

4 Mass Spectra

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1. Introduction

Although the method of mass spectrometry is relatively old (in 1910, J.J. Thomson was able to separate the ^{20}Ne and ^{22}Ne isotopes), it did not achieve recognition as an important analytical method in organic chemistry until 1960. Two features have helped to bring it to prominence. Firstly, it is possible to determine the relative molecular mass and even the elemental composition of a compound using only the smallest amount of substance. Furthermore, the fragmentation pattern (i.e. the decomposition of the material being analysed under the influence of electron bombardment or other techniques for ion formation) depicted in the mass spectrum allows one to make important deductions about the structure of the compound. In recent years, both of these aspects have been crucial to the development of the application of mass spectrometry in organic chemistry¹.

There are limits to the mass spectrometric determination of the relative molecular mass of a sample. The polarity of a substance is inversely proportional to its volatility. Also, the larger the relative molecular mass, the greater, in general, the number of functional groups and therewith the danger of thermal decomposition upon vaporisation. Therefore, various procedures were developed (e.g. chemical ionisation, field ionisation, field desorption, secondary ion mass spectrometry, fast atom bombardment, electrospray methods), which, compared with electron impact ionisation, allow the relative molecular masses of involatile compounds to be determined in many more cases. In more recent times, efforts have been made to further improve known procedures or to investigate new

promising possibilities (see Sec. 8.9, ionisation methods, p. 263). In routine operation, relative molecular masses up to ca. 1200 can be determined using mass spectrometry (see, however, Sec. 8.8, p. 261).

Another aspect that has also received considerable attention is the utilisation of the generally enormous amount of information contained in mass spectra. These efforts have led to significant advances in instrumentation. Today, fast and dependable spectrometers are available which permit the determination of the empirical formulae of fragment ions and additional equipment has been developed to measure metastable ions or record collision activation spectra. The results from all of these methods of measurement advance our knowledge about mass spectra and, furthermore, ease the deduction of the structures of the compounds being examined. Also, the results from measurements made using isotopically labelled derivatives have contributed strongly to the successful interpretation of spectra. The result is that today we know considerably more about the behaviour of substances in mass spectrometers. Nevertheless, the number of generally applicable rules in proportion to the number of exceptions and special cases has unfortunately become rather small. One can only hope that this proportion will change in the future.

In the following sections, an introduction will be given to the most important aspects of mass spectrometry. Except in those cases where it is specifically stated, the discussion will refer to the method most commonly employed today: electron impact mass spectrometry (abbr. EI).

2. Instrumentation and the Recording of Spectra

First of all, the principle of mass spectrometric separation will be discussed briefly. When accelerated, positively charged particles are in the gas phase, a homogeneous magnetic field will separate them by an amount proportional to their mass. The experimental means of achieving this is complicated and requires further explanation, which will only be given to the extent that is necessary for an adequate understanding of the method by an organic chemist.

2.1 The Principle of the Mass Spectrometer

As indicated in the schematic diagram in Fig. 4.1, the functions of a mass spectrometer may be divided into four sections: sample injection, ion generation, mass separation and ion detection. The ion generation and the processes in the magnetic analyser (mass separation and ion detection) occur under high vacuum in order to minimise undesired collisions between ions and molecules or atoms. In ordinary mass spectrometers, the following pressures are obtained: in the ion generator: 10^{-3} to 10^{-4} Pa; in the magnetic analyser: 10^{-6} to 10^{-7} Pa. A great deal of instrumentation is necessary for the

generation and control of the high vacuum, but this will not be discussed in any detail here.

Sample Injection

It follows from the above explanation that there is a problem in how one gets a sample of a substance at normal pressure into the high vacuum without breaking the vacuum. Principally, there are two types of injection systems. These are the gas inlet and the direct inlet systems.

Gas inlet. Application: for liquid or gaseous samples. A liquid can either be injected with a microsyringe through a septum directly into a previously evacuated reservoir or frozen in a glass vessel (e.g. with liquid nitrogen). The air above the frozen sample can be pumped away and the sample can then be vaporised into the reservoir. In order to minimise the inclusion of gases in the frozen material, it is advisable to thaw and re-freeze the sample at least once while it is under vacuum. The reservoir is fitted with various valves (e.g. to the vacuum pumps, inlet port and ion source), the internal surface is as inert as possible (e.g. glass or enamel) and it can be heated

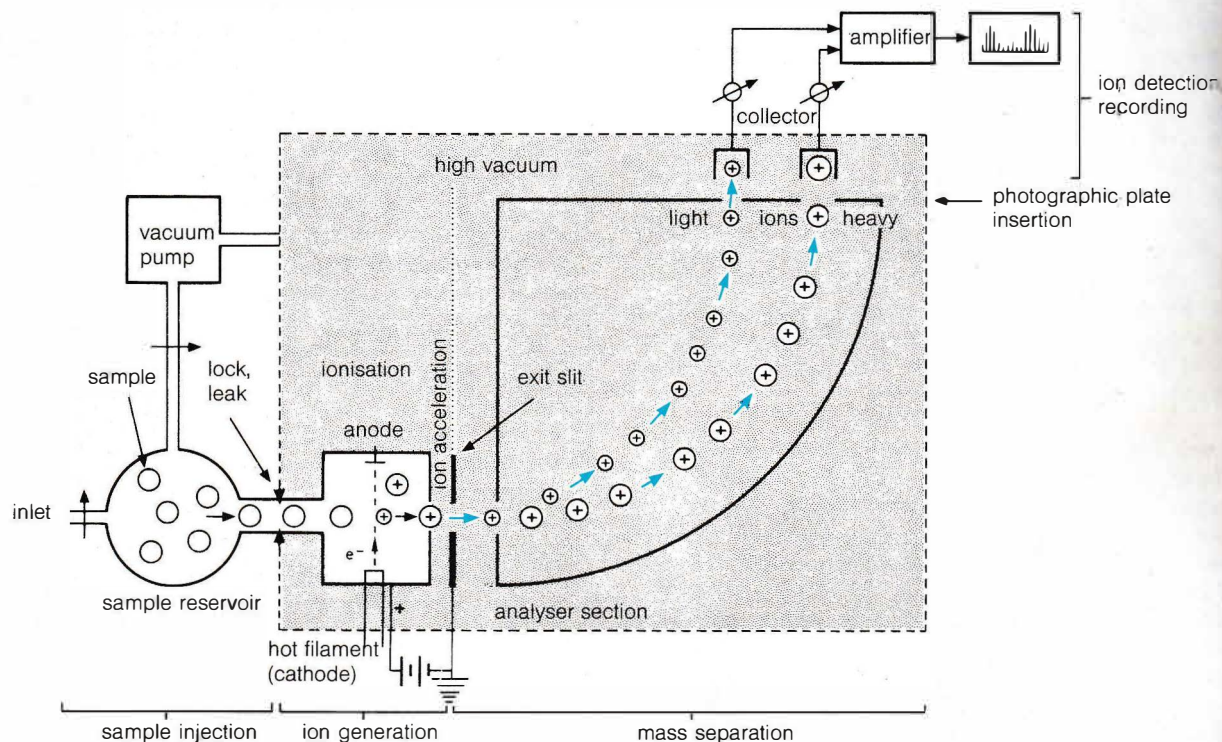


Fig. 4.1 Schematic representation of a mass spectrometer

(max. temperature during continuous service is usually 150°C). The reservoir is connected to the ion source *via* a leak (a hole of a specific size, e.g. a perforated gold foil melted into a glass tube [gold leak]). Gaseous samples can be introduced into the reservoir through a container fitted with a break-seal.

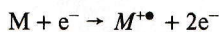
Volatile substances can also be injected directly into the mass spectrometer *via* a gas chromatograph (GC) or a liquid chromatograph (LC, HPLC) (see Sec. 8.11, p. 266).

Direct inlet. Application: crystalline, lacquer-like or viscous liquid samples. The sample is placed in a metal crucible (e.g. gold or aluminium; internal diameter 1 mm), which is fixed on the tip of a heatable probe and the tip of the probe is inserted into a lock-chamber. After evacuating the chamber, the cooled tip of the probe is brought into the ion source and slowly heated until the sample vaporises. In addition, the end of the probe that is in the ion source, which is at a high voltage, must be electrically isolated from the handle. The tip of the probe can also be cooled so as to enable the measurement of easily vaporised samples, to hinder the vaporisation of the sample by the (hot) ion source, or to quickly cool an overheated sample.

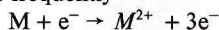
Sample requirements. *Via* gas inlet: 0.1 to 1 mg; *via* GC: in the region of 10^{-9} to 10^{-15} g; *via* direct inlet: 0.001 to 0.1 mg for normal measurements. The full amount of the given sample quantities must immediately be available to the instrument and should not, for example, be smeared out as a film on the surface of a large flask!

Ion Generation

From one of the inlet systems (gas or direct inlet), a fine and as constant as possible beam of molecules streams into the ion source where it intersects perpendicularly with an electron beam [between a hot filament (cathode) and an anode]. The potential difference between the cathode and the anode can be varied between 0 and, in general, 300 V, which means that the electrons can carry between 0 and 300 eV. For low-voltage spectra, 12 to 15 eV is used and for normal spectra, 60 to 100 eV can be used, although 70 eV is normally employed. The interaction between the electrons and the neutral molecules generates positively charged molecular ions (see pp. 223, 226) according to:



or less frequently



Other ionisation procedures are discussed in Sec. 8.9 (see p. 263).

The non-ionised particles are removed from the ion source chamber by the high vacuum pumps, whereas the molecular ions that have been generated are now accelerated and focused. The acceleration of the ions is achieved by applying a potential to the source (the acceleration potential varies with

the type of instrument from 2 to 10 kV) and the final speed is reached at the exit slit (0 V). The focusing of the ions is achieved by an additional electric field. The exit slit permits only the narrow, central, and therefore homogeneous region of the ion beam to pass into the magnetic analyser. The speed of the ions can be calculated as follows:

$$z \cdot U = \frac{m \cdot v^2}{2} \quad (1)$$

$$v = \sqrt{\frac{2 \cdot z \cdot U}{m}} \quad (2)$$

z ionic charge ($= n \cdot e$)

m ion mass

v ion speed

U acceleration potential

Mass Separation

The separation of the ions takes place in the magnetic analyser and is proportional to their mass. The separation occurs in a magnetic field (of the order of magnitude of 1 T), in which, with ions of the same charge, the paths of the lighter ions will be more strongly deflected than those of the heavier particles. In other words, the various ions move along circular trajectories with mass dependent radii. (With double-focusing mass spectrometers, an additional electrostatic analyser, which serves to focus the energy of the ions, is placed between the ion source and the magnetic analyser.) The radius of curvature can be expressed as:

$$r_m = \frac{m \cdot v}{z \cdot B} \quad (3)$$

B magnetic field strength

Equations (1) and (3) can be combined to give the fundamental equation of mass spectrometry (4):

$$\frac{m}{z} = \frac{r_m^2 \cdot B^2}{2 \cdot U} \quad (4)$$

The mass/charge ratio is therefore dependent upon the magnetic field strength, the radius of curvature and the acceleration potential. This equation has allowed the development of specific instrumentation for the direct detection of ions.

Ion Detection

If the acceleration potential and the magnetic field strength are kept constant, Eqn. (4) reduces to Eqn.(5):

$$\frac{m}{z} = k \cdot r_m^2. \quad (k = \text{const.}) \quad (5)$$

This means that the m/z ratio is directly proportional to the square of the radius of curvature. As a result of this behaviour, it is possible to use a large number of individual collectors for the ion detection, or a photographic plate on which the number of impinging particles will be indicated by a corresponding variation in the degree of darkening of the plate. The distance between the individual dark streaks is then related to the masses of the recorded particles.

If the acceleration potential and the radius of curvature in Eqn. (4) are kept constant, Eqn. (6) is obtained:

$$\frac{m}{z} = k \cdot B^2 \quad (k = \text{const.}) \quad (6)$$

Thus, for the determination of m/z (earlier m/e) ratios using a fixed radius of curvature it is only necessary to vary (scan) the magnetic field strength. In this case, only one ion detector is required at the exit to the magnetic analyser and an electron multiplier (EM) is used to amplify the very weak ion current.

A mirror galvanometer is used for the actual ion detection. This device directs a beam of UV light onto UV sensitive paper and as the paper moves forward, a spectrum is produced. Usually three traces are recorded simultaneously, which depict the same spectrum at different sensitivity ratios (usually 1:10:100). Additional traces are often also recorded, such as the ticked line of the mass marker. (Mass markers generally function very accurately, however it is necessary to calibrate them against a known mass from time to time. Normally a tick-mark is made at every fifth mass unit.) Another trace which can be drawn records the total ion current. This can be used as a record of the sample pressure developed during the measurement.

Today, the electrical signals are generally recorded during the measurement by a computer, which is directly coupled to the instrument. The data can subsequently be evaluated and printed as desired. Frequently the data are printed as a list of masses which contains both the mass number and the relative abundance of each mass. Furthermore, the computer can also display the data graphically as spectra, similar to those shown throughout this chapter. In contrast to the recording of spectra directly onto photosensitive paper, weak signals (<1%) are not registered under normal conditions. If such signals are to be made visible – e.g. for the recognition of molecular ions with a low relative abundance – additional manipulation of the spectrum must be performed by the operator (e.g. a signal of low abundance, rather than the most abundant one, can be chosen as the base peak).

3. Fragmentation of Organic Compounds

The following discussion contains **generalised remarks** about the behaviour of organic compounds under the influence of electron bombardment (70 eV). For the behaviour of inorganic or organometallic compounds, see the bibliography. With regard to other ionisation methods, which sometimes substantially reduce the fragmentation, see Sec. 8.9 (p. 263).

For reproduction in the literature, the recorded spectrum is drawn so that the most abundant peak of the spectrum (base peak) is set to 100% (relative %) and all other signals are scaled accordingly. If the most abundant signal appears at $m/z = 28$, or a similar mass number, it is advantageous to examine the sample of the compound for the presence of foreign substances (air, solvent). A convenient scale for displaying spectra has been found to be 1 rel. % = 1 mass number = 1 mm. Occasionally, one also finds the percentage fraction of the total ion current (% Σ) marked on the right hand side of the spectrum

(see Fig. 4.2, p. 223). For this, the abundances of all signals above a specific mass (e.g. $m/z=20$) can be added together and the sum (e.g. 335) set to 100%. If, for reasons of low abundance, an important signal (e.g. $M^{+\bullet}$) still does not appear in the displayed spectrum when either of these scales are used, the affected region can be drawn using an expanded scale. This section would then be indicated with the label $\times 0.1$ or $\times 0.01$ (equivalent to $\times 10$ or $\times 100$), see Fig. 4.2. Another possibility for the display of signals of low abundance is to use a logarithmic rendering of the abundance of the total spectrum, instead of rel. %. For various reasons (e.g. the over-weighting of weak signals), this latter method is used only rarely.

The determination of the mass numbers in a spectrum, i.e. the association of each signal with a mass, is achieved either from the output of an automatic mass marker (as in a spectrum recorded and stored by mass on a computer), or by the count

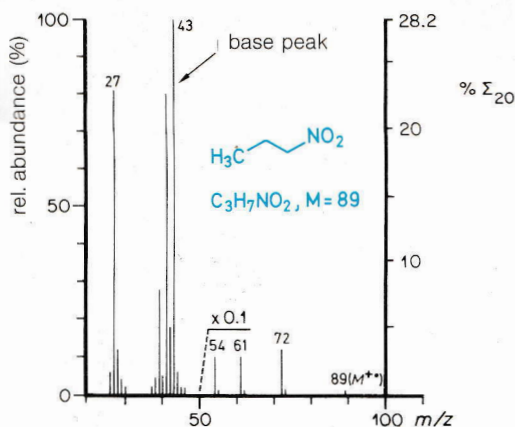


Fig. 4.2 The schematic representation of a spectrum, explained using the spectrum of 1-nitropropane as an example

ing out of easily identified and constantly appearing masses in a computed spectrum (stretched spectrum) [e.g. 12(C[•]), 18(H₂O[•]), 28(N₂[•]), 32(O₂[•]), 40(Ar[•])].

Molecular ion. Aside from a few exceptions (see Sec. 5, p. 246), the signal with the highest mass corresponds to the molecular ion peak.

Exception: so-called [M + 1]⁺ or [M + H]⁺ signals, which result from the tendency of H⁺ ions to accumulate on molecules (especially prevalent with amines and alcohols). Furthermore, the M[•] signal is sometimes not observed, but that from the [M - R]⁺ ion is seen instead. This occurs when the compound decomposes very easily.

Organic compounds generally consist of carbon, hydrogen, oxygen and nitrogen atoms and sometimes they also contain sulfur, phosphorus or halogen atoms. As shown in Tab. 4.13 (see p. 304), most of these elements are not monoisotopic, but are composed of a mixture of naturally occurring isotopes. Because most organic compounds have a natural origin, the ratio of this mixture of isotopes is also reflected in their mass spectra. Three categories can be defined for the most important elements, i.e. those most commonly occurring in organic compounds:

- **Monoisotopic elements:** ¹⁹F, ³¹P, ¹²⁷I;
- **Elements with one very abundant isotope:** (> 98%): H(¹H), C(¹²C), N(¹⁴N), O(¹⁶O);
- **Elements with two abundant isotopes:** S(³²S, ³⁴S), Cl(³⁵Cl, ³⁷Cl), Br(⁷⁹Br, ⁸¹Br).

