethene, from the $M^+$ of 1-decanol can undergo fragmentation via multiple pathways. This is the same type of fragmentation expected for a terminal olefin such as 1-octene.
An important consideration with respect to peaks representing substituted oxonium ions is that a peak at $m/z$ 59 could represent an ethyl-monosubstituted oxonium ion or a dimethyl-disubstituted oxonium ion. It should also be kept in mind that these mono- and disubstituted oxonium ions are the source of the primary oxonium ions with $m/z$ 31.

As pointed out earlier, the absence of a $M^+$ peak makes it difficult to identify the exact structure of an aliphatic alcohol from its mass spectrum. It is fairly easy to recognize a mass spectrum as being that of an aliphatic alcohol because of the characteristic peak at $m/z$ 31. Once it is suspected that the analyte is an aliphatic alcohol, an educated guess can be made as to its nominal mass. The nominal mass will be either 28 or 46 Da greater than the highest $m/z$ value peak representing an odd-electron ion of a significant abundance. To confirm the validity of this guess, it is possible to prepare the trimethylsilyl derivative (discussed later in this chapter) of the analyte for reanalysis by EI or to reanalyze the original sample by chemical ionization. It is also possible to confirm the nominal mass of an aliphatic alcohol by increasing the ion source pressure to promote the formation of an $[M + H]^+$ ion. When analyses are carried out in an internal ionization quadrupole ion trap $m/z$ analyzer, the $[M + H]^+$ peak is often observed because of the high propensity for analyte molecules to react with proton-donating ions formed from ion/molecule reactions involving the analyte in this type of instrument. In this case, the proton donor would be the primary or substituted oxonium ion.

One last comment about the mass spectra aliphatic alcohols: it should not have gone unnoticed that the numerical difference between the nominal mass of an aliphatic alcohol and the value of the highest $m/z$ peak of a reasonable intensity in the mass spectrum is often 46. The nominal mass of a natural alcohol, ethanol, is 46 Da. For some time it was thought that the $M^{**}$ of higher nominal mass alcohols expelled a molecule of ethanol; however, this is not the case. The apparent $[M – 46]^+$ peak is actually an $[(M – H_2O) – H_2C=CH_2]^+$ peak.

### B. Aliphatic Ethers

As was the case with aliphatic alcohols, the most probable site for the charge and the radical will be on the oxygen atom due to the loss of an electron from one of its two pairs of nonbonding electrons. As long as the two alkyl branches consist of three carbons or less, there will be an easily observable $M^+$ peak in the mass spectrum. When one of these branches consists of four or more carbon atoms, the $M^+$ peak may be very weak, although more intense than the (nearly nonexistent) $M^*$ peak in the mass spectra of aliphatic alcohols. Another feature of the mass spectra of aliphatic ethers (where one of the branches has two or more carbon atoms) that is similar to the mass spectra of aliphatic alcohols is a peak at $m/z$ 31 representing a primary oxonium ion. Aliphatic ethers and aliphatic alcohols are the only compounds that produce ions with $m/z$ 31. In the fragmentation of aliphatic ethers, this ion is produced by a hydride-shift rearrangement that occurs in a primary oxonium ion with an alkyl substituent on the oxygen. This is illustrated in Scheme 6-36.

\[
\begin{align*}
\text{Scheme 6-36}
\end{align*}
\]
The dissubstituted oxonium ion from an asymmetric ether will be formed by loss of a radical (via homolytic cleavage) from the longer chain (based on the loss of the largest moiety principle). Even if the short chain contains as few as three carbon atoms, the intensity of the m/z 31 peak is less than 5% of the base peak. Regardless of the number of carbon atoms in the long chain, if the short chain has only two carbon atoms, the intensity of the m/z 31 peak will be well above 50% of the base peak. This aspect probably is related to the stability of the ethene molecule compared with that of larger olefins that would be formed when this shorter chain contains more than two carbon atoms. Even though the mass spectra of alkyl ethers and alkyl alcohols both exhibit peaks at m/z 31, its intensity is far greater in the mass spectra of ethers than in the mass spectra of alcohols. Another very important fact is that the mass spectra of ethers do not exhibit the obvious [M – H2O]⁺ or [(M – H2O) – H2C=CH2]⁺ peak seen in the mass spectra of the aliphatic alcohols. Care must be taken with this assertion, because the mass spectra of some alkyl ethers (ones that have a chain length that will supply hydrogens on a γ carbon that is not an ω carbon) do exhibit peaks at even m/z values that represent odd-electron ions. However, these spectra of aliphatic ethers lack the clusters of peaks every 14 m/z units, distinguishing them from the spectra produced by the alcohols.

In addition to the intense peak representing the primary oxonium ion with m/z 31, spectra of ethers containing an ethyl moiety will have a discernible peak representing the dissubstituted oxonium ion (H2C=O−CH2−CH3) at m/z 59. Peaks can also be observed in the mass spectra of aliphatic ethers that represent ions produced by heterolytic cleavage due to the shift of the pair of electrons between the number 1 carbon and the oxygen atom in response to the charge on the oxygen atom. This heterolytic cleavage reaction is quite prevalent in symmetrical aliphatic ethers. In some cases, the spectra of ethers will exhibit peaks representing ions formed by cleavage of the carbon–oxygen bond of one branch where the charge is retained by the oxygen atom still attached to the other branch; i.e., the loss of a radical representing the entire chain (see Scheme 6-39 later).

As stated above, in cases where one of the chains has hydrogens on a γ carbon that is not an ω carbon, peaks representing odd-electron ions will be present in the mass spectra. Although not thoroughly documented in the literature, the formation of these odd-electron ions separated by 28 m/z units can be explained by a mechanism analogous to that for the loss of water from aliphatic alcohols followed by the subsequent loss of a molecule of ethene as shown in Scheme 6-36, which shows a possible mechanism for the formation of the ions represented by the peaks at m/z 84 and m/z 56 in the mass spectrum of ethyl hexyl ether (Figure 6-33).

![Figure 6-33. EI mass spectrum of ethyl hexyl ether; nominal mass of 130 Da.](image-url)
An explanation of the peaks representing the ions formed by the fragmentation of ethyl hexyl ether (Figure 6-33) is a good example of the various fragmentation pathways that can be involved in the mass spectrometry of aliphatic ethers. The origin of the ions represented by the peaks at \(m/z\) 84 and \(m/z\) 56 is explained in Scheme 6-37. The peaks at \(m/z\) 59 and \(m/z\) 115 represent oxonium ions with an alkyl substituent on the oxygen formed by homolytic (\(\alpha\) cleavage) taking place on either side of the oxygen in the \(M^+\) (see Scheme 6-38). The peak at \(m/z\) 31 represents a primary oxonium ion formed by the loss of an olefin through a process involving a hydride-shift rearrangement that occurs in the alkyl portion of the ion with \(m/z\) 59 or \(m/z\) 115 (Scheme 6-38).

![Scheme 6-37](image)

The peaks at \(m/z\) 29 and \(m/z\) 85 in Figure 6-33 could represent alkyl ions formed by heterolytic cleavage of the \(M^{++}\) at one of the two carbon–oxygen bonds with the charge and the radical sites on the oxygen atom (Scheme 6-39). The peak at \(m/z\) 69 could represent an ion formed by the loss of a molecule of methane (16 Da) from the hexyl ion (85 Da). The ions with \(m/z\) 45 and \(m/z\) 101 could be formed by the cleavage of the appropriate one-electron C–O bond created on either side of the oxygen by one-electron shifts within the \(M^{++}\) (mm = 130 Da), as illustrated in Scheme 6-40.
Scheme 6-39

The peak at m/z 47 could represent a CH₃–CH₂–O⁻H₂ ion formed by a double hydrogen rearrangement [27] described in Scheme 6-41. As pointed out in the source of this mechanism, “[the mechanism was] subject to verification by deuterium labeling experiments” in 1967 [28]. It is not known whether that verification was ever accomplished.

Scheme 6-40

The pattern formed by the peaks at m/z 43, 42, 41, and 39 in Figure 6-33 indicate that the m/z 43 peak represents a propyl ion. It is clear from the described possible origins of the ion with m/z 29 that this ion is an ethyl ion; the pattern of the peaks at m/z 29, 28, and 27 supports this assertion.

Scheme 6-41

A possible explanation for the formation of the propyl ion is shown in Scheme 6-42. This ion is produced by the secondary fragmentation of the hexyl ion with m/z 85 formed by the heterolytic cleavage of the M⁺.

An interesting point about the mechanism proposed in Scheme 6-42 is that it involves another of these virtual six-membered ring mechanisms. In this case, the mechanism was inspired by the mechanism shown for the formation of the ion with m/z 43 in the mass spectrum of cyclohexane (Figure 6-16 and Scheme 6-25). As was also seen in the formation of the ion with m/z 29 in the fragmentation of cyclohexane, these hydride-shift rearrangement fragmentations of an EE ion can also occur through five-membered ring transitions.
A good example of the mass spectrum of a symmetrical ether is shown in Figure 6-34. This spectrum also illustrates the effect of chain length on the formation of the primary oxonium ion represented by the peak at m/z 31, which is vanishingly small, but still perceptible. Also notice the peaks at m/z 70 and m/z 42 (neither of which is labeled) for odd-electron ions resulting from the molecular ion of dipentyl ether participating in a rearrangement mechanism analogous to that illustrated in Scheme 6-37.

![Figure 6-34. EI mass spectrum of dipentyl ether; nominal mass of 158 Da.](image)

**Scheme 6-42**

C. Aromatic Alcohols

Aromatic alcohols are phenols. These are compounds that have a hydroxyl group attached to a phenyl ring. Like other aromatic compounds, the mass spectra of phenols exhibit intense molecular ion peaks. These spectra will often exhibit a peak representing the loss of a molecule of carbon monoxide; a mechanism for this loss of CO is illustrated in Scheme 6-43.
Also as shown in Scheme 6-43, some of the distonic ions with m/z 66 resulting from the expulsion of CO from ionized phenol continue to fragment by losing a molecule of ethene via another heterolytic cleavage driven by the nascent charge site on carbon atom 2 that was adjacent to the carbon atom 1 (lost as CO). As can be seen in Figure 6-35, in addition to the intense M** peak, the mass spectrum of phenol also is characterized by peaks at m/z 65, 51, and 39 representing secondary fragmentations of the aromatic ring discussed earlier in this chapter.

An important differentiator between the mass spectra of aromatic alcohols and aliphatic alcohols is the fact that phenols do not lose water under normal circumstances. In cases where the phenolic hydroxyl is on a carbon atom adjacent to a carbon atom with a non-alkyl substitution, there will be an ortho effect, which promotes the loss of a molecule from the M**. The ortho effect is a rearrangement that will cause a molecule to be expelled from the M** of an aromatic compound that has two functionalities on adjacent carbon atoms. One of the functionalities can be a heteroatom. When one of the substituents is an alkyl group (CH₃, C₂H₅, etc.), the ortho effect is minimal. In the mass spectrum of o-chlorotoluene, there is an obvious [M − HCl]** peak (although very small) whereas no peak of significant intensity at the same m/z value appears in the mass spectrum of either the m- or p-isomer of this compound. The mass spectra of the o-isomers of chloroethylbenzene and chloroisopropylbenzene do not exhibit a peak representing the [M − HCl]** ion (none of these spectra is shown). The ortho effect was first reported in 1959 by McLafferty and Golke in a publication dealing with hydroxylated aromatic acids [29]. There are three other references in the literature that give a great deal more information on the ortho effect [30–32].

The ortho effect is pronounced with phenols in which the substituent is anything other than an alkyl group. All three regioisomers of methylphenol exhibit an [M − H₂O]** peak at m/z 90. None of the mass spectra of ethylphenol exhibits such a peak. The mass spectra of the regioisomers of dichlorophenol show the influence of the ortho effect; there are six possible isomers. For each isomer in which the chlorine moiety is on a carbon atom adjacent to the carbon atom with the hydroxyl group, the spectrum exhibits an [M − HCl]** and an [M − HCl − CO]** peak. In the mass spectra of the isomers that do not have at least one of the two chlorine atoms adjacent to the OH, these OE ion peaks do not exist (Figures 6-36 and 6-37). There is more on the ortho effect later in this chapter.
Returning to the point that phenols do not lose water as do aliphatic alcohols and cycloalkanols, a good example is available in a comparison of the mass spectra of two positional isomers, one constituting a cycloalkanol (1,2,3,4-tetrahydro-1-naphthalenol) (Figure 6-38), and the other a phenol (5,6,7,8-tetrahydro-1-naphthalenol) (Figure 6-39). Scheme 6-44 is a possible mechanism showing the loss of water from the M$^+$ to justify the peak at m/z 130 in the mass spectrum (Figure 6-38) of the cyclic aliphatic alcohol (1,2,3,4-tetrahydro-1-naphthalenol).

Scheme 6-44
Representative Fragmentations (Spectra) of Classes of Compounds

Figure 6-38. EI mass spectrum of 1,2,3,4-tetrahydro-1-naphthalenol.

Scheme 6-45
An intense peak at m/z 120 is observed in the spectra of both of these compounds; however, whereas this ion has the same elemental composition as the two possible ions that have m/z 120 formed by the phenolic compound, the structure of the m/z 120 ion formed by the cyclic alkanol is very different than the two proposed structures for the ion with the same m/z value formed by the phenolic compound. This is seen in Scheme 6-45.

Scheme 6-46
The phenolic isomer (5,6,7,8-tetrahydro-1-naphthalenol) exhibits a mass spectrum with a significantly different appearance (Figure 6-39). This is because the phenolic compound has two possible mechanistic pathways by which to lose ethene (Schemes 6-46 and 6-47).
Figure 6-39.  El mass spectrum of 5,6,7,8-tetrahydro-1-naphthalenol.

The two ions with \( m/z \ 120 \) in Schemes 6-46 and 6-47 have the same elemental composition and thus the same exact mass, but each of the structures could be validated by the use of stable isotope labeling by incorporating \(^{13}C\) for carbons 6 and 7.  The formation of the ion via the loss of ethene involving carbons 5 and 6 (as shown in Scheme 6-46) would result in an \([M - 29]^+\) peak at \( m/z \ 121 \) (loss of \( H_2^{13}C=CH_2 \) from a \( M^+ \) with \( m/z \ 150 \)).  The other ion resulting from a loss of a molecule of ethene involving carbons 6 and 7 would have an \( m/z \) value of 120 (loss of \( H_2^{13}C=^{13}CH_2 \)).

Scheme 6-47

In all of the examples of ionization and fragmentation of hydroxyl groups attached to phenyl rings described above, the mechanisms are based on the charge and radical sites being on the oxygen atom due to the loss of a nonbonding electron.  It is also possible to have molecular ions in which the charge and radical sites are associated with the aromatic ring due to the loss of a \( \pi \)-bond electron.  Because there are competing fragmentation pathways of the different types of molecular ions resulting from the loss of different types of electrons, both sets of mechanisms must be considered.

Two features differentiate the mass spectra of aromatic alcohols and aliphatic alcohols.  First, the intense \( M^{**} \) peak in the mass spectra of aromatic alcohols is in stark contrast to the vanishingly small \( M^+ \) peak in the mass spectra of aliphatic alcohols.  Second, aromatic alcohols do not expel water, whereas aliphatic alcohols do.  Even though the mass spectrum of 1,2,3,4-tetrahydro-1-naphthalenol exhibits a molecular ion peak, its intensity is only about half that of the molecular ion peak exhibited in the mass spectrum of 5,6,7,8-tetrahydro-1-naphthalenol.
D. Cyclic Ethers

The mass spectrum of tetrahydropyran is a good example of the complex nature of the mass spectra of cyclic ethers. The source of all the ions represented by the major peaks in this mass spectrum (Figure 6-40) is shown in Schemes 6-48 and 6-49. Because of the simplicity of the mechanisms in Scheme 6-49 and the stability of the resulting ions, it might be expected that the peak at m/z 31 and the one corresponding to $[M - 28]^+$ (m/z 58) would be more intense than observed in the mass spectrum (Figure 6-40). The peak at m/z 58 could be mistaken for an isotope peak associated with m/z 56; however, its intensity is too great to be explained away as representing a species containing two atoms of $^{13}$C or an atom of $^{18}$O. Also, the ion with m/z 58 appears to be a precursor of the ion represented by the peak at m/z 30 in the mass spectrum. Although the peaks at m/z 57 and 71 represent O-substituted oxonium ions of the form H$_2$C=O–C$_n$H$_{2n-1}$ (where n = 2 and 3, respectively), the peak at m/z 43 probably represents a propyl ion rather than a substituted oxonium ion (the fragmentation pathway is not shown).

