CHAPTER 5

Organic Mass Spectrometry

5.1 ACCURATE MASS MEASUREMENTS

The molecular weight of a compound alone can be useful for identifying an organic compound. Where this measurement is made with high mass resolution, the molecular weight of a compound can be measured to an accuracy of a few parts-per-million (ppm). The experiment is referred to as an *accurate mass measurement*.

As described in Chapter 1, Section 1.3.1, this information can be sufficient to derive the molecular formula for the compound provided that two or more possible elemental compositions are distinguishable by mass within the error of the measurement. Benzene, with an elemental formula of C_6H_6, will give rise to a singly-charged ion with a resolved monoisotopic mass of 78.0468 based on the masses for the ^{12}C and ^{1}H isotopes (12.0000 and 1.0078 respectively). A mass measurement of within 149 ppm would be required to distinguish this ion from that of chloropropane (C_3H_7Cl) with a monoisotopic mass of 78.0235. This value reflects one-half of the difference between the two masses divided by the mass of benzene.

5.1.1 Calibrating the Mass Scale

To measure the *m/z* ratio of an ion with high accuracy, it is necessary to calibrate the mass scale of the instrument by analysing a mixture of known compounds that produce ions across the mass scale. On a magnetic-based instrument, for a fixed accelerating voltage, the magnetic field strength (B) is corrected (to those values predicted from equation 3.22) to focus ions of known *m/z* onto the detector.

Synthetic polymers are useful calibration compounds for this purpose, since the samples are polydisperse and generate a series of ions separated in mass-to-charge by the repeating monomer unit. Compounds such as perfluorotributylamine are also commonly used since they are rich in

carbon and fluorine both of which have a mass for their lightest isotope close to an integral value (arbitrarily set to 12.0000 for carbon, and 18.9984 for fluorine, see Appendix 2).

Simple metal halide salts are also used to calibrate the mass scale because they produce many *ion clusters*. These cluster ions are also separated by a repeating elemental or chemical unit. The compounds CsI and CsF are common examples. In the case of FAB ionisation, clusters observed from the matrix compound (such as glycerol) provide a convenient means with which to calibrate the mass scale using ions that appear along with those of the sample.

5.1.2 Peak Matching

Peak matching is the one of the most accurate methods used to assign the mass of an ion to a value within 1 ppm from the calculated *exact mass*. This technique is usually performed on high-resolution magnet-based instruments, but instead of adjusting the magnetic field strength, it involves rapidly switching the accelerating voltage, V. The accelerating voltage applied to ions leaving the ion source is alternately switched between two values such that the ion peaks for an unknown and reference compound are merged or overlap at the detector.

For a fixed magnetic field strength (B), it can be seen from equation 3.22 (Chapter 3) that the two ions will have the same apparent m/z ratios when the value of V is adjusted to compensate for their different mass and charge. By knowing the values of V and the m/z of ion for the reference compound with high accuracy, one can calculate the m/z ratio of the unknown compound within a small error when the accelerating voltage required to converge the ion peaks is measured. In practice, because the two ions under comparison are unlikely to have equal abundances, particularly at accelerating voltages that are not optimal, the ion signals are amplified as necessary so that their peaks can be overlapped.

Accurate mass measurements are easily achieved for relatively low molecular weight compounds of the order of a few hundred Daltons. Now that ionisation methods are available for ionising much larger molecules, further information may be required to reliably establish a compound's identity.

5.2 FRAGMENTATION OF ORGANIC MOLECULES

5.2.1 Mass Spectral Databases

Traditionally, organic molecules were analysed by EI in conjunction with a magnetic-sector mass spectrometer. Since EI is a so-called *hard ionisation* method, most mass spectra contain a series of fragment ions in addition to the molecular ion $M^{+\bullet}$ formed within the ion source. The fragmentation pattern of a compound is most influenced by the nature of the molecule, such that organic molecules generate specific signatures that have been studied over several decades.

A number of collections of EI mass spectra of organic compounds have been assembled that allow one to compare a recorded spectrum of an unknown compound with those for known compounds. One such collection is the Eight-Peak Index, published by the Royal Society of Chemistry. This collection contains in excess of 80,000 mass spectra presented as a list of the first eight major or most intense ion peaks. The spectra are classified according to the name and chemical class of the compound, the compound's molecular weight, its elemental composition, and the mass-to-charge ratios and intensities for the eight most abundant ions of the spectrum.

5.2.2 Location of Charge and Predictive Bond Fission

The initial location of a charge in a molecular ion is of particular importance in driving the fragmentation of molecules. Not all molecular ions formed from a single compound will have their charge located at the same position in the molecule, nor necessarily will that charge remain localised during the fragmentation of the compound. Yet due to the relatively high ionisation energies (typically 70 eV) used to ionise organic molecules in EI mass spectrometry, we assume from the *quasi-equilibrium theory* (Section 2.2.4) that during the formation of the molecular ion $M^{+\bullet}$ an electron can be removed from anywhere within the molecule. This ion then has sufficient time and energy prior to decomposition to transfer electrons and produce a more stable species of lower energy.

For the purpose or predicting or interpreting the dissociation of organic molecules, only those fragment ions arising from the last-formed precursors are of concern. For the most part, charge location(s) in the precursor ions drives the dissociation process. However, the products of so-called *charge remote* fragmentation processes are detected to a lesser extent, particularly in molecules such as fatty acids that contain extended aliphatic groups.