Depending upon which of these elements are present in a substance, the molecular ion peak is accompanied by one or more

isotope peaks, which are always found at higher masses. Thus, the molecular ion of C₇H₆ClNO (M = 155) is composed as follows (Fig. 4.3):

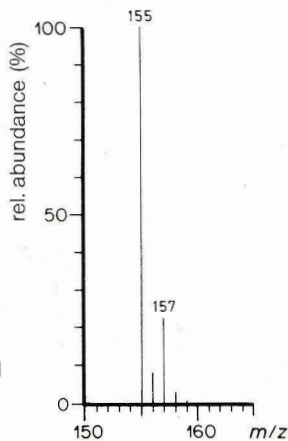
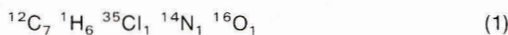
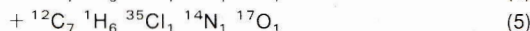
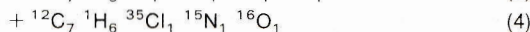
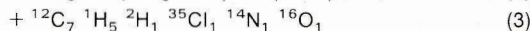
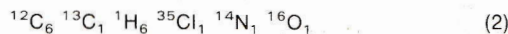


Fig. 4.3 Molecular region of the mass spectrum of C₇H₆ClNO

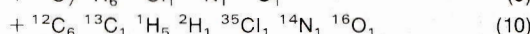
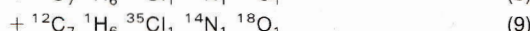
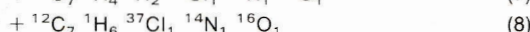
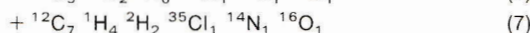
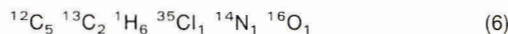
$m/z = 155$:



$m/z = 156$:

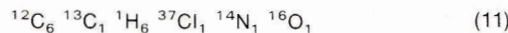


$m/z = 157$:



⋮

$m/z = 158$:



⋮

The isotope with the highest possible mass can be expected at $m/z = 173$ (¹³C₇ ²H₆ ³⁷Cl ¹⁵N ¹⁸O). As can be seen by estimation or calculation from the natural abundances of the individual isotopes (see Tab. 4.13, p. 304), the contribution given to the total abundance of an isotope peak by each of the various combinations can be very different. Whereas (1), (2), (8) and (11) represent the main contribution to the corresponding peaks, certain other combinations can be ignored because they have very low abundance; this is especially so in the case of $m/z = 173$.

It is a characteristic of compounds that possess elements with two common isotopes (e.g. Br and Cl), that the type and number of atoms of these elements can be deduced from the intensity ratios of the isotope peaks (see Tab. 4.13, p. 304 and 4.10, p. 294).

The mass number of the molecular ion in compounds of the type $C_xH_yN_wO_x(\text{halogen})_zS_z$ also permits certain information to be derived about the number of N -atoms that are present. When the mass of the molecular ion is an even number, the presence of an even number of N -atoms (N_0, N_2, N_4, \dots) is indicated. Conversely, an odd number for the mass of the molecular ion points to N_1, N_3, N_5, \dots (nitrogen rule).

Furthermore, the molecular ion represents that ion in a spectrum which possesses the smallest appearance potential (AP). In order to remove an electron from a neutral atom or molecule, a minimum amount of energy, the ionisation potential (IP), is required. For organic molecules, this energy lies between 7 and 14 eV ($1 \text{ eV} = 23.04 \text{ kcal} \cdot \text{mol}^{-1} = 96.3 \text{ kJ} \cdot \text{mol}^{-1}$). Some examples:

<i>n</i> -hexane	10.17 eV	ethanol	10.48 eV
cyclohexane	9.88 eV	acetaldehyde	10.21 eV
cyclohexene	8.95 eV	acetic acid	10.35 eV
benzene	9.25 eV	methylamine	8.97 eV
anthracene	7.23 eV	aniline	7.70 eV
		trifluoromethane	13.84 eV

Therefore, when just the ionisation energy is available, only the molecular ion can appear as a signal in the mass spectrum. For the creation of fragment ions, an additional dissociation energy must be provided, so that the appearance potential of the fragment ions lies above that of the molecular ion².

Clear information about the elemental composition of a molecular ion can be obtained by the determination of its exact mass. This can be achieved by the use of high resolution mass spectrometry. The resolving power, A , of a mass spectrometer is defined by

$$A = \frac{m}{\Delta m} \quad (7)$$

According to the 10%-valley definition, two neighbouring signals can be considered to be resolved when they do not overlap each other by more than 10%. (The alteration of the positions of both maxima caused by the 10% overlap will still be within a tolerable limit.) As an example, two signals of equal intensity are shown in Fig. 4.4. In order to separate, for example, $m/z = 950$ from 951, a resolving power of 950 is required: $A = 950/1 = 950$. Low resolution mass spectrometers have a resolving power of between 1000 and 2000. In contrast, the determination of the exact masses of ions requires a much larger resolving power, as can easily be seen in the following example.

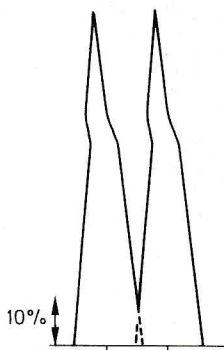


Fig. 4.4 Schematic representation of two neighbouring peaks of equal intensity with a 10% overlap (10%-valley definition)

The elemental compositions given in formulae (2)–(5) (p. 223) correspond, as which can easily be calculated with the help of Tab. 4.13, with the masses

156.017147	(2)
156.020069	(3)
156.010827	(4)
156.018008	(5).

For the separation of these masses, the following resolving powers are required:

$$A_{(2)/(3)} = \frac{156}{0.002922} = 53\,388$$

$$A_{(2)/(4)} = \frac{156}{0.006320} = 24\,684$$

$$A_{(2)/(5)} = \frac{156}{0.000863} = 180\,756$$

⋮

From this it can be deduced that in order to record all four signals, a resolving power of ca. 181 000 is needed. The resolving power of a mass spectrometer fitted with a magnetic analyser is particularly limited when the ions are generated by electron impact, because the translational energy of the ions (caused, for example, by the effect of charge) is too inhomogeneous. The insertion of an electrostatic analyser before the magnetic analyser converts a simple mass spectrometer into a double-focusing instrument (cf. Fig. 4.5). The electrostatic analyser focuses the speed and energy of the ions. High resolution mass spectra can only be produced with such equipment.

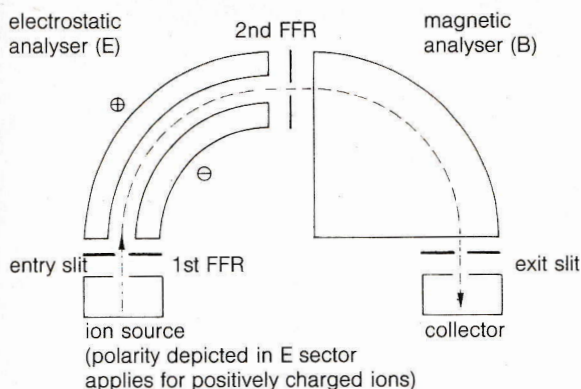


Fig. 4.5 Schematic representation of a double-focusing mass spectrometer with the EB-configuration (Nier-Johnson geometry).

FFR = field free region

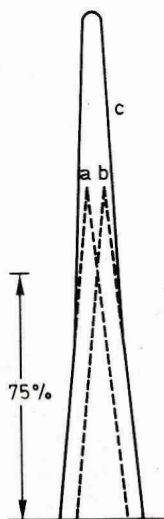


Fig. 4.6 The overlap of two peaks, a and b, of equal intensity when the resolving power is insufficient. The resultant peak, c, will be recorded

Today, commercially available double-focusing instruments guarantee a resolving power of up to 150 000. However, because instruments in routine service can only quickly reach half of this value (due to light contamination of the ion source by the samples), only three signals in our example will be recorded: (3), (4) and (2) + (5). The peaks for (2) and (5) overlap and, depending on their intensity, each influences the recorded mass of the other. If (2) and (5) have equal intensities, the mean of both masses will be recorded (Fig. 4.6). On the other hand, if the intensity of (2) \gg (5), the mass of (2) appears correctly, because (5) can be ignored. Such cases are to be reckoned with constantly and they can lead to the misinterpretation of spectra. Frequently, signals with different intensities overlap each other and the resolving power of the instru-

ment is just sufficient, so that the form of the peak displayed on the oscilloscope can be recognised visually as being either a singlet (ideal peak shape) or the result of two or more superimposed signals. In the latter case, the peaks can usually be separated by increasing the resolving power.

The elemental composition can be calculated from the exactly determined mass number either manually or, more realistically, with the time-saving help of a computer. If, for such calculations, no restrictions were made and it was assumed that any of the elements in the entire periodic table could be present in the unknown compound, then the number of possible combinations is very large. However, based on a knowledge of the origin of the substance and the chemical reactions used to synthesise it, it is nearly always possible to reduce the expected number of elements present in a compound to just a few, so that, in the ideal case, only the composition involving one set of elements must be determined. The same considerations are valid for the determination of the masses of fragment ions, whereby new selection rules, which can deliver additional details about the molecular ion, also come into play (e.g. fragment ions cannot contain any elements other than those in the molecular ion; the number of individual atoms in the fragment ion cannot exceed the number in the molecular ion; for typical fragment ions, it must be possible to find the corresponding cleavage sites, e.g. for $[M - 15]^+$, the fragment $[M - \text{CH}_3]^+$ must be used in the calculation and so a methyl group must be present in the molecular ion). In general, however, it is a valid concept that the number of combinations increases as the mass number rises.

Three different procedures are available for the production of high resolution mass spectra. For all of the following procedures, it is absolutely essential that the electric and magnetic fields of the instrument, as well as those from the surroundings, remain constant. Magnetic fields generated by nearby electric railways or tramways can have particularly disturbing effects.

Exposure of photographic plates. In a mass spectrometer tuned for high resolution (narrowed exit slit, see Sec. 2, p. 220), the sample being examined and a reference sample, usually perfluorokerosene (PFK), are vaporised simultaneously while the magnetic field and acceleration potential are held constant. The spectrum is recorded by making multiple exposures of a photographic plate. The high resolution mass numbers can be obtained by measuring the distances and intensities of the individual signals (the intensities are determined by comparing the darkness of the various streaks) with the aid of the reference spectrum. The advantage of the method is that all of the peaks are recorded simultaneously, which is important for thermal reactions, or when only a small amount of substance is available (e.g. for the investigation of metabolites and biological materials). The disadvantage is that the method requires additional instrumentation to conduct the measurement of the intensities and the distances.

Electrical recording (magnetic field scan). In this case the mass spectrum is not recorded on photographic paper (see Sec. 2), but three data channels (ion current, total ion current and variation in the magnetic field strength) are processed as a function of time by an interface and stored and displayed on a computer. The spectra of the sample and the reference substance (PFK) are recorded simultaneously and a "superimposed spectrum" is produced. The spectrum of PFK is recognised by the computer, because it is stored therein, and before it is eliminated, it is used as an aid for the subsequent calculations. A special computer program associates each signal with an exact mass (within a certain margin of error or uncertainty). (This calculation is based on the ratio of the distance between neighbouring PFK signals to the distance between a PFK signal and that of the substance whose mass is to be determined – both values are measured – as well as on the exact mass of the PFK signal, whose value is known.) In this way, the elemental composition of the corresponding ions can be determined and printed out as a list. It is advisable to record several spectra, one after the other, and compare the results. In this way it is possible to eliminate false spectra (e.g. due to the absence of any substance, the presence of only PFK, impurities, spikes, electronic noise or peak deformation).

The advantage of the method lies in its ability to rapidly record and evaluate high resolution spectra. The time required for one scan (ca. 20 s for measurements up to $m/z = 450$) can be a disadvantage when there is only the smallest amount of sample available or when the substance is thermally labile. This type of measurement also has a relatively low resolving power (ca. 10 000; multiplets). The computer printout of a high resolution spectrum is listed and explained on p. 325 (Chapter 5, 16, spectrum 19). One possibility for overcoming these disadvantages is to record Fourier-transform spectra.

Peak matching method. First of all, by varying the magnetic field, a signal from a reference substance with a known exact mass (m_1) is displayed on a cathode ray oscilloscope in such a way that the half-height width of the peak fills about one third of the oscilloscope screen and the peak is scanned repeatedly at fixed time intervals. By the application of a supplementary potential (by the alteration of the acceleration potential), a signal of known nominal mass, but unknown exact mass (m_2),

can also be projected onto the oscilloscope screen in a similar way. Both of the signals are displayed alternately. The supplementary potential is adjusted so that both signals appear at exactly the same point on the screen. This supplementary potential can be determined exactly and the exact mass can then be calculated from ($B = \text{const.}$):

$$m_1 : m_2 = U_2 : U_1 \quad (8)$$

$$m_2 = \frac{m_1 \cdot U_1}{U_2} \quad (9)$$

Because $U_1 = 1$ and m_1 are known, the unknown mass can be determined by division (uncertainty ± 3 ppm). There is a restriction that, depending on the type of instrument, the differences between the masses m_1 and m_2 must not exceed 10 to 20% of the mass of m_1 .

The advantage of the method is that the ion signals of the sample in question are visible to the operator, which means that multiplets can be recognised visually, even when the constituent peaks have significantly different intensities. The limits of uncertainty can also be checked and ions of predicted elemental composition can be verified by the suitable application of Eqn. (9). The mass numbers obtained in this way are very exact. Two disadvantages are evident: a longer time, and thus a greater amount of substance, is required for a measurement, which consequently leads to the ion source becoming contaminated more quickly.

General reference for high resolution mass spectrometry: ³.

The molecular ion that has been excited by electron bombardment can now undergo fragmentation reactions (i.e. decomposition reactions). In Sec. 4 it will be assumed that the charge is localised, at least at the moment of the onset of the fragmentation reaction. Preferred sites for localisation are primarily heteroatoms with lone pairs of electrons, although the sites of π -bonds and π -bonded systems are also favoured. The least preferred sites are α -bonds. As will easily be seen from the examples that are given, this concept is well suited to the interpretation of the spectra of organic compounds. There are also other theories applicable to fragmentation reactions, however, these will not be discussed here.

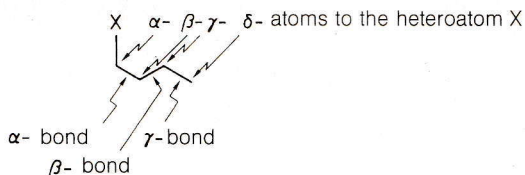
4. The Main Fragmentation Reactions of Organic Compounds

In this section, the most important, i.e. the most frequently observed, fragmentation reactions of organic compounds will be presented and discussed with the aid of examples.

4.1 α -Cleavage

Analogous reactions from other areas of chemistry (photochemistry): Norrish Type I reaction (α -cleavage).

α -Bonds adjacent to heteroatoms (such as N, O, S) are cleaved preferentially, because the ensuing charge is stabilised by the heteroatom.



Aside from a very few exceptions, α -cleavage can occur only once in a decomposition chain (sequential fragmentation reactions). This is because the homolytic cleavage in a cation that has been created by the α -cleavage of a radical cation requires too much energy^a.

The mass spectrum of 2-butanone (**1**, $M = 72$) is displayed in Fig. 4.7. Two characteristic fragment ions are present: $m/z = 43$ and 57. Their masses differ from that of the molecular ion by 29 and 15 amu^b, respectively, which means that the corresponding fragment ions have been formed by the loss of the radicals $C_2H_5^\bullet$ and CH_3^\bullet , respectively, from the molecular ion. (*A priori* it is conceivable that the loss of $C_2H_5^\bullet$ has occurred firstly by the loss of CH_3^\bullet ($m/z = 57$) followed by CH_2 (14 amu). The loss of CH_2 from molecular or fragment ions occurs extremely rarely, if at all. Therefore, we can exclude the two step process from consideration in this case. In contrast, the loss of CH_2 during collision activation reactions has been established using special compounds.) The creation of fragment ions

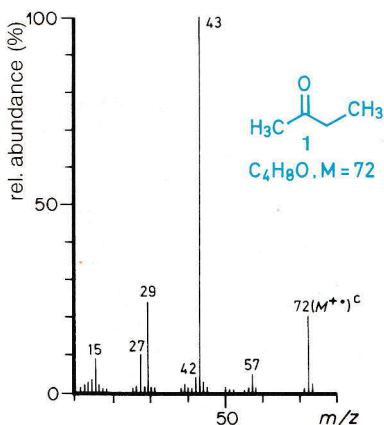
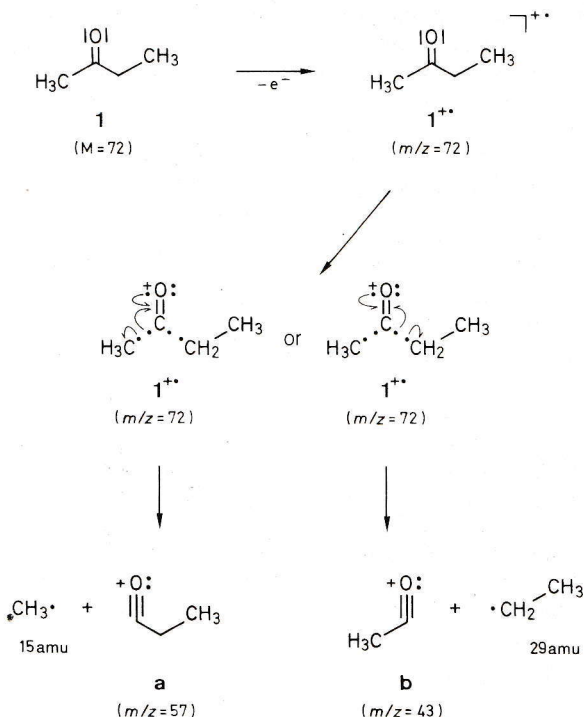


Fig. 4.7 Mass spectrum of 2-butanone (**1**)

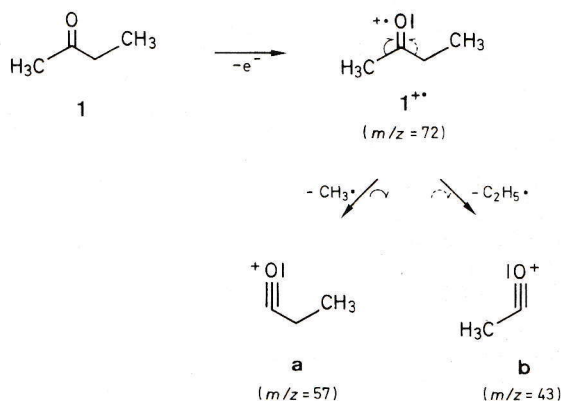
^a There are only a few cases described in the literature in which two consecutive α -cleavages have been observed. One example is the aromatic di-(*tert*-butyl) ether.

