# Mass Spectrometry

Bahaa El-Dien M. El-Gendy
Chemistry Department
Benha University
bahaaeldin.adli@fsc.bu.edu.eg

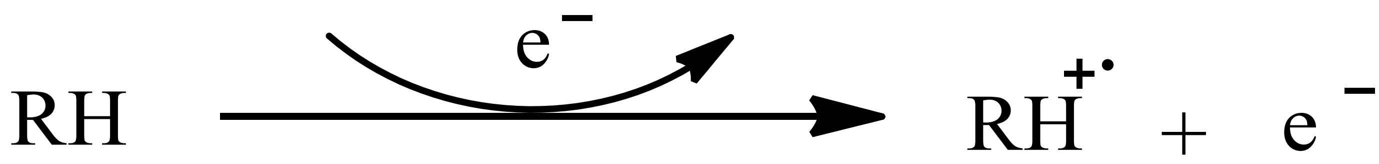
# Introduction

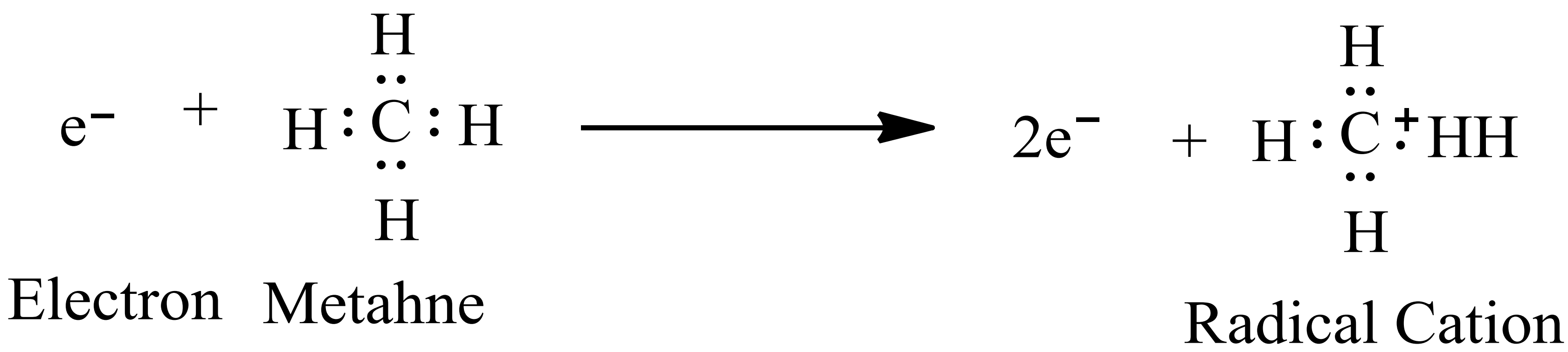
- Provides the molecular weight.
- Helps you to determine the molecular formula (MF).
- High Resolution Mass Spectrometry (HRMS) can provide an accurate molecular formula.
- Provides structural information that can confirm a structure derived from NMR and IR.
- Uses a very small sample.
- Used for protein profiling in proteomics.

# Mass Spectrometry NOT Spectroscopy

- Mass spectrometry doesn’t use light at all (doesn’t involve absorption or emission of light over a range of wavelengths like spectroscopic techniques).
- In the mass spectrometer: A sample is struck by high energy electrons, breaking the molecule apart. The masses of the fragments are measured and this information is used to reconstruct the molecule.

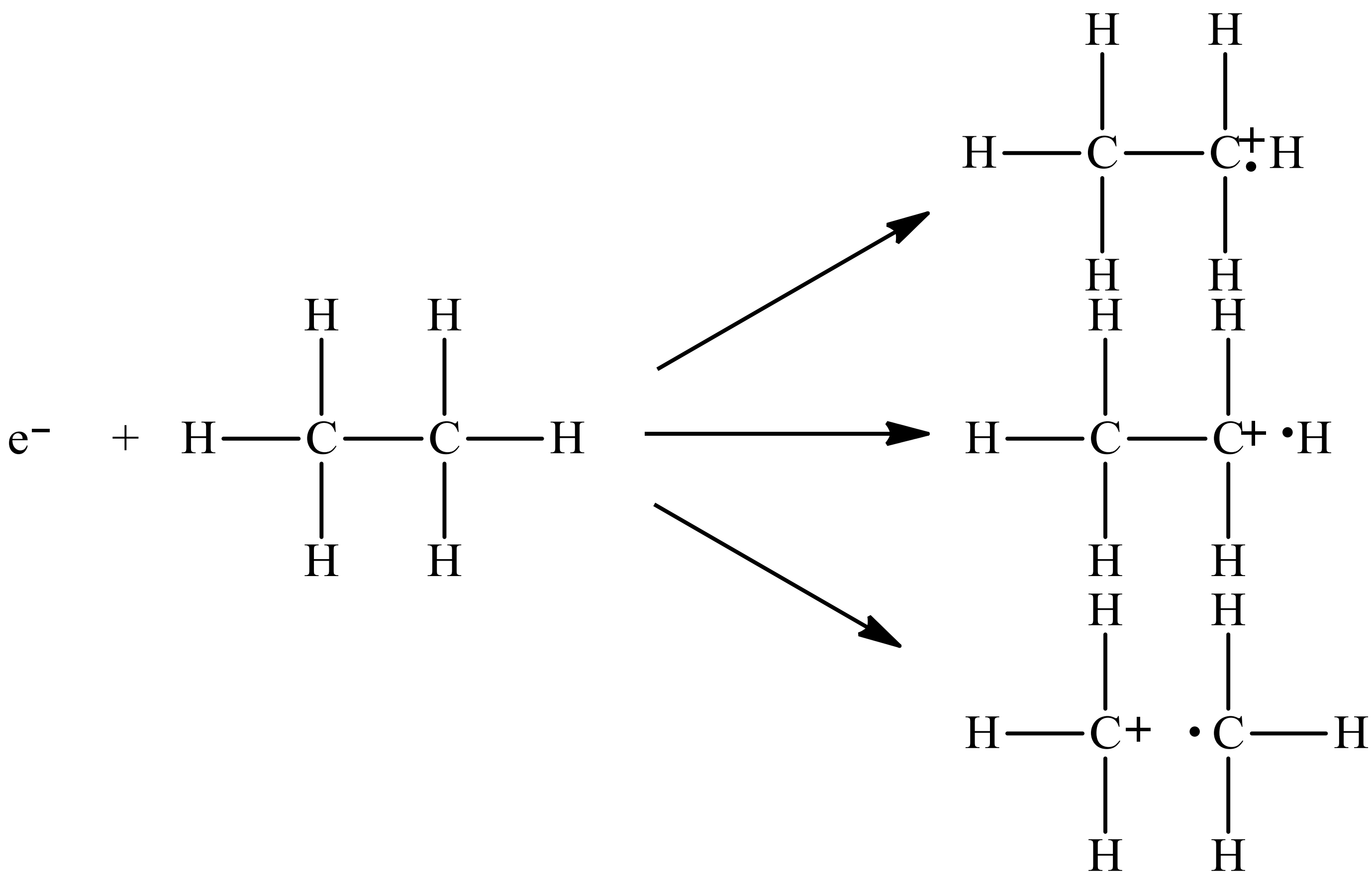
# Electron Impact Ionization




**Radical Cation**: A molecule that has a positive charge and an unpaired electron.

- During ionization, other fragments can be formed when C—C or C—H bonds are broken.
- In Mass: *<u>Only the positive fragments can be detected.</u>*



# Electron Impact Ionization

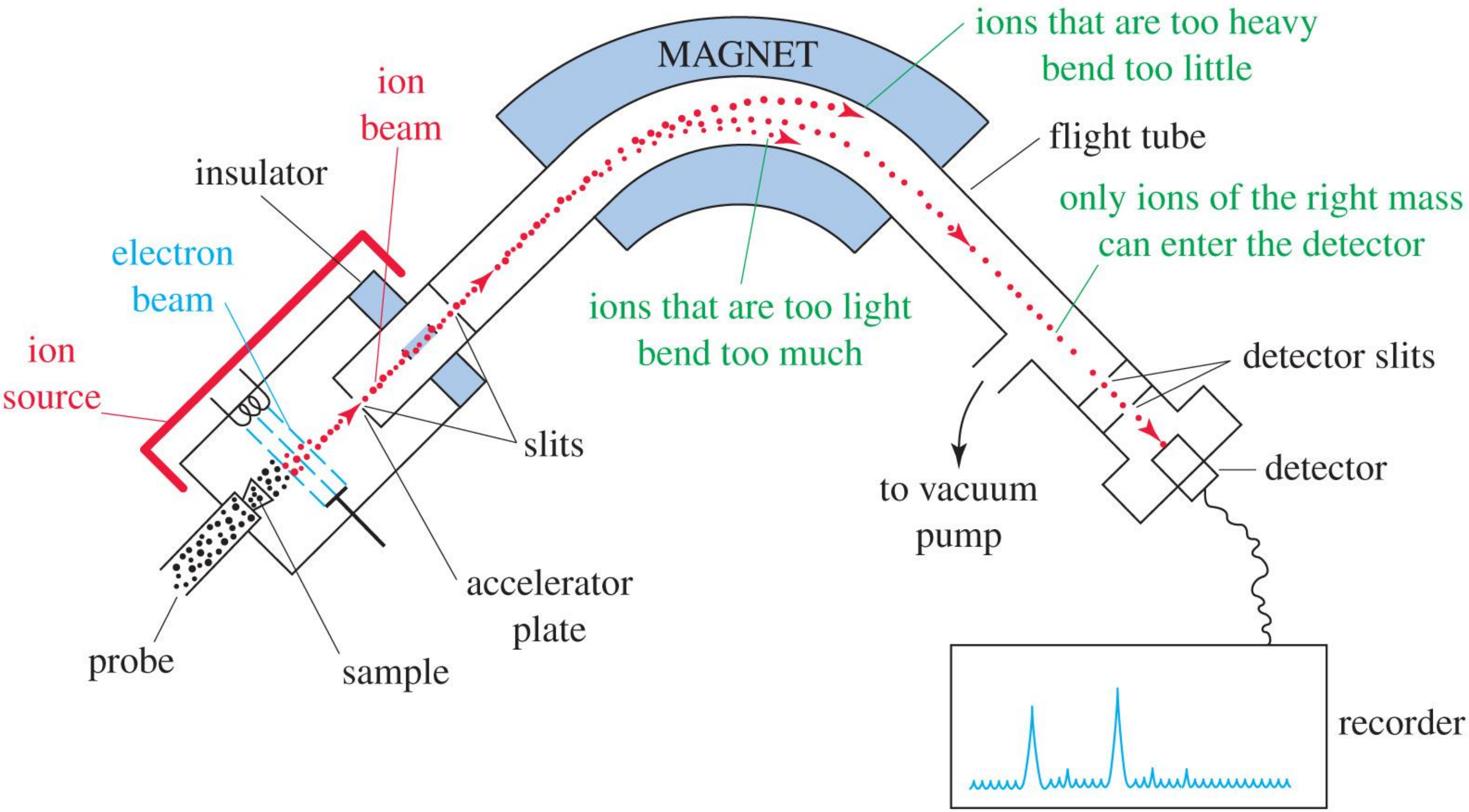
Advantages

- Well-established technique
- Fragmentation pattern gives structural information
- Databases (libraries) available for pattern identification

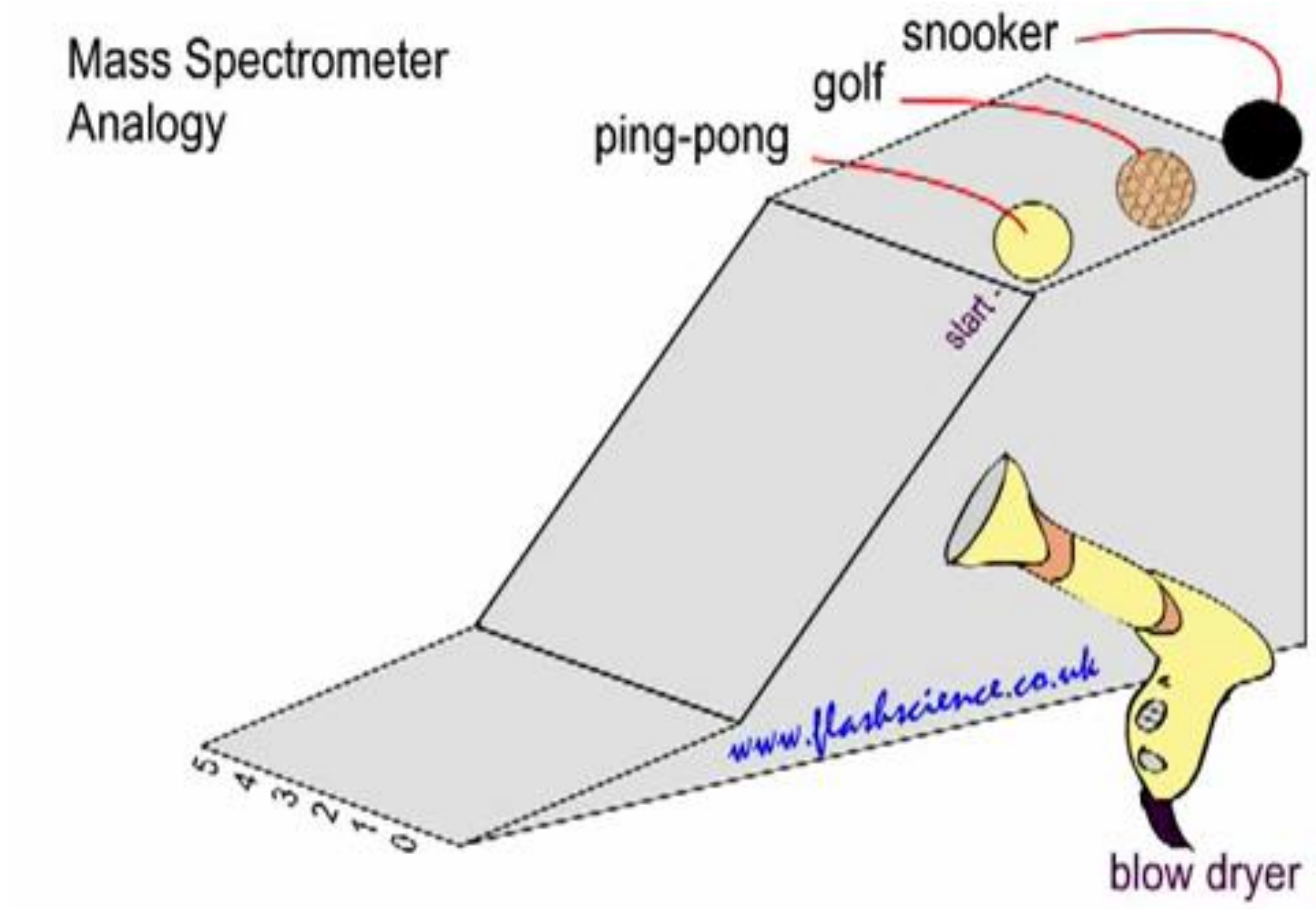
Disadvantages

- Sample must be volatile
- Only 10% of all molecules give the molecular ion peak
- Does not work well for large fragile or ionic species

