CHAPTER 8

MASS SPECTROMETRY

The principles that underlie mass spectrometry pre-date all of the other instrumental techniques described in this book. The fundamental principles date to the late 1890s when J. J. Thomson determined the mass-to-charge ratio of the electron, and Wien studied magnetic deflection of anode rays and determined the rays were positively charged. Each man was honored with the Nobel Prize (Thomson in 1906 and Wien in 1911) for their efforts. In 1912–1913, J. J. Thomson studied the mass spectra of atmospheric gases and used a mass spectrum to demonstrate the existence of neon-22 in a sample of neon-20, thereby establishing that elements could have isotopes. The earliest mass spectrometer, as we know it today, was built by A. J. Dempster in 1918. However, the method of mass spectrometry did not come into common use until about 50 years ago, when inexpensive and reliable instruments became available.

Development of ionization techniques for high molecular weight (MW) compounds and biological samples in the 1980s and 1990s introduced mass spectrometry to a new community of researchers. The introduction of lower-cost commercial instruments that provide high resolution and are maintained easily has made mass spectrometry an indispensable technique in numerous fields far removed from the laboratories of Thomson and Wien. Today, the biotechnology industry uses mass spectrometry to assay and sequence proteins, oligonucleotides, and polysaccharides. The pharmaceutical industry uses mass spectrometry in all phases of the drug development process, from lead compound discovery and structural analysis, to synthetic development and combinatorial chemistry, and to pharmacokinetics and drug metabolism. In health clinics around the world, mass spectrometry is used in testing blood and urine for everything from the presence and levels of certain compounds that are "markers" for disease states, including many cancers, to detecting the presence and quantitative analysis of illicit or performance-enhancing drugs. Environmental scientists rely on mass spectrometry to monitor water and air quality, and geologists use mass spectrometry to test the quality of petroleum reserves.

To date, no fewer than five Nobel Prizes have been awarded for work directly related to mass spectrometry: J. J. Thomson (Physics, 1906) for "theoretical and experimental investigations on the conduction of electricity by gases"; F. W. Aston (Chemistry, 1922) for "discovery, by means of a mass spectrograph, of isotopes, in a large number of non-radioactive elements"; W. Paul (Physics, 1989) "for the development of the ion trap technique"; and most recently J. B. Fenn and K. Tanaka (Chemistry, 2002) "for the development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules."

8.1 THE MASS SPECTROMETER: OVERVIEW

In its simplest form, the mass spectrometer has five components (Fig. 8.1), and each will be discussed separately in this chapter. The first component of the mass spectrometer is the **sample inlet** (Section 8.2), which brings the sample from the laboratory environment (1 atm) to the lower pressure of the mass spectrometer. Pressures inside the mass spectrometer range from a few millimeters of mercury in a chemical ionization source to a few micrometers of mercury in the mass analyzer and detector regions of the instrument. The sample inlet leads to the **ion source** (Section 8.3), where the sample molecules are transformed into gas phase ions. The ions are then accelerated by an electromagnetic field. Next, the **mass analyzer** (Section 8.4) separates the sample ions based on their **mass-to-charge (*m/z*) ratio**. The ions then are counted by the **detector** (Section 8.5), and the signal

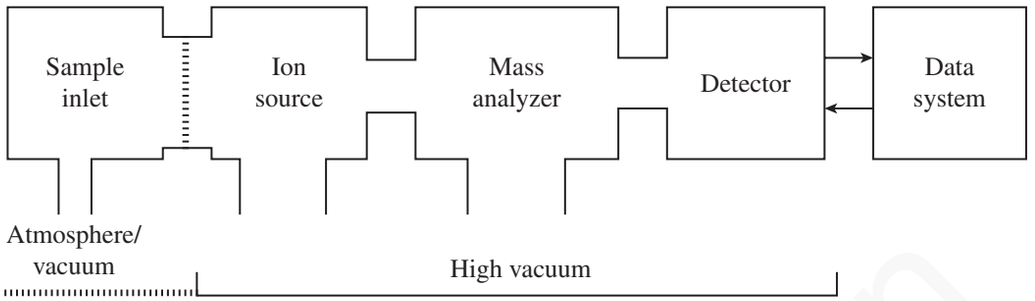


FIGURE 8.1 The components of a mass spectrometer. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

is recorded and processed by the **data system,** typically a personal computer (PC). The output from the data system is the **mass spectrum**—a graph of the number of ions detected as a function of their *m/z* ratio.

8.2 SAMPLE INTRODUCTION

When we examine each of these essential mass spectrometer functions in detail, we see that the mass spectrometer is somewhat more complex than just described. Before the ions can be formed, a stream of molecules must be introduced into the **ion source** (ionization chamber) where the ionization takes place. A **sample inlet** system provides this stream of molecules.

A sample studied by mass spectrometry may be a gas, a liquid, or a solid. Enough of the sample must be converted to the vapor state to obtain the stream of molecules that must flow into the ionization chamber. With gases, of course, the substance is already vaporized, so a simple inlet system can be used. This inlet system is only partially evacuated so that the ionization chamber itself is at a lower pressure than the sample inlet system. The sample is introduced into a larger reservoir, from which the molecules of vapor can be drawn into the ionization chamber, which is at low pressure. To ensure that a steady stream of molecules is passing into the ionization chamber, the vapor travels through a small pinhole, called a **molecular leak,** before entering the chamber. The same system can be used for volatile liquids or solids. For less-volatile materials, the system can be designed to fit within an oven, which can heat the sample to increase the vapor pressure of the sample. Care must be taken not to heat any sample to a temperature at which it might decompose.

With nonvolatile samples, other sample inlet systems must be used. A common one is the **direct probe** method. The sample is placed on a thin wire loop or pin on the tip of the probe, which is then inserted through a vacuum lock into the ionization chamber. The sample probe is positioned close to the ion source. The probe can be heated, thus causing vapor from the sample to be evolved in proximity to the ionizing beam of electrons. A system such as this can be used to study samples of molecules with vapor pressures lower than 10^{-9} mmHg at room temperature.

The most versatile sample inlet systems are constructed by connecting a chromatograph to the mass spectrometer. This sample introduction technique allows a complex mixture of components to be separated by the chromatograph, and the mass spectrum of each component may then be determined individually. A drawback of this method involves the need for rapid scanning by the mass spectrometer. The instrument must determine the mass spectrum of each component in the mixture *before* the next component exits from the chromatography column so that the first substance is not contaminated by the next before its spectrum has been obtained. Since high-efficiency columns are used in the chromatograph, in most cases compounds are completely separated before the eluent stream is analyzed. The instrument must have the capability of obtaining at least one scan per second in the range of 10 to 300 *m/z*. Even more scans are necessary if a narrower range of masses is to

be analyzed. The mass spectrometer that is coupled to the chromatograph should be relatively compact and capable of high resolution.

In **gas chromatography–mass spectrometry (GC-MS)**, the gas stream emerging from a gas chromatograph is admitted through a valve into a tube, where it passes over a molecular leak. Some of the gas stream is thus admitted into the ionization chamber of the mass spectrometer. In this way, it is possible to obtain the mass spectrum of every component in a mixture injected into the gas chromatograph. In effect, the mass spectrometer acts in the role of detector. Similarly, **high-performance liquid chromatography–mass spectrometry** (**HPLC-MS,** or more simply **LC-MS**) couples an HPLC instrument to a mass spectrometer through a special interface. The substances that elute from the HPLC column are detected by the mass spectrometer, and their mass spectra can be displayed, analyzed, and compared with standard spectra found in the computer library built into the instrument.

8.3 IONIZATION METHODS

A. Electron Ionization (EI)

Regardless of the method of sample introduction, once the stream of sample molecules has entered the mass spectrometer, the sample molecules must be converted to charged particles by the **ion source** before they can be analyzed and detected. The simplest and most common method for converting the sample to ions is **electron ionization** (**EI**). In EI-MS, a beam of high-energy electrons is emitted from a **filament** that is heated to several thousand degrees Celsius. These high-energy electrons strike the stream of molecules that has been admitted from the sample inlet system. The electron–molecule collision strips an electron from the molecule, creating a cation. A **repeller plate,** which carries a positive electrical potential, directs the newly created ions toward a series of **accelerating plates.** A large potential difference, ranging from 1 to 10 kilovolts (kV), applied across these accelerating plates produces a beam of rapidly traveling positive ions. One or more **focusing slits** direct the ions into a uniform beam (Fig. 8.2).

Most of the sample molecules are not ionized at all but are continuously drawn off by vacuum pumps that are connected to the ionization chamber. Some of the molecules are converted to negative ions through the absorption of electrons. The repeller plate absorbs these negative ions. It is possible to reverse the polarity of the repeller and accelerating plates in some instruments, thereby allowing for mass analysis of negative ions (anions) that are created by electron capture when the sample molecules are hit by the electron beam. A small proportion of the positive ions that are formed may have a charge greater than one (a loss of more than one electron). These are accelerated in the same way as the singly charged positive ions.

The energy required to remove an electron from an atom or molecule is its **ionization potential** or **ionization energy.** Most organic compounds have ionization potentials ranging between 8 and 15 electron volts (eV). However, a beam of electrons does not create ions with high efficiency until it strikes the stream of molecules with a potential of 50 to 70 eV. To acquire reproducible spectral features, including fragmentation patterns, that can be readily compared with electronic databases, a standard 70-eV electron beam is used.

EI-MS has distinct advantages for routine mass spectrometry of small organic molecules. Electron ionization hardware is inexpensive and robust. The excess kinetic energy imparted to the sample during the EI process leads to significant fragmentation of the molecular ion (Section 8.8). The fragmentation pattern of a compound is reproducible, and many libraries of EI-MS data are available. This allows one to compare the mass spectrum of a sample compound against thousands of data sets in a spectral library in a few seconds using a PC, thus simplifying the process of determining or confirming a compound's identity.

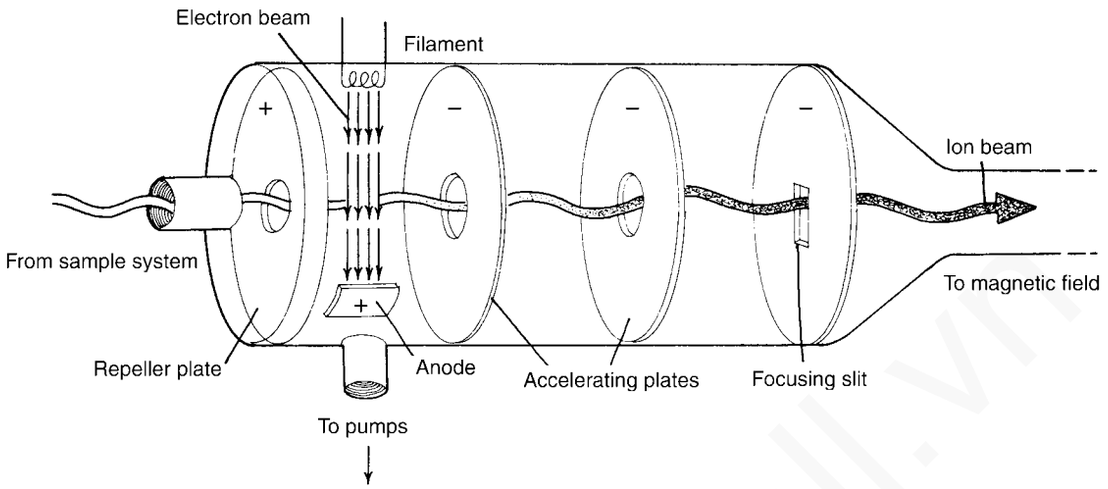


FIGURE 8.2 Electron ionization chamber.

The fragmentation of the molecular ion under EI conditions may also be considered a distinct disadvantage. Some compounds fragment so easily that the lifetime of the molecular ion is too short to be detected by the mass analyzer. Thus, one cannot determine a compound's molecular mass (Section 8.6) in such cases. Another drawback to EI-MS is that the sample must be relatively volatile so it can come into contact with the electron beam in the ionization chamber. This fact coupled with the fragmentation problem make it difficult to analyze high molecular weight (MW) compounds and most biomolecules using EI-MS.

B. Chemical Ionization (CI)

In **chemical ionization–mass spectrometry** (**CI-MS**), the sample molecules are combined with a stream of ionized reagent gas that is present in great excess relative to the sample. When the sample molecules collide with the preionized reagent gas, some of the sample molecules are ionized by various mechanisms, including proton transfer, electron transfer, and adduct formation. Almost any readily available gas or highly volatile liquid can be used as a reagent gas for CI-MS.

Common ionizing reagents for CI-MS include methane, ammonia, isobutane, and methanol. When methane is used as the CI reagent gas, the predominant ionization event is proton transfer from a CH_5^+ ion to the sample. Minor ions are formed by adduct formation between $C_2H_5^+$ and higher homologues with the sample. The methane is converted to ions as shown in Equations 8.l–8.4.

$$CH_4 + e^- \rightarrow CH_4^{\bullet+} + 2e^- \quad \textbf{Equation 8.1}$$

$$CH_4^{\bullet+} + CH_4 \rightarrow CH_5^+ + {\cdot}CH_3 \quad \textbf{Equation 8.2}$$

$$CH_4^{\bullet+} \rightarrow CH_3^+ + H{\cdot} \quad \textbf{Equation 8.3}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad \textbf{Equation 8.4}$$

The sample molecule M is then ionized through the ion–molecule reactions in Equations 8.5 and 8.6:

$$M + CH_5^+ \rightarrow (M+H)^+ + CH_4 \quad \textbf{Equation 8.5}$$

$$M + C_2H_5^+ \rightarrow (M + C_2H_5)^+ \quad \textbf{Equation 8.6}$$

The situation is very similar for CI with ammonia as reagent gas (Equations 8.7–8.9):

$$NH_3 + e^- \rightarrow NH_3^{\bullet +} + 2e^- \quad \textbf{Equation 8.7}$$

$$NH_3^{\bullet +} + NH_3 \rightarrow NH_4^+ + {\cdot}NH_2 \quad \textbf{Equation 8.8}$$

$$M + NH_4^+ \rightarrow (M + H)^+ + NH_3 \quad \textbf{Equation 8.9}$$

Using isobutane as reagent gas produces *tert*-butyl cations (Equations 8.10 and 8.11), which readily protonate basic sites on the sample molecule (Equation 8.12). Adduct formation is also possible using isobutane in CI-MS (Equation 8.l3).

$$(CH_3)_3CH + e^- \rightarrow (CH_3)_3CH^{\bullet +} + 2e^- \quad \textbf{Equation 8.10}$$

$$(CH_3)_3CH^{\bullet +} \rightarrow (CH_3)_3C^+ + H{\cdot} \quad \textbf{Equation 8.11}$$

$$M + (CH_3)_3C^+ \rightarrow (M + H)^+ + (CH_3)_2C{=}CH_2 \quad \textbf{Equation 8.12}$$

$$M + (CH_3)_3C^+ \rightarrow [M + C(CH_3)_3]^+ \quad \textbf{Equation 8.13}$$

Varying the reagent gas in CI-MS allows one to vary the selectivity of the ionization and degree of ion fragmentation. The choice of reagent gas should be made carefully to best match the **proton affinity** of the reagent gas with that of the sample to ensure efficient ionization of the sample without excessive fragmentation. The greater the difference between the proton affinity of the sample and that of the reagent gas, the more energy that is transferred to the sample during ionization. The excess energy produces an analyte ion in a highly excited vibrational state. If enough excess kinetic energy is transferred, the sample ion will fragment through the cleavage of covalent bonds. Therefore, using a reagent gas with a proton affinity matched closely to that of the sample will result in a greater number of intact molecular ions and smaller number of fragment ions. It is unlikely, of course, that one knows the precise proton affinity of the sample, but one can estimate the value by looking at tables of values determined for simple compounds with functional groups similar to the sample in question. A summary of common CI reagent gases and their ions/properties is presented in Table 8.l.

As one can see from Figure 8.3, CI-MS of lavandulyl acetate (MW 196) gives mass spectra with very different appearances depending on the regent gas used to ionize the sample. In the top spectrum,

TABLE 8.1
SUMMARY OF CHEMICAL IONIZATION (CI) REAGENT GASES

Reagent Gas	Proton Affinity (kcal/mole)	Reagent Ion(s)	Analyte Ion(s)	Comments
H_2	101	H_3^+	$(M + H)^+$	Produces significant fragmentation
CH_4	132	CH_5^+, $C_2H_5^+$	$(M + H)^+$, $(M + C_2H_5)^+$	Less fragmentation than H_2, can form adducts
NH_3	204	NH_4^+	$(M + H)^+$, $(M + NH_4)^+$	Selective ionization, little fragmentation, some adduct formation
$(CH_3)_3CH$	196	$(CH_3)_3C^+$	$(M + H)^+$, $[M + C(CH_3)_3)]^+$	Mild, selective protonation, little fragmentation
CH_3OH	182	$CH_3OH_2^+$	$(M + H)^+$	Degree of fragmentation observed between that of methane and isobutane
CH_3CN	188	CH_3CNH^+	$(M + H)^+$	Degree of fragmentation observed between that of methane and isobutane

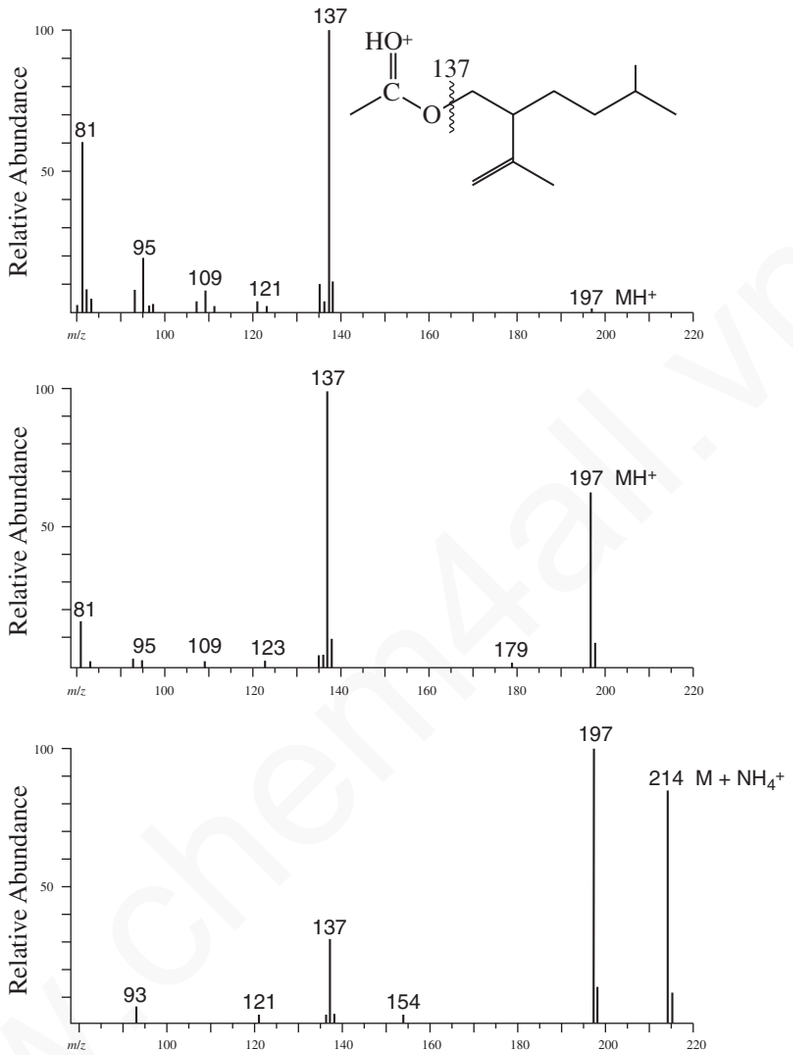


FIGURE 8.3 Comparison of CI-MS data of lavandulyl acetate using methane (top), isobutane (middle), and ammonia (bottom) as reagent gases. (From McLafferty, F. W. and F. Tureček, *Interpretation of Mass Spectra,* 4th ed., University Science Books, Mill Valley, CA, 1993. Reprinted with permission.)

the protonated molecular ion of lavandulyl acetate [$(M + H)^+$, m/z = 197] is barely visible, and the largest peak in the spectrum belongs to the fragment at m/z = 137. In the middle spectrum, acquired using isobutane as reagent gas, the protonated molecular ion at m/z = 197 is much more prominent, and there is less overall fragmentation. Fragmentation is still significant in this case, though, as the ion at m/z = 137 is still the most abundant in the spectrum. Finally, when lavandulyl acetate is ionized using ammonia, the protonated molecular ion is the most abundant ion (the base peak), and almost no fragmentation is observed. Note the presence of an adduct ion [$(M + NH_4)^+$, m/z = 214] present in this spectrum.

As a practical note, spectra acquired under CI conditions are usually acquired over a mass range above the m/z of the reagent gas ions. The ionized reagent gas is also detected by the spectrometer, and because the reagent gas is present in great excess relative to the sample, its ions would dominate the spectrum. Thus, CI (methane) spectra are typically acquired above m/z = 50 (CH_5^+ is m/z = 17,

of course, but $C_2H_5^+$ [m/z = 29] and $C_3H_5^+$ [m/z = 41] are also present), and CI (isobutane) spectra are typically acquired above m/z = 60 or 70.

The main advantage of CI-MS is the selective production of intact quasi-molecular ions [$(M + H)^+$]. Figure 8.4 shows the mass spectrum of butyl methacrylate acquired under different ionization conditions. The molecular ion (m/z = 142) is barely visible in the EI-MS, but the $(M + H)^+$ ion (m/z = 143) is prominent in the CI-MS spectra. The CI-MS acquired using isobutane has much less fragmentation than the CI-MS acquired using methane as the reagent gas. Other advantages to CI-MS include inexpensive and robust hardware. Like in EI-MS, however, the

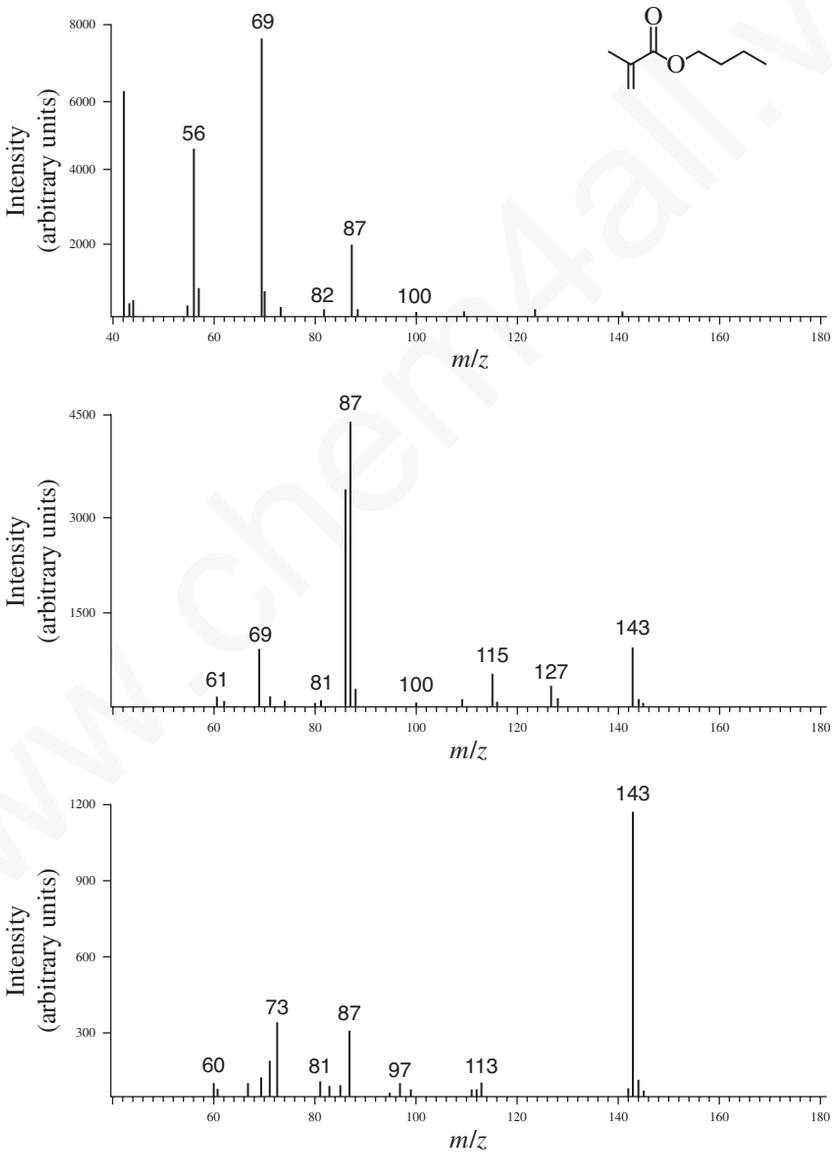


FIGURE 8.4 MS of butyl methacrylate acquired under EI (top) and CI (methane, middle; isobutane, bottom) conditions. (From DeHoffmann, E. and V. Stroobant, *Mass Spectrometry: Principles and Applications,* 2nd ed., John Wiley and Sons, New York, 1999. Reprinted with permission.)

sample must be readily vaporized to be subjected to chemical ionization, which precludes the analysis of high molecular weight compounds and many biomolecules. CI ion sources are very similar in design to EI sources, and most modern mass spectrometers can switch from EI to CI mode in a matter of minutes.

While protonation is the most commonly encountered ionization method in CI-MS, other ionization processes may be exploited. For example, use of methyl nitrite/methane mixtures as reagent gas produces CH_3O^- that abstracts a proton from the sample, leading to a $(M - H)_-$ parent ion. Similarly, use of NF_3 as reagent gas produces F^- ion as a proton abstraction agent, also leading to $(M - H)^-$ ions. It is also possible to form negatively charged adducts under CI conditions.

C. Desorption Ionization Techniques (SIMS, FAB, and MALDI)

Both EI and CI methods require a relatively volatile (low molecular weight) sample. More recently developed ionization techniques allow the analysis of large, nonvolatile molecules by mass spectrometry. Three of these methods, **secondary ion mass spectrometry** (**SIMS**), **fast atom bombardment** (**FAB**), and **matrix-assisted laser desorption ionization** (**MALDI**) are all **desorption ionization** (**DI**) techniques. In desorption ionization, the sample to be analyzed is dissolved or dispersed in a matrix and placed in the path of a high-energy (1- to 10-keV) beam of ions (SIMS), neutral atoms (FAB), or high-intensity photons (MALDI). Beams of Ar^+ or Cs^+ are often used in SIMS, and beams of neutral Ar or Xe atoms are common in FAB. Most MALDI spectrometers use a nitrogen laser that emits at 337 nm, but some applications use an infrared (IR) laser for direct analysis of samples contained in gels or thin-layer chromatography (TLC) plates. The collision of these ions/atoms/photons with the sample ionizes some of the sample molecules and ejects them from the surface (Fig. 8.5). The ejected ions are then accelerated toward the mass analyzer as with other ionization methods. Since FAB uses neutral atoms to ionize the sample, both positive-ion and negative-ion detection are possible. Molecular ions in SIMS and FAB are typically $(M + H)^+$ or $(M - H)^-$, but adventitious alkali

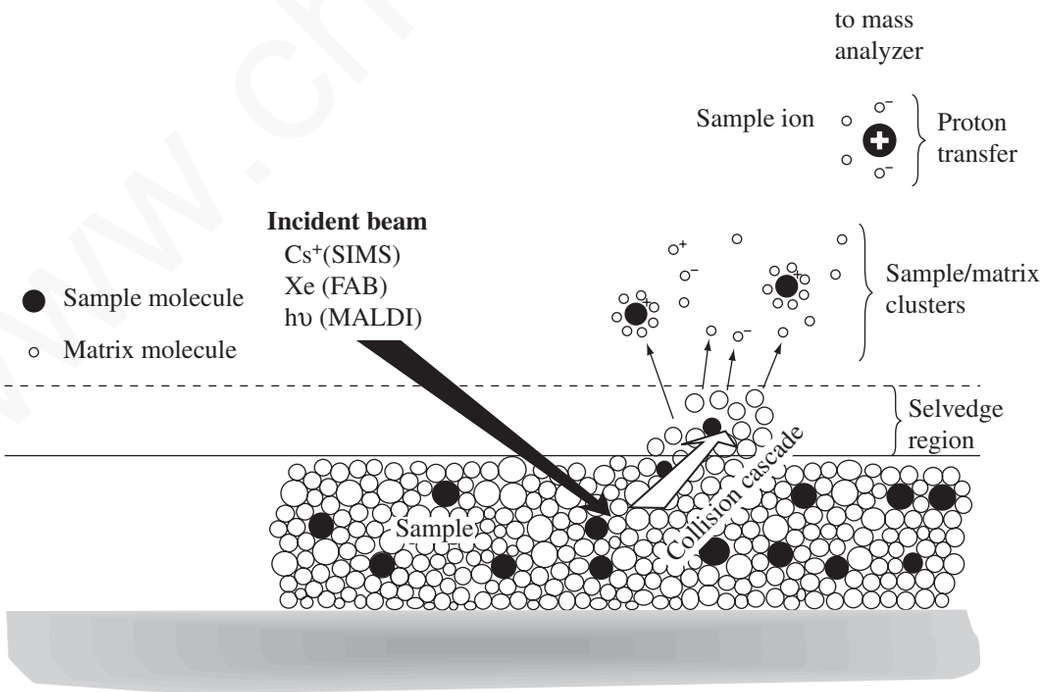


FIGURE 8.5 Schematic representations of desorption ionization techniques.

metals can create $(M + Na)^+$ and $(M + K)^+$ ions also. SIMS and FAB ionization methods may be used on sample compounds with molecular weights up to about 20,000, such as polypeptides and oligonucleotides.

The matrix should be nonvolatile, relatively inert, and a reasonable electrolyte to allow ion formation. If the matrix compound is more acidic than the analyte, then predominantly $(M + H)^+$ ions will be formed, while mostly $(M - H)^-$ ions will result when the matrix is less acidic than the analyte. The matrix absorbs much of the excess energy imparted by the beam of ions/atoms and produces ions that contribute a large amount of background ions to the mass spectrum. In fact, chemical reactions within the matrix during ionization can contribute background ions in most mass regions below about 600 *m/z*. Common matrix compounds for SIMS and FAB include glycerol, thioglycerol, 3-nitrobenzyl alcohol, di- and triethanolamine, and mixtures of dithiothreitol (DTT) and dithioerythritol (Fig. 8.6)

The matrix compounds used in MALDI are chosen for their ability to absorb the ultraviolet (UV) light from a laser pulse (337 nm for N_2 laser). Substituted nicotinic, picolinic, and cinnamic acid derivatives are often used in MALDI techniques (Fig. 8.7). The matrix absorbs most of the energy from the laser pulse, thus allowing for the creation of intact sample ions that are ejected from the matrix. MALDI mass spectrometry is useful for analytes spanning a wide range of molecular weights, from small polymers with average molecular weights of a few thousand atomic mass units (amu) to oligosaccharides, oligonucleotides and polypeptides, antibodies, and small proteins with molecular weights approaching 300,000 amu. Furthermore, MALDI requires only a few femtomoles (1×10^{-15} mole) of sample!

D. Electrospray Ionization (ESI)

An even more useful technique for studying high molecular weight biomolecules and other labile or nonvolatile compounds is **electrospray ionization** (**ESI**) and its cousin **thermospray ionization** (**TSI**). In ESI, a solution containing the sample molecules is sprayed out the end of a fine capillary into a heated chamber that is at nearly atmospheric pressure. The capillary through which the sample solution passes has a high voltage potential across its surface, and small, charged droplets are expelled into the ionization chamber. The charged droplets are subjected to a counterflow of a drying gas (usually nitrogen) that evaporates solvent molecules from the droplets. Thus, the charge density of each droplet increases until the electrostatic repulsive forces exceed the surface tension of the droplet (the Rayleigh limit), at which point the droplets break apart into smaller droplets. This process continues

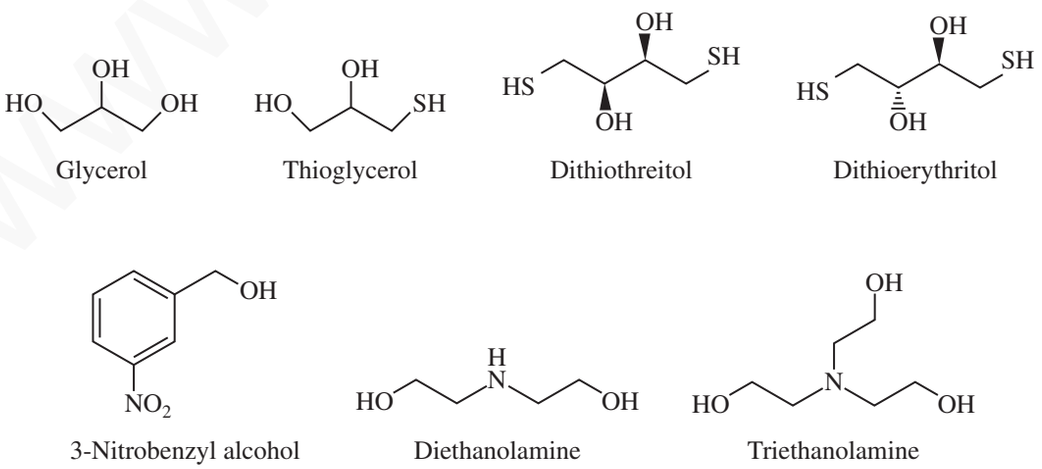


FIGURE 8.6 Common matrices for SIMS and FAB mass spectrometry.

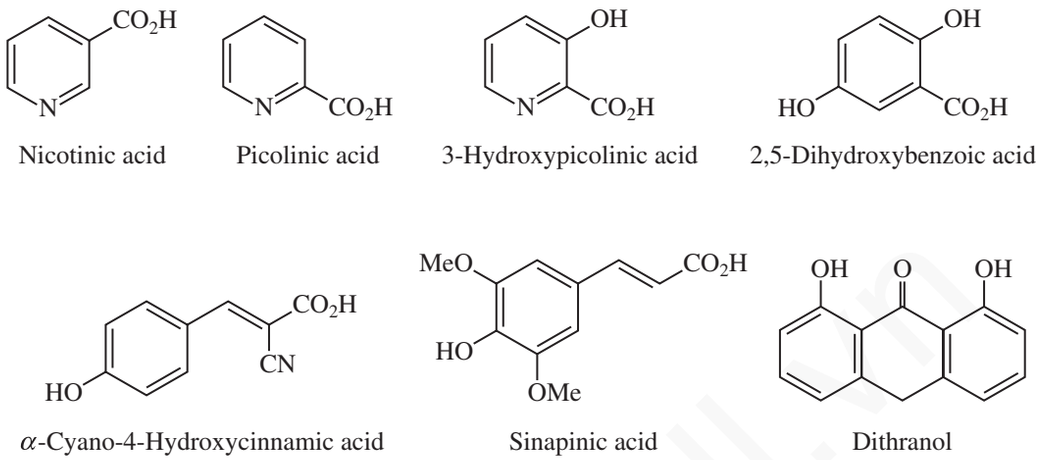


FIGURE 8.7 Common matrices for MALDI applications.

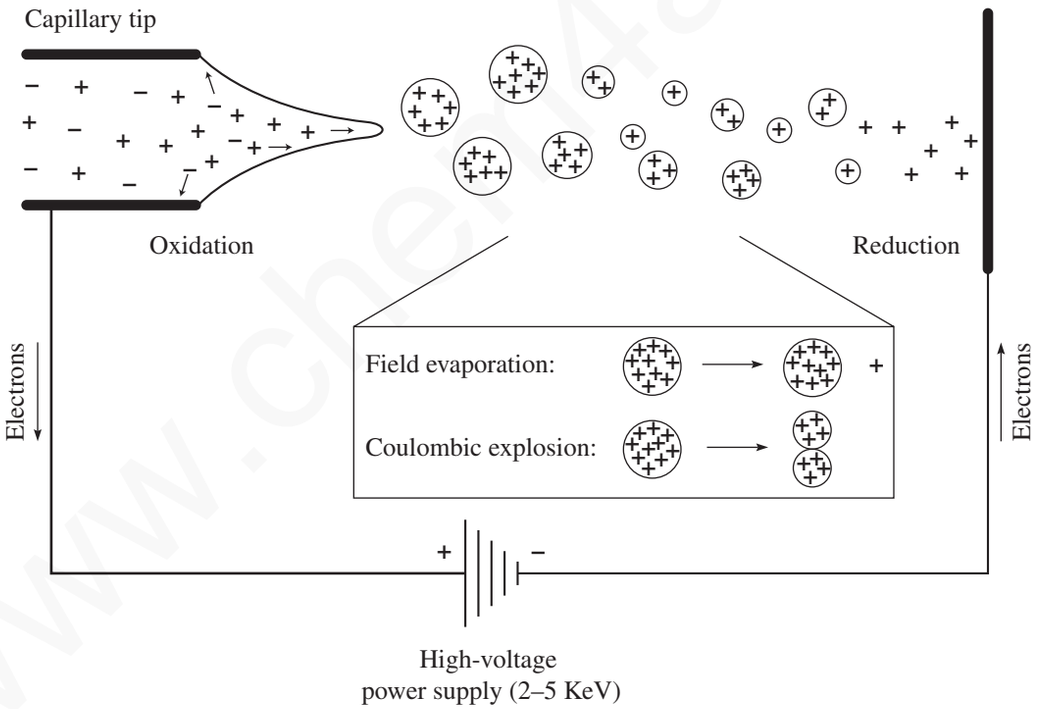


FIGURE 8.8 Schematic representation of electrospray ionization (ESI) showing both field evaporation and coulombic explosion. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

until solvent-free sample ions are left in the gas phase (Fig. 8.8). TSI occurs by a similar mechanism but relies on a heated capillary rather than one with an electrostatic potential to initially form the charged droplets. Negative ions may also be formed in ESI by loss of protons from the sample to basic species in solution. ESI has become much more common than TSI over the last decade or two, and because it relies on a sample in solution, ESI is the most logical method to be employed in LC-MS systems.

The charges of the ions generated using ESI do not necessarily reflect the charge state of the sample in solution. The charge transferred to the sample molecules (usually in the form of protons) arises from a combination of charge concentration in the droplets during evaporation of the aerosol and electrochemical processes stemming from the electrostatic potential of the capillary.

The sample ions may bear a single charge or multiple charges. Figure 8.9 shows the ESI-MS of lysozyme from chicken egg white in the absence and presence of dithiothreitol. In the first spectrum, ions are observed representing protein molecules bearing 10^+, 11^+, 12^+, and 13^+ charges. The latter spectrum shows even more highly charged ions—including a peak from protein bearing a 20^+ charge. The formation of multiply charged ions is particularly useful in the MS analysis of proteins. Typical proteins can carry many protons due to the presence of basic amino acid side chains, resulting in peaks at m/z = 600–2000 for proteins with a molecular weight that approaches 200,000 amu.

The data shown in Figure 8.9 can be used to calculate the molecular mass for lysozyme. The mass is calculated by multiplying the charge on the lysozyme by the m/z value shown on the chromatogram. For example:

$$(10)(1432) = 14{,}320 \text{ AMU}$$
$$(12)(1193) = 14{,}316$$
$$(15)(955) = 14{,}325$$

Thus, the molecular mass of lysozyme is about 14,320 AMU.

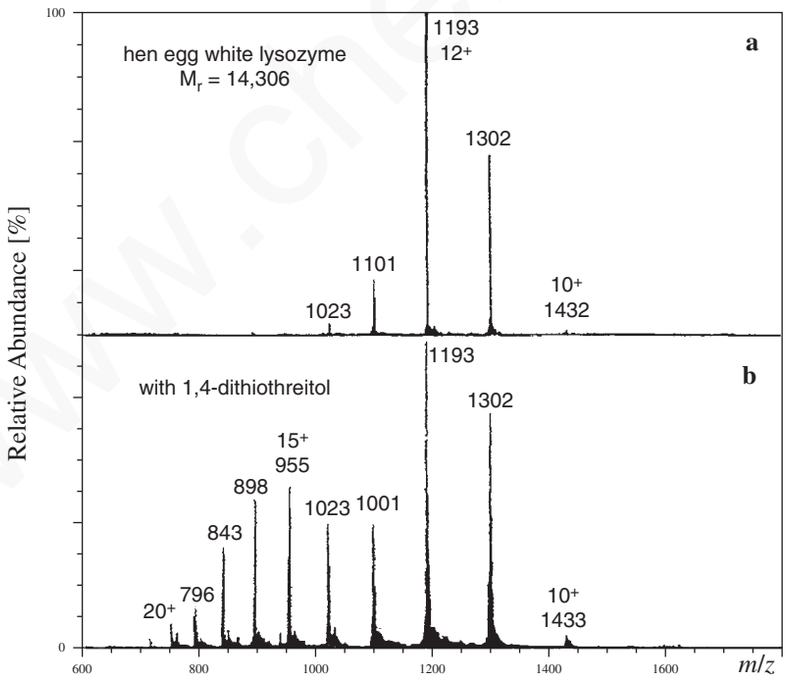


FIGURE 8.9 ESI-MS of proteins. Chicken egg white lysozyme in the absence (top) and presence (middle) of dithiothreitol. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Spinger, Berlin, 2004. Reprinted with permission.)

ESI-MS is not limited to the study of large biomolecules, however. Many small molecules with molecular weight in the 100–1500 range can be studied by ESI-MS. Compounds that are too nonvolatile to be introduced by direct probe methods or are too polar or thermally labile to be introduced by GC-MS methods are ideal for study by LC-MS using ESI techniques.

8.4 MASS ANALYSIS

Once the sample has been ionized, the beam of ions is accelerated by an electric field and then passes into the **mass analyzer,** the region of the mass spectrometer where the ions are separated according to their mass-to-charge (m/z) ratios. Just like there are many different ionization methods for different applications, there are also several types of mass analyzers.

A. The Magnetic Sector Mass Analyzer

The kinetic energy of an accelerated ion is equal to

$$\frac{1}{2}mv^2 = zV \qquad \textbf{Equation 8.14}$$

where m is the mass of the ion, v is the velocity of the ion, z is the charge on the ion, and V is the potential difference of the ion-accelerating plates. In the **magnetic sector** mass analyzer (Fig. 8.10), the ions are passed between the poles of a magnet. In the presence of a magnetic field, a charged particle describes a curved flight path. The equation that yields the radius of curvature of this path is

$$r = \frac{mv}{zB} \qquad \textbf{Equation 8.15}$$

where r is the radius of curvature of the path, and B is the strength of the magnetic field. If these two equations are combined to eliminate the velocity term, the result is

$$\frac{m}{z} = \frac{B^2r^2}{2V} \qquad \textbf{Equation 8.16}$$

As can be seen from Equation 8.16, the greater the value of m/z, the larger the radius of the curved path. The analyzer tube of the instrument is constructed to have a fixed radius of curvature. A particle

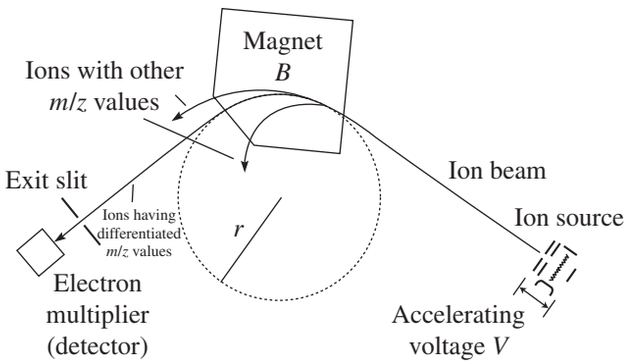


FIGURE 8.10 Schematic of a magnetic sector mass analyzer. (From Smith, R. M., *Understanding Mass Spectra, A Basic Approach*, 2nd ed., John Wiley and Sons, New York, 2004. Reprinted with permission.)

with the correct *m/z* ratio can negotiate the curved analyzer tube and reach the detector. Particles with *m/z* ratios that are either too large or too small strike the sides of the analyzer tube and do not reach the detector. The method would not be very interesting if ions of only one mass could be detected. Therefore, the magnetic field strength is continuously varied (called a *magnetic field scan*) so that all of the ions produced in the ionization chamber can be detected. The record produced from the detector system is in the form of a plot of the numbers of ions versus their *m/z* values.

An important consideration in mass spectrometry is **resolution,** defined according to the relationship

$$R = \frac{M}{\Delta M} \qquad \textbf{Equation 8.17}$$

where R is the resolution, M is the mass of the particle, and ΔM is the difference in mass between a particle of mass M and the particle of next higher mass that can be resolved by the instrument. A magnetic sector analyzer can have R values of 2000–7000, depending on the radius of curvature.

B. Double-Focusing Mass Analyzers

For many applications, much higher resolution is needed and can be achieved through modifications of this basic magnetic sector design. In fact, magnetic sector analyzers are used today only in **double-focusing mass spectrometers.** The particles leaving the ionization chamber do not all have precisely the same velocity, so the beam of ions passes through an electric field region before or after the magnetic sector (Fig. 8.11). In the presence of an electric field, the particles all travel at the same velocity. The particles describe a curved path in each of these regions, and the resolution of the mass analyzer improves—by a factor of 10 or more over the magnetic sector alone.