The mass spectrum of tetrahydropyran, along with the associated fragmentation schemes, is a good illustration of the complexity that is introduced by multiple EE-ion rearrangements that can occur in such compounds. The fragmentation of tetrahydropyran is consistent with the fragmentation of tetrahydrofuran reported by Smakman and De Boer [33].

E. Ketones and Aldehydes

Ketones and aldehydes are characterized by a carbonyl (C=O) moiety positioned between two R groups. In aldehydes, one of the R groups is a hydrogen atom; this hydrogen is located in a “special place” which promotes its expulsion to form a stable acylium ion as represented by the characteristic $[M - H]^+$ peak of significant intensity in the mass spectra of many aldehydes, regardless of whether the other R group is aliphatic or aromatic. Ketones have two non-hydrogen moieties attached to the carbonyl.

An intense $[M - 1]^+$ peak (>5% of the base peak) in the mass spectrum of an aliphatic compound indicates the possibility that the compound is an aldehyde. $[M - 1]^+$ peaks are also observed when an aldehydic group is attached to an aromatic ring.
Scheme 6-48

Scheme 6-49
Straight-chain aliphatic aldehydes are similar in their mass spectral behavior to aliphatic alcohols. The mass spectra of straight-chain aliphatic aldehydes, through C₄, exhibit a relatively intense M⁺ peak (>50% of the base peak) and the signature [M − 1]⁺ peak. Figure 6-41 is the mass spectrum of butyl aldehyde showing both an intense M⁺ peak and a readily discernible [M − H]⁺ peak. The mass spectra of straight-chain aldehydes larger than C₄ show no observable M⁺ peak. The mass spectra of straight-chain aliphatic aldehydes larger than C₃ exhibit a peak at m/z 44, which represents an OE ion formed by a γ-hydrogen-shift rearrangement fragmentation (a McLafferty rearrangement). This m/z 44 peak is the base peak in the mass spectra of C₄ through C₆ straight-chain aliphatic aldehydes. The intensity of this peak declines to about 25% of the base peak in the mass spectrum of octadecyl aldehyde.

Figure 6-41. El mass spectrum of butyl aldehyde (Wiley Registry 8th Ed).

Figure 6-42 is the mass spectrum of decyl aldehyde, which exhibits no discernible M⁺ peak. However, there are several OE ion peaks in the M⁺ peak region that appear in the mass spectra of all straight-chain aliphatic aldehydes that contain more than four carbon atoms. These are peaks representing [M − 18]⁺ (loss of H₂O), [M − 28]⁺ (loss of H₂C=CH₂), and [M − 44]⁺ ions. The loss of ethene from the M⁺ is explained in Scheme 6-50. The loss of water from the M⁺ can occur via two different pathways, as illustrated in Scheme 6-51. The loss of 44 Da from the M⁺ is more complex. This ion could be formed by the loss of a molecule of HC≡CH from the ion formed by the loss of a water molecule via an enol form of the aldehyde as shown in Scheme 6-51; alternatively, this [M − 44]⁺ ion could result from loss of the aldehydic H⁺ radical followed by the loss of CO followed by the loss of a CH₃ radical from the M⁺.

Figure 6-42. El mass spectrum of decyl aldehyde (Wiley Registry 8th Ed).
Scheme 6-50

Scheme 6-51
The mass spectrum of benzaldehyde (Figure 6-43) exhibits a very intense M* peak and an [M – 18]⁺ peak of almost equal intensity. The high intensity of the M* and [M – 1]⁺ peaks reflect the resonance stability of the corresponding ions. The peak at m/z 77 represents a phenyl ion formed by heterolytic cleavage of the molecular ion that was formed by the loss of one of the nonbonding electrons on oxygen.

Compared to the mass spectrum of benzaldehyde, the spectrum of cyclohexanol, the saturated analog (Figure 6-44), presents a very different picture. In the mass spectrum of cyclohexanal, there is an easily discernible M* peak, although the intensity is not a large value (<25% of the base peak). The intensity of the [M – 1]⁺ peak is very low, barely detectable in the graphics display. The intensity of the M* peak is as great as it is largely due to delocalization of the charge and radical sites among several structures, all of which have the same m/z value as rationalized in Scheme 6-52 through β cleavage resulting first in a cyclic oxonium ion with subsequent cleavage through a hydride-shift rearrangement, all of which may be reversible.
A good example of the fragmentation of aliphatic ketone molecular ions is the mass spectrum of 4-decanone (Figure 6-11). In addition to the formation of OE fragment ions with \( m/z \) 86 and 58, through the rearrangement fragmentation of the \( M^+ \) followed by the rearrangement fragmentation of that product (as illustrated in Scheme 6-18), a number of other fragmentations are initiated by the charge and radical sites on the carbonyl oxygen atom due to the loss of one of the nonbonding electrons. All \( M^+ \) fragmentations of 4-decanone can be rationalized through mechanisms starting with \( M^+ \) having the “plus/dot” on the oxygen, as will become clear in the next few pages.

One very important aspect of the rearrangement fragmentation of ketones that should be pointed out is the formation of the OE ion with \( m/z \) 58. This ion is present in the mass spectrum of all aliphatic ketones except those that have a substituent on the \( \alpha \) carbon. As was stated in the discussion on primary and secondary radicals in relation to formation of OE ions through \( \gamma \)-hydrogen-shift \( \beta \)-cleavage rearrangements, the intensity of the peak at \( m/z \) 58 will be significant as long as the process involves intermediate formation of a secondary radical. When the carbonyl is the number 3 carbon atom, the diagnostic peak at \( m/z \) 58 will be shifted by 14 \( m/z \) units to \( m/z \) 72. This arrangement can be considered as a special case of \( \alpha \)-substitution.

Schemes 6-53 and 6-54 illustrate the pathways of \( \alpha \) cleavage (a special case of homolytic cleavage) for the \( M^+ \) of 4-decanone to account for the ion current with \( m/z \) 113 and \( m/z \) 71 in Figure 6-11. Some of the resulting acylium ions subsequently expel a molecule of CO via a heterolytic-cleavage mechanism (not shown) to account for some of the ion current at \( m/z \) 85 and \( m/z \) 43.

The terms \( \alpha \) cleavage, \( \beta \) cleavage, or \( \gamma \) cleavage refer to special cases of homolytic cleavage where the carbon bond next to the \( \alpha \)-C, \( \beta \)-C, or \( \gamma \)-C atom, on the side closest to the moiety carrying the charge, is broken.
Some of the other 4-decanone molecular ions will undergo fragmentation via heterolytic cleavage to produce ions with $m/z$ 43 and $m/z$ 85, as illustrated in Schemes 6-55 and 6-56. The base peak at $m/z$ 43 in the mass spectrum (Figure 6-11) of this compound represents an ion formed by the M$^+$ losing a seven-carbon carbonyl radical, which is the largest possible moiety that can be lost. Although there are competing pathways for the formation of the ions with $m/z$ 43 and $m/z$ 85, the ion with $m/z$ 43 dominates because of the loss of the largest moiety during heterolytic cleavage.

The peaks at the lower $m/z$ values (42, 41, and 39) preceding the peak at $m/z$ 43 represent the secondary fragmentation ions so often associated with aliphatic ions. There are peaks with nominal $m/z$ values of 99, 57, and 29 that cannot be accounted for through the fragmentation mechanisms of homolytic cleavage, heterolytic cleavage, or rearrangements. From the peak pattern between $m/z$ 53 and $m/z$ 57, it appears that the peak at $m/z$ 57 represents a butyl ion. The difference between 156 (the nominal mass of 4-decanone) and 57 is 99, which is the $m/z$ value of one of the other yet unexplained peaks in the mass spectrum. It is possible that these two peaks represent ions produced by σ-bond fragmentation at a site of σ-bond ionization. This process of σ-bond fragmentation could also be an explanation for the peak at $m/z$ 29, although the peak at $m/z$ 27 appears to be too intense to be rationalized as representing an ion formed by the loss of a hydrogen molecule from an ethyl ion, which could be represented by the peak at $m/z$ 29.

Continuing with the interpretation of the mass spectrum (Figure 6-11) of 4-decanone, there is a problem with the relative intensities of the yet-to-be-explained peaks at $m/z$ 99, 29, and 27. The intensity of these peaks would suggest that a significant number of the molecular ions formed were due to σ-bond ionization, which is not reasonable based on the much lower ionization potential associated with the loss of one of the nonbonding electrons on the oxygen atom.

There are some other possibilities for fragmentation of molecular ions that have the charge and radical sites on the oxygen atom that could relate to the origin of the so-far-unexplained peaks. The peak at $m/z$ 57 could represent a butyl ion formed by the loss of a molecule of ethene via a secondary heterolytic cleavage of hexyl ion as shown in Scheme 6-57. The $n$-butyl ion would then expel another molecule of ethene to produce the ethyl ion with $m/z$ 29, also shown in Scheme 6-57.
The above description for the formation of the ion with \( m/z \) 29 does not explain why the intensity of the peak at \( m/z \) 27 is as great as it appears in Figure 6-11. The origins of the vinyl ion represented by the peak at \( m/z \) 27 could be from both the expulsion of a hydrogen molecule from the ethyl ion and the expulsion of a molecule of methane from the propyl ion. This leaves only the peak at \( m/z \) 99 in Figure 6-11 to be explained.

A possible source of the ion with \( m/z \) 99 is through a \( \gamma \) cleavage as illustrated in Scheme 6-56. According to Reference 26, page 138, this reaction may be the result of the rupture of an allylic bond in the enol form of the \( M^+ \) or, as illustrated in Scheme 6-58, may involve the formation of a cyclic oxonium ion. The reference points out that ions formed by this \( \gamma \) cleavage have a far greater abundance than those formed either by \( \beta \) cleavage or by \( \delta \) cleavage; however, the presence of these products would be masked by peaks at \( m/z \) 113 and at \( m/z \) 85 for the isobaric ions formed by homolytic and heterolytic cleavage, respectively. The dominance of \( \gamma \) cleavage (\( m/z \) 113) over \( \beta \) cleavage (\( m/z \) 99) and \( \delta \) cleavage (\( m/z \) 127) is reflected in the mass spectrum of di-\( n \)-butyl ketone (Figure 6-45).

![Scheme 6-58](image)

**Figure 6-45.** EI mass spectrum of di-\( n \)-butyl ketone with peaks representing \( \beta, \gamma, \) and \( \delta \) cleavages (left to right) marked with an *. The fragmentation of aromatic ketones can usually be explained by a mechanism starting with the most probable site for the charge and the odd electron in the \( M^+ \). The peaks at \( m/z \) 105 and \( m/z \) 77 in the mass spectrum of diphenyl ketone (Figure 6-46) represent ions formed by homolytic and heterolytic cleavages, respectively. It should be noted that the high abundance of the ion with \( m/z \) 105 is a result of resonance stabilization of the acylium moiety resulting from its being attached directly to the phenyl ring.
Peaks resulting from the charge and radical sites being only associated with the carbonyl oxygen are also seen in the mass spectrum of ethyl phenyl ketone (Figure 6-47). A significant change in the mass spectrum of a compound consisting of $\text{C}_{10}\text{H}_{10}\text{O}$ results from a slight change in the arrangement of the atoms to position the carbonyl between a benzyl carbon and a methyl carbon (in going from ethyl phenyl ketone to phenyl acetone); compare Figures 6-47 and Figure 6-48.
The change in fragmentation pattern illustrated in Figures 6-47 vs Figure 6-48 results from changing structural features in going from a resonantly stabilized $M^+$ to an aliphatic ketone $M^+$; in both cases, the charge and radical sites are on the carbonyl oxygen. From the structure of benzyl methyl ketone (a.k.a. phenyl acetone), a peak in the mass spectrum representing a benzyl acylium ion might be predicted. However, no such peak is observed. If the benzyl-acylium ion is formed, it will quickly lose CO to produce a tolyl ion, which has an $m/z$ value of 91. The mass spectrum of benzyl methyl ketone (Figure 6-48) also exhibits a peak at $m/z$ 43, which represents the very stable methyl acylium ion. There is also a peak at $m/z$ 92, which is far more intense than would be expected for a $^{13}$C-isotope peak of the ion with $m/z$ 91. This peak represents an ion formed by a rearrangement that can only be explained by the charge and radical sites resulting from the loss of an electron on the ring (Scheme 6-59).

Scheme 6-59

Although ethyl phenyl ketone also has hydrogens on a $\gamma$-carbon atom, the lack of the carbonyl next to this $\omega$ carbon makes the formation of the primary radical much less likely than is the case with benzyl methyl ketone. The mass spectrum of benzyl methyl ketone is a good example of the effect of having competing sites for the radical and the charge in molecular ions. The most probable sites of the charge and radical will be the result of the loss of a nonbonding electron from the oxygen; however, there will also be molecular ions formed by the loss of a $\pi$ electron from the phenyl ring.
It is also worth noting that the peak at m/z 91 in the mass spectrum of benzyl methyl ketone (Figure 6-48) probably represents tropylium ions formed through multiple fragmentation pathways [24]. A tropylium ion is formed via benzylic cleavage when the charge and radical sites are initially on the ring. Benzyl ions will be formed by the loss of CO from the benzyl-acylium ions (with m/z 119 in Scheme 6-60) formed via homolytic cleavage of those molecular ions produced with the charge and radical sites on the oxygen atom (analogous to that shown in Scheme 6-60). Benzyl ions can also be produced directly by heterolytic cleavage of the bond between the carbonyl carbon and the benzyl carbon in molecular ions with the charge and radical sites on the oxygen atom (not shown).

Scheme 6-60

Another interesting property of an alkyl-aryl ketone is reflected in the mass spectrum of dibenzyl ketone (Figure 6-49), which exhibits a peak at m/z 119 that is of much lower intensity than might be predicted. Also, there is an OE ion peak at m/z 118.

Scheme 6-60 illustrates the formation of an acylium ion of m/z 119 from homolytic fission of a molecular ion of dibenzyl ketone having the charge and radical sites on the carbonyl oxygen. Because this acylium ion is not resonantly stabilized through conjugation with the phenyl ring, it readily loses carbon monoxide to form the tolyl ion, which likely isomerizes to the tropylium ion of m/z 91 [24].
In addition to being formed via the loss of CO from the benzyl-acylium ion, the ion with \( m/z \) 91 may also be formed directly by heterolytic cleavage of the benzylic carbon–carbonyl carbon bond in the \( M^+ \) (Scheme 6-60). A third possibility for the formation of the ion with \( m/z \) 91 is through benzylic cleavage of molecular ions produced by loss of a \( \pi \) electron from the phenyl ring. This type of \( M^+ \) would also offer a possible explanation for the formation of the ion with \( m/z \) 118; this OE ion could be the result of a heterolytic cleavage following an initial \( \gamma \)-hydrogen shift, as illustrated in Scheme 6-61.

