NEPHAR 407 PHARMACEUTICAL CHEMISTRY III LAB

MASS SPECTROMETRY (MS)

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Analytical Chemistry

Instrumental Methods

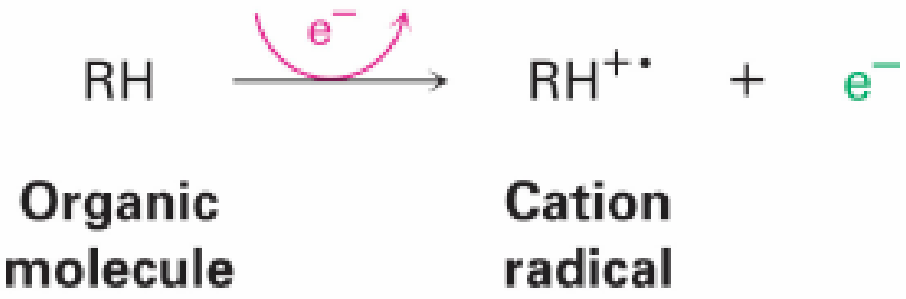
- Spectroscopy
  - NMR
  - Optical Absorption
  - Microwave
  - Optical Emission
- Mass Spectrometry
  - FT-ICR
  - TOF
  - Quadrupole
  - Ion Trap
  - Linear Trap
  - Magnetic Sector

Chemical Methods

- Titration
- Gravimetric Analysis
- Solution Chemistry

Mass Spectrometry (MS)

- Measures molecular weight
- Sample vaporized and subjected to bombardment by electrons that remove an electron
  - Creates a cation-radical
- Bonds in cation radicals begin to break (fragment)
- Charge to mass ratio is measured (see next page)



Mass Spectrometry

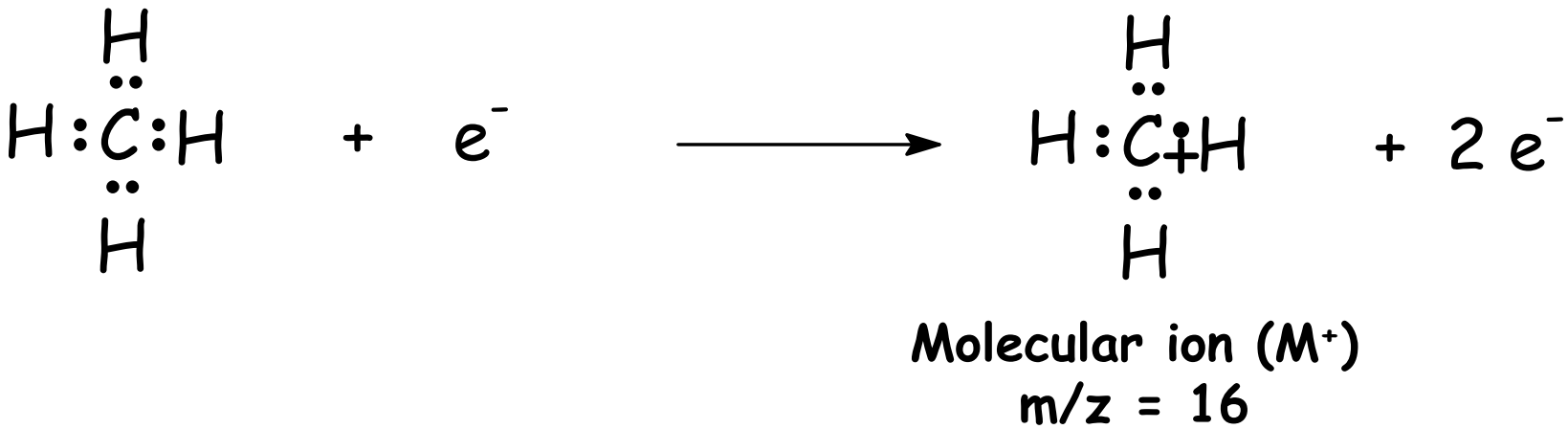
- Molecular weight can be obtained from a very small sample.
- Mass spectrometry (MS) is NOT a form of spectroscopy because it does not involve the absorption or emission of light.
- A beam of high-energy electrons breaks the molecule apart.
- The masses of the fragments and their relative abundance reveal information about the structure of the molecule.

Mass Spectrometry

- Molecules should enter the vapor phase (can be difficult)
- Produce ions from the molecules that enter the gas phase
- Separate the ions according to their mass-to-charge ratios (m/z)
- Measure and record relative abundance of theses ions

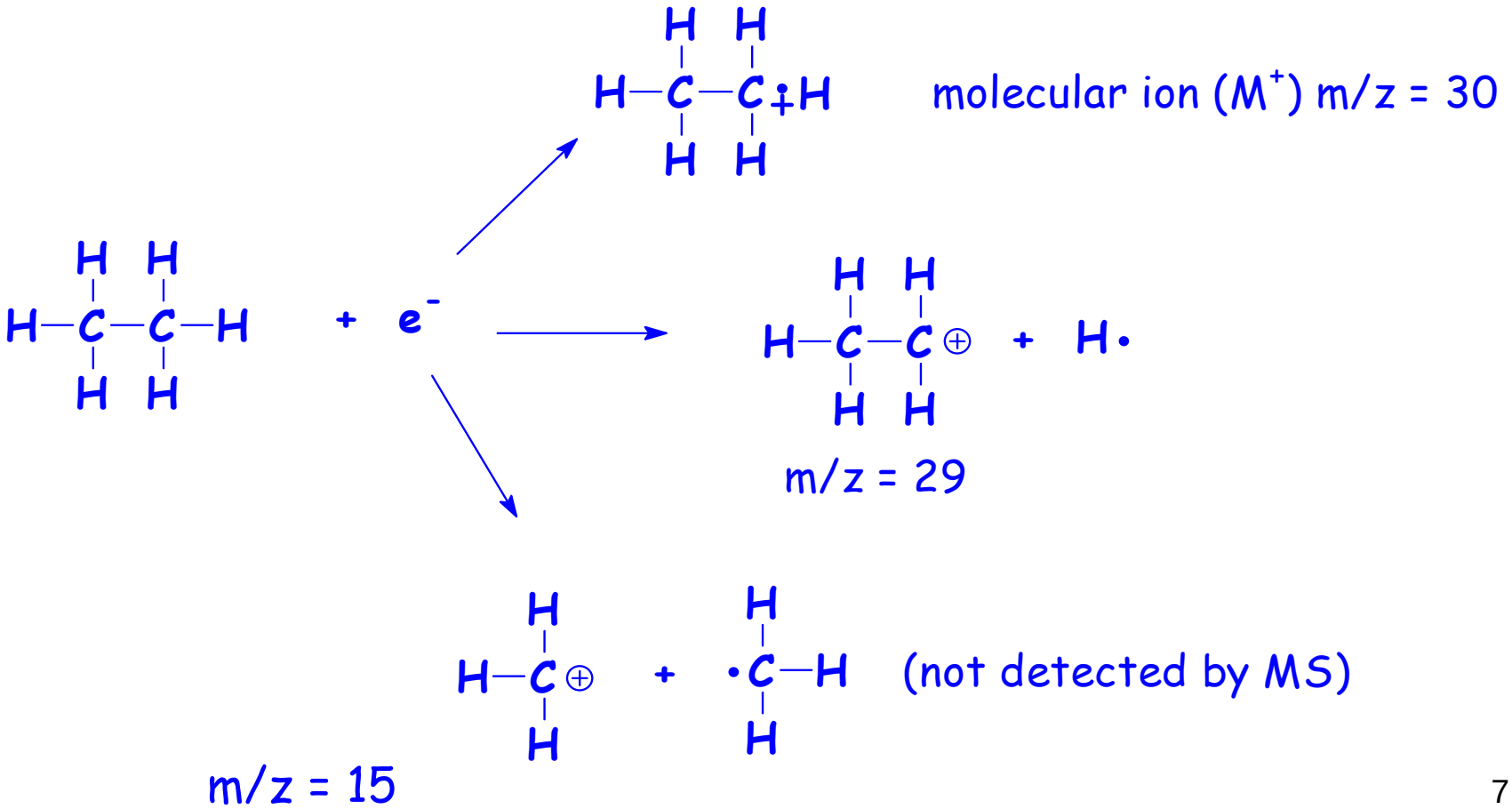
Background

- The impact of a stream of high energy electrons causes the molecule to lose an electron forming a **radical cation.**
  - A species with a positive charge and one unpaired electron

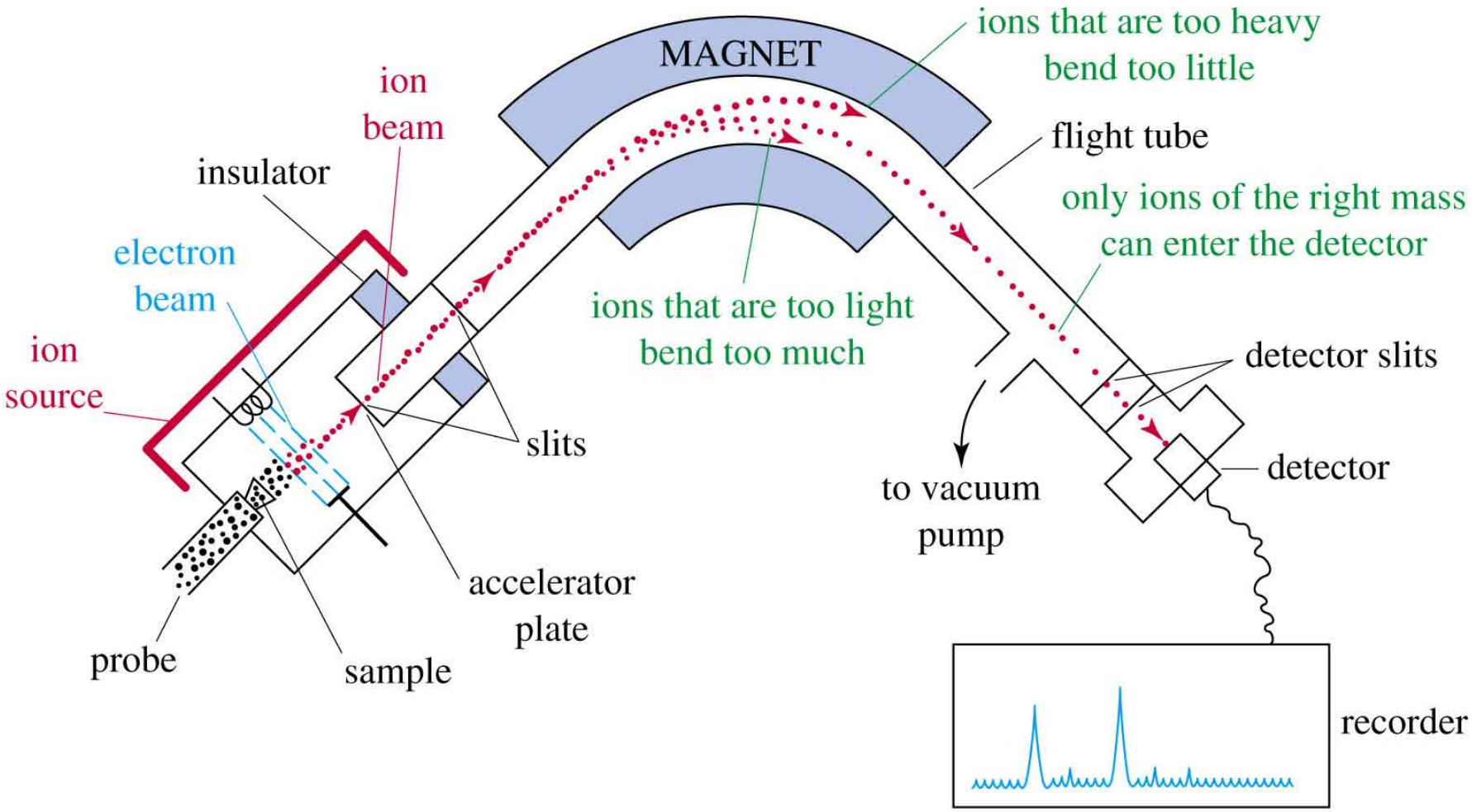


Background

- The impact of the stream of high energy electrons can also break the molecule or the radical cation into fragments.