C. Quadrupole Mass Analyzers

In a **quadrupole mass analyzer** (Fig. 8.12), a set of four solid rods is arranged parallel to the direction of the ion beam. The rods should be hyperbolic in cross section, although cylindrical rods may be used. A direct-current (DC) voltage and a radiofrequency (RF) is applied to the rods, generating an oscillating electrostatic field in the region between the rods. Depending on the ratio of the RF amplitude to the DC voltage, ions acquire an oscillation in this electrostatic field. Ions of an incorrect *m/z* ratio (too small or too large) undergo an unstable oscillation. The amplitude of the oscillation continues to increase until the particle strikes one of the rods. Ions of the correct mass-to-charge ratio undergo a stable oscillation of constant amplitude and travel down the quadrupole axis with a "corkscrew"-type trajectory. These ions do not strike the quadrupole rods but pass

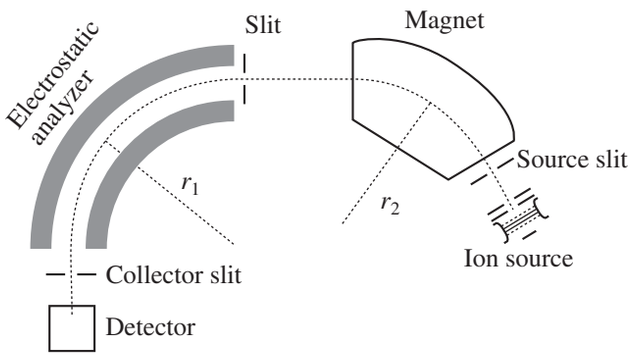


FIGURE 8.11 Schematic of a double-focusing mass analyzer. (From Smith, R. M., *Understanding Mass Spectra, A Basic Approach*, 2nd ed., John Wiley and Sons, New York, 2004. Reprinted with permission.)

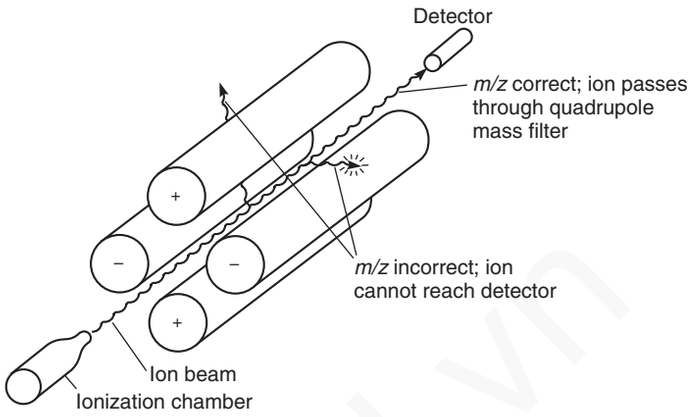


FIGURE 8.12 Quadrupole mass analyzer.

through the analyzer to reach the detector. Like the magnetic sector analyzer, the quadrupole can be scanned from high to low values of *m/z*. A quadrupole mass analyzer is found in most "benchtop" GC-MS systems and typically has a *m/z* range from 0 to 1000, although quadrupole analyzers are available on some LC-MS systems with *m/z* ranges that approach 2000. Quadrupole mass spectrometers are low-resolution instruments (R~3000) incapable of providing exact elemental composition of the sample.

The quadrupole **ion trap** mass analyzer operates by similar principles as the linear quadrupole described above and is a common mass analyzer found in GC-MS instruments. The ion trap consists of two hyperbolic endcap electrodes and a doughnut-shaped ring electrode (the endcap electrodes are connected). An alternating current (AC) (or DC) and an RF potential is applied between the endcaps and the ring electrode (Fig. 8.13). In the linear quadrupole analyzer, ions of different *m/z* values are allowed to pass in turn through the quadrupole by adjusting the RF and DC voltages. In the ion trap, ions of all *m/z* values are in the trap simultaneously, oscillating in concentric trajectories. Sweeping the RF potential results in the removal of ions with increasing *m/z* values by putting them in unstable trajectory that causes them to be ejected from the trap in the axial direction toward the detector. This process is called **resonant ejection.** Ion trap mass analyzers are somewhat more sensitive than linear quadrupole instruments, but they have similar resolution capabilities.

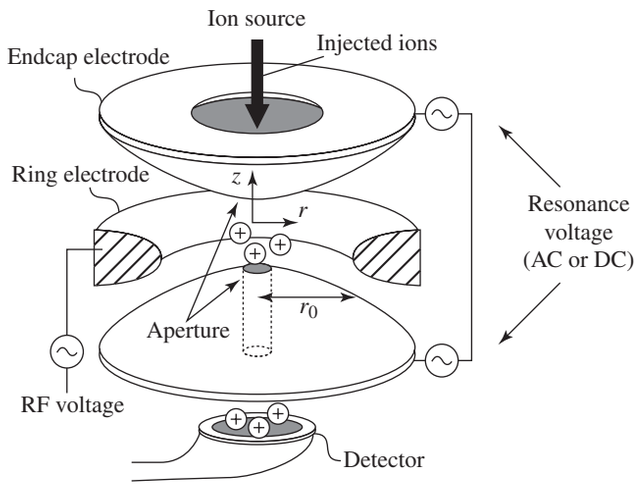


FIGURE 8.13 Quadrupole ion trap mass analyzer. (From Gross, J. H., *Mass Spectrometry: A Textbook,* Springer, Berlin, 2004. Reprinted with permission.)

Because the ion trap contains ions of all values of *m/z* at the same time (as well as neutral molecules that were not ionized prior to entering the trap), ion trap mass analyzers are also sensitive to overload and ion–molecule collisions that complicate the resulting spectrum. Recall that not all of the sample molecules get ionized—many remain uncharged. These neutral species move in a random path in the ion trap, resulting in collisions with ions as the ions oscillate in their stable trajectories. These collisions result in chemical ionization-type ionization events (Equation 8.18). This is sometimes referred to as *self-CI.*

$$\underset{\text{fragment ion}}{(R-H)^+} + \underset{\substack{\text{neutral}\\ \text{sample molecule}}}{M} \longrightarrow \underset{\substack{\text{neutral}\\ \text{or radical}}}{R} + \underset{\substack{\text{protonated}\\ \text{molecular ion}}}{(M+H)^+} \qquad \textbf{Equation 8.18}$$

The result is an abnormally large $(M + H)^+$ peak in the mass spectrum. This is observed in Figure 8.14, in which the base peak in the EI-MS of methyl dodecanoate under standard conditions has $m/z = 215$, representing an $(M + H)^+$ ion produced in the ion trap from ion–molecule conditions. This self-CI process can be minimized by increasing ionization efficiency, reducing the number of ions in the trap (injecting less sample), or both. The bottom spectrum in Figure 8.14 was acquired under optimized ion trap conditions with a longer ion residence time. Now, the M^+ ion is clearly visible, although the (M + 1) peak is still much larger than it should be based on isotopic contributions of ^{13}C alone (see Section 8.7). Fortunately, the presence of the larger (M + 1) peak rarely has an adverse effect on spectral library searches done by a computer. The visual inspection of a sample spectrum to a printed standard spectrum is quite another matter. The self-CI peak becomes quite problematic when one is attempting to characterize unknowns if one does not know the molecular formula or functional groups present ahead of time.

D. Time-of-Flight Mass Analyzers

The **time-of-flight** (**TOF**) mass analyzer is based on the simple idea that the velocities of two ions, created at the same instant with the same kinetic energy, will vary depending on the mass of the ions—the lighter ion will have a higher velocity. If these ions are traveling toward the mass spectrometer's detector, the faster (lighter) ion will strike the detector first. Examining this concept further, the kinetic energy of an ion accelerated through an electrical potential V will be

$$zV = \frac{mv^2}{2} \qquad \textbf{Equation 8.19}$$

and the velocity of the ion is the length of the flight path L divided by the time *t* it takes the ion to travel over that distance:

$$v = \frac{L}{t} \qquad \textbf{Equation 8.20}$$

Replacing this expression for v in Equation 8.19 gives

$$zV = \frac{mL^2}{2t^2} \qquad \textbf{Equation 8.21}$$

Thus, it follows that

$$\frac{m}{z} = \frac{2Vt^2}{L^2} \qquad \textbf{Equation 8.22}$$

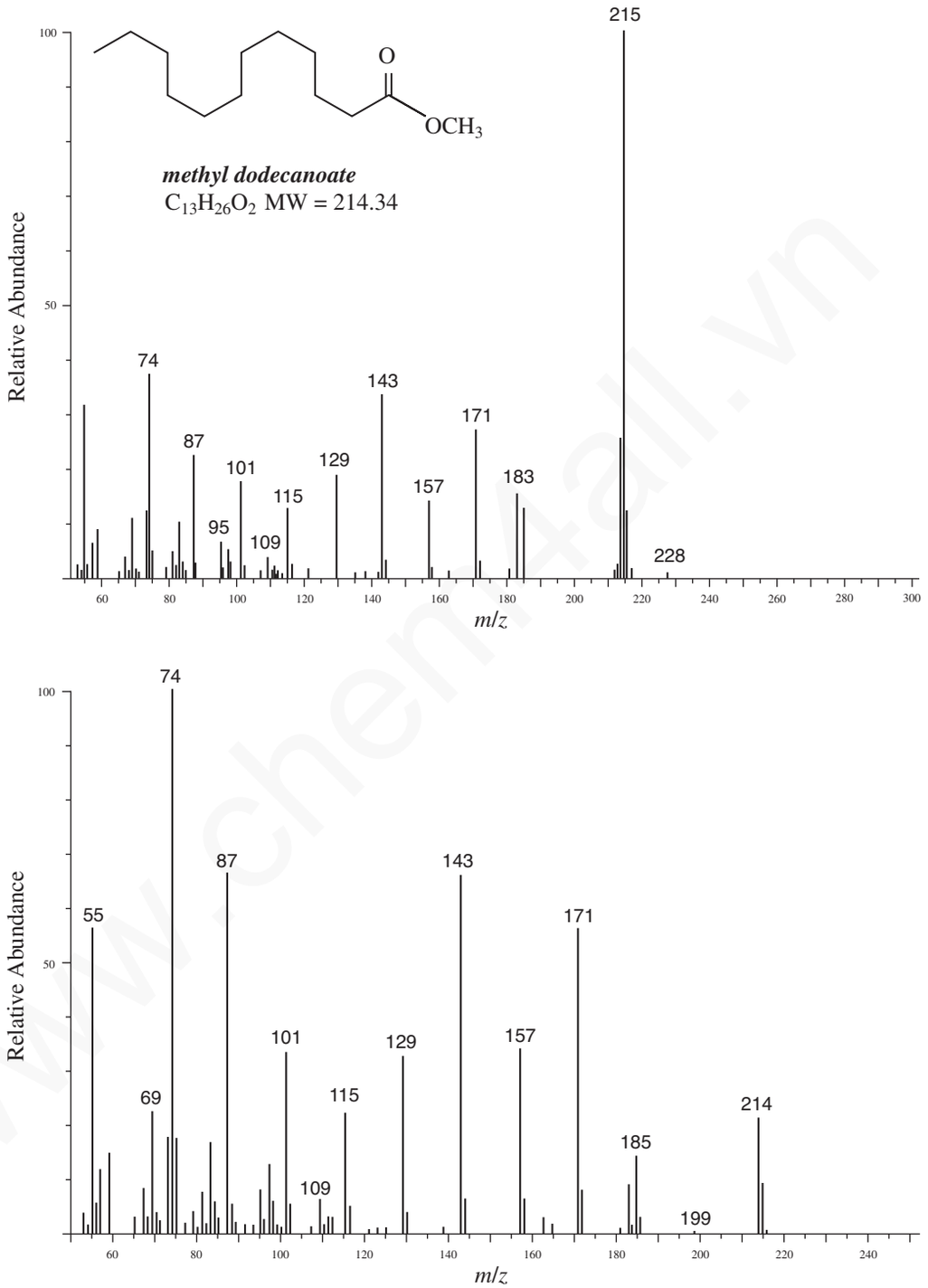


FIGURE 8.14 EI-MS of methyl dodecanoate using a quadrupole ion trap mass analyzer. Standard conditions (top) and optimized conditions to minimize ion–molecule collisions and self-CI (bottom). (Reproduced from Varian, Inc.)

The TOF mass analyzer (Fig. 8.15) requires very fast electronics to accurately measure ion flight times that may be submicrosecond. Furthermore, the ions in a TOF system must be created in short, well-defined pulses so that the ions all start their journey toward the detector at the same moment. The first requirement explains why TOF instrumentation (first developed in the 1940s and 1950s) did not become widely used until the 1980s and 1990s, when suitable circuitry became cost-effective. The last requirement is perfectly suited for the MALDI ionization technique, and MALDI/TOF mass spectrometers have found wide use in the analysis of biomolecules and synthetic polymers. In theory, TOF mass analyzers have no upper limit to their effective mass range, and these mass analyzers have high sensitivity. Unlike magnetic sector or quadrupole spectrometers, in which some of the ions are "thrown away" during the experiment, TOF instruments are able to analyze (in principle) every ion created in the initial pulse. Mass data have been obtained using MALDI/TOF from samples with molecular weights of 300,000 amu and as little as a few hundred attomoles of material.

The major disadvantage of the TOF analyzer is its inherently low resolution. The mass resolution (**R**, Eq. 8.17) of the TOF instrument is proportional to the ion's flight time, so using longer drift tubes increases resolution. Flight tubes a few meters long are commonly used in high-end instruments. With shorter drift tubes, R of only 200–500 is possible. A modification to the TOF analyzer that increases resolution is the ion reflector. The reflector is an electric field behind the free drift region of the spectrometer that behaves as an ion mirror. The reflector is able to refocus ions of slightly different kinetic energies and, if set at a small angle, sends the ions on a path back toward the original ion source. This essentially doubles the ion flight path as well. In reflector TOF instruments, a mass resolution of several thousand is possible.

Time-of-flight mass spectrometers are relatively simple, which makes it possible to use them in the field. During the 1991 Gulf War, concern arose that Iraqi troops might be releasing chemical warfare agents against American troops. To guard against that possibility, the U.S. Army deployed a number of tracked vehicles, each equipped with a mass spectrometer. The mass spectrometer was used to sample the air and provide advance warning should any poisonous gases be released into the air. Basic TOF mass spectrometers are also used to detect residue from explosives and illegal drugs at security screening stations in airports. Because of their value for studying short-lived species, TOF mass spectrometers are particularly useful in kinetic studies, especially with applications to very fast reactions. Very rapid reactions such as combustion and explosions can be investigated with this technique.

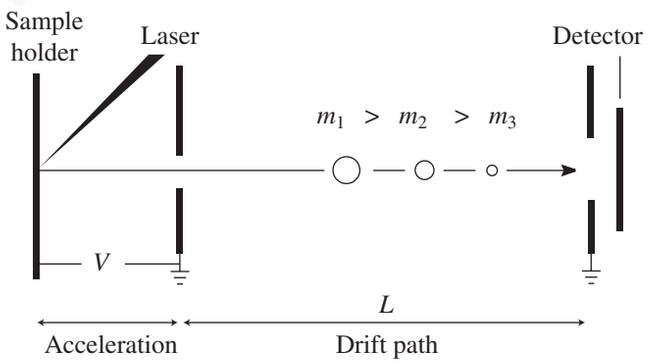


FIGURE 8.15 Schematic representation of a MALDI/TOF mass spectrometer.

8.5 DETECTION AND QUANTITATION: THE MASS SPECTRUM

The **detector** of a typical mass spectrometer consists of a counter that produces a current that is proportional to the number of ions that strike it. This sounds quite reasonable until one pauses to think about exactly how many ions will strike the detector in a typical experiment. Consider a typical application—analysis of a small organic molecule (MW = 250) by EI GC-MS. A 1.0-μL injection of a 1.0 mg/mL sample contains 3.6×10^{15} molecules. If the GC is running in split mode with a 1:100 ratio, only 3.6×10^{13} molecules enter the chromatographic column. A mass spectrum acquired at the top of the GC peak may only account for 10% of the material that elutes, and if only 1 in 1000 molecules is converted to an ion, just 3.6 billion ions are available. This still sounds like a lot of charged particles, but wait! In a scanning spectrometer, most of these ions never reach the detector; as the mass analyzer sweeps through the range of 35 to 300 *m/z*, most of the ions discharge on the quadrupole rods, for example. In a case like this, an ion of any given *m/z* value makes it through the analyzer only 1 time out of 300. Clearly, each peak in the mass spectrum represents a very small electrical signal, and the detector must be able to amplify this tiny current.

Through the use of **electron multiplier** circuits, this current can be measured so accurately that the current caused by just one ion striking the detector can be measured. When an ion strikes the surface of the electron multiplier (lead-doped glass coated with lead oxide), two electrons are ejected. The approximately 2-kV potential difference between the opening and end of the detector draws the electrons further into the electron multiplier, where each electron strikes the surface again, each causing the ejection of two more electrons. This process continues until the end of the electron multiplier is reached, and the electrical current is analyzed and recorded by the data system. The signal amplification just described will be 2^n, where n is the number of collisions with the electron multiplier surface. Typical electron multipliers provide a signal increase of 10^5–10^6. Two configurations of electron multipliers are shown in Figure 8.16. A curved electron multiplier shortens the ion path and results in a signal with less noise. Photomultiplier detectors operate on a similar principle as the electron multiplier, except ion collisions with the fluorescent screen in the photomultiplier result in photon emission proportional to the number of ion collisions. The intensity of the light (rather than electrical current) is then analyzed and recorded by the data system.

The signal from the detector is fed to a **recorder**, which produces the mass spectrum. In modern instruments, the output of the detector is fed through an interface to a computer. The computer can

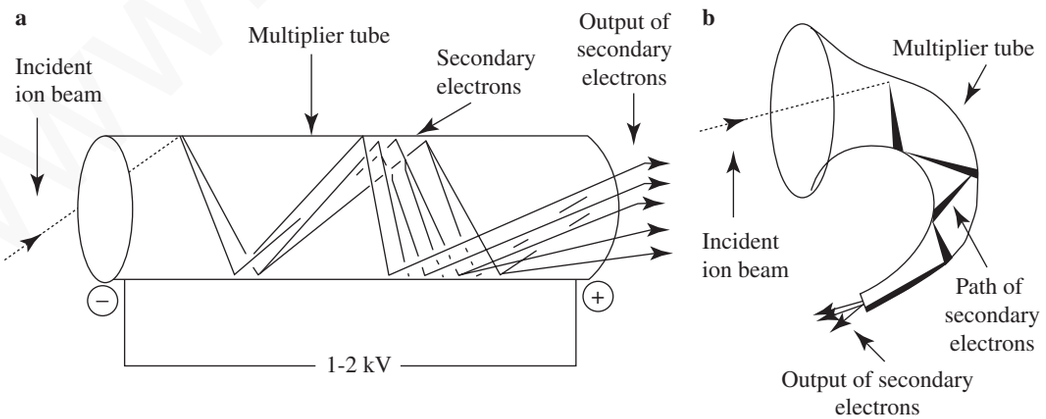


FIGURE 8.16 Schematic representation of a linear channel electron multiplier (a) and a curved channel electron multiplier (b). (From Gross, J. H., *Mass Spectrometry: A Textbook,* Spinger, Berlin, 2004. Reprinted with permission.)

store the data, provide the output in both tabular and graphic forms, and compare the data to standard spectra, which are contained in spectra libraries that are also stored in the computer.

Figure 8.17 is a portion of a typical mass spectrum—that of dopamine, a substance that acts as a neurotransmitter in the central nervous system. The *x*-axis of the mass spectrum is the *m/z* ratio, and the *y*-axis is ion abundance. Mass spectral results may also be presented in tabular form, as in Table 8.2. The most abundant ion formed in the ionization chamber gives rise to the tallest peak in the mass spectrum, called the **base peak.** In the mass spectrum of dopamine, the base peak is indicated at an *m/z* value of 124. The spectral intensities are normalized by setting the base peak to relative abundance 100, and the rest of the ions are reported as percentages of the base peak intensity. The low end of the *m/z* range is typically 35 or 40 to eliminate the very large peaks from low-mass fragments from background ions arising from gases and small alkyl fragments. When acquiring data under CI conditions, the low end of the *m/z* range is set higher to eliminate the large peaks from the reagent gas ions.

As discussed earlier, in EI-MS, the beam of electrons in the ionization chamber converts some of the sample molecules to positive ions. The simple removal of an electron from a molecule yields an ion with weight that is the actual molecular weight of the original molecule. This is the **molecular ion,** which is usually represented by $\mathbf{M^+}$ or $\mathbf{M^{\bullet +}}$. Strictly speaking, the molecular ion is a **radical cation** since it contains an unpaired electron as well as a positive charge. The value of *m/z* at which the molecular ion appears on the mass spectrum, assuming that the ion has only one electron missing, gives the molecular weight of the original molecule. If you can identify the molecular ion peak in the mass spectrum, you will be able to use the spectrum to determine the molecular weight of an unknown substance. Ignoring heavy isotopes for the moment, the molecular ion peak is the peak in the mass spectrum with the largest *m/z* value; it is indicated in the graphic presentation in Figure 8.17 (*m/z* = 153).

Molecules in nature do not occur as isotopically pure species. Virtually all atoms have heavier isotopes that occur in characteristic natural abundances. Hydrogen occurs largely as ^{1}H, but about 0.02% of hydrogen atoms are the isotope ^{2}H. Carbon normally occurs as ^{12}C, but about 1.1% of carbon atoms are the heavier isotope ^{13}C. With the possible exception of fluorine and a few other elements, most elements have a certain percentage of naturally occurring heavier isotopes.

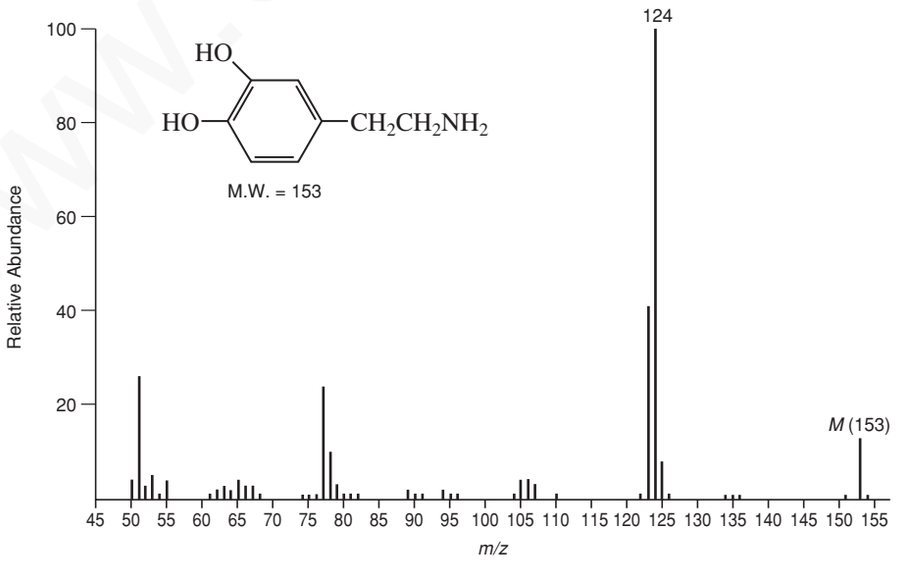


FIGURE 8.17 Partial EI-MS of dopamine.

TABLE 8.2
EI-MS OF DOPAMINE. TABULAR REPRESENTATION OF THE DATA IN FIGURE 8.17

m/z	Relative Abundance	*m/z*	Relative Abundance	*m/z*	Relative Abundance
50	4.00	76	1.48	114	0.05
50.5	0.05	77	24.29	115	0.19
51	25.71	78	10.48	116	0.24
51.5	0.19	79	2.71	117	0.24
52	3.00	80	0.81	118	0.14
52.5	0.62	81	1.05	119	0.19
53	5.43	82	0.67	120	0.14
53.5	0.19	83	0.14	121	0.24
54	1.00	84	0.10	122	0.71
55	4.00	85	0.10	123	41.43
56	0.43	86	0.14	124	100.00 (base peak)
56.5	0.05 (metastable peak)	87	0.14	125	7.62
57	0.33	88	0.19	126	0.71
58	0.10	89	1.57	127	0.10
58.5	0.05	89.7	0.10 (metastable peak)	128	0.10
59	0.05	90	0.57	129	0.10
59.5	0.05	90.7	0.10 (metastable peak)	131	0.05
60	0.10	91	0.76	132	0.19
60.5	0.05	92	0.43	133	0.14
61	0.52	93	0.43	134	0.52
61.5	0.10	94	1.76	135	0.52
62	1.57	95	1.43	136	1.48
63	3.29	96	0.52	137	0.33
64	1.57	97	0.14	138	0.10
65	3.57	98	0.05	139	0.10
65.5	0.05	99	0.05	141	0.19
66	3.14	100.6	0.19 (metastable peak)	142	0.05
66.5	0.14	101	0.10	143	0.05
67	2.86	102	0.14	144	0.05
67.5	0.10	103	0.24	145	0.05
68	0.67	104	0.76	146	0.05
69	0.43	105	4.29	147	0.05
70	0.24	106	4.29	148	0.10
71	0.19	107	3.29	149	0.24
72	0.05	108	0.43	150	0.33
73	0.14	109	0.48	151	1.00
74	0.67	110	0.86	152	0.38
74.5	0.05	111	0.10	153	13.33 (molecular ion)
75	1.00	112	0.05	154	1.48
75.5	0.14	113	0.05	155	0.19

Peaks caused by ions bearing those heavier isotopes also appear in mass spectra. The relative abundances of such isotopic peaks are proportional to the abundances of the isotopes in nature. Most often, the isotopes occur one or two mass units above the mass of the "normal" atom. Therefore, besides looking for the molecular ion (M^+) peak, one would also attempt to locate ***M* + 1** and ***M* + 2** peaks. As Section 8.6 will demonstrate, the relative abundances of the $M + 1$ and $M + 2$ peaks can be used to determine the molecular formula of the substance being studied. In Figure 8.17, the isotopic peaks are the low-intensity peaks at *m/z* values (154 and 155) higher than that of the molecular ion peak (see also Table 8.2).

We have seen that the beam of electrons in the ionization chamber can produce the molecular ion. This beam is also sufficiently powerful to break some of the bonds in the molecule, producing a series of molecular fragments. The positively charged fragments are also accelerated in the ionization chamber, sent through the analyzer, detected, and recorded on the mass spectrum. These **fragment ions** appear at *m/z* values corresponding to their individual masses. Very often, a fragment ion, rather than the parent ion, is the most abundant ion produced in the mass spectrum. A second means of producing fragment ions exists if the molecular ion, once it is formed, is so unstable that it disintegrates before it can pass into the accelerating region of the ionization chamber. Lifetimes less than 10^{-6} sec are typical in this type of fragmentation. The fragments that are charged then appear as fragment ions in the mass spectrum. A great deal of structural information about a substance can be determined from an examination of the fragmentation pattern in the mass spectrum. Section 8.8 will examine some fragmentation patterns for common classes of compounds.

Ions with lifetimes on the order of 10^{-6} sec are accelerated in the ionization chamber before they have an opportunity to disintegrate. These ions may disintegrate into fragments *while they are passing into the analyzer region* of the mass spectrometer. The fragment ions formed at that point have considerably lower energy than normal ions since the uncharged portion of the original ion carries away some of the kinetic energy that the ion received as it was accelerated. As a result, the fragment ion produced in the analyzer follows an abnormal flight path on its way to the detector. This ion appears at an *m/z* ratio that depends on its own mass as well as the mass of the original ion from which it formed. Such an ion gives rise to what is termed a **metastable ion peak** in the mass spectrum. Metastable ion peaks are usually broad peaks, and they frequently appear at nonintegral values of *m/z*. The equation that relates the position of the metastable ion peak in the mass spectrum to the mass of the original ion is

$$m_1^+ \longrightarrow m_2^+ \qquad \textbf{Equation 8.23}$$

and

$$m^* = \frac{(m_2)^2}{m_1} \qquad \textbf{Equation 8.24}$$

where m^* is the apparent mass of the metastable ion in the mass spectrum, m_1 is the mass of the original ion from which the fragment formed, and m_2 is the mass of the new fragment ion. A metastable ion peak is useful in some applications since its presence definitively links two ions together. Metastable ion peaks can be used to prove a proposed fragmentation pattern or to aid in the solution of structure proof problems.

8.6 DETERMINATION OF MOLECULAR WEIGHT

Section 8.3 showed that when a beam of high-energy electrons impinges on a stream of sample molecules, ionization of electrons from the molecules takes place. The resulting ions, called **molecular ions,** are then accelerated, sent through a magnetic field, and detected. If these molecular ions have lifetimes of at least 10^{-5}sec, they reach the detector without breaking into fragments. The user then

observes the *m/z* ratio that corresponds to the molecular ion to determine the molecular weight of the sample molecule.

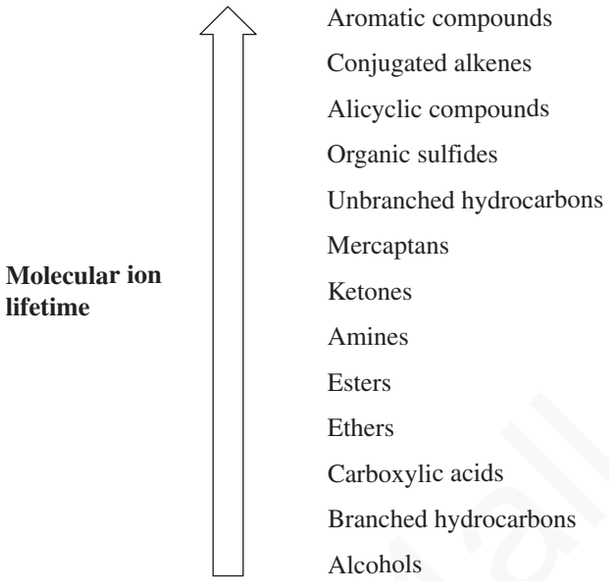
In practice, molecular weight determination is not quite as easy as the preceding paragraph suggests. First, you must understand that the value of the mass of any ion accelerated in a mass spectrometer is its true mass, the sum of the masses of each atom in that single ion, and not its molecular weight calculated from chemical atomic weights. The chemical scale of atomic weights is based on weighted averages of the weights of all of the isotopes of a given element. The mass spectrometer can distinguish between masses of particles bearing the most common isotopes of the elements and particles bearing heavier isotopes. Consequently, the masses that are observed for molecular ions are the masses of the molecules in which every atom is present as its most common isotope. In the second place, molecules subjected to bombardment by electrons may break apart into fragment ions. As a result of this fragmentation, mass spectra can be quite complex, with peaks appearing at a variety of *m/z* ratios. You must be quite careful to be certain that the suspected peak is indeed that of the molecular ion and not that of a fragment ion. This distinction becomes particularly crucial when the abundance of the molecular ion is low, as when the molecular ion is rather unstable and fragments easily. The masses of the ions detected in the mass spectrum must be measured accurately. An error of only one mass unit in the assignment of mass spectral peaks can render determination of structure impossible.

One method of confirming that a particular peak corresponds to a molecular ion is to vary the energy of the ionizing electron beam. If the energy of the beam is lowered, the tendency of the molecular ion to fragment lessens. As a result, the intensity of the molecular ion peak should increase with decreasing electron potential, while the intensities of the fragment ion peaks should decrease. Certain facts must apply to a molecular ion peak:

1. The peak must correspond to the ion of highest mass in the spectrum, excluding isotopic peaks that occur at higher masses. The isotopic peaks are usually of much lower intensity than the molecular ion peak. At the sample pressures used in most spectral studies, the probability that ions and molecules will collide to form heavier particles is quite low. Care must be taken, especially with GC-MS spectra, to recognize background ions that are a result of column bleed—small pieces of the silicone-based stationary phase of the capillary GC column.
2. The ion must have an odd number of electrons. When a molecule is ionized by an electron beam, it loses one electron to become a radical cation. The charge on such an ion is 1, thus making it an ion with an odd number of electrons.
3. The ion must be capable of forming the important fragment ions in the spectrum, particularly the fragments of relatively high mass, by loss of logical neutral fragments. Fragment ions in the range from (M – 3) to (M – 14) and (M – 21) to (M – 25) are not reasonable losses. Similarly, no fragment ion can contain a greater number of atoms of a particular element than the molecular ion. Section 8.8 will explain fragmentation processes in detail.

The observed abundance of the suspected molecular ion must correspond to expectations based on the assumed molecule structure. Highly branched substances undergo fragmentation very easily. Observation of an intense molecular ion peak for a highly branched molecule thus would be unlikely. The lifetimes of molecular ions vary according to the generalized sequence shown on page 440.

Another rule that is sometimes used to verify that a given peak corresponds to the molecular ion is the so-called **Nitrogen Rule.** This rule states that if a compound has an even number of nitrogen atoms (zero is an even number), its molecular ion will appear at an even mass value. On the other hand, a molecule with an odd number of nitrogen atoms will form a molecular ion with an odd mass. The Nitrogen Rule stems from the fact that nitrogen, although it has an even mass, has an odd-numbered valence. Consequently, an extra hydrogen atom is included as a part of the molecule, giving it an odd mass. To picture this effect, consider ethylamine, $CH_3CH_2NH_2$. This substance has



one nitrogen atom, and its mass is an odd number (45), whereas ethylenediamine, $H_2NCH_2CH_2NH_2$, has two nitrogen atoms, and its mass is an even number (60).

You must be careful when studying molecules containing chlorine or bromine atoms since these elements have two commonly occurring isotopes. Chlorine has isotopes of 35 (relative abundance = 75.77%) and 37 (relative abundance = 24.23%); bromine has isotopes of 79 (relative abundance = 50.5%) and 81 (relative abundance = 49.5%). When these elements are present, take special care not to confuse the molecular ion peak with a peak corresponding to the molecular ion with a heavier halogen isotope present. This is discussed further in Section 8.7B.

In many of the cases that you are likely to encounter in mass spectrometry, the molecular ion can be observed in the mass spectrum. Once you have identified that peak in the spectrum, the problem of molecular weight determination is solved. However, with molecules that form unstable molecular ions, you may not observe the molecular ion peak. Molecular ions with lifetimes less than 10^{-5} sec break up into fragments before they can be accelerated. The only peaks that are observed in such cases are those due to fragment ions. In many of these cases, using a mild CI method will allow for detection of the pseudomolecular ion $(M + H)^+$, and one can determine the molecular weight of the compound by simply subtracting one mass unit for the extra H atom present. If a molecular ion is not able to be detected by this method, then you will be obliged to deduce the molecular weight of the substance from the fragmentation pattern on the basis of known patterns of fragmentation for certain classes of compounds. For example, alcohols undergo dehydration very easily. Consequently, the initially formed molecular ion loses water (mass = 18) as a neutral fragment before it can be accelerated toward the mass analyzer. To determine the mass of an alcohol molecular ion, you must locate the heaviest fragment and keep in mind that it may be necessary to add 18 to its mass. Similarly, acetate esters undergo loss of acetic acid (mass = 60) easily. If acetic acid is lost, the weight of the molecular ion is 60 mass units higher than the mass of the heaviest fragment.

Since oxygen compounds form fairly stable oxonium ions and nitrogen compounds form ammonium ions, ion–molecule collisions form peaks in the mass spectrum that appear one mass unit *higher* than the mass of the molecular ion. This was referred to as self-CI in the discussion of the ion trap mass analyzer in Section 8.4. At times, the formation of ion–molecule products may be helpful in the determination of the molecular weight of an oxygen or nitrogen compound, but this self-CI can sometimes be confusing when one is trying to determine the true molecular ion in a spectrum of an unknown sample.

8.7 DETERMINATION OF MOLECULAR FORMULAS

A. Precise Mass Determination

Perhaps the most important application of high-resolution mass spectrometers is the determination of very precise molecular weights of substances. We are accustomed to thinking of atoms as having integral atomic masses—for example, H = 1, C = 12, and O = 16. However, if we determine atomic masses with sufficient precision, we find that this is not true. In l923, Aston discovered that every isotopic mass is characterized by a small "mass defect." The mass of each atom actually differs from a whole mass number by an amount known as the *nuclear packing fraction*. Table 8.4 gives the actual masses of some atoms.

Depending on the atoms contained in a molecule, it is possible for particles of the same nominal mass to have slightly different measured masses when precise determinations are possible. To illustrate, a molecule with a molecular weight of 60.1 g/mole could be C_3H_8O, $C_2H_8N_2$, $C_2H_4O_2$, or CH_4N_2O (Table 8.3). Thus, a **low-resolution mass spectrum** (**LRMS**) will not be able to distinguish between these formulas. If one calculates the precise masses for each formula using the mass of the most common isotope for each element, however, mass differences between the formulas appear in the second and third decimal places. Observation of a molecular ion with a mass of 60.058 would establish that the unknown molecule is C_3H_8O. An instrument with a resolution of about 5320 would be required to distinguish among these peaks. That is well within the capability of modern mass spectrometers, which can attain resolutions greater than one part in 20,000. A **high-resolution mass spectrum** (**HRMS**), then, not only determines the exact mass of the molecular ion, it allows one to know the exact molecular formula (see Appendix 11). Typical high-resolution instruments can determine an ion's *m/z* value to four or five decimal places. When the precise mass is measured to this degree of precision, only one formula (excluding isotopes) will fit the data. HRMS is extremely valuable to synthetic chemists as well as researchers doing natural product isolation/structure determination work or drug metabolism studies. It is interesting to compare the precision of molecular weight determinations by mass spectrometry with the chemical methods described in Chapter 1, Section 1.2. Chemical methods give results that are accurate to only two or three significant figures (±0.1% to 1%). Molecular weights determined by mass spectrometry have an accuracy of about ±0.005%. Clearly, mass spectrometry is much more precise than chemical methods of determining molecular weight. Precise mass values for some commonly encountered elements may be found in Table 8.4.

B. Isotope Ratio Data

The preceding section described a method of determining molecular formulas using data from high-resolution mass spectrometers. Another method of determining molecular formulas is to examine the relative intensities of the peaks due to the molecular ion and related ions that bear one or more

TABLE 8.3
SELECTED COMPARISONS OF MOLECULAR WEIGHTS AND PRECISE MASSES

Molecular Formula (MF)	Molecular Weight (MW) (g/mole)	Precise Mass
C_3H_8O	60.1	60.05754
$C_2H_8N_2$	60.1	60.06884
$C_2H_4O_2$	60.1	60.02112
CH_4N_2O	60.1	60.03242

TABLE 8.4
PRECISE MASSES OF SOME COMMON ELEMENTS

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00797	^{1}H	1.00783
		^{2}H	2.01410
Carbon	12.01115	^{12}C	12.0000
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.086	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.974	^{31}P	30.9738
Sulfur	32.064	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.453	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.909	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.904	^{127}I	126.9045

heavy isotopes (the molecular ion cluster). This method would not be commonly used by researchers who have a high-resolution mass spectrometer at their disposal or are able to submit their samples to a service laboratory for exact mass analysis. Use of the molecular ion cluster can be useful, though, for a relatively quick determination of the molecular formula that does not require the much more expensive high-resolution instrument. This method is useless, of course, when the molecular ion peak is very weak or does not appear. Sometimes the isotopic peaks surrounding the molecular ion are difficult to locate in the mass spectrum, and the results obtained by this method may at times be rendered ambiguous.

The example of ethane (C_2H_6) can illustrate the determination of a molecular formula from a comparison of the intensities of mass spectral peaks of the molecular ion and the ions bearing heavier isotopes. Ethane has a molecular weight of 30 when it contains the most common isotopes of carbon and hydrogen. Its molecular ion peak should appear at a position in the spectrum corresponding to $m/z = 30$. Occasionally, however, a sample of ethane yields a molecule in which one of the carbon atoms is a heavy isotope of carbon, ^{13}C. This molecule would appear in the mass spectrum at $m/z = 31$. The relative abundance of ^{13}C in nature is 1.08% of the ^{12}C atoms. In the tremendous number of molecules in a sample of ethane gas, one of the carbon atoms of ethane will turn out to be a ^{13}C atom 1.08% of the time. Since there are two carbon atoms in the molecule, an ethane with mass 31 will turn up (2×1.08) or 2.16% of the time. Thus, we would expect to observe a peak at $m/z = 31$ with an intensity of 2.16% of the molecular ion peak intensity at $m/z = 30$. This mass 31 peak is called the $M + 1$ peak since its mass is one unit higher than that of the molecular ion. You may notice that a particle of mass 31 could form in another manner. If a deuterium atom, ^{2}H, replaced one of the hydrogen

atoms of ethane, the molecule would also have a mass of 31. The natural abundance of deuterium is only 0.016% of the abundance of ^{1}H atoms. The intensity of the $M + 1$ peak would be (6×0.016) or 0.096% of the intensity of the molecular ion peak if we consider only contributions due to deuterium. When we add these contributions to those of ^{13}C, we obtain the observed intensity of the $M + 1$ peak, which is 2.26% of the intensity of the molecular ion peak. An ion with $m/z = 32$ can form if *both* of the carbon atoms in an ethane molecule are ^{13}C. The probability that a molecule of formula $^{13}C_2H_6$ will appear in a natural sample of ethane is $(1.08 \times 1.08)/100$, or 0.01%.

A peak that appears two mass units higher than the mass of the molecular ion peak is called the $M + 2$ peak. The intensity of the $M + 2$ peak of ethane is only 0.01% of the intensity of the molecular ion peak. The contribution due to two deuterium atoms replacing hydrogen atoms would be $(0.016 \times 0.016)/100 = 0.00000256\%$, a negligible amount. To assist in the determination of the ratios of molecular ion, $M + 1$, and $M + 2$ peaks, Table 8.5 lists the natural abundances of some common elements and their isotopes. In this table, the relative abundances of the isotopes of each element are calculated by setting the abundances of the most common isotopes equal to 100.

To demonstrate how the intensities of the $M + 1$ and $M + 2$ peaks provide a unique value for a given molecular formula, consider two molecules of mass 42, propene (C_3H_6) and diazomethane (CH_2N_2). For propene, the intensity of the $M + 1$ peak should be $(3 \times 1.08) + (6 \times 0.016) = 3.34\%$, and the intensity of the $M + 2$ peak should be 0.05%. The natural abundance of ^{15}N isotopes of nitrogen is 0.38% of the abundance of ^{14}N atoms. In diazomethane, we expect the relative intensity of the $M + 1$ peak to be $1.08 + (2 \times 0.016) + (2 \times 0.38) = 1.87\%$ of the intensity of the molecular ion peak, and the intensity of the $M + 2$ peak would be 0.01% of the intensity of the molecular ion peak. Table 8.6 summarizes these intensity ratios. It shows that the two molecules have nearly the same molecular weight, but the relative intensities of the $M + 1$ and $M + 2$ peaks that they yield are quite different.

As an additional illustration, Table 8.7 compares the ratios of the molecular ion, $M + 1$, and $M + 2$ peaks for three substances of mass 28: carbon monoxide, nitrogen, and ethene. Again, notice that the relative intensities of the $M + 1$ and $M + 2$ peaks provide a means of distinguishing among these molecules.

As molecules become larger and more complex, the number of possible combinations that yield $M + 1$ and $M + 2$ peaks grows. For a particular combination of atoms, the intensities of these peaks

TABLE 8.5
NATURAL ABUNDANCES OF COMMON ELEMENTS AND THEIR ISOTOPES

Element	Relative Abundance					
Hydrogen	^{1}H	100	^{2}H	0.016		
Carbon	^{12}C	100	^{13}C	1.08		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0
Iodine	^{127}I	100				

TABLE 8.6
ISOTOPE RATIOS FOR PROPENE AND DIAZOMETHANE

		Relative Intensities		
Compound	**Molecular Mass**	***M***	***M* + 1**	***M* + 2**
C_3H_6	42	100	3.34	0.05
CH_2N_2	42	100	1.87	0.01

TABLE 8.7
ISOTOPE RATIOS FOR CO, N_2, AND C_2H_4

		Relative Intensities		
Compound	**Molecular Mass**	***M***	***M* + 1**	***M* + 2**
CO	28	100	1.12	0.2
N_2	28	100	0.76	
C_2H_4	28	100	2.23	0.01

relative to the intensity of the molecular ion peak are unique. Thus, the isotope ratio method can be used to establish the molecular formula of a compound. Examination of the intensity of the $M + 2$ peak is also useful for obtaining information about elements that may be present in the molecular formula. An unusually intense $M + 2$ peak can indicate that sulfur or silicon is present in the unknown substance. The relative abundances of ^{33}S and ^{34}S are 0.78 and 4.40, respectively, and the relative abundance of ^{30}Si is 3.35. A trained chemist knows that a larger-than-normal $M + 2$ peak can be the first hint that sulfur or silicon is present. Chlorine and bromine also have important $M + 2$ isotopes, and they are discussed separately below.

Tables of possible combinations of carbon, hydrogen, oxygen, and nitrogen and intensity ratios for the $M + 1$ and $M + 2$ peaks for each combination have been developed. An example of this sort of table is found in Appendix 11. More extensive tables of intensity ratios for the $M + 1$ and $M + 2$ peaks may be found in specialized books on interpreting mass spectra. Accurate calculation of the relative intensities of isotope peaks in a molecular ion cluster for compounds containing several elements with isotopes is time consuming to do by hand as it requires polynomial expansions. Fortunately, many websites dealing with mass spectrometry have isotope calculators that make this a trivial task. Some of these sites may be found in the references at the end of this chapter.