While *bond dissociation energies* (BDE) influence fragmentation, their values within ions are very different from those of neutral molecules. Thus a BDE value for a neutral molecule may not indicate that its ion dissociates along a particular path. Rather, the proximity of the charge to the dissociating bond has a greater effect on the pathway followed. As in descriptions of organic synthesis, the cleavage and formation of bonds during the dissociation of ionised organic molecules is typically described by the use of half ("fish hook") and full-headed arrows showing the direction of electron movement. As molecular ions $M^{+\bullet}$ are deficient in a single electron, bond fission (and formation) is typically represented by a homolytic process, unless evidence for a heterolytic cleavage is otherwise available.

In the following sections, fragmentations that are common across many classes of organic compounds are reviewed.

5.2.3 Homolytic Cleavage

Homolytic cleavage occurs when the electron pair of a covalent bond is transferred to two different atom centres. The site of the radical within the molecular ion $[A–B]^{+\bullet}$ is undefined in the following equations. Odd-electron ions dissociate by homolytic bond cleavage to an even-electron fragment ion and a radical (equations 5.1 and 5.2).

$$[A–B]^{+\bullet} \rightarrow A^{+} + B^{\bullet} \tag{5.1}$$

$$[A–B]^{+\bullet} \rightarrow A^{\bullet} + B^{+} \tag{5.2}$$

The fragment ion A^+ or B^+ with the greatest tendency to support an unpaired electron will have a higher *appearance energy*. This ion will be less stable than a low energy fragment, and it should appear with a lower relative abundance in the mass spectrum.

The decompositions of odd-electron ions are influenced by the preference for even-electron ions according to the *even-electron rule*. This states that the production of the odd-electron fragment must be accompanied by the formation of a radical species. Thus it is assumed that reactions associated with the pairing of the radical site in the molecular ion have lower energy barriers to activation.

Less favourable dissociations involving the homolytic cleavage of *two* bonds can produce odd-electron products as illustrated in equation 5.3. The bond cleaved in this example is α to the charge localised on oxygen.

$$CH_2(H)–CH_2(O^{+\bullet})H \rightarrow CH_2 = CH_2^{+\bullet} + H_2O \tag{5.3}$$

This reaction is initiated at the radical site and is driven by an electron pairing process resulting in the formation of a new bond. It is accompanied by cleavage of the α-bond. Cleavage at the β-position to the charge site is less common than α-cleavage but can be an observed fragmentation within a mass spectrometer (equation 5.4).

$$CH_3–CH_2–C(=O^{+\bullet})–CH_3 \rightarrow CH_3–CH_2^+ + CH_3–C^\bullet = O \quad (5.4)$$

5.2.4 Heterolytic Cleavage

Heterolytic cleavage of a covalent bond occurs when both electrons that constitute the bond are transferred to a single atom centre. The driving force for this cleavage is the *induction effect* in which the electrons migrate to, and neutralise, the charge. Heterolytic cleavages can occur in both odd- and even-electron ions. An odd-electron ion will fragment to give a radical and an even-electron fragment ion (equation 5.5).

$$[A–B]^{+\bullet} \rightarrow A^+ + B^\bullet \quad (5.5)$$

Even-electron ions dissociate to an even-electron fragment and a neutral fragment (equation 5.6).

$$[A–B]^+ \rightarrow A^+ + B \quad (5.6)$$

5.2.5 σ-Bond Cleavage

When the bond depleted of an electron in the formation of a molecular ion $M^{+\bullet}$ is dissociated, the fragmentation is referred to as a *σ-bond cleavage* and gives rise to an even-electron product ion and a radical (equation 5.7).

$$A^{+\bullet}B \rightarrow A^+ + B^\bullet \quad (5.7)$$

An example of a σ-bond cleavage is shown in equation 5.8.

$$C_6H_5^{+\bullet}C(O)–CH_3 \rightarrow C_6H_5^+ + CH_3–C^\bullet = O \quad (5.8)$$

Many forms of a molecular ion may be generated during the ionisation process such that the site of the charge is not localised to a single bond. As such it can be difficult to characterise a σ-bond cleavage.

For saturated hydrocarbons, without heteroatoms that contain lone electron pairs, ionisation at a σ-bond is the lowest energy process. The

bond that fragments is that which results in the production of a more substituted cation according to equation 5.9.

$$(CH_3)_3C^{+\bullet}\ CH_3 \rightarrow (CH_3)_3C^+ + CH_3^\bullet \qquad (5.9)$$

$$\not\rightarrow (CH_3)_3C^\bullet + CH_3^+$$

5.2.6 Rearrangements

Fragment ions can also form by processes in which the initial bond connections in the molecular ion are reordered or arranged. Fortunately, many of these rearrangement processes have been characterised for organic molecules and therefore can be predicted based on an ion's structure. Rearrangement reactions occur with the movement of two or more sets of electron pairs.

A common rearrangement first reported by McLafferty and co-workers is referred to as the *McLafferty rearrangement*. In this homolytically-driven process, a hydrogen atom at a γ-centre migrates to the charge site through a cyclic intermediate resulting in the loss of an alkene or other stable molecule (equation 5.10).

$$CH_3{-}C(O^{+\bullet}){-}CH_2{-}CH_2{-}CH_2{-}H \rightarrow CH_3{-}C(OH^+){-}CH_2{-}CH_2{-}CH_2^\bullet$$

$$\rightarrow CH_3{-}C(OH^+){-}CH_2^\bullet + CH_2{=}CH_2 \qquad (5.10)$$

The rearrangement shown in equation 5.10 leads to the production of a *distonic ion*, namely one in which the charge and radical centres are remote from one another.