![Scheme 6-61](image)

The fragmentation of dibenzyl ketone (Schemes 6-60 and 6-61) supports the premise that isomeric molecular ions can be produced during the ionization process. It is obvious from the above explanation and the mass spectrum that molecular ions of dibenzyl ketone with the charge and radical sites on the carbonyl oxygen are formed as well as those with the charge and radical sites on the ring.

**F. Aliphatic Acids and Esters**

Like ketones and aldehydes, acids and esters are characterized by the presence of a carbonyl group. Just as an aldehyde can be viewed as an oxidation product of an alcohol, an organic acid (carboxylic acid) can be viewed as an oxidation product of an aldehyde. Organic acids are seldom encountered in EI mass spectrometry because of their polarity. These highly polar molecules are also difficult to separate by gas chromatography. When these acids also contain hydroxyl and amino groups, the resulting increase in polarity and thermal lability make vaporization of the compound difficult; for example, amino acids decompose upon heating. The analysis of an organic acid by EI usually involves preliminary conversion to a less polar, more volatile derivative such as methyl ester or a trisubstituted silyl ester (discussed later in this chapter).

The mass spectra of aliphatic carboxylic acids and esters of carboxylic acids are very similar. Like the EI mass spectra of aliphatic aldehydes and ethers, those of carboxylic acids and esters exhibit discernible \( M^+ \) peaks unless the molecule contains a \( \gamma \) carbon (i.e., a \( \gamma \) carbon on the carboxylic acid moiety). The presence of this \( \gamma \) carbon allows for \( \beta \) cleavage induced by a \( \gamma \)-hydrogen shift (a McLafferty rearrangement) in molecular ions of both acids and esters where the \( \gamma \) carbon is on the acid side. The resulting OE ion is characteristic of both of these compound types. The McLafferty rearrangement ion from a methyl ester has an \( m/z \) value of 74 and that for carboxylic acids has an \( m/z \) value of 60. If the alcohol moiety of an ester has a carbon that is gamma to the alkoxy oxygen, a rearrangement can occur when the charge and radical sites are associated with this alkoxy oxygen.

Even though \( n \)-butyl acetate and methyl butanoate both have a carbon atom that is a \( \gamma \) carbon, the geometry of the two molecular ions is different in that the virtual six-member ring is formed via the ether oxygen (sp\(^3\) hybrid) in the \( n \)-butyl acetate and the carbonyl
oxygen (sp$^2$ hybrid) in the methyl butanoate. The $\gamma$-hydrogen shift in the $n$-butyl acetate M$^{+\star}$ in response to the charge and radical sites on the alkoxy oxygen of the ester linkage results in the inductive cleavage of the sp$^3$ hybrid oxygen–aliphatic carbon bond to form the OE ion with m/z 56 (Scheme 6-62), whereas the $\gamma$-hydrogen shift in the methyl butanoate M$^{+\star}$ is in response to the charge and radical sites being on the carbonyl oxygen resulting in $\beta$ cleavage to form the ion of m/z 74 that characterizes the McLafferty rearrangement (Scheme 6-63).

Scheme 6-62

Scheme 6-63

The intensity of the peaks representing these OE ions formed from these rearrangement fragmentations is quite high, even though the $\gamma$ hydrogens are associated with terminal methyl groups and result in intermediate formation of primary radicals during the $\gamma$-hydrogen shift process. Examination of the mass spectra in Figures 6-50 and 6-51 shows that the peaks representing OE fragment ions account for more than 50% of the intensity of the base peak.

Figure 6-50. El mass spectrum of n-butyl acetate; the peak at m/z 56 represents an odd-electron ion formed by a $\gamma$-hydrogen shift through a virtual six-membered ring transition.
Figure 6-51. **EI mass spectrum of methyl butanoate; the peak at m/z 74 represents an OE ion formed by a \( \gamma \)-hydrogen shift.**

The mass spectra of acetates formed by esterification of acetic acid with a homologous series of straight-chain alcohols illustrate how fragmentation mechanisms can be supported. Scheme 6-62 proposes that the peak at \( m/z \) 56 in the mass spectrum of \( n \)-butyl acetate represents a distonic aliphatic ion with the charge on one terminal carbon atom and the radical site on the other terminal carbon atom. Examine the four spectra in Figures 6-50, 6-52, 6-53, and 6-54.

Unlike the mass spectrum of \( n \)-butyl acetate (Figure 6-50), the mass spectrum of \( n \)-pentyl acetate (Figure 6-52) does not exhibit a significant peak at \( m/z \) 56; however, there is an OE fragment ion peak at \( m/z \) 70 that is 14 \( m/z \) units greater than \( m/z \) 56. This means that a distonic ion containing one more \(-\text{CH}_2\-) is formed via a six-membered ring transition analogous to that shown in Scheme 6-62 with the radical site located on C-4.

In the mass spectrum of \( n \)-hexyl acetate (Figure 6-53), the OE ion peak at \( m/z \) 70 is no longer present; however, there are OE fragment ion peaks at \( m/z \) 84 and \( m/z \) 56. The ion with \( m/z \) 84 is a homolog of the ion with \( m/z \) 56 (contains two more \(-\text{CH}_2\-) units than does the ion with \( m/z \) 56) that was seen in the mass spectrum of \( n \)-butyl acetate. The ion with \( m/z \) 86, like the ion at \( m/z \) 56 formed from \( n \)-butyl acetate and the ion with \( m/z \) 70 formed from \( n \)-pentyl acetate, has the radical site located on C-4. The peak at \( m/z \) 56 observed in the mass spectrum of \( n \)-hexyl acetate represents an ion formed by the loss of a molecule of \( \text{H}_2\text{C}=\text{CH}_2 \) from the OE fragment ion with \( m/z \) 84 through heterolytic cleavage.

In the mass spectrum of \( n \)-heptyl acetate (Figure 6-54), there are OE ion peaks at \( m/z \) 98, \( m/z \) 70, and \( m/z \) 56. In the mass spectrum of \( n \)-octyl acetate (data not shown), there are OE ion peaks at \( m/z \) 112, \( m/z \) 84, \( m/z \) 70, and \( m/z \) 56. From the examination and description of these mass spectra of homologous aliphatic acetates, it is apparent that the distonic ion (homologs of the ion with \( m/z \) 56 in Scheme 6-62) represented by the high-mass OE ion peak undergoes secondary fragmentation to eliminate an olefin through inductive cleavage. For example, the ion with \( m/z \) 70 in the mass spectrum of \( n \)-heptyl acetate (Figure 6-54) results from inductive cleavage of the initial distonic ion with \( m/z \) 98 in response to the charge site to lose a molecule of \( \text{H}_2\text{C}=\text{CH}_2 \) as illustrated in Scheme 6-64. The OE ion peak at \( m/z \) 56 in Figure 6-54 represents an ion formed by hydrogen-shift-induced homolytic cleavage of the initial distonic ion with \( m/z \) 98 to expel propene, through the mechanism illustrated in Scheme 6-64. The OE fragment ion represented by the peak at \( m/z \) 56 in the mass spectrum of \( n \)-hexyl acetate (Figure 6-53) could also be formed as a result of the loss of a molecule of ethene from the ion with \( m/z \) 84 as a result of inductive cleavage (analogous to Scheme 6-64).
Figure 6-52. EI mass spectrum of n-pentyl acetate.

Figure 6-53. EI mass spectrum of n-hexyl acetate.

Figure 6-54. EI mass spectrum of n-heptyl acetate.
The EI mass spectra of the propionates of the same homologous series of alcohols show the same OE ion peaks. Even as the size of the carbon chain on the acid side of the ester grows, the rearrangement driven by the charge and radical sites on the alkoxy oxygen dominates. These same series of OE ion peaks dominate the spectra of the corresponding propionate compounds. The utility of these mass spectra in identifying the corresponding compounds is limited by the lack of a M$^+$ peak. Therefore, GC retention time becomes an important factor in distinguishing these compounds. Determination of nominal mass through derivatization is not an option in helping to distinguish this series of compounds because esters do not contain active hydrogens to react with derivatizing reagents.

Aliphatic carboxylic acids with 12–20 carbon atoms are known as fatty acids. These compounds have a great deal of biological significance and have been extensively studied by mass spectrometry [34–36]. As was stated at the beginning of this section, because of the polarity of organic acids, they are usually analyzed by gas chromatography and/or mass spectrometry in the form of their methyl esters.

A comparison of the mass spectra of stearic acid (Figure 6-55) and methyl stearate (Figure 6-56) shows a great number of similarities. Both spectra exhibit an intense peak at m/z 60 and m/z 74, respectively, representing the ion formed through a $\gamma$-hydrogen shift-induced $\beta$ cleavage (McLafferty rearrangement). In each spectrum, there is a peak 13 m/z units higher (at m/z 73 and m/z 87, respectively) representing a structurally diagnostic EE ion. Among the subsequent series of peaks appearing at 14 m/z unit intervals; i.e., 87, 101, 115, etc., there are those of more significant intensity appearing at intervals 56 m/z units; i.e., 73, 129, 185, etc. in Figure 6-55 and 87, 143, 199, etc. in Figure 6-56.
Spectra of both the acid and the ester also exhibit \([M - 29]^+\) and \([M - 43]^+\) peaks that have greater-than-expected relative intensities. The origin of the ions represented by all of these notable peaks is the same for both molecular ions. The formation of these ions has been extensively studied for methyl stearate. Based on stable isotope-labeling studies using both deuterium and \(^{13}\)C, Ryhage and Stenhagen proposed an explanation for the formation of the ions represented by the peaks at \(m/z\) 87, \(m/z\) 143, and \(m/z\) 199 in the mass spectrum of methyl stearate as shown in Scheme 6-65 [34, 37, 38].

Even though the logic of the mechanisms involving several distonic ions proposed in Scheme 6-65 is irrefutable, it has been argued that these proposed mechanisms follow from an initial six-membered ring transition [39] as opposed to the eight-membered ring transition shown here. Sparkman and Ren used the B3LYP/6-31+G(d) method for geometry and energy calculation with the Windows\textsuperscript{®} version of Gaussian 98 to model methyl heptanoate. Based on the results of these calculations, the ion with \(m/z\) 87 can be formed via an initial eight-membered ring hydrogen atom transfer with less energy requirement than that for the initial six-membered ring transition [40].
However, if the six-membered ring secondary transition philosophy shown in Scheme 6-65 for the formation of the ions with \( m/z \) 87, \( m/z \) 143, and \( m/z \) 199 is extended one more step, an alternative justification for the formation of the ion with \( m/z \) 255 can be made by proposing the mechanism in Scheme 6-66.

The species at the left in Scheme 6-66 is formed starting with the distonic ion (shown as the second structure in Scheme 6-65) initially formed through a hydrogen shift in response to the radical site associated with the carbonyl oxygen occurring through an eight-membered ring transition; the radical will be on C-6. Just as was the case with the formation of the ion with \( m/z \) 199 in Scheme 6-65, a six-membered transition involving a hydrogen atom on C-10 occurs, causing the radical site to now be located on C-10.
However, rather than a homolytic cleavage being initiated by the movement of one of the two electrons between C-11 and C-12 in response to the radical site on C-10, another six-membered transition involving a hydrogen shift from C-14 could occur. The new radical site at C-14 initiates homolysis by having one of the two electrons forming the bond between the C-15 and C-16 pair with the radical site at C-14. This would result in the loss of a propyl radical.

Hubert Budzikiewicz and colleagues [28] in Carl Djerassi’s laboratory at Stanford University suggested the mechanism shown in Scheme 6-67 based on the stable isotope-labeling studies carried out by Stenhagen. The Stenhagen studies showed that the [M − 43]+ peak in the mass spectrum of methyl stearate was due to the loss of carbon atoms 2, 3, and 4. This same work also showed that the [M − 29]+ ion was formed through the loss of carbon atoms 2 and 3. The Budzikiewicz et al. mechanism proposes the loss of the methylene units comprised of C-2, C-3, and C-4 plus an additional hydrogen atom that could come from any position along the chain beyond C-5. The [M − 29]+ ion is formed through a five-membered ring transition rather than the six-membered transition shown in Scheme 6-67.
One of the reasons for showing these somewhat bizarre mechanisms is to emphasize the point that with such ionic reactions and/or isomerizations apparently taking place inside the EI ion source, it is amazing that it is possible to extract so much information about the structure of an analyte from its EI mass spectrum. As was stated early on, all of these schemes are paper mechanisms. They are based on what is known about the behavior of organic molecules. However, even with the results of stable isotope-labeling studies, it is not always possible to describe fragmentation behavior accurately.

A significant difference in the mass spectra of methyl esters and those of the corresponding free fatty acids is the presence of a [M – 31]$^+$ ([M – OCH$_3$]$^+$) peak for the methyl esters, but not a [M – 17]$^+$ ([M – OH]$^+$) peak for the free acids. Alkyl esters lose an alkoxy radical via homolytic cleavage when the charge and radical sites are on the carbonyl oxygen atom (Scheme 6-68). The analogous M$^{**}$ of the free acid does not lose a hydroxy radical. A peak at m/z 74, together with a [M – 31]$^+$ peak, is a strong indication that the analyte is a methyl ester in which the acid moiety consists of at least four carbon atoms.

Scheme 6-68

A periodic series of peaks every 56 m/z units are observed in the mass spectra of all long-chain fatty acids and esters of higher mass alcohols. There are also peaks that stand out from the basic spectral pattern of peaks separated by 14 m/z units including those that designate the position of branch points and other functional groups. It is important to be able to recognize the periodic peaks that correspond to the above-described rearrangements and to differentiate these peaks from those that are structurally diagnostic.

The mass spectra of esterified fatty acids provide a good example of using general observations and knowledge of specific fragmentation mechanisms to elucidate the structure of an unknown from its EI mass spectrum.
Consider the mass spectrum in Figure 6-57 as that of an unknown. Reading the spectrum from the right to the left, the first observation is that the highest m/z value peak that is not due to background or an isotope peak is at m/z 312. This is an even number indicating, according to the Nitrogen Rule, that the analyte does not contain an odd number of N atoms. The base peak in the mass spectrum is at m/z 88, which could well represent an OE fragment ion. There is an obvious series of peaks separated by 56 m/z units (see m/z 101, 157, 213, etc.). It is known that the mass spectra of methyl esters of fatty acids exhibit a M^+ peak, a peak that represents an OE fragment ion at m/z 74, and periodic peaks separated by 56 m/z units that represent other rearrangement fragmentations of the M^+. Having noted such clues that are ester-like, the peak at m/z 88 might be expected to correspond to an ethyl ester considering the homologous shift of 14 from m/z 74. The formation of an ion of m/z 88 could be produced as illustrated in Scheme 6-69 from the M^+ of an ethyl ester.

Scheme 6-69

Further evidence that the unknown is likely to be an ester comes from noting that the difference between the OE ion peak (m/z 88) and the first peak (m/z 101) representing the 56-m/z unit periodic rearrangement ion is 13 m/z units just as in the mass spectrum of methyl stearate the peak (m/z 74) for the OE ion and the first (m/z 87) of the periodic series of peaks separated by 56 m/z units is 13 m/z units. Because the difference between the nominal mass of methyl stearate and the apparent nominal mass of the unknown is 14 Da, the evidence described to this point suggests that the unknown could be the ethyl ester of stearic acid.

However, examination of the high-mass end of the mass spectrum in Figure 6-57 shows no evidence of a peak at M – 45 (m/z 267) corresponding to loss of an ethoxy radical, as would be expected for an ethyl ester. Recall the important characteristic fragmentation of esterified fatty acids where some of the molecular ions tend to lose the alkoxy radical as shown in Scheme 6-68. Instead, there is a peak at m/z 281 in Figure 6-57 that could correspond to the loss of a methyl radical! This evidence suggests that the unknown is a methyl ester. A structural possibility that would be consistent with all the mass spectral evidence is a methyl ester of a C_{18} acid, which is substituted on the C-2 position (the α carbon) with a methyl group (α-methyl methyl stearate). Some of the molecular ions of this compound could produce an OE fragment ion with m/z 88 as illustrated in Scheme 6-70. The mass spectrum of ethyl stearate is shown in Figure 6-58.
Figure 6-58. **EI mass spectrum of ethyl stearate.**

The above example illustrates the importance of extracting several pieces of information from a mass spectrum to support the identification of an unknown. As was seen in this particular case, even though there was a low-

$m/z$ value OE ion peak that represented a specific behavior, its meaning was less than conclusive. It was necessary to look at the high-

$m/z$ end of the mass spectrum to obtain the unambiguous information.