For compounds containing only C, H, N, O, F, Si, P, and S, the relative intensities of $M + 1$ and $M + 2$ peaks can be estimated quickly using simplified calculations. The formula to calculate the $M + 1$ peak intensity (relative to $M^+ = 100$) for a given formula is found in Equation 8.25. Similarly, the intensity of an $M + 2$ peak intensity (relative to $M^+ = 100$) may be found by using Equation 8.26.

$$[M+1] = (\text{number of C} \times 1.1) + (\text{number of H} \times 0.015) + (\text{number of N} \times 0.37) + (\text{number of O} \times 0.04) + (\text{number of S} \times 0.8) + (\text{number of Si} \times 5.1) \quad \textbf{Equation 8.25}$$

$$[M+2] = \frac{(\text{number of C} \times 1.1)^2}{200} + (\text{number of O} \times 0.2) + (\text{number of S} \times 4.4) + (\text{number of Si} \times 3.4) \quad \textbf{Equation 8.26}$$

TABLE 8.8
RELATIVE INTENSITIES OF ISOTOPE PEAKS FOR VARIOUS COMBINATIONS OF BROMINE AND CHLORINE

	Relative Intensities			
Halogen	***M***	***M* + 2**	***M* + 4**	***M* + 6**
Br	100	97.7		
Br_2	100	195.0	95.4	
Br_3	100	293.0	286.0	93.4
Cl	100	32.6		
Cl_2	100	65.3	10.6	
Cl_3	100	97.8	31.9	3.47
BrCl	100	130.0	31.9	
Br_2Cl	100	228.0	159.0	31.2
Cl_2Br	100	163.0	74.4	10.4

When chlorine or bromine is present, the $M + 2$ peak becomes very significant. The heavy isotope of each of these elements is two mass units heavier than the lighter isotope. The natural abundance of ^{37}Cl is 32.5% that of ^{35}Cl, and the natural abundance of ^{81}Br is 98.0% that of ^{79}Br. When either of these elements is present, the $M + 2$ peak becomes quite intense. If a compound contains two chlorine or bromine atoms, a distinct $M + 4$ peak, as well as an intense $M + 2$ peak, should be observed. In such a case, it is important to exercise caution in identifying the molecular ion peak in the mass spectrum. Section 8.8V will discuss the mass spectral properties of the organic halogen compounds in greater detail. Table 8.8 gives the relative intensities of isotope peaks for various combinations of bromine and chlorine atoms, and Figure 8.18 illustrates them.

8.8 STRUCTURAL ANALYSIS AND FRAGMENTATION PATTERNS

In EI-MS, a molecule is bombarded by high-energy electrons in the ionization chamber. The collision between the sample molecules and the electrons initially results in the sample molecule losing one electron to form a radical cation. The molecule also absorbs a considerable amount of extra energy during its collision with the incident electrons. This extra energy places the molecular ion in a highly excited vibrational state. The vibrationally excited molecular ion may be unstable, and it may lose some of its extra energy by breaking apart into fragments. If the lifetime of the molecular ion is greater than 10^{-5} sec, a peak corresponding to the molecular ion will appear in the mass spectrum. However, molecular ions with lifetimes less than 10^{-5} sec break apart into fragments before they are accelerated within the ionization chamber and enter the mass analyzer. In such cases, peaks corresponding to the mass-to-charge ratios (m/z) for these fragments appear in the mass spectrum. For a given compound, not all of the molecular ions formed by ionization have precisely the same lifetime; some have shorter lifetimes than others. As a result, in a typical EI mass spectrum one observes peaks corresponding to both the molecular ion and the fragment ions.

For most classes of compounds, the mode of fragmentation is somewhat characteristic and hence predictable. This section discusses some of the more important modes of fragmentation for common organic functional groups. It is helpful to begin by describing some general principles that govern fragmentation processes. The ionization of the sample molecule forms a molecular ion that

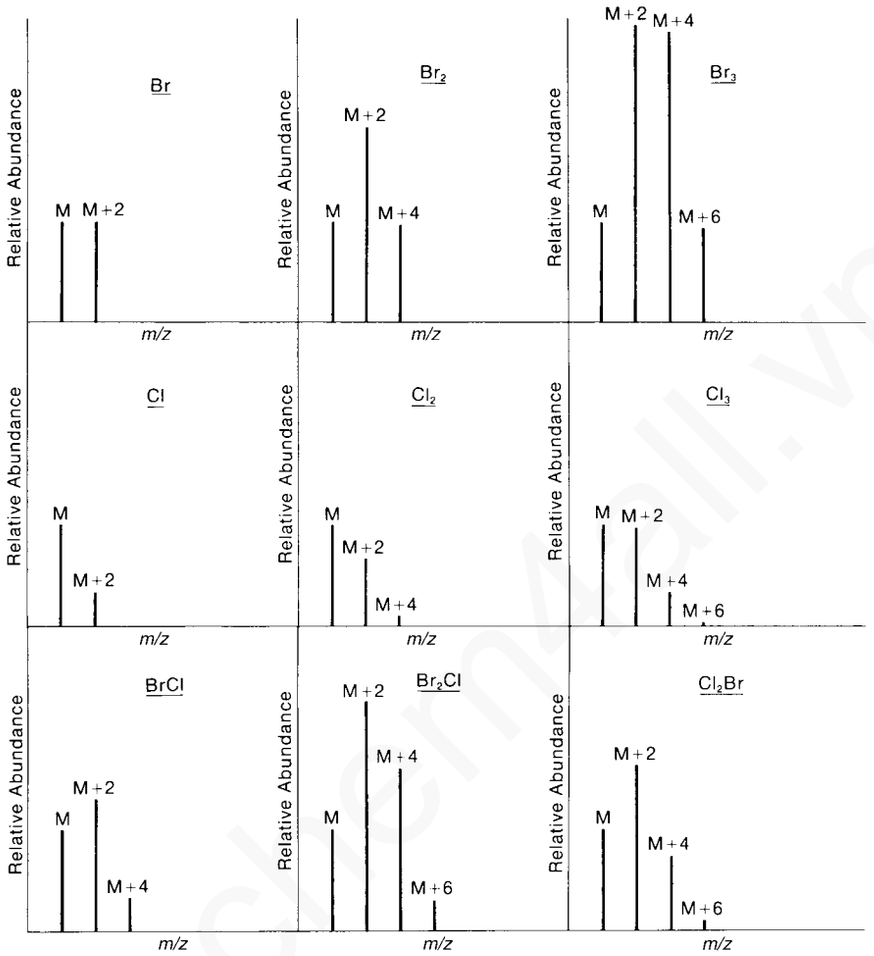


FIGURE 8.18 Mass spectra expected for various combinations of bromine and chlorine.

not only carries a positive charge but also has an unpaired electron. The molecular ion, then, is actually a radical cation, and it contains an odd number of electrons. Odd-electron ions ($\mathbf{OE^{\bullet +}}$) have even mass (if no nitrogen is present in the compound), and thus even-electron ions ($\mathbf{EE^{+}}$) will have odd mass.

A. Stevenson's Rule

When fragment ions form in the mass spectrometer, they almost always do so by means of unimolecular processes. The low pressure of the ionization chamber makes it unlikely a significant number of bimolecular collisions could occur. The unimolecular processes that are energetically most favorable give rise to the most fragment ions. This is the idea behind **Stevenson's Rule:** The most probable fragmentation is the one that leaves the positive charge on the fragment with the lowest ionization energy. In other words, fragmentation processes that lead to the formation of more stable ions are favored over processes that lead to less-stable ions. This idea is grounded in the same concepts as Markovnikov's Rule, which states that in the addition of a hydrogen halide to an alkene, the more stable carbocation forms the fastest and leads to the major product of the addition

reaction. In fact, a great deal of the chemistry associated with ionic fragmentation can be explained in terms of what is known about carbocations in solution. For example, alkyl substitution stabilizes fragment ions (and promotes their formation) in much the same way that it stabilizes carbocations. Other familiar concepts will help one predict likely fragmentation processes: electronegativity, polarizability, resonance delocalization, the octet rule, and so on.

Often, fragmentation involves the loss of an electrically neutral fragment. This fragment does not appear in the mass spectrum, but its existence can be deduced by noting the difference in masses of the fragment ion and the original molecular ion. Again, processes that lead to the formation of a more stable neutral fragment are favored over those that lead to less-stable neutral fragments.

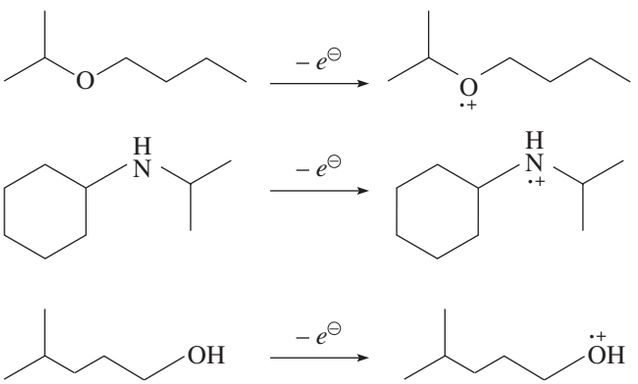
An $OE^{\bullet+}$ can fragment in two ways: cleavage of a bond to create an EE^+ and a radical ($R^\bullet$) *or* cleavage of bonds to create another $OE^{\bullet+}$ and a closed-shell neutral molecule (*N*). An EE^+, on the other hand, can only fragment in one way—cleavage of bonds to create another EE^+ and a closed-shell neutral molecule (*N*). This is the so-called **even-electron rule.** The most common mode of fragmentation involves the cleavage of one bond. In this process, the $OE^{\bullet+}$ yields a radical ($R^\bullet$) and an EE^+ fragment ion. Cleavages that lead to the formation of more stable carbocations are favored. When the loss of more than one possible radical is possible, a corollary to Stevenson's Rule is that the largest alkyl radical to be lost preferentially. Thus, ease of fragmentation to form ions increases in the order

$$\underset{\textbf{DIFFICULT}}{H_3C^+ < RCH_2^+ < R_2CH^+ < R_3^+ < H_2C=CHCH_2^+ \sim HC{\equiv}CCH_2^+ < C_6H_5CH_2^+}\quad\underset{\textbf{EASY}}{}$$

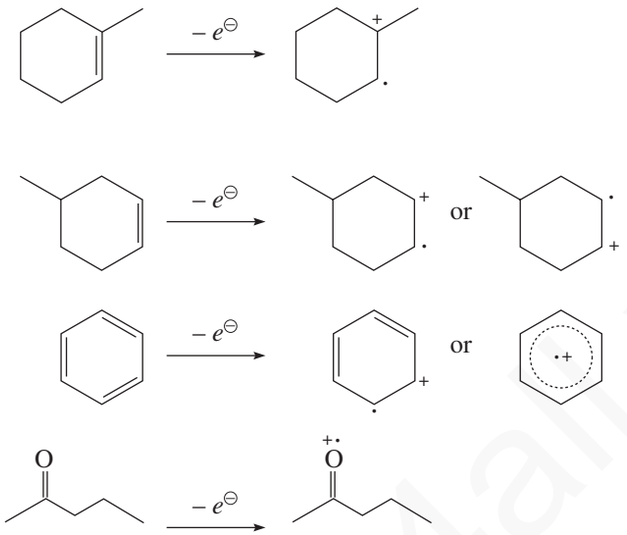
B. The Initial Ionization Event

One cannot get very far in the discussion of ion fragmentation without first considering which electron is lost in the initial ionization event to form $M^{\bullet+}$. The electrons most likely to be ejected during the ionization event are the ones that are in the highest potential energy molecular orbitals, that is, the electrons held least tightly by the molecule. Thus, it is easier to remove an electron from a nonbonding orbital *n* than it is to strip an electron from a π orbital. Similarly, it is much easier to eject an electron from a π orbital in comparison to a σ orbital. The molecular ion can be represented with either a localized or a nonlocalized charge site. Some examples of loss of an electron and the notation for the molecular ion are shown below.

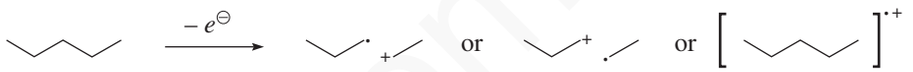
Loss of an electron from a nonbonding orbital:



Loss of an electron from a π orbital.



Loss of an electron from a σ orbital:



When drawing fragmentation mechanisms, it is essential that one tracks the charge and radical sites carefully to prevent either misassignment of which fragment is the ion and which is neutral or drawing highly improbable fragmentations. It is also important to keep in mind that the fragmentation is happening in the gas phase to an ion in a highly excited vibrational state. It is tempting to draw fragmentation mechanisms in the same way that one draws mechanisms for chemical reactions—with concerted bond-breaking or bond-making events. The vast majority of fragmentations in the mass spectrometer are likely stepwise in nature, although some processes like the retro Diels–Alder fragmentation are frequently drawn in a concerted fashion to emphasize the parallel to the chemical reaction more familiar to us. Finally, one needs to be consistent with the use of a single-headed arrow (fishhook, ↷) for movement of a single electron and a double-headed arrow (↷) for two-electron processes.

C. Radical-Site-Initiated Cleavage: α-Cleavage

Before examining the characteristic fragmentation patterns of common organic functional groups, let us consider some of the most common modes of fragmentation. **Radical-site**-initiated fragmentation is one of the most common one-bond cleavages and is more commonly called an α-cleavage. The term α-cleavage is confusing to some because the bond that is broken is not directly attached to the radical site but is rather the bond to the next neighboring atom (the α-position). α-Cleavages may occur at saturated or unsaturated sites that may or may not involve a heteroatom (Y in Fig. 8.19).

D. Charge-Site-Initiated Cleavage: Inductive Cleavage

Another common one-bond cleavage is **charge-site**-initiated or **inductive cleavage,** often indicated in a fragmentation mechanism by the symbol ***i***. Inductive cleavage involves the attraction of an electron pair by an electronegative heteroatom that ends up as a radical or as a closed-shell neutral

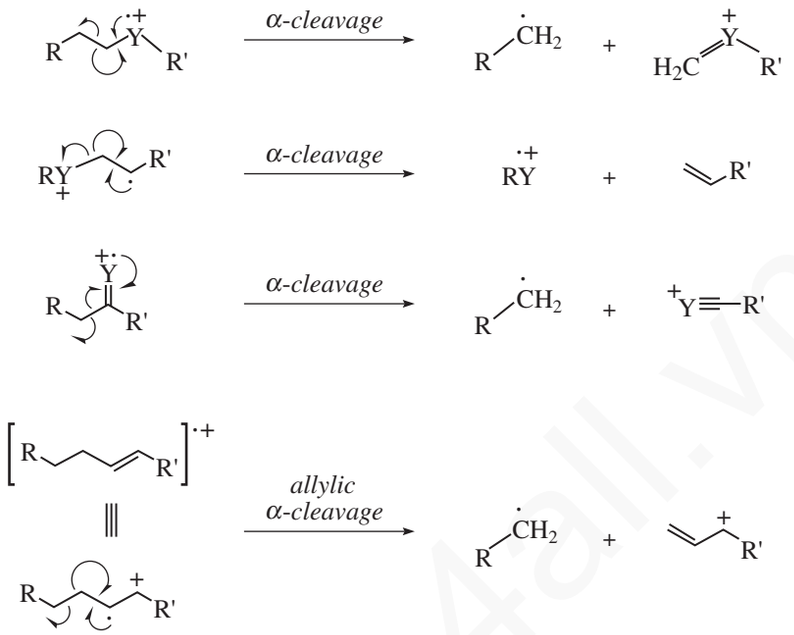


FIGURE 8.19 Representative α-cleavage fragmentations (Y = heteroatom).

molecule. While α-cleavage is a fragmentation of OE^+ only, inductive cleavage can operate on either an OE^+ or an EE^+, as seen in Figure 8.20.

E. Two-Bond Cleavage

Some fragmentations involve cleavage of two bonds simultaneously. In this process, an elimination occurs, and the odd-electron molecular ion yields an OE^+ and an even-electron neutral fragment *N*,

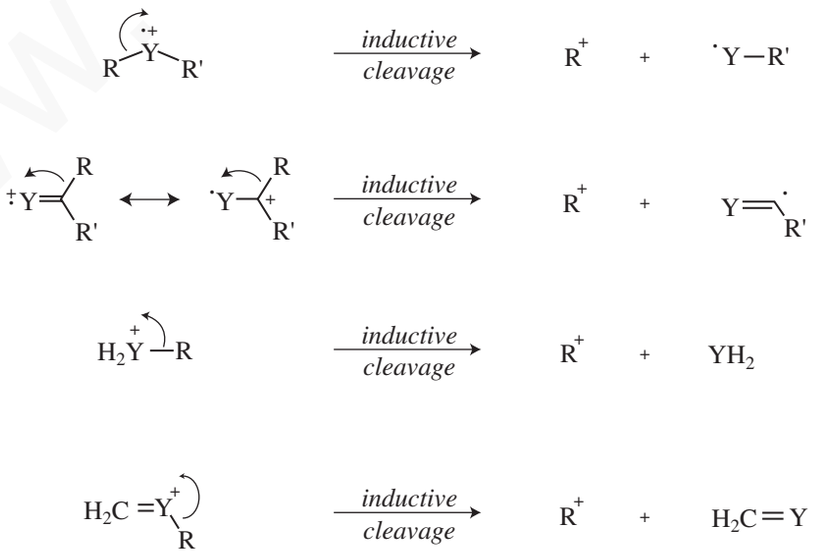


FIGURE 8.20 Representative inductive-cleavage fragmentations (Y = heteroatom).

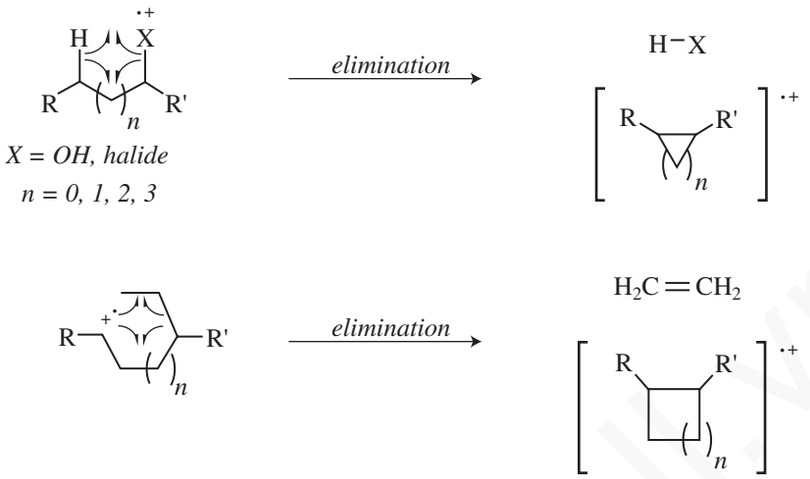


FIGURE 8.21 Common two-bond fragmentations (X = heteroatom).

usually a stable small molecule of some type: H_2O, a hydrogen halide, or an alkene. Some examples of two-bond cleavages of this type are shown in Figure 8.21.

F. Retro Diels-Alder Cleavage

Unsaturated six-membered rings can undergo a **retro Diels–Alder** fragmentation to produce the radical cation of a diene and a neutral alkene—the hypothetical precursors to the cyclohexene derivative if it had been prepared in the forward direction via the [4π + 2π] diene + dienophile cycloaddition known to every organic chemist as the Diels–Alder reaction. A schematic representation of the retro Diels–Alder fragmentation is shown in Figure 8.22 Note that the unpaired electron and charge remain with the diene fragment according to Stevenson's Rule.

G. McLafferty Rearrangements

Another very common fragmentation that can occur with many substrates is the **McLafferty rearrangement** (Fig. 8.23). This fragmentation was first described by Fred McLafferty in 1956 and is one of the most predictable fragmentations, next to the simple α-cleavage. In the McLafferty rearrangement, a hydrogen atom on a carbon 3 atom away from the radical cation of an alkene, arene, carbonyl, or imine (a so-called γ-hydrogen) is transferred to the charge site via a six-membered transition state, with concurrent cleavage of the sigma bond between the α and β positions of the tether. This forms a new radical cation and an alkene with a π bond between what

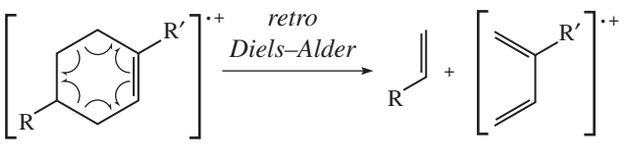


FIGURE 8.22 A retro Diels–Alder fragmentation.

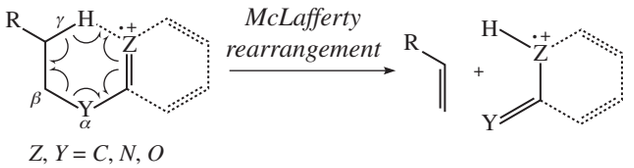


FIGURE 8.23 The McLafferty rearrangement.

were the original β and γ carbons. For simplicity, the mechanism of the McLafferty rearrangement is usually drawn as a concerted process, as in Figure 8.23. There is experimental evidence, however, that the fragmentation is in fact stepwise, and as a general rule fragmentations that involve breaking more than one bond are probably stepwise. The McLafferty rearrangement is readily observed in the mass spectra of many organic functional groups, and several examples will be shown in the remaining sections of this chapter.

H. Other Cleavage Types

In addition to these processes, fragmentation processes involving rearrangements, migrations of groups, and secondary fragmentations of fragment ions are also possible. These modes of fragmentation occur less often than the two cases already described, and additional discussion of them will be reserved for the compounds in which they are important. To assist you in identifying possible fragment ions, Appendix 12 provides a table that lists the molecular formulas for common fragments with *m/z* less than 105. More complete tables may be found in the books listed in the references at the end of this chapter.

I. Alkanes

For saturated hydrocarbons and organic structures containing large saturated hydrocarbon skeletons, the methods of fragmentation are quite predictable. What is known about the stabilities of carbocations in solution can be used to help us understand the fragmentation patterns of alkanes. The mass spectra of alkanes are characterized by strong molecular ion peaks and a regular series of fragment ion peaks separated by 14 amu.

For a straight-chain, or "normal," alkane, a peak corresponding to the molecular ion can be observed as in the mass spectra of butane (Fig. 8.24) and octane (Fig. 8.25). As the carbon skeleton

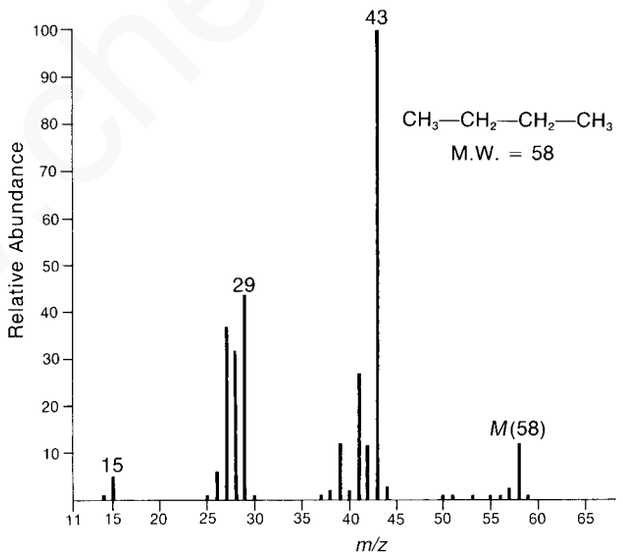


FIGURE 8.24 Mass spectrum of butane.

SPECTRAL ANALYSIS BOX — Alkanes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	Loss of CH_2 units in a series: $M - 14$, $M - 28$, $M - 42$, etc.

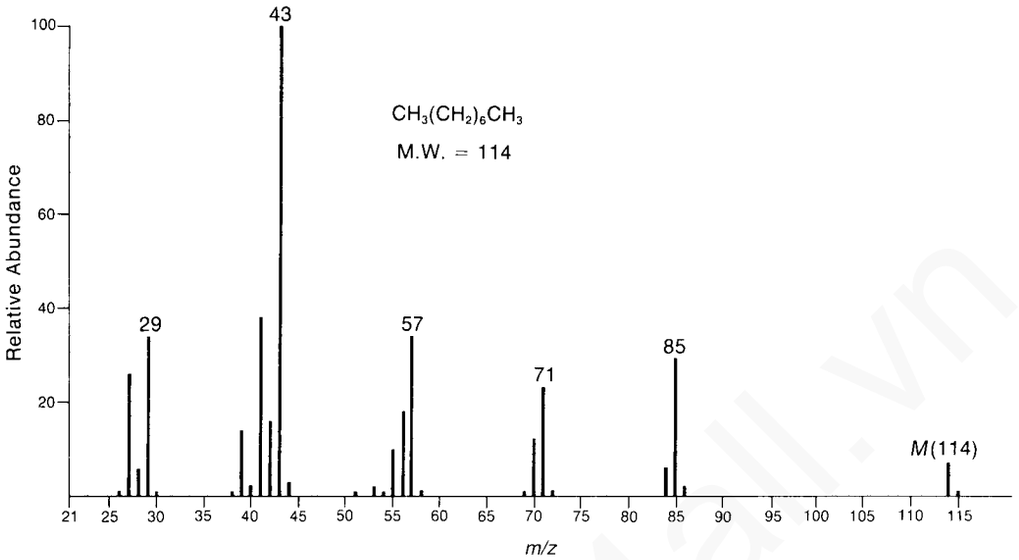


FIGURE 8.25 EI mass spectrum of octane.

becomes more highly branched, the intensity of the molecular ion peak decreases. Straight-chain alkanes have fragments that are always primary carbocations. Since these ions are rather unstable, fragmentation is not favored. A significant number of the original molecules survive electron bombardment without fragmenting. Consequently, a molecular ion peak of significant intensity is observed. You will see this effect easily if you compare the mass spectrum of butane with that of isobutane (Fig. 8.26). The molecular ion peak in isobutane is much less intense than that in butane. Comparison of the mass spectra of octane and 2,2,4-trimethylpentane (Fig. 8.27) provides a more dramatic illustration of the effect of chain branching on the intensity of the molecular ion peak.

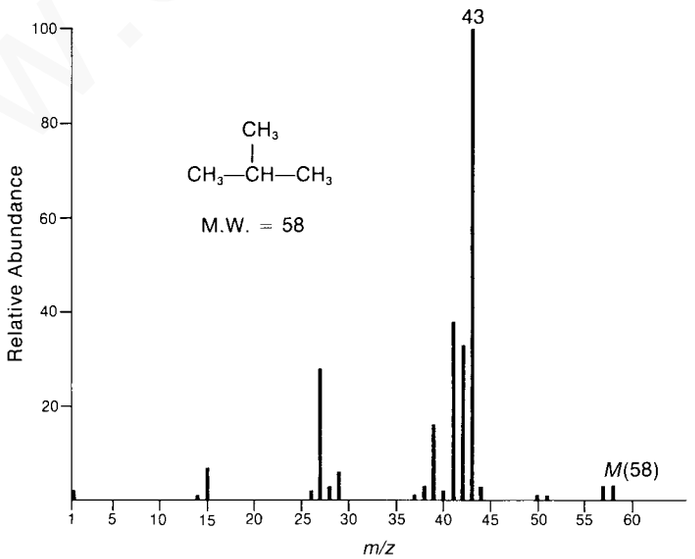


FIGURE 8.26 EI mass spectrum of isobutane.

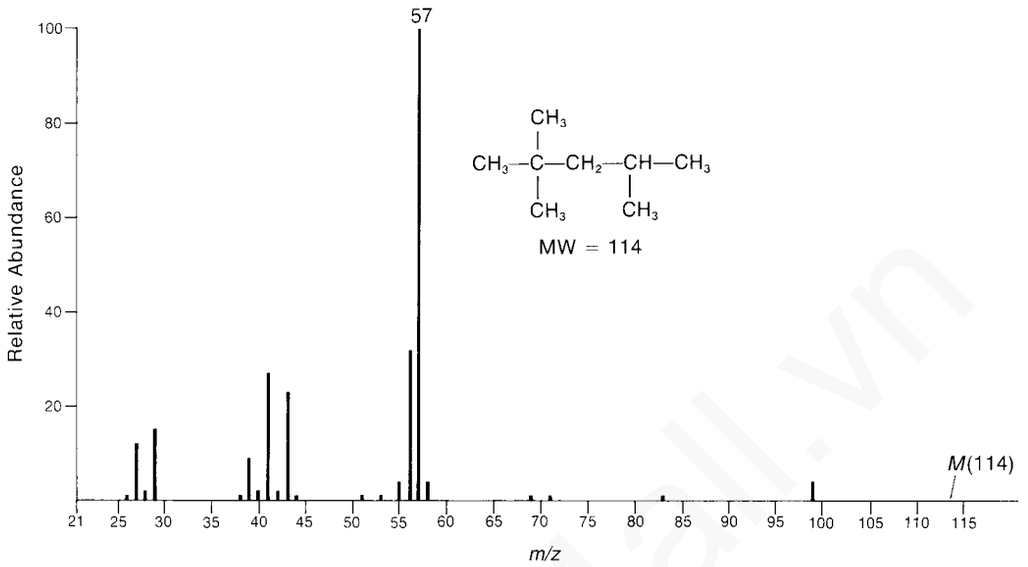


FIGURE 8.27 EI mass spectrum of 2,2,4-trimethylpentane (isooctane).

The molecular ion peak in 2,2,4-trimethylpentane is too weak to be observed, while the molecular ion peak in its straight-chain isomer is quite readily observed. The effect of chain branching on the intensity of the molecular ion peak can be understood by examining the method by which hydrocarbons undergo fragmentation.

Straight-chain hydrocarbons undergo fragmentation by breaking carbon–carbon bonds, resulting in a homologous series of fragmentation products. For example, in the case of butane, cleavage of the C1−C2 bond results in the loss of a methyl radical and the formation of the propyl carbocation ($m/z = 43$). Cleavage of the C2−C3 bond results in the loss of a ethyl radical and the formation of the ethyl carbocation ($m/z = 29$). In the case of octane, fragment peaks due to the hexyl ion ($m/z = 85$), the pentyl ion ($m/z = 71$), the butyl ion ($m/z = 57$), the propyl ion ($m/z = 43$), and the ethyl ion ($m/z = 29$) are observed. Notice that alkanes fragment to form clusters of peaks that are 14 mass units (corresponding to one CH_2 group) apart from each other. Other fragments within each cluster correspond to additional losses of one or two hydrogen atoms. As is evident in the mass spectrum of octane, the three-carbon ions appear to be the most abundant, with the intensities of each cluster uniformly decreasing with increasing fragment weight. Interestingly, for long-chain alkanes, the fragment corresponding to the loss of one carbon atom is generally absent. In the mass spectrum of octane, a seven-carbon fragment should occur at a mass of 99, but it is not observed.

Cleavage of the carbon–carbon bonds of branched-chain alkanes can lead to secondary or tertiary carbocations. These ions are more stable than primary ions, of course, so fragmentation becomes a more favorable process. A greater proportion of the original molecules undergoes fragmentation, so the molecular ion peaks of branched-chain alkanes are considerably weaker or even absent. In isobutane, cleavage of a carbon–carbon bond yields an isopropyl carbocation, which is more stable than a normal propyl ion. Isobutane undergoes fragmentation more easily than butane because of the increased stability of its fragmentation products. With 2,2,4-trimethylpentane, the dominant cleavage event is the rupture of the C2-C3 bond, which leads to the formation of a *tert*-butyl carbocation. Since tertiary carbocations are the most stable of the saturated alkyl carbocations, this cleavage is particularly favorable and accounts for the intense fragment peak at $m/z = 57$.

J. Cycloalkanes

Cycloalkanes generally form strong molecular ion peaks. Fragmentation via the loss of a molecule of ethene ($M - 28$) is common. The typical mass spectrum for a cycloalkane shows a relatively intense molecular ion peak. Fragmentation of ring compounds requires the cleavage of two carbon–carbon bonds, which is a more difficult process than cleavage of one such bond. Therefore, a larger proportion of cycloalkane molecules than of acyclic alkane molecules survives electron bombardment without undergoing fragmentation. In the mass spectra of cyclopentane (Fig. 8.28) and methylcyclopentane (Fig. 8.29), strong molecular ion peaks can be observed.

The fragmentation patterns of cycloalkanes may show mass clusters arranged in a homologous series, as in the alkanes. However, the most significant mode of cleavage of the cycloalkanes involves the loss of a molecule of ethene ($H_2C{=}CH_2$), either from the parent molecule or from intermediate OE^+. The peak at $m/z = 42$ in cyclopentane and the peak at $m/z = 56$ in methylcyclopentane result from the loss of ethene from the parent molecule. Each of these fragment peaks is the most intense in the mass spectrum. When the cycloalkane bears a side chain, loss of that side chain is a favorable mode of fragmentation. The fragment peak at $m/z = 69$ in the mass spectrum of methylcyclopentane is due to the loss of the CH_3 side chain, which results in a secondary carbocation.

SPECTRAL ANALYSIS BOX — Cycloalkanes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$M - 28$
	A series of peaks: $M - 15$, $M - 29$, $M - 43$, $M - 57$, etc.

Applying these pieces of information to the mass spectrum of bicyclo[2.2.1]heptane (Fig. 8.30), we can identify fragment peaks due to the loss of the side chain (the one-carbon bridge, plus an additional hydrogen atom) at $m/z = 81$ and the loss of ethene at $m/z = 68$. The fragment ion peak at $m/z = 67$ is due to the loss of ethene plus an additional hydrogen atom.

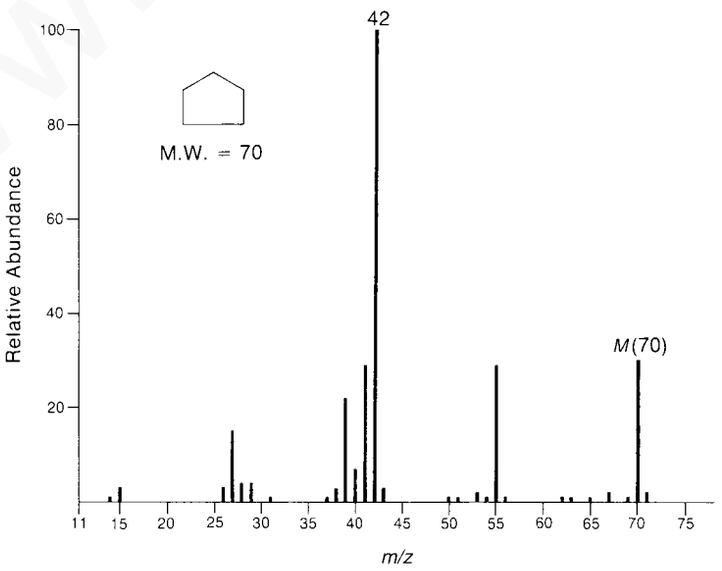


FIGURE 8.28 EI mass spectrum of cyclopentane.

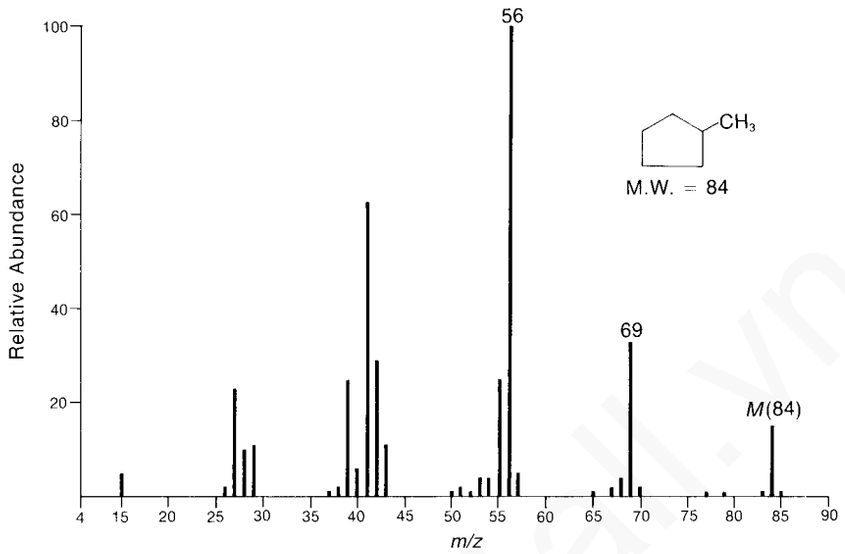


FIGURE 8.29 EI mass spectrum of methylcyclopentane.

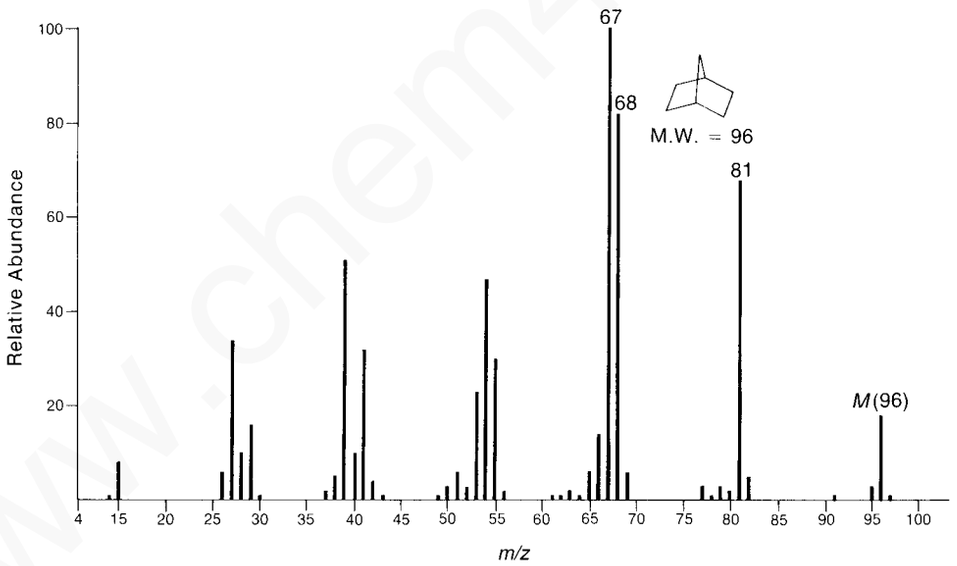


FIGURE 8.30 EI mass spectrum of bicyclo[2.2.1]heptane (norbornane).

K. Alkenes

The mass spectra of most alkenes show distinct molecular ion peaks. Naturally, the mass of the molecular ion should correspond to a molecular formula with an index of hydrogen deficiency equal to at least *one* (see Chapter 1). Apparently, electron bombardment removes one of the electrons in the π bond, leaving the carbon skeleton relatively undisturbed. When alkenes undergo fragmentation processes, the resulting fragment ions have formulas corresponding to $C_nH_{2n}^+$ and $C_nH_{2n-1}^+$. It is sometimes difficult to locate double bonds in alkenes since they migrate readily. The similarity of the mass spectra of alkene isomers is readily seen in the mass spectra of three isomers of the formula C_5H_{10} (Figs. 8.31, 8.32, and 8.33). The mass spectra are very nearly identical, with the only difference being a large fragment at $m/z = 42$ in the spectrum of 1-pentene. This ion likely forms via loss of ethylene through a McLafferty-type rearrangement of the molecular ion. The allyl carbocation

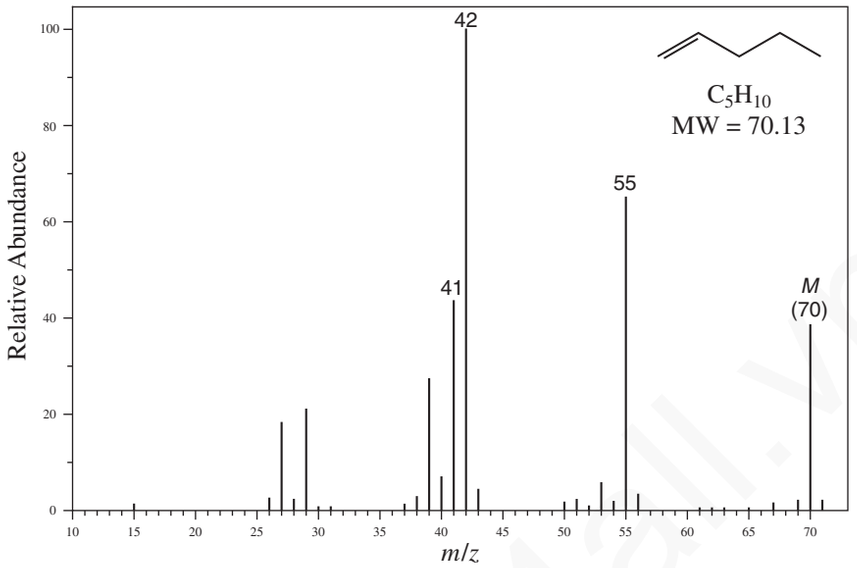


FIGURE 8.31 EI-MS spectrum of 1-pentene.

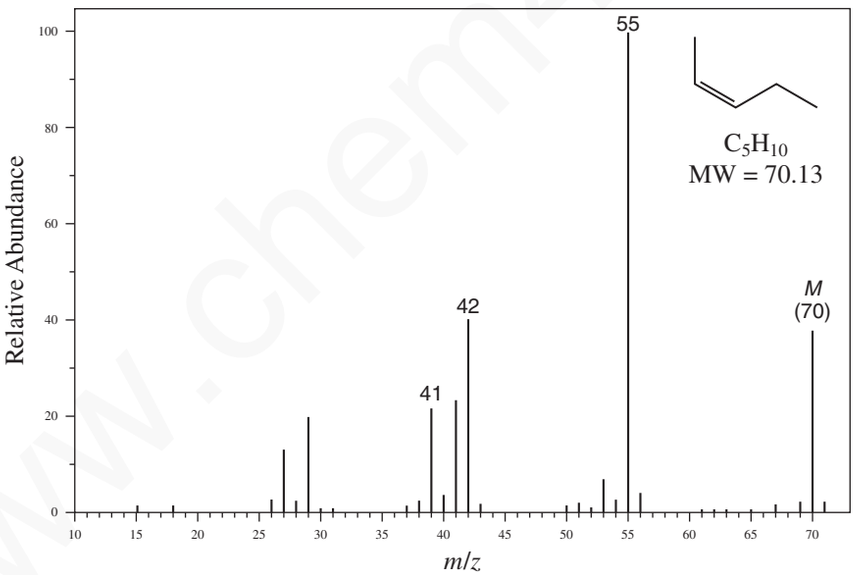


FIGURE 8.32 EI-MS spectrum of *Z*-2-pentene.

($m/z = 41$) is an important fragment in the mass spectra of terminal alkenes and forms via an allylic α-cleavage as shown in Figure 8.19. The fragment at $m/z = 55$ is from loss of a methyl radical. This fragment is the base peak in the spectra of the diastereomeric pentene isomers since loss of the methyl group distal to the alkene creates an allylic cation that is resonance stabilized.

SPECTRAL ANALYSIS BOX—Alkenes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$m/z = 41$
	A series of peaks: $M - 15$, $M - 29$, $M - 43$, $M - 57$, etc.

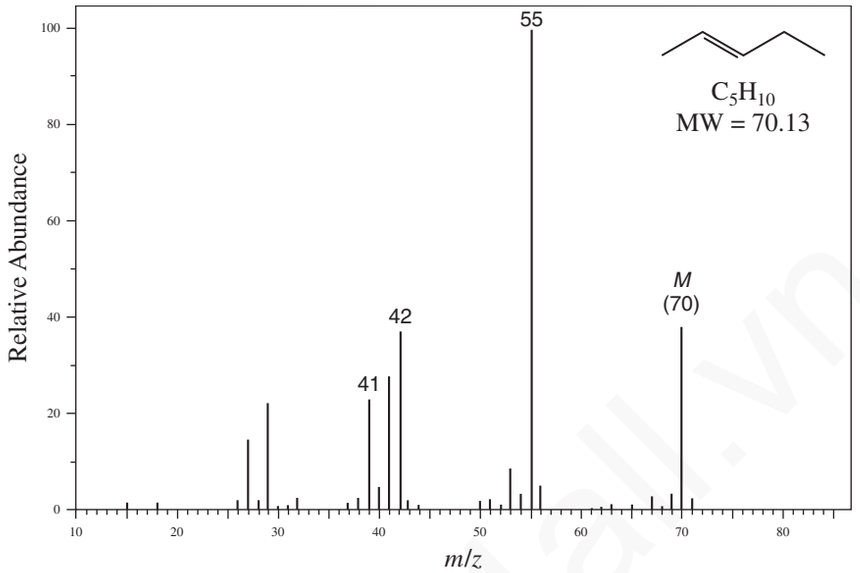


FIGURE 8.33 EI-MS spectrum of *E*-2-pentene.

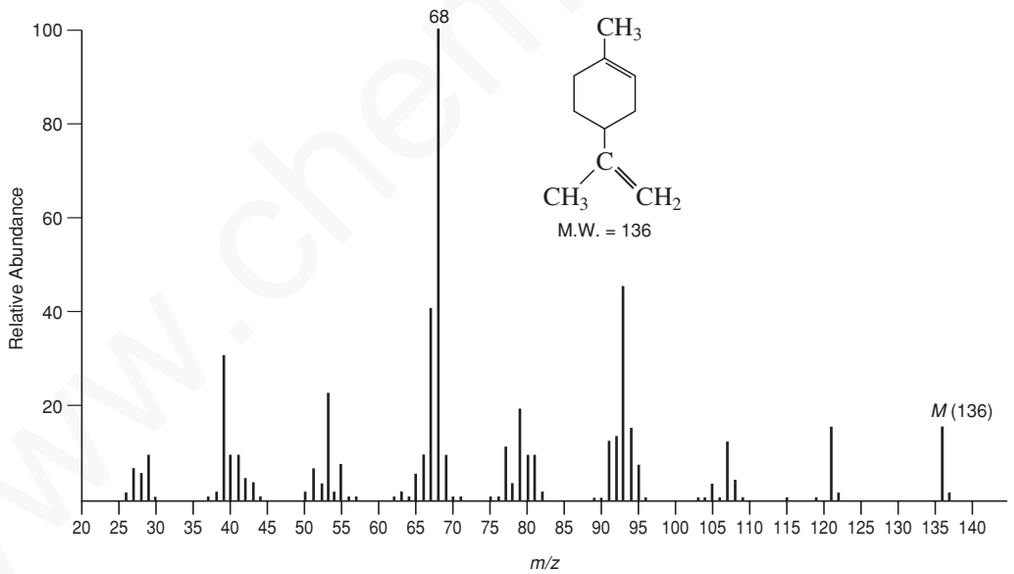


FIGURE 8.34 EI-MS spectrum of limonene.