Even-electron ions can also undergo rearrangement processes during fragmentation resulting in a reorganised structure accompanied by dissociation (see for example, equation 5.11).

$$CH_3{-}CH(H){-}CH_2{-}N^+H{=}CH_2 \rightarrow CH_3{-}CH{=}CH_2 + N^+H_2{=}CH_2 \qquad (5.11)$$

The next section of this chapter reviews the major fragmentation processes that are observed in EI mass spectra for organic compounds classified by their functional groups. Compounds with more than one functional group will fragment according to processes influenced by all such groups. This section is designed to assist the reader with interpreting the EI mass spectra of organic compounds. It is not necessary to learn every single dissociation pathway for every class of compound. Rather,

an understanding of the common and likely dissociation processes will aid in the interpretation of the mass spectra of any organic compound.

For a more detailed account of these processes, the reader is referred to the works of Budzikiewicz and McLafferty and their co-authors (see cited references at the end of this chapter). A summary of the fragmentation processes of organic compounds also appears in Appendix 6 at the rear of this book.

5.3 FRAGMENTATION OF ORGANIC MOLECULES BY COMPOUND CLASS

5.3.1 Hydrocarbons

The fragmentation of aliphatic hydrocarbons is usually characterised by the loss of an alkyl group and the formation of an even-electron ion (equation 5.12 and Figure 5.1).

$$[CH_3{-}CH_2{-}CH_2{-}CH_2{-}R]^{+\cdot} \rightarrow CH_3{-}CH_2{-}CH_2{-}CH_2^+ + R^{\cdot} \qquad (5.12)$$

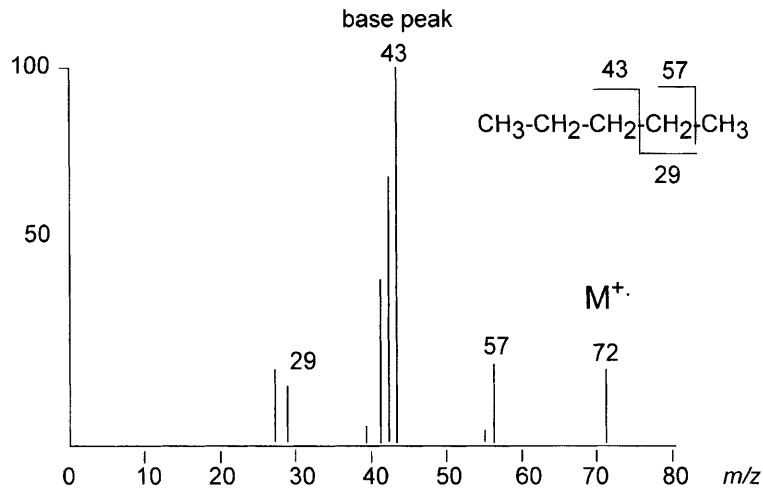
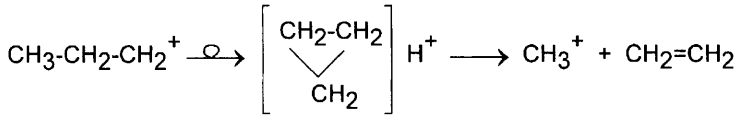


Figure 5.1 *Representation of the EI mass spectrum of pentane*

As illustrated in Section 2.2.3, fragmentation processes in which substituted carbocations are produced are favoured according to the stability of these ions ($(CH_3)_3C^+ > (CH_3)_2CH^+ > CH_3CH_2^+$).

The ability of hydrocarbons to undergo rearrangement reactions after ionisation is also well-known. Many large hydrocarbons fragment to ions corresponding to the formula $C_3H_7^+$ (*m/z* 43) (Figure 5.1) associated with an isopropyl species that subsequently loses ethylene to form the methyl cation CH_3^+ (equation 5.13).

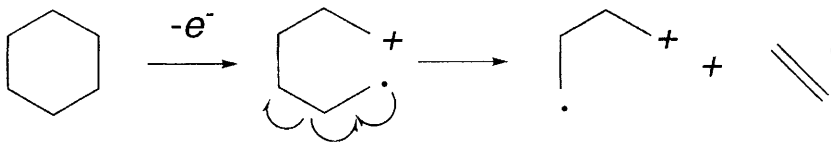


(5.13)

Hydrogen rearrangements are rarely detected in saturated hydrocarbons but are observed in the EI mass spectra of unsaturated forms. A McLafferty-type rearrangement is often encountered for unsaturated hydrocarbons with the elimination of an alkene (equation 5.14).

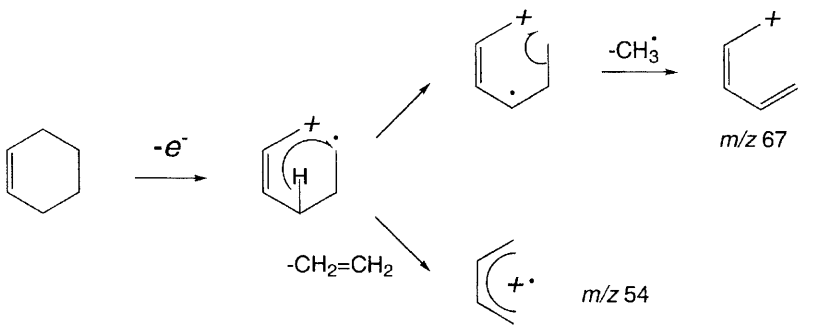
$$[H{-}CH_2{-}CH_2{-}CH_2{-}CH{=}CH_2]^{+\bullet} \longrightarrow CH_2{=}CH_2 + [CH_2{=}CH{-}CH_2]^{+\bullet} \qquad (5.14)$$

Despite these rearrangements, the fragmentations of straight-chain and branched aliphatic hydrocarbons can often be deciphered through a one-step dissociation pathway. The same is not true of cyclic hydrocarbons. Cyclohexane, for example, shows a loss of ethylene in its EI mass spectrum. Dissociation of a σ-bond cleavage followed by homolytic cleavage can account for the formation of a distonic ion at *m/z* 56 (equation 5.15) that represents the base peak in the spectrum.