# Mass Spectrometer

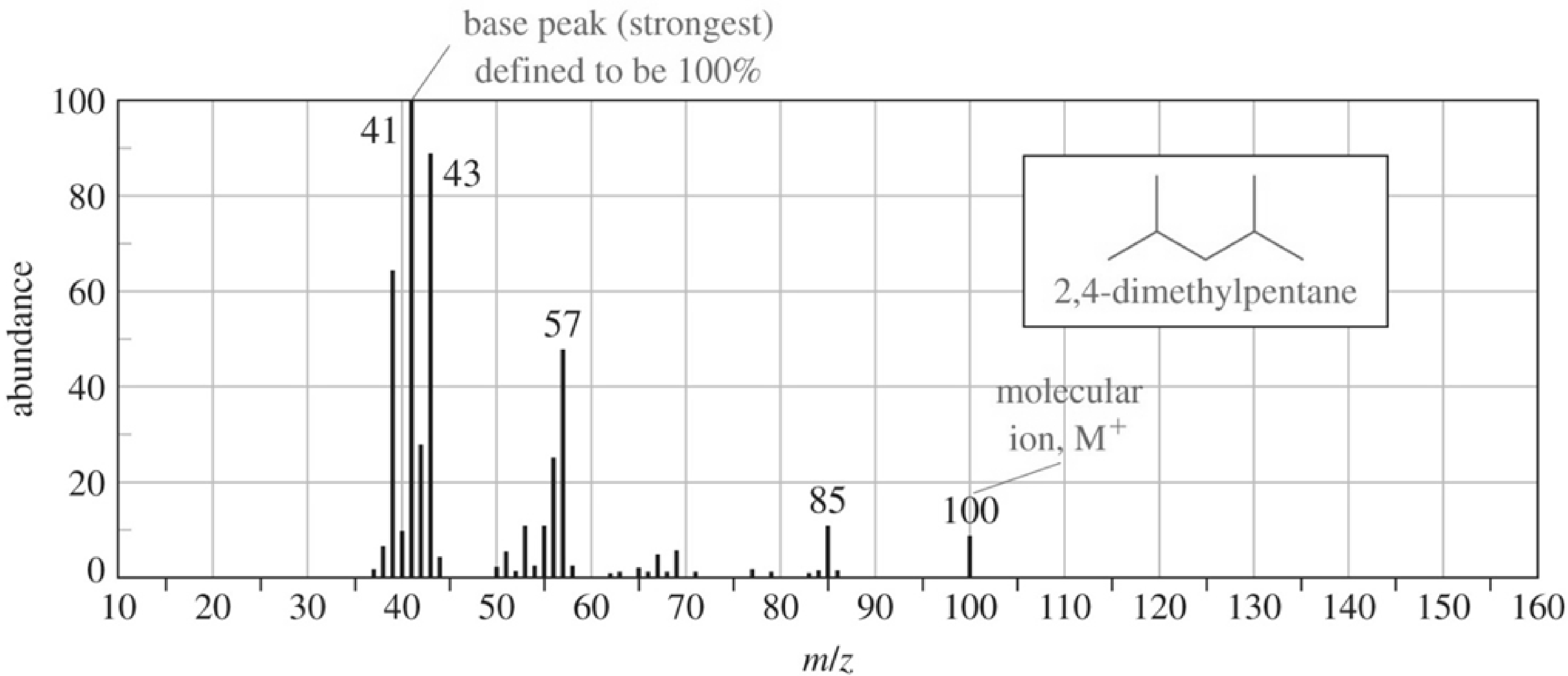


# Simple Explanation of Mass Spectrometer



# Mass Spectrometer

# The Mass Spectrum




- The Molecular Ion Peak or Parent Peak ($M^+$): Gives the molecular weight of the compound (means a detectable number of molecular ions reach the detector without further fragmentation).

- Base Peak: Is the tallest peak and corresponds to an abundance of 100%. The % abundance of all other peaks are given relative to the base peak.

# Isotopic Abundance

Isotopic Composition of Some Common Elements

| Element | M$^+$ | | M+1 | | M+2 | |
|---|---|---|---|---|---|---|
| hydrogen | $^{1}H$ | 100.0% | | | | |
| carbon | $^{12}C$ | 98.9% | $^{13}C$ | 1.1% | | |
| nitrogen | $^{14}N$ | 99.6% | $^{15}N$ | 0.4% | | |
| oxygen | $^{16}O$ | 99.8% | | | $^{18}O$ | 0.2% |
| sulfur | $^{32}S$ | 95.0% | $^{33}S$ | 0.8% | $^{34}S$ | 4.2% |
| chlorine | $^{35}Cl$ | 75.5% | | | $^{37}Cl$ | 24.5% |
| bromine | $^{79}Br$ | 50.5% | | | $^{81}Br$ | 49.5% |
| iodine | $^{127}I$ | 100.0% | | | | |

# High Resolution MS

- Detect particle masses to an accuracy of about 1 part in 20,000 by using extra stages of electrostatic or magnetic focusing to form a very precise beam.
- Exact Mass: A mass determined to several significant figures using an HRMS
- Example: A molecule with mass of 44 could be $C_3H_8$, $C_2H_4O$, $CO_2$, or $CN_2H_4$.
- For example, let's say the compound we are looking for has mass of 44.029, What is the correct structure?

| $C_3H_8$ | | $C_2H_4O$ | | $CO_2$ | | $CN_2H_4$ | |
|---|---|---|---|---|---|---|---|
| 3 C | 36.00000 | 2 C | 24.00000 | 1 C | 12.00000 | 1 C | 12.00000 |
| 8 H | 8.06260 | 4 H | 4.03130 | | | 4 H | 4.03130 |
| | | 1 O | 15.99491 | 2 O | 31.98983 | 2 N | 28.00610 |
| | 44.06260 | | 44.02621 | | 43.98983 | | 44.03740 |

# Exact Masses of Common Elements and Isotopes

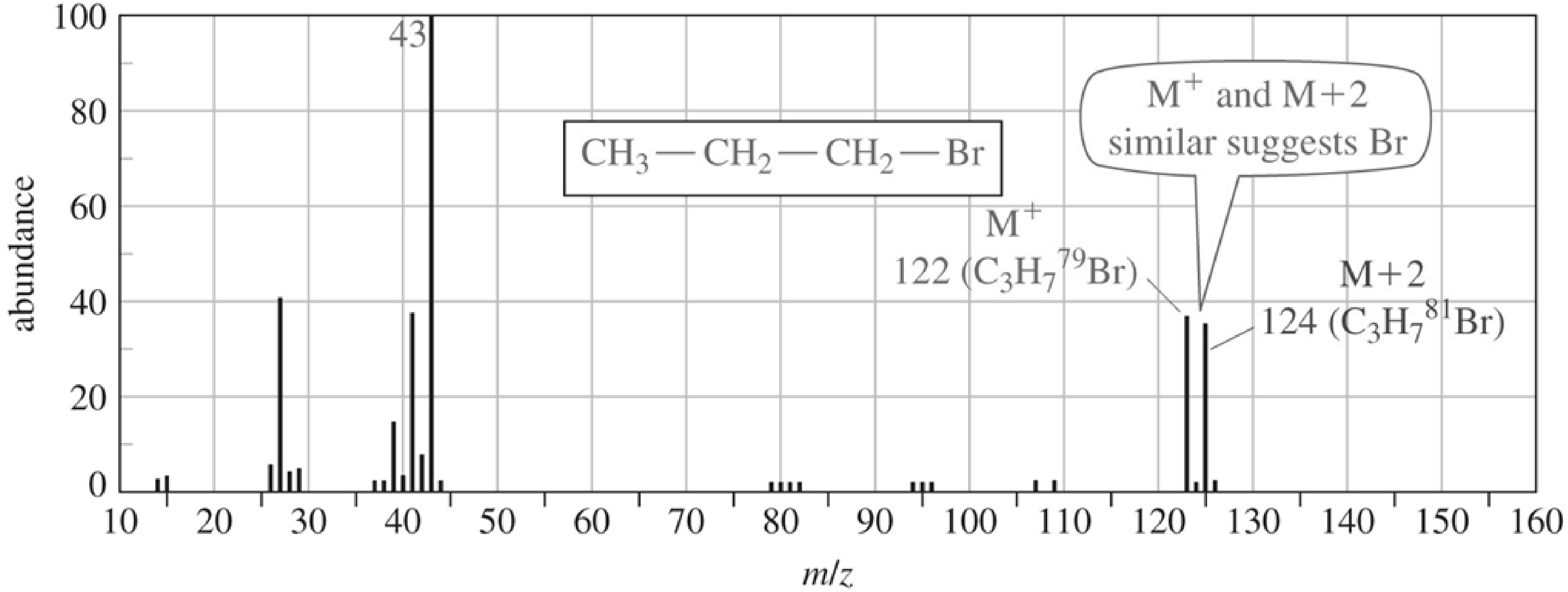
| isotope | mass | natural abundance |
|---|---|---|
| $^{1}H$ | 1.00782 | 99.985 |
| $^{2}H$ | 2.01410 | 0.015 |
| $^{12}C$ | 12.0000 | 98.892 |
| $^{13}C$ | 13.00335 | 1.108 |
| $^{14}N$ | 14.00307 | 99.634 |
| $^{15}N$ | 15.00010 | 0.366 |
| $^{16}O$ | 15.99491 | 99.763 |
| $^{17}O$ | 16.99913 | 0.037 |
| $^{18}O$ | 17.99916 | 0.200 |
| $^{19}F$ | 18.99840 | 100.000 |
| $^{35}Cl$ | 34.96885 | 75.77 |
| $^{37}Cl$ | 36.96590 | 24.23 |
| $^{79}Br$ | 78.91839 | 50.69 |
| $^{81}Br$ | 80.91642 | 49.31 |
| $^{127}I$ | 126.90447 | 100.000 |

mass= 58

| | |
|---|---|
| $N_3O$ | 58.0042 |
| $N_4H_2$ | 58.0280 |
| $CNO_2$ | 57.9929 |
| $CH_2N_2O$ | 58.0167 |
| $CH_4N_3$ | 58.0406 |
| $C_2H_2O_2$ | 58.0054 |
| $C_2H_4NO$ | 58.0293 |
| $C_2H_6N_2$ | 58.0532 |
| $C_3H_6O$ | 58.0419 |
| $C_3H_6N$ | 58.0657 |
| $C_4H_{10}$ | 58.0783 |

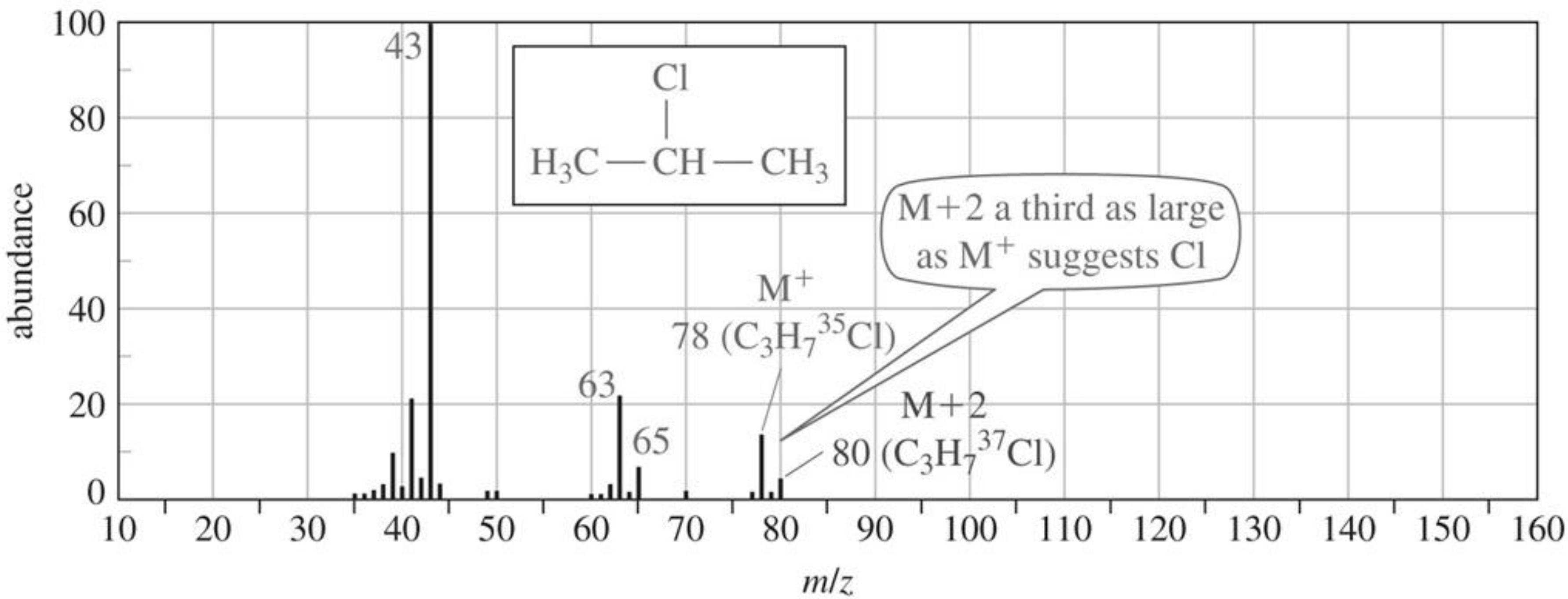
# Use of Heavier Isotope Peaks

Mass Spectrum of Bromine Containing Compounds



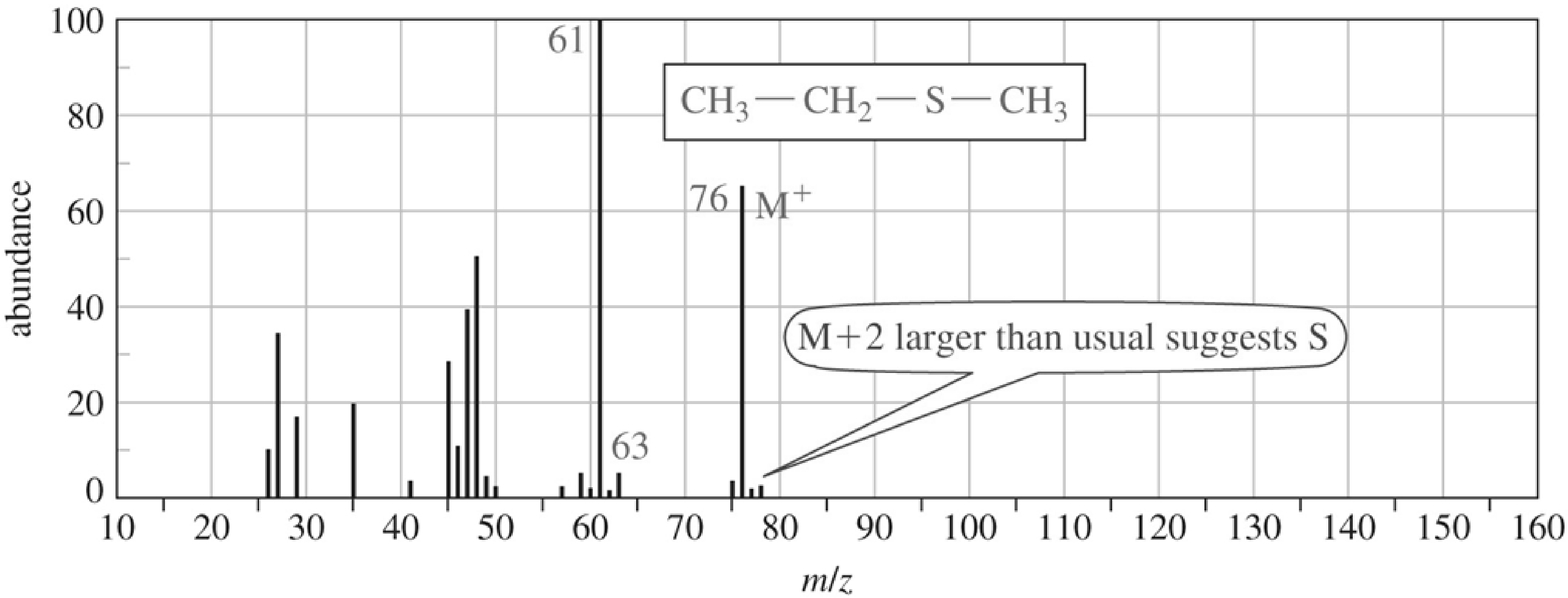

The molecular ion peak $M^+$ that has $^{79}$Br is almost as tall as the M+2 peak that has $^{81}$Br because bromine is a mixture of 50.5% $^{79}$Br and 49.5% $^{81}$Br.