^b 1 amu (atomic mass unit) is the constant of atomic mass which is defined as 1/12 of the mass of a ^{12}C atom. - The designation Dalton refers to a mass unit which is defined as the mass of a hypothetical atom of atomic weight 1 on the atomic weight scale.

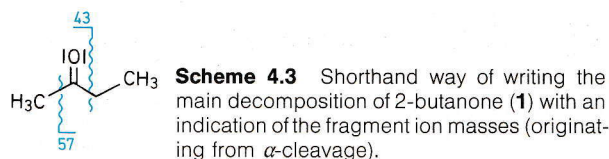
^c This is the usual style for representing the radical cation $M^{+\bullet}$.



Scheme 4.1 Detailed style for writing the main fragmentation of 2-butanone (**1**), see Fig. 4.7^d



Scheme 4.2 Shortened style for Scheme 4.1



^d The structures of the fragment ions are written such that the geometry and normal style of representing the original molecular ion is retained. As a result, the geometry of the fragment ions may sometimes be incorrectly represented.

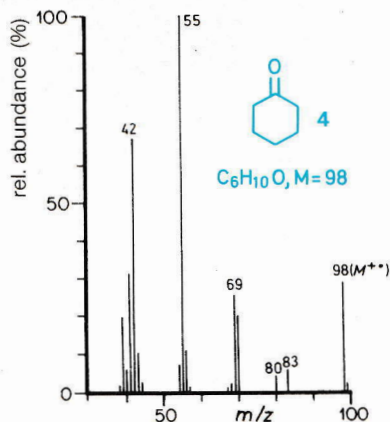
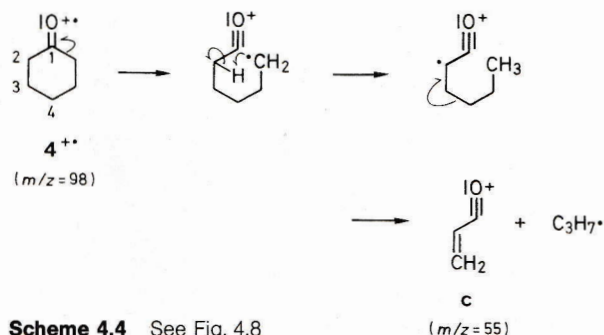


Fig. 4.8 Mass spectrum of cyclohexanone (4)

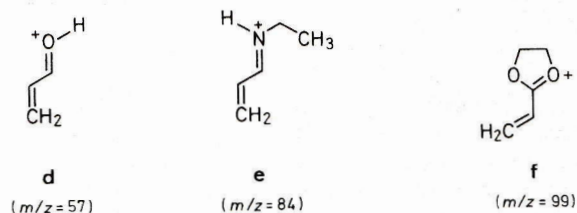
A primary radical is present in the isomeric molecular ion. This radical is stabilised by the transfer of an *H*-atom, *via* a six-membered transition state, over from the C-2 position, which has been activated by the C=O group. As a result, a resonance-stabilised radical is created, which is energetically more stable than the initial step. A radical cleavage reaction results in the formation of a propyl radical as well as the ion *c* ($m/z=55$), in which the multiple bonds are conjugated.



Depending upon the type of substituent and the site of substitution, alkyl derivatives of cyclohexanone show the ion *c* or a homologue thereof. If, for example, a methyl group is at the 4-position, not only is the molecular ion recorded at $m/z=112$,

but the ion *c* also appears at the same mass number. In the mass spectrum of 2-methyl- and 3-methylcyclohexanone, $m/z=69$ ($=55+14$) will be recorded in addition to $m/z=55$. For dimethylcyclohexanone, appropriate ions will be recorded at $m/z=55$ (no methyl substituents on the 2 and 3 or the 5 and 6 positions, respectively), 69 (one methyl group at one of these positions) and 83 (two methyl groups).

α -Cleavages of other alicyclic compounds occur in a similar manner and form ions that have a structure comparable with ion *c* from cyclohexanone. Thus, the corresponding ion in the mass spectrum of cyclohexanol (5; $M=100$, Fig. 4.9) is shifted by +2 amu to $m/z=57$ (*d*) and that from *N*-ethylcyclohexylamine (6; $M=127$, Fig. 4.10) is found at $m/z=84$ (*e*). The ethylene acetal of cyclohexanone shows $m/z=99$ (*f*) as the most abundant fragment ion signal.



If a carbonyl group is incorporated in larger alicyclic assemblages, fragment ions that originate from α -cleavage adjacent to the carbonyl group will still be detected, although their abundances will be weaker, because other cleavage reactions

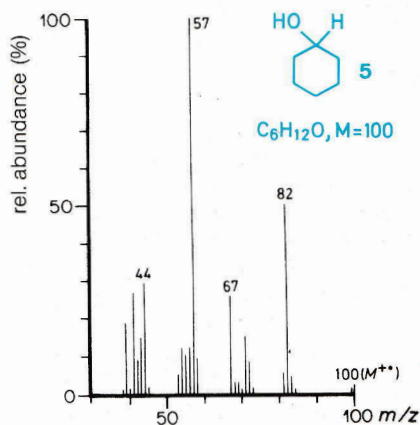


Fig. 4.9 Mass spectrum of cyclohexanol (5)

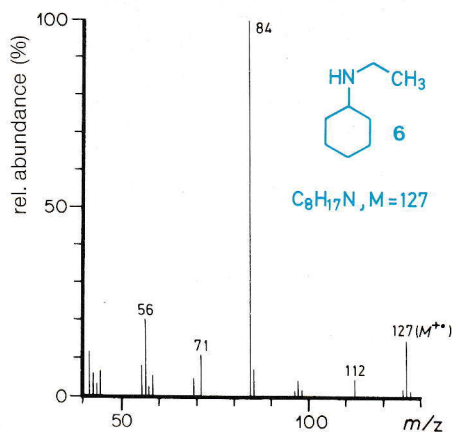
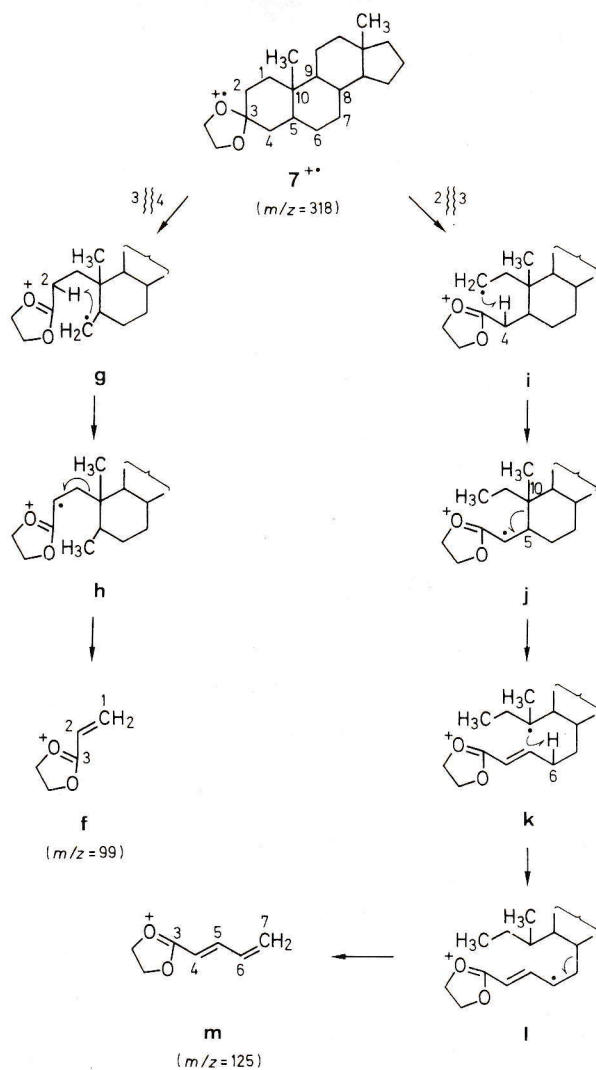


Fig. 4.10 Mass spectrum of *N*-ethylcyclohexylamine (6)

may occur with a similar probability. On the other hand, the incorporation of an ethylene acetal function instead of a carbonyl group causes a marked preference for α -cleavage at the new group. To illustrate this, the spectrum of 5 α -androstan-3-one ethylene acetal (7; M=318) is reproduced in Fig. 4.11. The primary main cleavage reaction is the α -cleavage that is controlled by the ethylene acetal residue. However, in contrast to the "model substance" behaviour of the ethylene acetal of cyclohexanone, the two α -bonds adjacent to the functional group (C-2–C-3 and C-3–C-4) are not equivalent, because the "cyclohexane ring" is substituted at C-5 and C-10. As a result, it is apparent that 7 can undergo two α -cleavages. Both of these possibilities have been confirmed by D-labelling experiments and are shown in Scheme 4.5. The cleavage of the α -positioned C-3–C-4 bond produces the isomeric molecular ion **g**, which is comparable with the primary cleavage ion from cyclohexanone (Scheme 4.4). (In Scheme 4.5, the α -cleavage of the bonds C-3–C-4 and C-2–C-3 is depicted by 3}}4 and 2}}3, respectively. This is an alternative to the use of various arrows to indicate cleavage possibilities, as was done in Scheme 4.2.) The resonance-stabilised and also isomeric molecular ion **h** is generated by the transfer of an *H*-atom from the 2-position. This is then converted by the breaking of the C-1–C-10 bond into the ion **f** ($m/z=99$), which possesses conjugated double bonds. In a similar way, the second α -positioned bond, C-2–C-3, cleaves to give **i** and then **j**. The cleavage of the C-5–C-10 bond in **j** does not, however, result in the loss of a radical, but once again an isomeric molecular ion (**k**) is formed, in which the tertiary radical once more accepts one of the C(6)-*H*-atoms *via* a 6-membered transition state. In the thereby obtained ion **l**, the possibility exists for an ideal radical cleavage: the cleavage of the C-7–C-8 bond yields the ion **m** ($m/z=125$) with three conjugated double bonds. The spec-



Scheme 4.5 See Fig. 4.11

trum in Fig. 4.11 clearly shows that the fragment ions **f** and **m** play a dominating role in the decomposition of 7.

Similarly, the *N,N*-dimethylamino group, which is frequently found in steroid alkaloids, possesses the same strong α -cleavage properties as the ethylene acetal group. Very abundant fragment ion signals, which correspond with ions of similar structure, are also observed for these compounds. The appearance of various very abundant signals in the spectra of these types of compounds permits the determination of the points of substitution of the α -cleavage directing groups and, as a result, important parts of their structures can be deduced.

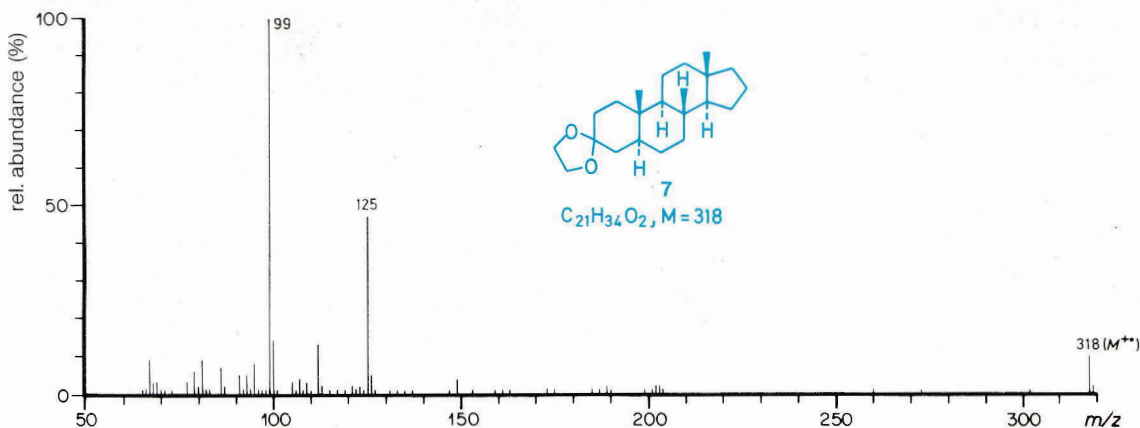


Fig. 4.11 Mass spectrum of 5 α -androstan-3-one ethylene acetal (**7**)

As already mentioned, the ethylene acetal group is able to direct decomposition reactions that start with α -cleavage to a much greater extent than the carbonyl group. By comparing the spectra of Figs. 4.8-4.10, it is evident that ketones, secondary alcohols and amines direct the α -cleavage to different degrees. With compounds of the type X-CH₂-CH₂-Y, where X and Y stand for different functional groups, it is possible to observe directly the effect of X and Y on the α -cleavage. The spectrum of 2-aminoethanol (**8**; M = 61, Fig. 4.12) can be used as an example. Cleavage of the C-C bond yields the ions with $m/z=30$ and 31. As can be seen in Fig. 4.12, the intensity of the signal due to the ion $m/z=30$, which is the nitrogen-containing fragment ion, exceeds by far that of the ion $m/z=31$. Hence it follows that the NH₂-group is significantly more charge stabilising and therefore directs the fragmentation more strongly than the aliphatic hydroxy group.

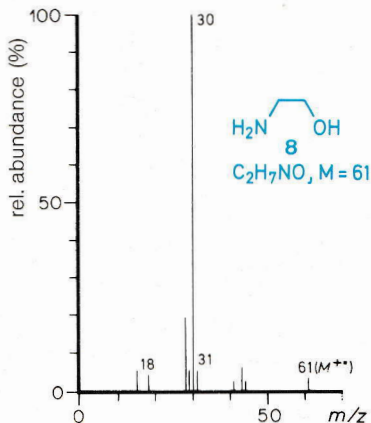


Fig. 4.12 Mass spectrum of 2-aminoethanol (**8**)

Tab. 4.3 A ranking of the relative degree to which substituents influence charge stabilisation (α -cleavage)




Functional group	Ion abundance	Functional group	Ion abundance
-COOH	1	-I	109
-Cl	8	-SCH ₃	114
-OH	8	-NHCOCH ₃	128
-Br	13	-NH ₂	990
-COOCH ₃	20		1600
	43		2100
-OCH ₃	100		

Table 4.3 lists some measured values, from which the individual functional groups can be compared with each other with respect to the relative abundances of the ions that arise from direct α -cleavage. When these values are only considered in relative terms, they actually give an order of effectiveness of substituents for inducing α -cleavage in equivalent structural entities.

The α -cleavage is the most important primary fragmentation reaction in mass spectrometry. Additional examples are included in the discussion of the other fragmentation reactions.

4.2 Benzyl and Allyl Cleavage

Aromatic centres, delocalised double bonded systems and even isolated double bonds have an activating influence on suitable benzylic or allylic bonds, in a similar way that a heteroatom influences a bond and leads to α -cleavage.

Benzyl cleavage. The mass spectrum of butylbenzene (**9**; $M = 134$) is shown in Fig. 4.13. The cleavage of the benzylic C—C bond results in the loss of a propyl radical to yield the main fragment ion, which is seen as the base peak of the spectrum $m/z = 91$ (**n**, **o**, **p**) (see Scheme 4.6).

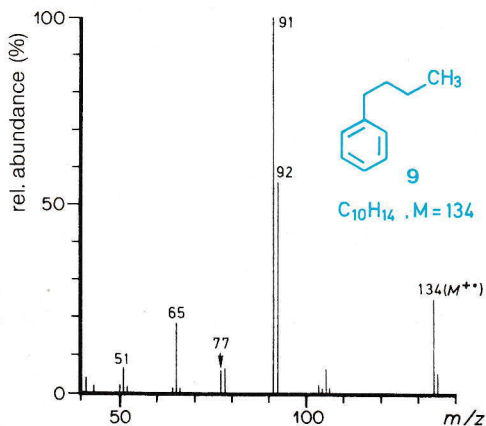
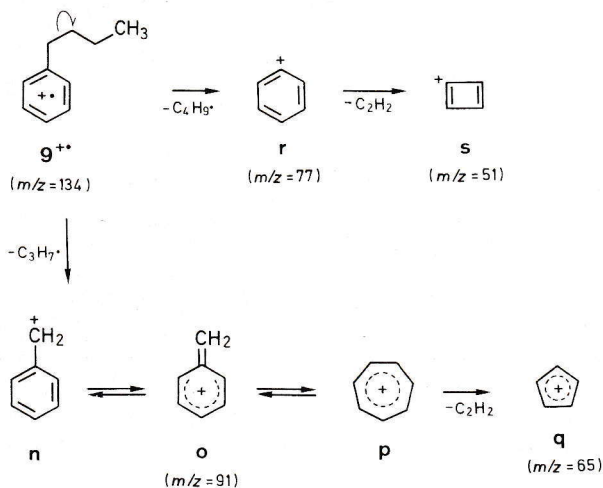


Fig. 4.13 Mass spectrum of butylbenzene (**9**)



Scheme 4.6 See Fig. 4.13

The high abundance of this signal indicates that the corresponding ion is very stable. The stability is not just a result of the formation of either of the tautomers **n** or **o**, but also originates from the formation of the tropylium ion (**p**, $C_7H_7^+$). The proof that **p** really is the deciding species comes from, among others, the subsequent reaction, which is the loss of C_2H_2 to yield **q** ($m/z = 65$). In the symmetrical tropylium ion (**p**), all of the C-atoms are equivalent to each other, as are all of the H-atoms. In contrast, the ions **n** and **o** possess at least three types of C-atom (CH_2 , CH, C) and at least two types of H-atom. If a ^{13}C - or D-labelled compound is now used, it must be possible in the case of the loss of C_2H_2 from **n** and **o** to determine a dependence of the labelled atoms on the original labelling positions, whereas this would not be possible for **p**. Analyses of alkylbenzenes have confirmed the equivalence of the C-atoms in the ion $m/z = 91$, which favours the acceptance of **p**. From the spectrum of butylbenzene, the reverse conclusion can also be drawn, which is not unimportant for structural analyses. That is, that an abundant signal at $m/z = 91$ indicates the presence of a benzyl residue in a compound of unknown structure. Weak signals, however, are less characteristic because the highly stable tropylium ion can also be formed by complicated rearrangements.