(5.15)

Unsaturated cyclic hydrocarbons also fragment through more complex dissociation pathways. Cyclohexene dissociates to yield major fragment ions at *m/z* 54 and 67. The latter is produced by the loss of a methyl radical after ring opening by σ-bond cleavage and hydrogen atom migration (equation 5.16). The ion at *m/z* 54 is associated with the loss of ethylene and corresponds to the ionized form of 1,4-butadiene (equation 5.17). This decomposition is a retro-Diels Alder reaction.



(5.16)

(5.17)

Aromatic hydrocarbons are more resistant to fragmentation and their EI mass spectra are usually dominated by the molecular ion, as well as its multiply-charged forms. In the case of benzene, in addition to the molecular ion at *m/z* 78 that appears as the base peak in the spectrum, other ions appear at *m/z* 51 and 39. The latter ion is the doubly-charged molecular ion $C_6H_6^{2+\bullet}$. Beynon and Fontaine measured the energy lost during the dissociation of $C_6H_6^{2+\bullet}$ to CH_3^+ and $C_5H_3^+$ as 2.8 eV consistent with the two charges in the parent being located over 5 Å apart. This observation suggests the doubly-charged form of benzene has an open-configuration with one of the following forms (Figure 5.2).

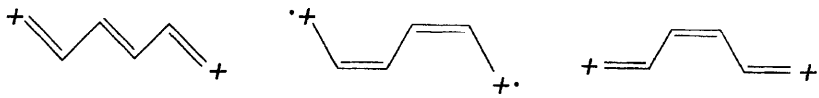
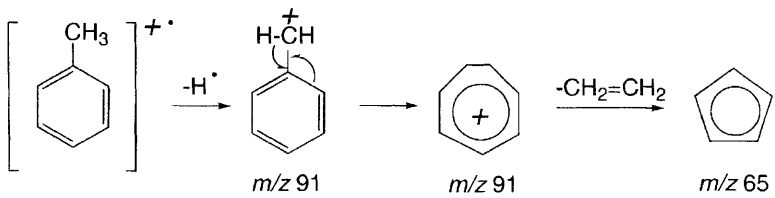


Figure 5.2 *Proposed structures for the ring-open configurations of the doubly-charged ion of benzene*

The fragment ion at *m/z* 51 can also be explained if dissociation of benzene is preceded by a ring opening reaction (equation 5.18).

$$[CH_2{=}CH{-}CH{=}CH{-}C{\equiv}CH]^{+\bullet} \rightarrow CH_2{=}CH^{\bullet} + {}^{+}CH{=}CH{-}C{\equiv}CH \qquad (5.18)$$

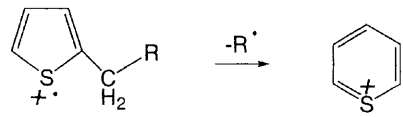
Toluene dissociates by the loss of a hydrogen atom to yield a fragment ion at *m/z* 91. Deuterium-labelling experiments have shown that further decomposition of this ion occurs through the loss of ethylene (*m/z* 65) suggesting all seven hydrogen are equivalent. This supports the formation of a cyclic tropylium structure as an intermediate (equation 5.19). The proposed rearrangement mechanism for the formation of the cyclic tropylium ion is shown in equation 5.19.



(5.19)

The cyclic tropylium ion often appears in the spectra of aromatic hydrocarbons, but not all alkylbenzenes necessarily give rise to a substituted tropylium ion $C_7H_6R^+$. Yet ring expansion processes have also

been proposed in the fragmentation of heteroaromatic compounds. Alkylthiophenes, for example, are proposed to lose alkyl radicals through a thiapyrylium ion (equation 5.20).

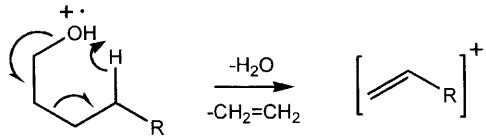


(5.20)

5.3.2 Alcohols

Aliphatic alcohols frequently give rise to EI spectra in which the ion signal for the molecular ion is generally weak or not observed. This is consistent with their lower ionisation efficiencies compared with the corresponding alkane. The absence or diminished intensity of the molecular ion is also associated with the susceptibility of alcohols to fragmention. It is convenient to visualise many of these fragmentation reactions as proceeding from a molecular ion first formed by the loss of an electron from one of the lone electron pairs from the oxygen of the hydroxyl group.

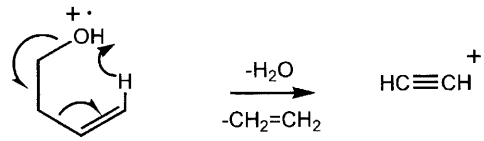
Aliphatic alcohols dissociate with the loss of a hydrogen atom, alkyl radical, water and alkenes such as ethylene. This is illustrated below where water loss proceeds through hydride ion migration to the hydroxyl group which neutralises the charge on oxygen.



(5.21)

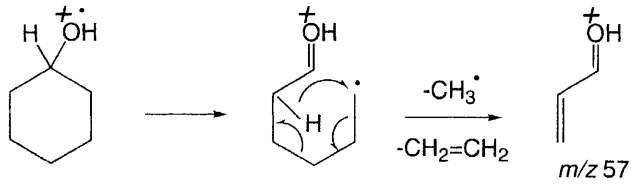
The loss of a hydroxyl radical, in contrast to water, is rarely observed. It is common, however, to detect ions corresponding to the loss of molecular hydrogen (H_2) from the molecular ion.

Unsaturated alcohols undergo similar processes. But-3-en-1-ol, and other long chain and branched unsaturated alcohols, also dissociate following a McLafferty rearrangment according to equation 5.22.