# Mass Spectrum of Chlorine Containing Compounds



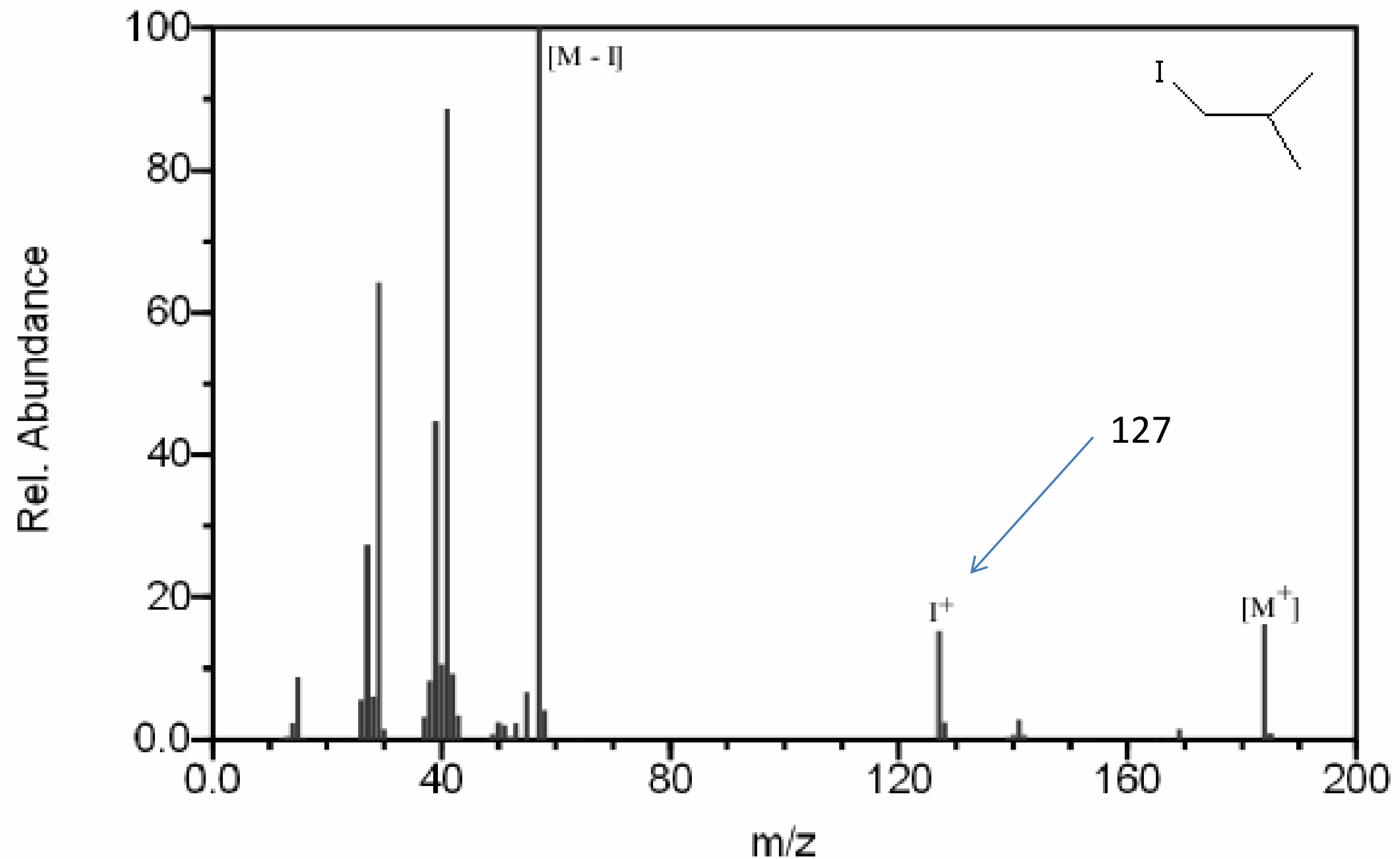

The molecular ion peak $M^+$ is 3 times higher than the M+2 peak because chlorine is a mixture of 75.5% $^{35}Cl$ and 24.5% $^{37}Cl$.

# Mass Spectrum of Sulfur Containing Compounds

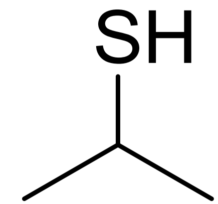


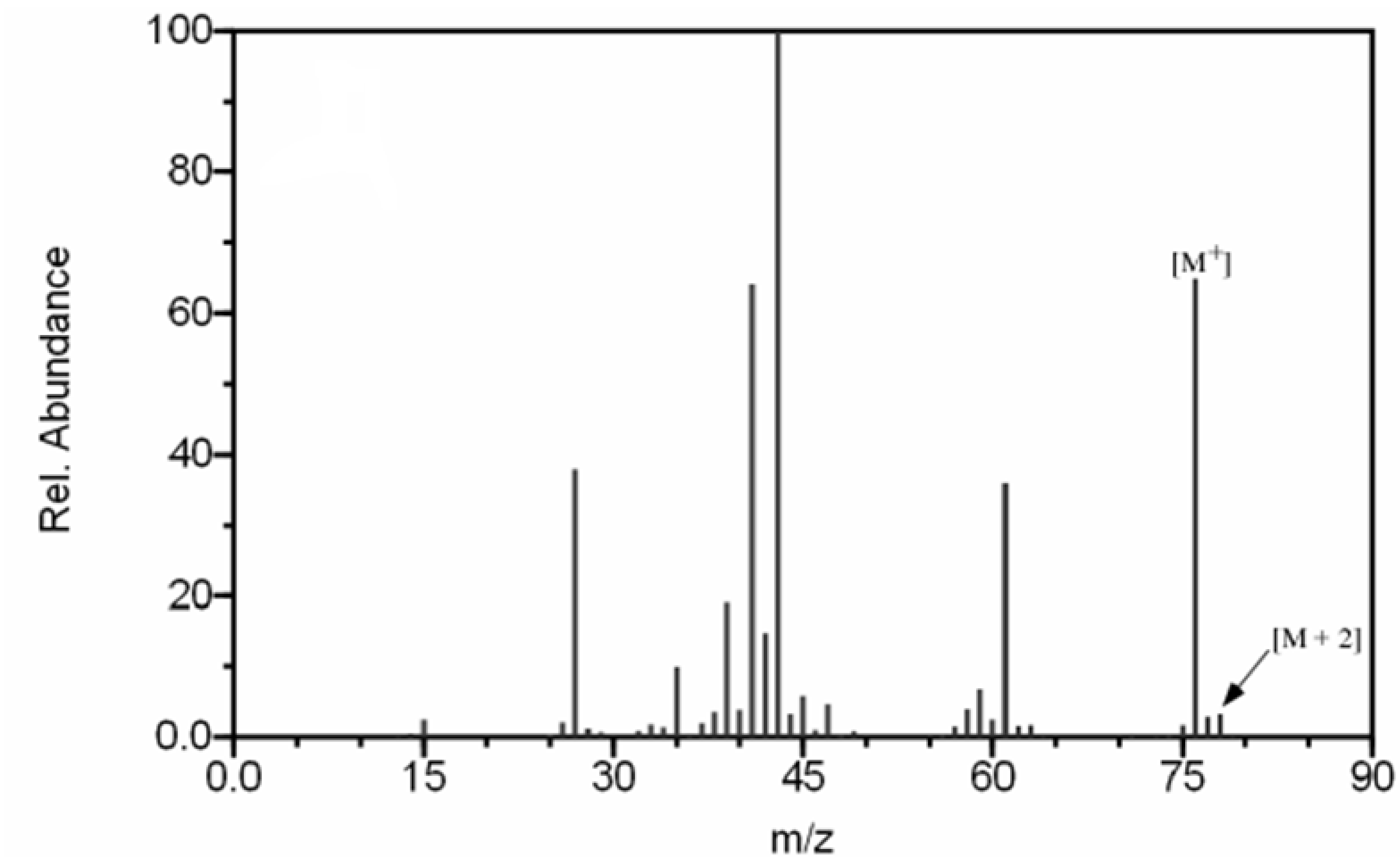

Sulfur has three isotopes: $^{32}S$ (95%), $^{33}S$ (0.8%), and $^{34}S$ (4.2%).

# Mass Spectrum of Iodine Containing Compounds

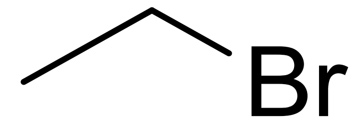


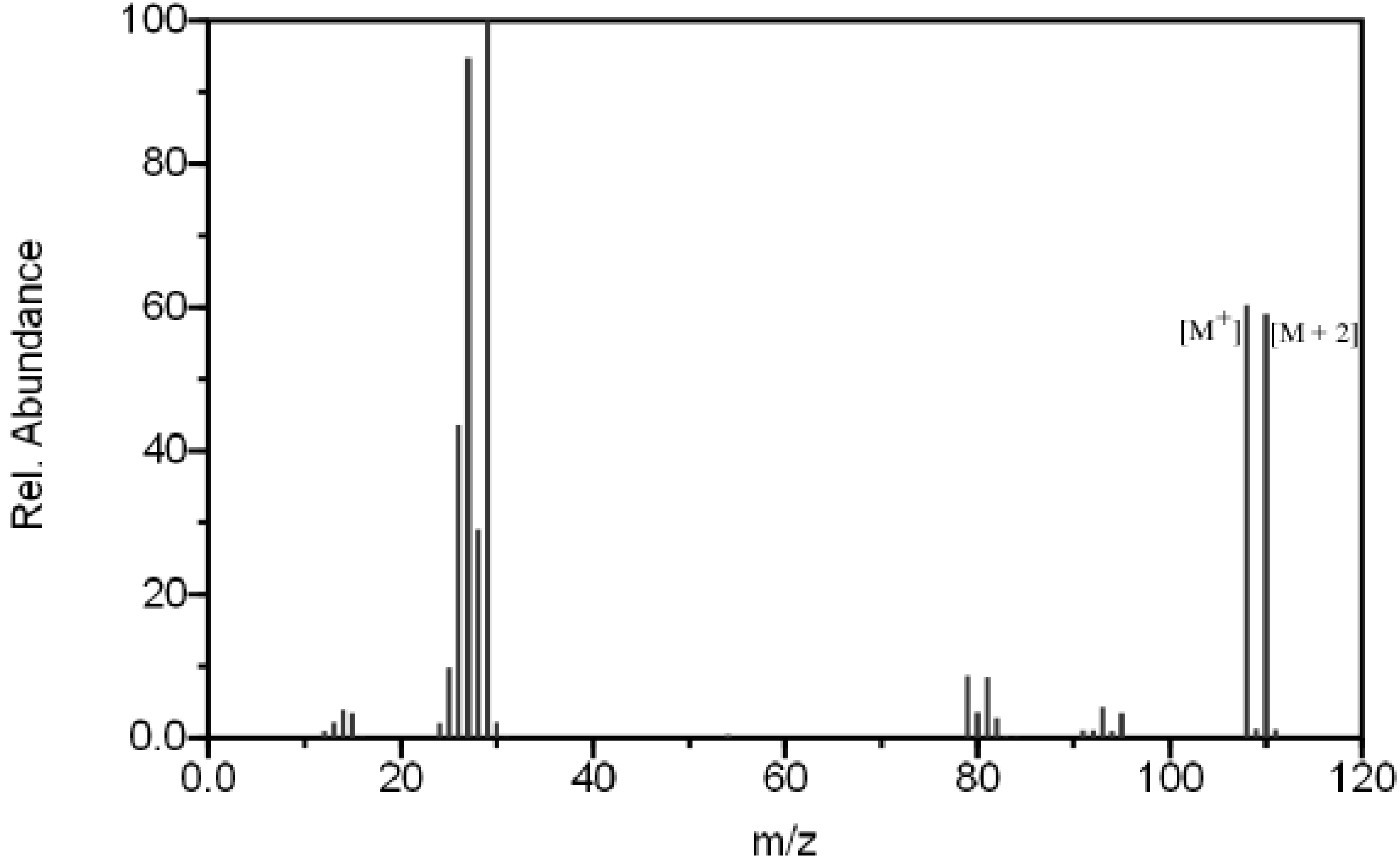
# Example1:



# Example 2:



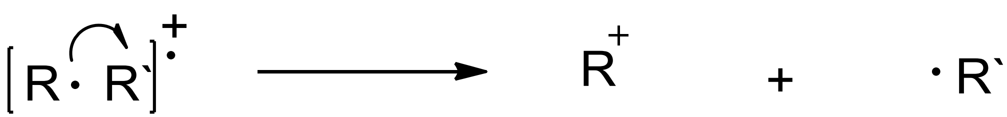
# Fragmentation Patterns in Mass Spectrometry

- ❑ Ionization

$$R:R^{`} + e^{-} \longrightarrow [R\cdot R^{`}]^{+\cdot} + 2e^{-}$$

Radical cation
(molecular ion)

- ❑ Fragmentation



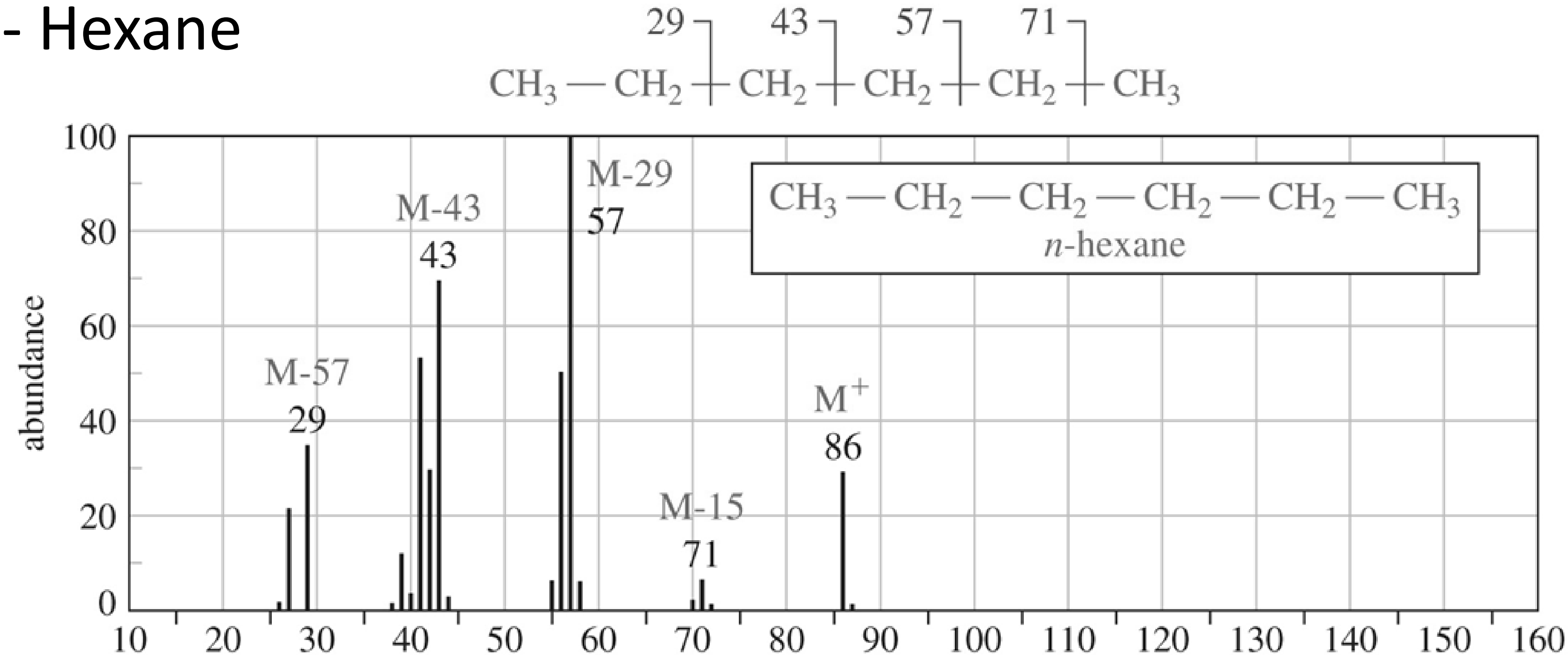
Cation fragment (observed)