G. Aromatic Acids and Esters

Two commercially significant compounds that fall into this category are acetylsalicylic acid (a.k.a. Aspirin) and methylsalicylate (2-hydroxymethylbenzoate, a.k.a. oil of wintergreen). The mass spectra of these compounds have the same general characteristics as the mass spectra of all aromatic compounds: (1) $M^+$ peaks of significant intensity, (2) ion current distributed throughout a few $m/z$ values, and (3) peaks representing secondary fragmentation ions with $m/z$ 39, 51, and 65. The best illustration of the behavior of these types of compounds is the fragmentation of the regioisomers of hydroxybenzoic acid. Examination of Scheme 6-71 shows how the ortho effect results in the expulsion of a molecule of water from the $M^+$ of salicylic acid. Some of these $[M - H_2O]^+$ ions with $m/z$ 120 undergo two successive losses of carbon monoxide to form the OE ions with $m/z$ 92 and 64 (see the corresponding peaks in Figure 6-59). Without the ortho effect, most of the molecular ions of the meta and para isomers lose a hydroxyl radical to form an EE ion ($m/z$ 121), some of which lose CO to form another EE ion ($m/z$ 93); some of these ions then lose another molecule of CO to form yet another EE ion ($m/z$ 65) (see the corresponding peaks in Figure 6-60).

If the acid moiety is esterified, the ortho effect will cause the $M^+$ to expel an alcohol molecule. If the hydroxyl moiety of 2-hydroxybenzoic acid is replaced with an –NH$_2$ group, the $M^+$ will still expel a molecule of water; however, if the –COOH group is replaced with a –CONH$_2$, the $M^+$ will expel a molecule of NH$_3$. There is no ortho effect observed in the mass spectrum of 2-chlorobenzoic acid (data not shown).

2-acetyloxybenzoic acid (acetylsalicylic acid, Aspirin) is another interesting compound in that it has both alkyl and aryl moieties. The mass spectra of both the 2- and 4- regioisomers of acetyloxybenzoic acid (Figures 6-61 and 6-62) exhibit discernible $M^+$ peaks. These two mass spectra also exhibit a peak at $m/z$ 138 (the same elemental composition and possibly the same structure as 2-OH benzoic acid), corresponding to the loss of a molecule of ketene from the $M^+$ (Scheme 6-72). Some of the resulting OE ions then fragment in the same way as the two isomers of hydroxybenzoic acid whose mass spectra are shown in Figures 6-59 and 6-60. In this case, the ortho effect functions during a secondary fragmentation process.
Figure 6-59. EI mass spectrum of salicylic acid (2-hydroxybenzoic acid).

Figure 6-60. EI mass spectrum of 4-hydroxybenzoic acid.
Because of the resonance structure of acetylsalicylic (acetyloxybenzoic) acid, the charge and radical sites may be transitory. The mass spectra of these compounds (Figures 6-61 and 6-62) both show a peak at \( m/z \) 138 corresponding to loss of ketene from the \( M^+ \). To support a mechanism for the loss of ketene, the charge and radical sites in the \( M^+ \) need to be on the position shown in Scheme 6-72.
4. Nitrogen-Containing Compounds

A. Aliphatic Amines

Aliphatic amines behave a lot like aliphatic alcohols in that the initial fragmentation occurs by cleavage of the bond between C-1 and C-2 due to homolysis driven by the charge and radical sites on the heteroatom ($\alpha$ cleavage). Structurally, aliphatic amines differ from aliphatic alcohols in that $1^\circ$, $2^\circ$, and $3^\circ$ amines are constituted by the number of non-hydrogen substituents on the N atom, whereas the number of non-hydrogen substituents on the hydroxylated carbon determines whether the alcohol is $1^\circ$, $2^\circ$, or $3^\circ$.

Depending on the number of carbon atoms in substituents on the nitrogen atom, a number of hydride-shift rearrangements resulting in secondary fragmentations can occur in the nascent EE fragment ions formed by the initial homolytic cleavage of the $M^\ast$. Once a radical site is no longer a factor due to its departure with the dark matter (the neutral loss), all of these secondary fragmentations are charge-site-driven with the charge still on the N atom.

The electrophilic character of oxygen is higher than that of nitrogen; therefore, in the mass spectra of ketones and ethers, peaks are observed representing ions that are due to heterolytic cleavage as well as homolytic cleavage. In the mass spectra of aliphatic amines, there are no peaks that represent ions formed by heterolytic cleavage of the $M^\ast$, only peaks representing ions formed by homolytic cleavage of the $M^\ast$.

The EI mass spectra of five isomers of the elemental composition $C_6H_{15}N$ will be examined on the following pages. The first three isomers represent $1^\circ$, $2^\circ$, or $3^\circ$ amines (1-hexylamine, Figure 6-64; dipropylamine, Figure 6-65; and triethylamine, Figure 6-66). Members of the second group are all primary amines, differing only in the position of the $-\text{NH}_2$ group on the hydrocarbon chain (1-hexylamine; 2-hexylamine, Figure 6-67; and 3-hexylamine, Figure 6-68); 1-hexylamine is common to both groups.

The mass spectra of 1-hexylamine, dipropylamine, and triethylamine shown in Figures 6-64, 6-65, and 6-66, respectively, illustrate an interesting property of the mass spectral display. Reading each spectrum from right to left, the relative intensity of the $M^\ast$ peak obviously increases from the primary (Figure 6-64) to the tertiary amine (Figure 6-66). The relative intensity of the peak at $m/z$ 30 is the same for the primary and secondary amines (the base peak). In reality, what is happening is that the abundance of the ion with $m/z$ 30 is decreasing. Therefore, the intensity of the $M^\ast$ peak relative to the base peak (at $m/z$ 30) in Figure 6-65 is greater than the intensity of the $M^\ast$ peak relative to the base peak (also at $m/z$ 30) in Figure 6-64. A determination of the percent abundance of the $M^\ast$ in all three spectra reveals that it is about the same (this numerical determination is not possible from the data provided in these three figures). It is important to remember that the physical dimensions of the mass spectral display are fixed. The y-axis shows the intensity of all the peaks relative to that for the most abundant ion (the base peak) in the mass spectrum. The separation of peaks along the x-axis is determined by the number of $m/z$ units required to show all the peaks within a selected display range.

The mass spectra of all five of these amines exhibit a peak at $m/z$ 30 representing a characteristic ion formed by aliphatic amines, just as the ion with $m/z$ 31 is a characteristic ion for aliphatic alcohols. Both of these ions are EE ions and both conform to the Nitrogen Rule in that the EE ion with $m/z$ 30 (an even number) has an odd number of N atoms ($H_2C=N\text{H}_2$) and the EE ion with $m/z$ 31 (an odd number) does not contain an odd number of N atoms ($H_2C=O\text{H}$). These important peaks will not be observed in a mass
spectrum unless the data acquisition starts at a sufficiently low m/z value. It is common practice to start data acquisition at m/z 35 to avoid ions due to air (m/z 28 and m/z 32) and water (m/z 18); therefore, special attention is required in performing mass spectral analyses of amines and alcohols. Compare the mass spectrum of 1-hexylamine where the acquisition began at m/z 35 (Figure 6-63) and that of the same compound where the data acquisition began at m/z 15 (Figure 6-64).

Returning to the comparison of the mass spectra of aliphatic alcohols and amines, another important difference between the oxonium ions formed by aliphatic alcohols and the immonium ions formed by aliphatic amines is their abundance. The primary immonium ion formed by an initial fragmentation of the M* of amines is far more abundant than the primary oxonium ion formed by the initial fragmentation of an aliphatic alcohol M*.

The mass spectrum of 1-hexylamine (Figure 6-64) is dominated by a single peak at m/z 30. There is a peak for the M* at m/z 101 (consistent with the Nitrogen Rule); however, it is of very low intensity. There is also a low intensity peak at m/z 100, which represents the loss of a hydrogen radical from the M*. The most probable positions for the charge and radical sites in an amine will be on the nitrogen atom as shown in Scheme 6-73, due the loss of an electron from the nonbonding orbital of nitrogen. The ion of m/z 30 is formed by homolytic cleavage of the bond between C-1 and C-2 (the α carbon) as illustrated in Scheme 6-73. The mass spectra of all aliphatic amines in which the –NH₂ group is located on a terminal carbon have the same general appearance: a base peak at m/z 30 (representing a primary immonium ion formed by α cleavage) and a barely discernible M* peak. When data are acquired using rapid scanning in GC/MS to accommodate narrow chromatographic peaks, the M* peak may not be observed due to poor ion counting statistics in the detection system.

The ion with m/z 30 is observed in the mass spectra of all aliphatic amines (1°, 2°, and 3°) and regardless of the position of the –NH₂ group on the chain. Ions with m/z 30 are not only formed by initial fragmentation of the M*, but also by secondary fragmentation of EE ions of higher m/z values produced by initial fragmentation of the M*, as described in detail in some of the following examples.

Aliphatic amines also undergo β cleavage (cleavage of the bond between C-2 and C-3) to produce ions with m/z 44 as illustrated in Scheme 6-74. This will be illustrated in more detail in the discussion of the fragmentation of dipropylamine and triethylamine. Like the ion with m/z 30, the ion with m/z 44 also can result from the secondary fragmentations of EE ions formed by an initial β cleavage.

![Figure 6-63. Mass spectrum of 1-hexylamine; acquisition started at m/z 35.](image-url)
Figure 6-64. EI mass spectrum of 1-hexylamine.

Figure 6-65. EI mass spectrum of dipropylamine.

Figure 6-66. EI mass spectrum of triethylamine.
Although β cleavage is an important factor in the explanation of the fragmentation of aliphatic amines, it is not the only source of ions with m/z 44. Peter Derrick and associates [41] used deuterium labeling to show that aliphatic amines with γ hydrogens on nonterminal carbon atoms form ions with m/z 44 through what Fred McLafferty refers to as a "pseudo α cleavage" involving a multistep hydrogen and skeletal rearrangement to produce an α-methyl-alkylamine, which then undergoes α cleavage to produce the methyl-substituted immonium ion (H₃C–CH=NH₂⁺) as illustrated in Scheme 6-75.
The ion with \( m/z \) 44 probably results both from the rearrangement (Scheme 6-75) and from \( \beta \) cleavage (Scheme 6-74). Examination of the mass spectra of a homologous series of aliphatic amines with the \( –NH_2 \) group located on a terminal carbon reveals that the relative intensity of the peak at \( m/z \) 44 increases with increasing chain length.

The mass spectrum (Figure 6-65) of the secondary aliphatic amine, dipropylamine, appears to be much more complex than that of 1-hexylamine. Initial fragmentation of the \( M^+ \) of dipropylamine via \( \alpha \) cleavage results in formation of the ion with \( m/z \) 72, a propyl-substituted immonium ion, and the loss of an ethyl radical (Scheme 6-76). Some of these EE ions of \( m/z \) 72, with the charge still on the N atom, will undergo a hydride-shift rearrangement fragmentation to produce the primary immonium ion with \( m/z \) 30 (Scheme 6-77).

Scheme 6-76

Some dipropylamine molecular ions will undergo \( \beta \) cleavage to produce ions with \( m/z \) 86 through the loss of a methyl radical (a methyl group in a special location) as shown in Scheme 6-78. Some of the ions with \( m/z \) 86 will then undergo a hydride-shift rearrangement to produce the ion with \( m/z \) 44 (Scheme 6-79).

Scheme 6-77

Scheme 6-78

Scheme 6-79
Examination of the starting ions in Schemes 6-75 (m/z 72) and 6-77 (m/z 86) reveals a strong structural similarity. Both ions have a propyl moiety attached to the nitrogen atom that carries the charge. Just as this propyl moiety can initiate a hydride-shift rearrangement fragmentation of the primary α-cleavage product (Scheme 6-77), it can do the same in the primary β-cleavage product as shown in Scheme 6-79. In both cases, a molecule of propene is expelled to produce a primary immonium ion.

Another interesting behavior of the M⁺⁺ ion of dipropylamine is the fragmentation of the ions represented by the peaks at m/z 58 and m/z 56 in Figure 6-65. This involves the loss of a hydrogen radical through a five-membered ring transition to form a propyl-substituted four-membered ring immonium ion (m/z 100) as shown in Scheme 6-80. Many of these ions lose a molecule of propene through a hydride-shift rearrangement forming the ion with m/z 58; some of these ions then lose a molecule of hydrogen (H₂) to form the ion with m/z 56.

Scheme 6-80

Just as the secondary amine required a two-step process to form the primary immonium ion with m/z 30, as illustrated through Schemes 6-76 and 6-77 in the explanation of the fragmentation of dipropylamine, the formation of this primary immonium ion with m/z 30 in the mass spectrum of a tertiary amine requires a three-step process as illustrated in Scheme 6-81. Not all of the initially formed ions are converted to the primary immonium ion; in fact, the more steps in the conversion process, the lower the abundance of the primary immonium ion formed as seen by the intensity of the peak at m/z 30 in the mass spectrum of the primary amine relative to the mass spectrum of the tertiary amine.

Scheme 6-81

The M⁺⁺ of triethylamine first undergoes α cleavage resulting in the loss of a methyl radical to produce the EE ion with m/z 86 as shown in Scheme 6-81. The base peak at m/z 86 in the mass spectrum of triethylamine (Figure 6-66) represents the only α-cleavage EE product ion regardless of which ethyl moiety loses a methyl radical. Some of the resulting EE ions with m/z 86 then undergo a hydride-shift rearrangement fragmentation to lose a molecule of ethene to produce the EE ion with m/z 58; some of these ions will also undergo a hydride-shift rearrangement to produce the primary immonium ion with m/z 30.
Some molecular ions of triethylamine will undergo β cleavage to produce an EE disubstituted immonium ion with m/z 100 through the loss of a hydrogen radical as illustrated in Scheme 6-82. The fact that there are eight other β hydrogens as well as six α hydrogens that could be expelled as a radical explains why the peak for [M – 1]+ has such a high intensity relative to that of the M+ peak in Figure 6-66 (compare with that in Figure 6-65). Most of the disubstituted immonium ions will undergo two iterations of hydride-shift rearrangements, each resulting in the loss of a molecule of ethene to finally form the ion with m/z 44 as shown in Scheme 6-82.

Another interesting observation in the mass spectra of aliphatic amines is the consistent presence of an [M – 1]+ peak. The ion represented by this peak was explained for the spectrum of triethylamine, but what about the [M – 1]+ peak in the mass spectra of dipropylamine (Figure 6-65), 1-hexylamine (Figure 6-64), 2-hexylamine (Figure 6-67), and 3-hexylamine (Figure 6-68)? The source of the [M – 1]+ ion in all of these mass spectra is the loss of a hydrogen radical through α cleavage from the carbon atom bonded to the nitrogen atom. The hydrogens attached to the carbon bonded to the nitrogen are in the α-position. The tendency of the radical site on a nitrogen atom to pair with another electron is so great that even though the hydrogen radical is not very stable, there will be some hydrogen loss through α cleavage.

Comparison of the mass spectra of the three primary amines, 1-, 2-, and 3-hexylamine, reveals how the position of the –NH₂ group on the carbon chain can cause significant differences in the appearance of the mass spectrum. The mass spectrum of 1-hexylamine was discussed earlier in this chapter. Its mass spectrum is dominated by the α-cleavage product with m/z 30 (Scheme 6-73 and Figure 6-64).