Mass Spectrometer



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Background

- Only cations are detected.
  - **Radicals are "invisible" in MS.**

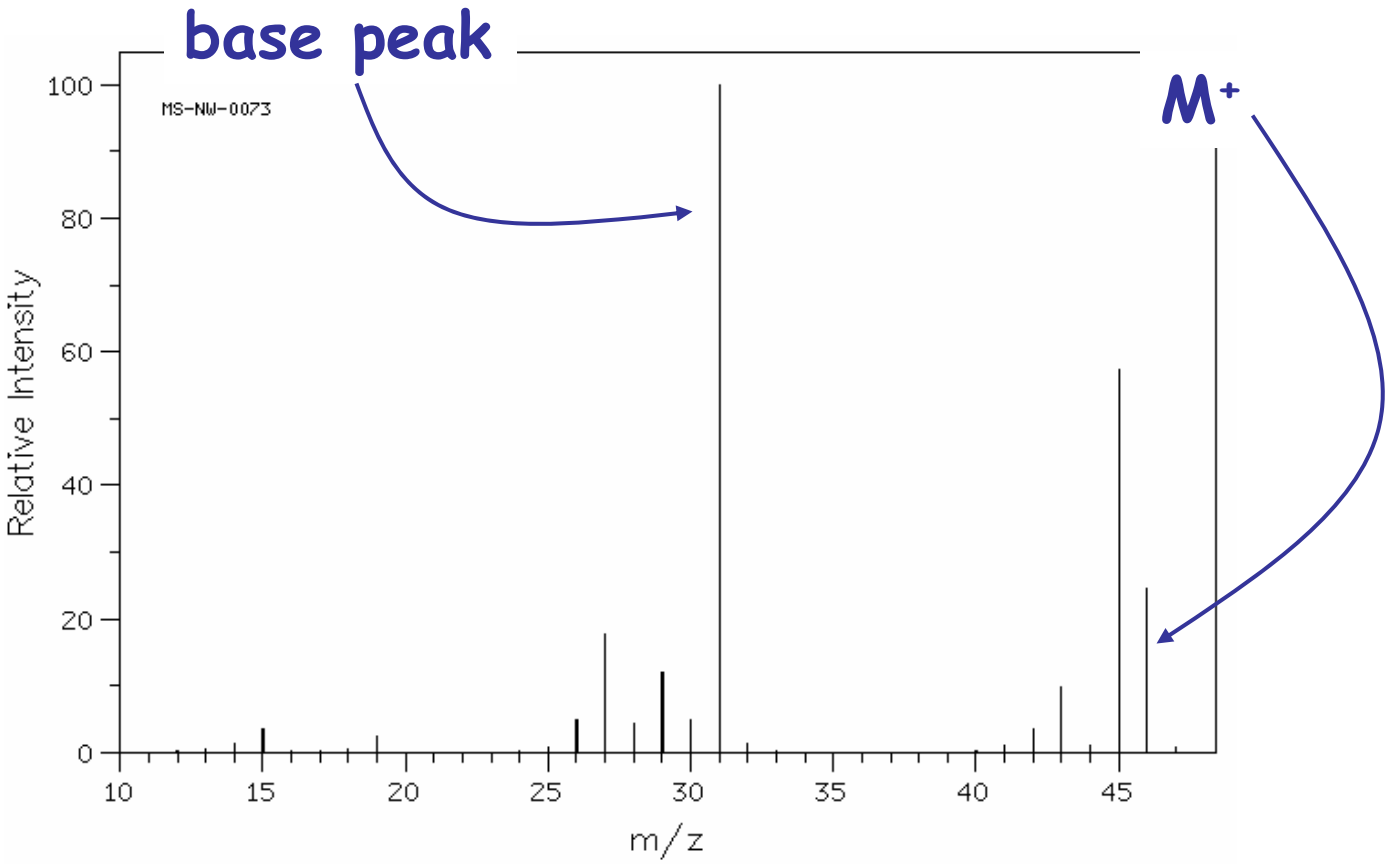
- The amount of deflection observed depends on the mass to charge ratio (m/z).
  - Most cations formed have a charge of +1 so the amount of deflection observed is usually dependent on the mass of the ion.
- The resulting mass spectrum is a graph of the mass of each cation vs. its relative abundance.

- The peaks are assigned an abundance as a percentage of the **base peak.**
  - **the most intense peak in the spectrum**

- <u>The base peak is not necessarily the same as the parent ion peak.</u>

Background

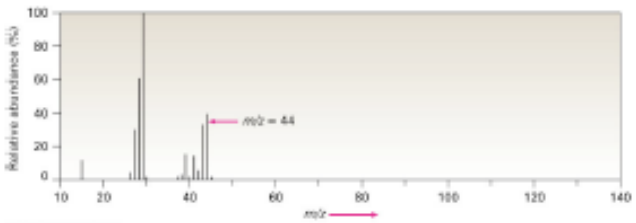
The mass spectrum of ethanol



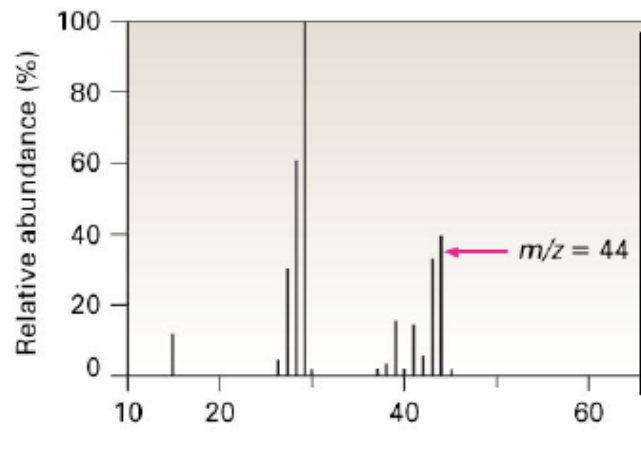
SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

The Mass Spectrum

- Plot mass of ions (m/z) (x-axis) versus the intensity of the signal (roughly corresponding to the number of ions) (y-axis)
- Tallest peak is **base peak** (100%)
  - Other peaks listed as the % of that peak
- Peak that corresponds to the unfragmented radical cation is **parent peak** or **molecular ion (M^+)**



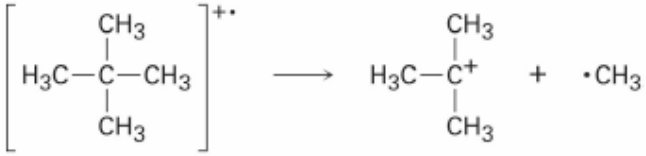
The Mass Spectrum



m/z	Relative abundance
45	0.33
44	30
43	34
41	14
39	18
29	100
28	60
27	30
15	10

Interpreting Mass Spectral Fragmentation Patterns

- The way molecular ions break down can produce characteristic fragments that help in identification
  - Serves as a "fingerprint" for comparison with known materials in analysis (used in forensics)
  - Positive charge goes to fragments that best can stabilize it



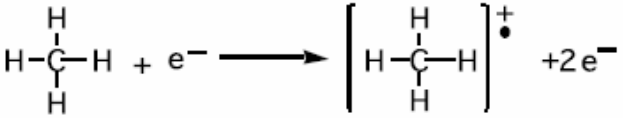
m/z = 57

Electron Ionization MS

In the ionization chamber, the sample is bombarded with a beam of high-energy electrons (70 eV).

1 eV = 96.49 kJ/mol (23.05 kcal/mol)
70 eV = 6754.3 kJ/mol (1613.5 kcal/mol)

This collision between electrons and the sample molecule results in the loss of an electron and in the formation of a radical cation.



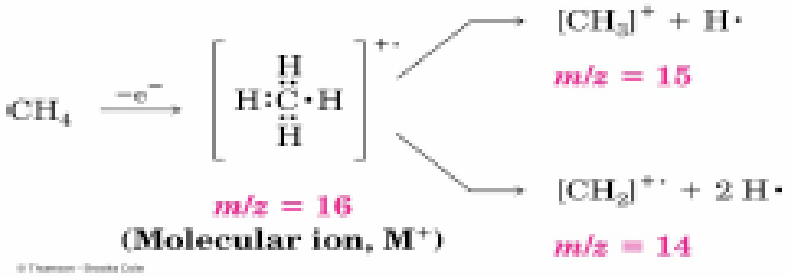
Molecular ion (a radical cation)

Molecular Ion

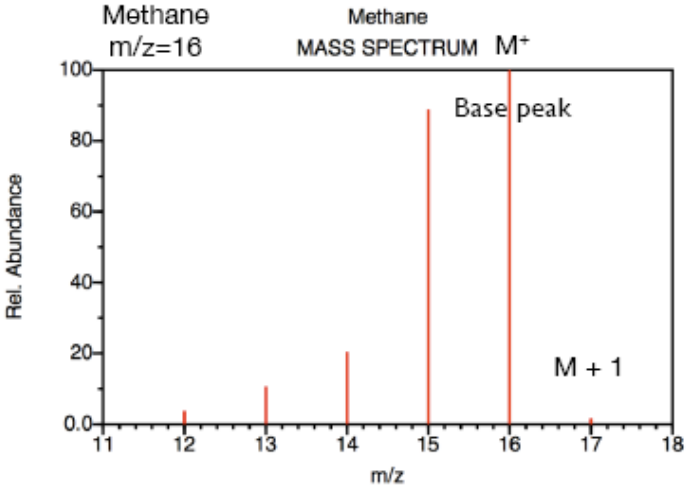
- **Molecular ion (M^+):** the species formed by removal of a single electron from a molecule
- For our purposes, it does not matter which electron is lost; radical cation character is delocalized throughout the molecule. Therefore, we write the molecular formula of the parent molecule in brackets with
  - a plus sign to show that it is a cation
  - a dot to show that it has an odd number of electrons

Example : Methane

- Methane produces a parent peak (m/z = 16) and fragments of 15 and 14
- The MS of propane is more complex since the molecule can break down in several ways

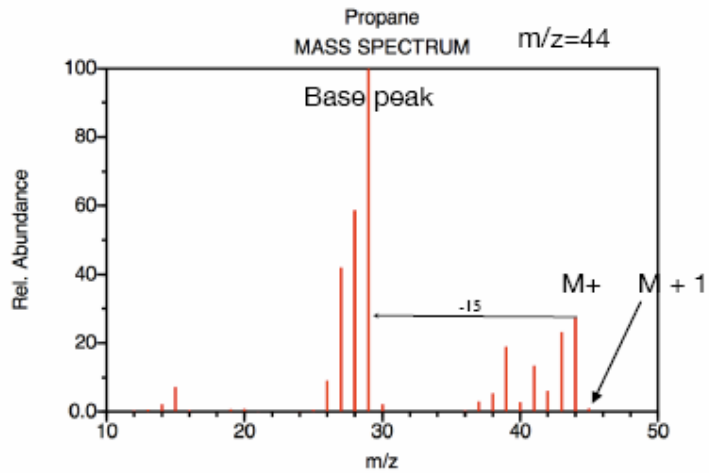


Methane



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Propane



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Mass Spectrum and Ion Fragments

In addition to stripping electrons, the energy used in MS is more than enough to break the bonds in organic molecules. (70 eV is actually a little overkill!)

This yields all combinations of **neutral and ionized fragments**.

These ionized fragments give rise to peaks in a mass spectrum (each representing a different mass), including the **molecular ion**.

In addition to the **molecular ion**, a **base peak** also appears in a mass spectrum. The base peak is the most INTENSE peak in the spectrum (normalized to a relative abundance of 100), and all other peaks/ fragments are described as a percentage of the base peak.

The **base peak** can be the same as the **molecular ion** or is a fragment of the **molecular ion**.

✓If some of the molecular (parent) ions remain intact long enough (about 10^{-6} seconds) to reach the detector, we see a molecular ion peak.

For example, methanol forms a molecular ion:

$$CH_3OH + e \longrightarrow CH_3OH^{+\cdot} + 2e$$

m/z= 32

Examples of possible fragmentation of methanol are given in below:

$$CH_3OH^{+\cdot} \longrightarrow CH_2OH^{+} + H^{\cdot}$$

m/z= 31

$$CH_3OH^{+\cdot} \longrightarrow CH_3^{+} + OH^{\cdot}$$

m/z= 15

$$CH_2OH^{+\cdot} \longrightarrow CHO^{+} + H_2$$

m/z= 29

- The positive ions are expelled from the ionisation chamber and resolved by means of a magnetic or an electric field.

- The mass spectrum is a record of the current produced by these ions as they arrive at a detector. The intensity of a peak in the spectrum is thus an indication of the relative number of ions; the larger peak the more abundant the ion producing it.

Interpreting Mass Spectra

- Molecular weight from the mass of the molecular ion
- Double-focusing instruments provide high-resolution "exact mass"
  - 0.0001 atomic mass units – distinguishing specific atoms
- Example MW "72" is ambiguous: C_5H_{12} and C_4H_8O but:
  - C_5H_{12} 72.0939 amu exact mass C_4H_8O 72.0575 amu exact mass
  - Result from fractional mass differences of atoms ^{16}O = 15.99491, ^{12}C = 12.0000, ^{1}H = 1.00783
- Instruments include computation of formulas for each peak

- If parent ion not present due to electron bombardment causing breakdown, "softer" methods such as chemical ionization are used
- Peaks above the molecular weight appear as a result of naturally occurring heavier isotopes in the sample
  - (M+1) from ^{13}C that is randomly present

Determination of molecular formula

- Most of the common elements found in organic compounds have naturally occurring heavier isotopes. For three of the elements –C, H, N, the principal heavier isotope is one mass unit greater than the most common isotope. The presence of these elements in a compound gives rise to a small isotopic peak with mass one unit greater than the molecular ion (at $\mathbf{M^{+\cdot}+1}$).

- For four of the elements (*oxygen, sulfur, chlorine, and bromine*) the principal heavier isotope is two mass units greater than the most common isotope. The presence of these elements in a compound gives rise to an isotopic peak at $\mathbf{M^{+\cdot}+2}$.