At the same time, the spectrum of **9** shows that the phenyl-cleavage (formation of **r**, $m/z = 77$) is significantly less favoured than the benzyl cleavage. **r** also loses acetylene (ion **s**, $m/z = 51$). The pairs of ions, $m/z = 91/65$ and $m/z = 77/51$, are typical for monosubstituted alkyl aromatics. The ion $m/z = 92$ will be discussed in Sec. 4.5 (p. 240).

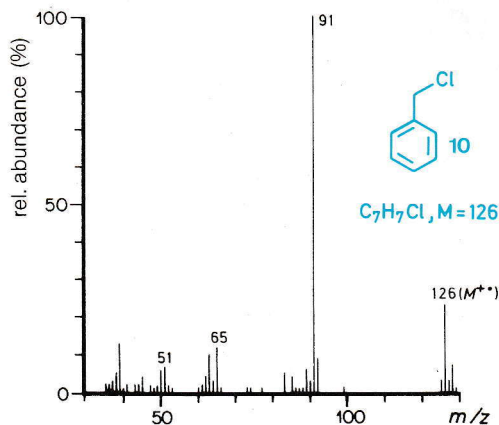


Fig. 4.14 Mass spectrum of benzyl chloride (**10**)

Other monosubstituted *n*-alkylbenzenes also show $m/z=91$ as the base peak (e.g. toluene, ethylbenzene, propylbenzene, pentylbenzene), as do *o*-, *m*- and *p*-xylene.

It is interesting to compare the spectra of benzyl chloride (**10**; $M=126$, Fig. 4.14) and *o*-chlorotoluene (**11**; $M=126$, Fig. 4.15). Aside from small differences in the relative abundances, the two spectra are the same.

In line with the above discussion, the spectrum of benzyl chloride appears as expected because a *Cl*-atom is more easily removed from the benzyl position than an *H*-atom. The spectrum of *o*-chlorotoluene, however, is surprising. The *Cl*-atom is directly attached to the phenyl ring and, as a result, the tendency for it to be ejected is small. Thus one would assume that a chlorosubstituted tropylium ion would be created by the loss of $H\cdot$ (from the CH_3 group) and that therefore an analogous behaviour to that of *p*-(chloro)ethylbenzene (**12**; Fig. 4.16)

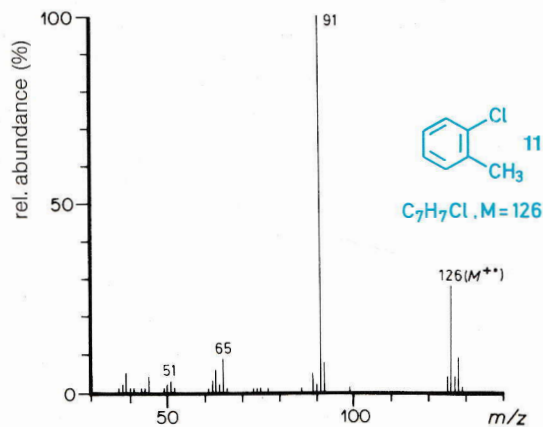


Fig. 4.15 Mass spectrum of *o*-chlorotoluene (**11**)

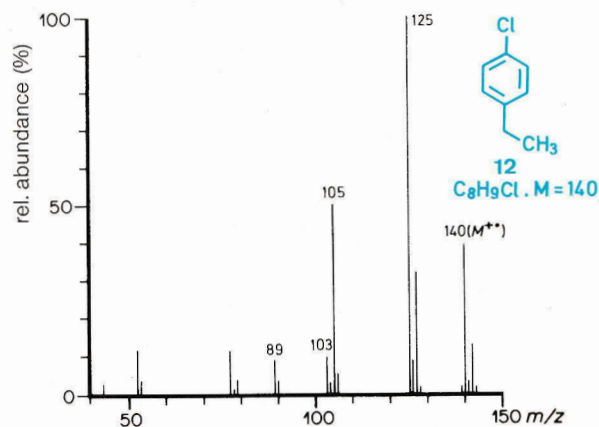
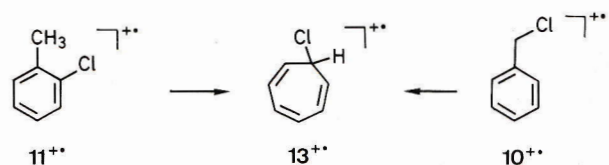


Fig. 4.16 Mass spectrum of *p*-(chloro)ethylbenzene (**12**)

would be expected. (The peak $m/z=125$ in the spectrum of *p*-(chloro)ethylbenzene is caused by the chlorotropylium or chlorobenzylum ion.)

However, because the spectra of **10** and **11** are the same, one can assume that **11** rearranges to **10** or, more likely, that they both isomerise to another common species, e.g. **13**, before they fragment (cf. Scheme 4.7). One would expect **13** to have the same mass spectrometric behaviour as **10**, but so far this investigation has not been carried out.

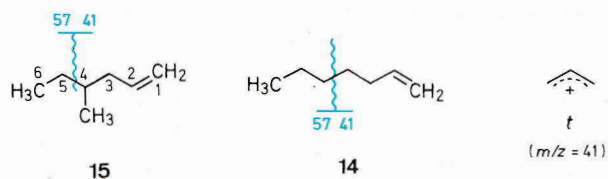
To what extent other systems, which show abundant $m/z=91$ signals (or corresponding derivatives thereof) in the mass spectrometer, isomerise after ionisation, but before their decomposition to cycloheptatriene derivatives, must be clarified in each particular case.



Scheme 4.7 See Figs. 4.14 and 4.15

Furthermore, the appearance of ions of mass 91, or their derivatives, does not automatically allow one to conclude that they are tropylium or benzylum ions. Usually this question must also be investigated in each case.

Allyl cleavages are less pronounced than benzyl cleavages, because the energy stabilisation through the formation of the resulting allyl cation is smaller. Fig. 4.17 shows the mass spectrum of 1-heptene (**14**; $M=98$) and Fig. 4.18 depicts that of 4-methyl-1-hexene (**15**; $M=98$). In both spectra, the base peak is at $m/z=41$ (**t**). Because the allyl-positioned bond in straight chain isomers is the weakest bond and, at the same time, the allyl cation is the most stable ion, $m/z=41$ will be recorded as the most abundant ion. The other cleavage fragment, $m/z=57$, which is also formed by an allyl cleavage, is, with 27% relative abundance, much weaker. Similarly, the allyl-positioned C-3-C-4 bond in the other isomer is also the most labile bond and so the spectrum of this isomer also shows the allyl cation as the most prominent ion. However, the peak $m/z=57$ is



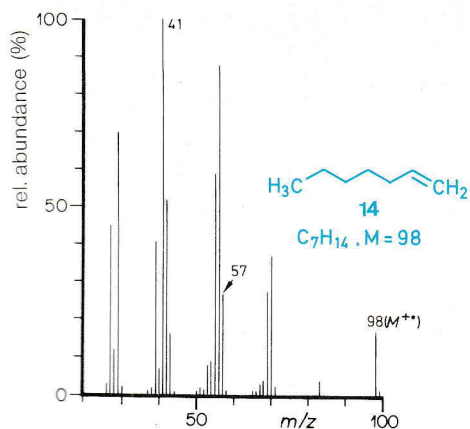


Fig. 4.17 Mass spectrum of 1-heptene (**14**)

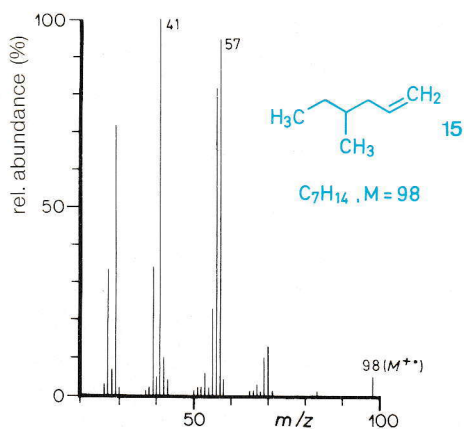


Fig. 4.18 Mass spectrum of 4-methyl-1-hexene (**15**)

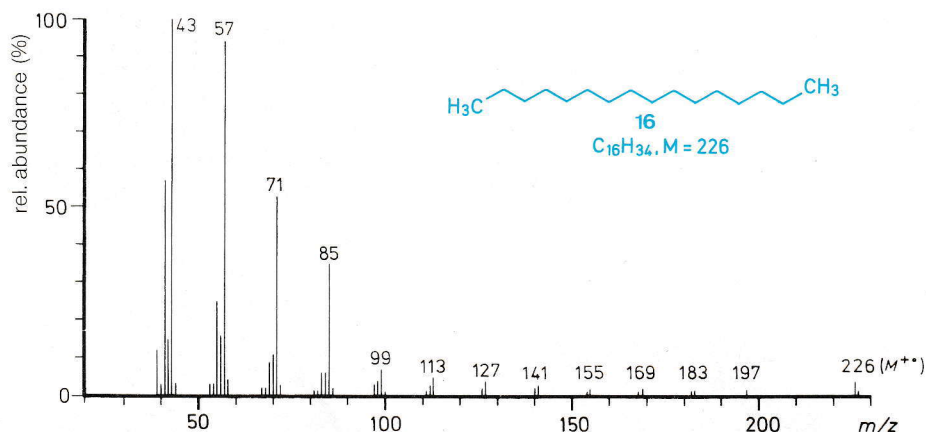


Fig. 4.19 Mass spectrum of hexadecane (**16**)

recorded as the second most abundant peak with 95% relative abundance, because of the greater stability of the secondary carbocation. (The ions $m/z = 42$ and 56 result from a McLafferty rearrangement, see Sec. 4.5, p. 240.) From these examples, it follows that cleavage at allyl positions is favoured over that at C–C bonds. However, the charge stabilisation of the resulting allyl cation is not reliable and, in addition, competing reactions can occur, which interfere with or even prevent the recognition of the C=C bonds. Particularly unpleasant, moreover, is the shifting of C=C bonds from their original sites. It is therefore better and safer to fix the positions of the C=C bonds by derivatisation and to analyse the derivatives mass spectrometrically. The acetyl compounds of the corresponding diols have proven themselves to be suitable derivatives. Similarly, for C≡C bonds, the analysis of derivatives (carbonyl compounds produced by the addition of water) is preferable.

4.3 The Cleavage of “Non-activated” Bonds

This section will summarise cleavage reactions in which the bond to be cleaved is not activated by heteroatoms (α -cleavage), phenyl groups (benzyl cleavage) or C=C bonds (allyl cleavage). Fig. 4.19 shows the mass spectrum of hexadecane (**16**; $M = 226$). This spectrum is typical for unbranched, straight-chain hydrocarbons. The most abundant signals lie in the region corresponding to fragments with three and four C-atoms, i.e. between $m/z = 40$ and 60 . With an increasing number of C-atoms, the abundance of the homologous ions decreases almost asymptotically. An $[M - 15]^+$ ion will not be

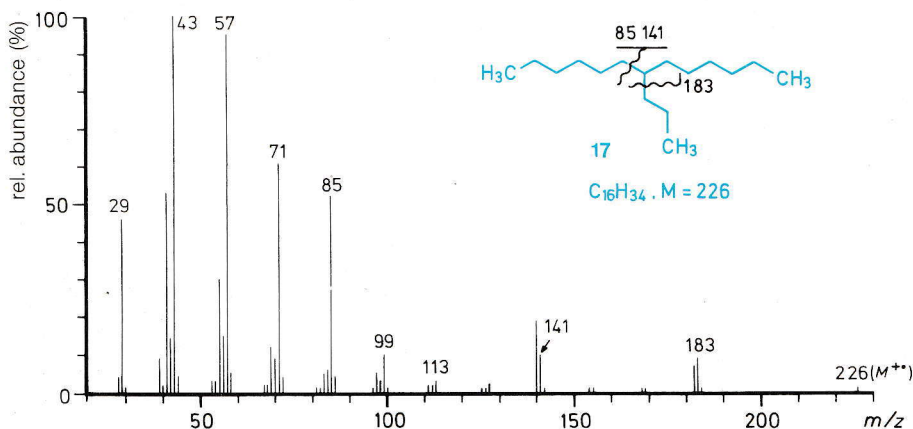


Fig. 4.20 Mass spectrum of 7-propyltridecane (17)

recorded, but, as a result of this, the signal due to the molecular ion is very easily recognised. The general shape of the profile of the spectrum (the connection lines between the highest peaks within each of the groups of signals) is typical. It is useful to memorise this profile, because hydrocarbons often appear as impurities in samples (see Sec. 6, p. 251). Signals that stand out by producing such a uniform picture must have a meaning for the structural analysis. The uniformity is indeed essentially the result of the fact that the cleavage of each C—C bond results in the formation of a primary carbocation and a primary radical. An exception exists only with the two terminal C—C bonds, where either CH_3^\bullet or CH_3^+ can be formed. If, however, the hydrocarbon is branched by an alkyl chain, the bonds to be cleaved are no longer equivalent. Additional secondary carbocations can now be formed and they are also more readily produced, so that the signals due to secondary carbocations stand clearly above the general profile of the spectrum. As an example, the spectrum of 7-propyltridecane (17; $M = 226$) is shown in Fig. 4.20.

With multiply branched hydrocarbons the spectra become unclear and the analysis turns out to be significantly more difficult.

The mass spectrometric analysis is of great importance, because, with the exception of ^{13}C NMR spectroscopy, other analytical techniques are not suitable for the determination of the structures of higher hydrocarbons. The larger the hydrocarbon part of monofunctional compounds, the more similar their mass spectra are to those of the pure hydrocarbons themselves. By altering the type of functional group, the number of CH_2 groups necessary before such a compound displays this spectral behaviour can be made higher or lower.

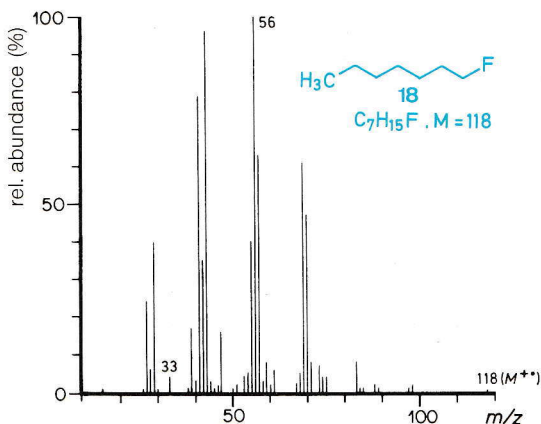


Fig. 4.21 Mass spectrum of 1-fluoroheptane (18)

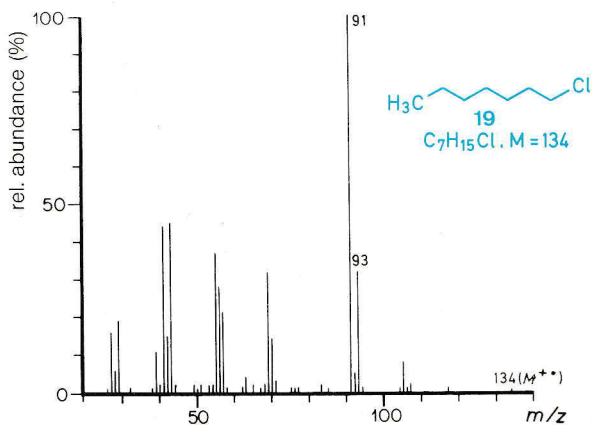


Fig. 4.22 Mass spectrum of 1-chloroheptane (19)

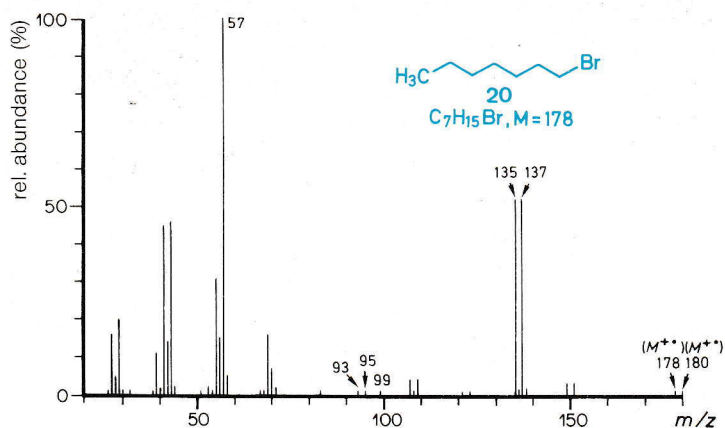


Fig. 4.23 Mass spectrum of 1-bromoheptane (20)

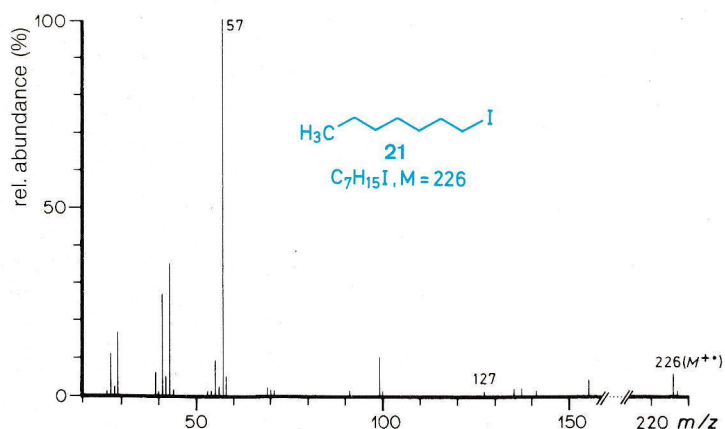
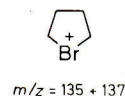
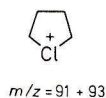


Fig. 4.24 Mass spectrum of 1-iodoheptane (21)

The decomposition of aliphatic haloalkanes is determined only to a very small extent by α -cleavage adjacent to the halogen atom. Fluoroalkanes show ions that have arisen through α -cleavage as single abundant peaks. Conversely, with iodoalkanes, the breaking of the C–X bond with charge localisation on the halogen atom occurs much more frequently and can easily be recognised in low resolution spectra by the large gap in the masses, which is due to the iodine. (If the charge is localised on the alkyl chain, the presence of iodine cannot be established.) Particularly characteristic is the behaviour of 1-chloro- and 1-bromoalkanes with at least five linearly ordered methylene groups. They usually form, as the most abundant signal of the spectrum, pentacyclic chloronium and bromonium ions with their characteristic isotopic abundance ratios (see Tab. 4.10, p. 294). To illustrate these

points, the mass spectra of four 1-haloheptanes are given in Figs. 4.21 to 4.24.