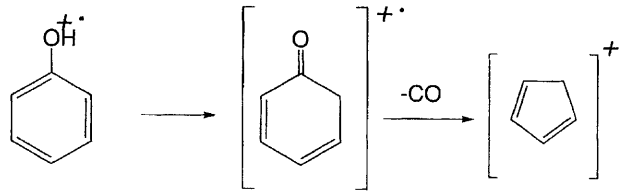
(5.22)

The EI mass spectrum of cyclohexanol is dominated by a fragment ion at *m/z* 57 (the base peak). This ion is formed following ring opening hydrogen atom migration, and the subsequent loss of a methyl radical and ethylene.



(5.23)

Many EI mass spectra of phenols have also been recorded. Like other aromatics, the spectra are dominated by intense ion signals for the molecular ion. Phenols, however, show the unique loss of carbon monoxide (–28 u) that has been proposed to occur through a cyclohexadienone intermediate followed by a compacting of the ring size to release CO (equation 5.24).



(5.24)

This loss is typically accompanied by the loss of 29 mass units corresponding to the CHO unit. Other substituents on the aromatic ring can stabilise the ionic products and hence can drive the fragmentation process. Alkyl substituents positioned *para* to the phenolic hydroxyl group can lose fragments from the benzylic position since the resulting ion has a stabilized oxonium ion form (Figure 5.3). An equivalent ion cannot form if the alkyl substituent is located *meta* to the phenolic hydroxyl group.

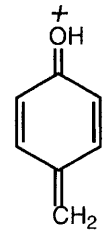
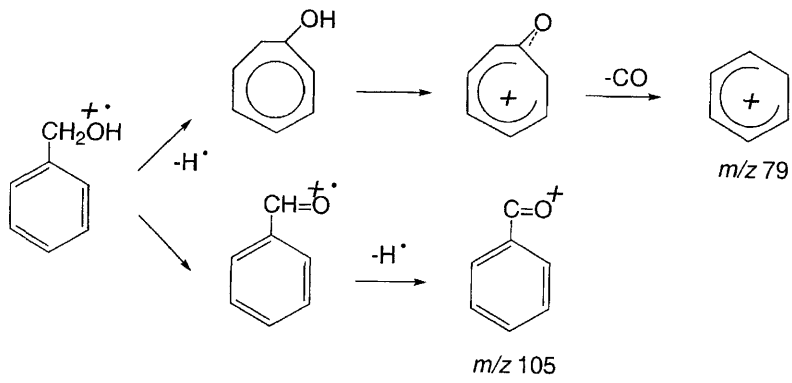


Figure 5.3 *Oxonium ion formed by the loss of alkyl substituents* para *to the phenolic hydroxyl group*

Shannon and others have studied in some detail the mass spectra of benzyl alcohols. These exhibit abundant ion signals associated with the molecular ion (*m/z* 108 in the case of benzyl alcohol) in addition to fragments formed by the loss of a hydrogen atom and carbon monoxide. This fragmentation has been proposed to occur through the formation of a seven-membered ring intermediate with the subsequent loss of CO to yield a benzenium ion (*m/z* 79) (equation 5.25). This ring can then lose molecular hydrogen to form $C_6H_5^+$ (*m/z* 77). An additional ion in the mass spectrum of benzyl alcohol is due to the loss of hydroxyl radical to yield the benzyl cation (*m/z* 91) that can be stabilised through the formation of a tropylium ion discussed earlier. A weaker fragment corresponding to the successive loss of molecular hydrogen and a hydrogen atom from the side chain gives rise to the benzoyl cation $C_6H_5CO^+$ (*m/z* 105) (equation 5.26).



5.3.3 Ethers

The molecular ion generally appears in greater abundance in the EI mass spectra of ethers relative to their corresponding alcohols. Like alcohols, however, a common cleavage observed for ethers involves fission of a α-bond. Asymmetrical ethers can give rise to two products by this process. The more substituted ions will tend to form preferentially as illustrated in equation 5.27 over 5.28.

$$(CH_3)_2CH\text{–}O^{+\cdot}\text{–}CH_2\text{–}CH_3 \rightarrow\rightarrow (CH_3)_2CH^+ + {}^{\cdot}O\text{–}CH_2CH_3 \qquad (5.27)$$

$$\rightarrow (CH_3)_2CH\text{–}O^{\cdot} + {}^{+}CH_2CH_3 \qquad (5.28)$$

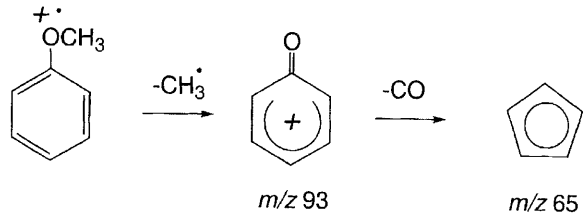
Another fragmentation process observed for ethers is hydrogen atom migration to the charge site with loss of an alkene (equation 5.29).

$$(CH_3)_2CH{-}O^{+\bullet}{-}CH_2{-}CH_3 \rightarrow CH_3{-}CH{=}O^{+}{-}CH_2{-}CH_3 + CH_3^{\bullet}$$
$$\rightarrow CH_3{-}CH{=}OH^{+} + CH_2{=}CH_2 \qquad (5.29)$$

Unsaturated ethers, such as alkyl vinyl ethers, can undergo a McLafferty rearrangement resulting in the loss of an alkene and a stable carbonyl cation (equation 5.30).

$$[H{-}CH_2CH_2{-}O{-}CH{=}CH_2]^{+\bullet} \rightarrow$$
$$CH_2{=}CH_2 + [O{=}CH{-}CH_3]^{+\bullet} \qquad (5.30)$$

Ethers possessing aromatic groups fragment through the simple α-cleavage processes shown above. Phenyl ethers also have been observed to undergo secondary fragmentations involving the loss of carbon monoxide as illustrated in equation 5.31.



m/z 93 *m/z* 65 (5.31)

This fragmentation is blocked when an additional methylene group is located between the oxygen atom and the aromatic ring. Such benzyl ethers have EI mass spectra that are dominated by the benzyl cation $C_6H_5{-}CH_2^{+}$ (*m/z* 91) formed by α-cleavage.