$M^{+\cdot}$+ 1 Elements: C, H, N;

$M^{+\cdot}$+ 2 Elements: O, S, Br, Cl

Interpreting Mass Spectra

- The only elements to give significant M + 2 peaks are Cl and Br. If no large M + 2 peak is present, these elements are absent
- Is the mass of the molecular ion odd or even?
- Nitrogen Rule: if a compound has
  - zero or an even number of nitrogen atoms, its molecular ion will appear as a even m/z value
  - an odd number of nitrogen atoms, its molecular ion will appear as an odd m/z value

Isotopes

- Carbon, for example, in nature is 98.90% ^{12}C and 1.10% ^{13}C. Thus, there are 1.11 atoms of carbon-13 in nature for every 100 atoms of carbon-12

$$\frac{(M+1)/M}{1.1} \times 100 = \text{maximum number of carbons (degree of unsaturation)}$$

- What is the maximum number of carbons a unknown substance can have if the relative abundance of the parent peak is 40 and the M + 1 peak, 1.4?

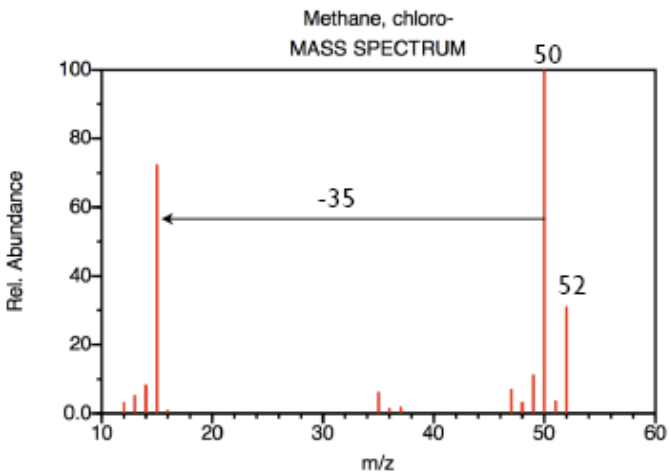
$$\frac{(M+1)/M}{1.1} \times 100 = \text{maximum number of carbons (degree of unsaturation)}$$

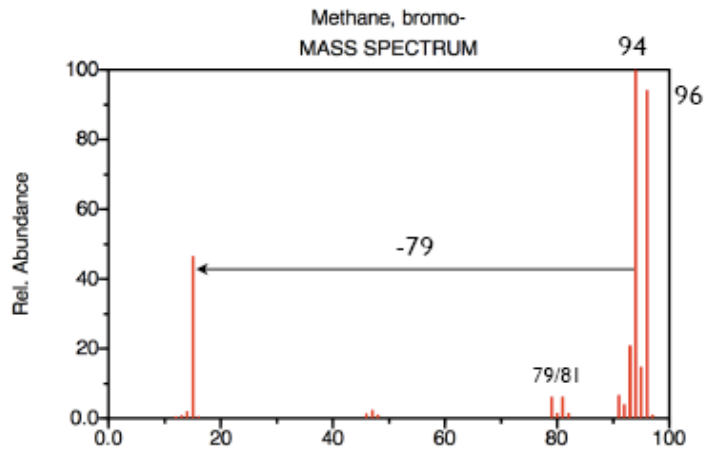
$$\frac{(1.4)/40}{1.1} \times 100 = 3.18$$

$\therefore$ maximum number of carbons = 3

M+1 and M+2 Peaks

- The most common elements giving rise to M + 2 peaks are chlorine and bromine
- Chlorine in nature is 75.77% ^{35}Cl and 24.23% ^{37}Cl
  - a ratio of M to M + 2 of approximately 3:1 indicates the presence of a single chlorine in a compound
- Bromine in nature is 50.7% ^{79}Br and 49.3% ^{81}Br
  - a ratio of M to M + 2 of approximately 1:1 indicates the presence of a single bromine in a compound





Principal Stable Isotopes of Common Elements

Element	Most common isotope		Natural Abundance of Other Isotopes (based on 100 atoms of most common isotope)			
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	0.016		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100	^{37}Cl	32.5		
Bromine	^{79}Br	100	^{81}Br	98.0		
Iodine	^{127}I	100				

➢If chlorine, bromine, sulfur or silicon is present, the M+2 peak will be more intense depending on the atom. A compound that contains one chlorine atom will have an M+2 peak approximately one-third the intensity of the molecular ion peak because of the presence of a molecular ion containing the ^{37}Cl isotope.

➢A compound that contains one bromine atom will have an M+2 peak almost equal in intensity to the molecular ion containing the ^{81}Br isotope. A compound that contains two chlorines, or two bromines, or one chlorine an one bromine will show a distinct M+4 peak, in addition to the M+2 peak.

➢Three chlorine atoms in a molecule will give peaks at M+2, M+4 and M+6. The relative abundances of the peaks (molecular ion, M+2, M+4, and so on) have been calculated by Beynon et al. For compounds containing chlorine and bromine. Similarly, the relative abundances of the isotope peaks can be calculated by equation shown below;

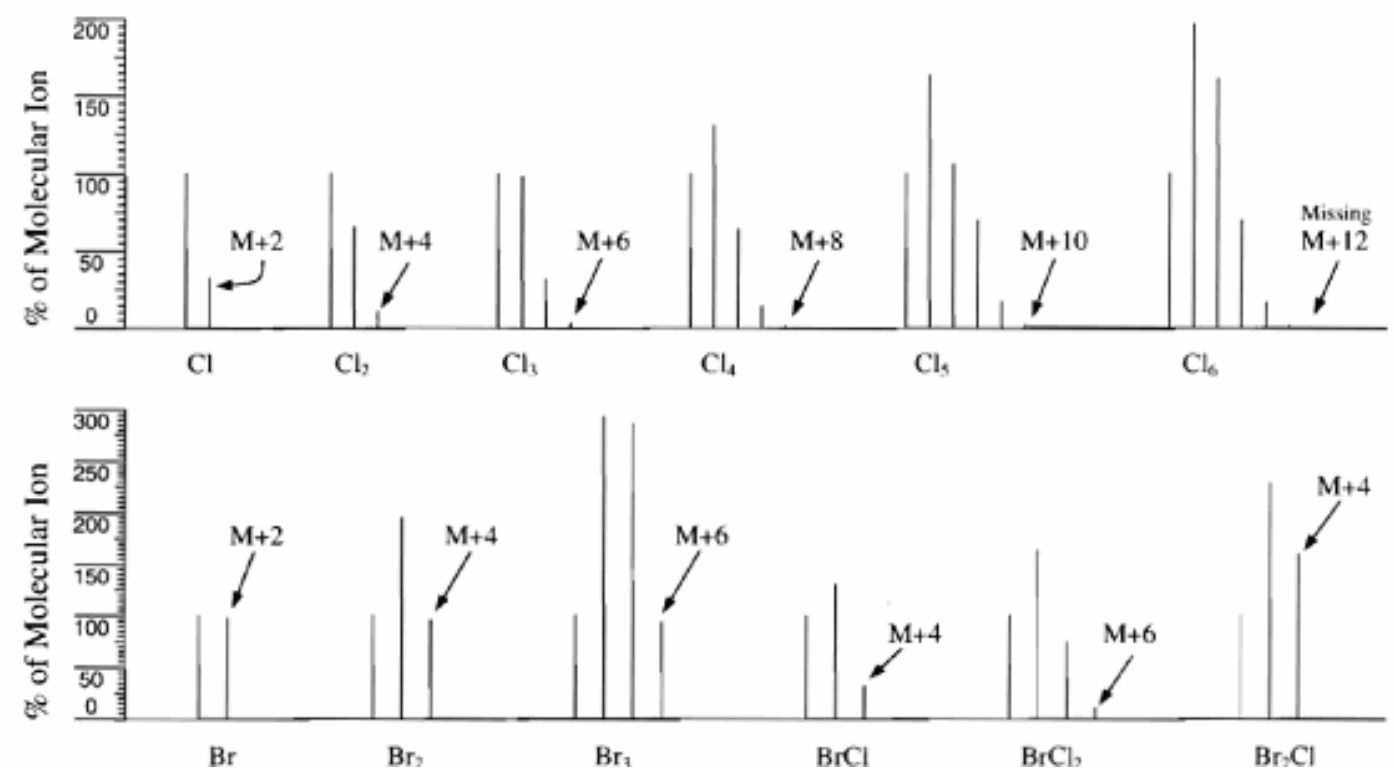
$$\mathbf{(a+b)^n}$$

a: the relative amount of heavier isotope,
b: the relative amount of lighter isotope, **n:** the number of halogen in the molecule.

Intensities of Isotope Peaks (Relative to the Molecular Ion) for Combination of Chlorine and Bromine

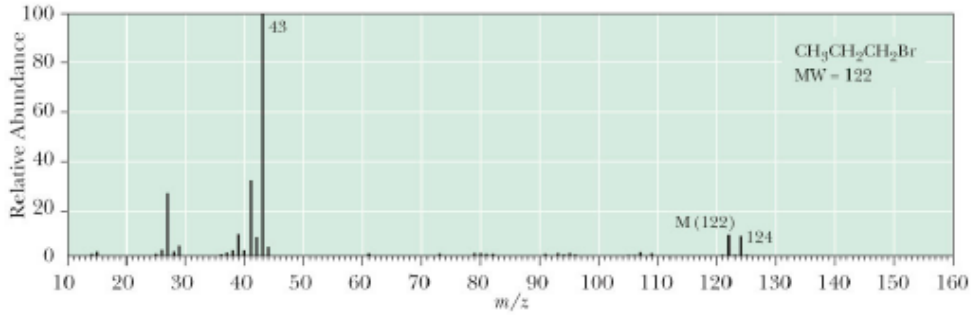
Halogen Present	% M+2	% M+4	% M+6	% M+8	% M+10	% M+12
Cl	32.6					
Cl_2	65.3	10.6				
Cl_3	97.8	31.9	3.5			
Cl_4	131.0	63.9	14.0	1.2		
Cl_5	163.0	106.0	34.7	5.7	0.4	
Cl_6	196.0	161.0	69.4	17.0	2.2	0.1
Br	97.9					
Br_2	195.0	95.5				
Br_3	293.0	286.0	93.4			
BrCl	130.0	31.9				
$BrCl_2$	163.0	74.4	10.4			
Br_2Cl	228.0	159.0	31.2			

➢Predicted patterns of M, M+2, M+4…… for compounds with various combinations of chlorine and bromine



Mass Spectrum of Bromopropane

Chlorine and bromine each have two naturally abundant isotopes that can be seen in mass spectra.



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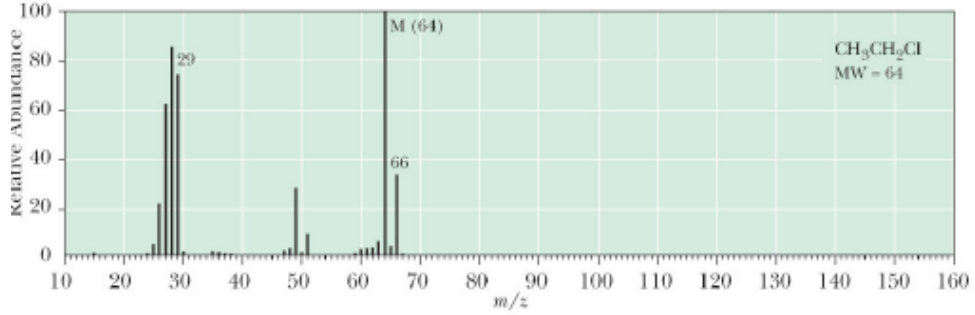
[M+2] Peaks

Commonly, the molecular ion created by the lesser abundant natural isotope is called the [M+2] peak.



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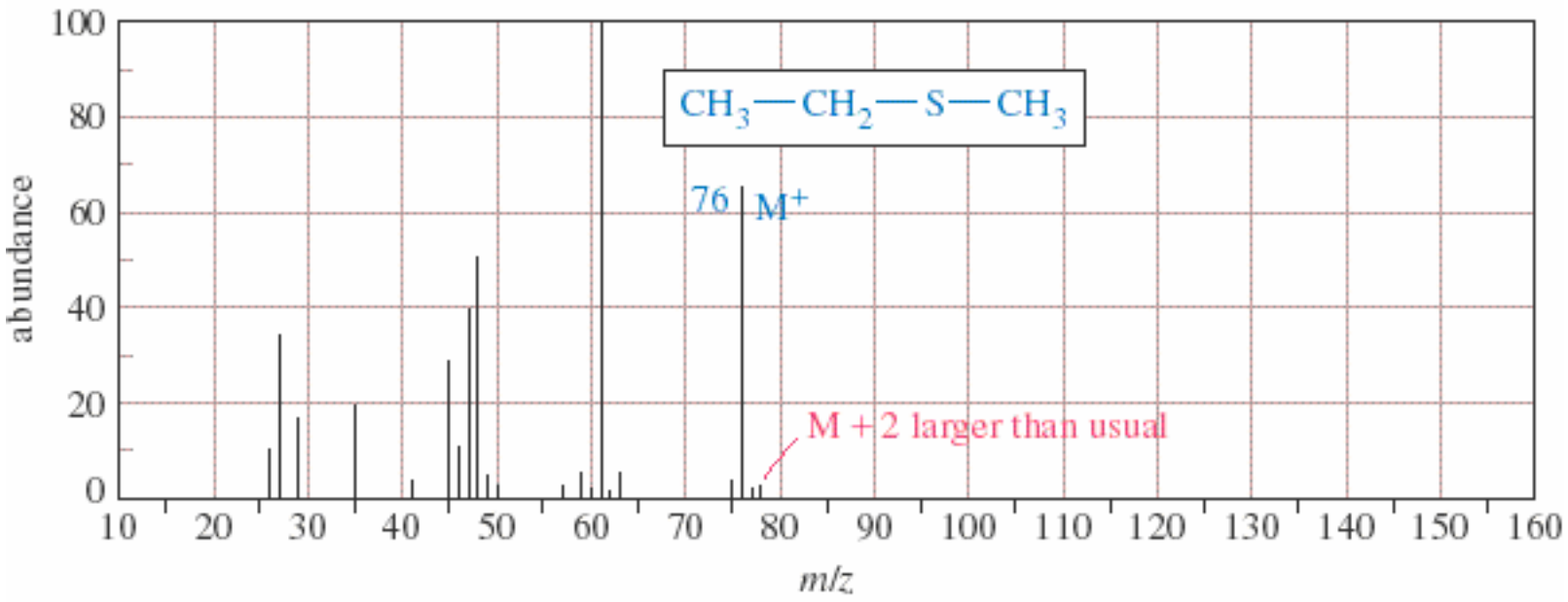
[M+1] and [M+2] Peaks

Sulfur is the only other element common to organic compounds that gives a significant [M+2] peak.