Figure 6-67. EI mass spectrum of 2-hexylamine.
Figure 6-68. EI mass spectrum of 3-hexylamine showing the structure of various ions and the origin of the ions represented by selected peaks.

The mass spectrum of 2-hexylamine (Figure 6-67) is dominated by a single peak at \( m/z \) 44. This mass spectrum exhibits the characteristic peaks of a diminishingly small \( M^+ \) peak followed by an \( [M - 1]^+ \) peak and a peak at \( m/z \) 30. The \( M^+ \) of 2-hexylamine will undergo fragmentation along three different pathways of \( \alpha \) cleavage as shown in Scheme 6-83. Most of the ions with \( m/z \) 100 will undergo a hydride-shift rearrangement to produce ions of \( m/z \) 44 analogous to the mechanism shown in Scheme 6-84 for the formation of the ion with \( m/z \) 44 from the ion with \( m/z \) 86. The same mechanism is employed by some of the EE ions with \( m/z \) 86 in the formation of ions with \( m/z \) 30 (Scheme 6-84). The resonance structures of the EE ions with \( m/z \) 100 (Scheme 6-83) and \( m/z \) 86 (Scheme 6-84) are keys to understanding these hydride-shift rearrangements that result in the primary immonium ions.

\( \beta \) cleavage (not shown) of some of the 2-hexylamine molecular ions will result in \( [M - 1]^+ \) ions due to the loss of a hydrogen radical from C-1 and other ions with \( m/z \) 58 having a methyl-substituted cyclic immonium structure. This latter ion, like the methyl-substituted primary immonium ion, cannot undergo further fragmentation. The \( [M - 1]^+ \) ion can undergo a hydride-shift rearrangement to produce an ion with \( m/z \) 58 in a manner analogous to that of the \( \alpha \)-cleavage product that produced the ion with \( m/z \) 44 from the \( [M - 1]^+ \) ion as shown in Scheme 6-82. Mechanisms for these fragmentations are purposefully omitted to challenge the reader, who may wish to retreat to review appropriate schemes described on the last few pages.

Molecular ions of 3-hexylamine can lose hydrogen, ethyl, or propyl radicals by \( \alpha \) cleavage and methyl or ethyl radicals by \( \beta \) cleavage. Some of the resulting EE ions will then undergo hydride-shift rearrangements as summarized in Figure 6-68. The mechanisms associated with the primary fragmentation of molecular ions of 3-hexylamine and subsequent secondary fragmentations of the resulting EE ions have been purposefully omitted to challenge the reader’s grasp of the subject, as was the case with the secondary fragmentations associated with \( \beta \)-cleavage products during interpretation of the mass spectrum of 2-hexylamine.
Not seen in many of the mass spectra of aliphatic amines shown in Figures 6-63 through 6-70, there is sometimes a peak that represents a distonic OE ion formed by the loss of a molecule of ammonia (NH₃) from the M⁺. The mechanism for this rearrangement is shown in Scheme 6-85. The peak for [M – NH₃]⁺⁺ is clearly visible in the mass spectrum of 3-hexylamine (Figure 6-68).
There is one exception to the statement, “The mass spectra of all aliphatic amines exhibit a peak at \( m/z \) 30.” This exception is when the \( –\text{NH}_2 \) group is on C-2. The methyl radical in the form of C-1 is lost, and there are no \( \beta \) hydrogens present to participate in a hydride-shift rearrangement. The smallest ion that can be formed from such a structure has an \( m/z \) value of 44. The ion with \( m/z \) 44 results from an \( \alpha \)-cleavage process that forms the methyl-substituted immonium ion in which the methyl substitution is C-1. A good example of this phenomenon is seen in the mass spectrum of amphetamine (Figure 6-69).

**B. Aromatic Compounds Containing Atoms of Nitrogen**

There are two types of aromatic amines. There are aromatic amines where an amino group is attached to an aromatic ring (aniline) and there are aromatic compounds with one or more nitrogen atoms in the ring (pyridine). Like the mass spectra of all aromatic compounds, these are characterized by intense peaks for the \( \text{M}^{+*} \), and not much fragmentation.

Molecular ions of compounds with an amine group attached to an aromatic ring, like aniline (Figure 6-70), behave somewhat like those of the corresponding aromatic alcohols. The mass spectra of both types of these compounds have intense \( \text{M}^{+*} \) peaks

![Figure 6-69. EI mass spectrum of amphetamine.](image-url)

![Figure 6-70. EI mass spectrum of aniline.](image-url)
reflecting the high resonance stability of the molecular ion. The molecular ions of aromatic alcohols do not lose water and those of aromatic amines do not lose ammonia. Just as the M⁺ of phenol loses CO, the M⁺ of aniline loses HCN. This loss of HCN from the M⁺ of aniline results in an OE ion of cyclopentadiene, which has an m/z value of 66. Some of these ions will then lose a hydrogen radical to form the ion with m/z 65. This loss of HCN is not observed in the mass spectra of ring-substituted anilines. The peak representing the [M – 1]⁺ ion in the mass spectrum of aniline is formed by the loss of a hydrogen radical from the amino group [42]. When there are alkyl substituents on the ring of aniline, the M⁺ behaves as if the charge and radical sites were on the ring. Benzylic cleavage occurs, which results in the formation of an amino-tropylium ion.

When a substituent is on the nitrogen atom of an aniline analog, the M⁺ behaves as if the charge and radical sites were on the nitrogen atom. However, the mass spectra of o-, m-, and p-methylaniline and N-methylaniline do not allow for differentiation between N-substituted and ring-substituted compounds. This is not true for dimethyl-substituted anilines in which the substituents are both on the ring, one on the ring and one on the nitrogen, or both on the nitrogen. Examine the three mass spectra in Figures 6-71, 6-72, and 6-73. It is also interesting to note that the ortho effect is not observed for haloanilines, 2,6-dihaloanilines, hydroxyaniline, or amino aniline; however, a strong ortho effect is observed in the mass spectrum of 2-aminobenzoic acid. This lack of the ortho effect in the mass spectra of certain anilines, which have analogous structures to phenols whose mass spectra do exhibit the ortho effect, is a good example of the necessity to look at appropriate spectra before jumping to a conclusion of similar behavior.

Once there is an alkyl group on the nitrogen that can undergo a hydride-shift rearrangement fragmentation of a substituted immonium ion with the loss of an alkene, differentiation between ring-substitution and N-substitution is possible. An example is the mass spectrum of N,N-diethylaniline (Figure 6-74). In addition to the M⁺ peak at m/z 149, there is an [M – CH₃]⁺ peak at m/z 134 due to the loss of a methyl radical from a M⁺ with the charge and radical sites on the nitrogen atom. Some of these EE ions with m/z 134 will then undergo a hydride-shift rearrangement fragmentation to lose a molecule of ethane, as shown in Scheme 6-86, to produce the EE ion with m/z 106, which still contains the single atom of nitrogen.

Figure 6-71. El mass spectrum of 2,3-dimethylaniline.
Figure 6-72. El mass spectrum of N,N-dimethylaniline.

Figure 6-73. El mass spectrum of 3-methyl-N-methylaniline.

Figure 6-74. El mass spectrum of N,N-diethylaniline.
The peak at m/z 120 in the mass spectrum of N,N-diethylaniline (Figure 6-74) illustrates that fragmentation can also be initiated with the charge and radical sites associated with the ring (also shown in Scheme 6-86).

Like the mass spectrum of aniline, the mass spectrum of pyridine has the M* peak as the base peak and exhibits a strong [M – HCN]* peak. There can be no substituents on the nitrogen in pyridine compounds, only on the carbon atoms in the ring; there are three regioisomers of a substituted pyridine. The mass spectra of three methyl isomers are almost identical and cannot be differentiated; however, the spectra of the three ethyl isomers are distinguishable. The spectrum of 2-ethylpyridine shows no peak at m/z 92 ([M – CH₃]* or [M – 15]*). The base peak in the mass spectrum of 3-ethylpyridine is at m/z 92. The peak at m/z 92 in the mass spectrum of 4-ethylpyridine is about 55% of the intensity of the base peak, which is the M* peak at m/z 107. All three spectra exhibit a weak [M – HCN]* peak and a strong [M – 1]* peak, which is the base peak in the mass spectrum of 2-ethylpyridine. (No spectra of the methyl- or the ethyl-substituted pyridines are shown.)

The mass spectra of the three regioisomers of butylpyridine exhibit significant differences (Figures 6-75, 6-76, and 6-77). All three spectra exhibit an OE ion peak at m/z 93, which represents the product of a γ-hydrogen-shift rearrangement (McLafferty rearrangement) resulting in a β cleavage with the loss of a molecule of propene (analogous to Scheme 6-29). This reaction is common for the M* of aromatic compounds where there are γ hydrogens associated with a nonterminal carbon atom. The intensity of the M* peak is almost nonexistent in the mass spectrum of the 2-butyl isomer, but it is >70% of the intensity of the base peak in the mass spectrum of the 3-butyl isomer; the M* peak has an intensity of ~30% of the base peak in the mass spectrum of the 4-butyl isomer. Of the mass spectra compared here, that of the 3-butyl isomer (Figure 6-76) shows the most intense peak (m/z 92) representing loss of a propyl radical via benzylic cleavage. All three spectra have peaks representing [M – 15]* and [M – 29]* ions. Another challenge for the reader is to propose mechanisms to explain the formation of these ions.
**Figure 6-75.** El mass spectrum of 2-butylpyridine.

**Figure 6-76.** El mass spectrum of 3-butylpyridine.

**Figure 6-77.** El mass spectrum of 4-butylpyridine.
The mass spectra of substituted pyridines and other aromatic compounds that contain two and three atoms of nitrogen in the ring often reveal much more information about the position of substitution than is forthcoming from the mass spectra of alkylbenzenes. Any time a class of compounds is to be analyzed, it is a good idea to look for examples of the mass spectra of these compounds in a mass spectral database like the NIST/EPA/NIH (NIST05) Database. Compare various spectra in a class such as those provided in Figures 6-75, 6-76, and 6-77.

C. Heterocyclic Nitrogen-Containing Compounds

There are saturated heterocyclic compounds that contain nitrogen atoms. The molecular ions of these compounds behave much like those of cyclic alkyl ethers. Compare the spectra in Figure 6-40 (tetrathydropyran) and Figure 6-78 (piperidine). The mass spectra of both compounds exhibit an intense M⁺ peak because α cleavage results only in the opening of the ring. There is a strong [M − 1]⁺ peak because α cleavage can also result in the loss of a hydrogen radical. To understand the origins of the other peaks in the mass spectrum of piperidine, look at Scheme 6-48 and draw the necessary parallels.

Many compounds that contain atoms of nitrogen are too nonvolatile or thermally labile for analysis by EI. These compounds, such as dimethyltryptamine, require derivatization. Because of rapid data acquisition rates used in GC/MS, a detectable molecular ion peak may not be observed in the mass spectra of nitrogen-containing compounds. Such compounds may require analysis by CI or by EI following derivatization or both to determine their nominal mass.

D. Nitro Compounds

Nitro compounds are unusual in that the nitrogen atom has a valence state greater than three. As would be expected, some the molecular ions of these compounds will fragment with the loss of the −NO₂ group. However, there are also peaks that represent the loss of oxygen and the loss of NO from other molecular ions; see peaks at m/z 107 and m/z 93, respectively, in the mass spectrum of nitrobenzene (Figure 6-79). The mechanisms for these losses are shown in Scheme 6-87. It is because of the high valence state of nitrogen that the loss of the −NO₂ group results in an EE-fragment ion. The NO₂ loss (even though NO₂ has an even mass and contains an odd number of nitrogen atoms) is not the loss of a molecule, but the loss of a radical. NO₂ is a radical, two of which exist as the molecule N₂O₄. In the mass spectra of both aromatic- and aliphatic-nitro compounds, the base peak is the [M − NO₂]⁺ peak.
When a mass spectrum is examined, it is always prudent to be mindful of the Nitrogen Rule. Many of the mass spectra of compounds containing a single atom of nitrogen as presented here exhibit the classic Nitrogen Rule spectrum with a $M^+$ peak at an odd $m/z$ value and several peaks at even $m/z$ values representing EE ions containing the single nitrogen atom. However, it must be remembered that if an EE-fragment ion is formed that does not contain the nitrogen atom, its $m/z$ value will be odd. Also be heedful of the fact that molecular ions containing an even number of nitrogen atoms (2, 4, 6, etc.) can fragment to form EE ions containing an odd number of nitrogen atoms, which will have an even $m/z$ value.

Earlier in this chapter, it was pointed out a peak at $m/z$ 58 indicated the presence of an OE ion that could well be the result of a rearrangement fragmentation of an aliphatic ketone. However, a peak at $m/z$ 58 also could possibly represent an EE fragment ion containing an odd number of nitrogen atoms.
5. Multiple Heteroatoms or Heteroatoms and a Double Bond

Throughout this chapter, the emphasis has been placed on whether the fragmentation is charge-site or radical-site-driven and on designating the probable sites of the charge and odd electron in the $M^+$. In compounds that contain a single functional group like ketones, aromatic hydrocarbons, chloroalkanes, etc., designating these sites is easy. When dealing with analytes that have more than one functional group that is readily ionizable, the situation becomes more complex. During the discussion of ketones, it was clear from an examination of the mass spectrum of benzyl methyl ketone (Figure 6-48) that there were peaks that represented ions formed by a fragmentation initiated with the charge and radical sites in the $M^+$ on the carbonyl oxygen (peak at $m/z$ 43) and those with the charge and radical sites in the $M^+$ on the aromatic ring ($m/z$ 92) as shown in Scheme 6-59. Ions formed by the charge and radical sites being associated with either the aromatic ring or the carbonyl oxygen in the $M^+$ were also pointed out as being represented in the mass spectrum of dibenzyl ketone (Figure 6-49 and Schemes 6-60 and 6-61).

There are some examiners of mass spectra who say that there can be only one site from which an electron can be lost in the formation of the $M^+$ and that site is the site having the lowest ionization potential. Even when there are heteroatoms, double bonds, and areas of resonance due to double-bond conjugation, any type of electron can be lost to form a molecular ion. This includes the possibility of initially losing a sigma-bond electron (unlikely, but possible) in the formation of a $M^+$. Thus, the molecular ion peak is likely to represent a distribution of different molecular ions, which are distinguished by the location of the charge and radical sites.

Ethanol amine is a simple compound that is both a primary alcohol and a primary amine. Examination of the mass spectrum (Figure 6-80) of this compound clearly illustrates that some molecular ions are formed with the charge and radical sites on the oxygen of the alcohol and other molecular ions are formed with the charge and radical sites on the nitrogen. The mass spectrum of ethanol amine has the base peak at $m/z$ 30, which represents the primary immonium ion $\text{H}_2\text{C}=\text{N}^+\text{H}_2$. The peak at $m/z$ 31 is far too intense relative to the peak at $m/z$ 30 to represent just the $^{13}\text{C}$-isotopic contribution of the immonium ion. This peak at $m/z$ 31 also (and primarily) represents the primary oxonium ion ($\text{H}_2\text{C}=\text{O}^+\text{H}$) characteristic of aliphatic alcohols.

There are many other examples of the role of multiple possible sites for the charge and odd electron. An area in which this phenomenon is especially important is the mass spectral analysis of illicit drugs. R. Martin Smith, in his book *Understanding Mass Spectra:*
A Basic Approach, 2nd ed. (John Wiley & Sons, Inc., New York, 2004), uses the mass spectra of illicit drugs to show that multiple fragmentation pathways exist and that not all are initiated by the charge and radical sites being on the same atom.