Radical fragment (not observed)

- ❑ The most stable fragments usually formed (bond breaking doesn't take place randomly)

# A. Mass Spectra of Alkanes

n- Hexane




*m/z* of the charged fragment on this side of the broken bond

57

$[CH_3CH_2CH_2CH_2 \mid CH_2CH_3]^{+\cdot} \longrightarrow CH_3CH_2CH_2CH_2^{+} + \cdot CH_2CH_3$

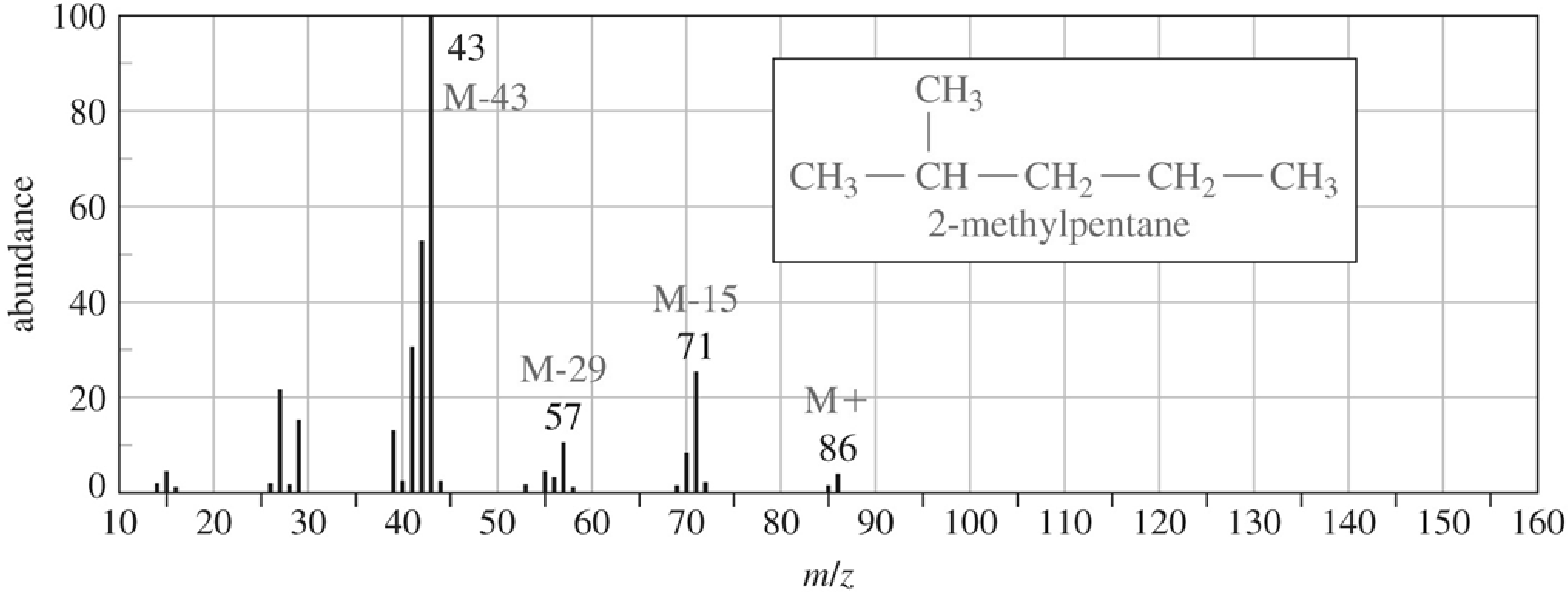
hexane radical cation, $M^+$ 86 → 1-butyl cation, detected at *m/z* 57 + ethyl radical (29), not detected

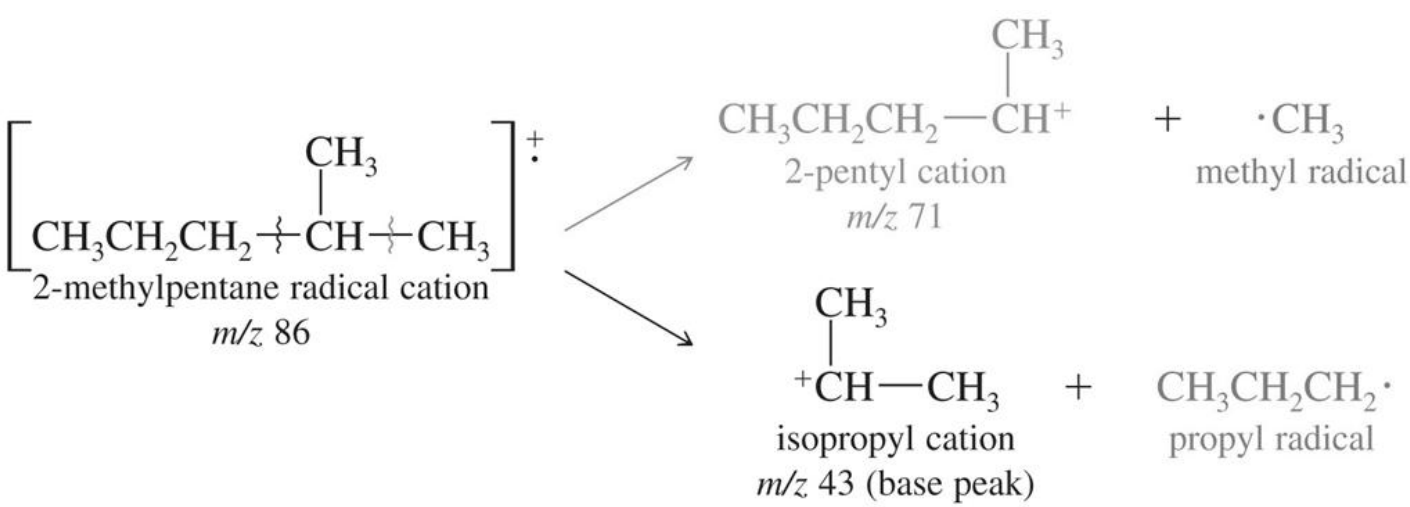
29

$[CH_3CH_2CH_2CH_2 \mid CH_2CH_3]^{+\cdot} \longrightarrow CH_3CH_2CH_2CH_2\cdot + {}^{+}CH_2CH_3$

hexane radical cation, $M^+$ 86 → 1-butyl radical (57), not detected + ethyl cation, detected at *m/z* 29

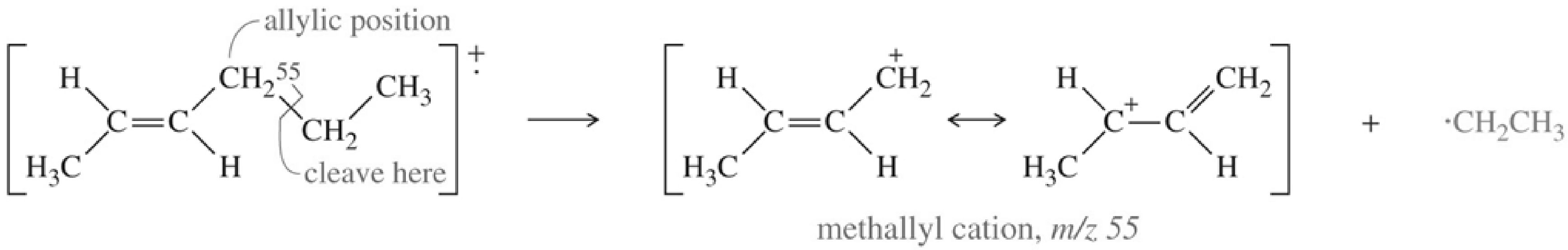
# Fragmentation of Branched Alkanes

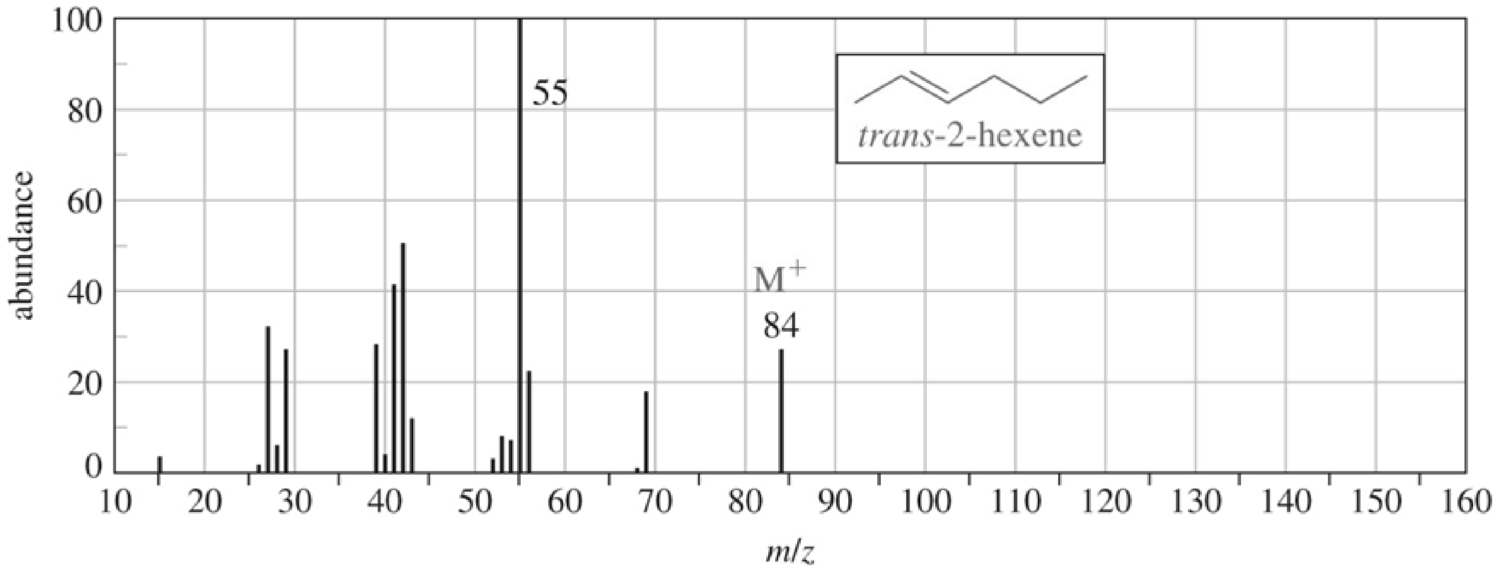



Fragmentation occurs to give the most stable cation and radical

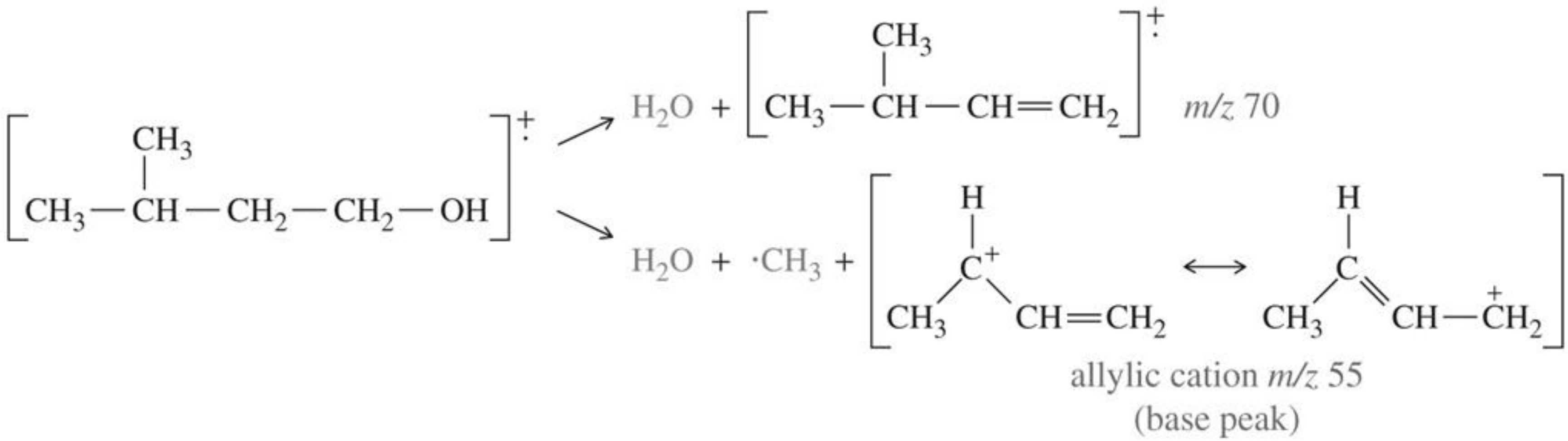
# B. Mass Spectra of Alkenes

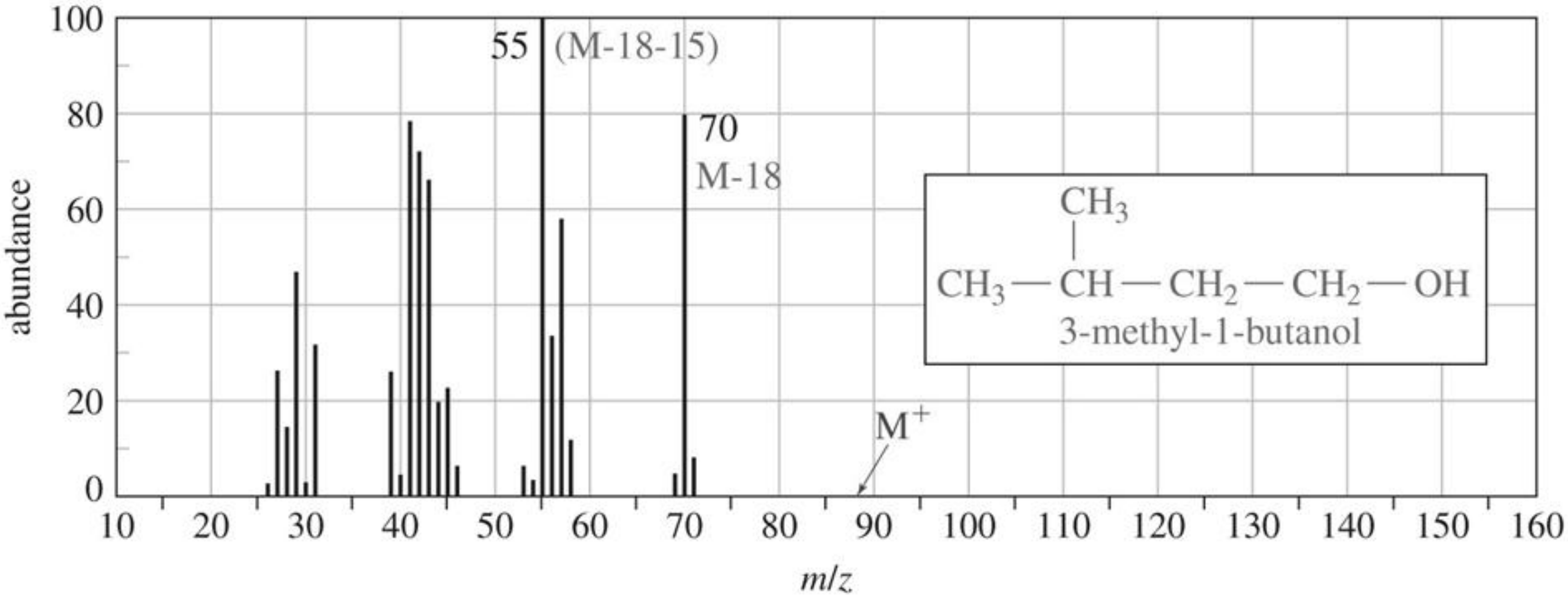



Resonance-stabilized cations are favored.