The mass spectra of cycloalkenes show quite distinct molecular ion peaks. For many cycloalkenes, migration of bonds gives virtually identical mass spectra. Consequently, it may be impossible to locate the position of the double bond in a cycloalkene, particularly a cyclopentene or a cycloheptene. Cyclohexenes do have a characteristic fragmentation pattern that corresponds to a retro Diels–Alder reaction (Fig. 8.22). In the mass spectrum of the monoterpene limonene (Fig. 8.34), the intense peak at $m/z = 68$ corresponds to the diene fragment arising from the retro Diels–Alder fragmentation.

The mere presence of a cyclohexene moiety does not guarantee that a retro Diels–Alder fragmentation will be observed in the mass spectrum. Consider the mass spectra of α- and β-ionone

(Fig. 8.35). The spectrum of α-ionone shows much more fragmentation in general and a peak at $m/z = 136$ in particular that is created by a retro Diels–Alder fragmentation of the cyclohexene ring and loss of isobutene. Retro Diels–Alder fragmentation of β-ionone should give a peak at $m/z = 164$ from loss of ethene, but the peak at that position is miniscule. In the case of β-ionone, loss of a methyl radical via α-cleavage adjacent to the ring double bond yields a relatively stable tertiary allylic cation. This fragmentation is not available to α-ionone.

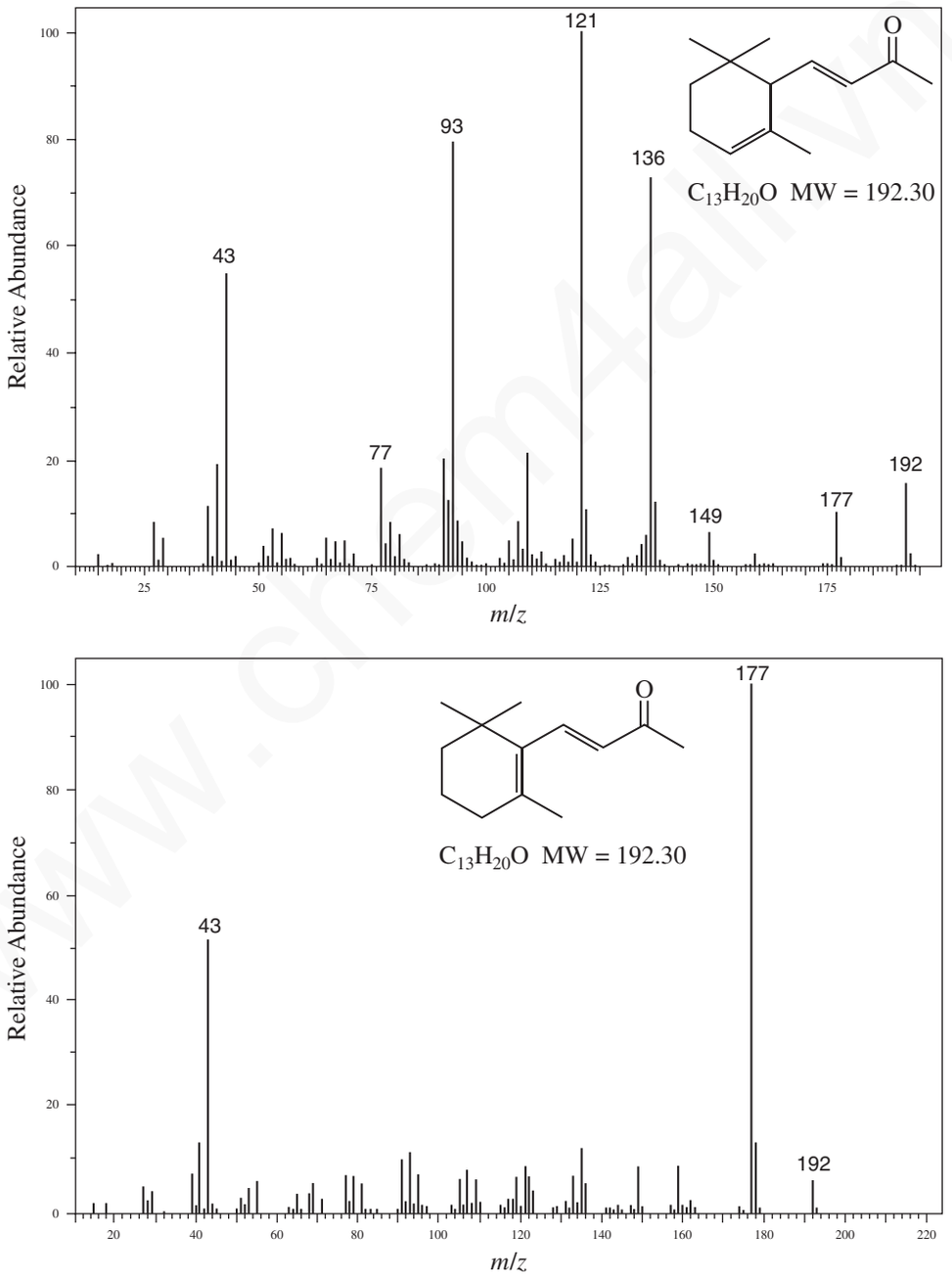


FIGURE 8.35 EI-MS spectra of α-ionone (top) and β-ionone (bottom).

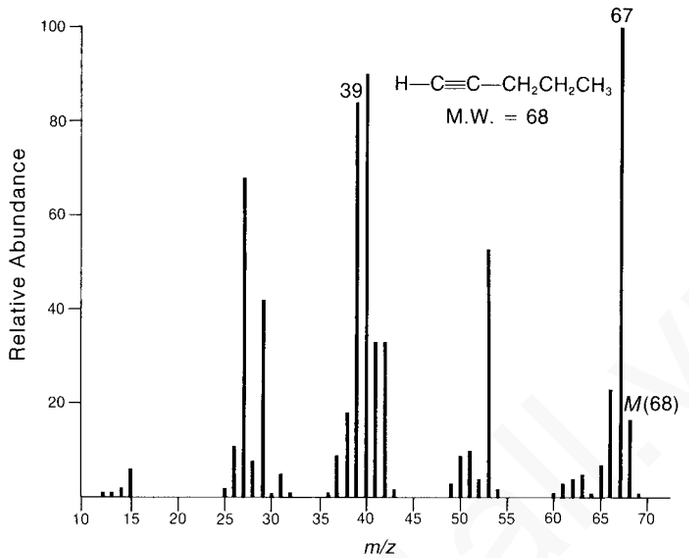


FIGURE 8.36 EI-MS spectrum of 1-pentyne.

L. Alkynes

The mass spectra of alkynes are very similar to those of alkenes. The molecular ion peaks tend to be rather intense, and fragmentation patterns parallel those of the alkenes. As can be seen from the mass spectrum of l-pentyne (Fig. 8.36), an important fragmentation is the loss of an ethyl radical via an α-cleavage to produce the propargyl ion ($m/z = 39$). Similarly, loss of methyl radical in an α-cleavage of 2-pentyne produces a resonance-stabilized propargylic cation at ($m/z = 53$) (Fig. 8.37). Another important mode of fragmentation for terminal alkynes is the loss of the terminal hydrogen, yielding a strong $M - 1$ peak. This peak appears as the base peak ($m/z = 67$) in the spectrum of 1-pentyne.

SPECTRAL ANALYSIS BOX — Alkynes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$m/z = 39$
	Strong $M - 1$ peak

M. Aromatic Hydrocarbons

The mass spectra of most aromatic hydrocarbons show very intense molecular ion peaks. As is evident from the mass spectrum of benzene (Fig. 8.38), fragmentation of the benzene ring requires a great deal of energy. Such fragmentation is not observed to any significant extent. In the mass spectrum of toluene (Fig. 8.39), loss of a hydrogen atom from the molecular ion gives a strong peak at $m/z = 91$. Although it might be expected that this fragment ion peak is due to the benzyl carbocation ($C_6H_5CH_2^+$), isotope-labeling experiments suggest that the benzyl carbocation actually rearranges to form the aromatic delocalized **tropylium ion** ($C_7H_7^+$, Figure 8.43). When a benzene ring contains larger side chains, a favored mode of fragmentation is cleavage of the side chain to form initially a **benzyl cation,** which spontaneously rearranges to the tropylium ion. When the side chain attached to a benzene ring contains three or more carbons, ions formed by a McLafferty rearrangement can be observed.

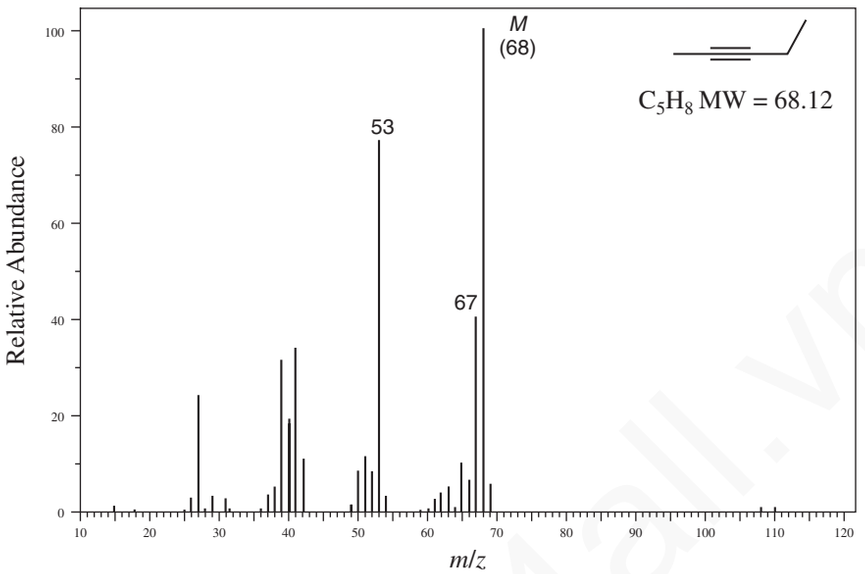


FIGURE 8.37 EI-MS spectrum of 2-pentyne.

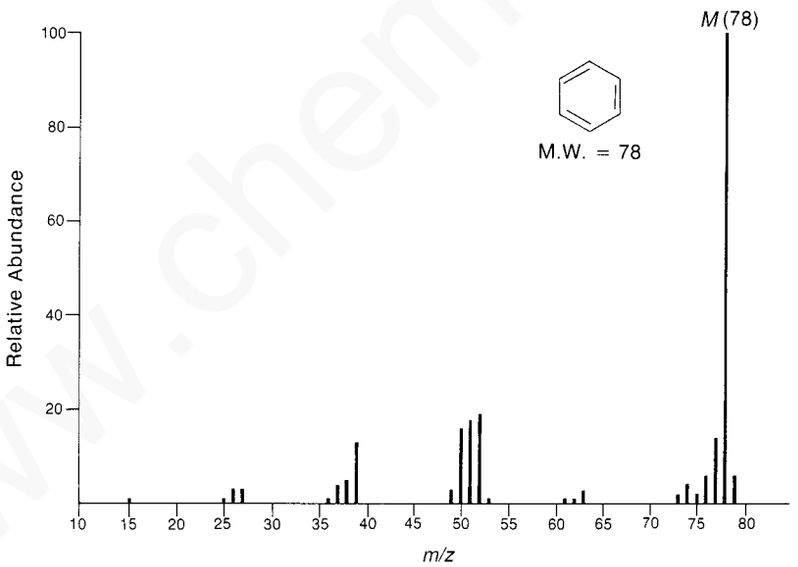


FIGURE 8.38 EI-MS spectrum of benzene.

SPECTRAL ANALYSIS BOX — Aromatic Hydrocarbons

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$m/z = 91$
	$m/z = 92$

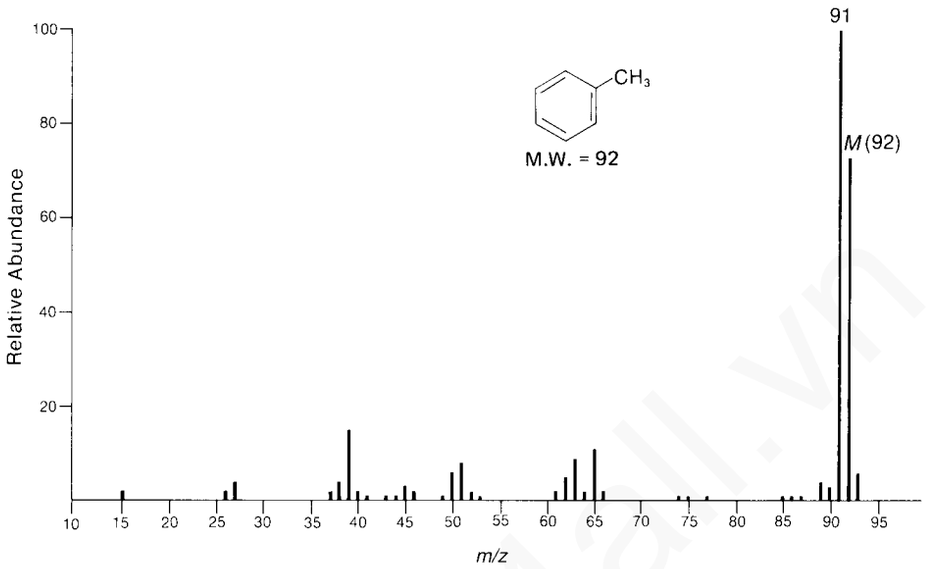


FIGURE 8.39 EI-MS spectrum of toluene.

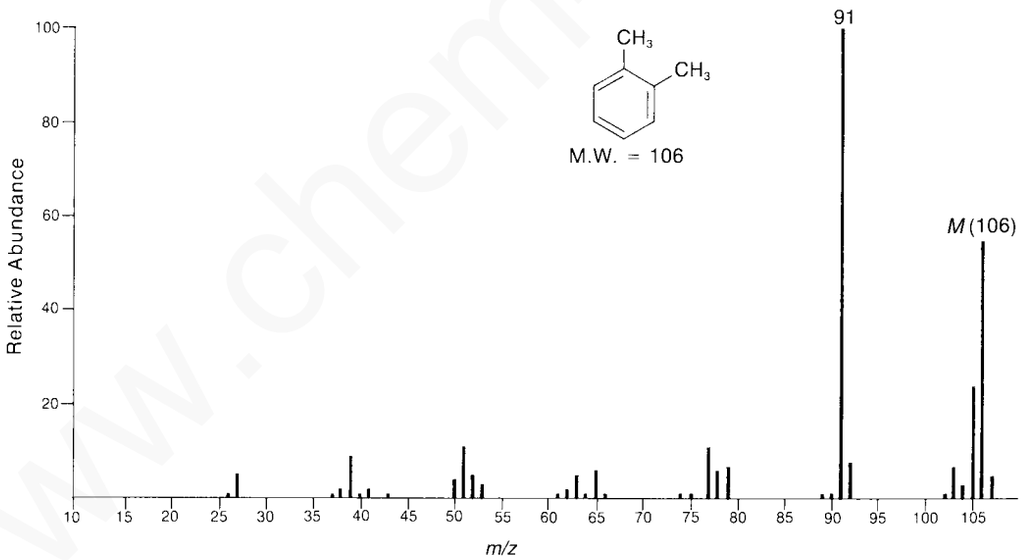


FIGURE 8.40 EI-MS spectrum of *ortho*-xylene.

The mass spectra of the xylene isomers (Figs. 8.40 and 8.41 for example) show a medium peak at $m/z = 105$, which is due to the loss of a hydrogen atom and the formation of the methyltropylium ion. More importantly, xylene loses one methyl group to form the tropylium ($m/z = 91$). The mass spectra of *ortho*-, *meta*-, and *para*-disubstituted aromatic rings are essentially identical. As a result, the substitution pattern of polyalkylated benzenes cannot be determined by mass spectrometry.

The formation of a substituted tropylium ion is typical for alkyl-substituted benzenes. In the mass spectrum of isopropylbenzene (Fig. 8.42), a strong peak appears at $m/z = 105$. This peak corresponds to loss of a methyl group to form a methyl-substituted tropylium ion. The tropylium ion has characteristic fragmentations of its own. The tropylium ion can fragment to form the aromatic

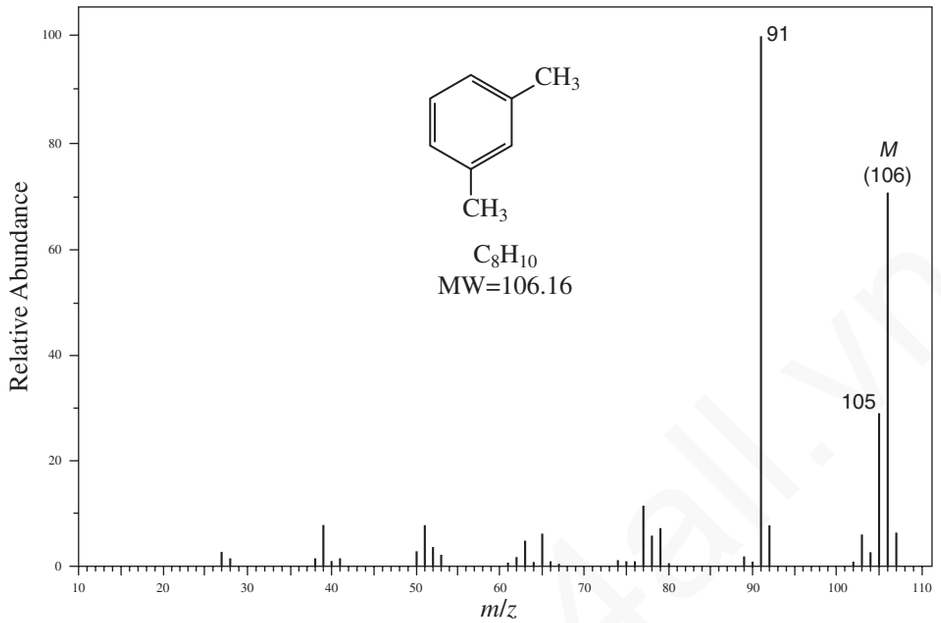


FIGURE 8.41 EI-MS spectrum of *meta*-xylene.

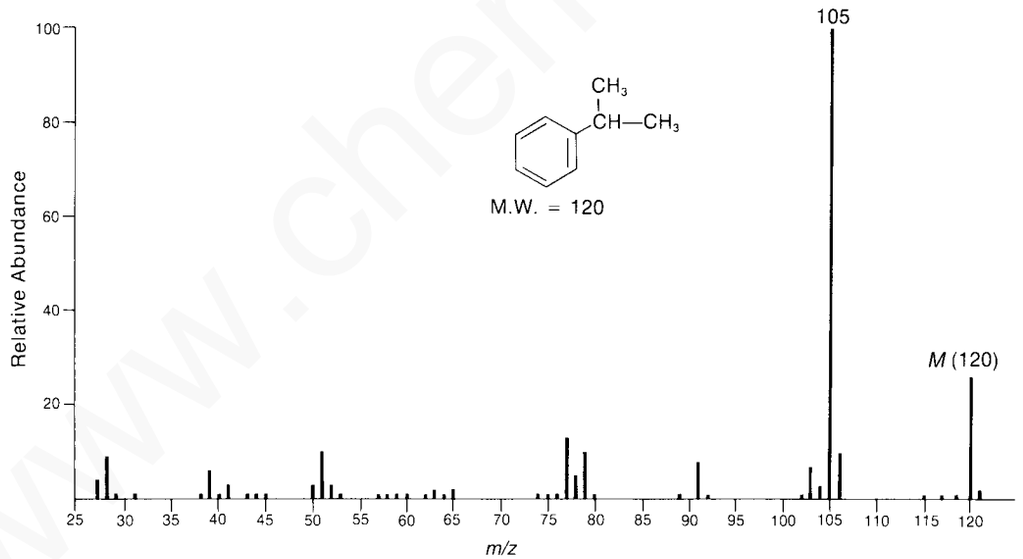


FIGURE 8.42 EI-MS of isopropylbenzene (cumene).

cyclopentadienyl cation ($m/z = 65$) plus ethyne (acetylene). The cyclopentadienyl cation in turn can fragment to form another equivalent of ethyne and the aromatic cyclopropenyl cation (m/z = 39) (Fig. 8.43).

In the mass spectrum of butylbenzene (Fig. 8.44), a strong peak due to the tropylium ion appears at m/z = 91. When the alkyl group attached to the benzene ring is a propyl group or larger, a McLafferty rearrangement is likely to occur, producing a peak at $m/z = 92$. Indeed, all alkylbenzenes bearing a side chain of three or more carbons and at least one hydrogen on the γ-carbon will exhibit a

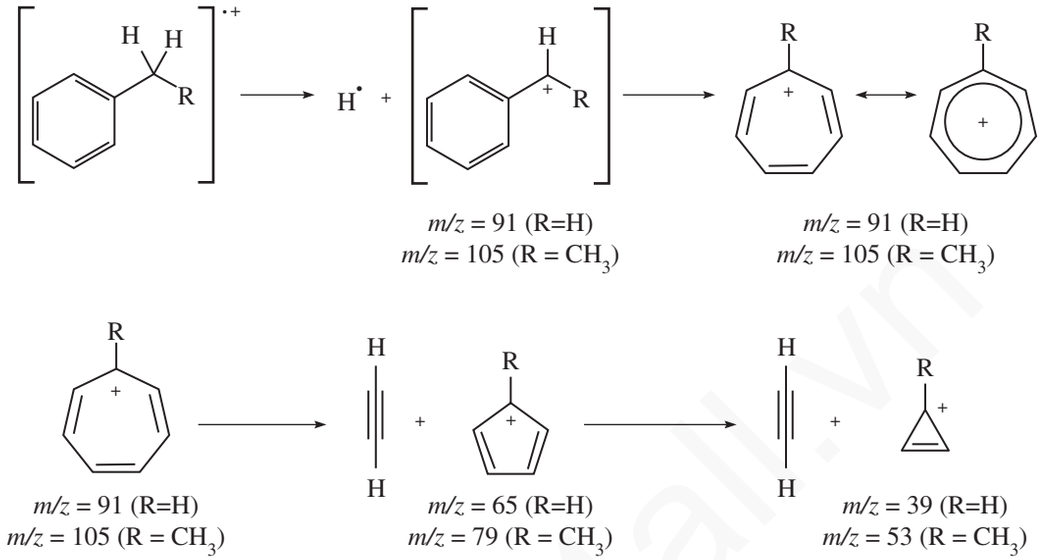


FIGURE 8.43 Formation and fragmentation of the tropylium ion.

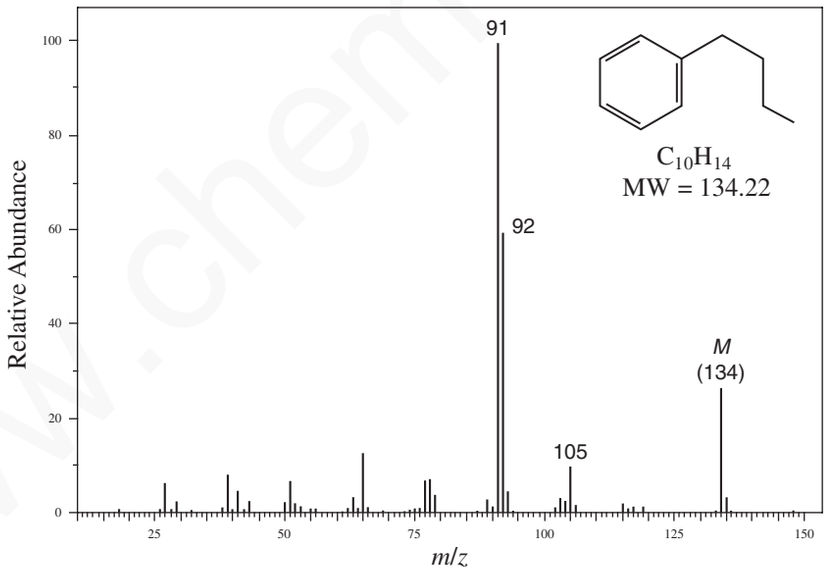
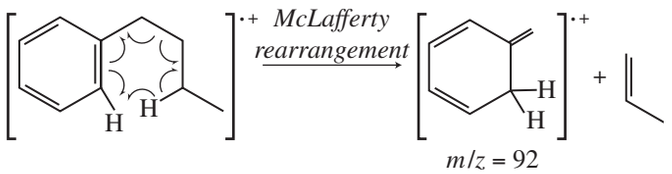


FIGURE 8.44 EI-MS of butylbenzene.

peak at $m/z = 92$ in their mass spectra from the McLafferty rearrangement. Using butylbenzene as an example, this rearrangement is depicted below.



N. Alcohols and Phenols

The intensity of the molecular ion peak in the mass spectrum of a primary or secondary alcohol is usually rather low, and the molecular ion peak is often entirely absent in the mass spectrum of a tertiary alcohol. Common fragmentations of alcohols are α-cleavage adjacent to the hydroxyl group and dehydration.

SPECTRAL ANALYSIS BOX — Alcohols

MOLECULAR ION	FRAGMENT IONS
M^+ weak or absent	Loss of alkyl group $M - 18$

The mass spectrum of straight-chain pentanol isomers, 1-pentanol (Fig. 8.45), 2-pentanol (Figure 8.46), and 3-pentanol (Fig. 8.47) all exhibit very weak molecular ion peaks at $m/z = 88$, while the molecular ion in the mass spectrum of the tertiary alcohol 2-methyl-2-butanol (Fig. 8.48) is entirely absent. The most important fragmentation reaction for alcohols is the loss of an alkyl group via α-cleavage. As discussed earlier, the largest alkyl group is most readily lost. In the spectrum of 1-pentanol (Fig. 8.45), the peak at $m/z = 31$ is due to the loss of a butyl group to form an $H_2C{=}OH^+$ ion. 2-Pentanol (Fig. 8.46) loses either a propyl group to form the $CH_3CH{=}OH^+$ fragment at $m/z = 45$ or a methyl radical to form the relatively small peak at $m/z = 73$ corresponding to $CH_3CH_2CH_2CH{=}OH^+$. 3-Pentanol loses an ethyl radical to form the $CH_3CH_2CH{=}OH^+$ ion at $m/z = 59$. The symmetry of 3-pentanol means there are two identical α-cleavage paths, making the peak corresponding to that ion even more prevalent. 2-Methyl-2-butanol (Fig. 8.48) undergoes α-cleavage to lose a methyl radical two different ways, creating a considerable size peak at $m/z = 73$ in addition to the peak at $m/z = 59$ corresponding to the $(CH_3)_2C{=}OH^+$ ion formed by loss of an ethyl radical.

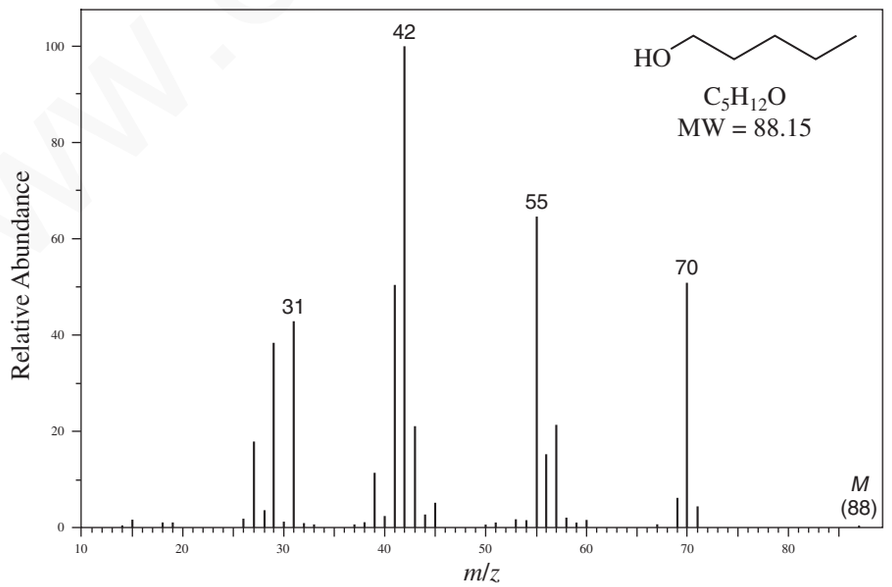


FIGURE 8.45 EI-MS of 1-pentanol.

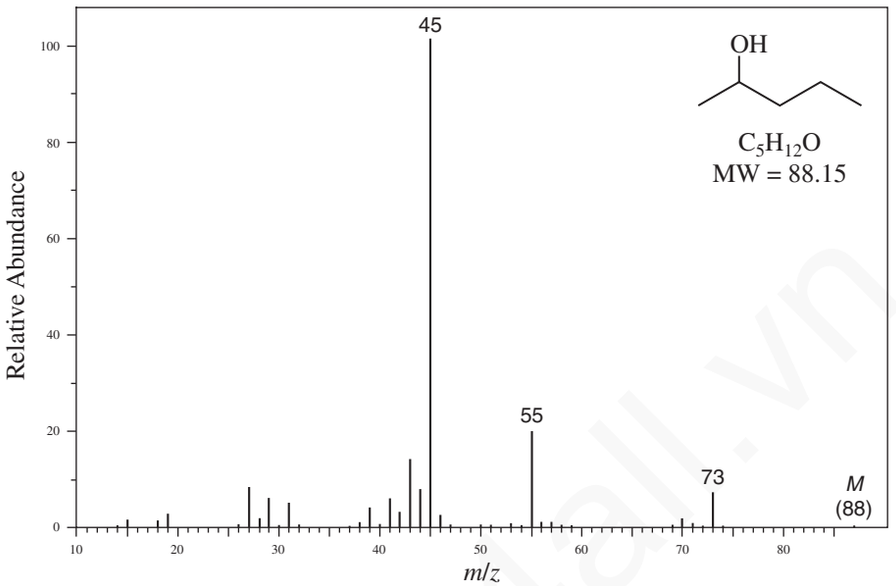


FIGURE 8.46 EI-MS of 2-pentanol.

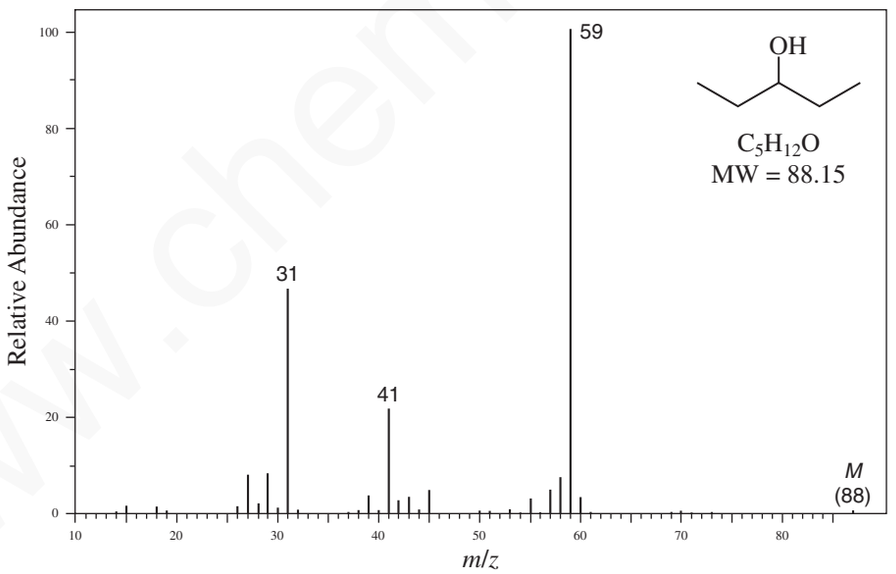


FIGURE 8.47 EI-MS of 3-pentanol.

A second common mode of fragmentation involves dehydration. The importance of dehydration increases as the chain length of the alcohol increases. While the fragment ion peak resulting from dehydration ($m/z = 70$) is very intense in the mass spectrum of 1-pentanol, it is quite weak in the other pentanol isomers. Dehydration may occur by either **thermal dehydration** prior to ionization or by fragmentation of the molecular ion. Thermal dehydration is especially troublesome for alcohol samples analyzed by GC-MS. The injection port of the gas chromatograph is usually maintained at more than 200°C, and many alcohols, especially tertiary or allylic/benzylic, will dehydrate before the sample molecules even reach the GC column and certainly before the molecules reach the ion

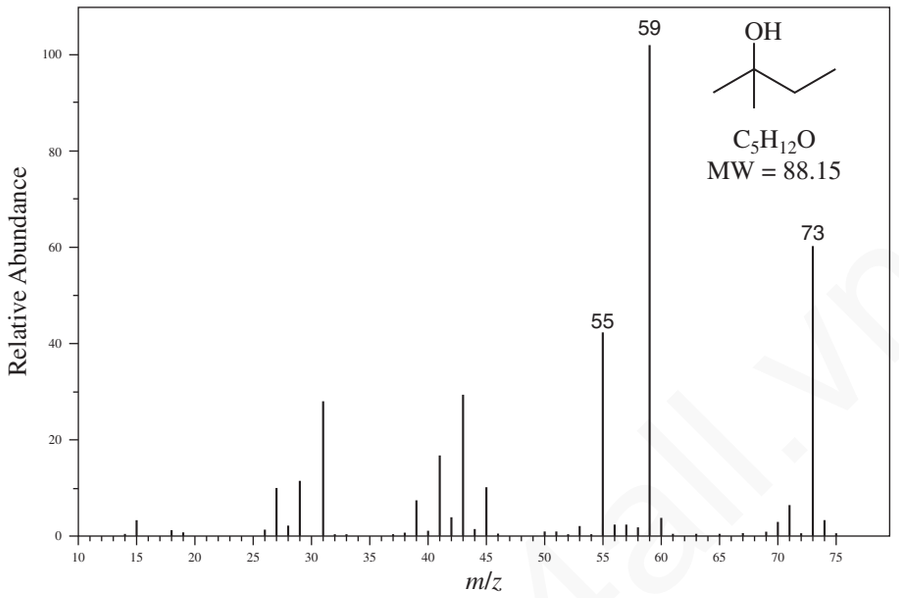
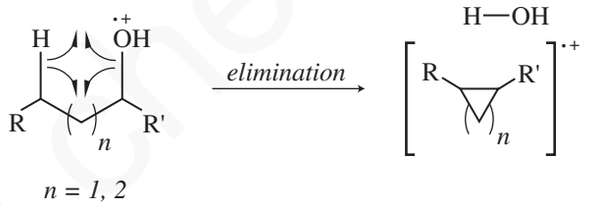
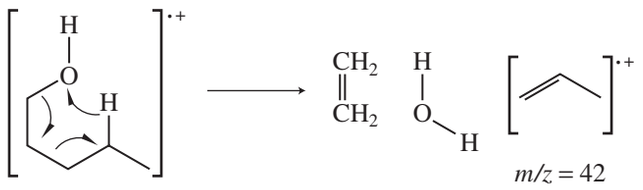


FIGURE 8.48 EI-MS of 2-methyl-2-butanol.

source of the mass spectrometer. Thermal dehydration is a **1,2-elimination** of water. If the alcohol molecules reach the ion source intact, however, dehydration of the molecular ion can still occur, but in this case it is a **1,4-elimination** of water via a cyclic mechanism:



Alcohols containing four or more carbons may undergo the *simultaneous* loss of both water and ethylene. This type of fragmentation is not prominent for 1-butanol but is responsible for the base peak at $m/z = 42$ in the mass spectrum of 1-pentanol (Fig. 8.45).



Cyclic alcohols may undergo fragmentation by at least three different pathways, and these are illustrated for the case of cyclohexanol in Figure 8.49. The first fragmentation is simply an α-cleavage and loss of a hydrogen atom to yield an $M - 1$ fragment ion. The second fragmentation path begins with an initial α-cleavage of a ring bond adjacent to the hydroxyl-bearing carbon, followed by a 1,5-hydrogen migration. This moves the radical site back to a resonance-stabilized position adjacent to the oxonium ion. A second α-cleavage results in the loss of a propyl radical and formation of a protonated acrolein ion with $m/z = 57$. This fragmentation path is nearly identical to

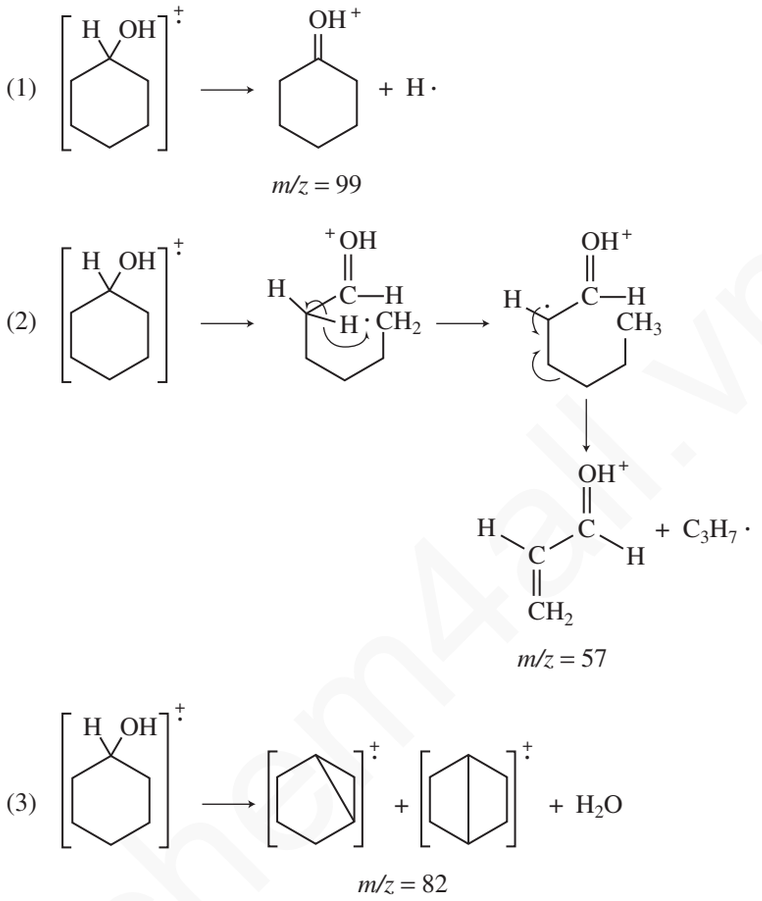
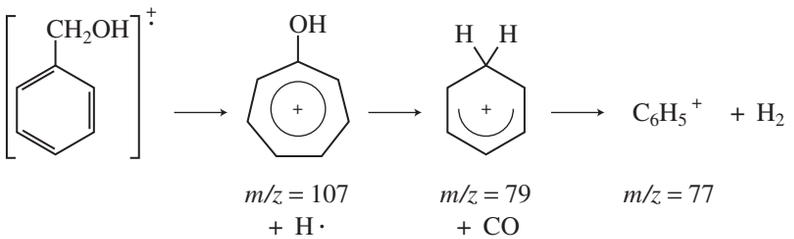


FIGURE 8.49 Fragmentation pathways for cyclohexanol.

one that operates on cyclohexanone derivatives (Section 8.8Q). The third fragmentation path of cyclic alcohols is dehydration via abstraction of a hydrogen atom from three or four carbons away (the hydrogen atom is transferred in a five- or six-membered cyclic transition state) to produce a bicyclic radical cation with $m/z = 82$. A peak corresponding to each of these fragment ions can be observed in the mass spectrum of cyclohexanol (Fig. 8.50).

Benzylic alcohols usually exhibit strong molecular ion peaks. The following sequence of reactions illustrates their principal modes of fragmentation. Loss of a hydrogen atom from the molecular ion leads to a hydroxytropylium ion (m/z = 107). The hydroxytropylium ion can lose carbon monoxide to form a resonance-delocalized cyclohexadienyl cation ($m/z = 79$). This ion can eliminate molecular hydrogen to create a phenyl cation, $C_6H_5^+$, $m/z = 77$. Peaks arising from these fragment ions can be observed in the mass spectrum of benzyl alcohol (Fig. 8.51).



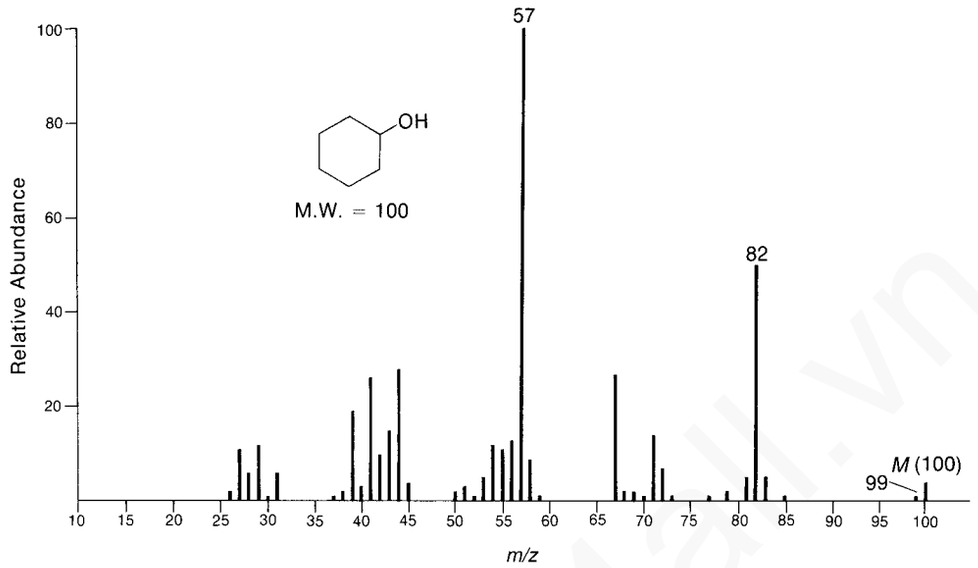


FIGURE 8.50 EI-MS of cyclohexanol.

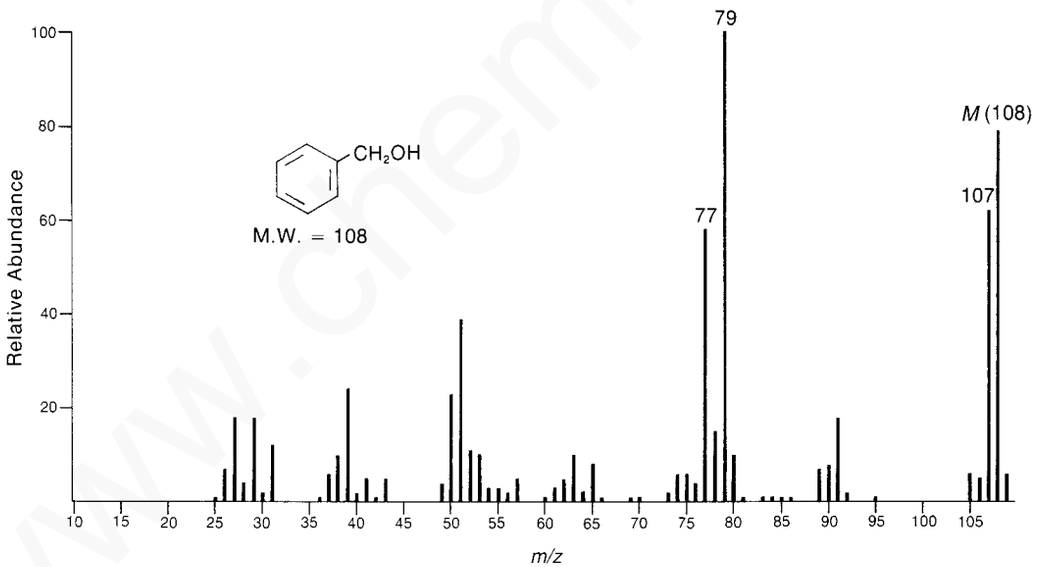


FIGURE 8.51 EI-MS of benzyl alcohol.

The mass spectra of phenols usually show strong molecular ion peaks. In fact, the molecular ion at $m/z = 94$ is the base peak in the EI-MS of phenol (Fig. 8.52). Favored modes of fragmentation involve loss of a hydrogen atom to create an $M - 1$ peak (a small peak at $m/z = 93$), loss of carbon monoxide (CO) to produce a peak at $M - 28$ ($m/z = 66$), and loss of a formyl radical (HCO·) to give a peak at $M - 29$. In the case of phenol itself, this creates the aromatic cyclopentadienyl cation at $m/z = 65$. In some cases, the loss of 29 mass units may be sequential: initial loss of carbon monoxide followed by loss of a hydrogen atom. The mass spectrum of *ortho*-cresol (2-methylphenol) exhibits a much larger peak at $M - 1$ (Fig. 8.53) than does unsubstituted phenol. Note also the peaks at $m/z = 80$ and $m/z = 79$ in the *o*-cresol spectrum from loss of CO and formyl radical, respectively.