^{32}S = 95.02% and ^{34}S = 4.21%

[M+1] peaks are relatively low in intensity compared to the molecular ion peak and are often difficult to measure with any precision. They are not commonly used for accurate determinations of molecular weight. If an [M+1] peak does appear, most likely it is caused by the presence of ^{13}C in the molecule.

Mass Spectrum with Sulfur



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Example: MS Data of an unknown compound

m/z	Intensity (as percent of base peak)	m/z	Intensity (as percent of $M^{+\cdot}$)	
27	59.0	**72**	**$M^{+\cdot}$**	73.0 / 73 x 100 = **100.0**
28	15.0	**73**	**$M^{+\cdot}$+1**	3.3 / 73 x 100 = **4.5**
29	54.0	**74**	**$M^{+\cdot}$+2**	0.2 / 73 x 100 = **0.3**
39	23.0		***Recalculated to base on $M^{+\cdot}$***	
41	60.0			
42	12.0			
43	79			
44	100.0 (base)			
72	73.0 ($M^{+\cdot}$)			
73	3.3			
74	0.2			

Then we use following guides to determine the molecular formula:

- Is $M^{+\cdot}$ odd or even?

According to ***nitrogen rule, if it is even, then the compound must contain an even number or no nitrogen atoms or if it is odd, then the compound must contain an odd number of nitrogen atoms.***

- For unknown, M+. is even. The compound must have an even number or zero nitrogen.

The relative abundance of the $M^{+\cdot}$ peak indicates the number of carbon atoms.

Number of carbon atoms = relative abundance of ($M^{+\cdot}$ + 1) / 1.1.

For our unknown, number of C atoms= 4.5 / 1.1 = 4

(This formula works because ^{13}C is the most important contributor to the $M^{+\cdot}$ + 1 peak and the approximate natural abundance of ^{13}C is 1.1%).

- The relative abundance of the $M^{+\cdot}$ + 2 peak indicates the presence (or absence) of S (4.4%), Cl (33%) or Br (98%)

For unknown, $M^{+\cdot}$ + 2 = 0.3%; thus, we can assume that S, Cl, and Br are absent.

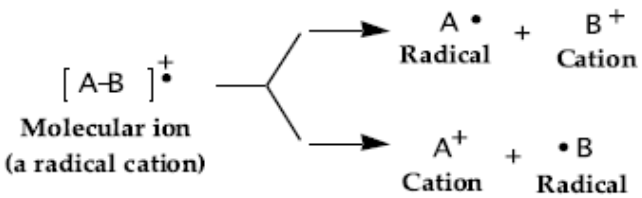
The molecular formula can now be established by determining the number of hydrogen atoms and adding the appropriate number of oxygen atoms, if necessary

Molecular Formula C4H8O

Fragmentation of the Molecular Ion

The energy used (~70 eV) in MS is sufficient to not only dislodge one or more electrons from a molecule, but also cause extensive fragmentation. These fragments may be unstable and in turn break apart to even smaller fragments.

Fragmentation of a molecular ion, $M^{+\bullet}$, produces a radical and a cation. Only the cation is detected by MS -- <u>not the neutral radical</u>.



Fragmentation and Structure

How fragments are formed can be partially explained by the relative **stabilities of carbocations**!

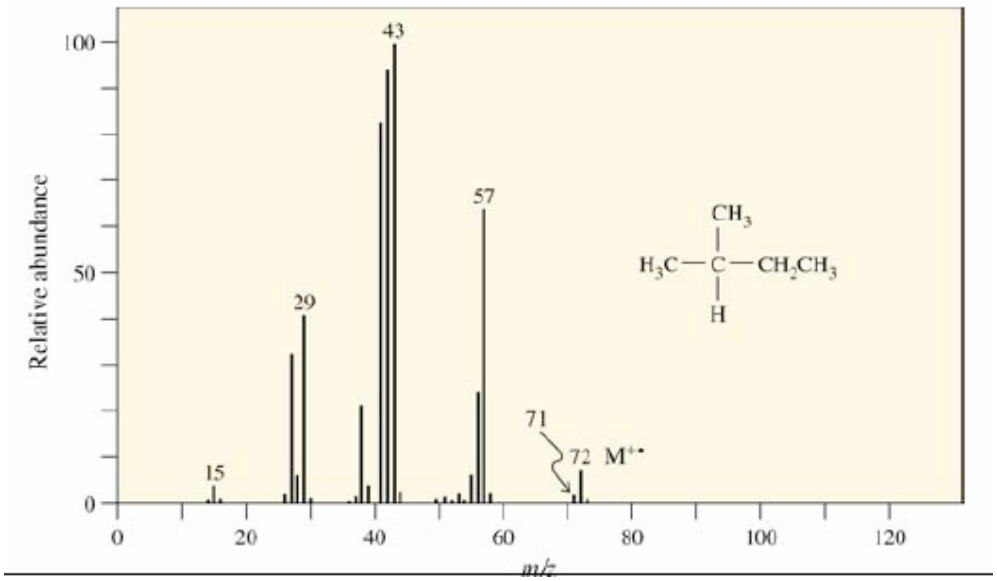
Where fragmentation can occur to form a new cation, the mode that gives the most stable cation is favored!

In case you forgot...
... the trend for carbocation stability is as follows...

$$CH_3^+ < 1° < \begin{matrix} 2° \\ 1° \text{ allylic} \\ 1° \text{ benzylic} \end{matrix} < \begin{matrix} 3° \\ 2° \text{ allylic} \\ 2° \text{ benzylic} \end{matrix} < \begin{matrix} 3° \text{ allylic} \\ 3° \text{ benzylic} \end{matrix}$$

Fragmentation Pattern of 2-methylbutane

Fragmentation frequently occurs at substituted carbons due to the stability of the resulting cation.

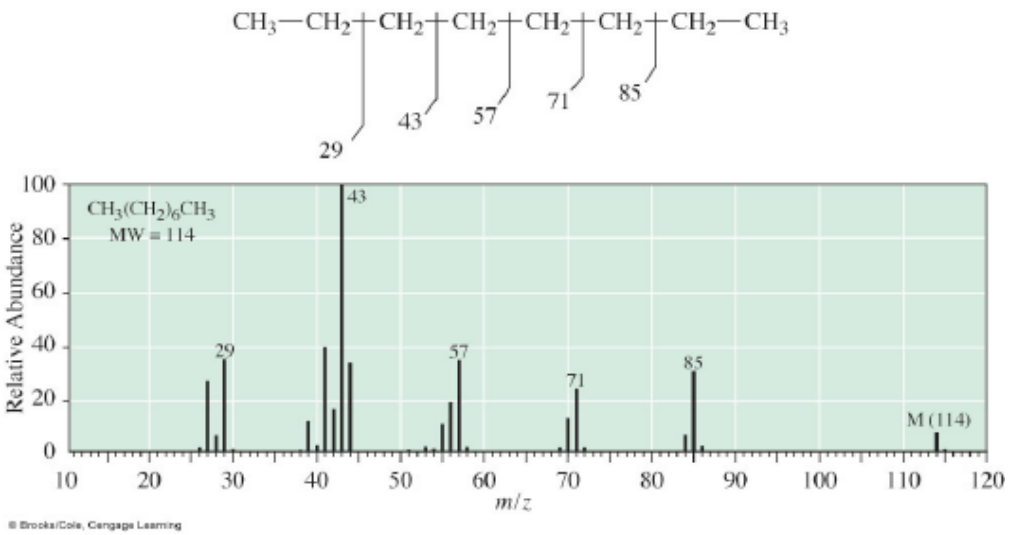


1. The relative height of the molecular ion peak is greatest for the straight-chain compound and decreases as the degree of branching increases (look at rule 3)
2. The relative height of the molecular ion peak usually decreases with increasing molecular weight in a homologous series. Fatty esters appear to be an exception.
3. Cleavage is favored at alkyl-substituted carbon atoms: the more substituted, the more likely is cleavage. This is a consequence of the increased stability of a tertiary carbocation over a secondary, which in turn is more stable than a primary.

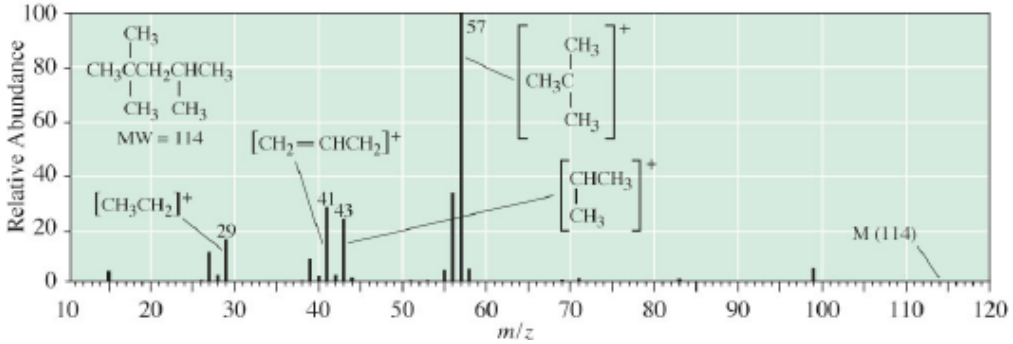
Fragmentation Patterns

- Alkanes
  - Fragmentation often splits off simple alkyl groups:
    - Loss of methyl $M^+ - 15$
    - Loss of ethyl $M^+ - 29$
    - Loss of propyl $M^+ - 43$
    - Loss of butyl $M^+ - 57$
  - Branched alkanes tend to fragment forming the most stable carbocations.

Fragmentation of Alkanes: MS of Octane



Fragmentation of Alkanes: MS of 2,2,4-trimethylpentane

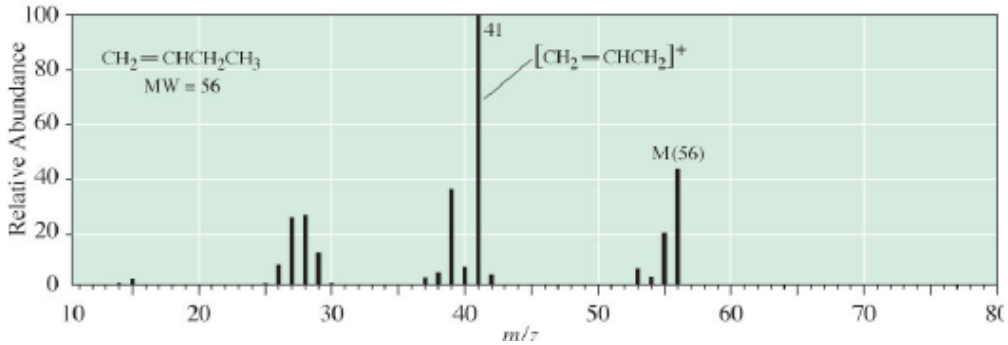


Fragmentation of Alkenes

Alkenes normally exhibit the following during fragmentation:

- a strong molecular ion peak
- cleavage to form resonance stabilized-allylic cations

$$[CH_2{=}CHCH_2CH_3]^{+\bullet} \longrightarrow [CH_2{=}CHCH_2]^+ + \bullet CH_3$$

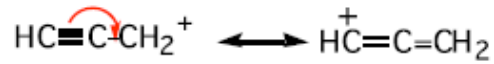


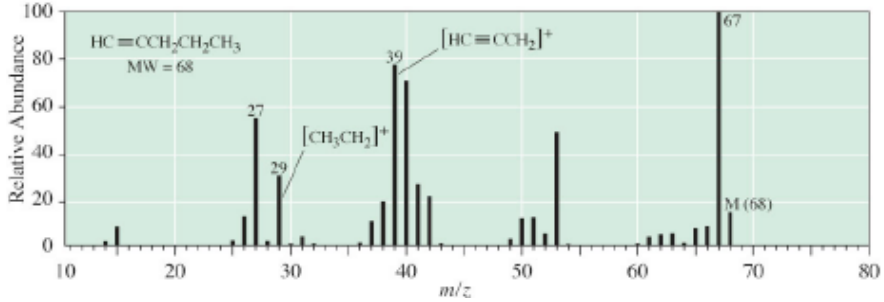
Fragmentation of Alkynes

Alkynes normally exhibit the following during fragmentation:

- shows a strong molecular ion peak
- cleavage to form resonance-stabilized propargyl cation

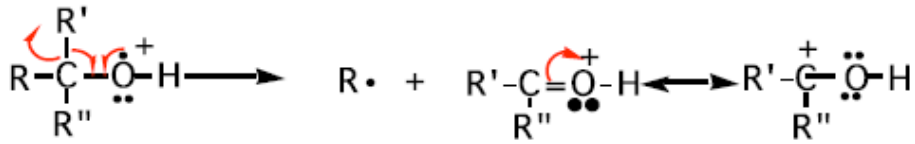
3-Propynyl cation (Propargyl cation)





Fragmentation of Alcohols

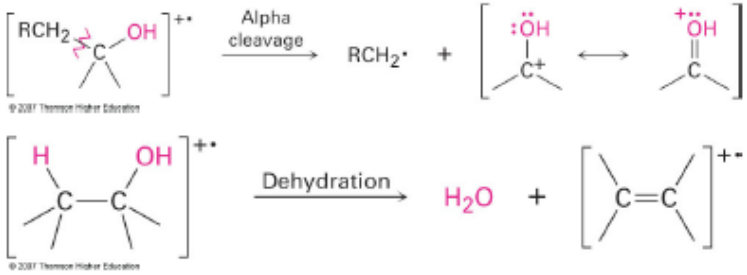
- One of the most common fragmentation patterns is the loss of H_2O to give a peak which corresponds to [M-18]
- Another common pattern is loss of an alkyl group from the carbon bearing the OH to give a resonance-stabilized oxonium ion and an alkyl radical.