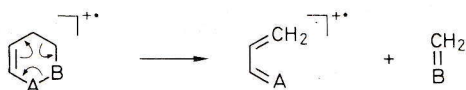


4.4 The Retro Diels-Alder Reaction (RDA Reaction)

Review article: ⁴.

Six-membered cyclic systems that contain a double bond can, via a concerted decyclisation reaction, dissociate into two frag-

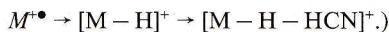
ments, the ene and diene components. The diene component is favoured as the charge carrier, however the ene part is also frequently observed in mass spectra. The cyclohexene ring need not contain any heteroatoms, but it can possess one or more of them. It may also form part of a larger ring system. The RDA reaction can occur in molecular ions as well as in fragment ions in which the double bond in the ring has been formed initially as a result of another fragmentation reaction (e.g. through an α -cleavage). The RDA reaction is a so-called neutral process in which a radical cation is formed either when the starting ion is a radical cation, or when a cation loses a non-radical fragment to form another cation.



This reaction type will be clarified further by using the mass spectrum of 1,2,3,4-tetrahydrocarbazole (**22**; $M = 171$) as an example. At the same time, the term “shift technique” should be introduced and the procedure for the clarification of a reaction mechanism demonstrated. Both are important working techniques in mass spectrometry.

The most abundant ion in the mass spectrum of **22** (Fig. 4.25) is $m/z = 143$. Firstly, it was determined by high resolution mass spectrometry that the main fragment ion $m/z = 143$ ($C_{10}H_9N$) differs from the molecular ion by the deficiency of C_2H_4 (28 amu). Furthermore, a metastable signal (m^* , cf. Sec. 8.25, p. 277) could be found at $m/z = 119.6$, which confirms the transition $171 \rightarrow 143$, i.e. the ion of mass 143 is formed directly from the molecular ion. (Based on the empirical formula of the molecular ion, another formulation of the fragment ion

(namely $C_{11}H_{11}$) would also be possible; this could, for example, have been formed in the following way:



By using measurements from low-voltage and field ionisation spectra, it could be confirmed that the compound did not contain any impurities. Based on the 70 eV spectrum alone, one would have the right to surmise that **22** is contaminated by dehydrogenation products, as evidenced by the signals at $m/z = 169$ ($M - 2H$) and 167 ($M - 4H$). However, because only $m/z = 171$ (and the corresponding isotope peaks) is found in the field ionisation spectrum, the dehydrogenation products must result from mass spectrometric processes. (If the dehydrogenation products had been present in the sample of the compound, their presence would also be apparent in the UV spectrum. However, the UV spectrum is in agreement with the literature spectrum of a pure sample, which provides an additional confirmation of the results from the field ionisation spectrum.) The methods of analysis described above can be carried out on the unlabelled compound and can therefore be completed without additional synthetic effort. They should always be done before the investigation of the reaction mechanism.

Useful information can also be delivered by the mass spectra of derivatives which exhibit mass spectrometric behaviour analogous to that of the molecule of interest. In the present case, the mass spectra of *N*-methyl-1,2,3,4-tetrahydrocarbazole (**23**; $M = 185$ corresponding to $171 + 14$) and 1,2,3,4-tetrahydrocarbazol-3-ol (**24**; $M = 187$ corresponding to $171 + 16$) were drawn into the investigation. Both spectra exhibit great similarity with that from **22**, i.e. one intense fragment ion signal is present (for **23**: $m/z = 157$, for **24**: $m/z = 143$), which in both cases is the base peak. The molecular ion peaks are about

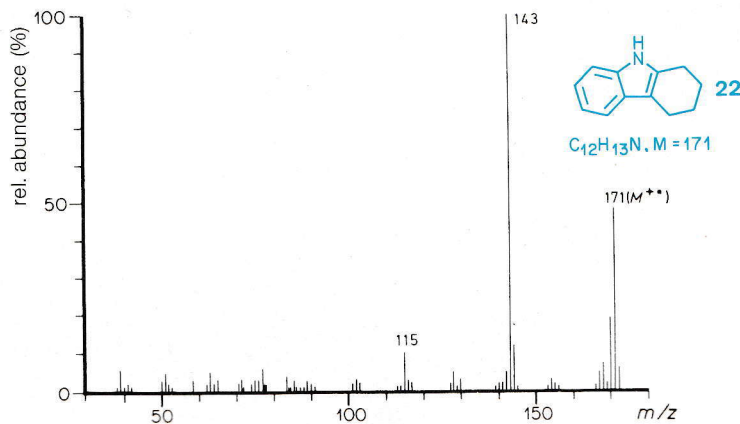
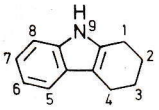
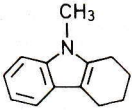
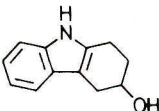
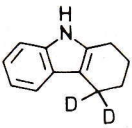
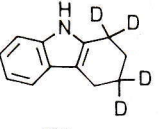
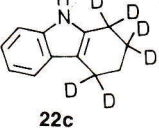


Fig. 4.25 Mass spectrum of 1,2,3,4-tetrahydrocarbazole (**22**)

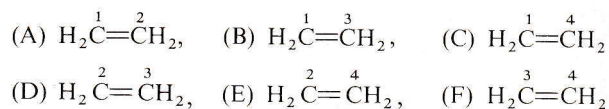
half as abundant as these fragment ion signals (see Tab. 4.4). In the spectrum of the methyl derivative **23**, not only is M^{\bullet} shifted by the mass of the substituent, but the fragment ion signal is also shifted by a similar amount; i.e. the fragment ion contains the methyl group and therefore also the *N*-atom. The situation is different with the hydroxy compound. Indeed the molecular ion is also shifted by +16 amu, however, the fragment ion signal is found at the same mass number as in the

Tab. 4.4 Compounds used in the analysis of the RDA reaction of 1,2,3,4-tetrahydrocarbazole (**22**)

Compound	M^{\bullet}			Fragment ion		
	<i>m/z</i>	difference from M^{\bullet}	rel. abundance (%)	<i>m/z</i>	difference from <i>m/z</i> = 143	rel. abundance (%)
	171	0	63	143	0	100
22						
	185	+14	50	157	+14	100
23						
	187	+16	43	143	0	100
24						
	173	+2	62	145	+2	100
22a						
	175	+4	62	145	+2	100
22b						
	177	+6	80	147	+4	100
22c						

spectrum of 1,2,3,4-tetrahydrocarbazole (**22**) itself. This allows the conclusion to be drawn that during the transition from $M^{\bullet} \rightarrow m/z=143$ the atom C-3 is also lost. This method of analysis is known as the Shift Technique (or Biemann Shift). It can be employed when compounds possess the same skeleton, but have different substituents and, aside from small differences in the abundances of the signals, exhibit similar mass spectra. Based on the shifts in the signals (or the absence thereof), conclusions can be drawn about the substituents^f.

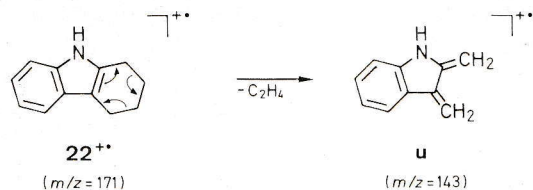
For the unequivocal clarification of a reaction mechanism, it is, however, necessary to investigate labelled derivatives. Therefore the following deuterated compounds were synthesised: 4,4-dideutero-1,2,3,4-tetrahydrocarbazole (**22a**; $M = 173$; by reduction of 1,2,3,4-tetrahydrocarbazol-4-one with LiAlD_4 and working up in the presence of H_2O); 1,1,3,3-tetra-deutero-1,2,3,4-tetrahydrocarbazole (**22b**; $M = 175$; by boiling the vinylogous amide, 1,2,3,4-tetrahydrocarbazol-4-one, with $\text{CD}_3\text{OD}/\text{CH}_3\text{ONa}$, neutralisation of the reaction solution with $\text{DCl}/\text{D}_2\text{O}$ and reduction of the thus formed 1,1,3,3,9-pentadeutero-1,2,3,4-tetrahydrocarbazol-4-one with LiAlH_4) and 1,1,2,3,4,4-hexadeutero-1,2,3,4-tetrahydrocarbazole [**22c**; $M = 177$; by boiling 1,2,3,4-tetrahydrocarbazol-1-one with $\text{DCl}/\text{D}_2\text{O}$ and reduction of the product with zinc amalgam/ $\text{DCl}/\text{D}_2\text{O}$ (\rightarrow 1,1,2,2,4,4,5,6,7,8,9-unadecadeutero-1,2,3,4-tetrahydrocarbazole) followed by boiling with $\text{HCl}/\text{H}_2\text{O}$]. The results of the mass spectra of the three compounds are summarised in Table 4.4. (The determination of the D-content of the molecular ions was, because of the strong $[M-1]^+$ signal, carried out by comparing the field ionisation spectra. The D-content of the fragment ions was determined using the 70 eV spectra (see Sec. 8.6, p. 261). The correct positioning of the isotope insertion was confirmed with ^1H NMR spectra.) If one assumes *a priori* that for the loss of ethylene from (**22**) the six combinations



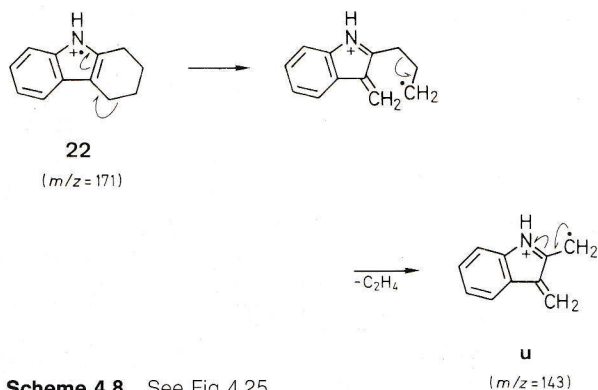
are possible, then the spectrum of **22a** permits the elimination of possibilities (C), (E) and (F), the spectrum of **22b** additionally eliminates (B) and that of **22c** eliminates (A), whereby the possibility (D), i.e. the elimination of $\text{H}_2\text{C}(2)=\text{C}(3)\text{H}_2$, can be taken as proven. Based on these results, two mechanisms can be brought into consideration (Scheme 4.8).

^f In the case of the two compounds **23** and **24**, when the sites of both substituents are unknown, but the mechanism of the fragmentation reaction is, however, known, the following conclusions can be drawn: the methyl group could be attached at the positions 1,4,5,6,7,8 or 9 and the hydroxy group at positions 2 or 3, without expecting the spectra to be significantly different.

mechanism I

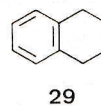
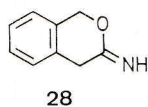
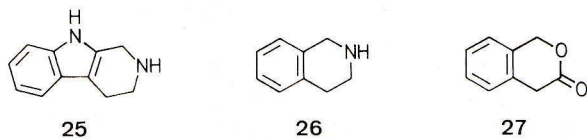


mechanism II

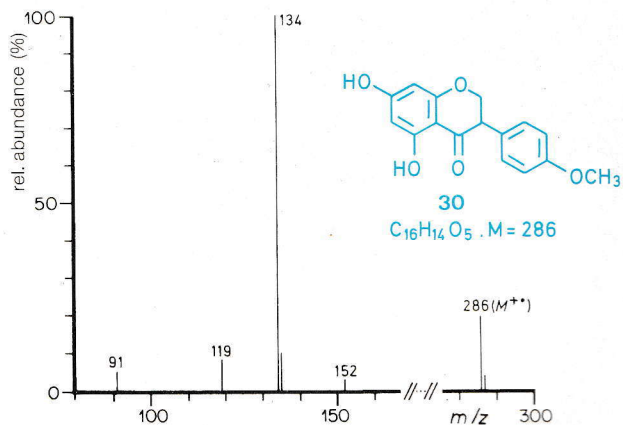
**Scheme 4.8** See Fig. 4.25

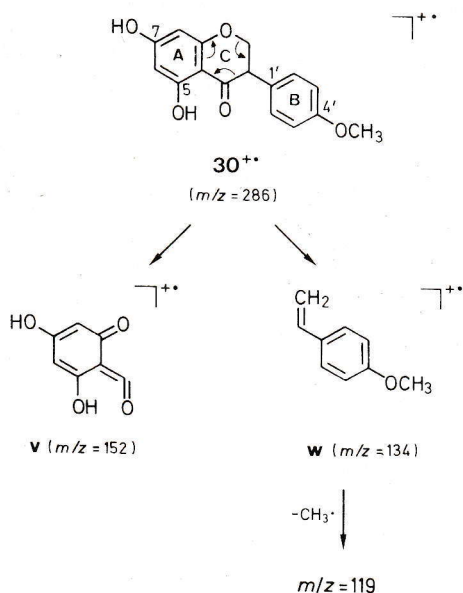
Mechanism I is a concerted RDA reaction, whereas mechanism II is a stepwise process that begins with a vinylogous α -cleavage and leads, *via* a free radical elimination reaction, to the same structural ion **u**. On the basis of the above results, it is not possible to decide between these mechanisms.

The RDA reaction has been demonstrated for a series of additional systems, e.g. 1,2,3,4-tetrahydro- β -carboline (**25**) and 1,2,3,4-tetrahydroisoquinoline (**26**). In all of these cases the ions $m/z = 143$ (from **25**) and 104 (from **26**, **27** and **28**), respectively, form the base peaks of the spectra. Conversely, only about one third of the intensity of the main fragment ion signal $m/z = 104$ of the unsubstituted 1,2,3,4-tetrahydronaphthalene (tetralin, **29**) results from an RDA reaction, the remaining two thirds result from a rearrangement of the CH_2 groups before the loss of ethylene.



Many organic natural substances contain these types of ring systems and the elucidation of their structures is achieved frequently by using the mass spectrometric RDA reaction. Particular examples of this are indole alkaloids (with **22** and **25** as part of the structure) and tetrahydroisoquinoline alkaloids (with **26** as part of the structure). In addition, many natural substances that belong to the flavonoid family (e.g. flavones, isoflavones, rotenoids) also have a central ring which can undergo the RDA reaction. The mass spectrum of 5,7-dihydroxy-4'-methoxyisoflavanone (**30**; $M = 286$) is reproduced in Fig. 4.26. The molecular ion is cleaved *via* an RDA reaction in ring C into two parts, each of which can carry the charge (Scheme 4.9). This reaction produces **v** ($m/z = 152$) as the diene component and, as the ene part, **w** ($m/z = 134$), which forms the base peak of the spectrum. **w** can also lose CH_3^\bullet to give $m/z = 119$. From the masses of both fragment ions, one can deduce that ring A (mass of the unsubstituted ion: 120) carries two hydroxy groups and ring B (mass of the unsubstituted ion: 104) has either a methoxy group or, and this cannot be differentiated mass spectrometrically, one hydroxy and one methyl group. This method of determining the distribution of substituents between rings A and B proves to be very useful for the elucidation of structurally unknown compounds. Additional conclusions about the points of substitution are, however, not possible. These must be determined by additional spectroscopic or chemical analyses.

**Fig. 4.26** Mass spectrum of 5,7-dihydroxy-4'-methoxyisoflavanone (**30**)

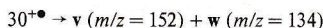
Scheme 4.9 See Fig. 4.26^g

4.5 The McLafferty Rearrangement

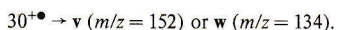
Analogous reactions: photochemistry: Norrish Type II reaction; ester pyrolysis, Tschugaev reaction, ene reaction.

This reaction type is also designated as a β -cleavage with an *H*-atom migration. In this reaction, an *H*-atom is transferred *via* a 6-membered transition state from the γ -position to another atom which must be at least double-bonded. Simultaneously, a migration of the double bond occurs and a neutral fragment containing the β - and γ -positioned atoms is ejected. The process can occur either in a concerted (I) or stepwise (II) fashion. The acceptor double bond that is necessary for reaction can be either already present in the starting molecule or formed in another fragmentation reaction (e.g. α -cleavage). Among the groups that can undergo a McLafferty rearrangement, a few are: C=O (e.g. carboxylic acids, esters, aldehydes, ketones, amides, lactams, lactones), C=N (e.g. azomethines or Schiff bases, hydrazones, oximes, semicarbazones), S=O (e.g.