SPECTRAL ANALYSIS BOX — Phenols

MOLECULAR ION	FRAGMENT IONS
M^+ strong	$M - 1$
	$M - 28$
	$M - 29$

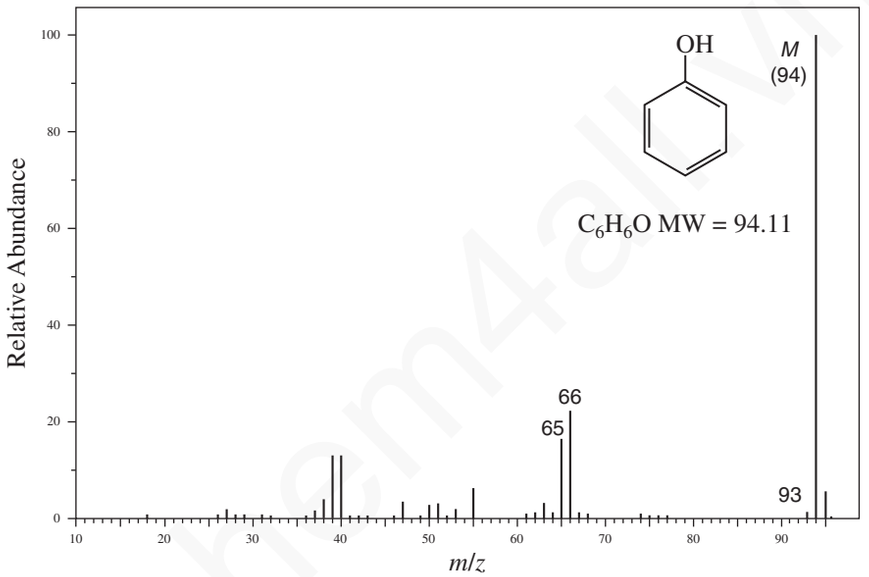


FIGURE 8.52 EI-MS of phenol.

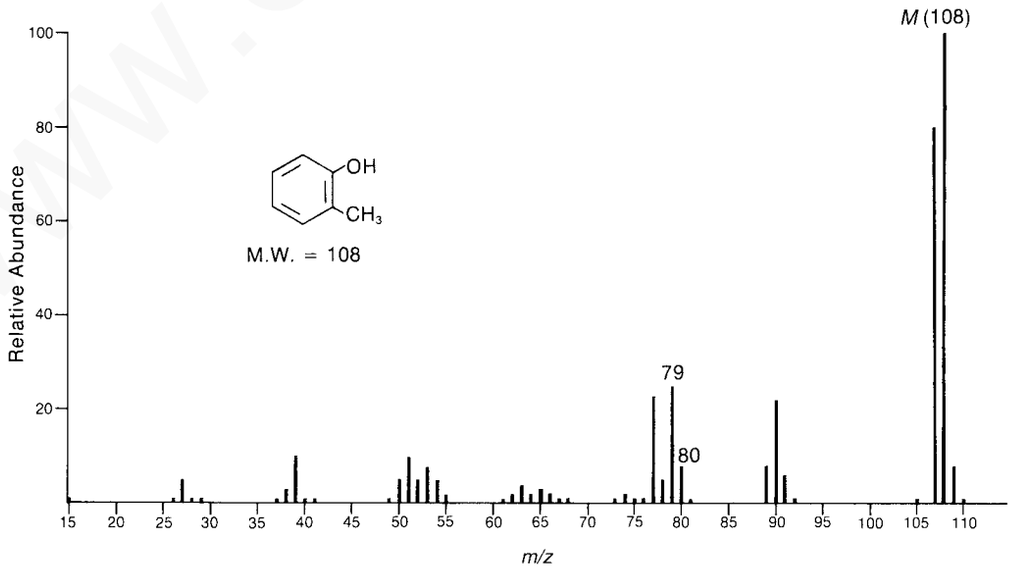


FIGURE 8.53 EI-MS of 2-methylphenol (*ortho*-cresol).

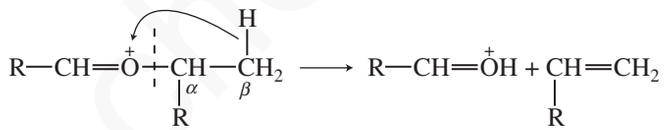
O. Ethers

Aliphatic ethers tend to exhibit molecular ion peaks that are stronger than those of alcohols with the same molecular weights. Nevertheless, the molecular ion peaks of ethers are still rather weak. Principal modes of fragmentation include α-cleavage, formation of carbocation fragments through inductive cleavage (β-cleavage), and loss of alkoxy radicals.

SPECTRAL ANALYSIS BOX — Ethers

MOLECULAR ION	FRAGMENT IONS
M^+ weak, but observable	α-Cleavage
	$m/z = 43, 59, 73$, etc.
	$M - 31$, $M - 45$, $M - 59$, etc.

The fragmentation of the ethers is somewhat similar to that of the alcohols. In the mass spectrum of diisopropyl ether (Fig. 8.54), an α-cleavage gives rise to a peak at $m/z = 87$ due to the loss of a methyl radical. A second mode of fragmentation involves cleavage of the carbon–oxygen bond of an ether to yield an isopropoxyl radical and a isopropyl carbocation. Cleavage of this type in diisopropyl ether is responsible for the $C_3H_7^+$ fragment at $m/z = 43$. A third type of fragmentation occurs as a rearrangement reaction of one of the fragment ions rather than on the molecular ion itself. The rearrangement involves transfer of a hydrogen β to the oxonium ion with concurrent formation of an alkene. This type of rearrangement is particularly favored when the α carbon of the ether is branched. In the case of diisopropyl ether, this rearrangement gives rise to a $(HO{=}CHCH_3)^+$ fragment at $m/z = 45$.



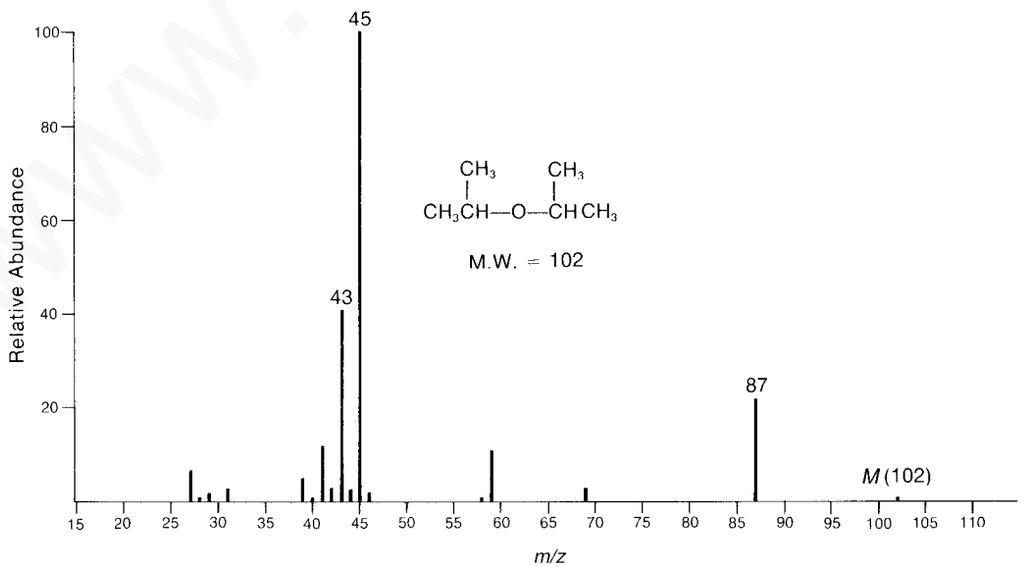


FIGURE 8.54 EI-MS of diisopropyl ether.

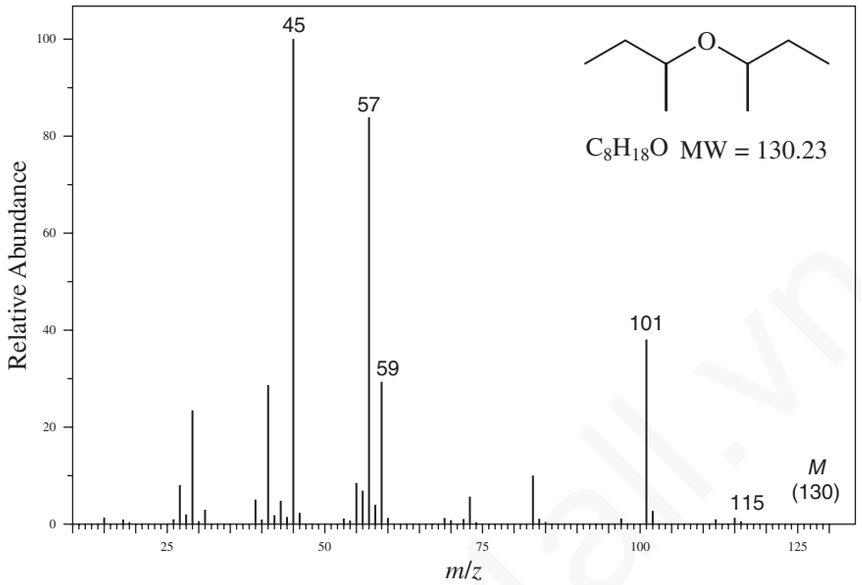


FIGURE 8.55 EI-MS of di-*sec*-butyl ether.

The mass spectrum of di-*sec*-butyl ether (Fig. 8.55) shows the same fragmentations. There are two possible α-cleavages in this compound, however. Loss of a methyl radical gives the very small $M - 15$ peak at $m/z = 115$, but loss of the larger ethyl radical gives the substantially larger peak at $m/z = 101$. Inductive cleavage of the C—O bond creates a *sec*-butyl cation at $m/z = 57$. Further rearrangement of the α-cleavage products produce ions at $m/z = 45$ and 59, corresponding to $(HO{=}CHCH_3)^+$ and $(HO{=}CHCH_2CH_3)^+$, respectively.

Acetals and ketals behave very similarly to ethers. However, fragmentation is even more favorable in acetals and ketals than in ethers, so the molecular ion peak of an acetal or ketal may be either extremely weak or totally absent. For example, in the mass spectrum of 2-ethyl-2-methyl-1, 3-dioxolane (the ethylene ketal of methyl ethyl ketone), the molecular ion is not visible (Fig. 8.56).

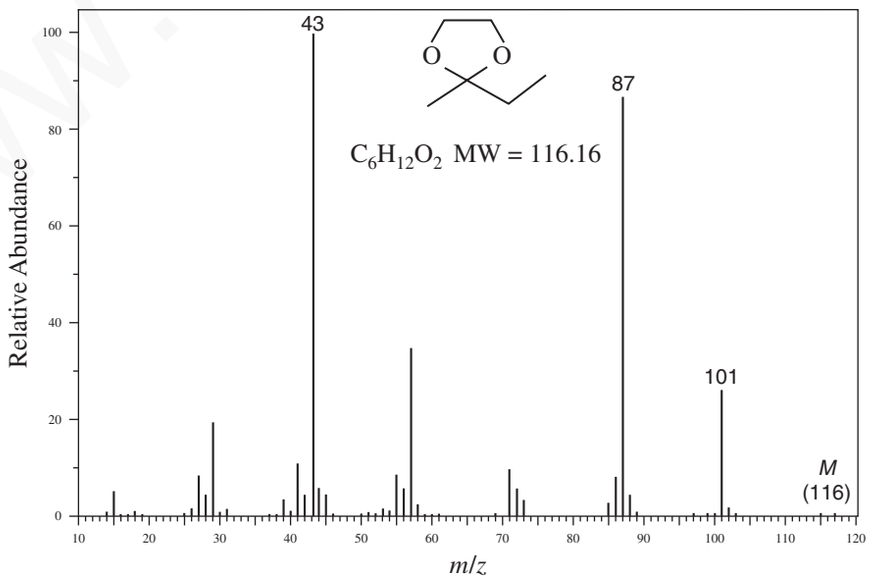


FIGURE 8.56 EI-MS of 2-ethyl-2-methyl-1,3-dioxolane.

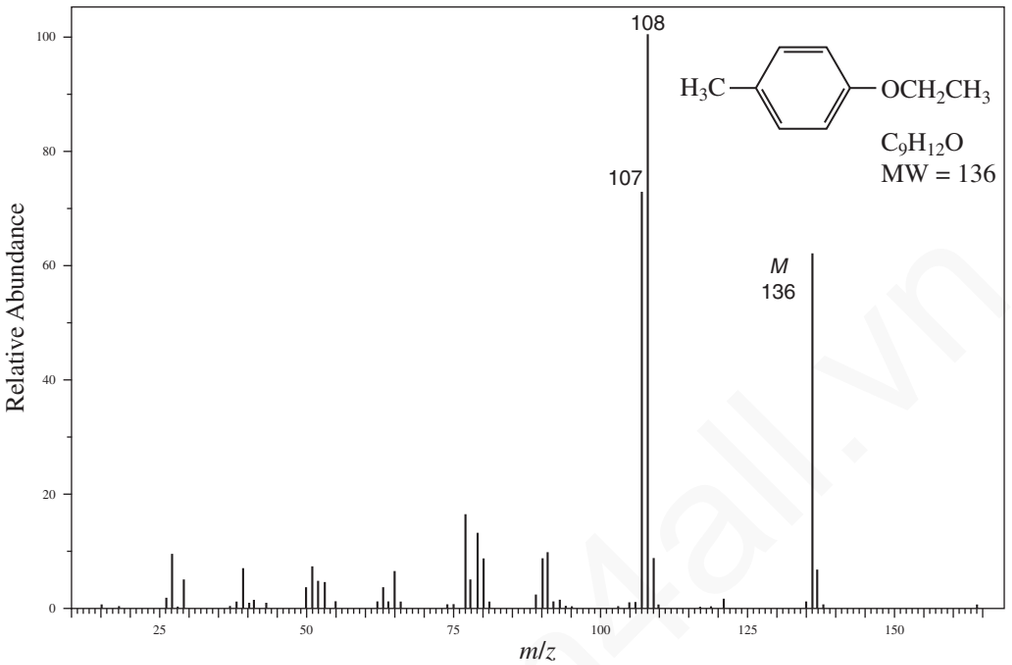


FIGURE 8.57 EI-MS of 4-methylphenetole.

The highest mass peak is at $m/z = 101$ from loss of a methyl radical via α-cleavage, and an alternative α-cleavage produces the large peak at $m/z = 87$ formed by loss of an ethyl radical. The base peak in the spectrum is found at $m/z = 43$, typical of 2-methyl-1,3-dioxolanes.

Aromatic ethers may undergo cleavage reactions that involve loss of the alkyl group to form $C_6H_5O^+$ ions. These fragment ions then lose carbon monoxide to form cyclopentadienyl cations ($C_5H_5^+$). In addition, an aromatic ether may lose the entire alkoxy group to yield phenyl cations ($C_6H_5^+$). The mass spectrum of ethyl 4-methylphenyl ether (*p*-methylphenetole) exhibits a strong molecular ion at $m/z = 136$ as well as a fragment at $m/z = 107$ from loss of an ethyl radical (Fig. 8.57). The base peak at $m/z = 108$ arises from loss of ethene via a McLafferty rearrangement.

P. Aldehydes

The molecular ion peak of an aliphatic aldehyde is usually observable, although at times it may be fairly weak. Principal modes of fragmentation include α-cleavage and β-cleavage. If the carbon chain attached to the carbonyl group contains at least three carbons, McLafferty rearrangement is also commonly observed.

SPECTRAL ANALYSIS BOX—Aldehydes

MOLECULAR ION	FRAGMENT IONS
M^+ weak, but observable (aliphatic)	Aliphatic:
M^+ strong (aromatic)	$m/z = 29$, $M - 29$,
	$M - 43$, $m/z = 44$
	Aromatic:
	$M - 1$, $M - 29$

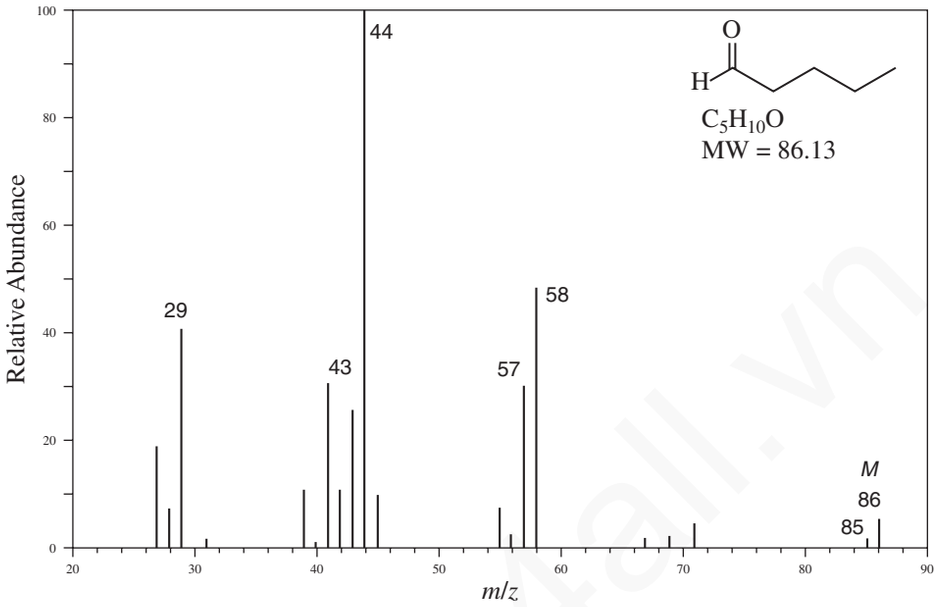


FIGURE 8.58 EI-MS of valeraldehyde.

The appearance of an $M - 1$ peak due to the loss of one hydrogen atom is very characteristic of aldehydes. This peak is observed at $m/z = 85$ in the mass spectrum of valeraldehyde (Fig. 8.58). The peak due to the formation of HCO^+ can be observed at $m/z = 29$; this is also a very characteristic peak in the mass spectra of aldehydes. The second important mode of fragmentation for aldehydes is known as **β-cleavage** (inductive cleavage). In the case of valeraldehyde, β-cleavage creates a propyl cation ($m/z = 43$).

The third major fragmentation pathway for aldehydes is the McLafferty rearrangement. The fragment ion formed in this rearrangement has $m/z = 44$ and is the base peak in the spectrum of valeraldehyde. The $m/z = 44$ peak is considered to be quite characteristic for aldehydes. As with all McLafferty rearrangements, of course, this rearrangement occurs only if the chain attached to the carbonyl group has three or more carbons.

Aromatic aldehydes also exhibit intense molecular ion peaks, and the loss of one hydrogen atom via α-cleavage is a very favorable process. The resulting $M - 1$ peak may in some cases be more intense than the molecular ion peak. In the mass spectrum of benzaldehyde (Fig. 8.59), the $M - 1$ peak appears at $m/z = 105$. Note also the peak at $m/z = 77$, which corresponds to the phenyl cation formed by loss of the formyl radical.

Q. Ketones

The mass spectra of ketones show an intense molecular ion peak. Loss of the alkyl groups attached to the carbonyl group is one of the most important fragmentation processes. The pattern of fragmentation is similar to that of aldehydes. Loss of alkyl groups by means of α-cleavage is an important

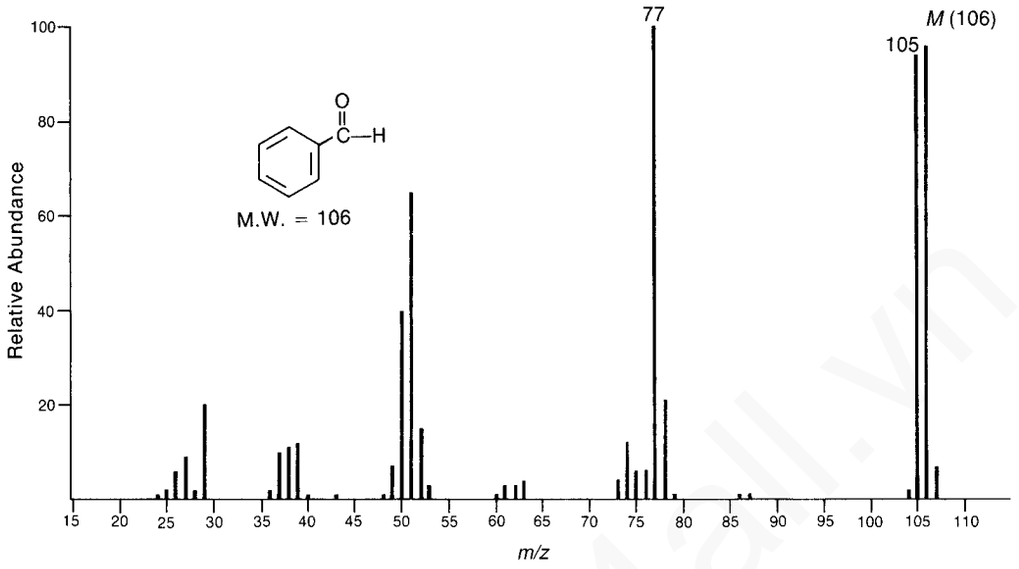


FIGURE 8.59 EI-MS of benzaldehyde.

mode of fragmentation, and the larger of the two alkyl groups attached to the carbonyl group appears more likely to be lost, in keeping with Stevenson's Rule. The ion formed from this type of α-cleavage in ketones (and aldehydes) is the acylium ion ($RC{\equiv}O^+$). In the mass spectrum of 2-butanone (Fig. 8.60), the peak at $m/z = 43$ is more intense than the peak at $m/z = 57$, which is due to the loss of the methyl group. Similarly, in the mass spectrum of 2-octanone (Fig. 8.61) loss of the hexyl group, giving a peak at $m/z = 43$, is more likely than loss of the methyl group, which gives the weak peak at $m/z = 113$.

SPECTRAL ANALYSIS BOX — Ketones

MOLECULAR ION	FRAGMENT IONS
M^+ strong	Aliphatic:
	$M - 15$, $M - 29$, $M - 43$, etc.
	$m/z = 43$
	$m/z = 58$, 72, 86, etc.
	$m/z = 42$, 83
	Aromatic:
	$m/z = 105$, 120

When the carbonyl group of a ketone has attached to it at least one alkyl group that is three or more carbon atoms in length, a McLafferty rearrangement is possible. The peak at $m/z = 58$ in the mass spectrum of 2-octanone is due to the fragment ion that results from this rearrangement.

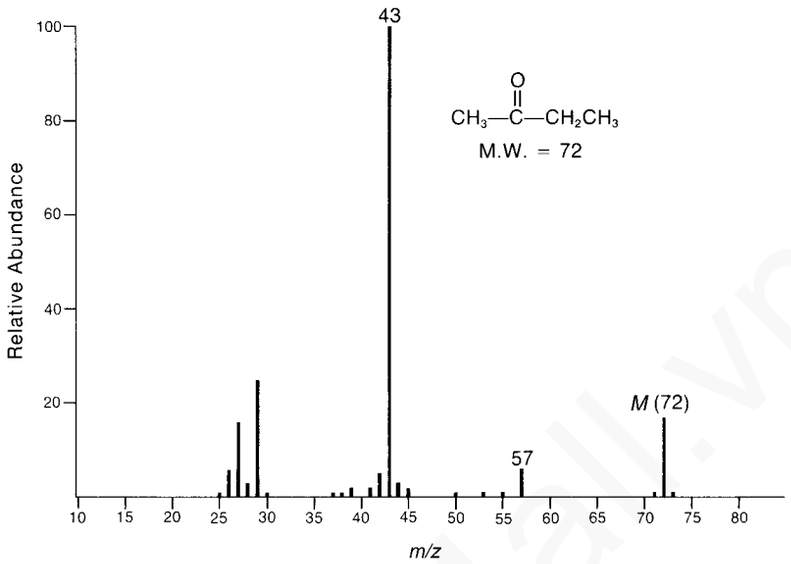


FIGURE 8.60 EI-MS of 2-butanone.

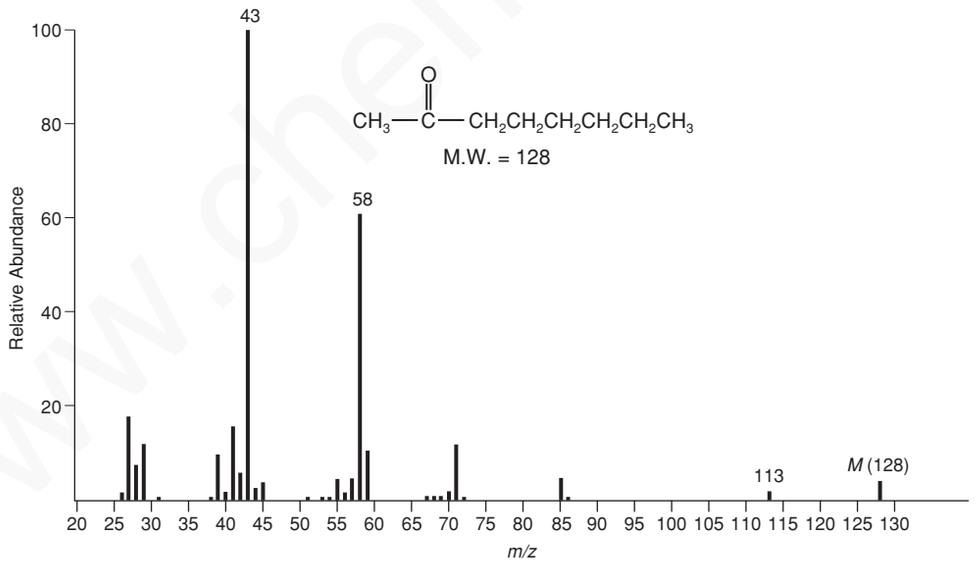


FIGURE 8.61 EI-MS of 2-octanone.

Cyclic ketones may undergo a variety of fragmentation and rearrangement processes. Outlines of these processes for the case of cyclohexanone follow. A fragment ion peak corresponding to each process appears in the mass spectrum of cyclohexanone (Fig. 8.62).

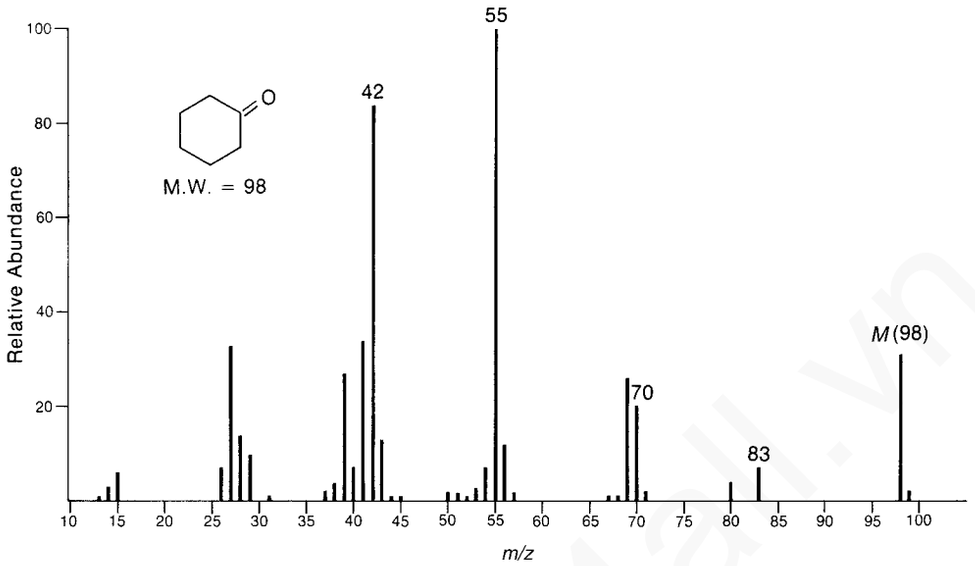
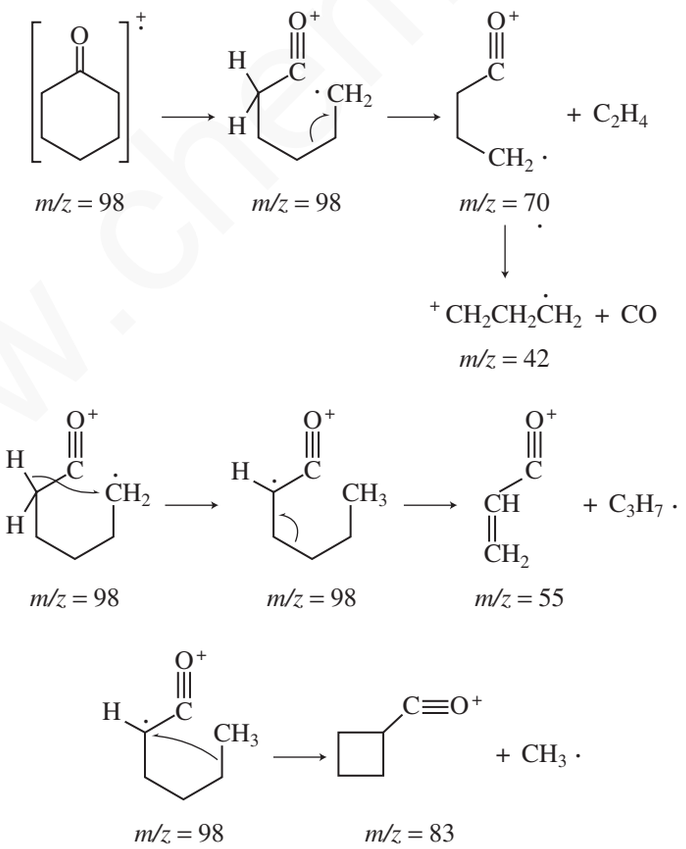


FIGURE 8.62 EI-MS of cyclohexanone.



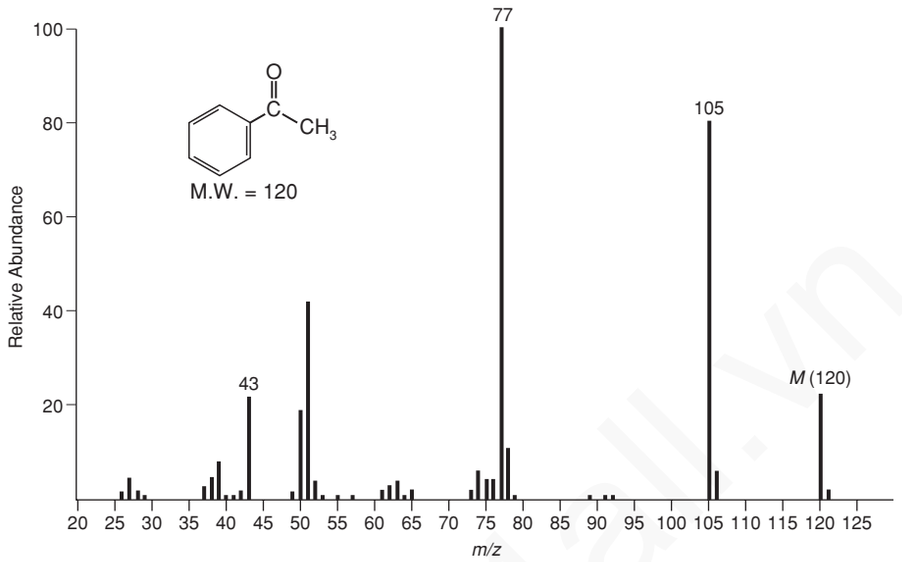
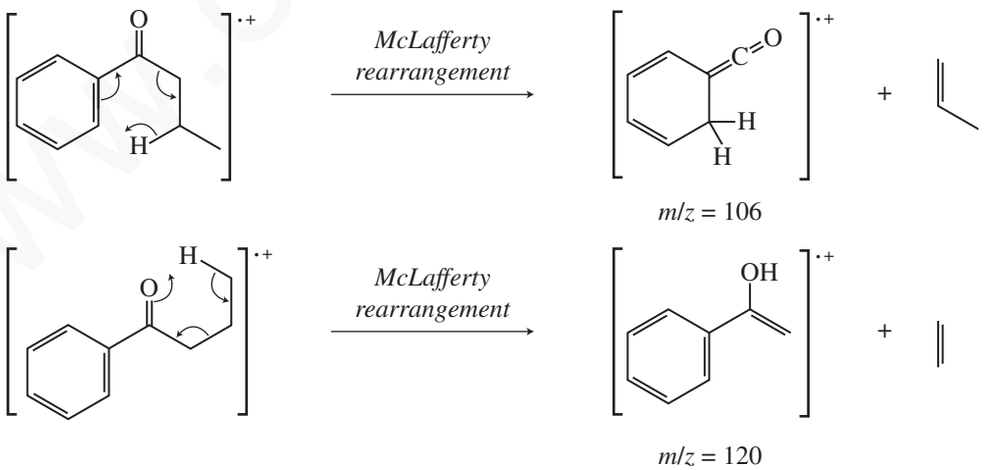


FIGURE 8.63 EI-MS of acetophenone.

Aromatic ketones undergo α-cleavage to lose the alkyl group and form the phenylacylium ($C_6H_5CO^+$, $m/z = 105$) ion. This ion can undergo secondary fragmentation to lose carbon monoxide, forming the $C_6H_5^+$ ion ($m/z = 77$). These peaks appear prominently in the mass spectrum of acetophenone (Fig. 8.63). With larger alkyl groups attached to the carbonyl group of an aromatic ketone, a rearrangement of the McLafferty type is likely, and the rearrangement can occur to the carbonyl and to the aromatic ring. In the case of butyrophenone, McLafferty rearrangement to the aromatic ring yields the fragment seen at $m/z = 106$, and the rearrangement to the carbonyl gives the fragment at $m/z = 120$ (Fig. 8.64). The $m/z = 120$ fragment ion may undergo additional α-cleavage to yield the $C_6H_5CO^+$ ion at $m/z = 105$.



R. Esters

Fragmentation of esters is especially facile, but it is usually possible to observe weak molecular ion peaks in the mass spectra of methyl esters. The esters of higher alcohols form much weaker

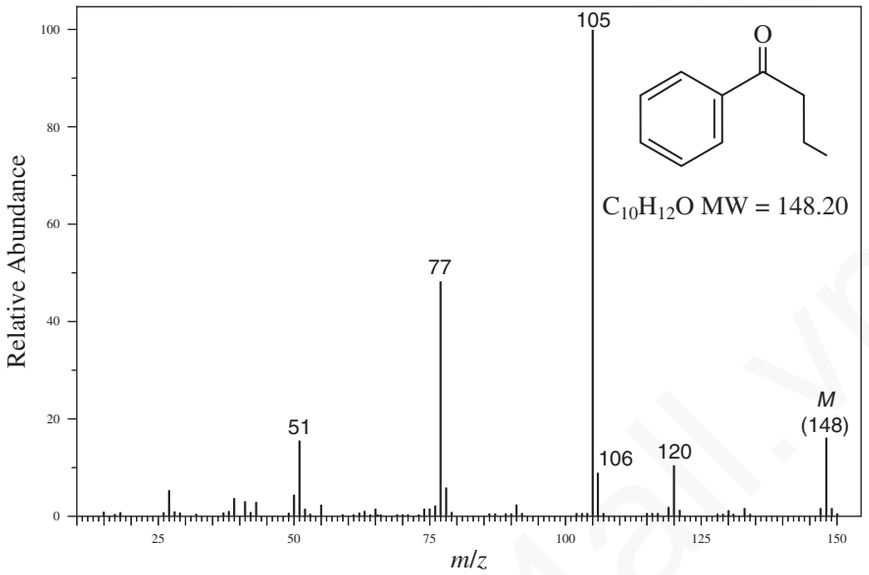


FIGURE 8.64 EI-MS of butyrophenone.

molecular ion peaks, and esters of alcohols larger than four carbons may form molecular ion peaks that fragment too quickly to be observed. The most important fragmentation of esters is an α-cleavage that involves the loss of the alkoxy group to form the corresponding acylium ion, $RC{\equiv}O^+$ The acylium ion peak appears at m/z = 71 in the mass spectrum of methyl butyrate (Fig. 8.65). A second useful peak results from the loss of the alkyl group from the acyl side of the ester, leaving a fragment $H_3C{-}O{-}C{=}O^+$ that appears at m/z = 59 in the mass spectrum of methyl butyrate. Other fragment ion peaks include the $^+OCH_3$ fragment (m/z = 31) and the R^+ fragment from the acyl portion of the ester molecule, $CH_3CH_2CH_2^+$ in the case of methyl butyrate, at m/z = 43.

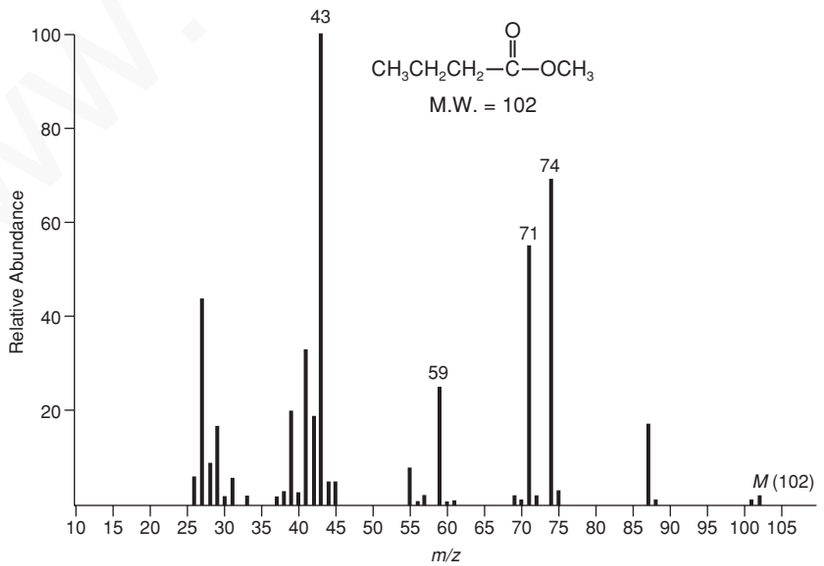


FIGURE 8.65 EI-MS of methyl butyrate.

SPECTRAL ANALYSIS BOX — Esters

MOLECULAR ION	FRAGMENT IONS
M^+ weak, but generally observable	Methyl esters: $M - 31$, $m/z = 59, 74$ Higher esters: $M - 45$, $M - 59$, $M - 73$ $m/z = 73, 87, 101$ $m/z = 88, 102, 116$ $m/z = 61, 75, 89$ $m/z = 77, 105, 108$ $M - 32$, $M - 46$, $M - 60$

Another important fragmentation of esters is the McLafferty rearrangement that produces the peak at m/z = 74 (for methyl esters). Ethyl, propyl, butyl, and higher alkyl esters also undergo α-cleavage and McLafferty rearrangements typical of the methyl esters. In addition, however, these esters may undergo an additional rearrangement of the alkoxy portion of the ester that results in fragments that appear in the series $m/z = 61, 75, 89$, and so on. This process is illustrated on page 480 for butyl butyrate and is commonly referred to as the **McLafferty + 1 rearrangement** or the McLafferty rearrangement with double-hydrogen transfer (Fig. 8.66) Several other peaks in the mass spectrum of butyl butyrate are readily assigned by considering the common fragmentations. Loss of a propyl radical through α-cleavage forms the butoxyacylium ion at m/z = 101, McLafferty rearrangement on the acyl side of the ester creates the ion observed at m/z = 73, and loss of butoxy radical from the molecular ion yields the acylium ion seen at m/z = 71.

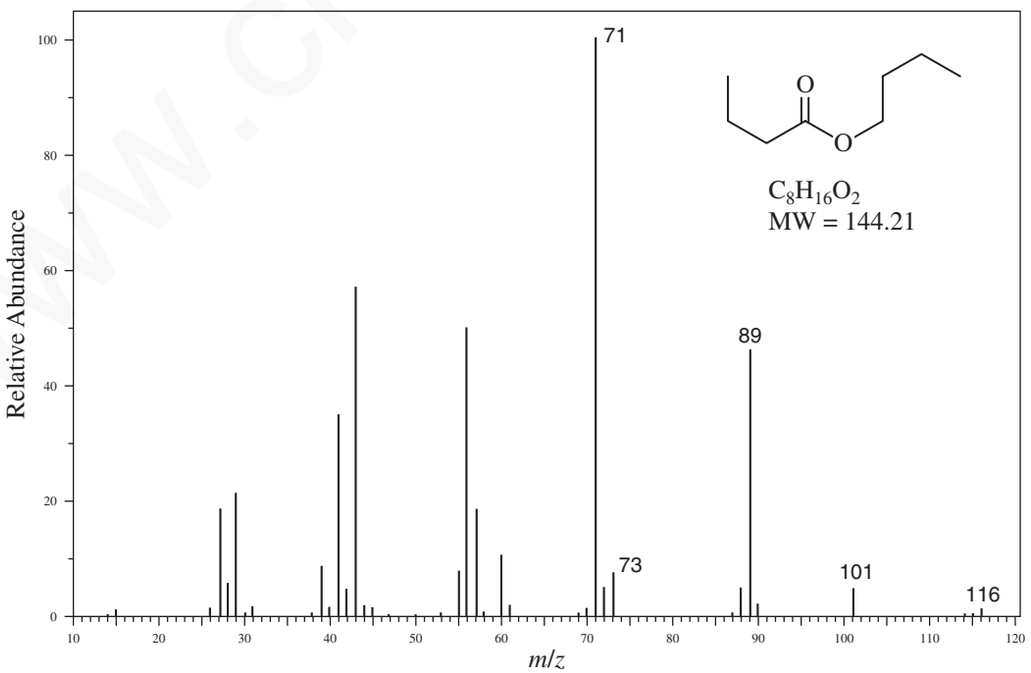
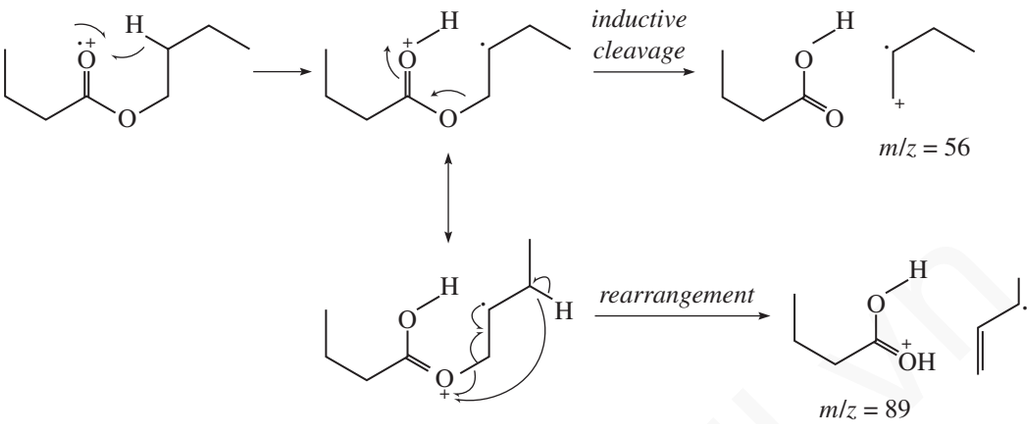


FIGURE 8.66 EI-MS of butyl butyrate.



Benzyl esters undergo rearrangement to eliminate a neutral ketene molecule and the radical cation of benzyl alcohol at $m/z = 108$. The resulting ion is often the most intense peak in the mass spectrum of such a compound. This fragmentation is dominant in the mass spectrum of benzyl laurate, along with the benzyl cation/tropylium ion at $m/z = 91$ (Fig. 8.67). Other high-mass fragments in the benzyl laurate spectrum include a peak at $m/z = 199$ from loss of a benzyl radical and the peak at $m/z = 183$ from loss of benzyloxy radical via α-cleavage.

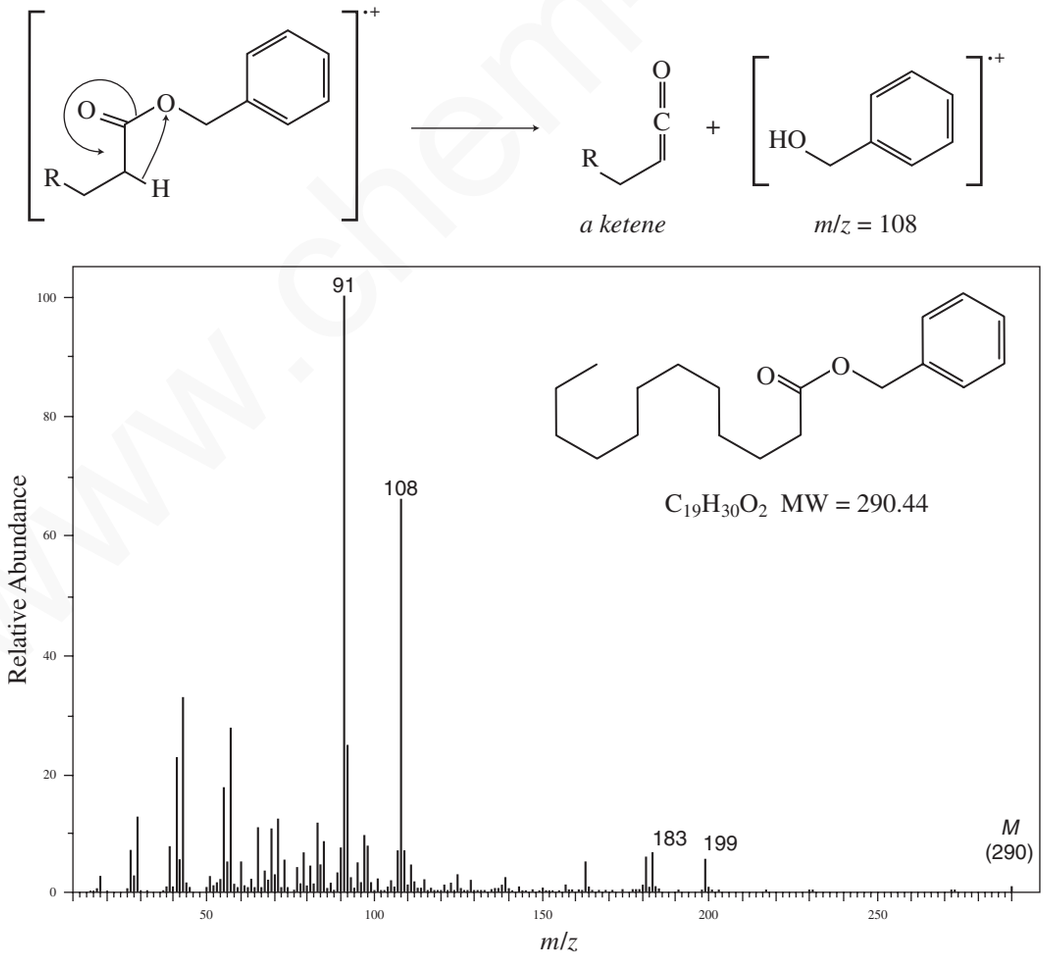


FIGURE 8.67 EI-MS of benzyl laurate.