Another interesting aspect of the Smith book is that a significant number of the mass spectral displays have amplified peak intensities for clarity. Many of the mass spectra presented are of aliphatic amines with oxygen- and aromatic-containing moieties. Such mass spectra often show no peak for the M⁺, but are dominated by a single peak representing a product of an initial homolytic cleavage promoted by the charge and radical sites being on the nitrogen atom. This use of expanded-intensity spectra is an important reminder that a significant amount of important information may be present in the low-intensity peaks that are often dismissed as being part of the background (the grass).

6. Trimethylsilyl Derivative

Derivatization can be important in EI mass spectrometry. As already mentioned, fatty acids are usually analyzed by GC/MS as the methyl ester derivative due to the high polarity of the free acids. Derivatives are prepared to make the analyte more volatile (by chemical modification to replace active hydrogens that would otherwise participate in hydrogen bonding) or, as in the case of aliphatic amines and alcohols, to aid in a determination of the analyte’s nominal mass. Besides methylation agents, other useful derivatizing reagents are those that form trimethylsilyl (TMS) derivatives [43, 44]. The TMS reagents shown in Figure 6-81 react with compounds that have reactive hydrogen atoms, like all alcohols (including phenols), organic acids (R–CO₂H), all 1° and 2° amines, and some amides. Other alkyl-silicon compounds also can be used as derivatization reagents.

As seen in Figure 6-82, these silylating reagents do not react with ketones or esters. One way of quickly determining the classification of an unknown analyte is to analyze some of it by GC/MS before and after reacting it with a silylating reagent. If there is no change in the mass spectral data or retention time of the chromatographic peaks, the analyte is probably a ketone or an ester. New retention times and differences in the mass spectra mean that the analyte could be a compound with reactive hydrogens.

![Figure 6-81. Four common silylating reagents.](image-url)
The mass spectra of TMS derivatives usually do not exhibit a $M^+$ peak. The highest $m/z$ value peak observed in the mass spectrum may be the $[M - CH_3]^+$ ($[M - 15]^+$) peak. There will always be a peak at $m/z$ 73, which represents the $(CH_3)_3Si^+$ ion. If the derivatized moiety is on a nonterminal carbon atom in an aliphatic structure, there will be ions representing secondary carbenium ions with the $-Si(CH_3)_3$ group attached. Formation of the TMS derivative of a primary aliphatic alcohol and subsequent fragmentation of the $M^+$ to form the $[M - 15]^+$ ion is illustrated in Scheme 6-88. The silyloxonium, such as the $[M - 15]^+$ ion, are very stable and unless there is some overriding factor such as the possibility of secondary carbenium ion formation, there always will be a discernible $[M - 15]^+$ peak in the mass spectrum. The analogous silylimmonium ions formed by fragmentation of the $M^+$ of the TMS derivative of amines are also quite stable and is likely to be represented by discernible peaks in the mass spectrum.

Scheme 6-88
As can be seen in the mass spectrum of the TMS ether formed from \textit{n}-decanol, there are other peaks worth noting (Figure 6-83). The peak at \(m/z\) 75 is found in the mass spectra of TMS derivatives of all aliphatic alcohols. Peaks at \(m/z\) 89 and 103 are found in the mass spectra of TMS derivatives of all primary alcohols. The structures of these three ions are:

\[
\begin{align*}
H^+\text{O} &= \text{Si(CH}_3\text{)}_3 & H_2\text{C} &= \text{SiO}^+(\text{CH}_3)_2 & H_2\text{C} &= \text{O}^+\text{Si(CH}_3\text{)}_3
\end{align*}
\]

\(m/z\) 75 \hspace{2cm} m/z 89 \hspace{2cm} m/z 103

The peaks at \(m/z\) 89 and \(m/z\) 103 will shift to higher \(m/z\) values in the mass spectra of TMS derivatives of \(2^\circ\) and \(3^\circ\) alcohols consistent with the mass of the substituents on the alcohol carbon. For example, the peak at \(m/z\) 89 in Figure 6-83 will shift to \(m/z\) 145 in the mass spectrum of the TMS derivative of 5-tetradecanol (a \(2^\circ\) alcohol with an \(n\)-butyl group on the alcohol carbon); similarly, the peak at \(m/z\) 103 in Figure 6-83 will shift to \(m/z\) 159. The peaks at \(m/z\) 73 and \(m/z\) 75 in Figure 6-83 would not shift to higher \(m/z\) values in the mass spectrum of the TMS derivative of 5-tetradecanol.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure683}
\caption{EI mass spectrum of the TMS derivative of decanol.}
\end{figure}

Examination of the mass spectrum of the TMS derivative of 1,10-decandiol (Figure 6-84) reveals an interesting, and what turns out to be a characteristic, pair of peaks at \(m/z\) 147 and \(m/z\) 149 [45]. Peaks at these \(m/z\) values are common in the mass spectra of TMS derivatives of \textit{vic}-diols; i.e., compounds in which there are hydroxyl groups on adjacent carbon atoms. Even though the two –OH groups are well separated in this particular example, the molecular ions are in the gas phase and the structure is free to fold so that the two trimethylsilyl ether groups can approach one another with sufficient proximity to form these diagnostic ions indicating the presence of at least two silyl groups in the analyte molecule [45]. The formation of these two diagnostic ions from their precursors is shown in Scheme 6-89. The presence of these diagnostic peaks in a mass spectrum only indicates that more than one group was derivatized; it does not indicate the total number of groups that were derivatized.
The formation of the TMS derivative results in a mass change of 72 Da. The trimethylsilyl group has a mass of 73 Da, but it replaces an active hydrogen atom in the analyte molecule. Therefore, the derivatization process results in a net change of 72 Da between the mass of the underivatized and mass of the derivatized analyte.

The number of sites derivatized in a given analyte molecule can be determined by analyzing derivatives prepared in parallel using unlabeled and stable isotope-labeled analogs of the derivatization reagent [45]. A commercially available stable isotope-labeled analog of the N,O-bis(trimethylsilyl)acetamide reagent has eighteen deuterium atoms, three on each of the three methyl groups in each of the two TMS groups, and is designated as \( d_{18} \)-BSA. The mass spectrum in Figure 6-85 is that of an unknown derivatized with \( d_{0} \)-BSA; i.e., the unlabeled reagent containing no deuterium atoms. The mass spectrum in Figure 6-86 is that of another aliquot of the same unknown after derivatization using \( d_{18} \)-BSA.

The upper left-hand structure in Figure 6-81 is that of BSA. When BSA reacts with an analyte with one active hydrogen, only one TMS ((CH₃)₃Si) group is added.
The net change in mass between the derivatized and the underivatized analyte is again 72 Da when the $-\text{Si(CH}_3\text{)}_3$ group replaces the active hydrogen by the use of $d_0$-BSA. However, when $d_{18}$-BSA is used, each hydrogen atom (mass 1 Da) in the $-\text{Si(CH}_3\text{)}_3$ group is replaced with a deuterium atom (D or $^2\text{H}$, mass 2 Da), resulting in a net increase between the mass of the underivatized analyte and that of the derivatized form of 81 Da: Si (28 Da) plus that of three $-\text{CD}_3$ groups (18 Da each or 54 Da) minus that of the replaced active hydrogen atom ($-1$ Da) in the analyte molecule ($28 + 54 - 1 = 81$). Note that the net shift in mass of the derivatized analyte molecule prepared using $d_{18}$-BSA is 9 Da more for each derivatizable functional group than that effected using $d_0$-BSA ($81 - 72 = 9$).

Examination of the mass spectrum (Figure 6-85) of the $d_0$-BSA-derivatized unknown shows that there are peaks at m/z 271 (which can be presumed to be the peak representing $[M - \text{CH}_3]^+$) and at m/z 73, 75, 89, and 103, indicating that the unknown is a primary alcohol. What is not known is how many $-\text{Si(CH}_3\text{)}_3$ groups were added.

In the spectrum (Figure 6-86) of the $d_{18}$-BSA-derivatized unknown, it appears that the $\text{(CH}_3\text{)}_3\text{Si}^-$ EE ion with m/z 73 in the spectrum of the unlabeled derivative prepared with $d_0$-BSA has shifted to m/z 82. The other diagnostic ions represented in the mass spectrum of a primary-alcohol TMS derivative have also shifted to new m/z values as follows:
Representative Fragmentations (Spectra) of Classes of Compounds

\[
\begin{align*}
H^+O &= Si(CH_3)_2 & H_2C &= SiO^+(CH_3)_2 & H_2C &= O^+Si(CH_3)_3 \\
\text{m/z} &= 75 & \text{m/z} &= 89 & \text{m/z} &= 103 \\
H^+O &= Si(CD_3)_2 & H_2C &= SiO^+(CD_3)_2 & H_2C &= O^+Si(CH_3)_3 \\
\text{m/z} &= 81 & \text{m/z} &= 95 & \text{m/z} &= 112 \\
\end{align*}
\]

The peak at the highest m/z value in the mass spectrum (Figure 6-86) of the derivative prepared with the \( d_{18}\)-BSA is at m/z 277. It is presumed that this peak at m/z 277 represents \([M - CD_3]^+\) (therefore \(M^+ = 296 = 277 + 18\)) just as it is presumed that the peak at m/z 271 in the mass spectrum (Figure 6-85) of the derivative prepared with \( d_0\)-BSA represents \([M - CH_3]^+\) (therefore \(M^+ = 286 = 271 + 15\)). This means that the nominal mass of the derivative formed with the labeled reagent has a nominal mass 9 Da greater than the derivative formed with the unlabeled reagent. This difference in the nominal mass of the labeled and unlabeled derivative is the same as the difference in mass of a single –Si(CD_3)_3 unit and a single –Si(CH_3)_3 unit. This difference of 9 Da indicates that only a single active hydrogen existed and that only a single TMS group exists in the derivatized compound. If two TMS groups had been incorporated into the derivative, the difference in the nominal mass of the labeled and unlabeled derivative would be 18 Da; if three TMS groups had been incorporated, the mass difference would be 27 Da, and so forth.

What feature in the mass spectrum reveals the number of sites that have been derivatized? Because there is a shift of 6 m/z units \((277 - 271 = 6)\) for a high-mass peak in the mass spectra of the unknown after parallel treatments with \( d_0\) and \( d_{18}\)-BSA, it can be inferred that the unknown contains only one derivatizable functional group. This inference is based on the fact that the peak at m/z 271 represents a dimethylsilyloxy ion (similar in structure to the ions with m/z 89 as shown above and having the structure shown in the second line of Scheme 6-88); the ion with m/z 271 is shifted by only 6 Da in the mass spectrum of the labeled derivative because the fragment ion contains only two –CD_3 \((d_{18}-CH_3)\) groups. If the unknown had contained more than one derivatizable functional group, this high-mass dimethylsilyloxy ion, corresponding to \([M - CH_3]^+\), would contain another –Si(CD_3)_3 group for each additional functional group. In an example where the unknown had contained more than one derivatizable functional group, the high-mass dimethylsilyloxy ion, corresponding to \([M - CD_3]^+\), would contain one intact –Si(CD_3)_3 group (net shift of 9 Da) in addition to the two CD_3 groups (net shift of 6 Da) comprising the dimethylsilyloxy moiety (analogous to the structure with m/z 95 directly above); in such a case, the peaks representing \([M - CH_3]^+\) and \([M - CD_3]^+\) in the corresponding mass spectra of the unknown after parallel treatments with \( d_0\) and \( d_{18}\)-BSA would be separated by 15 m/z units \((6 + 9 = 15)\).

Based on the information obtained from the peaks at the low end of the m/z scale of the mass spectrum (Figure 6-85) of the \( d_0\)-BSA-derivatized unknown, the analyte is a primary alcohol. Only one –Si(CH_3)_3 group was added. This means that there is only one OH group and it is on a –CH_2 moiety. The nominal mass of the TMS derivative is 286 Da; therefore, the nominal mass of the unknown is 214 Da \((286 - 72 = 214)\). To determine the mass of hydrocarbon backbone, subtract 17 Da for the –OH group and 1 Da for a hydrogen on the terminal carbon atom. The remainder is 196 Da. This number is evenly divisible by 14, which means that the unknown consists of 14 –CH_2– units. Neither the mass spectrum of the deuterated nor of the unlabeled TMS derivative of the unknown showed any peaks that would indicate branch points in the chain or that the –CH_2OH group was at a location other than at the end of the chain. Therefore, there is a good probability that the unknown is tetradecanol:

\[
\begin{array}{c}
\text{OH} \\
\end{array}
\]
Figure 6-87. El mass spectrum of methyl oleate (methyl 9-octadecenoate).

Figure 6-88. El mass spectrum of methyl linoleate (methyl 9,12-octadecdienoate).

Figure 6-89. El mass spectrum of methyl linolenate (methyl 9,12,15-octadecatrienoate).
7. Determining the Location of Double Bonds

The use of derivatization can facilitate a determination of the nominal mass of an analyte when there is no $M^+$ peak in the EI mass spectrum of the underivatized compound. Derivatization also can solve the issue of high polarity, nonvolatility, and thermal lability. Derivatization can be used to determine the position of substitution or sites of unsaturation in aliphatic compounds. However, care must be taken with regard to relying on the results of a derivatization reaction. Derivatizing an analyte is a chemical synthesis and it is necessary to practice this process in order to develop the confidence required for an unambiguous result.

Another use of derivatization is in the determination of the location of double bonds in a molecule. In some cases, interpretation of an EI mass spectrum can reveal the position of functional groups in a molecule; however, recognition of the position of a double bond in a molecule through the use of EI mass spectrometry requires some chemical means of marking the original site of unsaturation. Whereas the mass spectra of unsaturated fatty acid esters differ significantly from those of their saturated analogs, the mass spectra of isomeric unsaturated fatty esters exhibit no distinguishing peaks [34, 35, 46]. Derivatives must be prepared that mark the location of the original double bond and that generate fragmentation to facilitate recognition of its position in the carbon chain [47–52]. McCloskey [35] has reviewed several derivatization procedures that have been used to characterize unsaturated esters by GC/MS.

The mass spectra of esters of unsaturated fatty acids usually exhibit discernible $M^+$ peaks. Some of these molecular ions usually produce an abundant ion resulting from the loss of CH$_3$OH ([$M - 32]^+$) [46] and, in many cases, the important $[M - 31]^+$ ion corresponding to the loss of the methoxy radical. Unfortunately, as can be seen from the three mass spectra in Figures 6-87, 6-88, and 6-89, peaks in the remainder of the mass spectrum provide very little information. None of the peaks in these three mass spectra corresponds to fragmentation at the locus of a double bond in the long carbon chain of an unsaturated methyl ester. The peak at $m/z$ 222 in the spectrum of methyl oleate (Figure 6-87) represents an OE ion formed by the loss of H$_2$CC(O)OCH$_3$ (methyl acetate) which is expelled from the ester moiety [46]. The other obvious OE ion peaks in this mass spectrum are at $m/z$ 180, 166, and 152, none of which would correspond to the charge and radical site existing between C-9 and C-10, which is the site of the double bond. Scheme 6-90 illustrates fragmentation pathways that produce the ions that would be expected regardless of which of the two carbon atoms that originally constituted the double bond carry the charge and radical site. Close examination of the mass spectrum reveals that there are peaks representing the ions with $m/z$ 143 and possibly at $m/z$ 157; however, there is no appreciable ion current evidenced at $m/z$ 99, other than that due to a $^{13}$C-isotope ion of the ion with $m/z$ 98, or at $m/z$ 197. The ion current at $m/z$ 143 could well be due to a rearrangement fragmentation brought about by a hydrogen-shift rearrangement in the $M^+$.

It is also interesting to note that unlike the mass spectra of their saturated analogs, these unsaturated straight-chain methyl esters do not have $m/z$ 74 as the base peak. In some cases, finding the peak at $m/z$ 74 can be difficult because of the significant ion current at other $m/z$ values in that region. It should also be noted that the intensity of the $m/z$ 74 peak decreases as the degree of unsaturation increases.