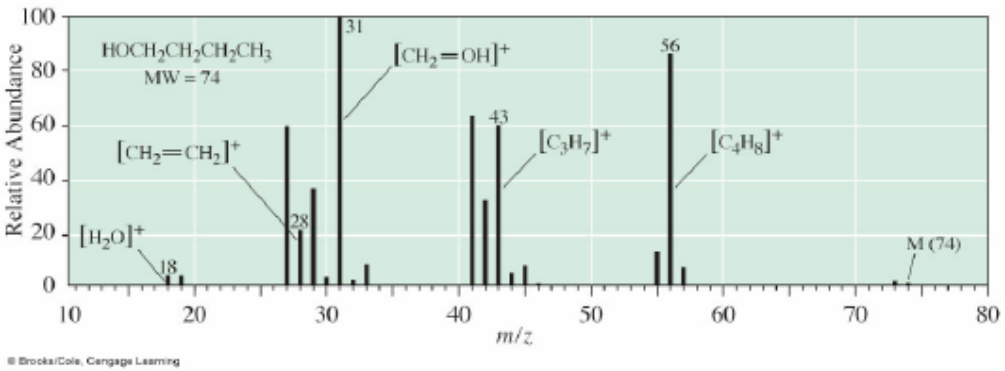
Molecular ion (a radical cation) **A radical** **A resonance-stabilized oxonium ion**

Mass Spectral Cleavage Reactions of Alcohols

- Alcohols undergo α-cleavage (at the bond next to the C-OH) as well as loss of H-OH to give C=C

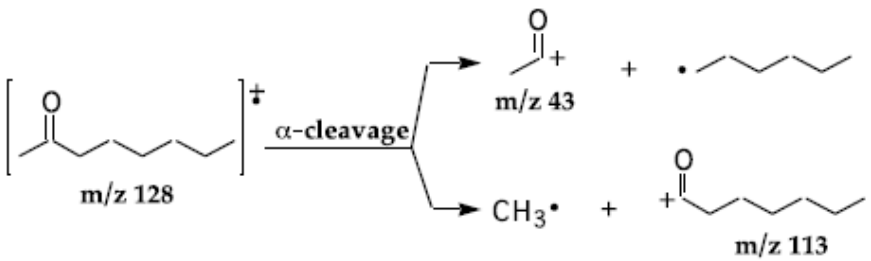


Fragmentation of Alcohols: MS of 1-butanol

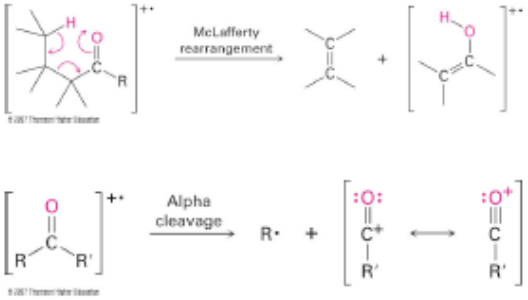


Fragmentation of Aldehydes & Ketones

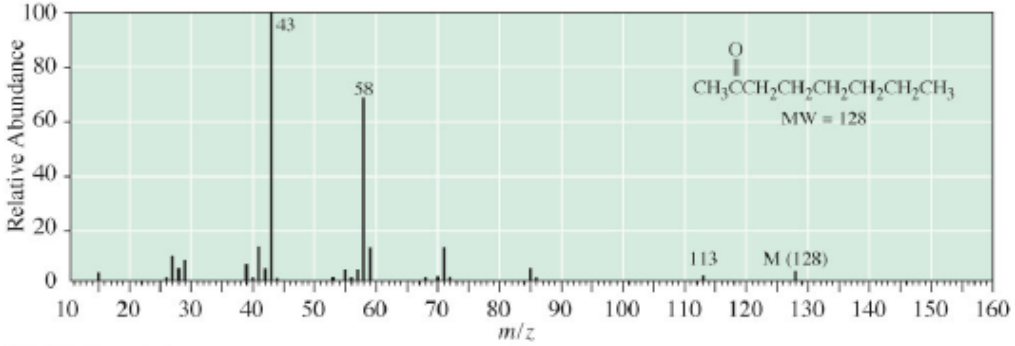
One of the most common fragmentation patterns is the cleavage of the bond alpha to the carbonyl (**alpha-cleavage**). This results in the formation of a carbonyl cation.



- A C-H that is three atoms away leads to an internal transfer of a proton to the C=O, called the McLafferty rearrangement
- Carbonyl compounds can also undergo α cleavage

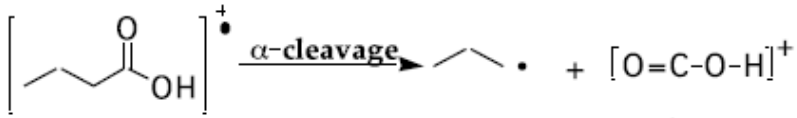


MS of 2-octanone



Fragmentation of Carboxylic Acids

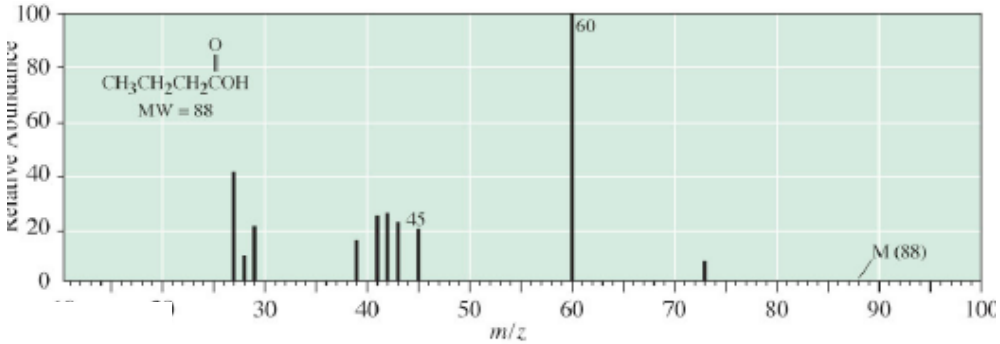
Similar to ketones and aldehydes, alpha-cleavage also occurs with carboxylic acids. This results in the formation of a $[CO_2H]^+$ cation equal to a m/z of 45.



Molecular ion m/z 88

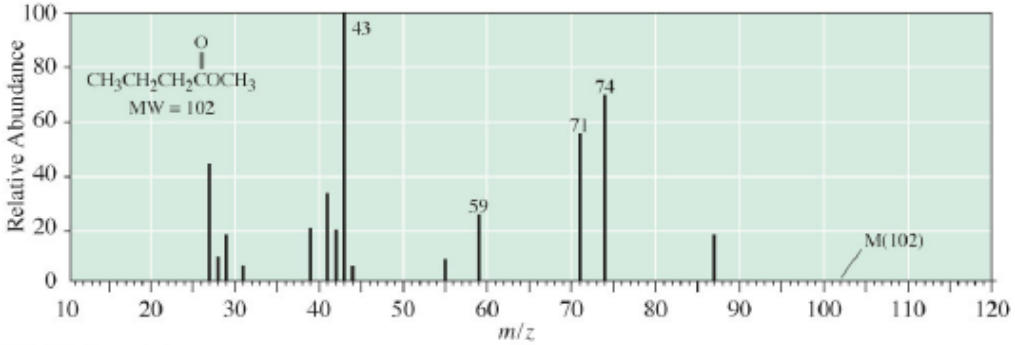
m/z 45

Fragmentation of 2-butanoic acid



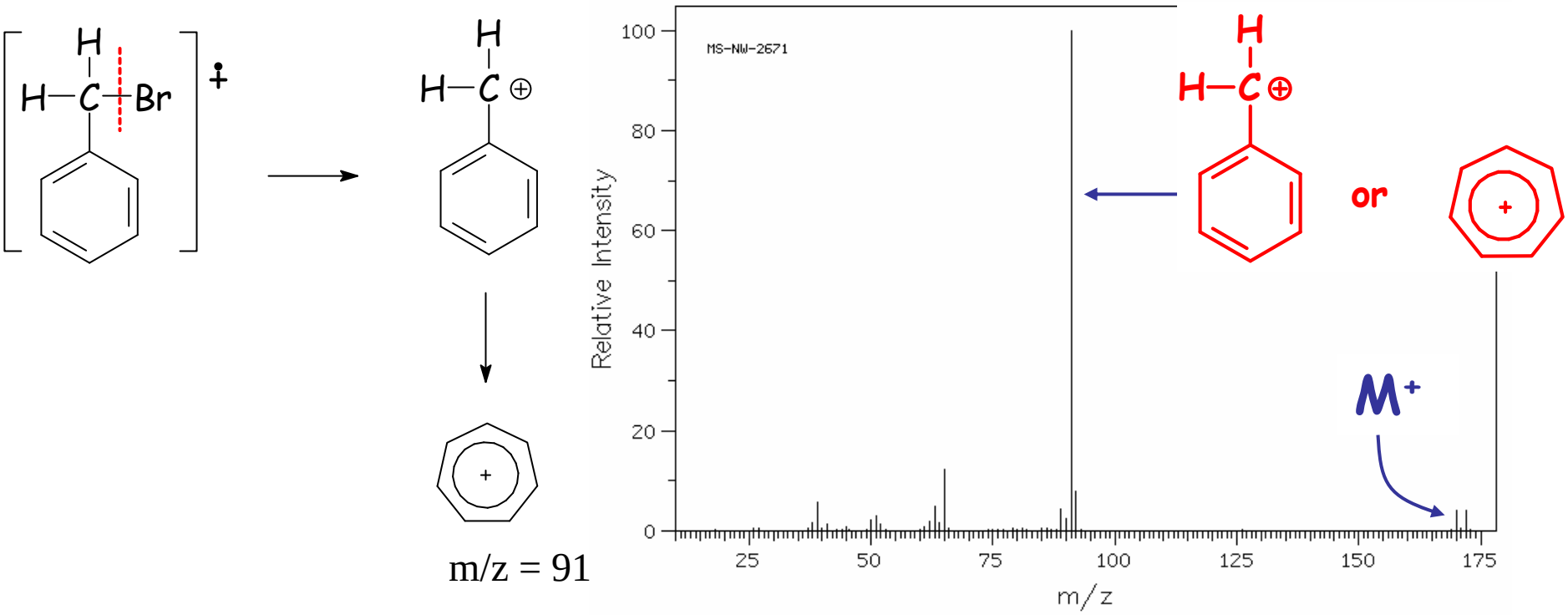
Fragmentation of methyl butanoate

Esters also can under alpha-cleavage during MS.



Fragmentation Patterns of Aromatics

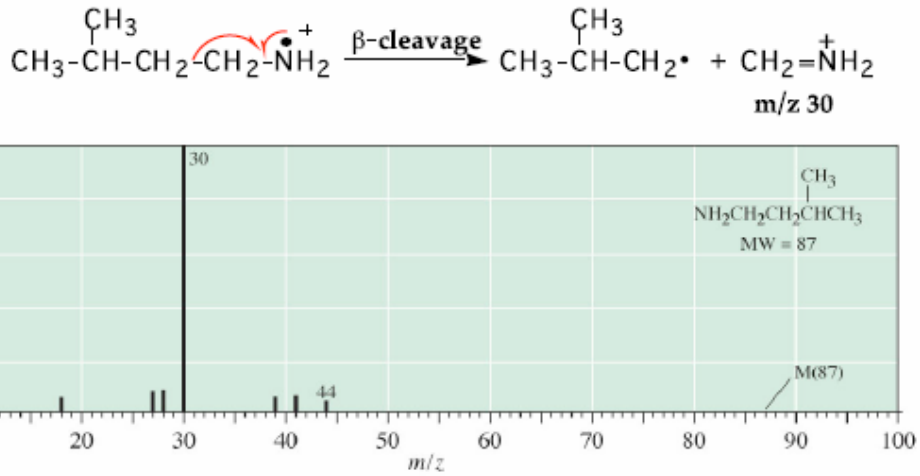
– Fragment at the benzylic carbon, forming a resonance stabilized benzylic carbocation (which rearranges to the tropylium ion)

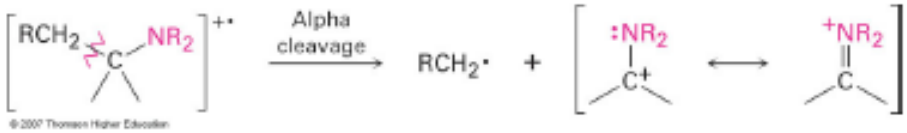


Fragmentation of Amines

The most common fragmentation pattern results from beta-cleavage.