Alkyl benzoate esters prefer to lose the alkoxy group to form the $C_6H_5C{\equiv}O^+$ ion (m/z = 105). This ion may lose carbon monoxide to form the phenyl cation ($C_6H_5^+$) at m/z = 77. Each of these peaks appears in the mass spectrum of methyl benzoate (Fig. 8.68). Alkyl substitution on benzoate esters appears to have little effect on the mass spectral results unless the alkyl group is in the *ortho* position with respect to the ester functional group. In this case, the alkyl group can interact with the ester function, with the elimination of a molecule of alcohol. This is observed in the mass spectrum of isobutyl salicylate (Fig. 8.69). The base peak at m/z = 120 arises from elimination of isobutyl alcohol via this *ortho* effect. The fragment at m/z = 121 comes from loss of isobutoxyl radical via standard α-cleavage, and the peak at m/z = 138 likely arises by elimination of isobutene from the molecular ion.

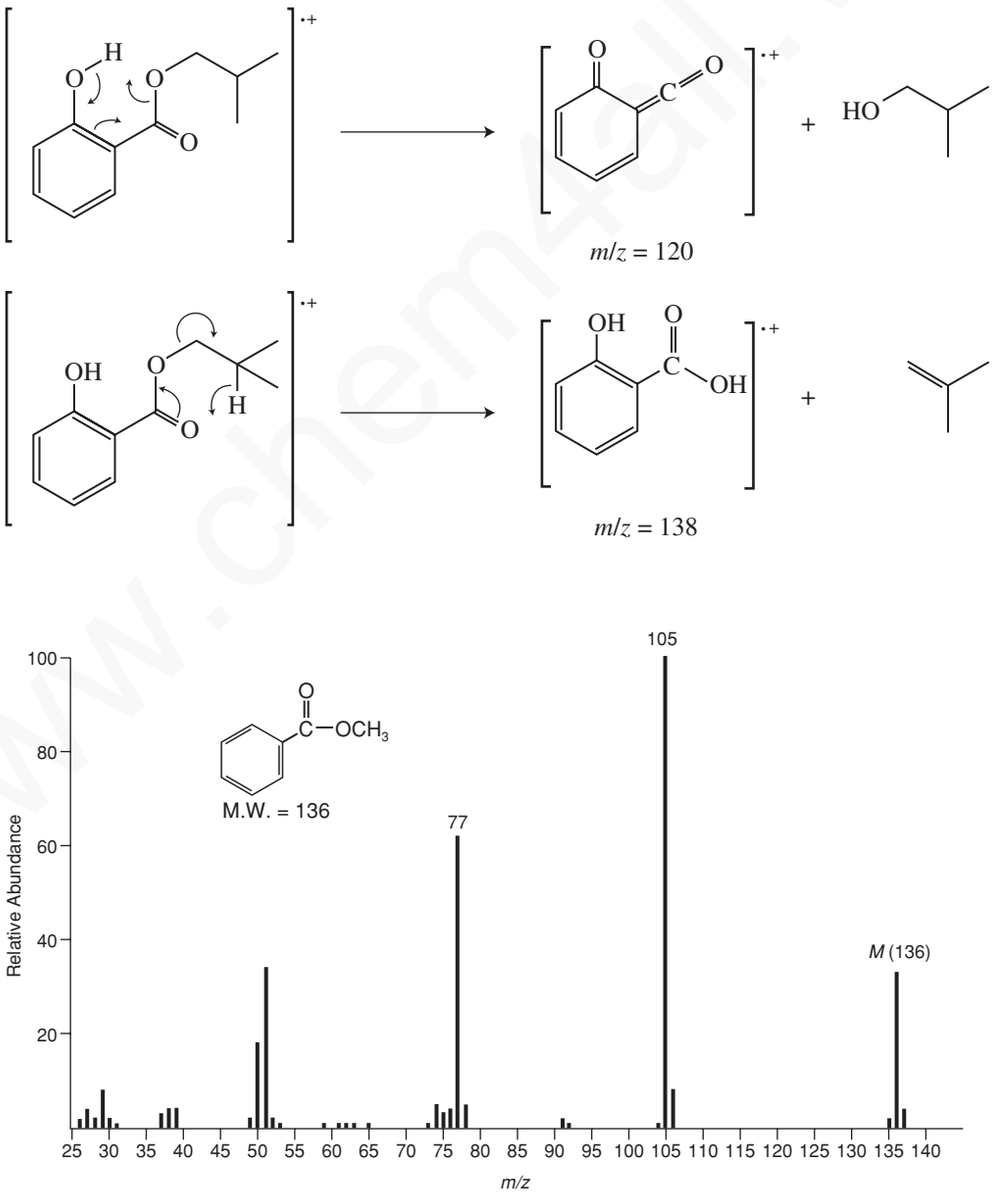


FIGURE 8.68 EI-MS of methyl benzoate.

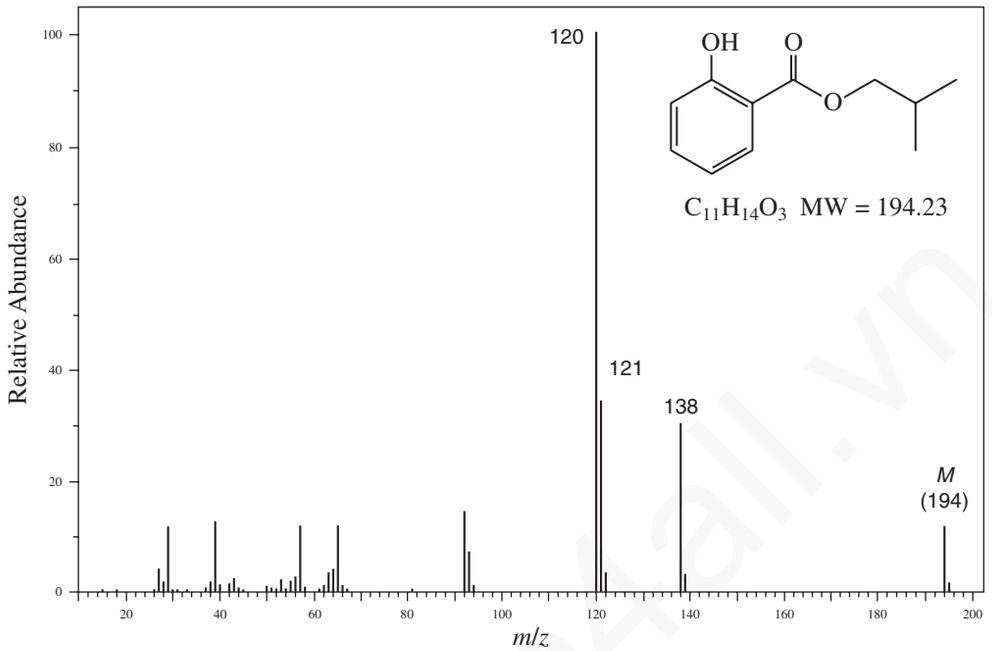


FIGURE 8.69 EI-MS of isobutyl salicylate.

S. Carboxylic Acids

Aliphatic carboxylic acids generally show weak, but observable, molecular ion peaks. Aromatic carboxylic acids, on the other hand, show strong molecular ion peaks. The principal modes of fragmentation resemble those of the methyl esters.

SPECTRAL ANALYSIS BOX — Carboxylic Acids

MOLECULAR ION	FRAGMENT IONS
Aliphatic carboxylic acids:	Aliphatic carboxylic acids:
M^+ weak, but observable	$M - 17$, $M - 45$
	$m/z = 45$, 60
Aromatic carboxylic acids:	Aromatic carboxylic acids:
M^+ strong	$M - 17$, $M - 45$
	$M - 18$

With short-chain acids, the loss of OH and COOH through α-cleavage on either side of the C=O group may be observed. In the mass spectrum of butyric acid (Fig. 8.70) loss of ·OH gives rise to a small peak at $m/z = 71$. Loss of COOH gives rise to a peak at $m/z = 45$. Loss of the alkyl group as a free radical, leaving the $COOH^+$ ion ($m/z = 45$), also appears in the mass spectrum and is characteristic of the mass spectra of carboxylic acids. With acids containing γ-hydrogens, the principal pathway for fragmentation is the McLafferty rearrangement. In the case of carboxylic acids, this rearrangement produces a prominent peak at $m/z = 60$.

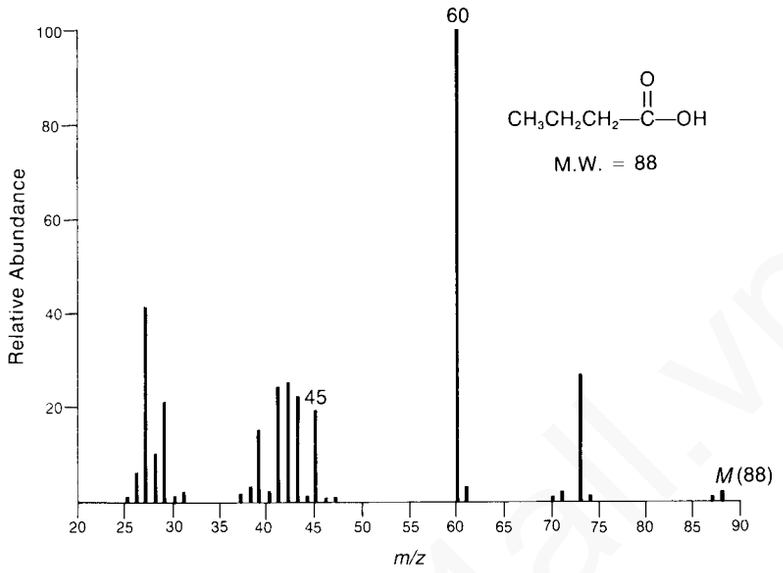


FIGURE 8.70 EI-MS of butyric acid.

Aromatic carboxylic acids produce intense molecular ion peaks. The most important fragmentation pathway involves loss of ·OH to form the $C_6H_5C{\equiv}O^+$ (m/z = 105), followed by loss of CO to form the $C_6H_5^+$ ion (m/z = 77). In the mass spectrum of *para*-anisic acid (Fig. 8.71), loss of ·OH gives rise to a peak at m/z = 135. Further loss of CO from this ion gives rise to a peak at m/z = 107. Benzoic acids bearing *ortho* alkyl, hydroxy, or amino substituents undergo loss of water through a rearrangement reaction similar to that observed for *ortho*-substituted benzoate esters, as illustrated at the end of Section 8.8R.

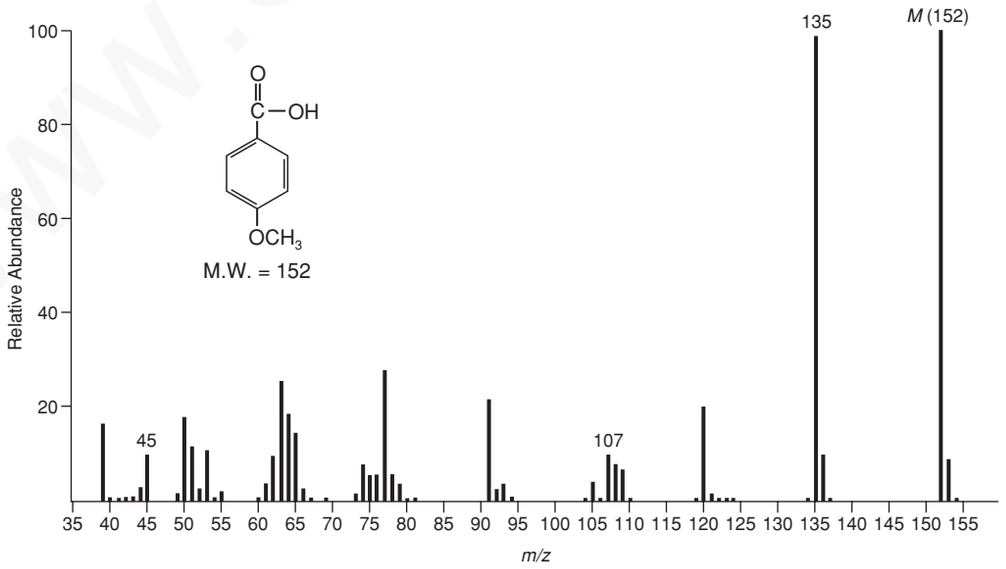


FIGURE 8.71 EI-MS of *para*-anisic acid.

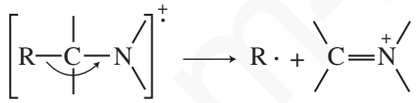
T. Amines

The value of the mass of the molecular ion can be of great help in identifying a substance as an amine. As stated in Section 8.6, a compound with an odd number of nitrogen atoms must have an odd-numbered molecular weight. On this basis, it is possible to quickly determine whether a substance could be an amine. Unfortunately, in the case of aliphatic amines, the molecular ion peak may be very weak or even absent.

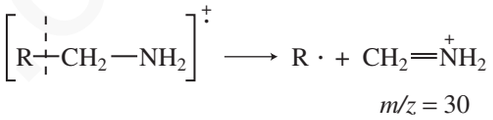
SPECTRAL ANALYSIS BOX—Amines

MOLECULAR ION	FRAGMENT IONS
M^+ weak or absent	α-Cleavage
Nitrogen Rule obeyed	$m/z = 30$

The most intense peak in the mass spectrum of an aliphatic amine arises from α-cleavage:

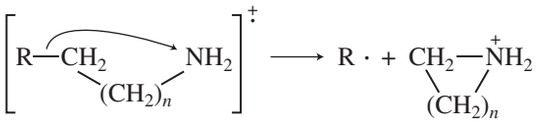


When there is a choice of R groups to be lost through this process, the largest R group is lost preferentially. For primary amines that are not branched at the carbon next to the nitrogen, the most intense peak in the spectrum occurs at $m/z = 30$. It arises from α-cleavage:



The presence of this peak is strong, although not conclusive, evidence that the test substance is a primary amine. The peak may arise from secondary fragmentation of ions formed from the fragmentation of secondary or tertiary amines as well. In the mass spectrum of ethylamine (Fig. 8.72), the $m/z = 30$ peak can be seen clearly.

The same β-cleavage peak can also occur for long-chain primary amines. Further fragmentation of the R group of the amine leads to clusters of fragments 14 mass units apart due to sequential loss of CH_2 units from the R group. Long-chain primary amines can also undergo fragmentation via the process



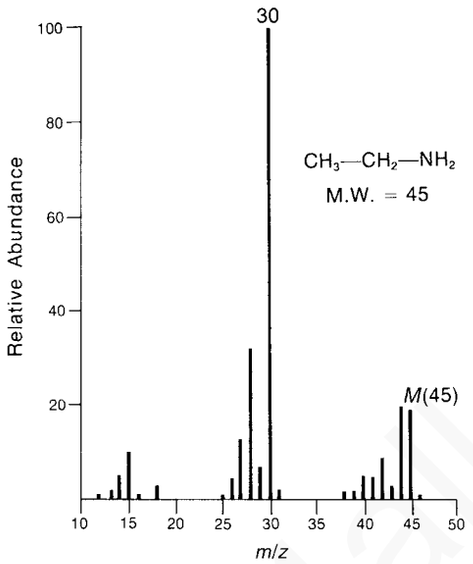


FIGURE 8.72 Mass spectrum of ethylamine.

This is particularly favorable when $n = 4$ since a stable six-membered ring results. In this case, the fragment ion appears at $m/z = 86$.

Secondary and tertiary amines also undergo fragmentation processes as described earlier. The most important fragmentation is β-cleavage. In the mass spectrum of diethylamine (Fig. 8.73), the intense peak at $m/z = 58$ is due to loss of a methyl group. Again, in the mass spectrum

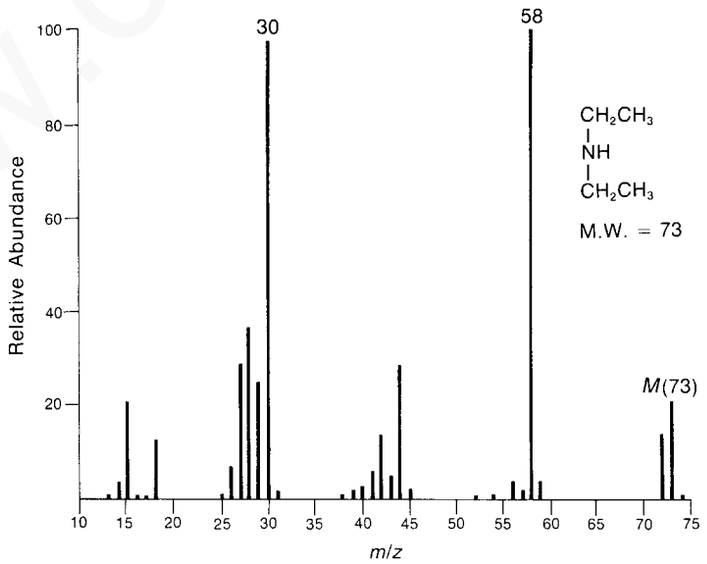


FIGURE 8.73 Mass spectrum of diethylamine.

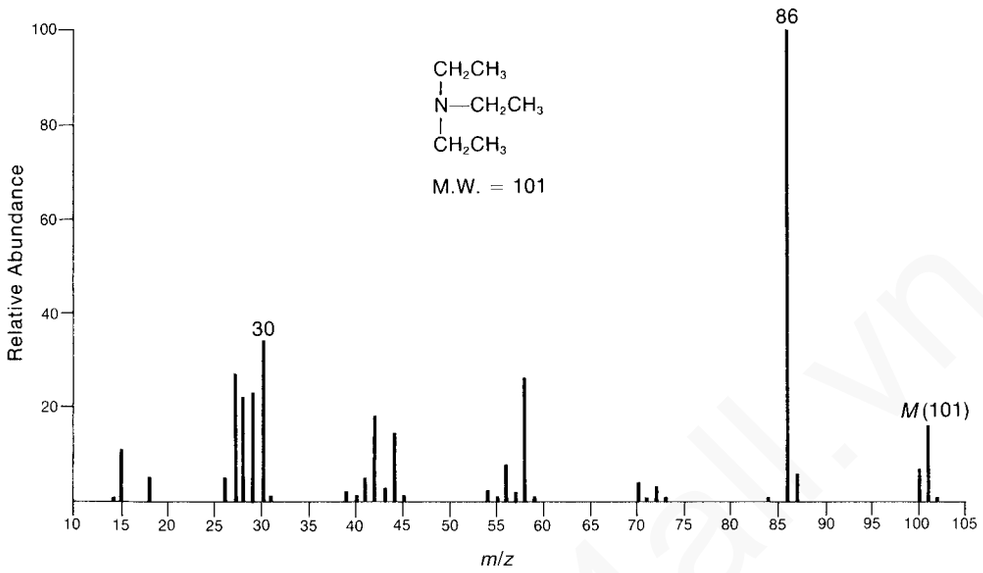
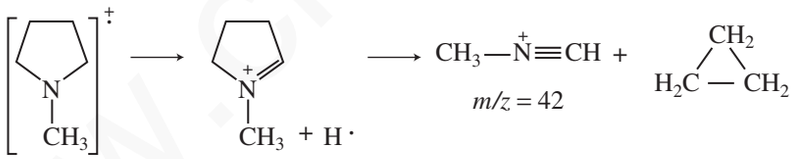
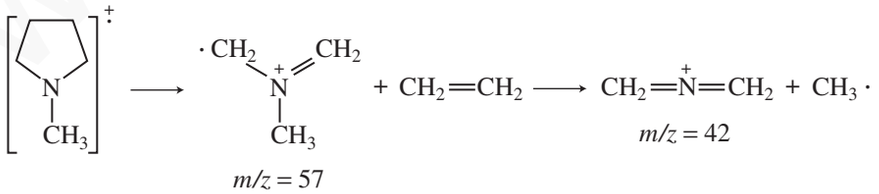


FIGURE 8.74 Mass spectrum of triethylamine.

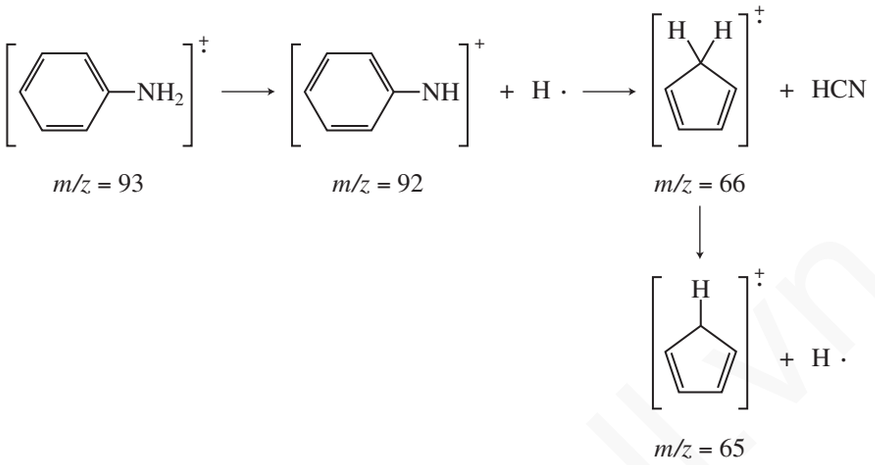
of triethylamine (Fig. 8.74), loss of methyl produces the most intense peak in the spectrum, at $m/z = 86$. In each case, further fragmentation of this initially formed fragment ion produces a peak at $m/z = 30$.

Cyclic aliphatic amines usually produce intense molecular ion peaks. Their principal modes of fragmentation are as follows:





Aromatic amines show intense molecular ion peaks. A moderately intense peak may appear at an m/z value one mass unit less than that of the molecular ion due to loss of a hydrogen atom. The fragmentation of aromatic amines can be illustrated for the case of aniline:



Very intense molecular ion peaks characterize substituted pyridines. Frequently, loss of a hydrogen atom to produce a peak at an *m/z* value one mass unit less than the molecular ion is also observed.

The most important fragmentation process for the pyridine ring is loss of the elements of hydrogen cyanide. This produces a fragment ion that is 27 mass units lighter than the molecular ion. In the mass spectrum of 3-methylpyridine (Fig. 8.75), you can see the peak due to loss of hydrogen ($m/z = 92$) and the peak due to loss of hydrogen cyanide ($m/z = 66$).

When the alkyl side chain attached to a pyridine ring contains three or more carbons arranged linearly, fragmentation via the McLafferty rearrangement can also occur.

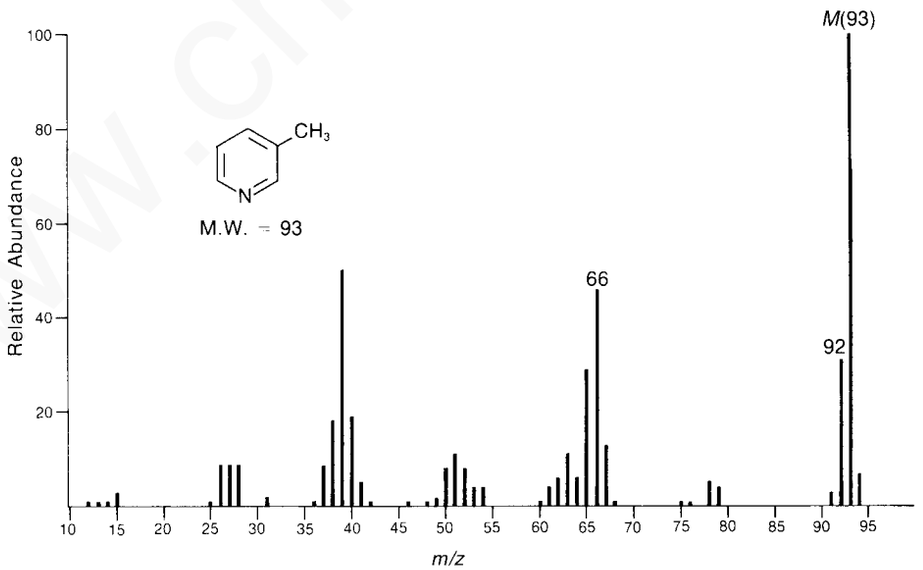
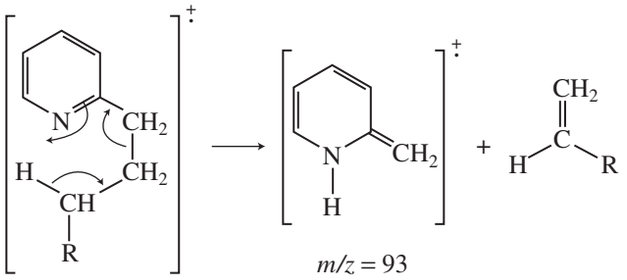


FIGURE 8.75 Mass spectrum of 3-methylpyridine.



$m/z = 93$

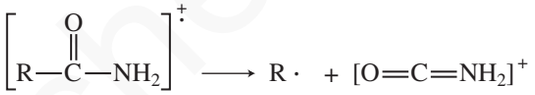
This mode of cleavage is most important for substituents attached to the number 2 position of the ring.

U. Selected Nitrogen and Sulfur Compounds

As is true of amines, nitrogen-bearing compounds such as amides, nitriles, and nitro compounds must follow the Nitrogen Rule (explained more completely in Section 8.6): If they contain an odd number of nitrogen atoms, they must have an odd-numbered molecular weight.

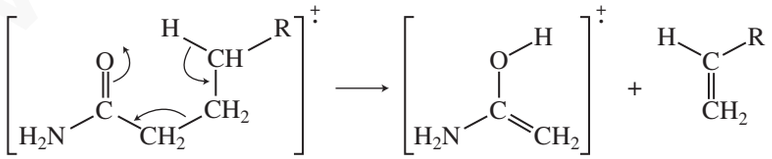
Amides

The mass spectra of amides usually show observable molecular ion peaks. The fragmentation patterns of amides are quite similar to those of the corresponding esters and acids. The presence of a strong fragment ion peak at $m/z = 44$ is usually indicative of a primary amide. This peak arises from α-cleavage of the following sort.



$m/z = 44$

Once the carbon chain in the acyl moiety of an amide becomes long enough to permit the transfer of a hydrogen attached to the γ position, McLafferty rearrangements become possible. For primary amides, the McLafferty rearrangement gives rise to a fragment ion peak at $m/z = 59$. For *N*-alkylamides, analogous peaks at m/z values of 73, 87, 101, and so on often appear.



$m/z = 59$

Nitriles

Aliphatic nitriles usually undergo fragmentation so readily that the molecular ion peak is too weak to be observed. However, most nitriles form a peak due to the loss of one hydrogen atom, producing an ion of the type $R{-}CH{=}C{=}N^+$. Although this peak may be weak, it is a useful diagnostic peak in characterizing nitriles. In the mass spectrum of hexanenitrile (Fig. 8.76), this peak appears at $m/z = 96$.

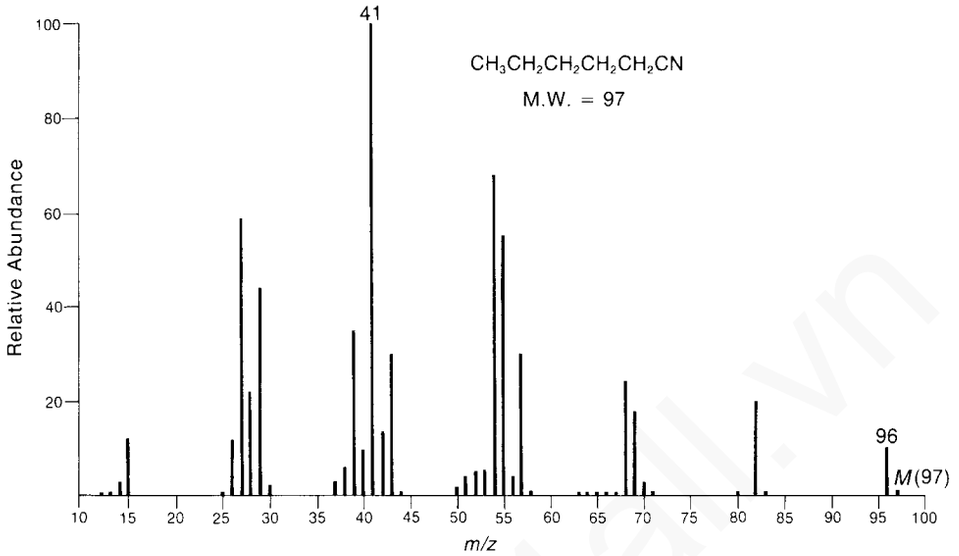
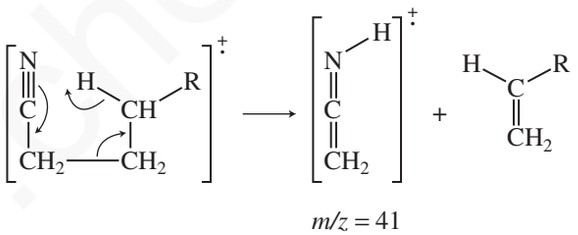


FIGURE 8.76 Mass spectrum of hexanenitrile.

When the alkyl group attached to the nitrile functional group is a propyl group or some longer hydrocarbon group, the most intense peak in the mass spectrum results from a McLafferty rearrangement:



This peak, which appears in the mass spectrum of hexanenitrile, can be quite useful in characterizing an aliphatic nitrile. Unfortunately, as the alkyl group of a nitrile becomes longer, the probability of formation of the $C_3H_5^+$ ion, which also appears at $m/z = 41$, increases. With high molecular weight nitriles, most of the fragment ions of mass 41 are $C_3H_5^+$ ions rather than ions formed as a result of a McLafferty rearrangement.

The strongest peak in the mass spectrum of an aromatic nitrile is the molecular ion peak. Loss of cyanide occurs, giving, in the case of benzonitrile (Fig. 8.77), the $C_6H_5^+$ ion at $m/z = 77$. More important fragmentation involves loss of the elements of hydrogen cyanide. In benzonitrile, this gives rise to a peak at $m/z = 76$.

Nitro Compounds

The molecular ion peak for an aliphatic nitro compound is seldom observed. The mass spectrum is the result of fragmentation of the hydrocarbon part of the molecule. However, the mass spectra of nitro compounds may show a moderate peak at $m/z = 30$, corresponding to the NO^+ ion, and a

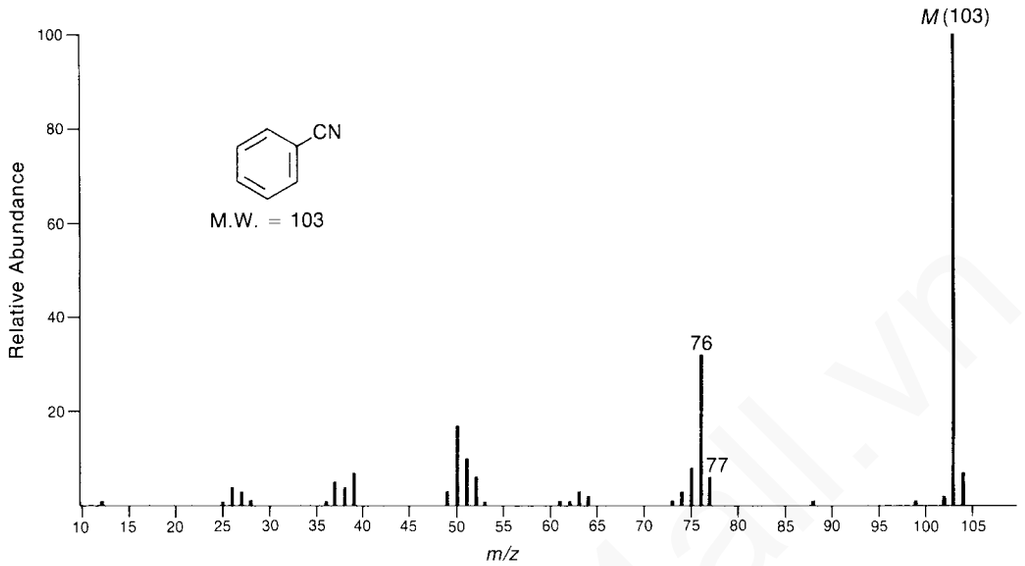


FIGURE 8.77 Mass spectrum of benzonitrile.

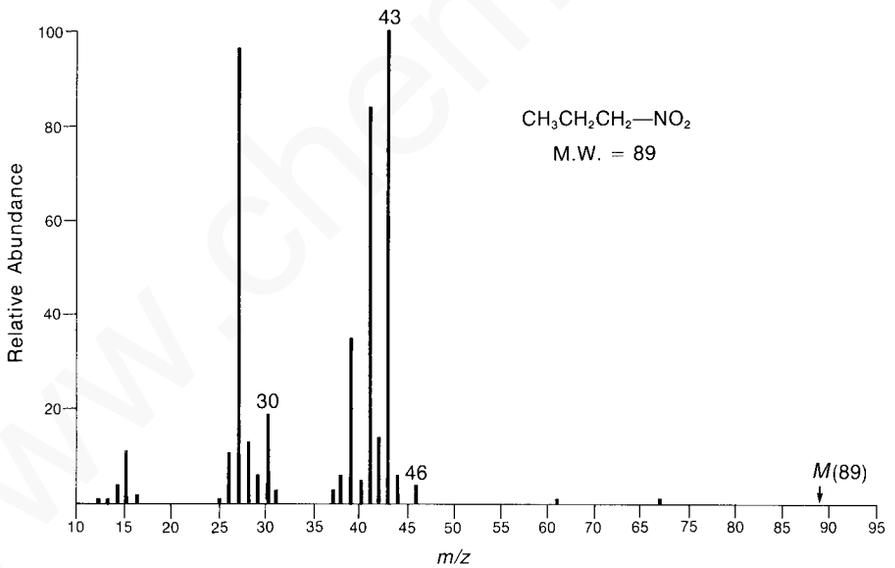


FIGURE 8.78 Mass spectrum of 1-nitropropane.

weaker peak at $m/z = 46$, corresponding to the NO_2^+ ion. These peaks appear in the mass spectrum of 1-nitropropane (Fig. 8.78). The intense peak at $m/z = 43$ is due to the $C_3H_7^+$ ion.

Aromatic nitro compounds show intense molecular ion peaks. The characteristic NO^+ ($m/z = 30$) and NO_2^+ ($m/z = 46$) peaks appear in the mass spectrum. The principal fragmentation pattern, however, involves loss of all or part of the nitro group. Using nitrobenzene (Fig. 8.79) as an example, this fragmentation pattern may be described as follows:

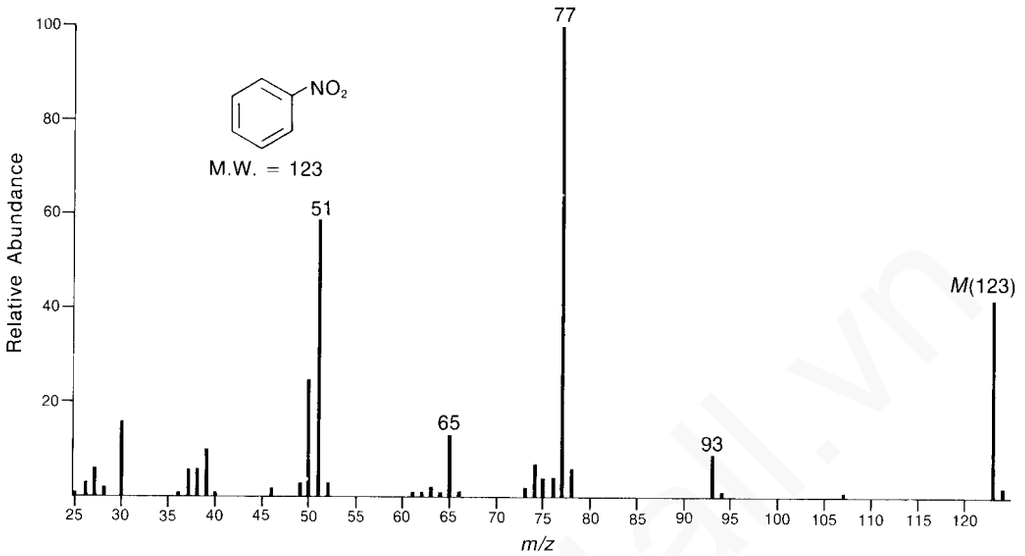
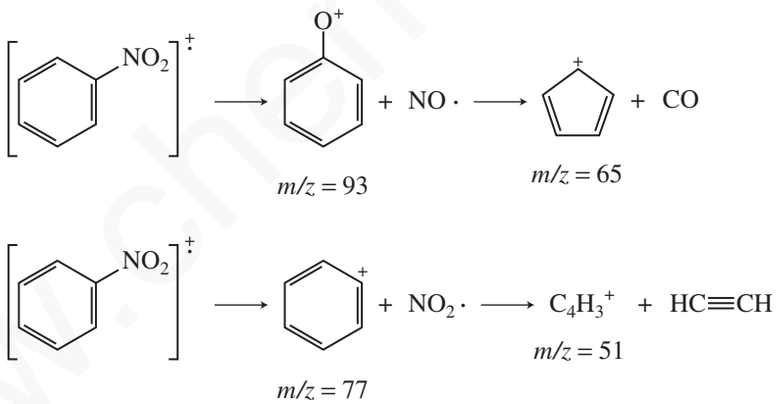


FIGURE 8.79 Mass spectrum of nitrobenzene.



Thiols and Thioethers

Thiols show molecular ion peaks that are more intense than those of the corresponding alcohols. A characteristic feature of the mass spectra of sulfur compounds is the presence of a significant $M + 2$ peak. This peak arises from the presence of the heavy isotope, ^{34}S, which has a natural abundance of 4.4%.

The fragmentation patterns of the thiols are very similar to those of the alcohols. As alcohols tend to undergo dehydration under some conditions, thiols tend to lose the elements of hydrogen sulfide, giving rise to an $M - 34$ peak.

Thioethers show mass spectral patterns that are very similar to those of the ethers. As in the case of the thiols, thioethers show molecular ion peaks that tend to be more intense than those of the corresponding ethers.

V. Alkyl Chlorides and Alkyl Bromides

The most dramatic feature of the mass spectra of alkyl chlorides and alkyl bromides is the presence of an important $M + 2$ peak. This peak arises because both chlorine and bromide are present in nature in two isotopic forms, each with a significant natural abundance.

For aliphatic halogen compounds, the molecular ion peak is strongest with alkyl iodides, less strong for alkyl bromides, weaker for alkyl chlorides, and weakest for alkyl fluorides. Furthermore, as the alkyl group increases in size or as the amount of branching at the α position increases, the intensity of the molecular ion peak decreases.

SPECTRAL ANALYSIS BOX — Alkyl Halides

MOLECULAR ION	FRAGMENT IONS
Strong $M + 2$ peak	Loss of Cl or Br
(for Cl, $M/M + 2 = 3:1$;	Loss of HCl
for Br, $M/M + 2 = 1:1$)	α-Cleavage

There are several important fragmentation mechanisms for the alkyl halides. Perhaps the most important is the simple loss of the halogen atom, leaving a carbocation. This fragmentation is most important when the halogen is a good leaving group. Therefore, this type of fragmentation is most prominent in the mass spectra of the alkyl iodides and the alkyl bromides. In the mass spectrum of 1-bromohexane (Fig. 8.80), the peak at $m/z = 85$ is due to the formation of the hexyl ion. This ion undergoes further fragmentation to form a $C_3H_7^+$ ion at $m/z = 43$. The corresponding heptyl ion peak at $m/z = 99$ in the mass spectrum of 2-chloroheptane (Fig. 8.81) is quite weak.

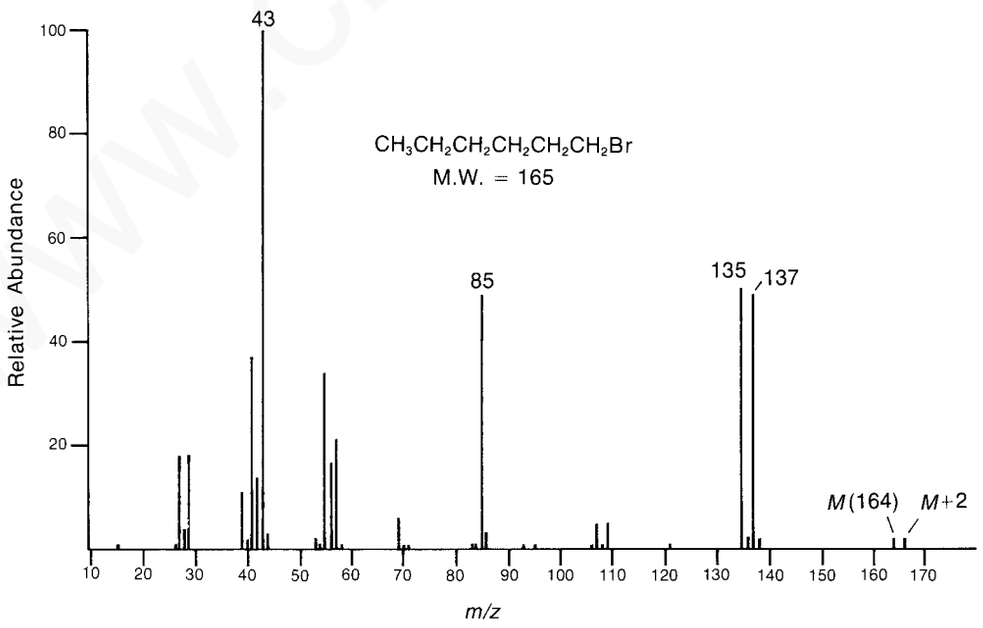


FIGURE 8.80 Mass spectrum of 1-bromohexane.

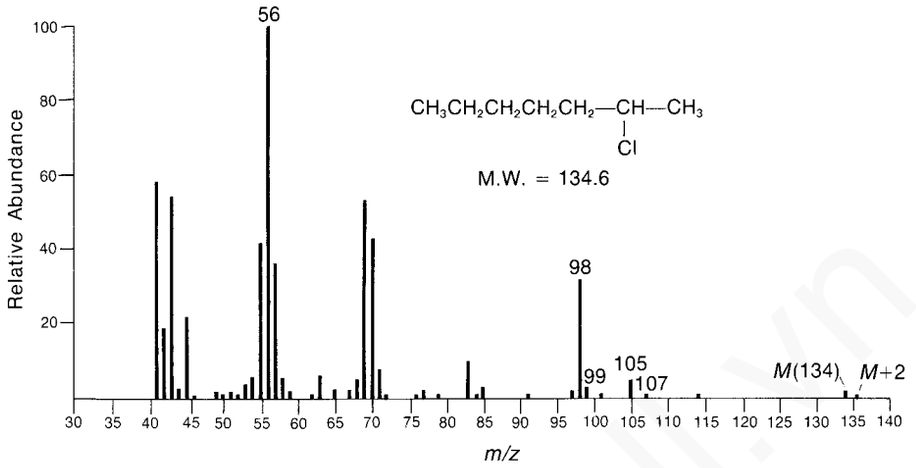


FIGURE 8.81 Mass spectrum of 2-chloroheptane.

Alkyl halides may also lose a molecule of hydrogen halide according to the process

$$[R{-}CH_2{-}CH_2{-}X]^{+\cdot} \longrightarrow [R{-}CH{=}CH_2]^{+\cdot} + HX$$

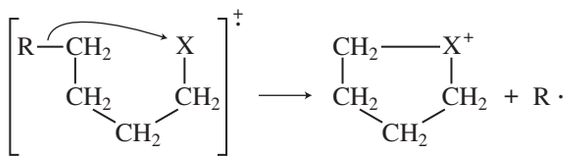
This mode of fragmentation is most important for alkyl fluorides and chlorides and is less important for alkyl bromides and iodides. In the mass spectrum of 1-bromohexane, the peak corresponding to the loss of hydrogen bromide at $m/z = 84$ is very weak. However, for 2-chloroheptane, the peak corresponding to the loss of hydrogen chloride at $m/z = 98$ is quite intense.