5.3.4 Amines

Aliphatic amines generally ionise poorly by EI. However, due to the basic nature of the amino groups, stable $[M+H]^{+}$ ions can be produced in high yield during chemical ionization. α-Cleavage is the predominate fragmentation pathway (shown for ethyl amine in equation 5.32) with β-cleavage, and to a lesser extent γ-cleavage, becoming more important as the size of the carbon chain increases.

$$[CH_3CH_2{-}NH_2]^{+\bullet} \rightarrow CH_3^{\bullet} + CH_2{=}N^{+}H_2 \ (m/z\ 30) \qquad (5.32)$$

One of the most dominant rearrangment processes observed in the

spectra of aliphatic amines, arises from the transfer of an alkyl group from the carbon α to the amine group to the equivalent carbon on the opposite side followed by cleavage of the N-C bond (equation 5.33). This fragmentation process is only possible for secondary and tertiary amines.

$$[(CH_3)_2CH{-}NH{-}CH_3]^{+\bullet} \rightarrow CH_3CH{=}NH^+ + {}^{\bullet}CH_2CH_3 \qquad (5.33)$$

Aromatic amines, in contrast to aliphatic amines, give rise to dominant molecular ions in their EI spectra. In many cases, as in the spectrum of aniline, the molecular ion is the base peak of the spectrum. The formation and stability of the molecular ion is attributed to electron transfer with the π-electrons of the ring (Figure 5.4).

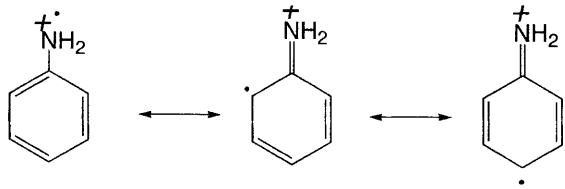


Figure 5.4 *Proposed structures for the molecular ions of aniline that contribute to its stability by electron transfer with the aromatic ring*

5.3.5 Aldehydes and Ketones

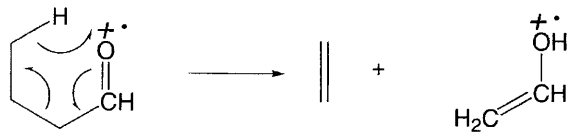
The addition of a carbonyl group to an alkane considerably lowers the ionisation energy of the molecule. The lowest energy form is that in which an electron is removed from one of the lone pairs of the carbonyl oxygen. Removal of an π-electron from the C=O bond requires more energy (typically 10.6 eV) than that required to remove an electron from a α-bond (11.5 eV). Since most EI spectra are recorded at 70 eV, all forms of the molecular ion are possible.

A common dissociation pathway in both aldehydes and ketones is that which results from cleavage of the bond α to the carbonyl group. This results in the loss of a hydrogen atom in the case of aldehydes and alkyl groups in both systems with the formation of the resonance stabilized ion $R{-}C^+{=}O$ (equation 5.34).

$$[R{-}CO{-}R']^{+\bullet} \rightarrow R{-}C^+{=}O + H^{\bullet} \text{ or } R'^{\bullet} \qquad (5.34)$$

The ion $H{-}C^+{=}O$ (*m/z* 29) is a signature fragment in the case of aldehydes and often appears as the base peak of the spectrum.

A McLafferty rearrangement can arise in the case of long chain aldehydes, as illustrated for the butyraldehyde ion in equation 5.35. This results in the cleavage of the β-bond through a cyclic transition state and the loss of an alkene. The mass of the latter provides an indication of the degree of branching in the molecule.

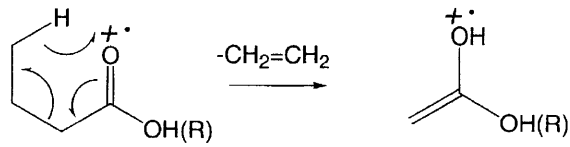


(5.35)

The aromatic ketone benzophenone is dominated by a strong molecular ion (*m/z* 182). The base peak of the spectrum at *m/z* 105 arises from the benzoyl ion C_6H_5–C=O$^+$ with a corresponding benzyl ion detected at *m/z* 77. The latter is generated as a secondary fragment of the benzoyl cation in the case of alkylphenones such as acetophenone.

5.3.6 Carboxylic Acids, Esters and Amides

The spectra of aliphatic carboxylic acids and esters, like aldehydes and ketones, are dominated by ions associated with a McLafferty rearrangement and the loss of an alkene.



(5.36)

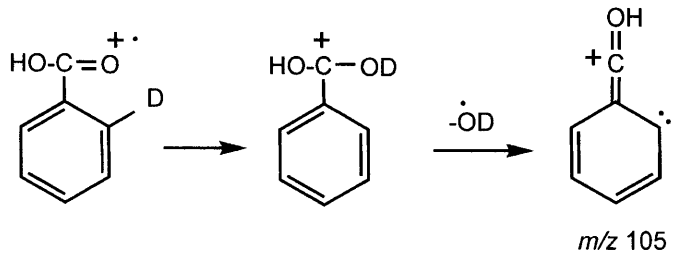
Both the resonance-stabilised O=C$^+$–OH ⇔ $^+$O≡C–OH (*m/z* 45) and O=C$^+$–OR ions predominate in the EI spectra of acids and esters respectively. Cleavage β to the carbonyl group gives rise to the resonance-stabilised ions $^+CH_2$–C(=O)–OH and $^+CH_2$–C(=O)–OR.