Compounds with an odd molecular ion may contain nitrogen!

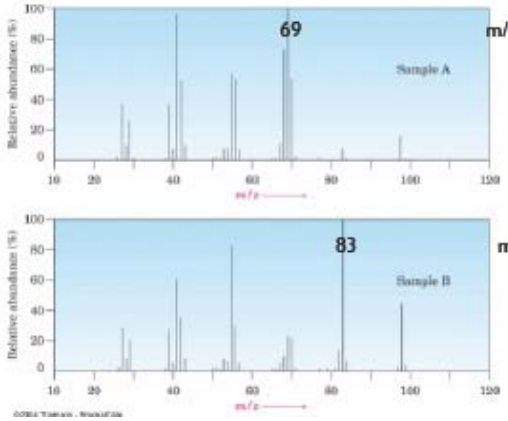




➢ Cleavage is often associated with elimination of small, stable, neutral molecules, such as carbon monoxide, olefins, water, ammonia, hydrogen sulfide, hydrogen cyanide, mercarptans, ketene, or alcohols, often with rearrangement.

Example

- Assume you have two unlabeled samples, one of methylcyclohexane and the other of ethylcyclopentane.
- How could you use mass spectrometry to tell them apart?



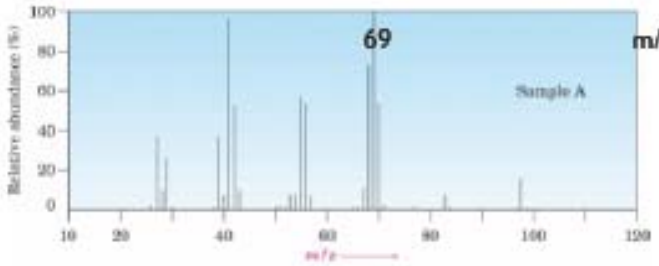
m/e 69 = loss of CH_2CH_3

m/e 83 = loss of CH_3

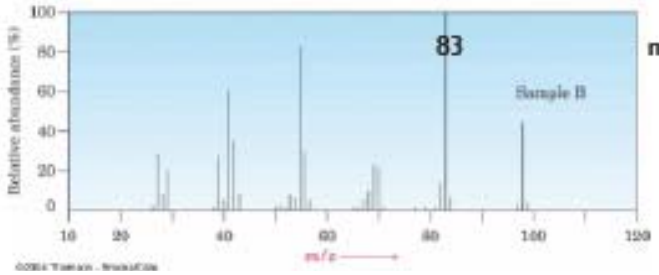
Strategy

- Look at the two possible structures and decide how they differ.
- Then think about how any of these differences might give rise to differences in mass spectra. Methylcyclohexane has a CH_3 group, and ethylcyclopentane has a CH_2CH_3 group, which should affect the fragmentation patterns.

Solution



m/e 69 = loss of CH_2CH_3



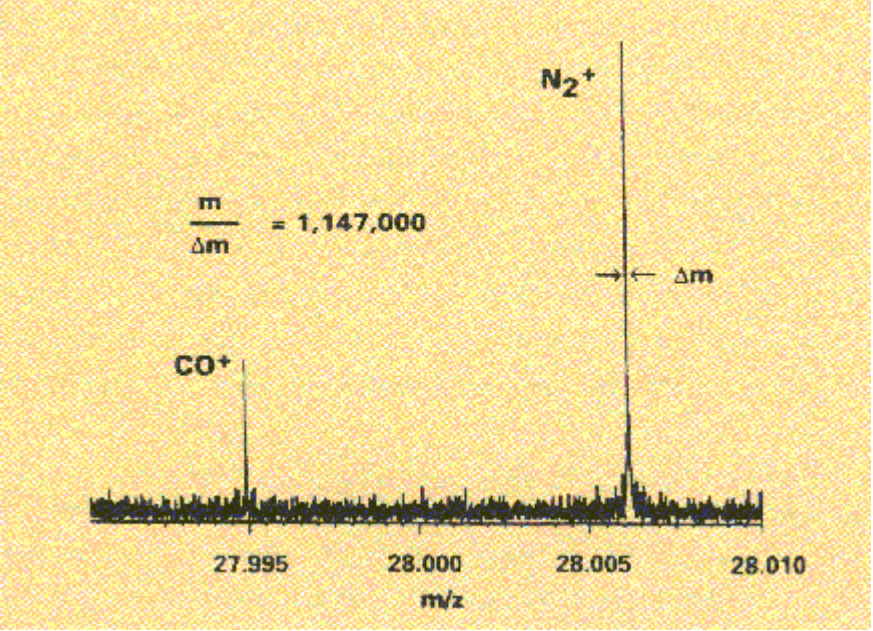
m/e 83 = loss of CH_3

- Both samples have a $M^+ = 98$, corresponding to C_7H_{14}.
- Sample B has a base peak at m/z = 83, corresponding to the loss of a CH_3 group from M^+.
- Sample A has a small peak at m/z = 83. Sample A has a base peak at m/z = 69, corresponding to the loss of a CH_2CH_3 group.
- However, B has a rather small peak at m/z = 69.
- Therefore, we can assume that B is methyl cyclohexane and A, ethylcyclopentane.

Low and High Resolution MS

- "high resolution" mass spectrometers can measure m/z values to three or four decimal places and thus provide an extremely accurate method for determining molecular weights

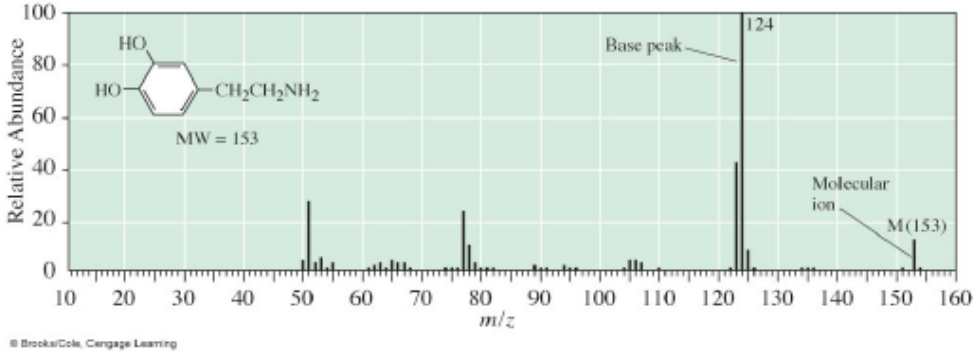
High resolution mass spectrum of CO and N_2



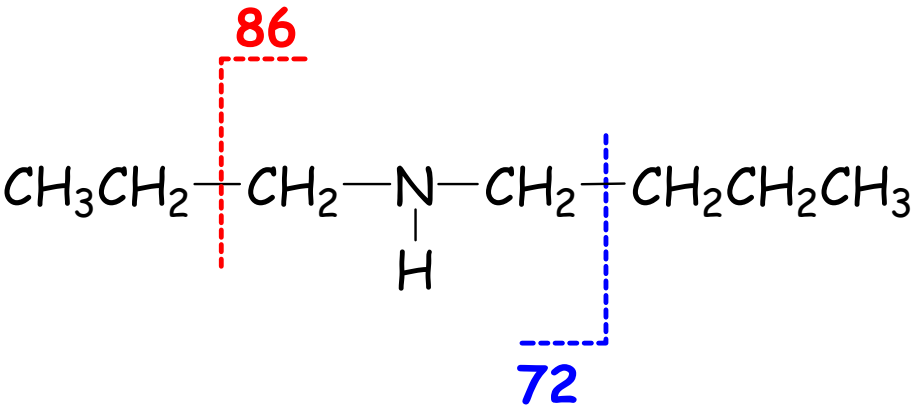
- Carbon monoxide (CO) and nitrogen (N_2), both have an integral mass of 28, but the accurate masses are m/z 27.995 and m/z 28.006, respectively

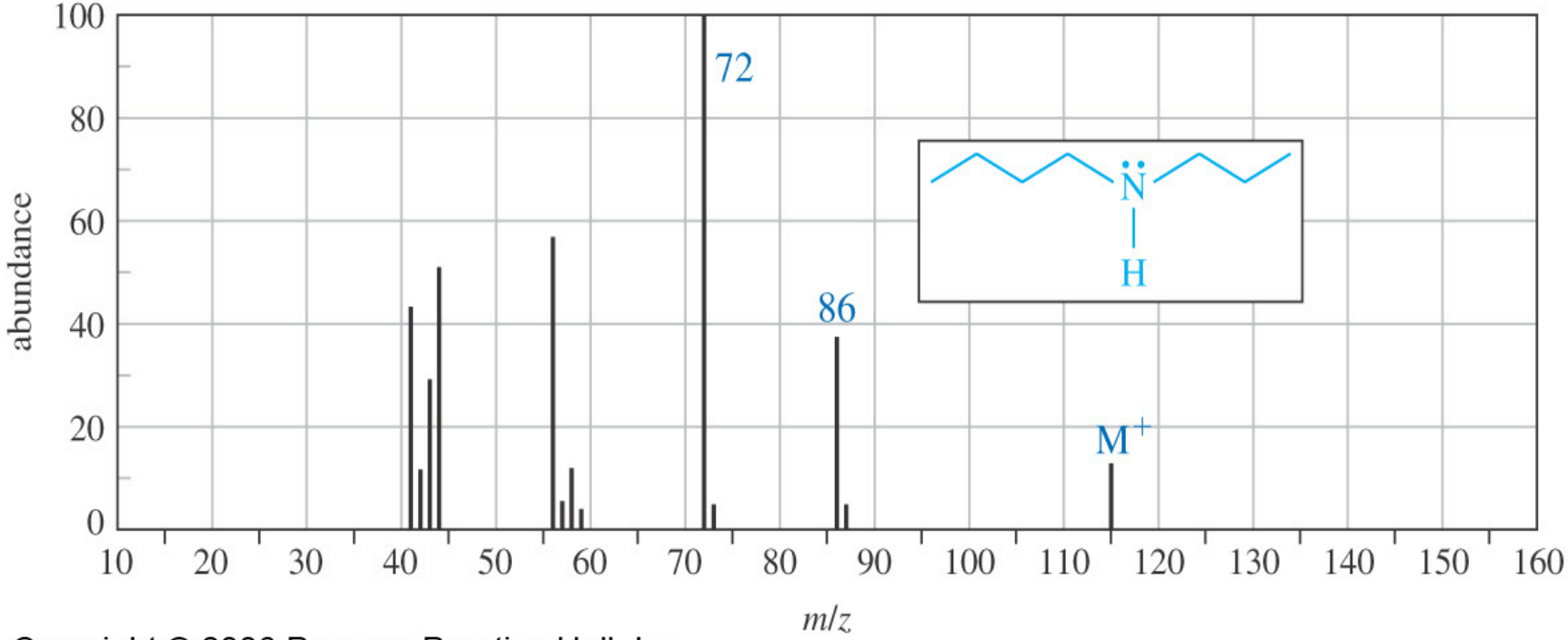
Some Examples of MS Spectra

MS of Dopamine



Some Examples of MS Spectra





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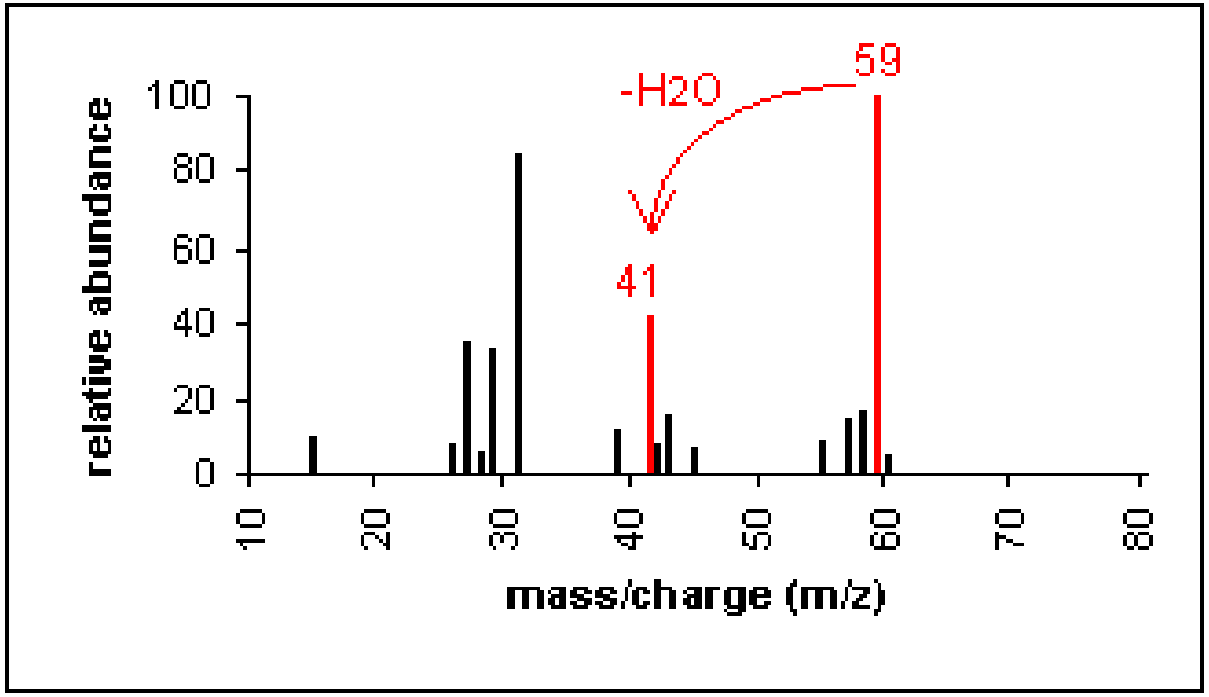
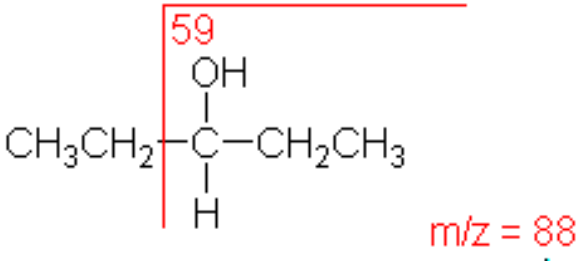
Alcohol:

An alcohol's molecular ion is small or non-existent. Cleavage of the C-C bond next to the oxygen usually occurs. A loss of H_2O may occur as in the spectra below.