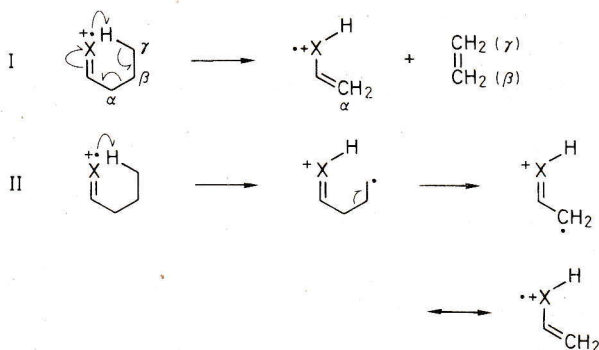
^g At this point one should allude to the occasional errors in formulation that appear in the literature: if both the ene and diene components appear as ions from an RDA reaction, as in the case of **30**, then the formulation



is wrong, because it implies the nonsensical physical condition of $+\bullet = +\bullet + +\bullet$. However, it is acceptable to write

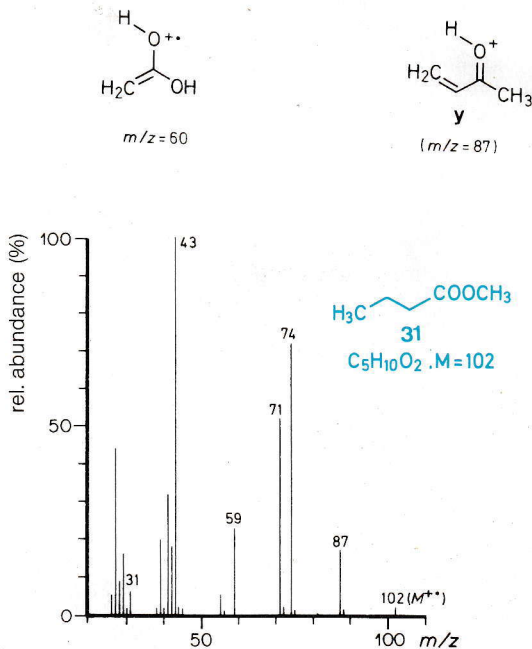


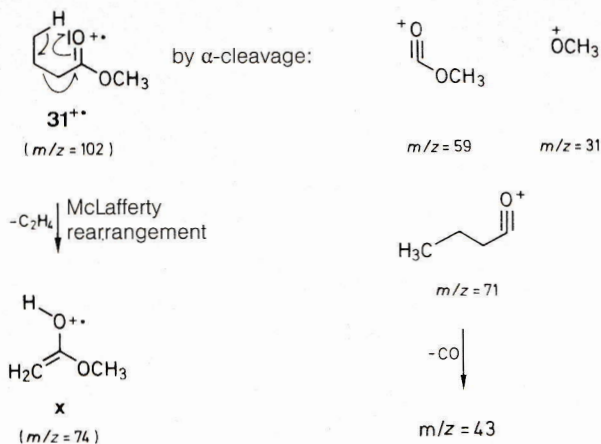
sulfonic acid esters) and C=C (e.g. alkylarenes, alkylheterocycles, benzyl ethers, olefins).



The McLafferty rearrangement can be illustrated with a couple of examples. Methyl butanoate (**31**; *M* = 102, Fig. 4.27) forms the ion *m/z* = 74 (**x**) through a McLafferty rearrangement involving the loss of ethylene. Other fragment ions emanating from **31** (*m/z* = 31, 59, 71) can be explained by α -cleavages (Scheme 4.10). The ejection of CO from the ion *m/z* = 71 results in *m/z* = 43.

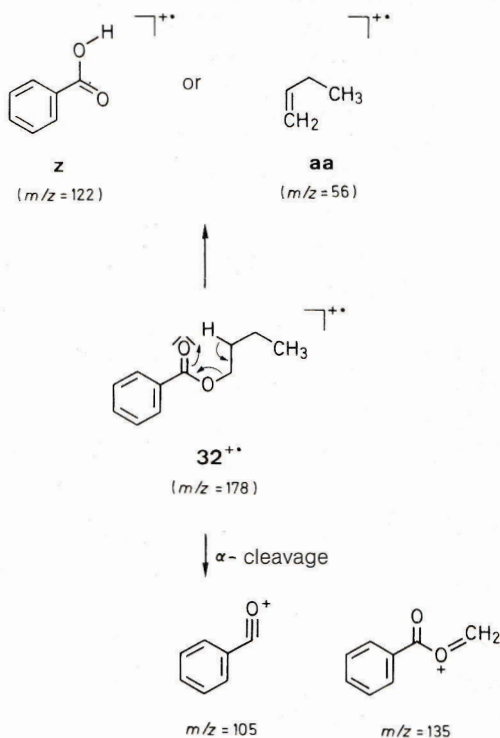
The signals at *m/z* = 74 (McLafferty rearrangement) and 59 (α -cleavage) are characteristic for methyl esters; for ethyl esters, the signals are found at 88 and 73, respectively, etc. The ion from the McLafferty rearrangement of aliphatic carboxylic acids is *m/z* = 60.

Fig. 4.27 Mass spectrum of methyl butanoate (**31**)



Scheme 4.10 See Fig. 4.27

For higher fatty acid esters, there is an increase in the abundance of the fragment ion **y**, which appears +13 amu higher than the fragment ion from the McLafferty rearrangement [for methyl esters: $m/z = 74$ (**x**) + 87 (**y**)]. Esters of higher alcohols and of aromatic carboxylic acids, e.g. butyl benzoate (**32**; $M = 178$, Fig. 4.28), also show, in addition to the ions from



Scheme 4.11 See Fig. 4.28

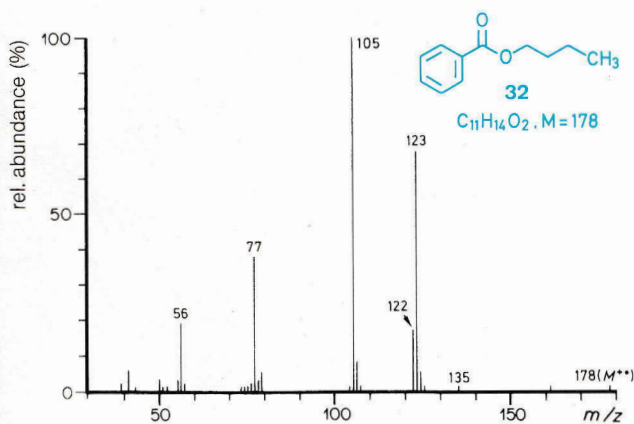


Fig. 4.28 Mass spectrum of butyl benzoate (**32**)

the McLafferty rearrangement, the ions of the protonated carboxylic acid. For **32**, the former are **z** ($m/z = 122$) and **aa** ($m/z = 56$) and the protonated benzoic acid ($\text{C}_6\text{H}_5\text{COOH}_2^+$) has the mass 123 (see Scheme 4.11). The ion $m/z = 105$, which is the

most abundant signal of the spectrum, originates from the α -cleavage adjacent to the carbonyl group and is typical for derivatives with a phenylcarbonyl group, such as benzylic acid esters, phenyl ketones, etc. From $m/z = 105$, the ion $m/z = 77$ is formed by the loss of CO and, following that, the ejection of acetylene results in $m/z = 51$ (see Sec. 4.1, p. 226).

McLafferty rearrangement reactions that occur under the influence of C=C bonds are represented, for example, by $m/z = 92$ in the spectrum of butylbenzene (**9**; Fig. 4.13) and by $m/z = 42$ and 56 in those of 1-heptene (**14**; Fig. 4.17) and 4-methyl-1-hexene (**15**; Fig. 4.18).

McLafferty rearrangements in which an alkyl or another residue is rearranged, instead of a γ -H-atom, are rare processes.

4.6 The Onium Reaction

Behind this name lies a reaction type that is observed mainly with fragment cations in which the heteroatom is the charge carrier. Fragment ions of this type are the **oxonium**, **ammonium**, **phosphonium** and **sulfonium** ions.

Under electron bombardment, *N*-isopropyl-*N*-methylbutylamine (33; $M = 129$, Fig. 4.29) loses CH_3^\bullet or $\text{C}_3\text{H}_7^\bullet$ through α -cleavage, whereby the ammonium ions **ab** ($m/z = 114$) and **ac** ($m/z = 86$) are formed.

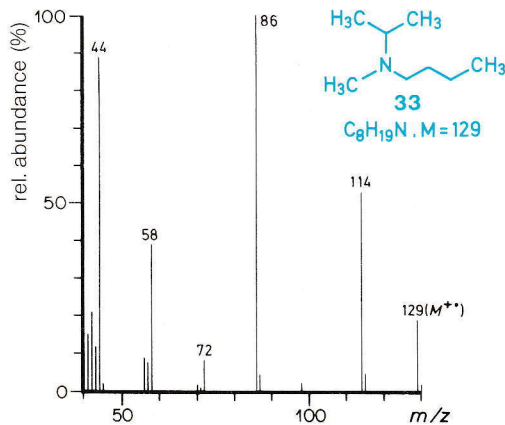
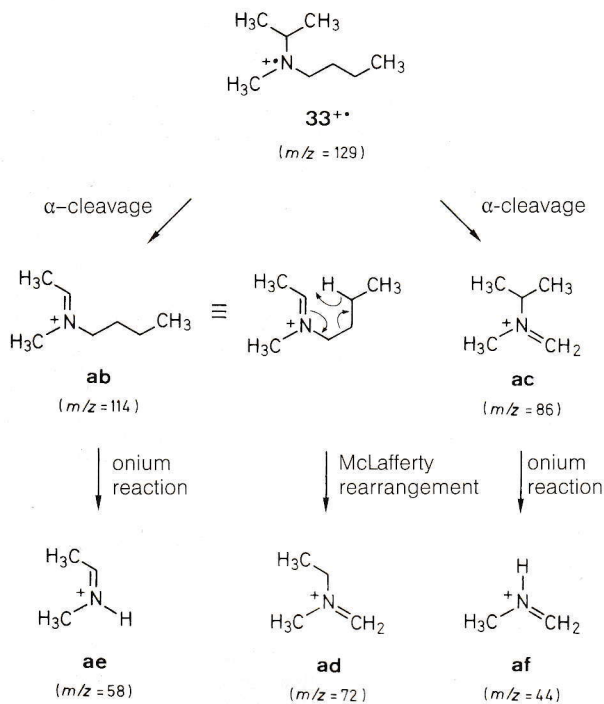
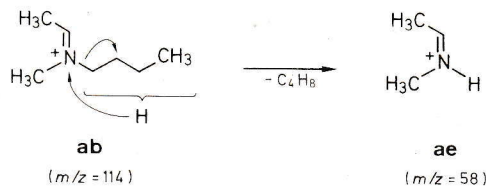


Fig. 4.29 Mass spectrum of *N*-isopropyl-*N*-methylbutylamine (33)



Scheme 4.12 See Fig. 4.29

The double bond in ion **ab** has a γ -positioned H -atom which can rearrange according to McLafferty. The fragment ion thereby formed is **ad** ($m/z = 72$). The other ion from the α -cleavage (**ac**) is unable to undergo a similar decomposition reaction (no γ - H -atom). Conversely, both ammonium ions can undergo the onium reaction in which an H -atom is transferred from the alkyl residue to the N -atom and the alkyl residue is eliminated. Thus C_4H_8 (56 amu) is eliminated from **ab** to give the ion **ae** ($m/z = 58$), while the elimination of C_3H_6 (42 amu) from **ac** produces **af** ($m/z = 44$) (see Scheme 4.12). Because the precise origin of the H -atom that is transferred to the heteroatom is usually unknown (no regiospecificity; deuteration experiments have shown that the various H -atoms along an alkyl chain will be transferred to varying degrees), one chooses, for example for the further decomposition of **ab**, the following manner of representation:



Similar decomposition sequences can be established for ethers [see Fig. 4.30, Scheme 4.13, butyl ethyl ether (34; $M = 102$)] and thioethers.

In addition to alkyl substituents (other than CH_3), acyl residues can also undergo the onium reaction. In the mass spectrum of *N*-butylacetamide (*N*-acetylbutylamine, 35; $M = 115$, Fig. 4.31), $m/z = 30$ (**ag**) is the base peak. The same base peak is found for the *n*-alkylamine itself. However, the primary amino group in 35 is acetylated and therefore is not free. According to

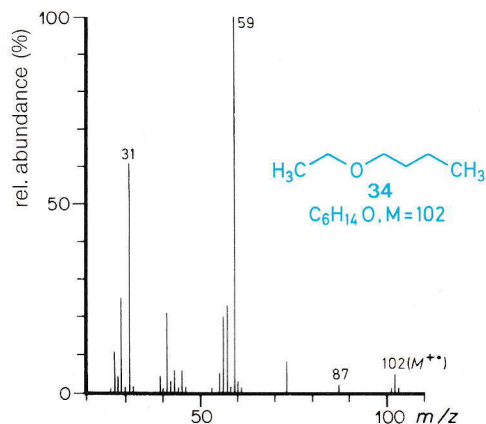
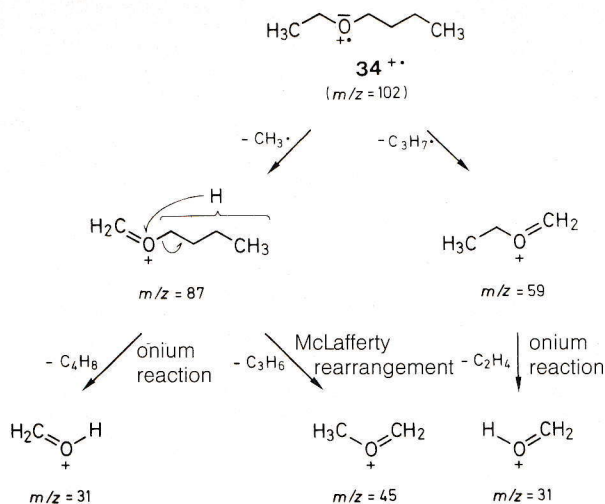
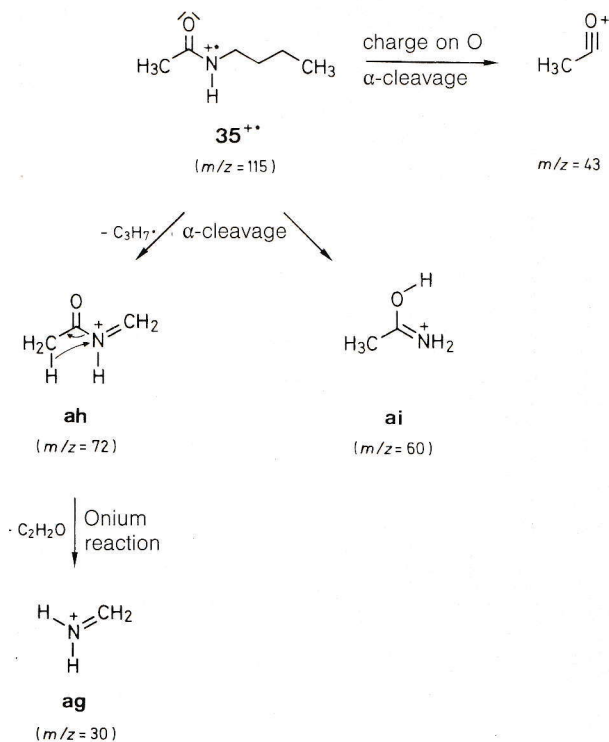
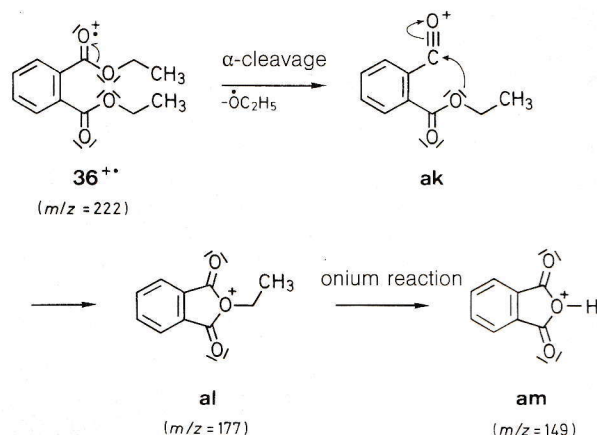
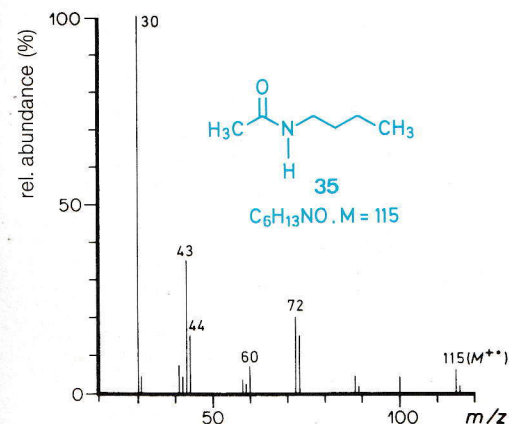


Fig. 4.30 Mass spectrum of butyl ethyl ether (34)


Scheme 4.13 See Fig. 4.30

Scheme 4.14 See Fig. 4.31

The most abundant ion $m/z = 149$ (**am**) in the spectra of phthalic acid dialkyl esters, e.g. diethyl phthalate (**36**; $M = 222$, Fig. 4.32), also has an onium reaction to thank for its existence: the ion that is produced by the ejection of an alkoxy residue, $m/z = 177$ (**ak**), cyclises. The cyclisation results from


Scheme 4.15 See Fig. 4.32

Fig. 4.31 Mass spectrum of *N*-butylacetamide (**35**)

Scheme 4.14, **ah** ($m/z = 72$) is formed first of all from the molecular ion through an α -cleavage adjacent to the *N*-atom. Subsequently, **ag** is produced by an onium reaction in which ketene is lost. At this point it is worth mentioning that *N*- and *O*-acetyl compounds can be recognised by the signal at $m/z = 43$. Furthermore, the spectra of *N*-substituted acetamides are characterised by a signal at $m/z = 60$ (**ai**), which results from a McLafferty rearrangement with an additional *H*-atom transfer.