The loss of water from a carboxylic acid usually requires an aliphatic chain of at least four carbon atoms long with hydrogen atom transfer from the γ-position accompanied by the cleavage of C–H and C–O bonds.

The loss of a hydroxyl or alkoxide radical from a carboxylic acid or ester is favoured if the resulting product ion is stable. As an example, the EI mass spectrum of benzoic acid is dominated by fragments associated with the loss of the hydroxy radical and the subsequent loss of CO (equation 5.37).

$$[C_6H_5\text{–}C(=O)\text{–}OH]^{+\bullet} \rightarrow C_6H_5\text{–}C{\equiv}O^+ \rightarrow C_6H_5^+ \qquad (5.37)$$

Deuterium-labelling studies, however, have shown that the loss of HO$^{\bullet}$ to form the ion at *m/z* 105 does not solely involve the hydrogen atom of the carboxylic acid group. The EI spectrum of *ortho*-d-benzoic acid, for example, exhibits ions at both *m/z* 105 and 106 due to what is known as the *ortho-effect*. The former product arises from transfer of a proximal hydrogen atom of the ring to the carboxylic acid group prior to hydroxyl radical loss (equation 5.38).



m/z 105

(5.38)

As one would expect, the EI mass spectra of amides resemble that of their corresponding acid and ester. An additional process, in the case of secondary and tertiary amides, results from the cleavage of the N–C bond and the transfer of one or two hydrogen atoms to produce a neutral loss (R–C(=O)–NH_2) and the ion R–C(=O)–NH_3^+.

5.3.7 Halides

The halogen atoms F, Cl, Br and I have a relatively small effect on the fragmentation processes of organic compounds. Molecular ions are detected in the EI mass spectra of halides though the proportion of charge residing at the halogen atom will vary (I>Br>Cl>F) counter to the electronegativity of the atoms. As a result, α-bond cleavage (equation 5.39) occurs preferentially adjacent to I.

$$R\text{–}CH_2\text{–}X^{+\bullet} \rightarrow R^{\bullet} + CH_2{=}X^{+} \qquad (5.39)$$

The intensity of the X^+ ion also is observed to increase as the electronegativity of the atom decreases. Hence the I^+ ion appears in greater abundance than the F^+ ion. This is consistent with the formation of R^+ from the fragmentation of R–X where the loss of X$^{\bullet}$ is observed to a greater extent in iodides and bromides, over chlorides and fluorides. This process is evident in the mass spectra of alkylhalides but not aromatic halides due to the stability of the C-X bond when the halogen is attached directly to the ring.

The abundant isotopes of some halogen atoms provide a unique signature that aids in the determination of an unknown compound. All fragments containing one chlorine atom for instance should appear as two ions differing by two mass units in an approximate ratio of 3:1 due to the natural abundance of ^{35}Cl and ^{37}Cl. The corresponding ions containing a bromine atom will appear two mass units apart in a ratio of 1:1 due to the natural abundance of ^{79}Br and ^{81}Br (see Appendix 2).

The loss of hydrogen halide is a common fragmentation pathway in the case of chlorides and fluorides (equation 5.40).

$$R{-}CH_2{-}CH_2{-}X^{+\bullet} \rightarrow [R{-}CH{=}CH_2]^{+\bullet} + HX \qquad (5.40)$$

5.4 QUANTITATIVE ANALYSIS OF ORGANIC COMPOUNDS

Having covered many of the common fragmentation processes observed for organic molecules with various functional groups, we turn our attention to the quantitative analysis of such compounds. Since organic compounds are used widely in prescription drugs and for agricultural, food and industrial purposes, the quantitation of such compounds in living systems, extracts and the environment is of particular importance. While the identification of an organic compound's structure provides valuable information, the absolute or relative levels of that compound in the sample may also be critical. For example, the identification of a performance-enhancing compound in the blood or urine of an athlete prior to competition may alone be sufficient to ban the athlete from competing. However, where the compound (such as a hormone) occurs naturally in the body it may be necessary to establish that a higher than usual dose has been administered. Mass spectrometry plays a major role in the quantitation of organic compounds in this, and many other, applications.

5.4.1 Role and Choice of Quantitation Standards

Quantitative analysis of any compound by mass spectrometry first requires that the detector response be calibrated as a function of the concentration of a compound at a particular set of operating conditions (*e.g.* ionisation conditions, ion source settings, tuning parameters, *etc.*). This allows the ion current detected by the mass spectrometer to be reliably correlated with the amount of compound in a particular sample. Note that this may involve a reasonably large number of measurements, since the detector response does not necessarily vary in a linear manner as the sample concentration changes across several orders of magnitude.

To perform the calibration optimally, the quantitation standards should have the same structural characteristics and be of a similar (though not the same) size to the compounds of interest. This ensures that the ionisation and detection efficiencies of the quantitation standards and the compounds under investigation are essentially identical. Ideally, the standards should be added to the sample; that is, they should be *internal quantitation standards*. This prevents any fluctuation in the performance of the instrument during the analysis of the standards, and subsequently the sample of interest, from adversely influencing the quantitation measurement. It is important that the internal standards be added to the sample at the earliest possible stage, so that they are subjected to the same potential losses prior to and during the analysis.