An explanation for the lack of discrete fragmentation relating to the position of the double bond in the molecule has been postulated by Biemann [4]. Formation of the $M^+$ with the site of electron deficiency at the original location of the double bond (as illustrated in Scheme 6-91) produces species A, which is interconvertible with species B via hydrogen...
and hydride shifts. This interconversion of molecular ion species corresponds to shifts or migration of the original double bond; thus, no discrete fragmentation relating to the original position of the double bond is observed.

Scheme 6-90

Scheme 6-91
The position of a double bond can be “marked” by oxidizing it with OsO₄ to a vicinal diol followed by silylation (with bis-trimethylsilylacetamide, BSA) of the hydroxyl groups to produce a derivative (as shown in Scheme 6-92) suitable for analysis by GC/MS [53]. The mass spectrum (Figure 6-90) of the bis-trimethylsilyloxy derivative of the vicinal diol derived from methyl oleate shows two intense peaks that can be related to the position of the double bond in the original molecule.

Based on the nominal mass of the derivative and the \( m/z \) values of the two ions represented by the intense peaks in Figure 6-90, it can be deduced that the two peaks represent moieties that can be joined to form the intact molecule. Although fragmentation studies with labeled compounds have shown that production of the ion of \( m/z \) 259 involves preliminary migration of a trimethylsilyl moiety to the ester moiety [53], the simple scheme of bond fission in Figure 6-90 is essentially correct. It would be expected that fission of the C-9–C-10 bond would produce two secondary carbenium ions that would be more stable than isobaric primary carbenium ions produced through cleavage resulting from sigma-bond ionization.

If the location of the double bond were not known in the example relating to Figure 6-90, the following logic would result in an answer. One of the two secondary carbenium ions represented by the two peaks at \( m/z \) 215 and \( m/z \) 259 will have the structure \((\mathrm{CH}_3)_3\mathrm{Si}=\mathrm{CH}(\mathrm{CH}_2)_x\mathrm{CO}_2\mathrm{CH}_3\) and the other structure will be \(\mathrm{H}_2\mathrm{C}(\mathrm{CH}_2)_y\mathrm{CH}=\mathrm{OSi}(\mathrm{CH}_3)_3\). Both ions have an \(-\mathrm{OSi}(\mathrm{CH}_3)_3\) moiety [nominal mass of 89 Da (\(16 + 28 + 3(15) = 89\)). Subtract the nominal mass of the \(-\mathrm{OSi}(\mathrm{CH}_3)_3\) moiety from the \( m/z \) value of each of the two secondary carbenium ions and divide by 14 (the mass of a \(-\mathrm{CH}_2–\) unit) to determine the number of methylene groups. In this case, 215 – 89 divided by 14 = 9 (an integer) and 259 – 89 divided by 14 = 12.14 (a fractional number). The integer (9) equals the number of methylene units in the hydrocarbon-containing TMS moiety, \(\mathrm{H}_3\mathrm{C}(\mathrm{CH}_2)_y\mathrm{CH}=\mathrm{OSi}(\mathrm{CH}_3)_3\). The noninteger value (12.14) corresponds to the ester-containing TMS moiety, \((\mathrm{CH}_3)_3\mathrm{Si}=\mathrm{CH}(\mathrm{CH}_2)_x\mathrm{CO}_2\mathrm{CH}_3\); if the mass of the \(-\mathrm{CO}_2\mathrm{CH}_3\) moiety (59 Da) is subtracted along with the mass of the \(-\mathrm{OSi}(\mathrm{CH}_3)_3\) moiety from 259, the resulting value will be one less than the sum of the masses of the internal \(-\mathrm{CH}_2–\) units in the ester-containing TMS moiety; i.e., 259 – (89 + 59) + 1 = 112, which divided by 14 = 8. This means that the double bond is between carbon atoms 9 and 10. This solution was arrived at using the same logic that was used to determine a branch point in a branched alkane as shown earlier in Figure 6-15.

![Figure 6-90. El mass spectrum of methyl 9,10-bis[(trimethylsilyl)oxy]octadecanoate.](image)
The derivatization approach to marking the site of unsaturation can be extended to polyunsaturated fatty acids. The spectra of derivatives of fatty acids having more than two double bonds are much more complex than that shown in Figure 6-90; however, with careful interpretation, the fragmentation pattern can be related to the locations of the double bonds in the original molecule [54, 55].

Similar approaches can be used to determine the positions of cyclopropane [35] or cyclopropene [35, 56] moieties in the main chain. The presence and positions of oxygen functions (hydroxy and keto groups) also can be determined by shifts in mass spectral data obtained from normal-chain fatty acid derivatives [35, 57]. The mass spectra of pyrrolidide [58, 59] and picolinyldimethylsilyl ether [60, 61] derivatives of unsaturated fatty acids also provide a generally reliable means of recognizing the site of the double bond. The use of substituted cyclopropylimines [62, 63], halocarbenes [64], or Diels–Alder adduct formation [65] also have been employed in determining the position and geometry of olefins.

Dimethyl disulfide (DMDS) is an example of a reagent that reacts directly with a double bond as shown in Scheme 6-93. The covalently modified hydrocarbon backbone of the analyte (after reaction with dimethyl disulfide) produces a mass spectrum characterized by a discernible \( M^+ \) peak and fragment ions that can be correlated with the position of the double bond in the original olefin [48, 66–68]. Formation of both prominent fragment ions is shown in Scheme 6-93; homolytic cleavage of the sulfur–carbon bond with charge retention on either sulfur atom is equally probable. From the mass spectrum of the dimethyl disulfide reaction product of 3-dodecene in Figure 6-91, it appears that fragmentation only occurs to produce ions with a single sulfur atom. Although theoretically possible, it appears that there is no cleavage that would result in a secondary carbenium ion that contains both sulfur atoms.

Reaction of olefins with 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene modifies the double bond in a manner similar to that shown in Scheme 6-93. However, in this case, the mass spectrum provides information not only on the position of the double bond in the olefin, but also on the geometry [66].

Double-bond location in fatty acids can also be accomplished by collisionally activated dissociation (CAD) of ions formed during chemical ionization (CI). When using acetonitrile as the CI reagent gas in an internal ionization 3D QIT mass spectrometer, an ion is formed by the reaction of the protonated adduct of acetonitrile with the methyl ester of...
the fatty acid. When this adduct ion is subjected to CAD, fragments related to the location of double bonds are produced [69]. The locations of as many as eight double bonds in a single analyte have been reported [69]. It is also possible to determine whether the analytes are *cis* or *trans* isomers using this technique, which is more of a direct-analysis method than those involving formation of derivatives.

VI. Library Searches and EI Mass Spectral Databases

1. Databases

One of the most powerful aids in the identification of an unknown compound is the comparison of the compound’s mass spectrum with those in a database of mass spectra acquired under the same conditions. There are four large commercially available databases of EI mass spectra: NIST05 (NIST/EPA/NIH Database of 190,825 spectra), Wiley Registry 8th Ed. (399,383 spectra), the Wiley/NIST (combination of the Wiley 8th Ed. and NIST05) (532,573 spectra), and the Palisade Complete Mass Spectral Library (composed of all the spectra in the Wiley Registry 7th Ed., NIST02, and another ~150K spectra for an advertised total of over 600,000 spectra). The NIST and Wiley databases are provided with chemical structures in a format that can be used in various structure search routines. The Palisade Complete Mass Spectral Library has a limited number of structures in a bit-map format that makes them unusable for structure searching.

There are several much smaller commercially available databases of EI mass spectra that are application specific, such as the Wiley Database of Designer Drugs and the Allurred 4th Ed. of the Robert P. Adams database of Essential Oil Compounds. These “boutique” databases, as well as the major ones listed above, are available in multiple
formats to accommodate various proprietary Database Search Engines. Table 6-4 contains a list of the known commercially available mass spectral databases.

There are also some EI databases that can be downloaded without charge. These are usually in the Agilent GC/MS ChemStation format; however, the NIST Mass Spectral (MS) Search Program has a utility that will convert mass spectral databases in the ChemStation format to the NIST MS Search Program’s format. Two of the better known free-downloadable databases are the ones from the American Academy of Forensics Sciences, Toxicology Section (AAFS) “Comprehensive Drug Library”, and The International Association of Forensic Toxicologists (TIAFT) “Derivatives of Drugs”.

The American Academy of Forensics Sciences (AAFS), Toxicology Section and “Comprehensive Drug Library” (http://www.ualberta.ca/~gjones/mslib.htm) of 2200 spectra, including replicates, was last updated in 2004. This database does not include structures, but it does include CAS registry numbers, molecular weights (nominal mass), and elemental compositions. If an NIST structure is present for a given CASrn, that structure will be displayed with that spectrum.

The current TIFF collection contains 205 EI mass spectra, including 122 spectra of TMS derivatives of drugs, provided by Aldo Polettini and 82 examples of various derivatives of relevant doping substances provided by Klaus Müller and Detlef Thieme. The majority of these spectra are of TMS derivatives. This database can be downloaded from (http://www.tiaft.org/main/mslib.html).

Table 6-4. Commercially available databases sold for use with GC/MS data systems.

<table>
<thead>
<tr>
<th>Publisher</th>
<th>Database Name</th>
<th>Number of Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Institute of Standards and Tech.</td>
<td>NIST05</td>
<td>190,825 spectra of 163,198 compounds</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Wiley Registry 8th Ed.</td>
<td>399,383 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Wiley 8N combination of Wiley 8th</td>
<td></td>
</tr>
<tr>
<td>Palisade MS</td>
<td>Ed. and the NIST05</td>
<td>532,573 spectra</td>
</tr>
<tr>
<td>Palisade MS</td>
<td>Palisade Complete</td>
<td>&gt;600,000 spectra</td>
</tr>
<tr>
<td>Allured Publishing</td>
<td>Compounds, 4th Ed.</td>
<td>2205 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Designer Drugs 2005</td>
<td>3437 spectra of 2959 compounds</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Geochemical, Petro Chemical, and Biomarkers</td>
<td>1110 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Volatiles in Food</td>
<td>1620 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Androgens, Estrogens and Other Steroids</td>
<td>2979 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Alexander Yarkov Organic Cpd Data, 4th Ed.</td>
<td>37,055 spectra</td>
</tr>
<tr>
<td>John Wiley &amp; Sons</td>
<td>Data, 4th Ed.</td>
<td>&gt;40,000 spectra</td>
</tr>
<tr>
<td>Agilent Technologies</td>
<td>Stan Pesticide Database</td>
<td>340 spectra</td>
</tr>
<tr>
<td>Agilent Technologies</td>
<td>RTL Pesticide Database</td>
<td>927 spectra</td>
</tr>
<tr>
<td>Agilent Technologies</td>
<td>RTL Hazardous Chemical DB</td>
<td>731 spectra</td>
</tr>
<tr>
<td>Not applicable Pfleger/Maurer/Weber (PMW)</td>
<td>&gt;6300 spectra</td>
<td></td>
</tr>
</tbody>
</table>
2. Library Search Programs

The comparison made between the sample spectrum and the spectra in the database is carried out by a Database Search Engine. The various search engines are the underlying structure of the Library Search Program. Even though the look and feel of various Library Search Programs may be different, the underlying search engine can be the same. The most widely used search engine today is that of the NIST MS Spectral Search Program, developed under the direction of Steve Stein at the Mass Spectrometry Data Center of the National Institute of Standards [70], part of the United States Department Commerce. This search engine is incorporated into the mass spectral application software of Varian, Thermo Electron, Perkin Elmer, Shrader/JEOL, and LECO. Agilent Technologies and Waters Corporation have direct links to the NIST MS Search Program through their software as well as their proprietary search systems. With some manipulation on the part of the user, spectra can be imported into the NIST MS Search Program from the Applied Biosystems/MSD Sciex, Shimadzu, and Bruker Daltonics mass spectral data applications. The Waters proprietary search engine uses the same base INCOS algorithm [71] used as the foundation of the NIST MS Search Program. In addition to their own library search routines Varian, Thermo Electron, Perkin Elmer, Shrader/JEOL, LECO, Agilent Technologies, and Waters Corporation provide the NIST MS Search Program as a standard addition to their software or when the NIST/EPA/NIH Mass Spectral Database is purchased for use with their software.

A copy of the NIST Mass Spectral Search Program along with a database of about 2,400 spectra can be downloaded from http://www.nist.gov/srd/nist1a.htm by clicking on the link “The NIST 05 Demo version may be downloaded here”, which is just under the graphic display of the NIST MS Search Program on this cited Web page. This download includes MS Interpreter and the Lib2NIST program used to create NIST MS Search user databases from GC/MS ChemStation libraries and JACMP and text files.

The search engine used by Shimadzu and Agilent in their proprietary mass spectral software is the Probability Based Matching (PBM) algorithm developed by Fred McLafferty and Bockhoff [72]. The problem with this search engine is that it is dependent on the base peak for an identified spectrum in the database, being at the same $m/z$ value as the base peak of the unknown’s spectrum.

The remainder of the discussion regarding the Library Search of mass spectra will use examples and terminology relating to the NIST Mass Spectral (MS) Search Program.

In order to obtain the best results from a library search, a good-quality spectrum should be submitted for identification. Issues of spectral skewing and background subtraction discussed in Chapter 10 (GC/MS) are very important in preparing the spectrum that will be used in the search. As pointed in Chapter 10 under the discussion of AMDIS (Automated Mass spectral Deconvolution and Identification System), it is best to use spectra that have been deconvoluted by AMDIS for a Library Search because AMDIS addresses both background subtraction and spectral skewing.
Figure 6-92. The NIST MS Search Program Lib Search tab. There are a total of eight visible panes; the Compare pane is not displayed. The vertical and horizontal slider bars can be adjusted according to user preference. The orientation of the bar between the display of the bar-graph spectrum and the text data can be switched from horizontal (the displayed orientation) to vertical.

Figure 6-92 is an example of a typical result obtained when an Identity Search is performed using the NIST MS Search Program. An Identity Search involves a presearch based on a limited number of the most intense peaks in the sample spectrum along with some other features explained in detail in the User’s Manual for the NIST MS Search Program. The presearch is followed by a peak-by-peak comparison between the sample spectrum and a subset of the database determined by the presearch. The peaks are rated according to their $m/z$ values and relative intensities with the peaks at the high end of the $m/z$ scale given the most weight. The best 100 matches (Hits) are listed according to the degree of match in the Hit List.

The Hit List (a list of the database spectra that most closely match the sample spectrum) is found in the lower left section of Figure 6-92. The first column on the left side of this panel is the ranking value, with “1” being the best match. The second column is an up-to-two letter code (first two letters) of the database’s name that contains the Hit. The single letter M and R are reserved for the NIST/EPA/NIH “main library” (mainlib) and
"replicate library" (replib), respectively. Designation of the library corresponding to the Hit is necessary because multiple databases can be searched simultaneously. The next three columns relate to how closely the sample spectrum matches that of the Hit. From the left, the first of these columns has the Match Factor (Match). This is a number between 0 and 999 that indicates how closely the sample spectrum matches the library spectrum of the Hit based on a peak-by-peak comparison of the two spectra. Values greater than 700 are considered to be good matches. However, even very high values (>900) do not guarantee that the sample spectrum and the Hit spectrum were produced by the same compound.

The next value is the Reverse Match Factor (R.Match). This is a match factor that is calculated disregarding any peaks in the sample spectrum that are not in the spectrum of the Hit. The Reverse Match Factor has the same numeric range as the Match Factor. These nonconforming peaks in the sample spectrum are considered to be from a source other than a single analyte. These spurious peaks may be due to background or a substance that coeluted during the chromatographic process.