# C. Mass Spectra of Alcohols

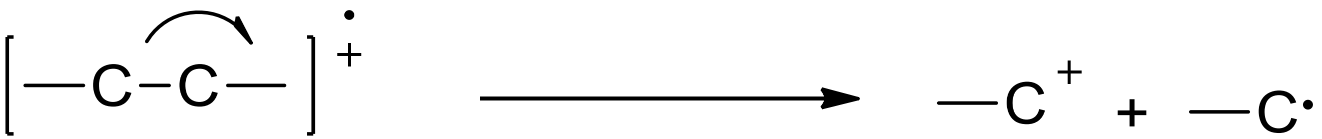


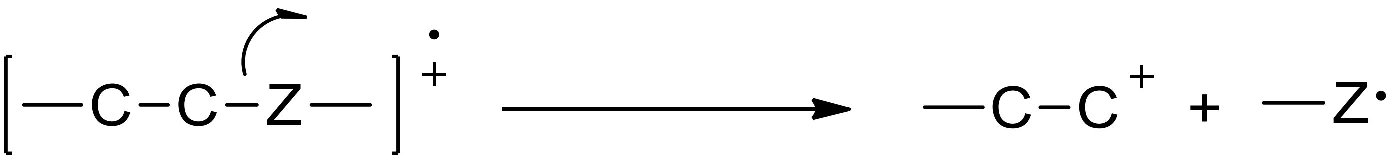
# Categories of Fragmentation

## A. One-bond σ-cleavages

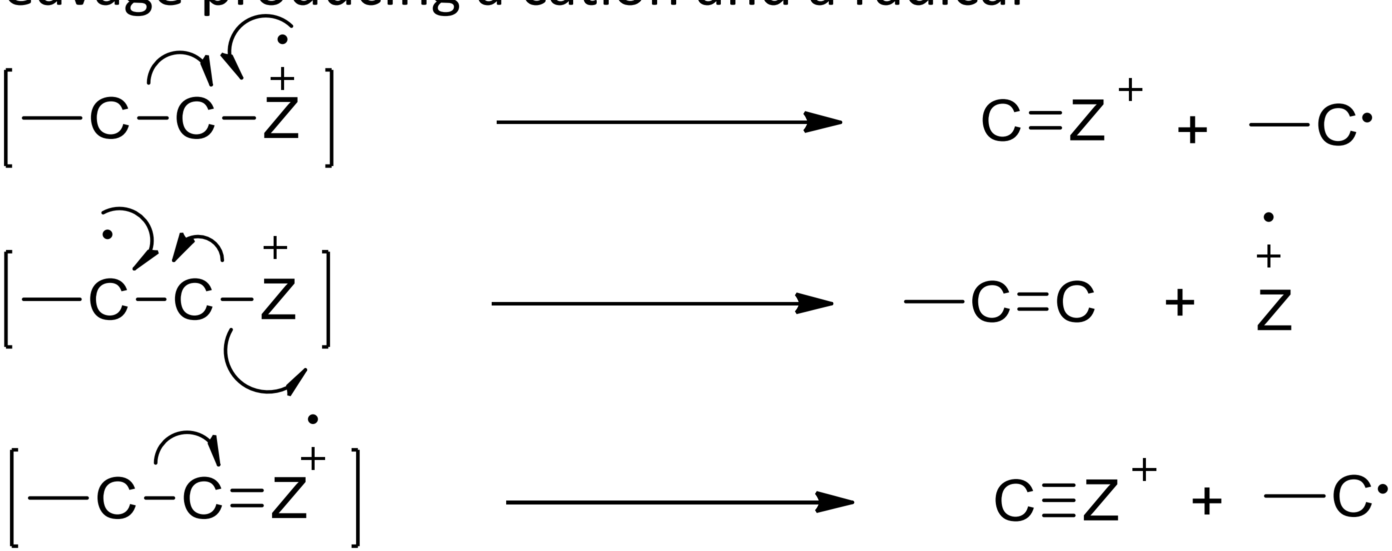
(a) Cleavage at C-C producing a cation and radical



(b) Cleavage at C-heteroatom producing a cation and a radical

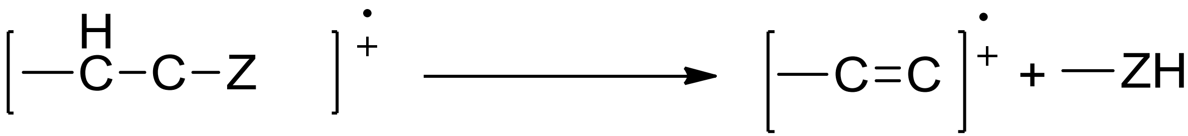


(c) α-Cleavage producing a cation and a radical

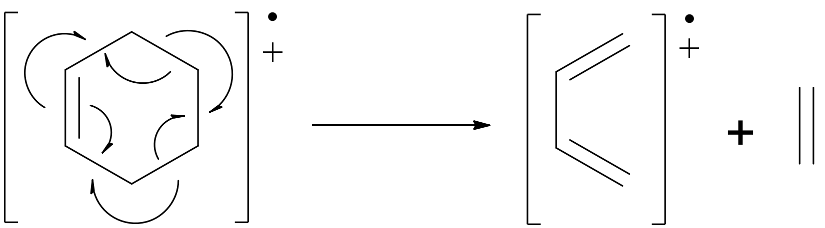


# B. Two-bond σ-cleavages or rearrangements

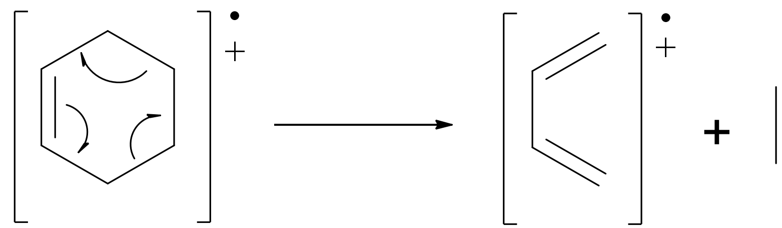
## (d) Elimination of a vicinal H and heteroatom:




## (e) Retro-Diels-Alder cleavage:

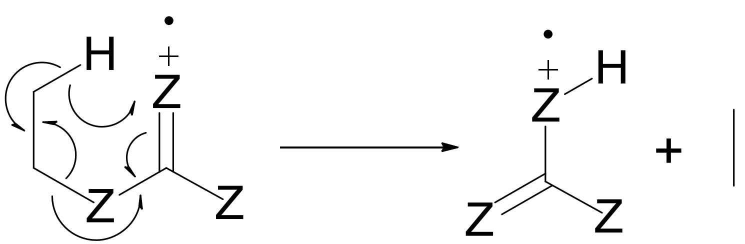


Full Mechanism

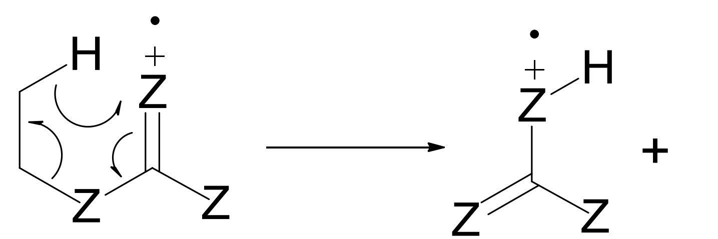


Abbreviated Mechanism

## (c) McLafferty type rearrangements:

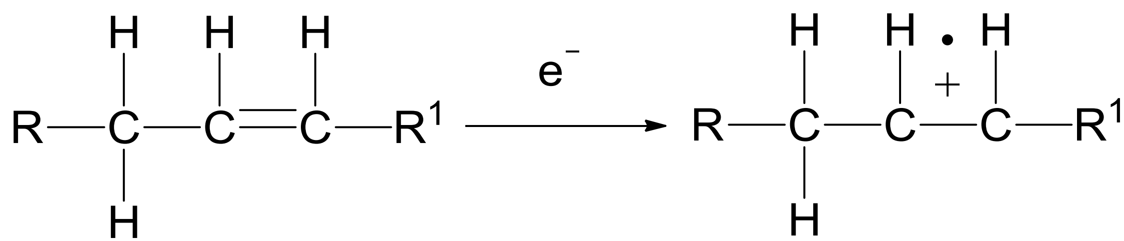


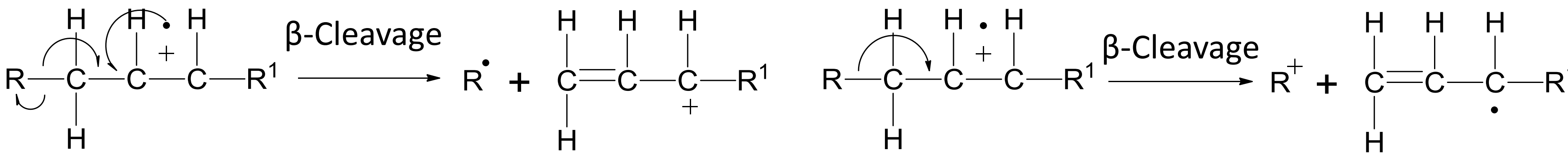

Full Mechanism

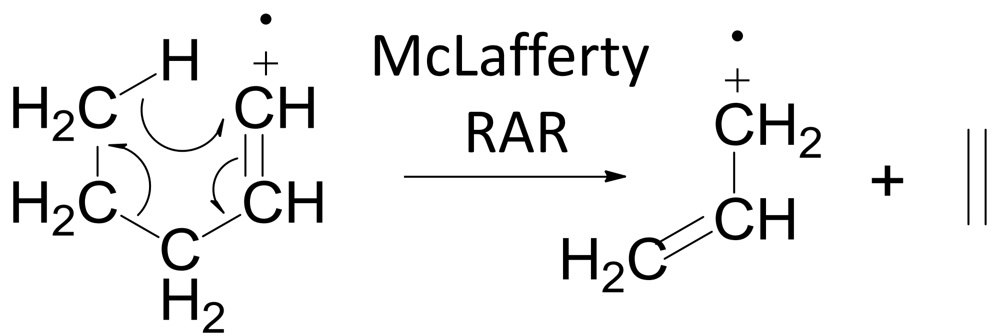


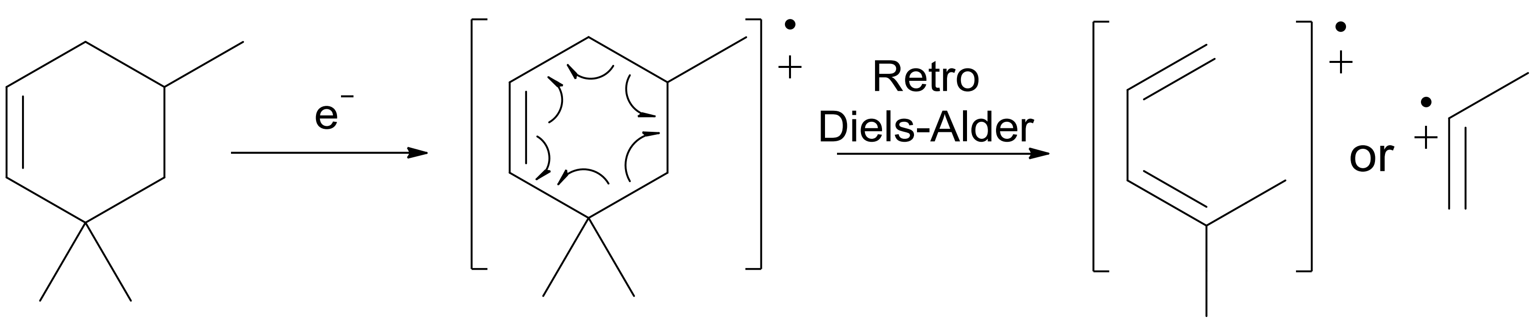

Abbreviated Mechanism

# Alkenes

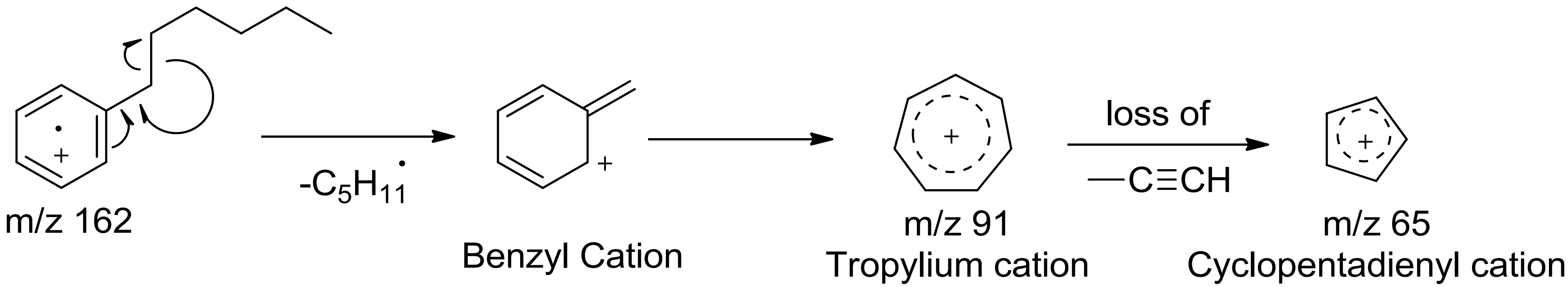

e⁻

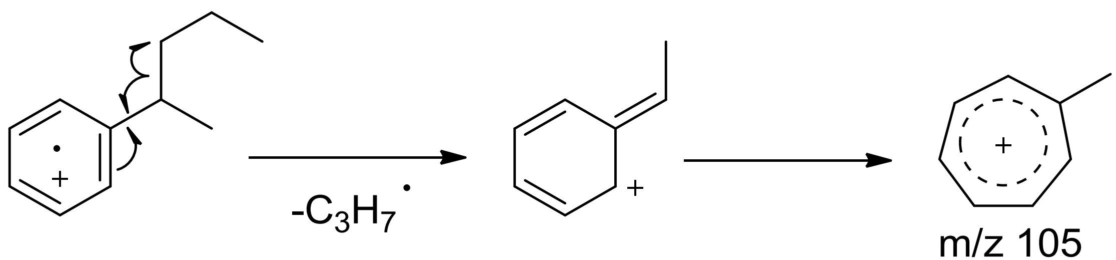

β-Cleavage
β-Cleavage

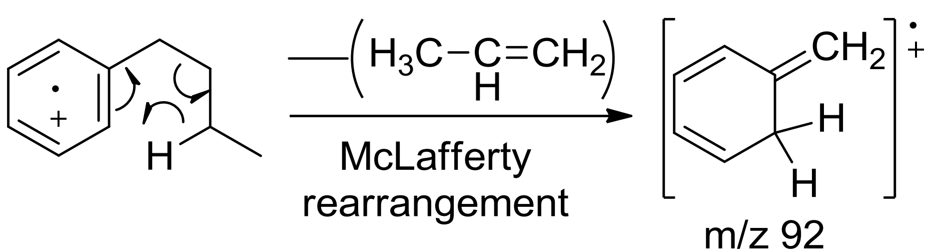

McLafferty
RAR


e⁻
Retro
Diels-Alder
or

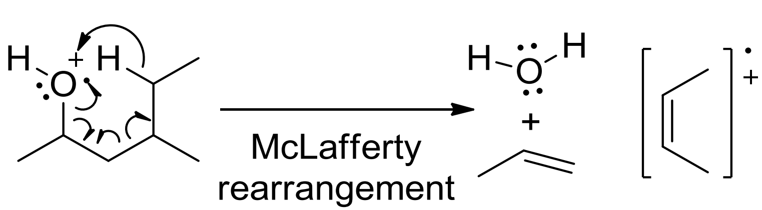
# Benzenoid Aromatics

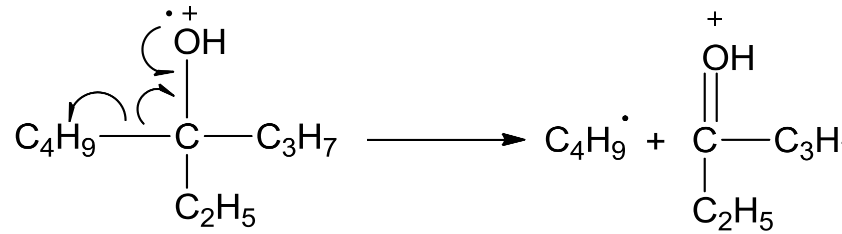


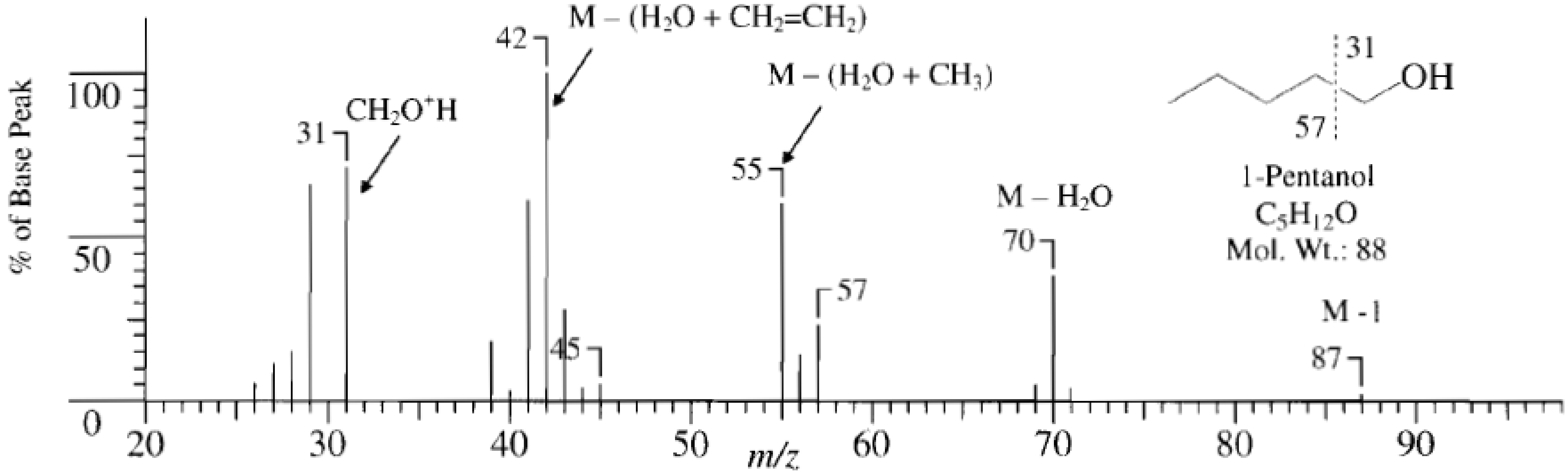



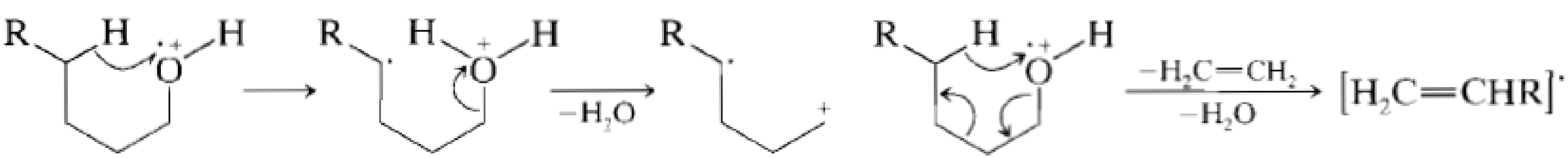
# Alcohols



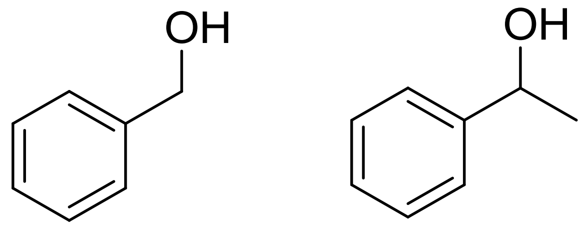




## Example:

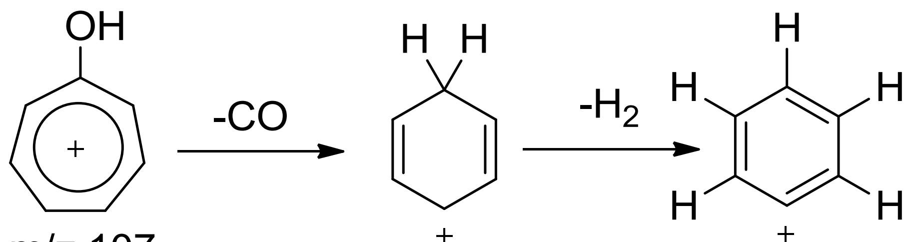


# Benzyl alcohols and substituted homologs




Benzyl alcohol 1-Phenylethanol

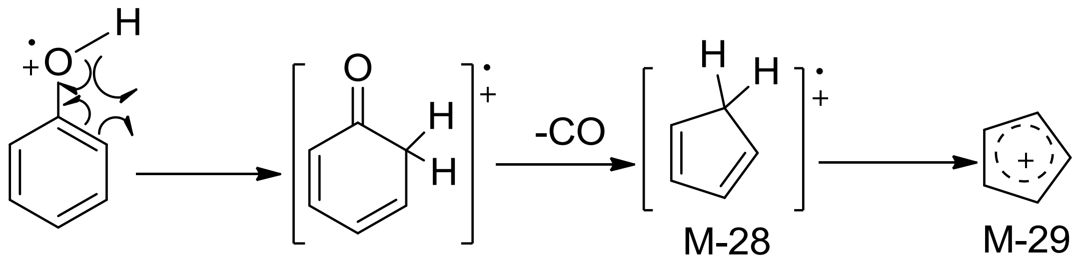


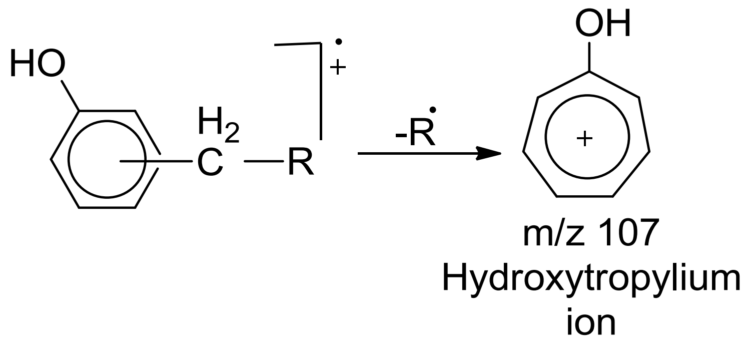

m/z 107
Hydroxytropylium ion

m/z 79

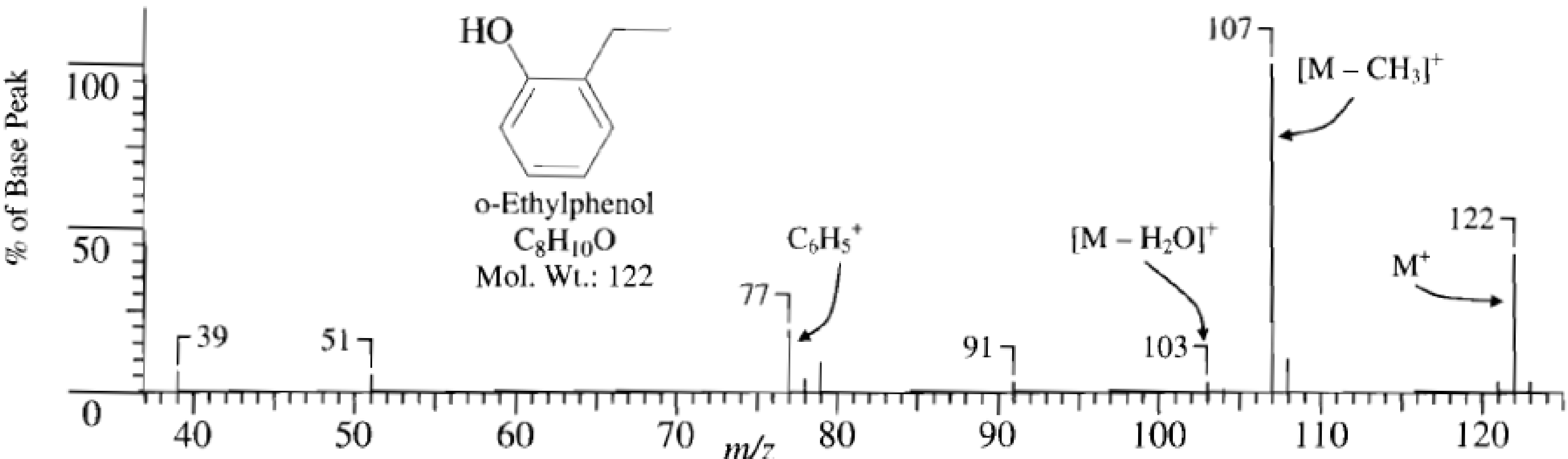
m/z 77

# Phenols



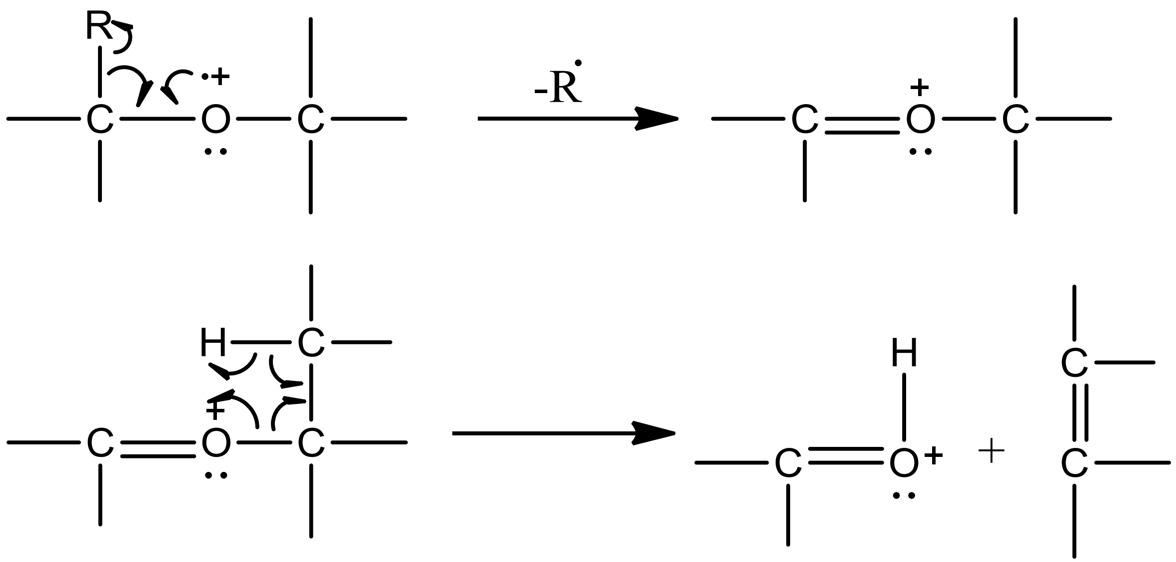

Example:



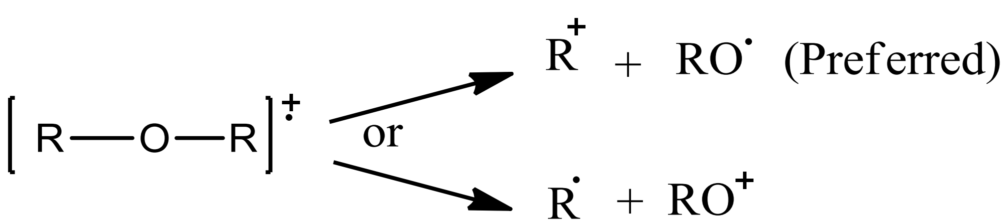
# Ethers

## Aliphatic Ethers

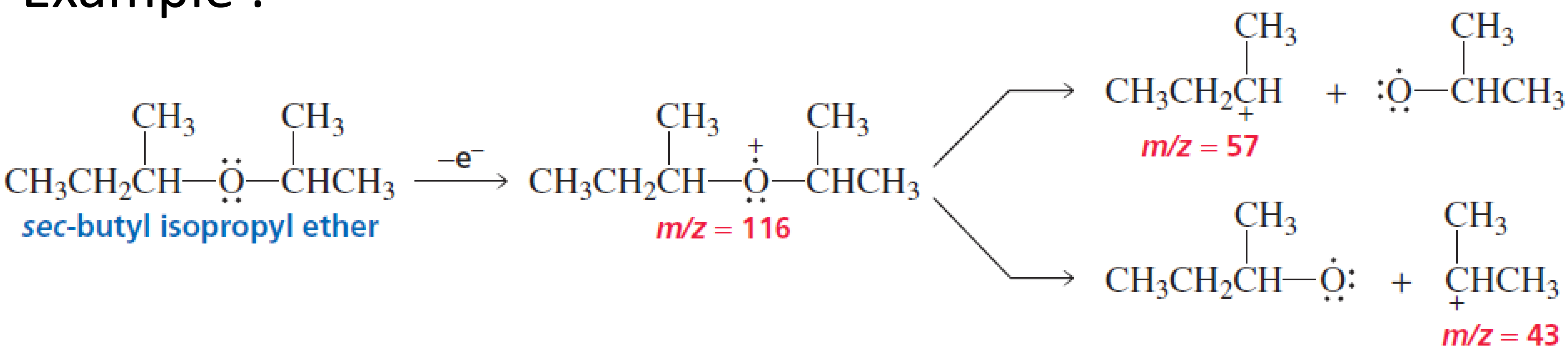
1. Cleavage of the C-C bond next to the oxygen:



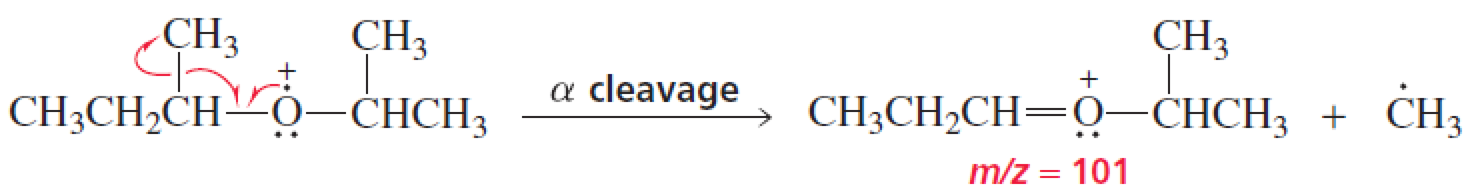

2. C-O Bond cleavage:

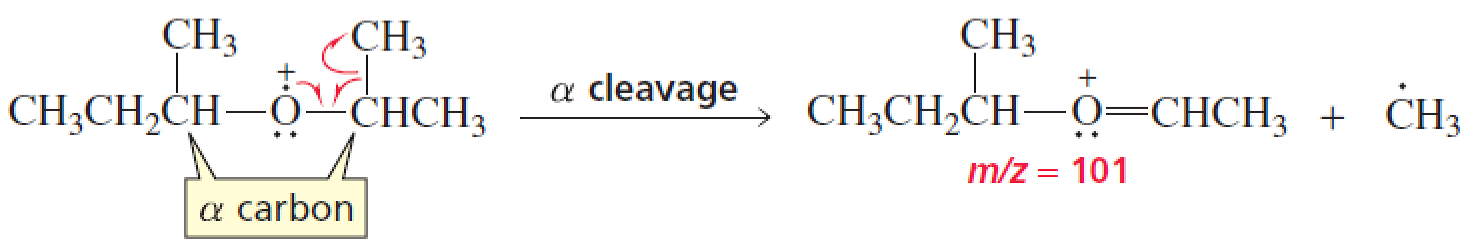


# Example :

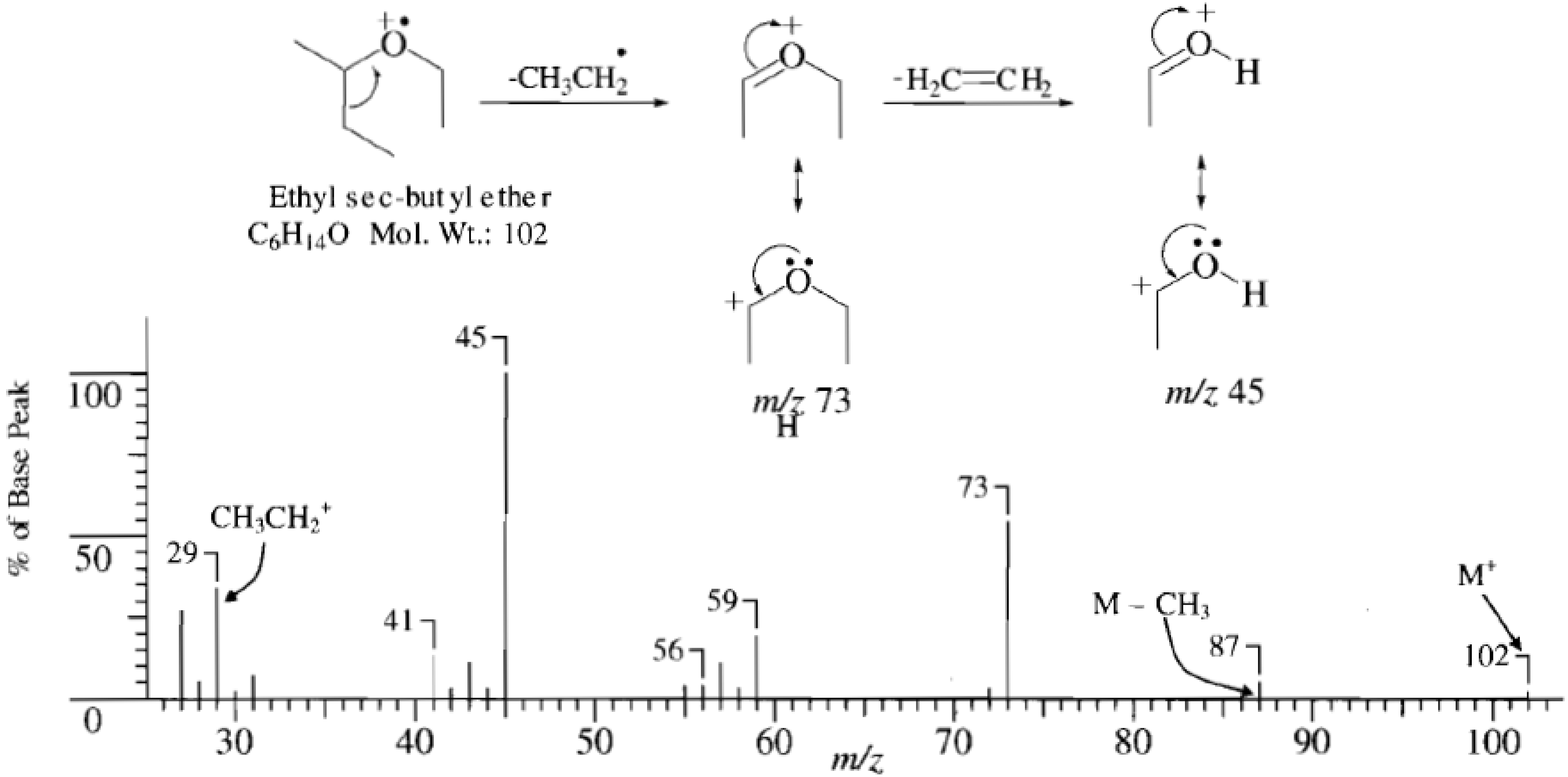

sec-butyl isopropyl ether
−e⁻
m/z = 116
m/z = 57
m/z = 43


α cleavage
m/z = 87


α cleavage
m/z = 101

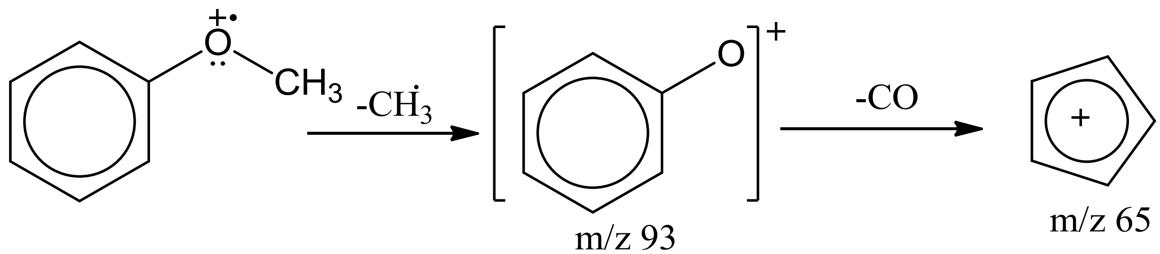

α carbon
α cleavage
m/z = 101

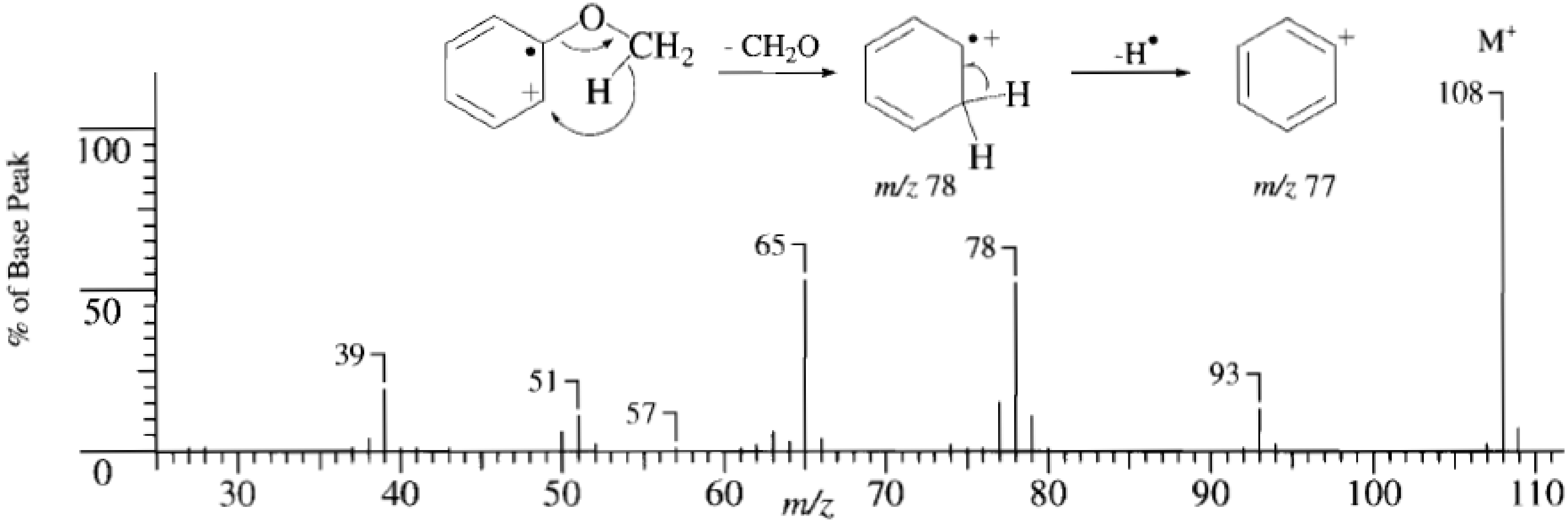
Example :



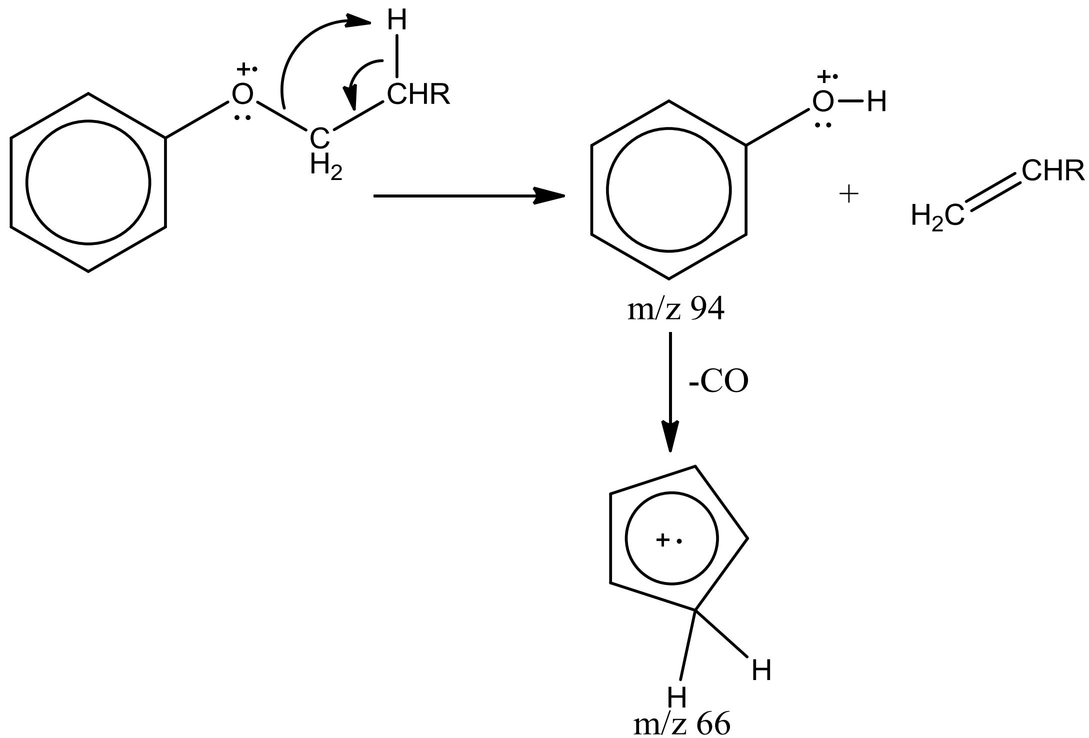
-CH3CH2•
-H2C=CH2
H
Ethyl sec-butyl ether
C6H14O Mol. Wt.: 102
H
m/z 73
H
m/z 45
% of Base Peak
100
50
0
45
73
CH3CH2+
29
41
59
56
M − CH3
87
M+
102
30
40
50
60
70
80
90
100
m/z