5.4.2 Calibration of the Detector Response

This stage of the analysis involves measuring the ion current of a particular standard or set of standard compounds as a function of their concentration. Depending on the concentration variations predicted for the samples that are to be analysed, the concentration of the standards might be varied over one or several orders of magnitude. Where the measurements are performed on a mass spectrometer featuring a scanning mass analyser (magnetic or quadrupole-based, including ion traps), the instrument is operated in the *selected ion monitoring* (SIM) mode. In this mode, the mass analyser is scanned over very small *m/z* ranges (about the ion signals of the standard(s)) to detect the majority of (if not all) the ions present. If a larger *m/z* range were scanned, the ions produced from the standard compound(s) would be passed to the detector during only part of the scan; the time the ions are detected being dependent on the scan rate of the instrument. In other words, during most of the scan the mass analyser would attempt to transmit ions of different *m/z* ratios than those of the standard(s) to the detector. In the SIM mode, many scans and thus mass spectra are obtained within a particular time interval. Where ions from several quantitation standards are to be detected, the mass analyser is scanned segmentally over several small *m/z* ranges about each ion.

Once the detector responses are measured, the area under the ion signals can be plotted as a function of the concentration of the standard(s). Any deviation in the areas obtained in subsequent runs from a line-of-best-fit establishes the error of the analysis. It is desirable to use the area under, rather than the height of, an ion signal for quantitation because the latter is highly influenced by the mass resolution of the measurement. The height of an ion peak corresponding to a

particular set of isotopes is less than that where the isotopes remain unresolved.

It is typical in selecting the quantitation standards to choose compounds that have a range of molecular weights so as to produce ions across the *m/z* range of the instrument. This is because ion detectors do not detect all ions across the full *m/z* range of a particular mass analyser with equal efficiency. Low *m/z* ions are generally detected with much greater efficiency than high *m/z* ions, irrespective of whether they are produced in equal quantities in the ion source.

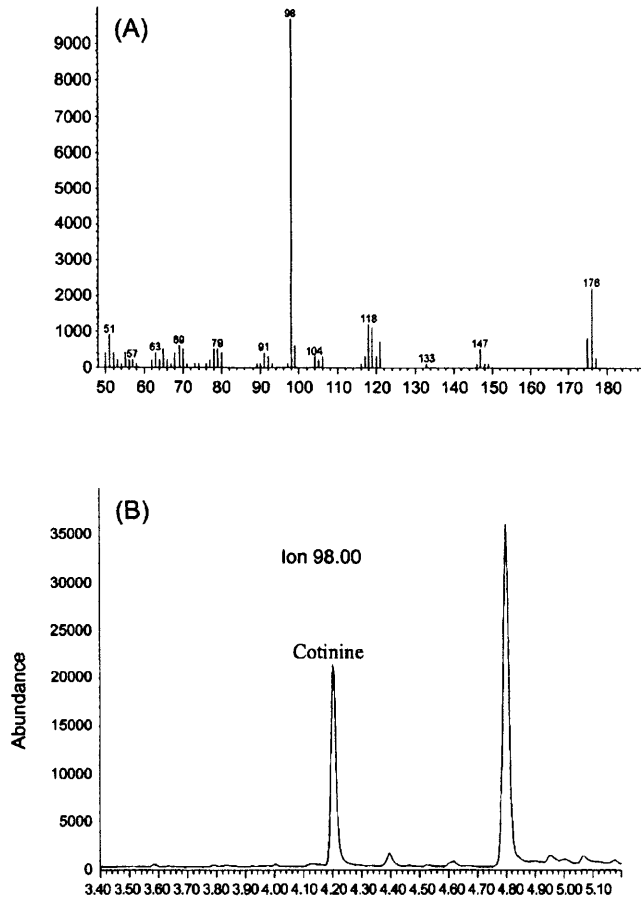


Figure 5.5 *EI mass spectrum of cotinine (Figure 5.5A) and selected ion chromatogram of fragment ion m/z 98 (Figure 5.5B) in the saliva of an active smoker*
(Source: J.-G. Kim, U.-S. Shin and H.-S. Shin, Rapid Monitoring Method of Active and Passive Smoker with Saliva Cotinine by Gas Chromatography-Mass Spectrometry, *Bull. Korean Chem. Soc.*, 2002, **23(10)**, p. 1497)

5.4.3 Quantitative Analysis of Cotinine; Example of Selected Ion Monitoring

Cotinine is a metabolite of nicotine that has been detected in both smokers and non-smokers by selected ion monitoring to assess the risk of passive exposure to cigarette smoke. The levels of cotinine were monitored in the blood, urine and saliva of both smokers and non-smokers. The EI mass spectrum of cotinine exhibits a fragment ion *m/z* 98 (the base peak) and a molecular ion at *m/z* 176 (Figure 5.5A). Selected ion monitoring of the base peak at *m/z* 98 thus affords optimal sensitivities for these experiments. A typical SIM ion chromatogram for the ion at *m/z* 98 is shown in Figure 5.5B at a cotinine concentration of 128 ng ml^{-1}.

Cotinine levels were measured based upon the ratio of the fragment ion peak area of cotinine at *m/z* 98 relative to that of the internal standard d_3-deuterocotinine (*m/z* 101) by interpolation from the regression line of the standard curve. Detection limits of 5–50 ng ml^{-1} were achieved among the biological matrices. The precision of the quantitation measurements was reported to be between 83.9–99.8%.

FURTHER READING

H. Budzikiewicz, C. Djerassi and D.W. Williams, *Mass Spectrometry of Organic Compounds*, John Wiley & Sons, New York, 1967.

Q.N. Porter, *Mass Spectrometry of Heterocyclic Compounds*, John Wiley & Sons, New York, 1985.

F.W. McLafferty and F. Turecek, *Interpretation of Mass Spectra*, University Science Books, 1993.