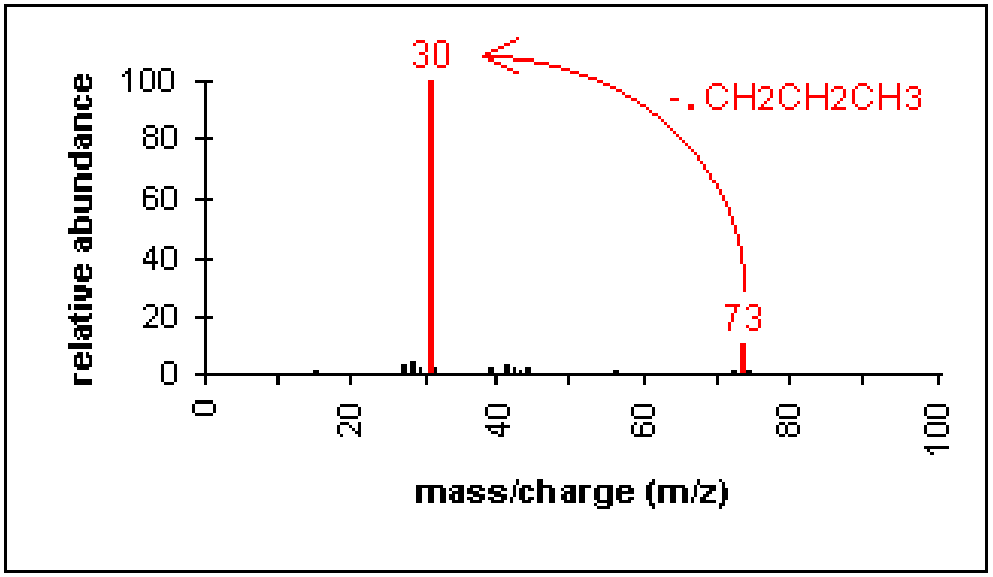


3-Pentanol
C5H12O
MW = 88.15

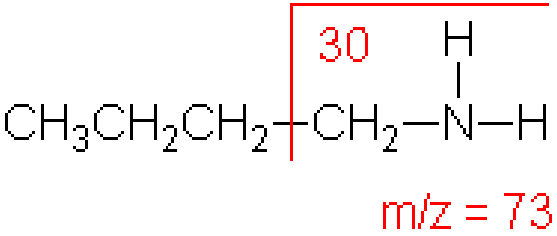


Amine

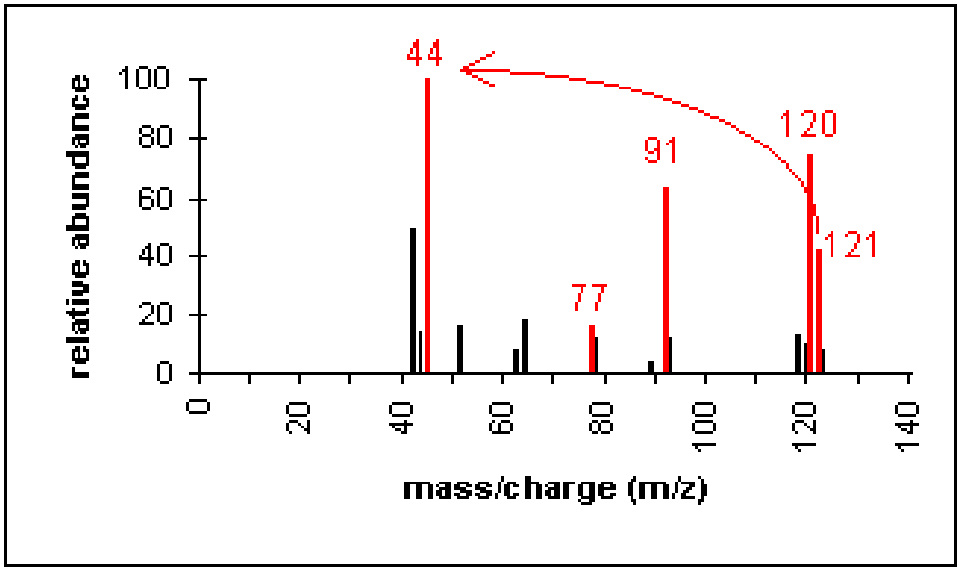
Molecular ion peak is an odd number. Alpha-cleavage dominates aliphatic amines.

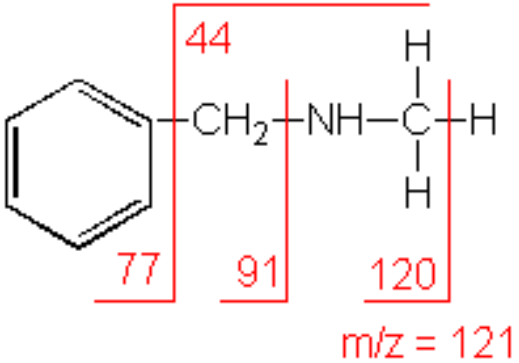


n-Butylamine
C4H11N
MW = 73.13



Another example is a secondary amine shown below.
Again, the molecular ion peak is an odd number.
The base peak is from the C-C cleavage adjacent to the C-N bond.

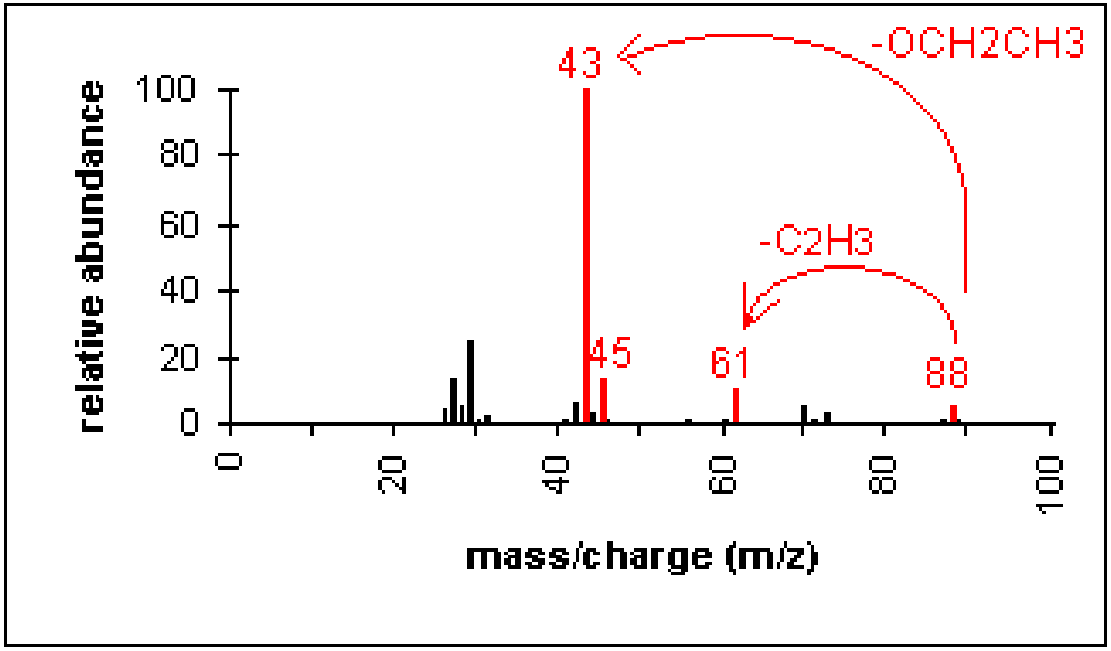




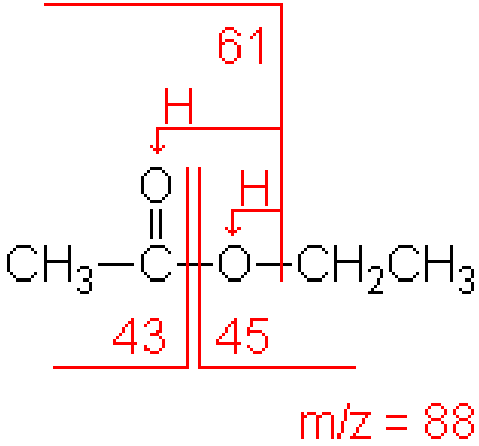
n-Methylbenzylamine
C8H11N
MW = 121.18

Ester

Fragments appear due to bond cleavage next to C=O (alkoxy group loss, -OR) and hydrogen rearrangements.

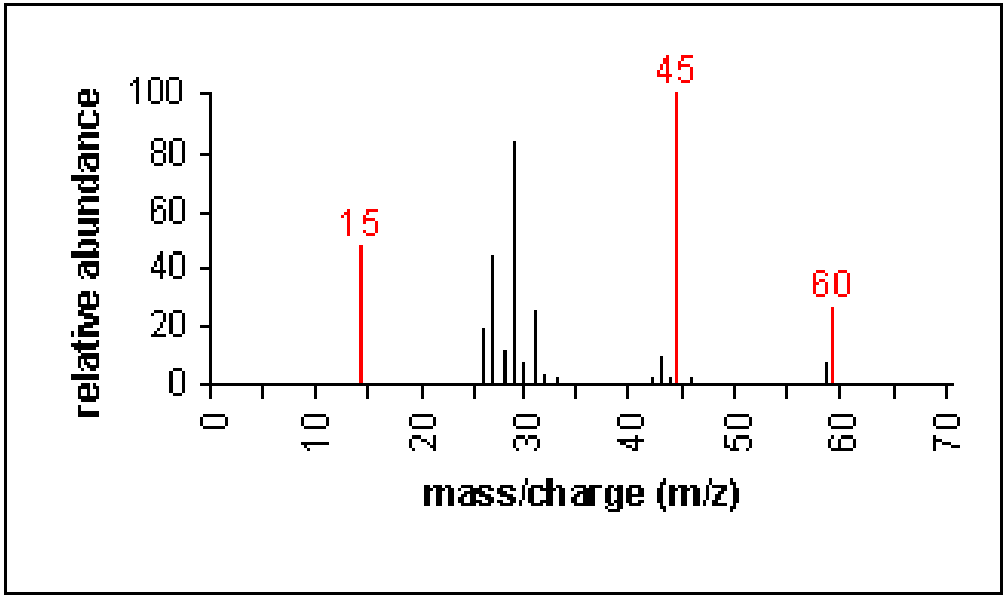


Ethyl acetate
$C_4H_8O_2$
MW = 88.11

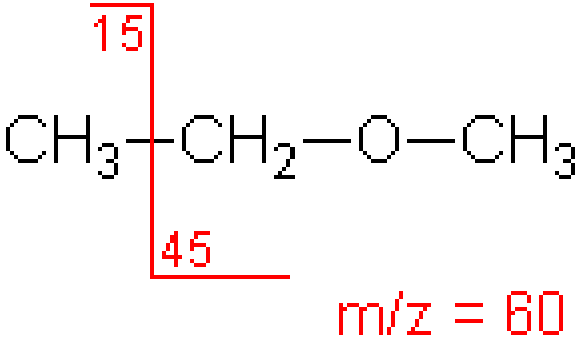


Ether

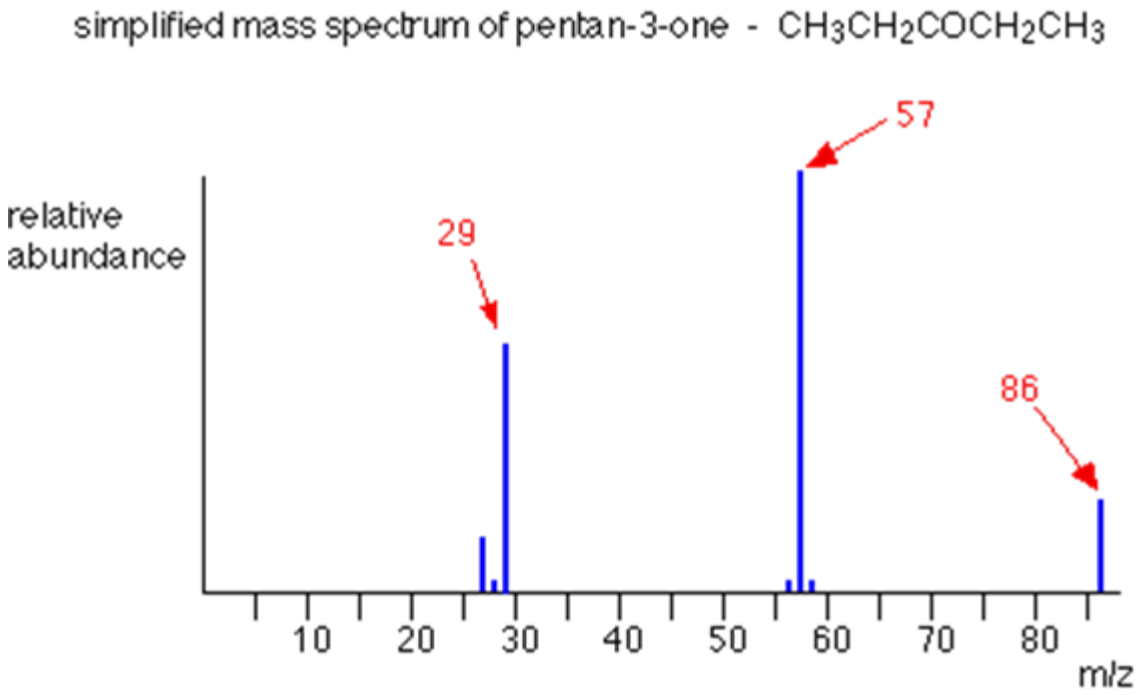
Fragmentation tends to occur alpha to the oxygen atom (C-C bond next to the oxygen).