All library search programs have numeric indicators with respect to how good a match has been achieved; however, reliance on these numeric values should be approached with caution. More reliance should be placed on a visual comparison of the graphical presentation of the two spectra. This visual comparison of the graphical displays may be enhanced by a comparison of the two spectra in a tabular format.

The third value with respect to the quality of the Hit is the Probability (Prob), which is unique to the NIST MS Search Program. This number is expressed as a percentage. This number represents the probability that the sample spectrum and the spectrum of the Hit come from the same compound if a spectrum of the analyte is in the database. This value is dependent on the uniqueness of the spectrum of a particular compound. For example, if the sample spectrum is that of m-xylene and the Match Factor for m-xylene is over 900, the Probability of a match may be low because of the similarity of the mass spectra of the xylene isomers. For example, in the case where the sample spectrum is m-xylene, which is compared (searched) against the NIST05 database, the top five Hits will be the spectra of o-xylene, p-xylene, and ethylbenzene, all of which are nearly the same as the spectrum of m-xylene. On the other hand, if the sample is phenylbutazone, the Probability value will be high because of the uniqueness of this compound's mass spectrum compared to all the mass spectra in the NIST05 database. The degree of uniqueness of a spectrum can be determined by comparing the appearance of a spectrum in the Hit List with other spectra close to it.

Match Factors and Reverse Match Factors are calculated based on individual spectra regardless of how many spectra of a particular compound are found in a search of multiple databases. The Probability is based on the compound, and will be the same for each spectrum of the same compound in a multiple-database search or in a search of a database with multiple spectra for the same compound.

It is very important to remember that chemists (people) identify unknown analytes, not Library Search Programs. This is why much more than just reliance on the numerical values for Match Factors must be used. First, it should be clearly understood that the various commercially available databases do not contain all the mass spectra of all analytes that can pass through a gas chromatograph and/or that can undergo electron ionization in the gas phase. This is why companies like Eastman Chemical maintain their own databases of mass spectra, which may contain mass spectra of tens of thousands of compounds that are not in any commercially available database. It may even be that there are not mass spectra of compounds similar to the specific analyte under investigation in any of the available databases.
If the analyte has what can be referred to as a “public origin” (e.g., a contaminant in a packaged substance such as a packaged food, a compound in a substance found to be associated with a crime against a person or property, a material associated with an equipment failure, etc.), that material is probably going to be known and there could well be a mass spectrum of it in the NIST/EPA/NIH Mass Spectral Database, if it is suitable for electron ionization mass spectrometry. Such substances, compounds, and materials will also probably be listed in some other database, like the one maintained by the U.S. EPA or the European Index of Commercial Chemical Substances. Compounds in the NIST/EPA/NIH Database are indexed according to their inclusion in the nine databases listed in Table 6-5. Given a choice for the identity of a substance of a public origin between a compound that is not listed in any of these nine databases and one that is in several of them, the latter would be the best choice. Another indicator as to which of several possibilities should be selected from a list is whether the Synonyms List contains several common or trade names for the compound. Compare the entries of the two suggested compounds (Hits) with very similar mass spectra that appear in the top 15 Hits of a mass spectral database search (comparison) of an unknown against the NIST05 Database: 3-aminoisonicotinic acid (CASrn 7579-20-6), which is not listed in any of the nine other databases and has no synonyms other than one other chemical name and aspirin (CASrn 50-78-2), which is also found in seven of the nine other databases and has 141 synonyms, many of which are common names. It is obvious which of these would be the most likely candidate to be a compound of public origin.

**Table 6-5. Other databases that may contain NIST/EPA/NIH database compounds.**

<table>
<thead>
<tr>
<th>NIST MS Search Program Designation</th>
<th>Database Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine</td>
<td>Commercially Available Fine Chemical Index</td>
</tr>
<tr>
<td>TSCA</td>
<td>Toxic Substances Control Act Inventory</td>
</tr>
<tr>
<td>RTECS</td>
<td>Registry of Toxic Effects of Chemical Substances</td>
</tr>
<tr>
<td>EPA</td>
<td>EPA Environmental Monitoring Methods Index</td>
</tr>
<tr>
<td>USP</td>
<td>U. S. Pharmacopoeia/U.S.A.N.</td>
</tr>
<tr>
<td>HODOC</td>
<td>CRC Handbook of Data of Organic Compounds</td>
</tr>
<tr>
<td>NIH</td>
<td>NIH-NCI Inventory File</td>
</tr>
<tr>
<td>EINECS</td>
<td>European Index of Commercial Chemical Substances</td>
</tr>
<tr>
<td>IR</td>
<td>NIST/EPA Gas Phase IR Database</td>
</tr>
</tbody>
</table>

Only 28,297 of the 163,198 compounds found in the NIST05 Mass Spectral Database are in one or more of the nine other databases. The remaining compounds are not so common and may have only been seen inside a mass spectrometer once under some special circumstances. Therefore, if substances associated with natural products, new drug metabolites, etc., are being analyzed, the “public origin” hypothesis may not be applicable.
3. When the Spectrum of the Unknown Is Not in the Database(s)

When there is a high confidence that a mass spectrum of the analyte is not present in one or more of the available mass spectral databases, the NIST MS Search Program has two other search modes. These are the Similarity Search (which compares the sample spectrum against the spectra of the database(s) to see which spectra are similar, even if they do not match) and the Neutral Loss Search (which looks for spectra in the database(s) that exhibit the same neutral losses from the M⁺). A hydride Neutral Loss and Similarity Search can also be carried out. The Hit List from any one of these three searches can be evaluated using the Substructure Identification Option accessed from the Tools menu on the Main Menu Bar of the Lib Search tab of the NIST MS Search Program. The functionality of this Substructure Identification utility [73] is explained in more detail in the Library Search section of Chapter 3 on MS/MS.

It should be noted that when this Substructure Identification utility is used with EI mass spectra of compounds whose spectra are not in the database(s), the only information that can be developed is the probable presence and probable absence of various substructures. The analyst will have to construct a probable structure. Once a structure has been proposed and drawn using some structural drawing program, the structure can be associated with the sample spectrum. The spectrum, along with the proposed structure, can be sent to MS Interpreter Program.

Figure 6-93. There is a peak at m/z 163 in this mass spectrum. Through the use of alternating colors in the program’s actual display (shown in grayscale), the loss of an isopropyl radical is shown from the structure in the top portion of the display. The resulting ion and the neutral loss are explained in the text boxes of the display.
The MS Interpreter Program predicts at what $m/z$ values there will be a mass spectral peak based on the structure. The position of these peaks can be compared with those in the spectrum that was imported with the proposed structure. If there is too much variation, then the structure can be adjusted, reassociated with the spectrum, and the two reimported into the program. An example of the MS Interpreter Program applied to the structure and mass spectrum of ibuprofen is shown in Figure 6-93.

In addition to the MS Interpreter Program that is provided with the NIST MS Search Program and is also part of the free download of the NIST MS Search Program, there are some other commercially available programs that perform similarly. There are the Mass Spec Calculator Pro from ChemSW (http://www.chemsw.com), the Mass Frontier (a Thermo Fisher product that requires Xcalibur Mass Spectral Software and components of Microsoft Office, http://www.highchem.com), and the ACD/MS Fragmentor Program from Advanced Chemistry Development (ACD Labs), all of which can be integrated with ACD/MS Manager: (http://www.acdlabs.com/products/spec_lab/exp_spectra/ms_fragmentor/).

4. Searching Multiple Databases

Care must be taken in decisions to search multiple mass spectral databases at the same time. When multiple databases are searched using the NIST MS Search Program, the 100 best matches are listed in the Hit List without indicating the database source(s). Each database is searched sequentially, but the order of presentation in the Hit List is based on the Match Factor or the Reverse Match Factor, depending on how the search was structured. This is not the case with PBM Search in the Agilent Technologies GC/MS ChemStation, which is one of the most widely used GC/MS library searches. Multiple libraries (up to three) are listed along with a "Match Quality" (a number between 0 and 100) for the first two. If the search finds a Hit that has a Match Quality greater than the minimum specified for the first library, the other two libraries are not searched. If the search program goes to the next library and the search finds a Hit that is greater than the minimum specified Match Quality for that library, that Hit will be reported and the third library will not be searched; however, a Hit with a higher Match Quality than that of the reported spectrum from the first database, but below that of the first database's minimum Match Quality, will not be reported.

This unusual behavior of the GC/MS ChemStation internal library search means that if the minimum Match Quality for the first database is set at 90 and the minimum Match quality for the second database is set at 70 and no Hits are found in the first library with $>90$ Match Quality and no Hits are found in the second library with a Match Quality $>70$, the third library will be searched where the Hit with the best Match Quality may be only 50. This Hit will be listed as the best match for the search even though there was a possible Hit in the first database that would have had a Match Quality of 89, which will not be reported at all.

5. Database Size and Quality

It certainly has not gone unnoticed that the Wiley Registry 8th Ed. has about twice as many spectra as does the NIST05, and the Palisade Complete has over three times as many spectra as are in the NIST05 Database. Is bigger better? If the bigger comes from quality spectra of more compounds, then there is no question that bigger is better when it comes to a library of mass spectra. Although the Wiley Registry and Palisade Complete do contain spectra of compounds that are not in the NIST Database,
both also contain many more replicate² spectra and they also have a number of
duplicate³ spectra. As an example, the Wiley Registry has 13 spectra with the name
“phenylbutazone” and at least 13 spectra for “hexachlorobenzene”, whereas the NIST05
has only four spectra for these two common and public origin compounds. The NIST05
can be searched without inclusion of the replicate spectra, whereas the Wiley Registry
and the Palisade Complete cannot. The importance of being able to search a database
separately without replicates (or duplicates) is in trying to identify compounds whose
spectra are not in the database. Too many spectra for a certain compound can limit the
number of different Hits that may provide the necessary clues for elucidation of a
structure.

Another important factor about a mass spectral database is spectral quality. All
the spectra in the NIST05 have been individually examined to assure their correctness
with the name, provided structure, and the spectrum itself [74]. Spectra in both the Wiley
Registry and the Palisade have been added as provided to the publisher without any
evaluation. As an example, there two spectra in the Wiley Registry that are labeled
Maneba, which is a herbicide that is a polymer of manganese containing a polymer of an
ethylene carbonoditioate. Examination of these two spectra shows that there are no
similarities between them. Even though the listed chemical names for the two spectra
are exactly the same and the elemental compositions are the same (no structure
provided), they have different CAS registry numbers. Examination of the spectrum of
CASrn 12427-38-2 reveals that it is clearly a mass spectrum of elemental sulfur (S8).
When the mass spectrum of elemental sulfur is searched against the Wiley Registry,
Maneba is one of the Hits.

The search of the NIST05 mass spectrum of elemental sulfur against the Wiley
Registry using the NIST MS Search Program points out another problem. Many of the
top 15 Hits are spectra that are reported to be of S8; however, five of these top 15 Hits
are for compounds whose spectra only have peaks that represent the molecular ion and
its isotopes. The Wiley Registry (and the Palisade Complete because it incorporates the
7th Ed. of the Wiley Registry) contain a significant number of these single-peak spectra.
These spectra can cause problems with a search because these single peaks are also
the base peak for these spectra and there is significant weighting given to them based on
their high m/z values and the fact that this peak has 100% relative intensity.

Because there are compounds in the Wiley Registry that are not in the NIST05
Database, it is probably best to have both. The advantage of having both, as opposed to
the Wiley/NIST combination, is that NIST05 can be searched without the possible
interferences from duplicate and replicate spectra. This is not the case with the
Wiley/NIST combination.

6. Concluding Remarks on the NIST Mass Spectral Search Program

The reason for the popularity of the NIST MS Search Program with so many
mass spectrometer manufacturers and third-party database publishers like John Wiley &
Sons, Inc. is its versatility. Not only can sample spectra be searched against the NIST05
and other mass spectral databases (up to 12 databases in addition to the NIST mainlib
and replib) using several different search algorithms but constraints regarding the number

² A replicate spectrum is another spectrum of the same compound; this spectrum is usually acquired by a
different laboratory.

³ A duplicate spectrum is a second copy of the same spectrum of the same compound. Duplicate spectra
originate during the combining of various databases, more than one of which contains the same spectrum.
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of specific elements, name fragments, molecular (nominal) mass ranges, presence of peaks based on their type (normal or loss), and m/z value can be placed on these searches. The databases (NIST05, other commercial databases, and user-generated databases) can be searched according to the ID number (position in the database) of a spectrum, the CAS registry number of the spectrum, and the spectrum’s NIST number (a unique number given to each spectrum in the NIST archive), as well as by any of the previously mentioned constraints. It is also possible to submit a structure for purposes of retrieving its spectrum or spectra of similar structures from the database. Spectra can easily be exported to other applications like Microsoft Word. The NIST MS Search Program can also be linked to structure-drawing programs to allow the easy import and export of structures in MOL format.

Used in combination with AMDIS (described in Chapter 10), it is easy to import spectra from nearly all commercial mass spectrometer data file formats into the NIST MS Search Program for searching or building user databases.

VII. Summary of Interpretation of EI Mass Spectra

There are many excellent reference books on the interpretation of EI mass spectra and rationalizations of fragmentation schemes for molecular ions of organic compounds. Budzikiewicz et al. [28] and Porter [75] offer systematic approaches to the interpretation and rationalization of the mass spectra of many classes of organic compounds; several graphic mass spectra and mechanisms of fragmentation are presented for representative compounds in each class.

Introductory approaches to the interpretation of mass spectra of organic molecules have been written by Biemann [4], McLafferty and Tureček [26], Hill [76], and Shrader [77]. Many other systematic studies of the mass spectra and fragmentation mechanisms of different classes of compounds are available in the literature, such as those of sulfur-containing compounds [78]. For example, much of the information characterizing the mass spectra of steroids gathered from two decades of studies of fragmentation pathways has been reduced to computer algorithms for investigations of marine sterols [79, 80]. In other exhaustive studies of steroids [81], deuterium labeling has been used to delineate fragmentation in Δ4- and Δ1,4-3-ketosteroids [82, 83] and stereochemical correlations in other steroids [84, 85]. Gaskell [86] has reviewed the mass spectrometry of steroids. General methods based on mass spectrometry have been reported for bile acids [87] and glycerolipids [88]. Spliter and Tureček [7] have addressed the general application of mass spectrometry to stereochemical features of molecules; a specific example of stereochemical differentiation has been reported for 1,3-amine acids [89]. Smith [90] has provided a great deal of information on general fragmentation and specific data regarding the fragmentation of molecular ions of illicit drugs.

Mass spectrometry of fatty acids [35, 36] has been well documented, including that of the eicosanoids [91, 92]. Other fields of application in natural products include studies of terpene derivatives [93] and alkaloids [94]. Mass spectrometry has also been useful in environmental [95–98] chemistry and toxicology studies of dioxins [99]. Fundamental studies of fragmentation mechanisms of other compounds, such as those of alkylbenzenes [100, 101], were important contributions to the current understanding of mass spectra. Detailed investigations of specific types of ions such as aliphatic oxonium ions [102], ion–dipole complexes [103], and 1,2-hydrogen shifts in allylic ions [104] help to advance the field and lay the groundwork for applications such as recognizing the location of double bonds [105] or branch points [106] in hydrocarbons and fatty acids.

The best approach to understanding fragmentation mechanisms in EI or those associated with any other type of ionization is to continue to practice the interpretation
process. Draw the structure of the molecular ion of the compound (or putative analyte) with the sites of the charge and the odd electron at the most probable location (heteroatom, in a \( \pi \) bond, or lastly in a sigma bond). Try to identify driving forces that would cause electrons to move in a way that would result in the apparent bond cleavage, and always keep in mind the valence rules that allow for logical losses.
References

References


“Most people would rather die than think; in fact, they do so.”

~Bertrand Russell