A less important mode of fragmentation is α-cleavage, for which a fragmentation mechanism might be

$$\left[R{\dot{-}}CH_2{-}X\right]^{+\cdot} \longrightarrow R\cdot + CH_2{=}X^+$$

When the α position is branched, the heaviest alkyl group attached to the α carbon is lost with greatest facility. The peaks arising from α-cleavage are usually rather weak.

A fourth fragmentation mechanism involves rearrangement and loss of an alkyl radical:



The corresponding cyclic ion can be observed at m/z = 135 and 137 in the mass spectrum of 1-bromohexane and at $m/z = 105$ and 107 in the mass spectrum of 2-chloroheptane. Such fragmentation is important only in the mass spectra of long-chain alkyl chlorides and bromides.

The molecular ion peaks in the mass spectra of benzyl halides are usually of sufficient intensity to be observed. The most important fragmentation involves loss of halogen to form the $C_7H_7^+$ ion. When the aromatic ring of a benzyl halide carries substituents, a substituted phenyl cation may also appear.

The molecular ion peak of an aromatic halide is usually quite intense. The most important mode of fragmentation involves loss of halogen to form the $C_6H_5^+$ ion.

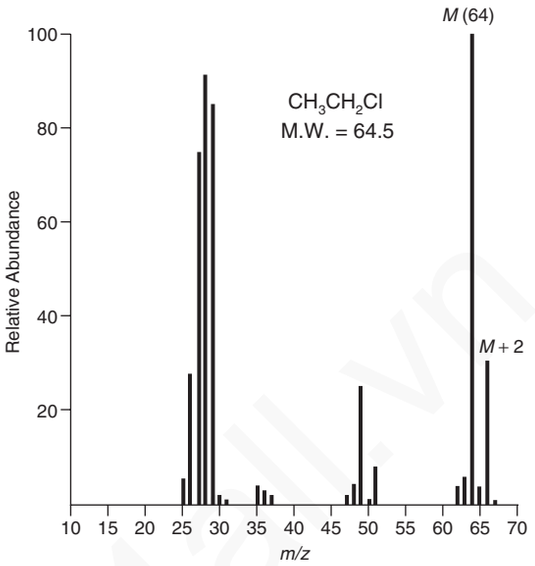


FIGURE 8.82 Mass spectrum of ethyl chloride.

Although the fragmentation patterns we have described are well characterized, the most interesting feature of the mass spectra of chlorine- and bromine-containing compounds is the presence of two molecular ion peaks. As Section 8.7 indicated, chlorine occurs naturally in two isotopic forms. The natural abundance of chlorine of mass 37 is 32.5% that of chlorine of mass 35. The natural abundance of bromine of mass 81 is 98.0% that of ^{79}Br. Therefore, the intensity of the $M + 2$ peak in a chlorine-containing compound should be 32.5% of the intensity of the molecular ion peak, and the intensity of the $M + 2$ peak in a bromine-containing compound should be almost equal to the intensity of the molecular ion peak. These pairs of molecular ion peaks (sometimes called doublets) appear in the mass spectra of ethyl chloride (Fig. 8.82) and ethyl bromide (Fig. 8.83).

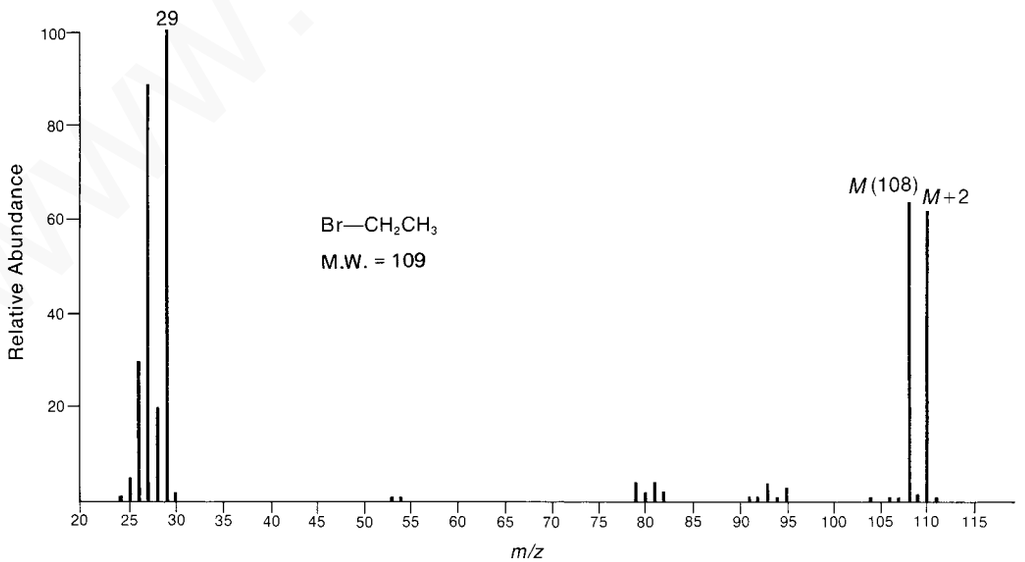


FIGURE 8.83 Mass spectrum of ethyl bromide.

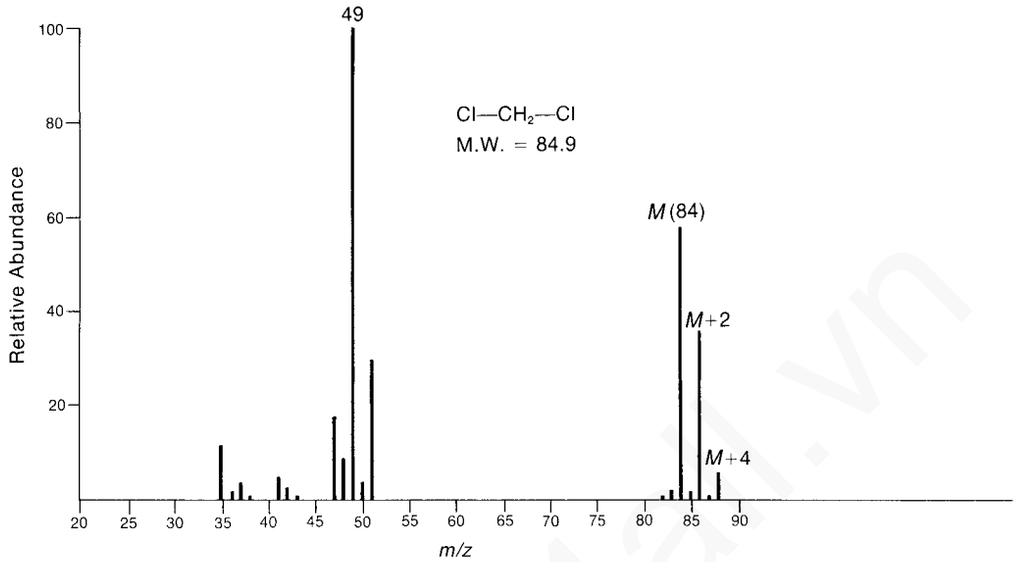


FIGURE 8.84 Mass spectrum of dichloromethane.

Table 8.8 in Section 8.7 can be used to determine what the ratio of the intensities of the molecular ion and isotopic peaks should be when more than one chlorine or bromine is present in the same molecule. The mass spectra of dichloromethane (Fig. 8.84), dibromomethane (Fig. 8.85), and 1-bromo-2-chloroethane (Fig. 8.86) are included here to illustrate some of the combinations of halogens listed in Figure 8.18.

Unfortunately, it is not always possible to take advantage of these characteristic patterns to identify halogen compounds. Frequently, the molecular ion peaks are too weak to permit accurate measurement of the ratio of the intensities of the molecular ion and isotopic peaks. However, it is often possible to make such a comparison on certain fragment ion peaks in the mass spectrum of a halogen compound.

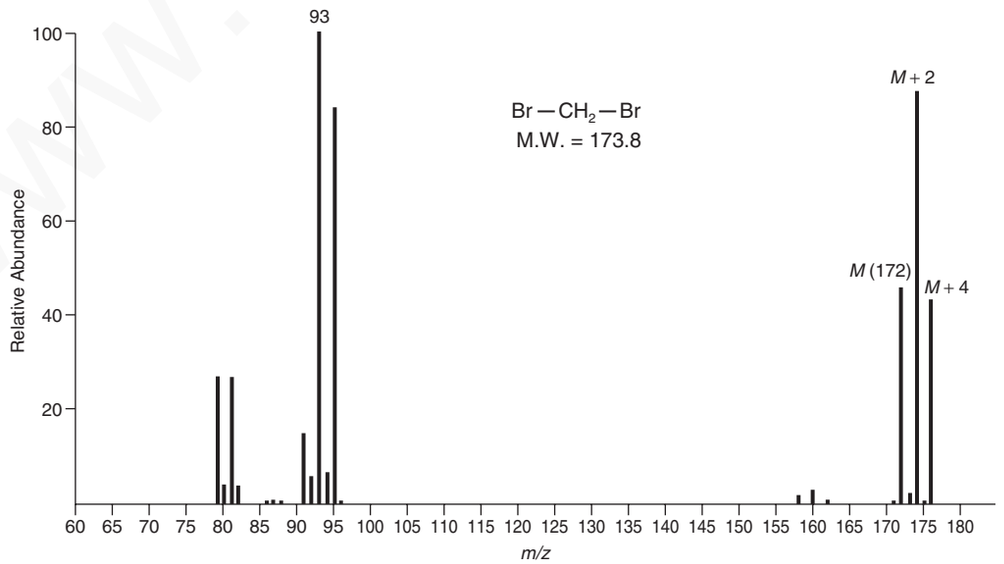


FIGURE 8.85 Mass spectrum of dibromomethane.

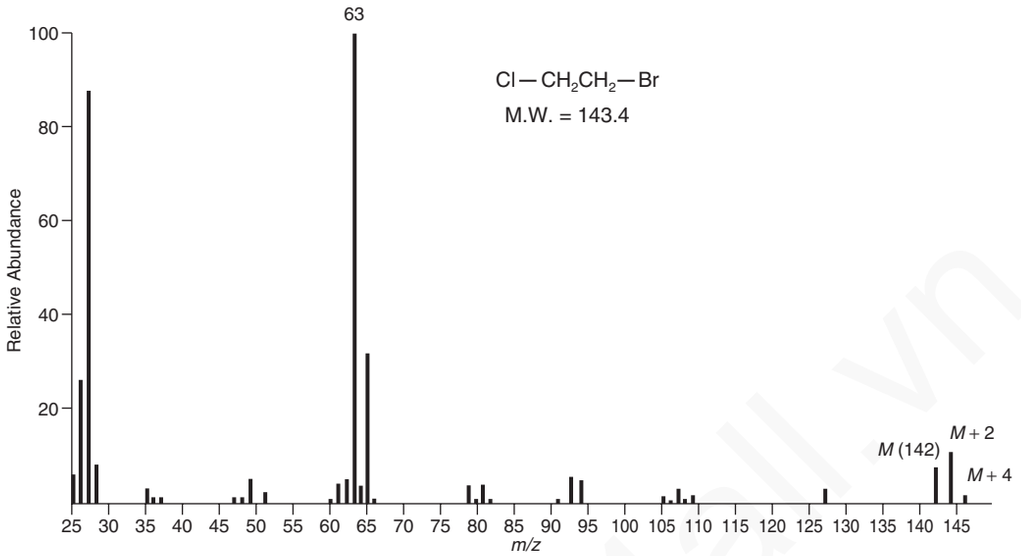


FIGURE 8.86 Mass spectrum of 1-bromo-2-chloroethane.

The mass spectrum of 1-bromohexane (Fig. 8.80) may be used to illustrate this method. The presence of bromine can be determined using the fragment ion peaks at *m*/*z* values of 135 and 137.

Since iodine and fluorine exist in nature in the form of only one isotope each, their mass spectra do not show isotopic peaks. The presence of halogen must be deduced either by noting the unusually weak $M + 1$ peak or by observing the mass difference between the fragment ions and the molecular ion.

8.9 STRATEGIC APPROACH TO ANALYZING MASS SPECTRA AND SOLVING PROBLEMS

Like any other problem involving the correlation of spectral data with structure, having a well-defined strategy for analyzing mass spectra is the key to success. It is also true that chemical intuition plays an important role as well, and of course there is no substitute for practical experience. Before diving into the mass spectrum itself, take an inventory of what is known about the sample. Is the elemental composition known? Has the molecular formula been determined by exact mass analysis? What functional groups are present in the compound? What is the sample's "chemical history"? For example, how has the sample been handled? From what sort of chemical reaction was the compound isolated? And the questions can continue.

The first step in analyzing the mass spectrum itself is identifying the molecular ion. See Section 8.6 to review the requirements for a molecular ion. Once the molecular ion is identified, note its nominal mass and examine the isotope cluster (if the formula is not already known) for the presence of Cl, Br, and other $M + 2$ elements. Depending on whether the *m*/*z* value of the molecular ion is odd or even, the nitrogen rule will tell you how many nitrogens, if any, to incorporate into your analysis. If the molecular ion is not visible, consider running the sample under CI conditions to determine the molecular mass of the sample. If acquiring more data is not an option, consider what logical losses could have created the high mass peaks in the spectrum you have (loss of water from an alcohol, for example).

After analysis of the molecular ion cluster, examine the high mass peaks in your spectrum and determine the whether the mass losses are odd or even. If an even number of nitrogens are present (zero is even), odd mass losses correspond to simple homolytic cleavages, and even mass losses are from rearrangements (this is reversed if there are an odd number of nitrogens present). Try to assign these mass losses to a radical fragment or neutral molecule. Next, look for readily identifiable fragments: phenylacylium ions, tropylium ions, phenyl cations, cyclopentadienyl cations, and so on.

Finally, use the fragmentation information to piece together a proposed structure. More than one potential structure may be reasonable pending further analysis. In some cases, it may only be possible to come up with a partial structure. Although tempting at times, remember that it is very risky to propose structures (or eliminate possible structures) on the *absence* of data: "That structure should give a peak at m/z = Q from a McLafferty rearrangement, but there is no peak there—therefore that structure is wrong." When you have assembled a potential structure, reanalyze the fragmentation of that structure and see if it agrees with the experimental data. Comparison of your data to reference spectra from compounds with similar structures and functional groups can be very informative, and conducting a mass spectral library search of your spectrum against a database will likely provide some clues to the compound's identity, if not an exact match.

8.10 COMPUTERIZED MATCHING OF SPECTRA WITH SPECTRAL LIBRARIES

Once a digitized mass spectrum is in hand, a basic PC can compare that data set to a library of tens of thousands of mass spectra within seconds and provide a list of potential matches. Each peak in a spectrum is categorized by the search program by uniqueness and relative abundance. Higher mass peaks are usually more characteristic of the compound in question than are commonly encountered low mass peaks, so the peaks with larger m/z may be weighted more heavily in the search algorithm. The output of a library search is a table that lists the names of possible compounds, their molecular formulas, and an indicator of the probability that the spectrum of the test compound matches the spectrum in the database. The probability is determined by the number of peaks (and their intensities) that can be matched. This type of table is often called a *hit list*. Figure 8.87 is the mass spectrum of an unknown liquid substance with an observed boiling point of 158°C to 159°C. Table 8.9 reproduces the type of information that the computer would display as a hit list. Notice that the information

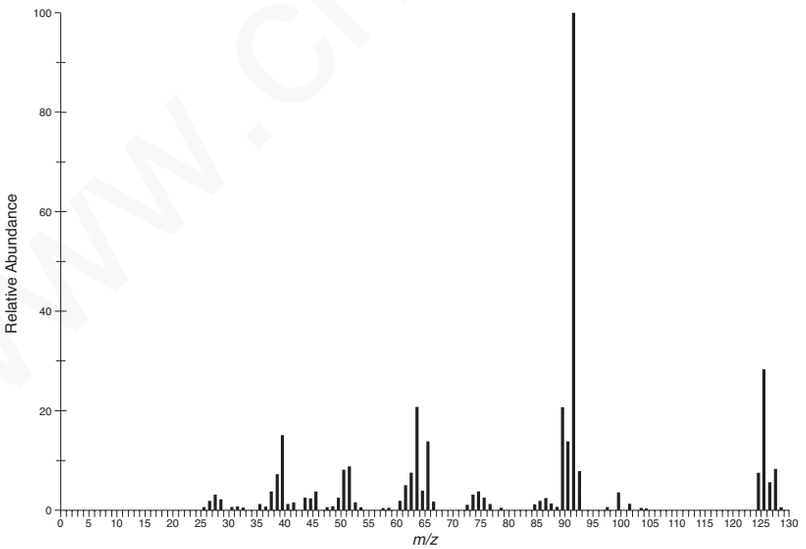


FIGURE 8.87 EI-MS of an unknown liquid.

TABLE 8.9
RESULT OF LIBRARY SEARCH FOR UNKNOWN LIQUID

Name	Molecular Weight	Formula	Probability	CAS No.
1. Benzene, 1-chloro-2-methyl-	126	C_7H_7Cl	94	000095-49-8
2. Benzene, 1-chloro-3-methyl-	126	C_7H_7Cl	70	000108-41-8
3. Benzene, 1-chloro-4-methyl-	126	C_7H_7Cl	60	000106-43-4
4. Benzene, (chloromethyl)-	126	C_7H_7Cl	47	000100-44-7
5. 1,3,5-Cycloheptatriene, 1-chloro-	126	C_7H_7Cl	23	032743-66-1

includes the name of each compound that the computer has used for matching, its molecular weight and molecular formula, and its Chemical Abstracts Service (CAS) Registry number.

The information in Table 8.9 indicates that the unknown liquid is most likely **1-chloro-2-methylbenzene** since the probability of a correct match is placed at 94%. It is interesting to note that the *meta* and *para* isomers show probabilities of 70% and 60%, respectively. It is tempting to simply accept the results of the computer-based library search as correct, but the method is not an absolute guarantee that the identity of a sample has been correctly determined. A visual inspection of the experimental and library spectra must be included as part of the process. A computer can compare a mass spectrum it has determined with the spectra in these databases.

PROBLEMS

***1.** A low-resolution mass spectrum of the alkaloid vobtusine showed the molecular weight to be 718. This molecular weight is correct for the molecular formulas $C_{43}H_{50}N_4O_6$ and $C_{42}H_{46}N_4O_7$. A high-resolution mass spectrum provided a molecular weight of 718.3743. Which of the possible molecular formulas is the correct one for vobtusine?

***2.** A tetramethyltriacetyl derivative of oregonin, a diarylheptanoid xyloside found in red alder, was found by low-resolution mass spectrometry to have a molecular weight of 660. Possible molecular formulas include $C_{32}H_{36}O_{15}$, $C_{33}H_{40}O_{14}$, $C_{34}H_{44}O_{13}$, $C_{35}H_{48}O_{12}$, $C_{32}H_{52}O_{14}$, and $C_{33}H_{56}O_{13}$. High-resolution mass spectrometry indicated that the precise molecular weight was 660.278. What is the correct molecular formula for this derivative of oregonin?

***3.** An unknown substance shows a molecular ion peak at $m/z = 170$ with a relative intensity of 100. The $M + 1$ peak has an intensity of 13.2, and the $M + 2$ peak has an intensity of 1.00. What is the molecular formula of the unknown?

***4.** An unknown hydrocarbon has a molecular ion peak at $m/z = 84$, with a relative intensity of 31.3. The $M + 1$ peak has a relative intensity of 2.06, and the $M + 2$ peak has a relative intensity of 0.08. What is the molecular formula for this substance?

***5.** An unknown substance has a molecular ion peak at $m/z = 107$, with a relative intensity of 100. The relative intensity of the $M + 1$ peak is 8.00, and the relative intensity of the $M + 2$ peak is 0.30. What is the molecular formula for this unknown?

***6.** The mass spectrum of an unknown liquid shows a molecular ion peak at $m/z = 78$, with a relative intensity of 23.6. The relative intensities of the isotopic peaks are as follows:

$m/z = 79$	Relative intensity = 0.79
80	7.55
81	0.25

What is the molecular formula of this unknown?

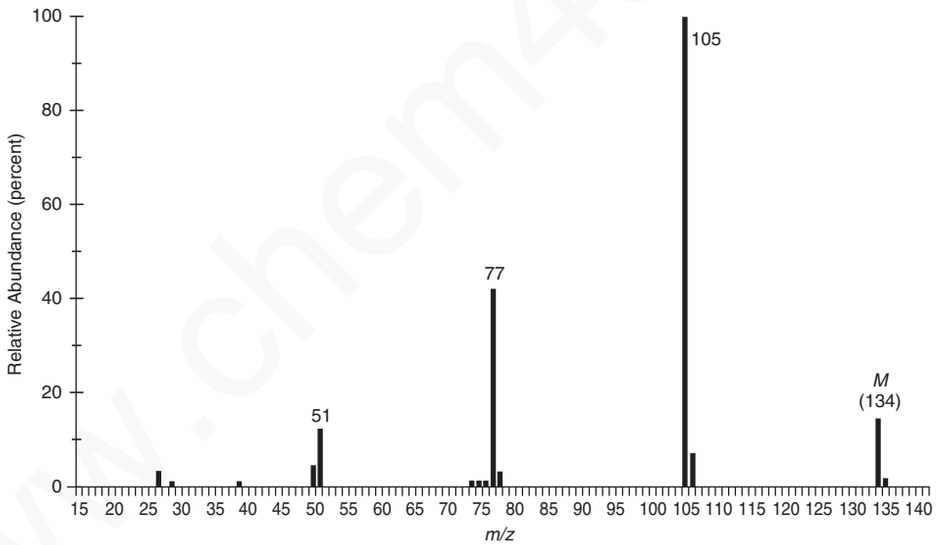
7. Assign a structure that would be expected to give rise to each of the following mass spectra. *Note*: Some of these problems may have more than one reasonable answer. In some cases, infrared spectral data have been included in order to make the solution to the problems more reasonable. We recommend that you review the index of hydrogen deficiency (Section 1.4) and the Rule of Thirteen (Section 1.5) and apply those methods to each of the following problems. To help you, we have provided an example problem with solution.

■ SOLVED EXAMPLE

An unknown compound has the mass spectrum shown. The infrared spectrum of the unknown shows significant peaks at

3102 cm^{-1}	3087	3062	3030	1688
1598	1583	1460	1449	1353
1221	952	746	691	

There is also a band from aliphatic C−H stretching from 2879 to 2979 cm^{-1}.



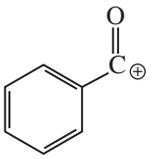
■ SOLUTION

1. The molecular ion appears at an *m/z* value of 134. Applying the Rule of Thirteen gives the following possible molecular formulas:

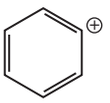
$C_{10}H_{14}$	U = 4
$C_9H_{10}O$	U = 5

2. The infrared spectrum shows a C=O peak at 1688 cm^{-1}. The position of this peak, along with the C−H stretching peaks in the 3030–3102 cm^{-1} range and C=C stretching peaks in the 1449–1598 cm^{-1} range, suggests a ketone in which the carbonyl group is conjugated with a benzene ring. Such a structure would be consistent with the second molecular formula and with the index of hydrogen deficiency.

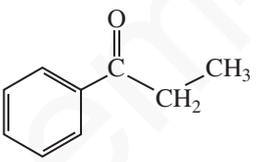
3. The base peak in the mass spectrum appears at $m/z = 105$. This peak is likely due to the formation of a benzoyl cation.



Subtracting the mass of the benzoyl ion from the mass of the molecular ion gives a difference of 29, suggesting that an ethyl group is attached to the carbonyl carbon. The peak appearing at $m/z = 77$ arises from the phenyl cation.

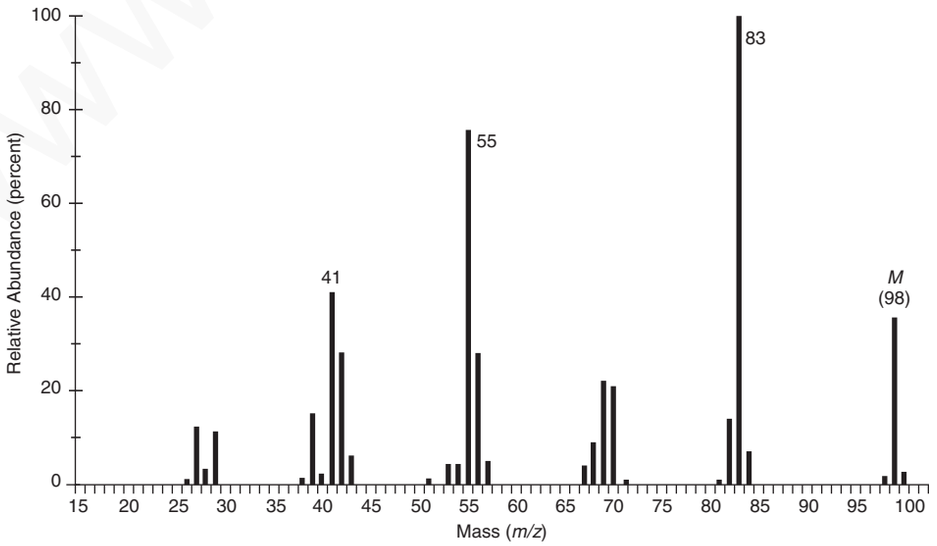


4. If we assemble all of the "pieces" suggested in the data, as described above, we conclude that the unknown compound must have been **propiophenone (1-phenyl-1-propanone).**

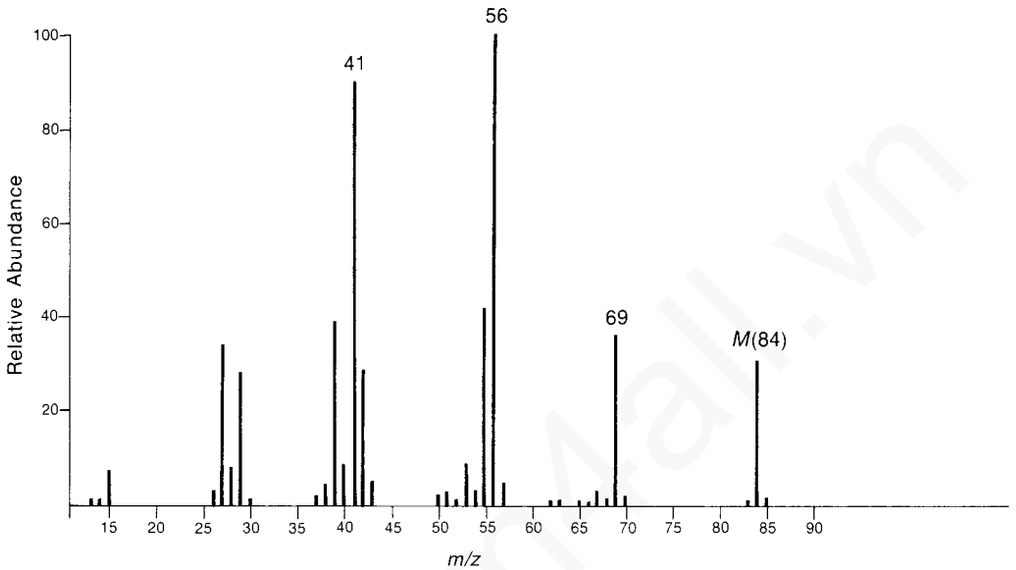


Problem 7 (continued)

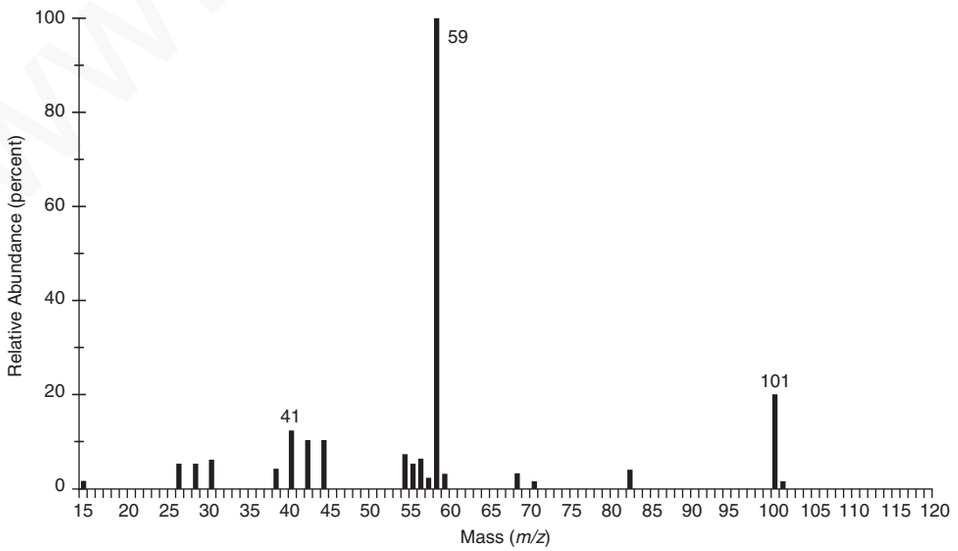
*(a) The infrared spectrum has no interesting features except aliphatic C—H stretching and bending.



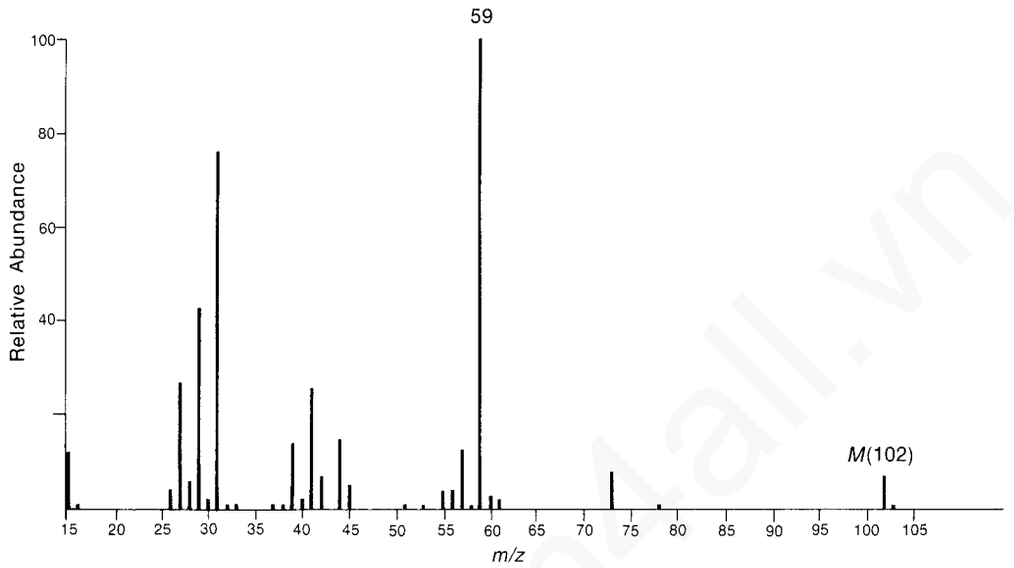
*(b) The infrared spectrum has a medium-intensity peak at about 1650 cm^{-1}. There is also a C—H out-of-plane bending peak near 880 cm^{-1}.



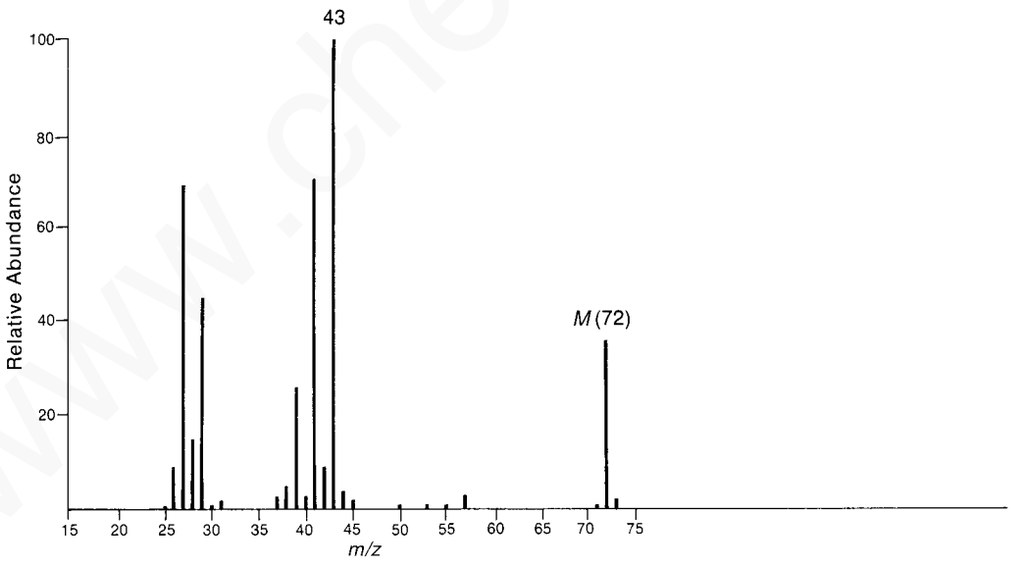
*(c) The infrared spectrum of this unknown has a prominent, broad peak at 3370 cm^{-1}. There is also a strong peak at 1159 cm^{-1}. The mass spectrum of this unknown does not show a molecular ion peak. You will have to deduce the molecular weight of this unknown from the heaviest fragment ion peak, which arises from the loss of a methyl group from the molecular ion.



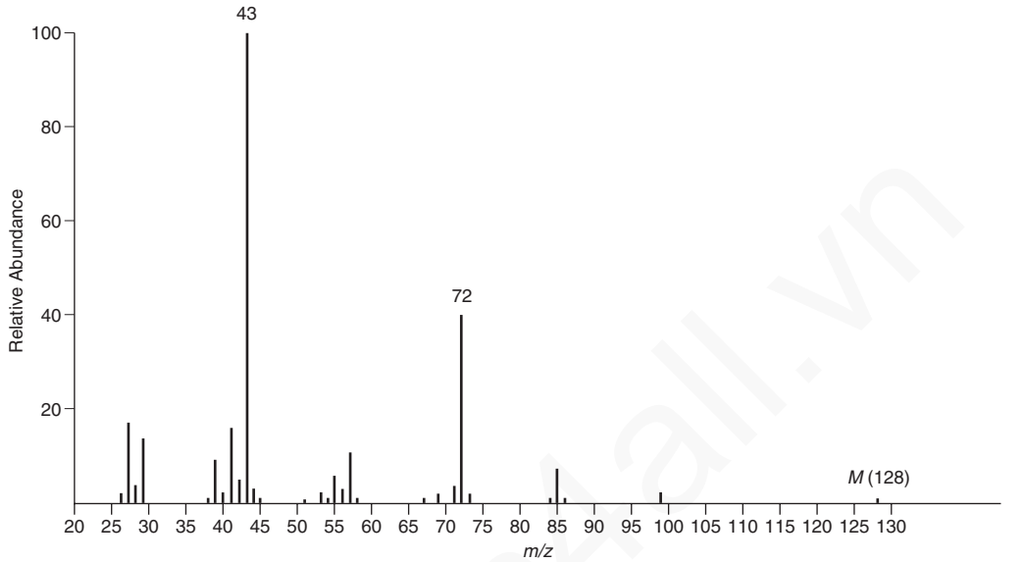
*(d) This unknown contains oxygen, but it does not show any significant infrared absorption peaks above 3000 cm^{-1}.



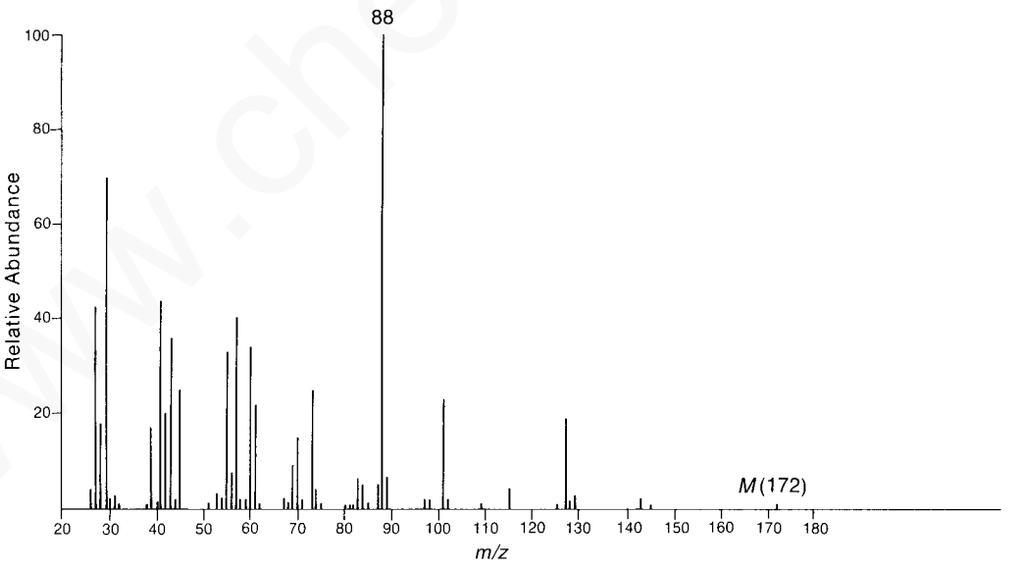
*(e) The infrared spectrum of this unknown shows a strong peak near 1725 cm^{-1}.



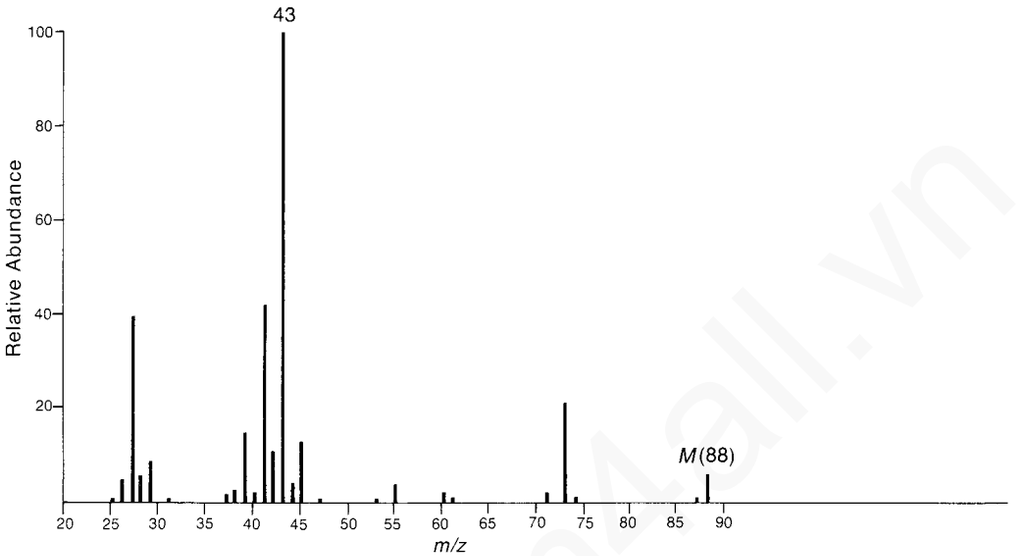
*(f) The infrared spectrum of this unknown shows a strong peak near 1715 cm^{-1}.



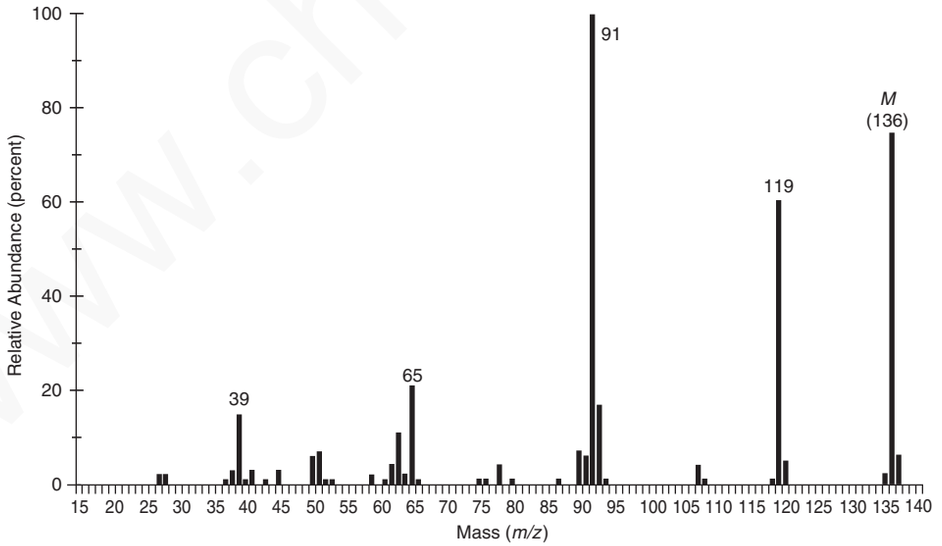
*(g) The infrared spectrum of this compound lacks any significant absorption above 3000 cm^{-1}. There is a prominent peak near 1740 cm^{-1} and another strong peak near 1200 cm^{-1}.



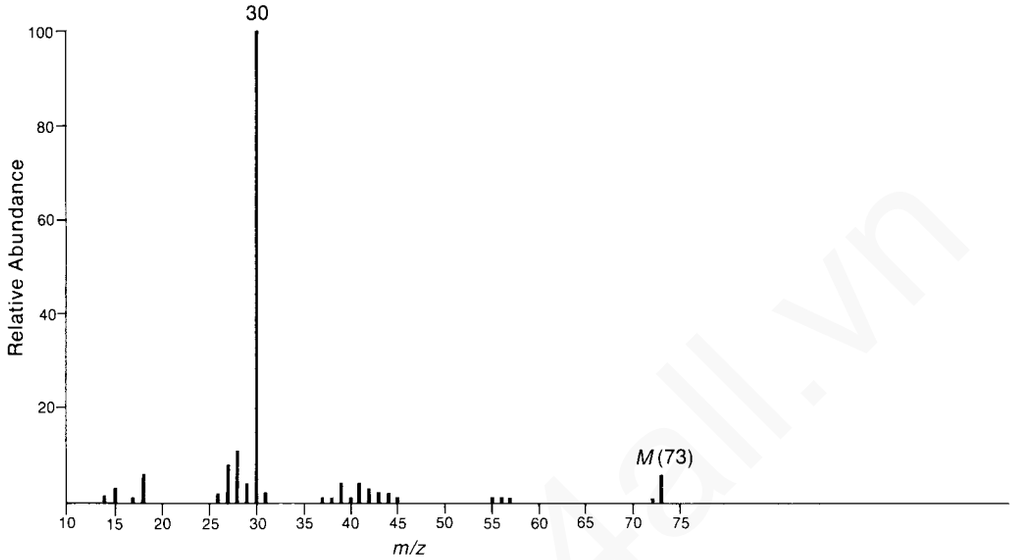
*(h) The infrared spectrum of this substance shows a very strong, broad peak in the range of 2500–3000 cm^{-1}, as well as a strong, somewhat broadened peak at about 1710 cm^{-1}.



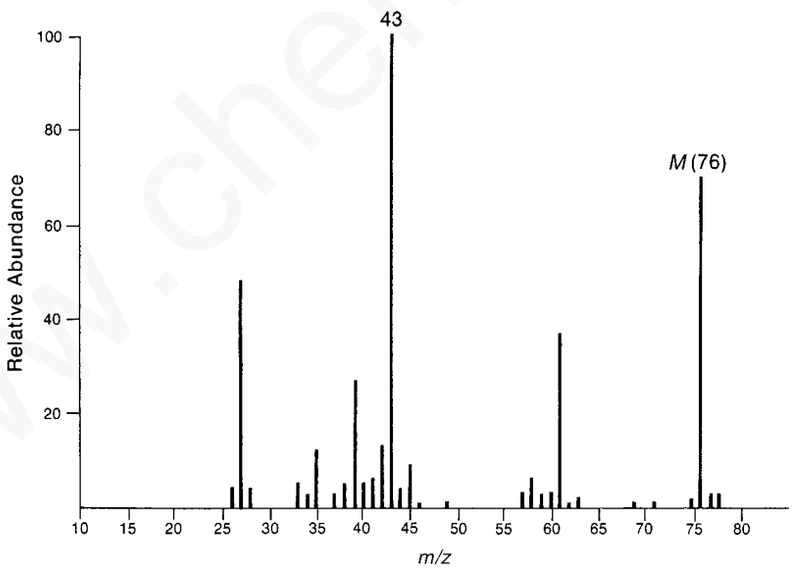
*(i) The ^{13}C NMR spectrum of this unknown shows only four peaks in the region 125–145 ppm. The infrared spectrum shows a very strong, broad peak extending from 2500 to 3500 cm^{-1}, as well a strong and somewhat broadened peak at 1680 cm^{-1}.



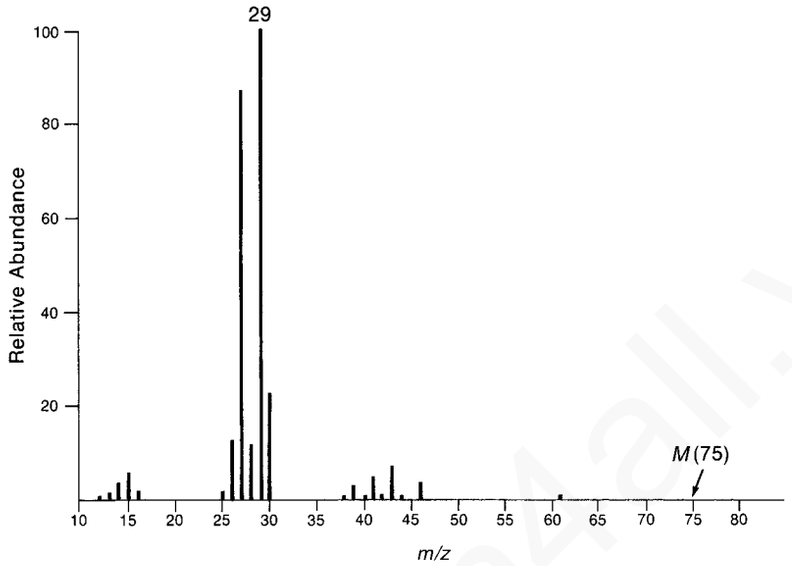
*(j) Note the odd value of mass for the molecular ion in this substance.