Other *N*-acetyl compounds behave similarly to **35**. It is worth remarking that acyl residues, which do not contain any aliphatic bonded *H*-atoms, can also be ejected through an onium reaction (with an *H*-migration!); such residues are, e.g., benzoyl-, benzenesulfonyl-, *p*-toluenesulfonyl- (= tosyl-).

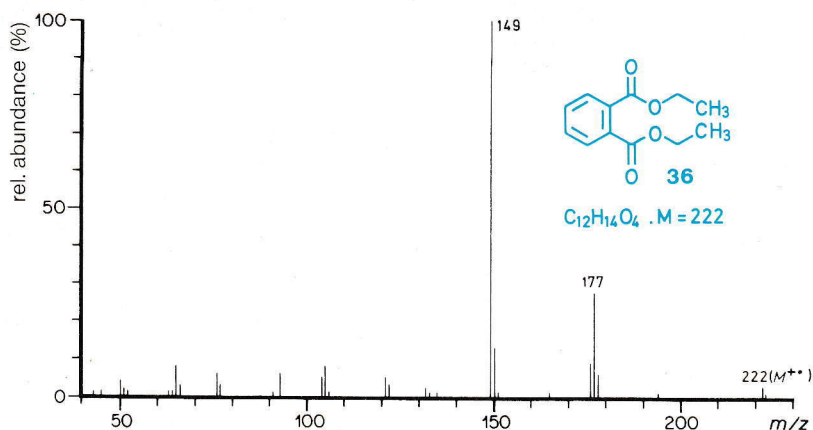


Fig. 4.32 Mass spectrum of diethyl phthalate (36)

the influence of the adjacent (*o*-positioned) second ethoxycarbonyl group (**al**) and enables the charge from the charge-carrying carbonyl group to be transferred to the ether *O*-atom. The ion **am** ($m/z = 149$, Scheme 4.15) is then produced via an *H*-transfer from the alkyl residue to the *O*-atom (see pp. 253, 302). Frequently, *o*-disubstituted benzene derivatives, when compared with the *m*- and *p*- isomers, show a special mass spectrometric behaviour, which is known as the *ortho* effect, see Sec. 8.15 (p. 269).

In a few cases, the ejection of acyl residues that are bound to *O*- or *N*-atoms occurs directly from the molecular ion. In particular, acyloxybenzenes and *N,N*-diacetylalkylamines belong to such compounds. It must be remembered, however, that these substances are very easily hydrolysed and that, because of this, it is very easy for mixtures of substances (e.g. formation of phenols, *N*-acylalkylamines) to find their way into the analysis.

4.7 Loss of CO

Cyclic, highly unsaturated compounds and even ions that have been formed by α -cleavage adjacent to a carbonyl group (see p. 228), have the tendency to eject CO (28 amu). If several CO groups are present in a molecule, they can be eliminated one after another. Usually such a fragmentation reaction is indicated by an (intense) metastable signal (cf. Sec. 8.25, p. 277).

The mass spectrum of tropone (**37**; $M = 106$) is given in Fig. 4.33. It clearly documents the favoured fragmentation reaction in such systems. The remaining part of the spectrum of **37** resembles that of benzene, which means that the ion resulting from the loss of CO is cyclic.

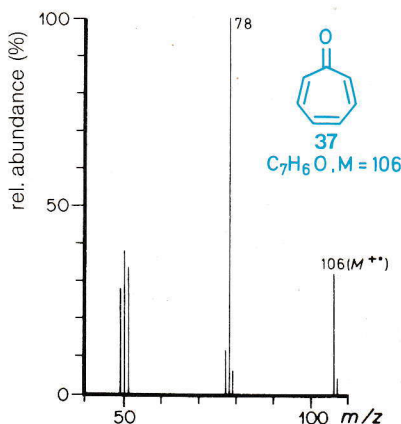
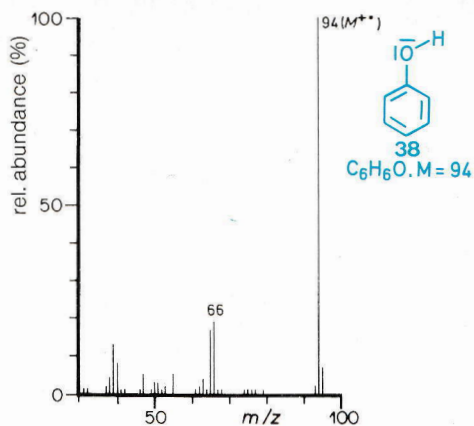
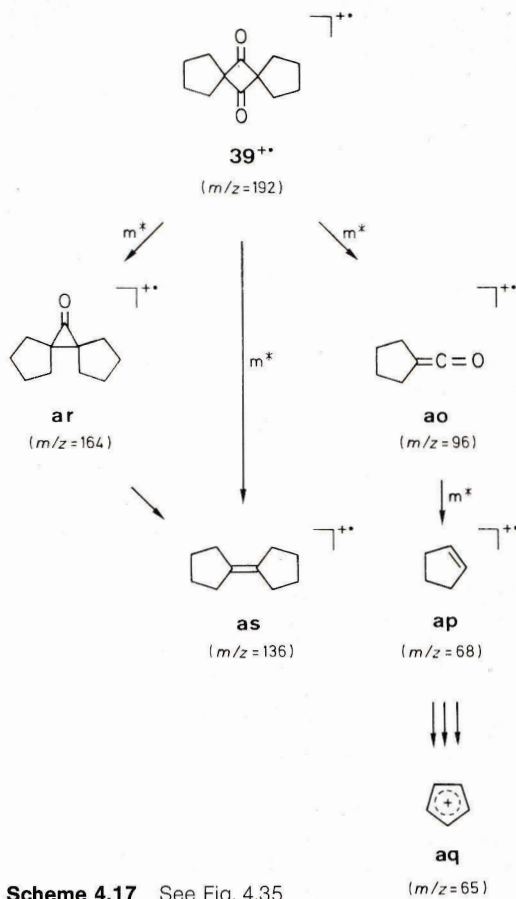
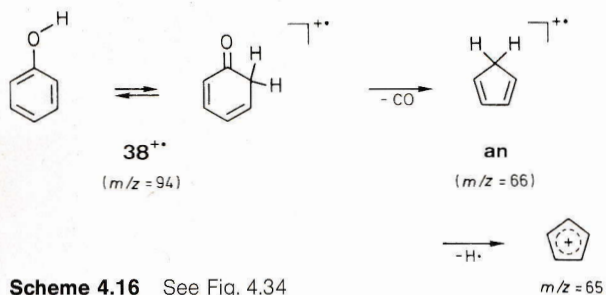
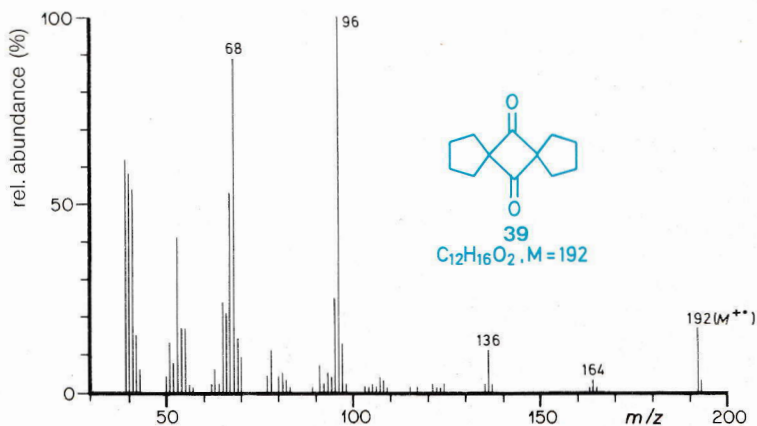


Fig. 4.33 Mass spectrum of tropone (37)

Compounds, whose enol form in solution is much more abundant than the keto form, also lose CO in the mass spectrometer. Phenols are typical of this substance class.

Phenol (**38**; $M = 94$) itself gives the spectrum shown in Fig. 4.34. The most abundant fragment ion of the spectrum is $m/z = 66$ (**an**), which is formed by the loss of CO. Because this ion is still a pseudo-molecular ion, it can transform to the cyclopentadienyl cation ($m/z = 65$) through the loss of an H^\bullet (Scheme 4.16). As an example of a compound with two CO groups, di-spiro[4.1.4.1]dodecane-6,12-dione (**39**; $M = 192$, Fig. 4.35) will be discussed. The base peak of the spectrum is at $m/z = 96$, which is half of the mass of the molecular ion. This peak is not due to a doubly charged molecular ion (isotope peaks at half mass numbers are not present), but represents the fragment ion **ao**. This fragment then loses CO and presumably produces the cyclopentene ion **ap** ($m/z = 68$), which can convert to the cyclopentadienyl cation **aq** ($m/z = 65$) by the ejection of a total


 Fig. 4.34 Mass spectrum of phenol (**38**)

 Fig. 4.35 Mass spectrum of dispiro[4.1.4.1]dodecane-6,12-dione (**39**)


of three *H*-atoms. In a second decomposition pathway, one CO can initially be ejected from M^{\bullet} to give **ar** ($m/z = 164$), followed by a second CO to give **as** ($m/z = 136$). Metastable sig-

nals show that the formation of **as** can also occur directly from the molecular ion (Scheme 4.17). In this step C_2O_2 can be eliminated either as one fragment or in two rapid consecutive

reactions. The loss of both CO groups also occurs to some extent with other diketones (e.g. anthraquinone).

The α -cleavage with its variants of the benzyl and allyl cleavages and the cleavage of non-activated C—C bonds, the RDA reaction, the McLafferty rearrangement, the onium reaction and also, because of the frequent occurrence of carbonyl groups, the loss of CO, very often play a deciding role in the

decomposition of organic molecules. However, the range of decomposition reactions is not completely exhausted. Special functional groups or a particular arrangement of atoms can sometimes bring about special fragmentation reactions. Some examples of such reactions are: loss of water, S_N1 reactions and reactions with neighbouring group participation (see Sec. 8.15, p. 269).

5. Thermal Reactions in the Mass Spectrometer

In order to record an electron impact mass spectrum, it is necessary, as described earlier, that the sample of the substance be in the gas phase. Because most organic compounds are liquids or solids at room temperature, they must first be brought into the vapour phase. Furthermore, for particular measurements (e.g. spectra of gases and liquids), it can be important to maintain the samples in the gas phase while they are held in sample reservoirs for relatively long periods of time. Not only does the vaporisation process require an elevated temperature, but the gaseous molecules can also undergo collisions with the walls of the reservoir and with parts of the ion source, which must be maintained at higher temperatures in order to prevent condensation. Collisions of this type lead to an increase in the energy of the molecules and can give rise to catalysed thermal decomposition reactions.

The inlet sections of the mass spectrometer have been improved markedly in recent years. Materials that promoted thermal reactions have been replaced by more inert components. The vaporisation device has also been improved significantly. The result of this is a reduction in the number of observed thermal decomposition processes in the mass spectrometer (for this reason, among others, older and newer spectra frequently show characteristic differences). However, many reactions of this type are still observed, so that a knowledge of them is absolutely necessary for the interpretation of mass spectra. Thermal decomposition reactions occur especially when high temperatures are necessary for the vaporisation of the sample, which is the case for compounds with large relative molecular masses (> 400) and/or multiple polar functional groups (e.g. $-\text{COOH}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{SH}$). Furthermore, impurities in the sample (e.g. silica gel, aluminium oxide or even activated charcoal) can catalyse thermal reactions.

These thermal reactions have nothing to do with the actual mass spectrometric fragmentation reactions. They occur

before the ionisation and lead, in comparison with the substance being analysed, to heavier, lighter or equally heavy (i.e. isomeric) particles. If two or more particles result from such processes, they will be ionised independently of each other and give superimposed mass spectra.

5.1 The Most Important Types of Thermal Reactions⁵

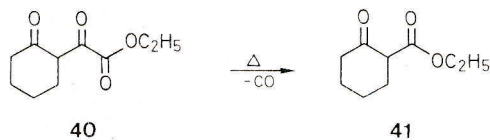
It can happen that organic substances decompose in an unspecific way into many small and large pieces. This usually occurs with high molecular weight compounds that are introduced into the mass spectrometer *via* the direct inlet system and ionised under electron impact conditions. If one records several mass spectra from such samples, one after the other, these spectra generally bear no great resemblance to one another. One gets the impression that a mixture of several substances must be present whose ion abundances decrease with increasing mass, without there being an obvious end to the spectrum. Aside from this type of general, more or less unspecific decomposition reaction, a few frequently observed general cases are described below with the aid of examples.

Thermal Loss of Smaller Fragments

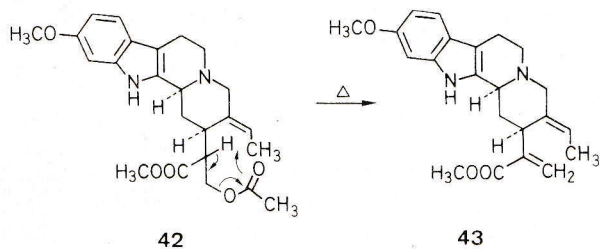
CO₂ (Decarboxylation). In particular, β -oxocarboxylic acids, but also aromatic and other compounds with multiple carboxylate groups, are readily inclined to lose CO₂. This is especially so when the β -oxocarboxylic acid is part of a larger molecular assemblage.

CO (Decarbonylation). α -Oxocarboxylic acids and their alkyl esters lose CO to some extent at their distillation temperature.

Such reactions can also occur under the conditions used to record mass spectra. Thus the spectra of ethyl (2-oxocyclohexyl)glyoxylate (**40**; $M = 198$) and its decarbonylation product, ethyl 2-oxocyclohexanoate (**41**; $M = 170$), are the same, aside from one signal with only a low abundance at $m/z = 28$ (gas inlet, 200°C).



CH₃COOH. The thermal loss of acetic acid from acyloxy derivatives can have its origin in an ester pyrolysis. This occurs particularly when the newly formed double bond can conjugate with an existing one and causes a reduction in the decomposition temperature. As an example, the mass spectrum of the indole alkaloid, *O*-acetylhervine (**42**; $M = 426$, ion source temperature 250°C, direct inlet), is described. At the beginning of the measurement, the ratio of $M^{+\bullet}/[M - 60]^{+\bullet h}$ was found to be 0.72. After just 3 min $M^{+\bullet}$ had disappeared and only $m/z = 366$ (**43**) was still observed. Hence it follows that the cause of the behaviour is a thermal ester pyrolysis and not its mass spectrometric equivalent, the McLafferty rearrangement.



Other functional groups can also display a similar behaviour under certain circumstances.

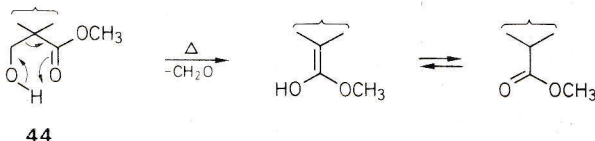
HX (H₂O, HCl, etc.). There are so many examples of the loss of water, hydrochloric acid, etc., that one should not need to go into this here. Not only are hydroxy groups responsible for the loss of water, but also certain *N*-oxides (after an ensuing rearrangement) and amides (lactams), which, for example, can transform into amidines when a transannularly disposed amine *N*-atom is present.

When compounds that contain water of crystallisation, other solvents of crystallisation, or inclusions are heated or vaporised, these included components are released and ionised independently of the compound itself. If a common mo-

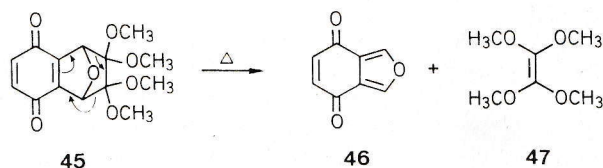
lecular ion is recorded, a (thermal) reaction between both molecules must have taken place.

Retro Reactions

Retro Aldol reaction. Special arrangements of atoms must be present in a molecule in order for a retro Aldol reaction to occur. Compounds that possess the general structural element **44** can decompose thermally with the loss of CH₂O (or its equivalent). Examples of this kind have been observed for basic natural substances (alkaloids).



Retro Diels–Alder reaction. One of the most frequently observed reactions of alicyclic and heterocyclic six-membered ring systems containing a double bond is the retro Diels–Alder reaction. Sometimes it is not easy to distinguish a thermal reaction, which takes place in the mass spectrometer (inlet), from a mass spectrometric reaction, or a mixture of both reactions. An example of this is the quinone **45** ($M = 296$) which, when introduced into the mass spectrometer through the direct inlet system (170°C), produces a signal corresponding to the molecular ion. In contrast, when the gas inlet system (200°C) is used, a thermal retro Diels–Alder reaction takes place and the mass spectra of both fragments **46** ($M = 148$) and **47** ($M = 148$) are superimposed upon one another. Preparatively, **46** and **47** can be obtained from **45** by distillation. The addition mass spectrum from **46** and **47** is very similar to the spectrum of **45** obtained by introducing the sample through the gas inlet system and not dissimilar (larger differences in the abundances of the main signals) to that of **45** which was obtained when the sample was introduced into the apparatus through the direct inlet system. Presumably this is an example of the parallel occurrence of a thermal and a mass spectrometric retro Diels–Alder reaction. A large number of similar cases have been observed.