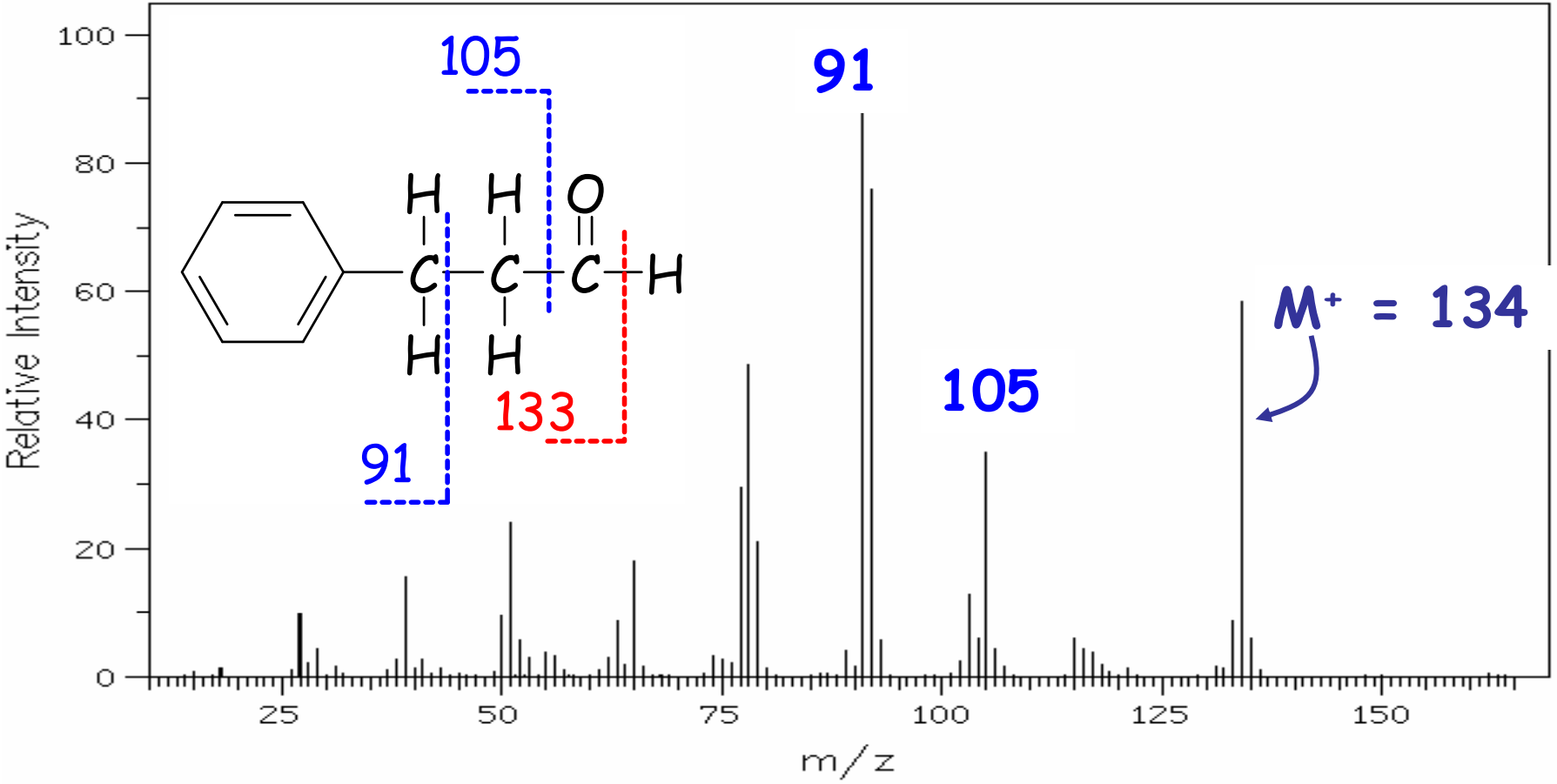
Ethyl methyl ether
C3H8O
MW = 60.10



The mass spectrum of pentan-3-one

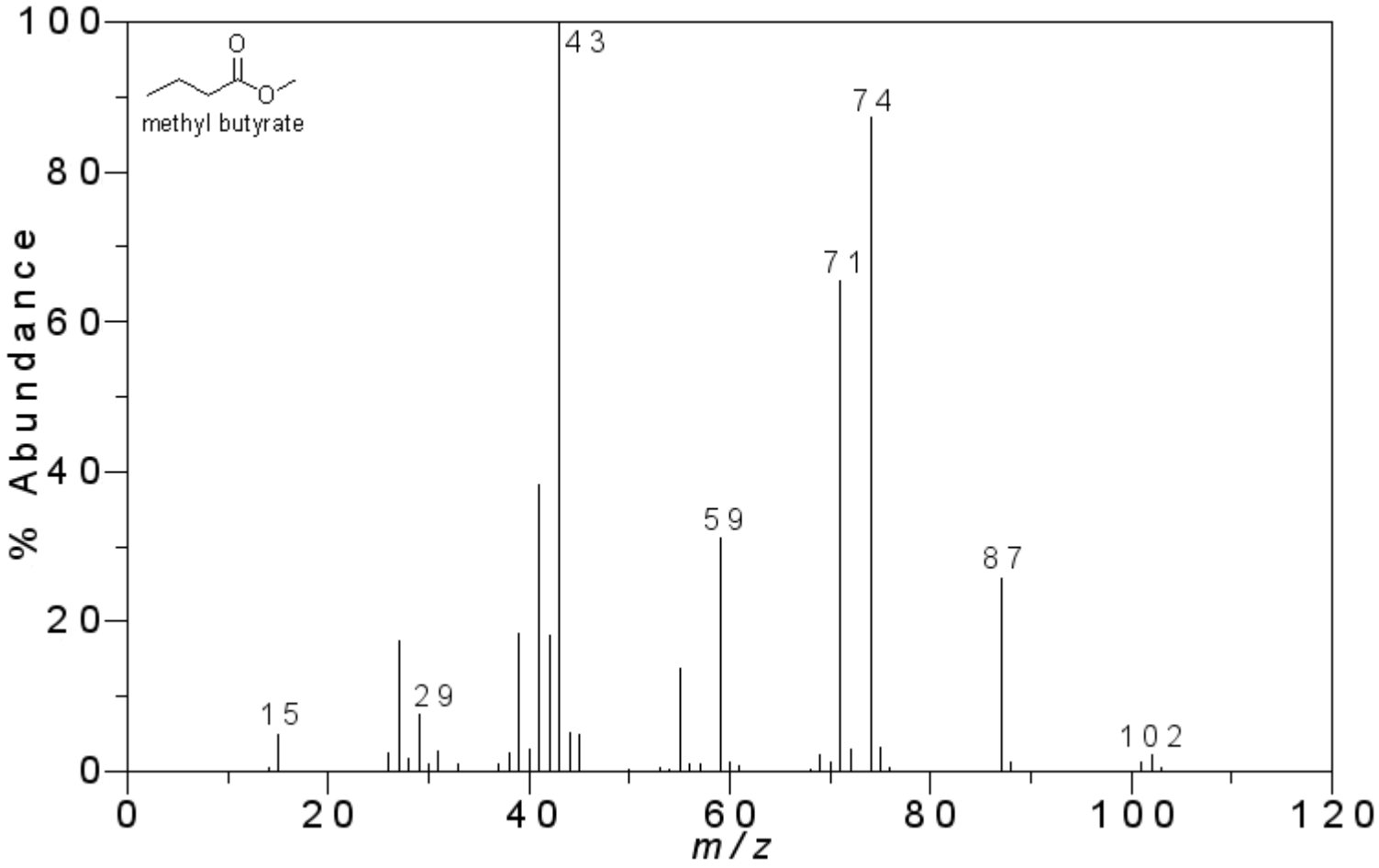
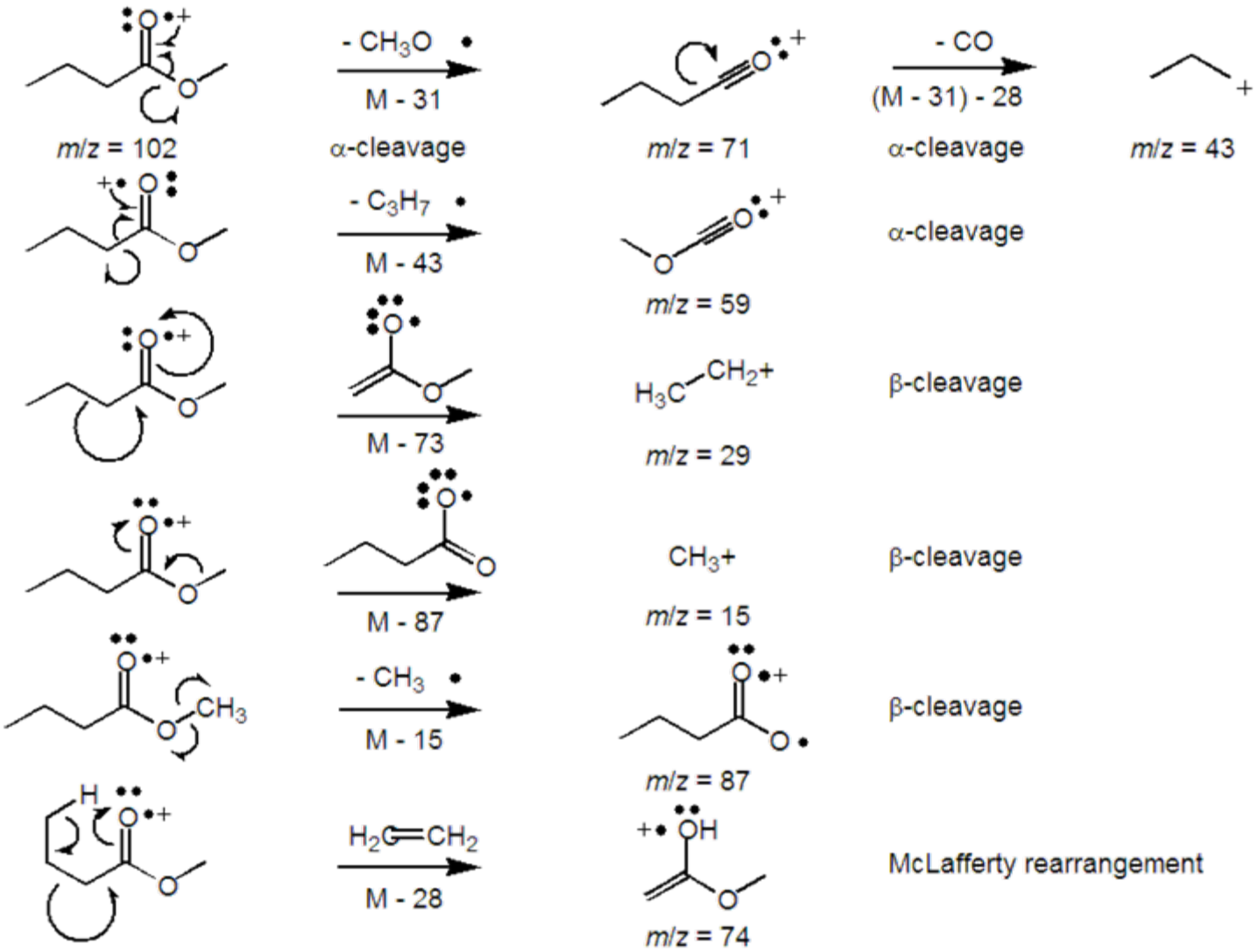


MS for hydrocinnamaldehyde



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

MS Spectrum of Methyl butrate



Mechanism of fragmentation for methyl butyrate