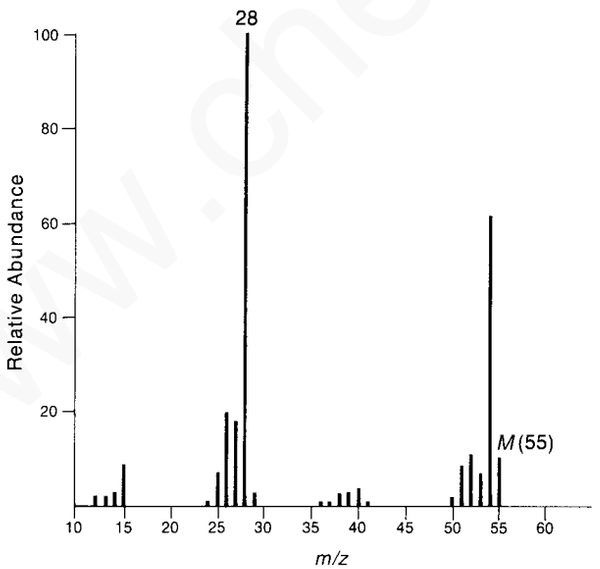
*(k) Notice the ***M* + 2** peak in the mass spectrum.



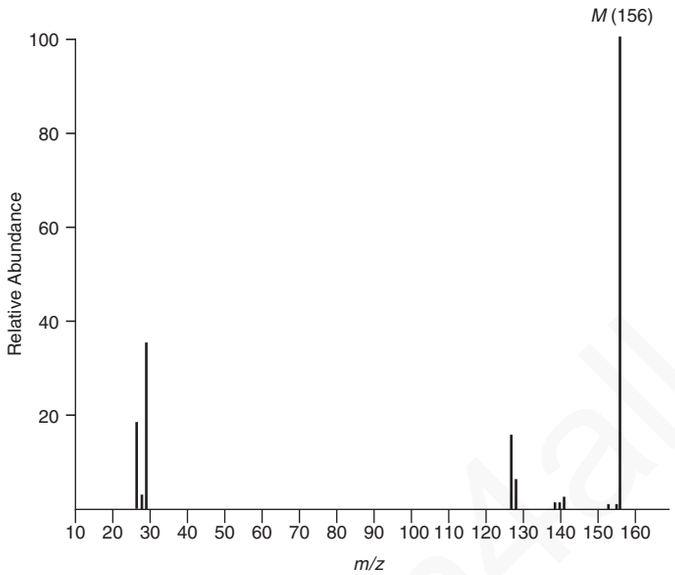
*(l) The infrared spectrum of this unknown shows two strong peaks, one near 1350 cm^{-1} and the other near 1550 cm^{-1}. Notice that the mass of the molecular ion is *odd*.



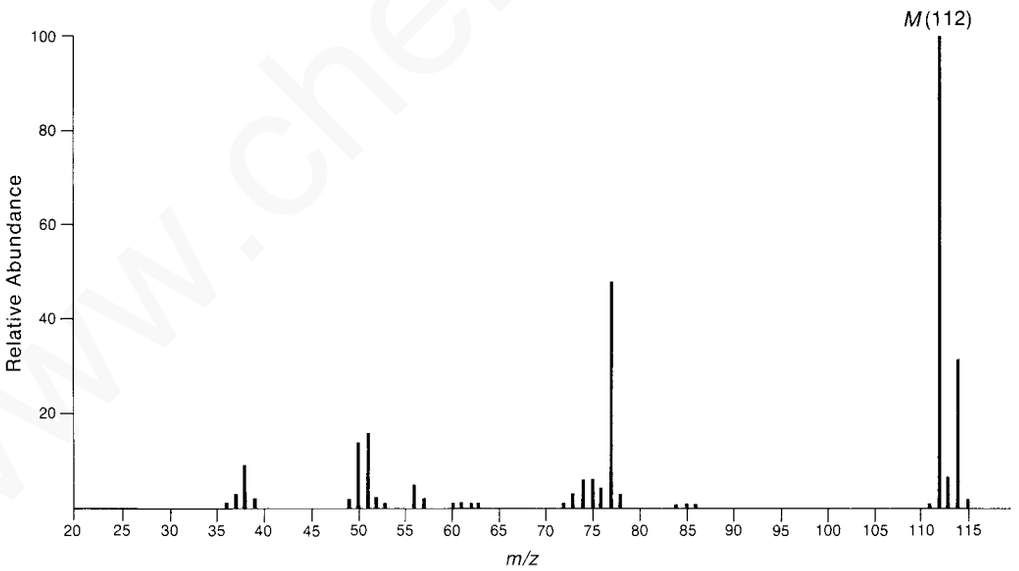
*(m) There is a sharp peak of medium intensity near 2250 cm^{-1} in the infrared spectrum of this compound.



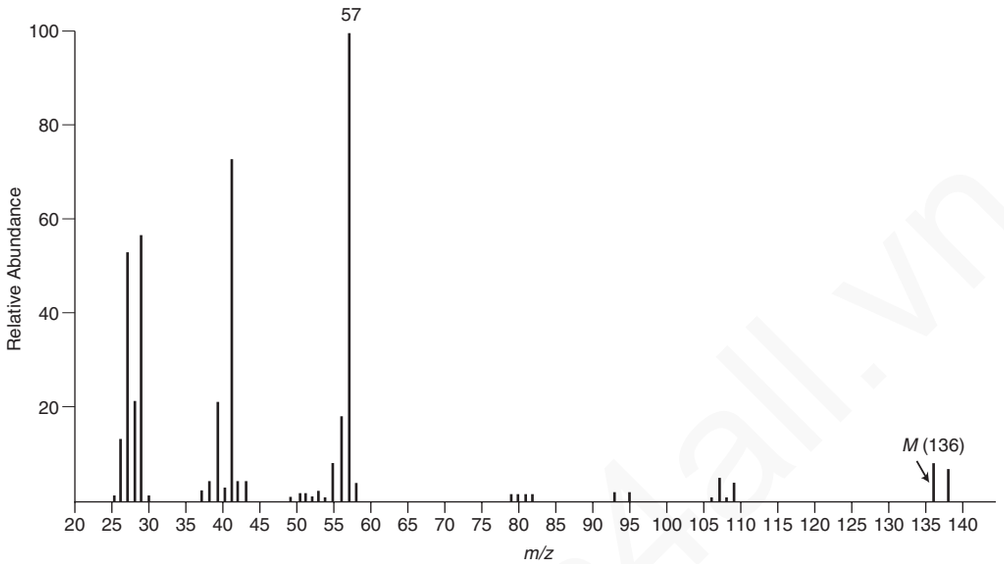
*(n) Consider the fragment ions at m/z = 127 and 128. From what ions might these peaks arise?



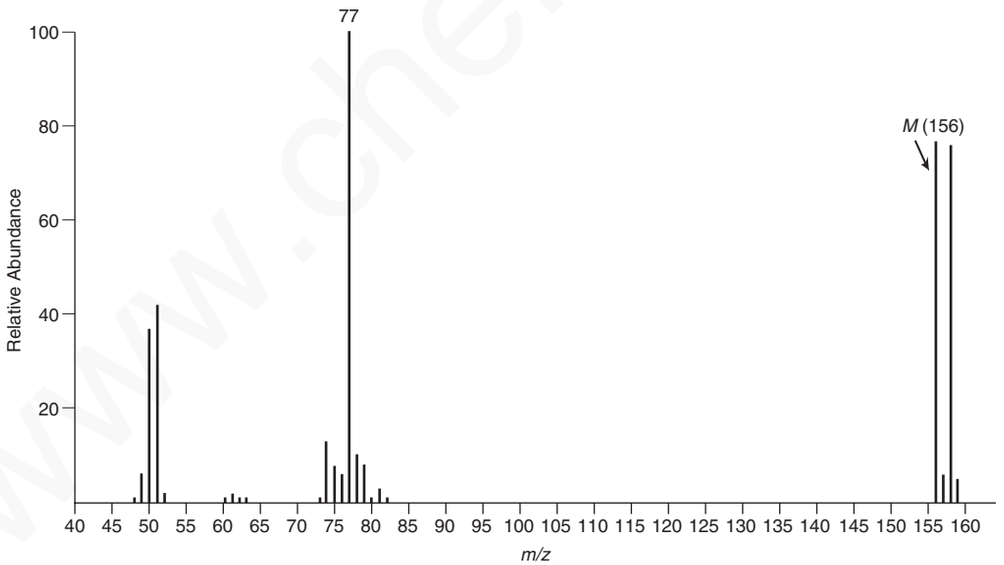
*(o)



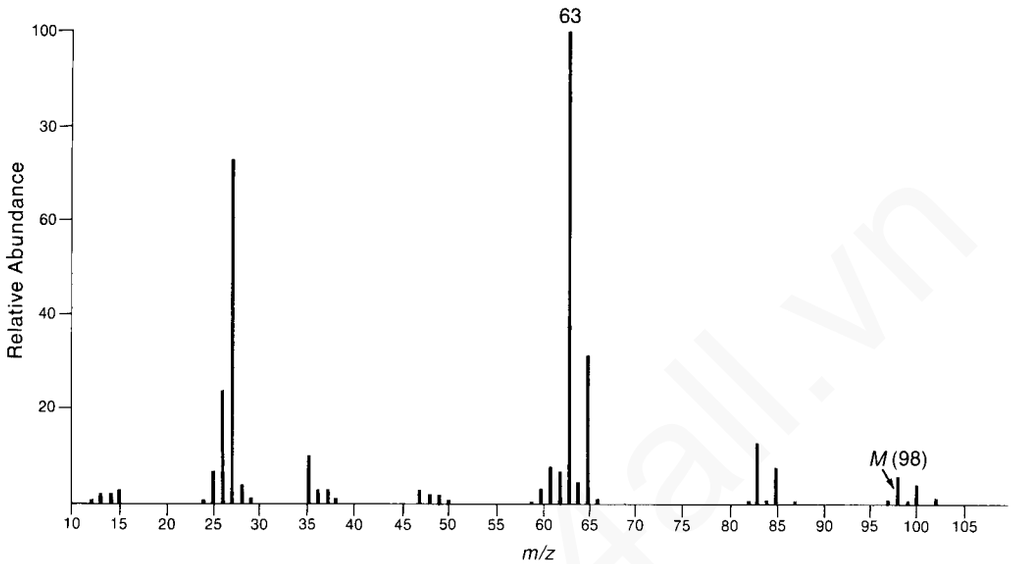
*(p)



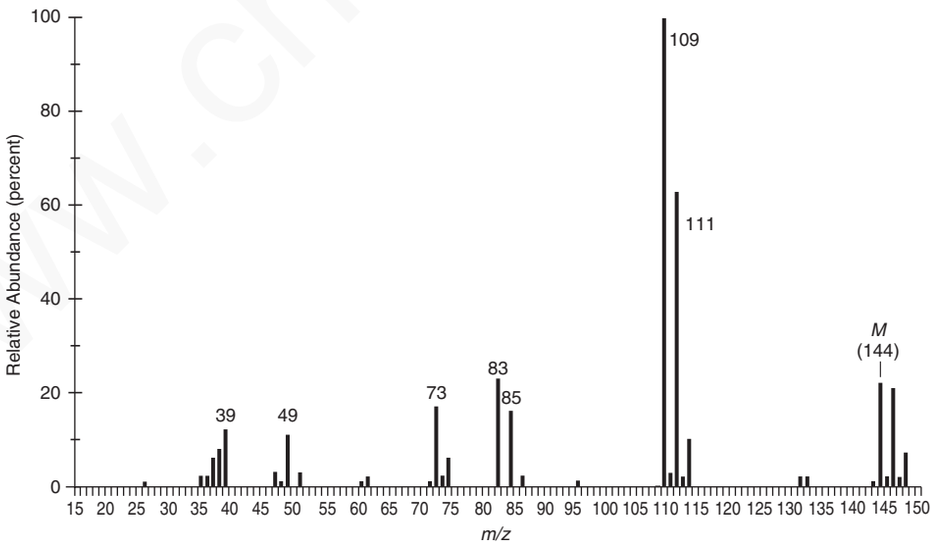
*(q)



*(r)

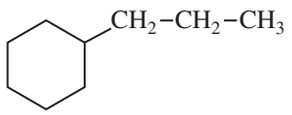
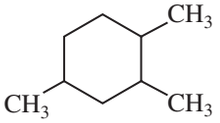


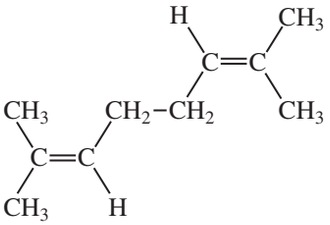
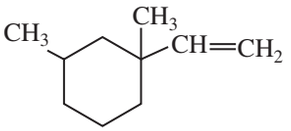
*(s) The infrared spectrum of this unknown shows a sharp peak at 3087 cm^{-1} and a sharp peak at 1612 cm^{-1} in addition to other absorptions. The unknown contains chlorine atoms, but some of the isotopic peaks ($M + n$) are too weak to be seen.

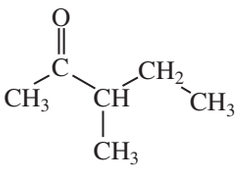
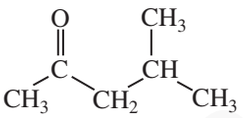


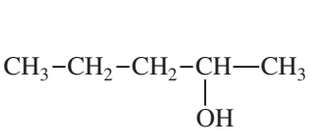
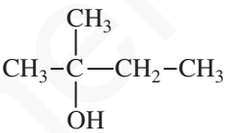
8. The mass spectrum of 3-butyn-2-ol shows a large peak at $m/z = 55$. Draw the structure of the fragment and explain why it is particularly stable.

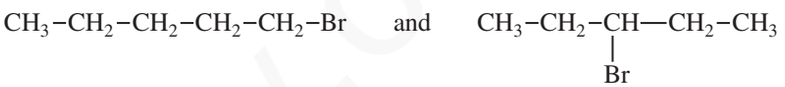
9. How could the following pairs of isomeric compounds be differentiated by mass spectrometry?

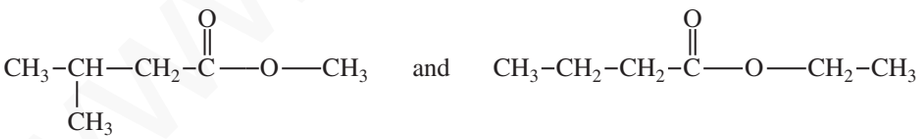
(a)  and 

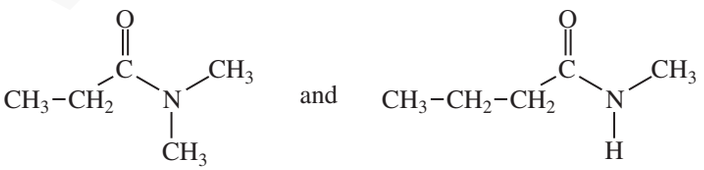
(b)  and 

(c)  and 

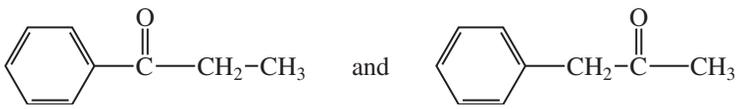
(d)  and 

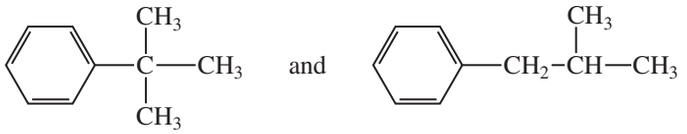
(e) 

(f) 

(g) 

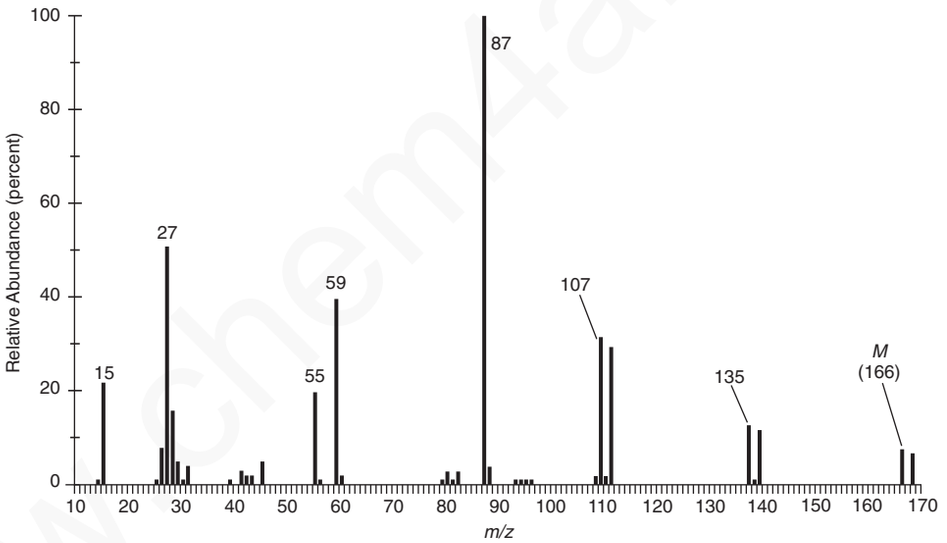
(h) $CH_3-CH_2-CH_2-CH_2-NH_2$ and $CH_3-CH_2-CH_2-NH-CH_3$

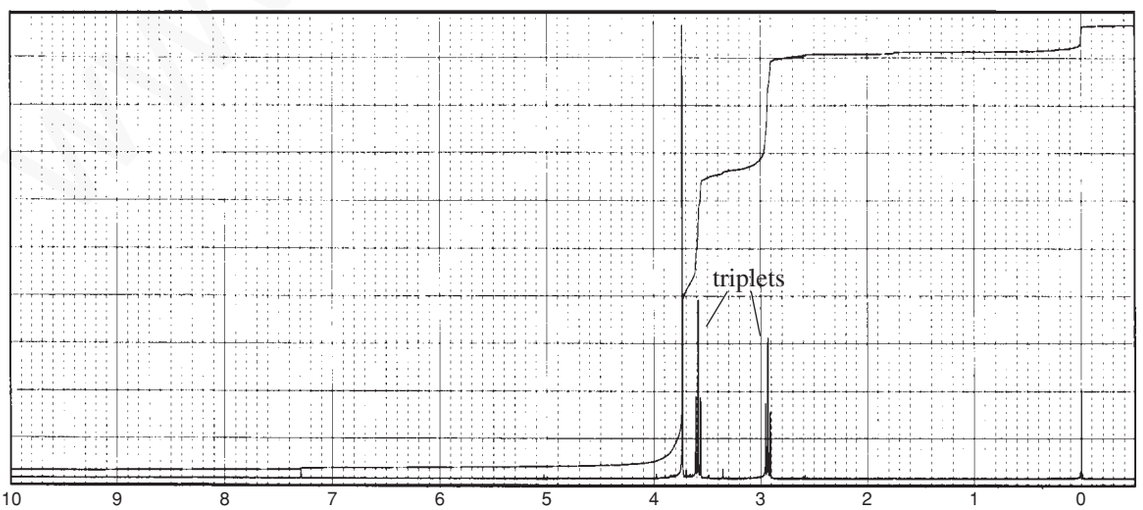
(i) 

(j) 

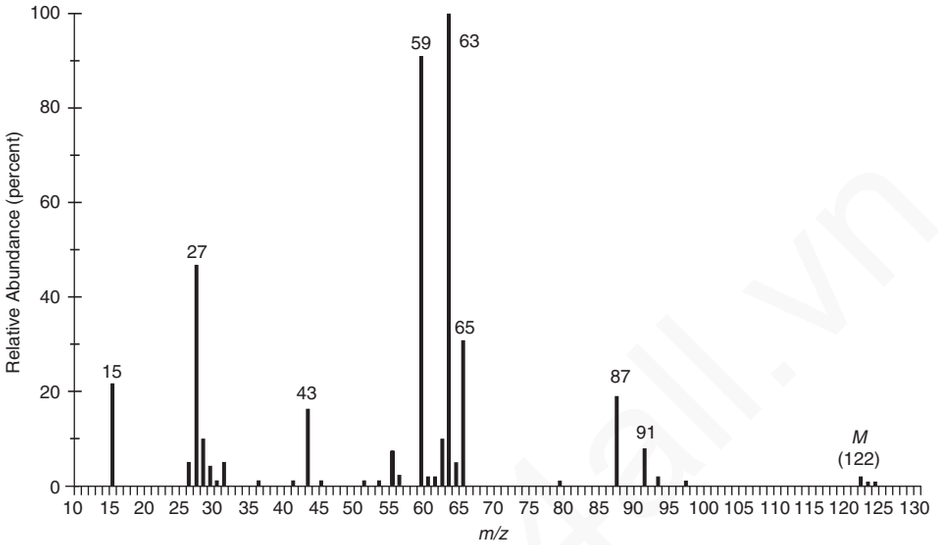
10. Use the mass spectrum and the additional spectral data provided to deduce the structure of each of the following compounds:

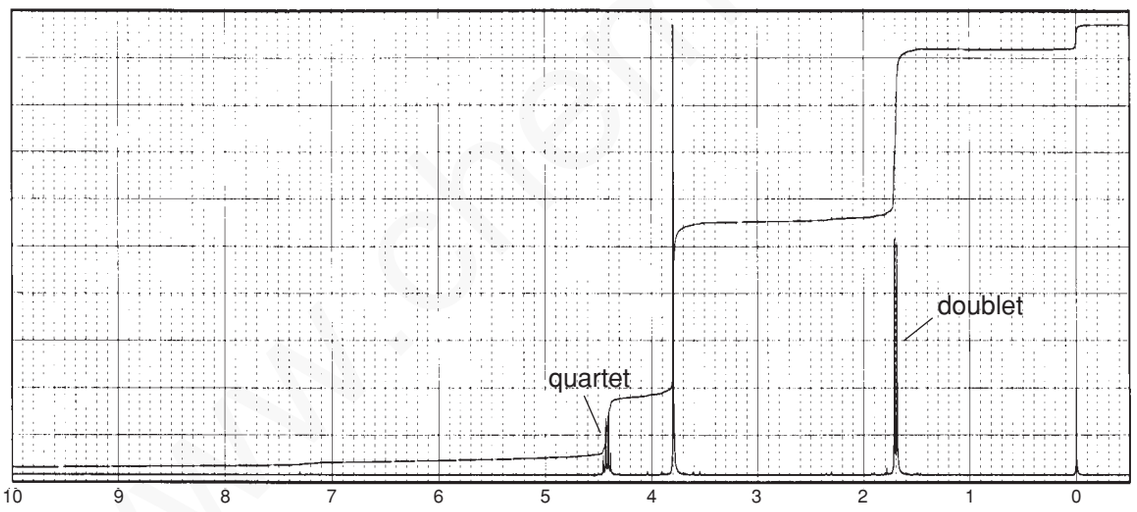
(a) $C_4H_7BrO_2$



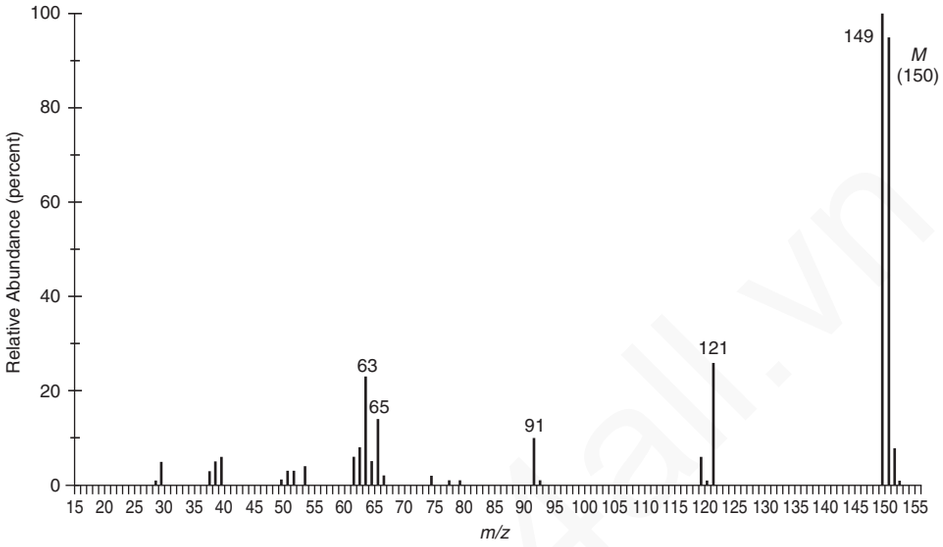


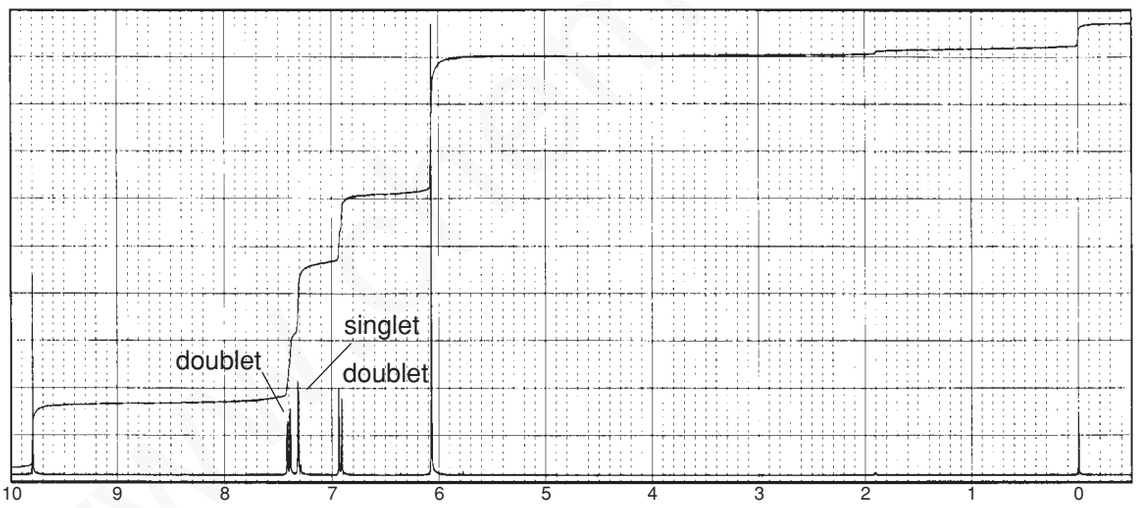
(b) $C_4H_7ClO_2$


Relative Abundance (percent)
100
80
60
40
20
0
59
63
27
65
15
43
87
91
M
(122)
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
m/z

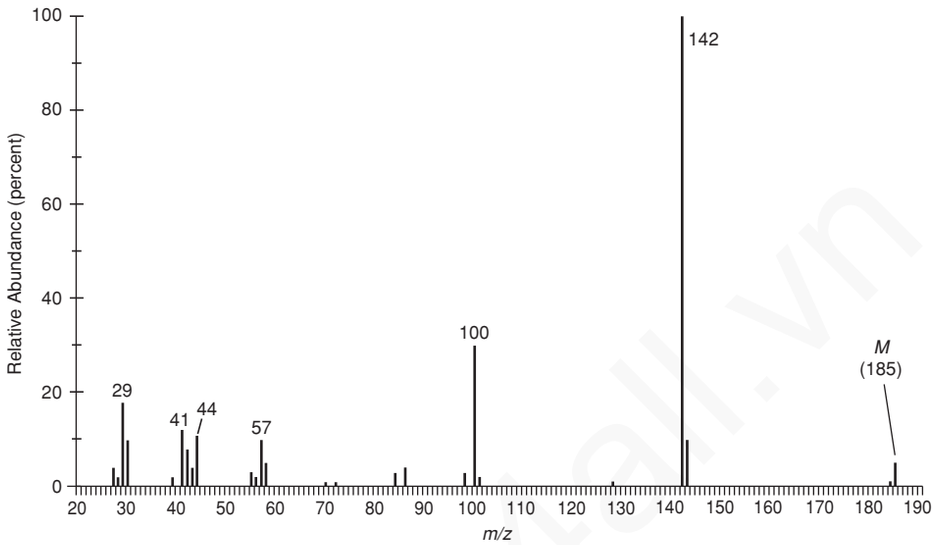

doublet
quartet
10
9
8
7
6
5
4
3
2
1
0

(c) $C_8H_6O_3$

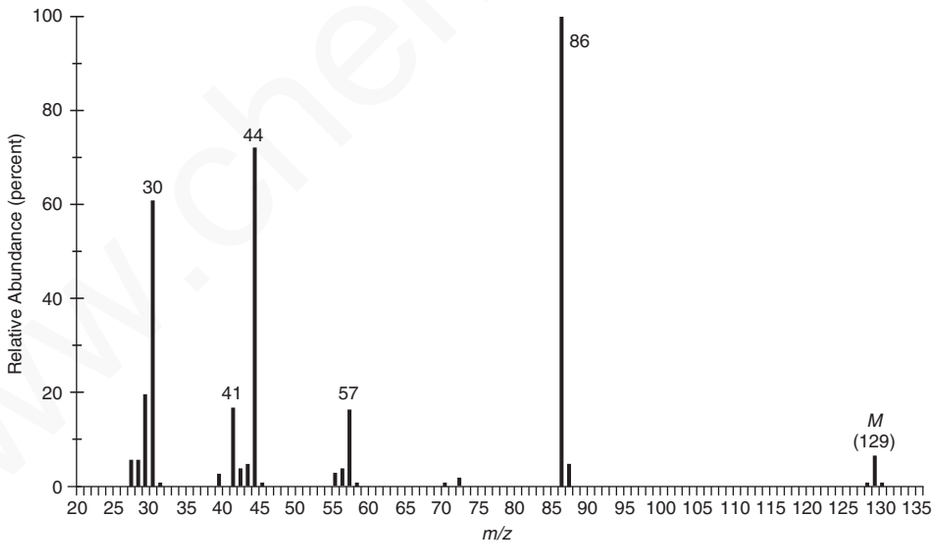




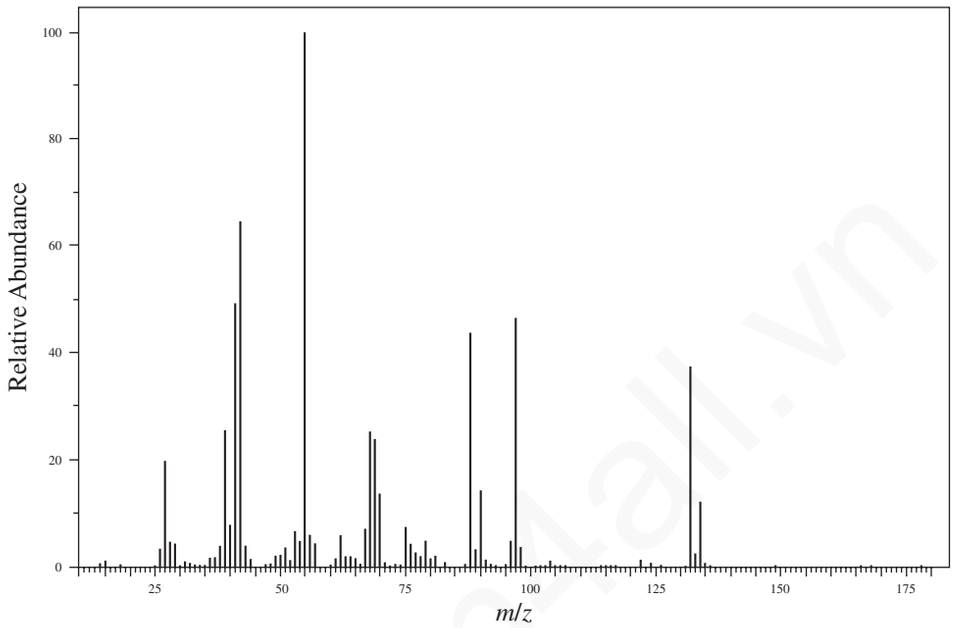
(d) The infrared spectrum lacks any significant peaks above 3000 cm^{-1}.



(e) The infrared spectrum contains a single, strong peak at 3280 cm^{-1}.



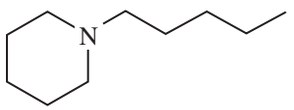
(f) The infrared spectrum contains a single, strong peak at 1723 cm^{-1}.



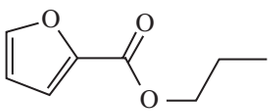
11. For each structure shown below

- Identify the site of initial ionization under EI conditions.
- Determine the structure of the ion indicated by the *m*/*z* value(s).
- Draw a fragmentation mechanism that accounts for the formation of the fragment ions.

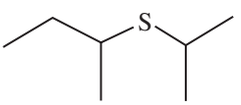
(a) Fragment ion at *m*/*z* = 98 (base peak in spectrum)



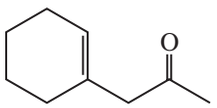
(b) Fragment ion at *m*/*z* = 95 (base peak in spectrum)



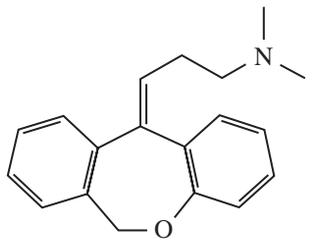
(c) Fragment ions at *m*/*z* = 103 and 61 (base peak)



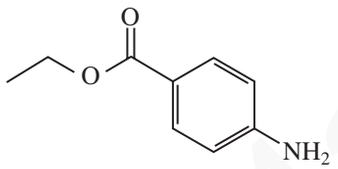
(d) Fragment ions at *m*/*z* = 95 (base peak) and 43



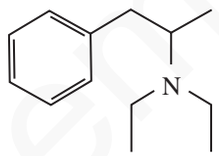
(e) Fragment ion at $m/z = 58$ (base peak)



(f) Fragment ion at $m/z = 120$ (base peak)

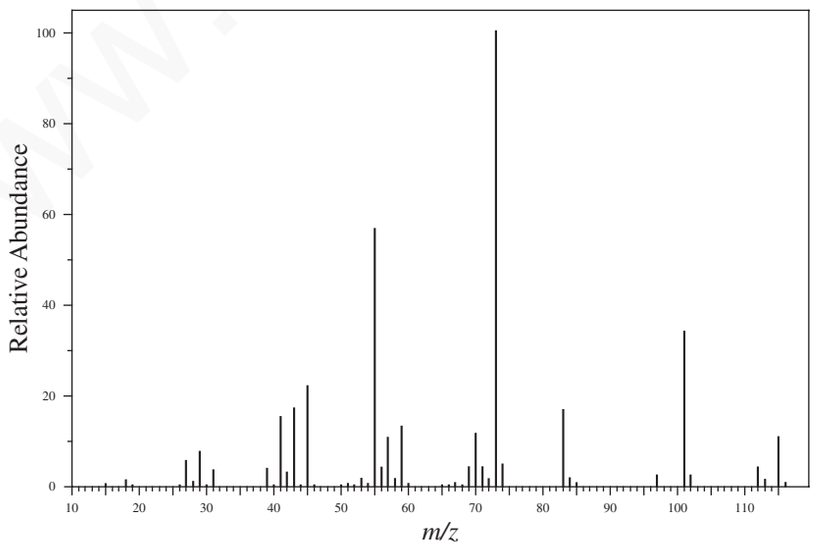


(g) Fragment ions at $m/z = 100$ (base peak), 91, 72, and 44

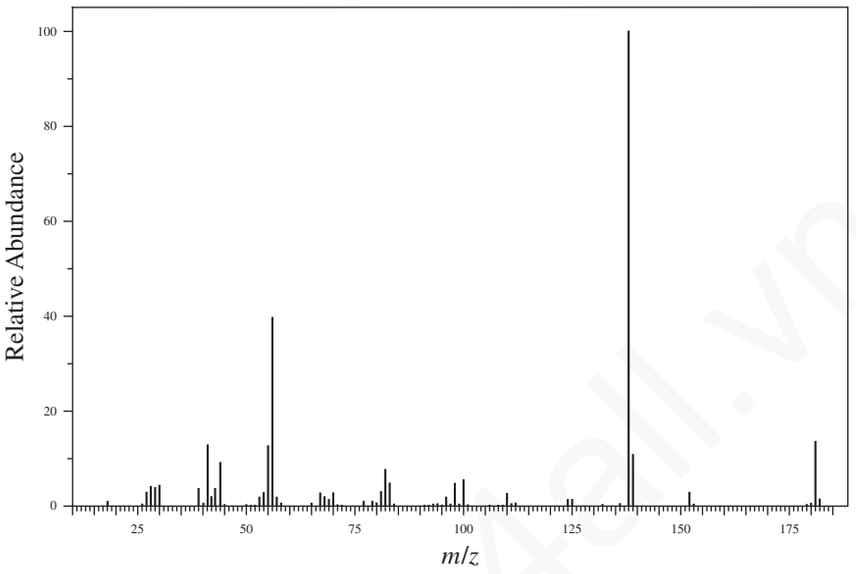


12. For each mass spectrum below, determine the structure of the prominent fragment ions and draw a fragmentation mechanism to explain their formation.

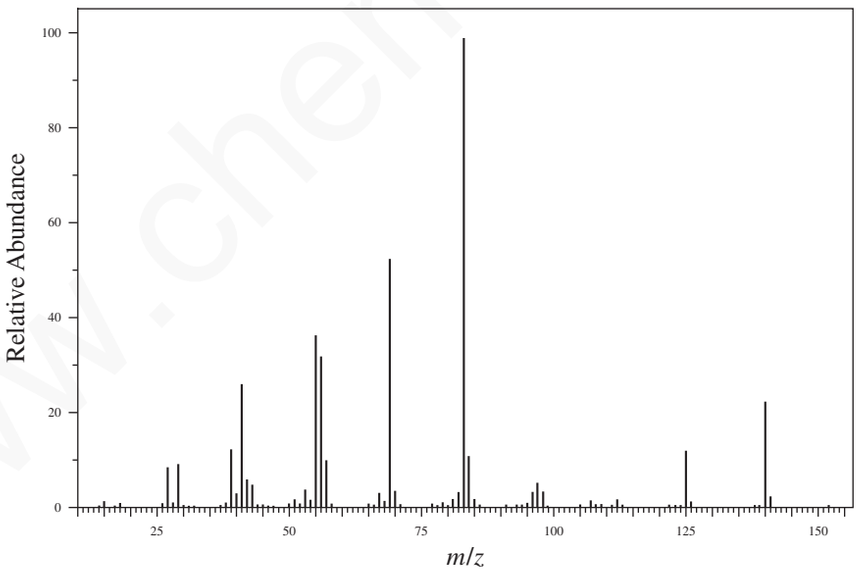
(a) 3-Methyl-3-heptanol



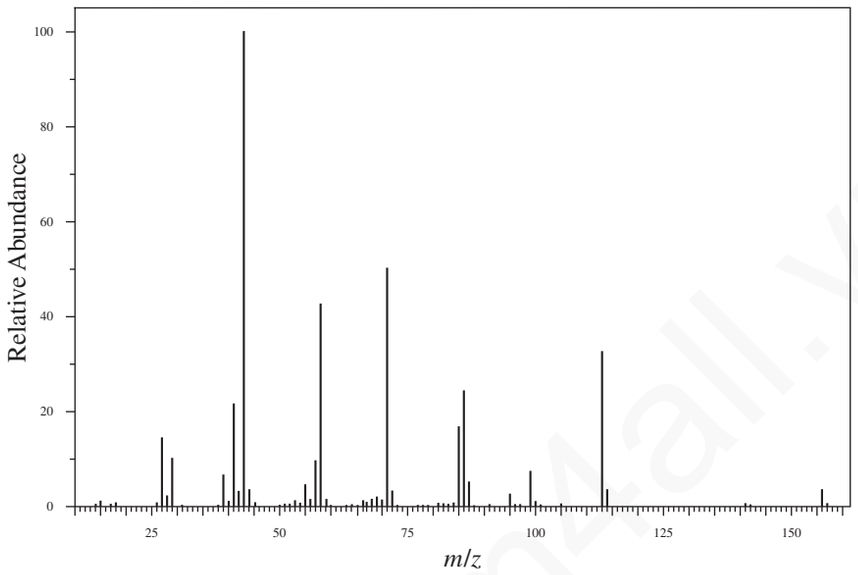
(b) Dicyclohexylamine



(c) 3,3,5-Trimethylcyclohexanone



13. While cleaning out old samples from your lab, you come across a vial labeled simply "decanone." You run an EI GC-MS of the material in the vial and obtain the mass spectrum shown below. Use the fragmentation pattern to determine which isomer of decanone is in the vial.

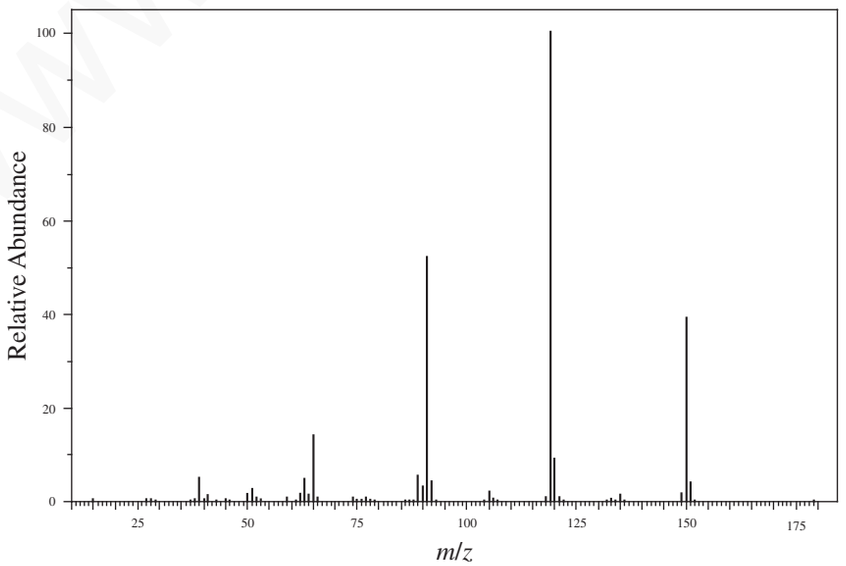


14. All dialkyl phthalate esters exhibit a base peak at m/z = 149. What is the structure of this fragment ion? Draw a mechanism that accounts for its formation from diethyl phthalate.

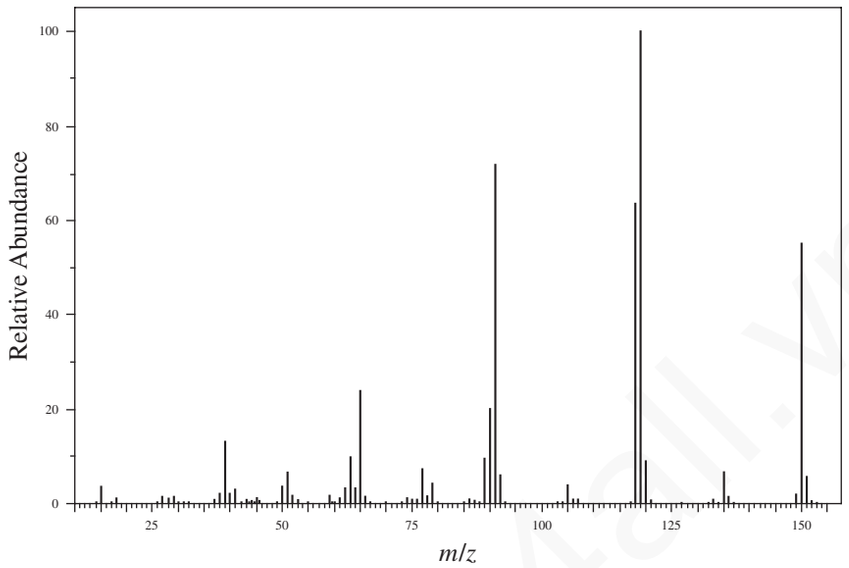
15. (a) The EI-MS of *ortho*-nitrotoluene (MW = 137) shows a large fragment ion at m/z = 120. The EI-MS of α,α,α-trideutero-*ortho*-nitrotoluene does **not** have a significant fragment ion at m/z = 120 but does have a peak at m/z = 122. Show the fragmentation process that explains these observations.

(b) The EI mass spectra for methyl 2-methylbenzoate and methyl 3-methylbenzoate are reproduced below. Determine which spectrum belongs to which isomer and explain your answer.

Spectrum 1



Spectrum 2



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Selected Web Sites

http://www.aist.go.jp/RIODG/SDBS/menu-e.html
National Institute of Materials and Chemical Research, Tsukuba, Ibaraki, Japan, *Integrated Spectra Data Base System for Organic Compounds (SDBS)*

http://webbook.nist.gov/chemistry/
National Institute of Standards and Technology, *NIST Chemistry WebBook*

http://winter.group.shef.ac.uk/chemputer/

http://www.sisweb.com/mstools.htm