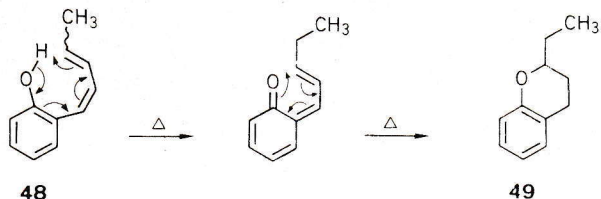


Isomerisation Reactions

It is clearly apparent that the partial mass spectrum of the *E/Z* isomeric mixture (**48**; $M = 160$, gas inlet, 200°C) shown in Fig. 4.36 is in stark contradiction to its structure: the loss of $\bullet\text{CH}_3$ from the molecular ion can readily be explained, but the very

^h 60 amu = CH₃COOH; the relative abundances of $m/z = 366$ and 426 are 95 and 68%, respectively.

strong loss of $\bullet\text{C}_2\text{H}_5$ ($m/z = 131$) is not foreseeable (it would mean the cleavage of a C=C bond with an *H*-migration). However, if one assumes that, preparatively, **48** can be isomerised thermally to **49** (this can actually be done by heating for 20 hours in octane), which is possible *via* an aromatic [1,7]-sigmatropic hydrogen shift followed by a cyclisation, then the mass spectrum can be explained very well. Indeed the mass spectrum of **49**, prepared synthetically, is in exceptional agreement with that of **48**.



Other isomerisation reactions are also known (e.g. double bond shifts)⁵.

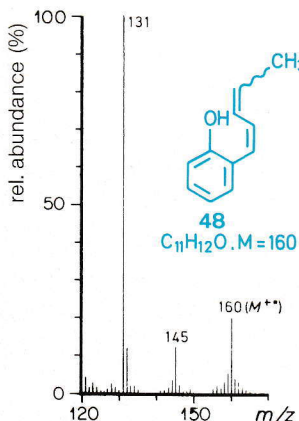
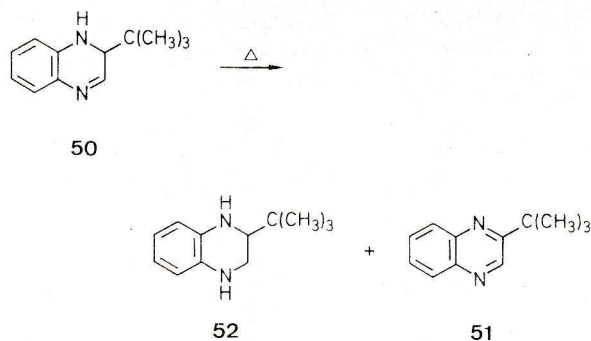


Fig. 4.36 Part of the mass spectrum of 2-(1,3-pentadienyl)phenol (**48**)

Disproportionation, Dehydrogenation and Hydrogenation Reactions

During the measurement of certain classes of compounds (e.g. dihydroquinoxalines, dihydroquinolines), it can happen that the expected molecular ion is not recorded, but, instead, those of the compound's hydrogenation **and** dehydrogenation products. Thus, for 2-(*tert*-butyl)-1,2-dihydroquinoxaline (**50**; $M = 188$), only the molecular ions of **51** ($M = 186$) and **52** ($M = 190$) are found. The reason for this is a thermal disproportionation reaction, whereby one molecule functions as a donor and the other as an acceptor of two *H*-atoms.

The appearance of relatively abundant $[M + 2]^{+\bullet}$ signals is characteristic of the mass spectra of quinones. Conversely,



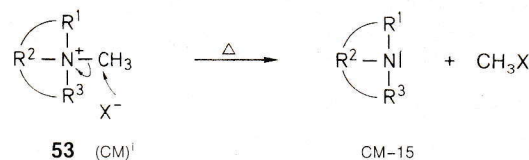
$[M - 2]^{+\bullet}$ signals are observed in the spectra of hydroquinones. Such peaks have not only been identified in the spectra of *o*-quinones, but also in those of *p*-quinones and quinone monoimines. It can be shown (by administering D_2O) that H_2O , which is present in the mass spectrometer, acts as an *H*-donor.

Pyrolysis of Quaternary Nitrogen Compounds

Protonated salts of organic bases easily decompose thermally into the bases and acids from which they were constituted. These compounds are then ionised independently under electron bombardment in the mass spectrometer. However, the analogous deprotonation of the thermally more stable quaternary nitrogen compounds cannot occur, because, in these compounds, the substituent bound to the nitrogen atom is an alkyl residue.

Under the conditions necessary for the recording of EI mass spectra, a salt cannot be brought into the vapour phase without first transforming the salt into neutral particles. This thermal transformation into neutral molecules occurs according to specific rules, which permit conclusions to be drawn about the relative molecular mass of the salt in question. Quaternary nitrogen compounds of the general formula **53** ($X = \text{halogen}$) can be transformed into neutral molecules in three ways.

Dealkylation. The anion attacks at the alkyl group of the quaternary *N*-atom with the formation of a tertiary amine (*nor-base*) and an alkyl halide:

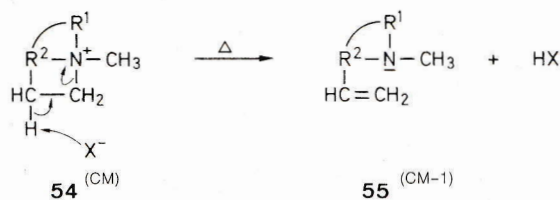


This type of degradation is particularly favoured by iodides, but occurs only rarely with fluorides.

¹ CM = cation mass

Thermal Hofmann elimination. The anion attacks at an *H*-atom which is β -positioned with respect to the quaternary *N*-atom **54**, whereby a tertiary amine (Hofmann base, **55**) and a hydrogen halide is formed.

Although with the thermal dealkylation of quaternary nitrogen compounds of the general type **53** only one possibility exists for demethylation, four Hofmann eliminations can, in principle, take place under favourable conditions (at least four *H*-atoms β -positioned with respect to the *N*⁺-atom and bound to different *C*-atoms). This number can be increased by vinyloous or ethyloous decomposition reactions. The thermal Hofmann elimination occurs primarily with fluorides and much less frequently with bromides and iodides.



A side reaction, which is frequently observed with thermal Hofmann eliminations, is the dimerisation of the Hofmann base, which sometimes gives rise to abundant $[2M]^+$ signals.

Substitution reactions. The anion attacks another *C*-substituent of the quaternary *N*-atom and forms the tertiary base **56**, which contains the anion. This rather rare reaction is related to an isomerisation, because the formula weight of the salt is exactly the same as the relative molecular weight of the pyrolysis product. [Under soft ionisation conditions, as, for example, with the field desorption or electrospray ionisation methods, the cations from onium compounds (ammonium-, sulfonium-, phosphonium salts) can be measured directly⁶.]

Transalkylation and Alkylation Reactions

When certain structural prerequisites are satisfied, the thermal transfer of an alkyl group (methyl, ethyl) from one functional group to another can occur.

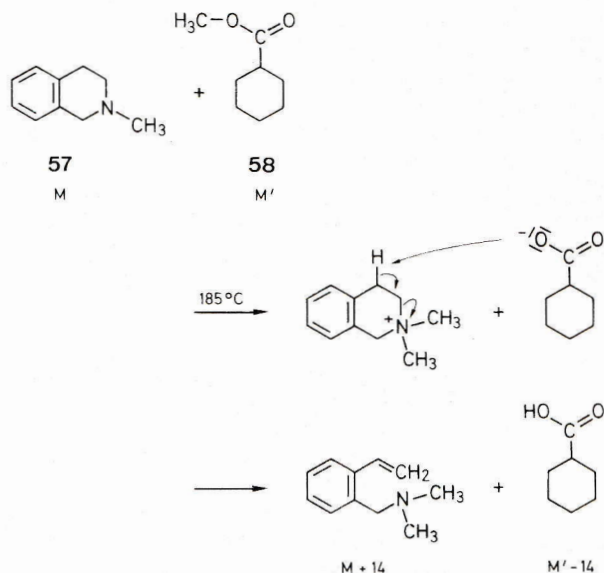
Examples of alkyl donors:



Examples of acceptors:



The reaction can proceed inter- or intramolecularly. In the case of intermolecular methylation, the $[M + 14]^+$, $[M' - 14]^+$ and, less frequently, the $[M + 28]^+$ and $[M - 28]^+$ signals are found, which correspond to a single and double reaction, respectively. The transfer of a CH_3 (15 amu) is then followed by the reverse transfer of an *H*-atom (or another alkyl group), as illustrated in the following example of the reaction of 2-methyl-1,2,3,4-tetrahydroisoquinoline (**57**; $M = 147$) with methyl cyclohexanoate (**58**; $M = 142$). Structural elements similar to **57** and **58** can be part of larger molecules and can therefore cause isomerisation reactions within the same molecule or between two molecules which have the same structure.



Once again it should be pointed out that thermal reactions are less often observed with small molecules; they occur more frequently with heavier molecules, especially when additional polar groups in the molecule hinder its vaporisation in the mass spectrometer, or when the decomposition is accelerated by accompanying impurities.

Furthermore, it should be mentioned that organometallic, organoboron and organosilicon compounds sometimes decompose easily, however their decomposition reactions are of a different nature to those described above.

5.2 Recognition of Thermal Reactions

As infrequent as it is that thermal reactions exhibit uniform behaviour, one can still give a generally valid procedure for their recognition. The fundamental aspects will be discussed below.

Preparative high vacuum distillation or sublimation. If the sample of the substance to be analysed is purified by a bulb-to-bulb distillation (or sublimation) in a glass vessel under high vacuum (at least 0.1 Pa) and the distillate (or sublimate) shows itself in thin layer chromatography to be identical with the undistilled material, then there is no reason to assume that the sample will decompose thermally upon introduction into the mass spectrometer. However, if this treatment of the sample results in the formation of one or more products that are different from the undistilled material, then this reaction can also occur in the mass spectrometer, although not necessarily so, because the vaporisation conditions in the mass spectrometer are considerably more favourable than in the distillation vessel (lower pressure, no condensation necessary, shorter flight path and therefore fewer wall reactions). If one also records the mass spectra of the distillation products and all of the signals due to the distillation products can be detected with the same relative abundance in the spectrum of the undistilled material, then it is probable (but not certain) that the same thermal reaction has also occurred in the mass spectrometer. If the reaction is, for example, a retro Diels-Alder reaction, which is a reaction that can occur thermally as well as mass spectrometrically, then the mass spectrum of the undistilled material can arise from different causes: pure thermal decomposition, pure mass spectrometric decomposition, or a mixture of both processes.

Recording of other mass spectra. If, instead of recording a spectrum at the usual 70 eV, it is recorded at 12 to 15 eV (a low-voltage spectrum), which is just above the ionisation potential of organic compounds, then, for energetic reasons, fragmentation reactions will be reduced and partially suppressed, whereas thermal reactions will remain unaffected (because the conditions necessary for such reactions have remain unchanged). If, for example, two new molecules arise from the thermal reaction, then these will be recorded as molecular ions. (However, this behaviour is not necessarily observed in all cases, because particularly energetically favourable fragmentation reactions can also occur at this low ionisation potential.) When soft ionisation methods are employed (see Sec. 8.9, p. 263), the ionisation conditions are significantly gentler on substances than those used for electron impact ionisation spectra. As a result, considerably fewer thermal reactions are observed under these conditions. However, it should be emphasised that even under the soft conditions required to record, for example, field ionisation and field desorption spectra, thermal reactions have still been found to occur.

Measurement of metastable transition signals. If the thermal reaction leads to the formation of two or more products, then it is not possible to find a transition signal (cf. Sec. 8.25, p. 277) between the "molecular ions" of the pyrolysis products, except if the process also occurs mass spectrometrically.

Recording of multiple spectra. If several mass spectra are recorded one after another under the same measurement conditions and the sample has decomposed in the direct inlet part of the mass spectrometer into at least two different transient compounds, then, when compared with each other, these spectra can exhibit significant differences in the abundances of the signals. The behaviour is similar to that of a mixture of substances in which the components possess different vaporisation temperatures. Newer instruments are equipped with an automatic temperature programmed mode, which allows a partial separation of components that have different volatilities.

Derivatisation. If the functional groups that are suspected of being in the substance are derivatised, then the molecular ion peak must shift by a definite mass difference. Examples: $-\text{COOH} \rightarrow -\text{COOCH}_3$ (+ 14 amu), $-\text{OH} \rightarrow \text{O}-\text{COCH}_3$ (+ 42 amu), $-\text{CH}=\text{CH} \rightarrow -\text{CH}_2-\text{CH}_2-$ (+ 2 amu), see Tab. 4.8 (p. 279). If the relative molecular mass of the starting material is determined to be, for example, $M^{+\bullet}$ and one finds $[M + 58]^{+\bullet}$ for the methyl ester, then the starting material has lost CO_2 (44 amu) during a thermal reaction (decarboxylation). The hydrogenation of a double bond can, for example, prevent a (thermal) retro Diels-Alder reaction.

Analysis of the fragmentation pattern. Sometimes a mass spectrum is obtained which does not appear to be in agreement with the structure of the compound under investigation (see, e.g., 48, p. 248), or there are mass differences between abundant signals and the heaviest ion (possibly the molecular ion) which cannot be explained by fragmentation reactions (e.g. $M-14$, $M-20$), or which can only be explained with great difficulty. In such cases a mixture is probably present, which was either already present from the outset or has arisen through a thermal reaction.

5.3 Prevention of Thermal Reactions in the Mass Spectrometer

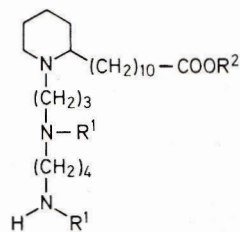
For the prevention of thermal reactions, it is important to recognise their cause. In a few cases, the purification of a sample (recrystallisation, filtration) is sufficient to stabilise it. Frequently, however, functional groups are responsible, either directly (e.g. $-\text{COOH}$, $-\text{CH}=\text{CH}-$) or indirectly (by increasing the vaporisation temperature), for the occurrence of a thermal reaction. If this is the case, a derivatisation (which thereby also alters the molecular weight) is unavoidable. The methods for the modification of functional groups are well known (e.g. esterification, reduction, hydrogenation, ether formation). In addition, the procedures to aid the volatilisation of organic substances will be assumed to be known, because

they are also required for gas chromatographic analysis. Suitable derivatives are, among others, for **hydroxy groups**: methyl ethers, trimethylsilyl ethers, acetanides, acetates; for **carboxyl groups**: methyl esters; for **amino groups**: acetamides, trifluoroacetamides, *N,N*-dimethylamides.

As an example of the prevention of thermal decomposition reactions by increasing the volatility, the triaminocarboxylic acid **59** ($M = 397$) is described.

In the spectrum of **59**, one can only observe a general decomposition of the compound. After esterification ($\text{CH}_3\text{OH}/\text{HCl}$) and acetylation [$(\text{CH}_3\text{CO})_2\text{O}/\text{pyridine}$], one obtains the derivative **60** ($M = 495$), which produces a mass spectrum that can be analysed readily.

The following methods, among others, should also be referred to: the CI (see Sec. 8.1, p. 258), FI (see Sec. 8.6, p. 261), FD



59 $R^1 = R^2 = \text{H}$

60 $R^1 = \text{COCH}_3$, $R^2 = \text{CH}_3$

(see Sec. 8.5, p. 261) and FAB (see Sec. 8.4, p. 260) techniques and the method of cation addition spectroscopy (see Sec. 8.10, p. 265). These soft ionisation methods allow compounds to be analysed with a lower probability of thermal decomposition.

6. Mass Spectra of Contaminated Samples and Mixtures

Because there is no fundamental difference, but rather a quantitative one, between a contaminated sample and a mixture, these concepts will not be discussed separately. In order to be able to obtain clear and correct evidence from the mass spectrum of a compound, the sample of the substance must be homogeneous, i.e. pure. This statement is valid without exception, although the majority of samples analysed in a mass spectrometry laboratory unfortunately do not meet these requirements. If contaminated samples are submitted, it is important to know what consequences this can have for the mass spectrometric analysis.

If, for reasons of its volatility, a sample is introduced into the instrument through the gas inlet system, then all volatile components in the sample will be transferred into the reservoir of the mass spectrometer. This has the result that each of the individual components of the sample are ionised independently from one another, but simultaneously, in the ion source and produce superimposed mass spectra. Certain conclusions can indeed be drawn from the spectra, however a quantitative analysis of the composition of the mixture is not possible as long as the structures of the components are unknown and a calibration has not been carried out. With a two component mixture, for example, no conclusions can be drawn about the ratio of the quantities of the individual components, because of the different ionisation probabilities and partial pressures and, as a result, the very different relative abundances of the molecular ion signals. To illustrate this, Fig. 4.37 shows the mass spectrum of *N,N*-diethyl-1,3-propanediamine (**61**) with "traces" of 1,3-diethylperhydropyrimidine (**62**) and 1,3-diethyl-2-methylperhydropyrimidine (**63**). This mixture of substances resulted when purest *N,N*-diethyl-1,3-propanediamine (**61**) was dissolved in denatured ethanol (methylated spirits) and

finally evaporated to dryness. The residue produced the illustrated spectrum in which 0.3% of compound **62** gives a molecular ion signal with the same abundance as that from 99.4% of **61**. See also Sec. 6.2, p. 252.

The GC/MS combination is a great help for the analysis of volatile mixtures (see Sec. 8.11, p. 266). For various reasons, however, it is often unavoidable that analyses have to be carried out on sample mixtures that are either difficult to vaporise or cannot be vaporised. For this purpose, in addition to the LC/MS technique, tandem mass spectrometry (see Sec. 8.22, p. 275) coupled with a suitable ionisation procedure can be useful.

If the direct inlet system is used for mixtures of substances, one obtains spectra that are different from those acquired when the gas inlet system is employed. Because the sample is heated in a crucible, the more volatile components will be vaporised first, followed by the less volatile and finally the least volatile components. If two components have the same or very similar vaporisation characteristics, they will be ionised at the same time. Mass spectra are recorded at regular time intervals until all of the components have been vaporised. Depending on the vaporisation characteristics of the components, these spectra overlap to a greater or lesser degree. In the ideal case one can obtain spectra of the pure components, however mixed spectra are normally observed. Because a certain amount of time is necessary to record a mass spectrum and this time can coincide with a sharp decline in the presence of a component, these mixed spectra frequently do not correspond to a clean addition of the spectra of the individual components. Neither quantitative statements nor calibrations are possible in this case. In connection with this it is perhaps