# Aromatic Ethers

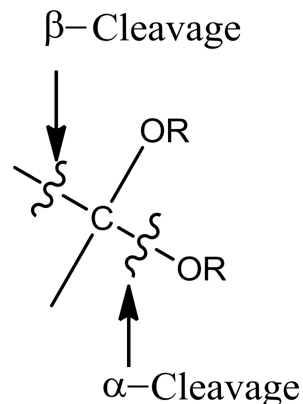


# Aromatic Ethers



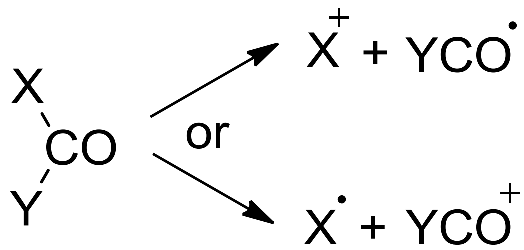

# Acetals and Ketals



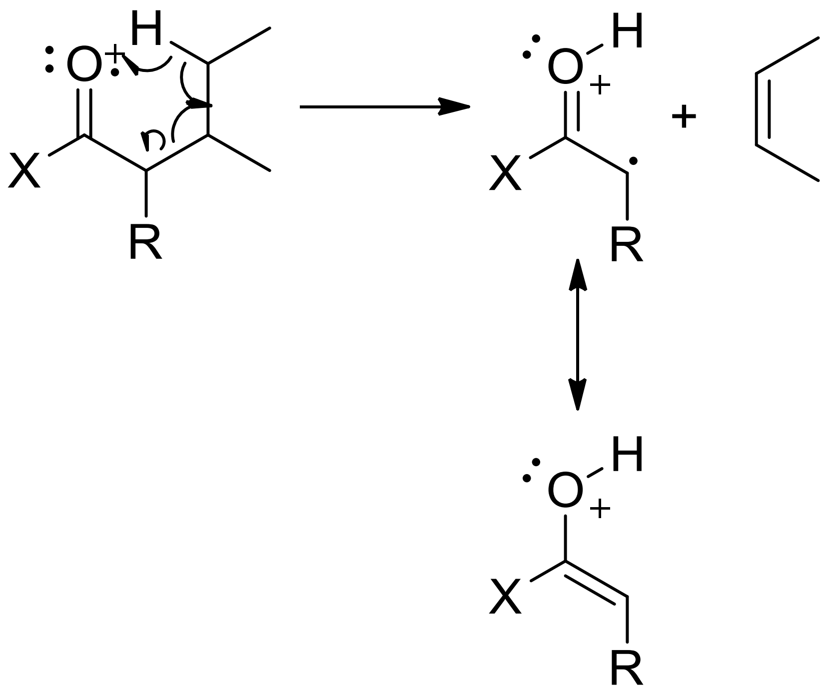

β-Cleavage is favored over α-cleavage

# Carbonyl Compounds

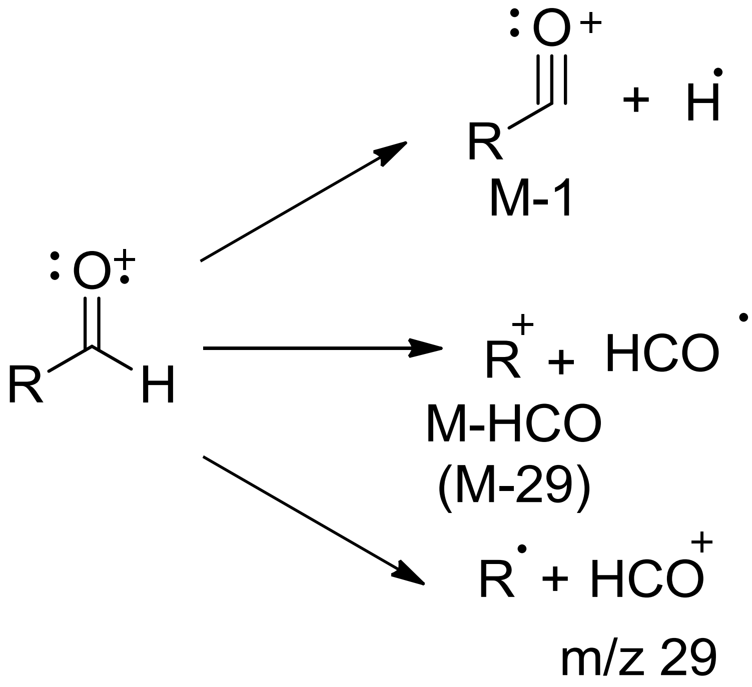
α-Cleavage



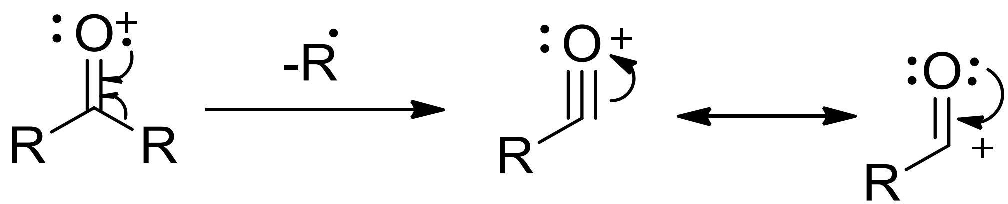

β-Cleavage with McLafferty RAR

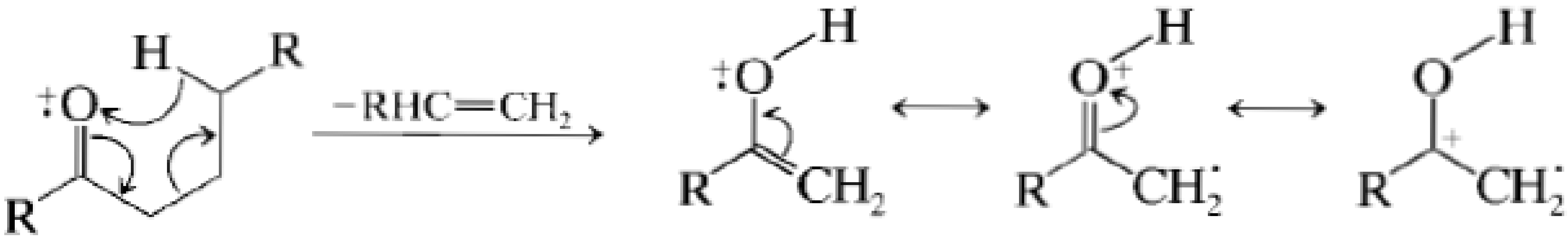


# Aldehydes

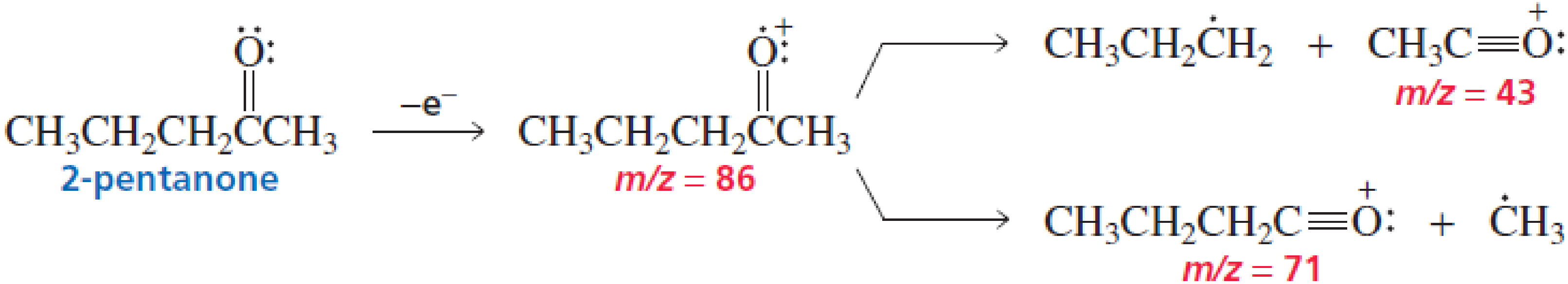


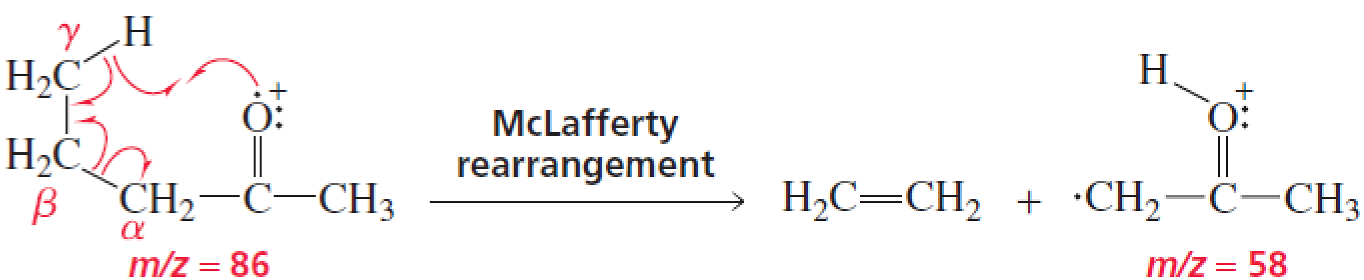
# Ketones



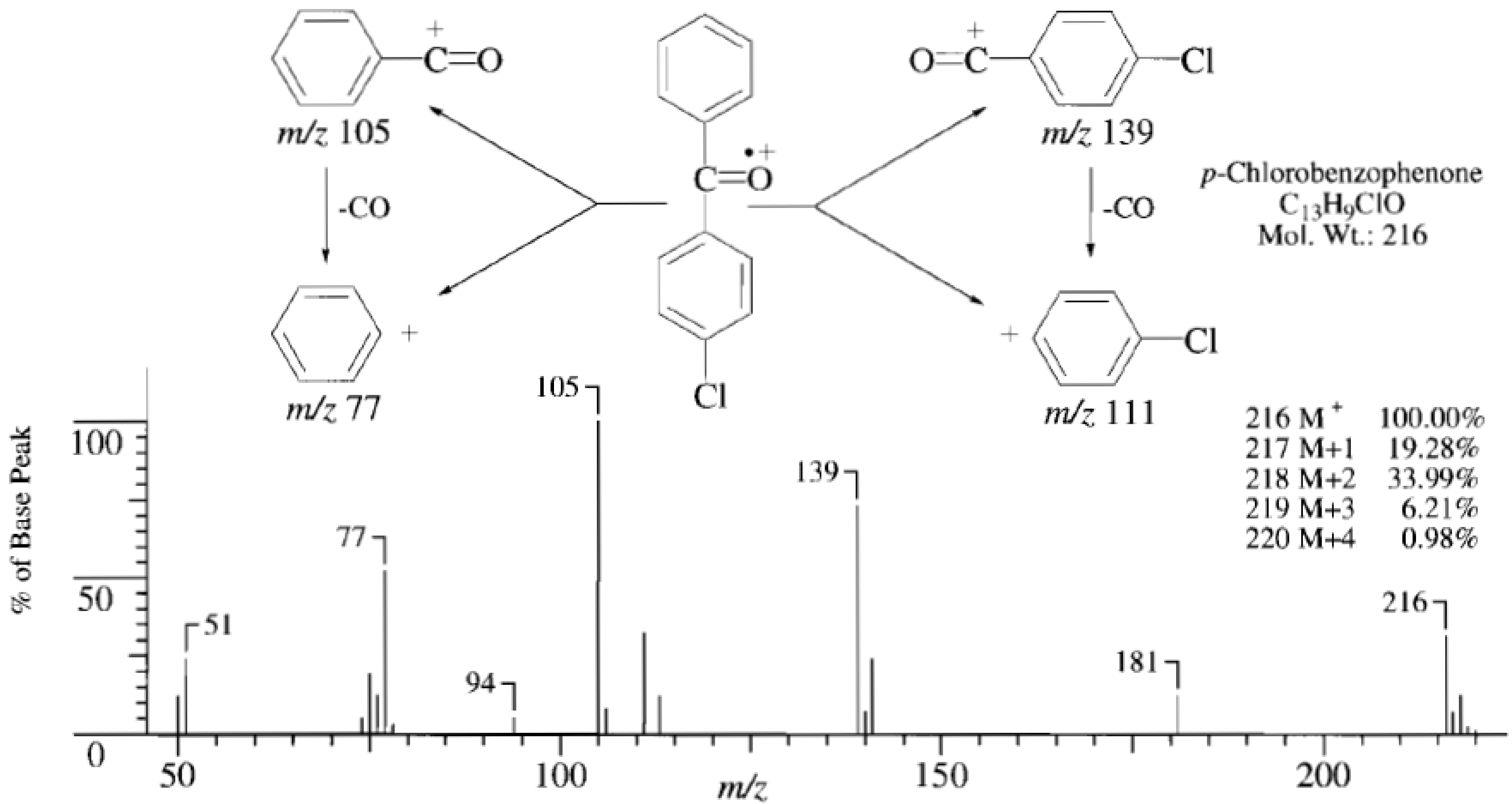




## Example 1:



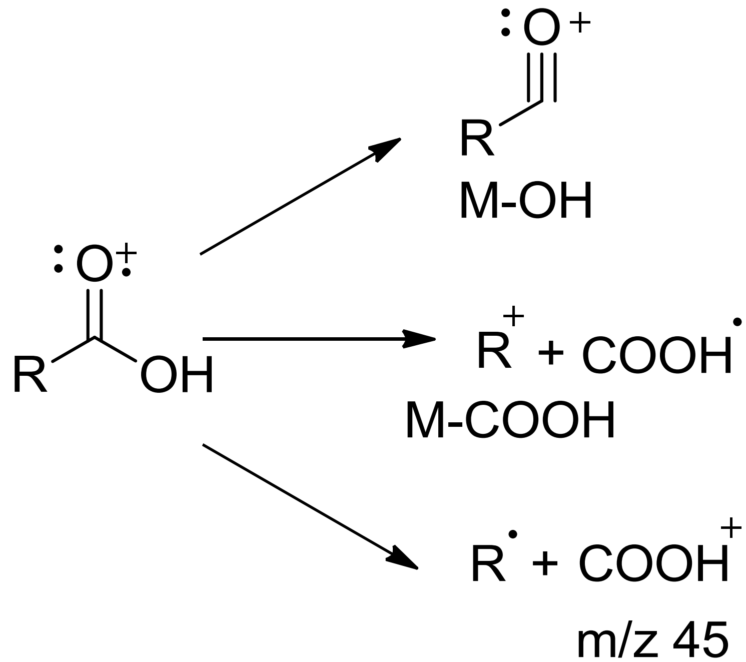



# Example 2:

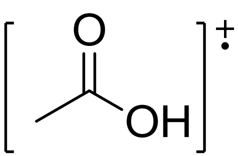


m/z 105
-CO
m/z 77
m/z 139
-CO
m/z 111
p-Chlorobenzophenone
C13H9ClO
Mol. Wt.: 216
216 M+ 100.00%
217 M+1 19.28%
218 M+2 33.99%
219 M+3 6.21%
220 M+4 0.98%
% of Base Peak
m/z
105
139
77
51
94
181
216

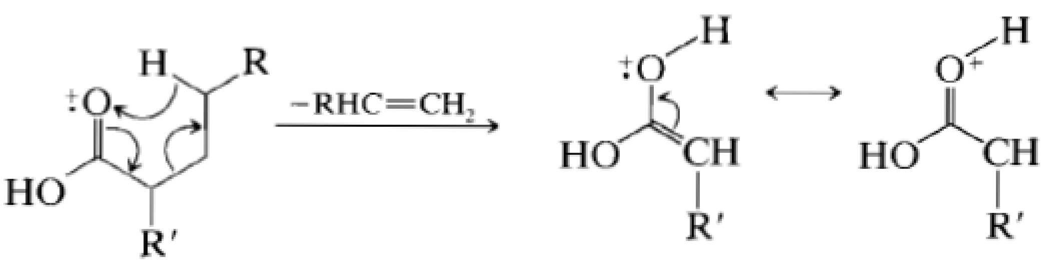
# Carboxylic Acids



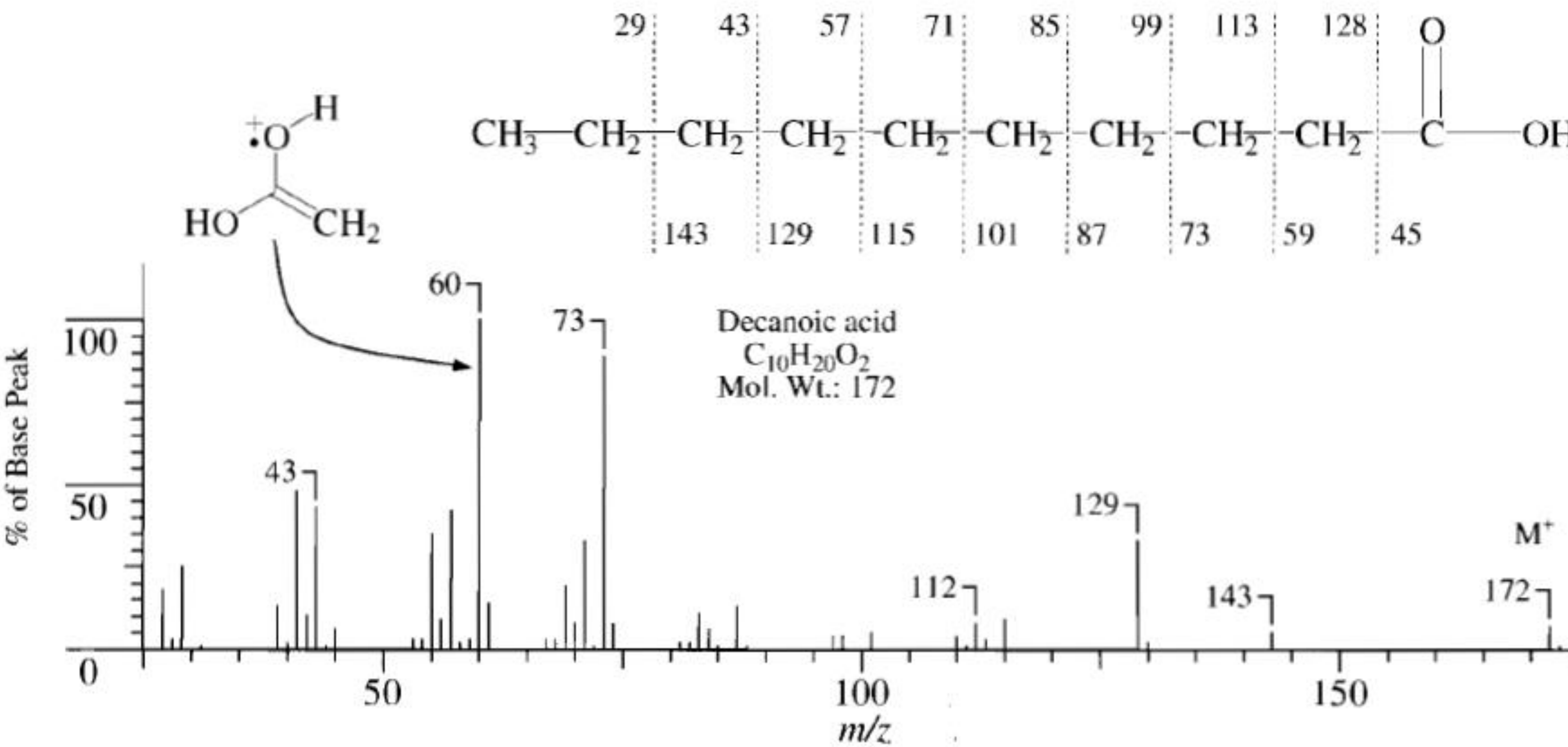

- Loss of 44 is loss of $CO_2$
- m/z = 45 suggests $[O{=}C{-}OH]^+$
- γ Hydrogen present? McLaffert rearrangement to alkene plus:



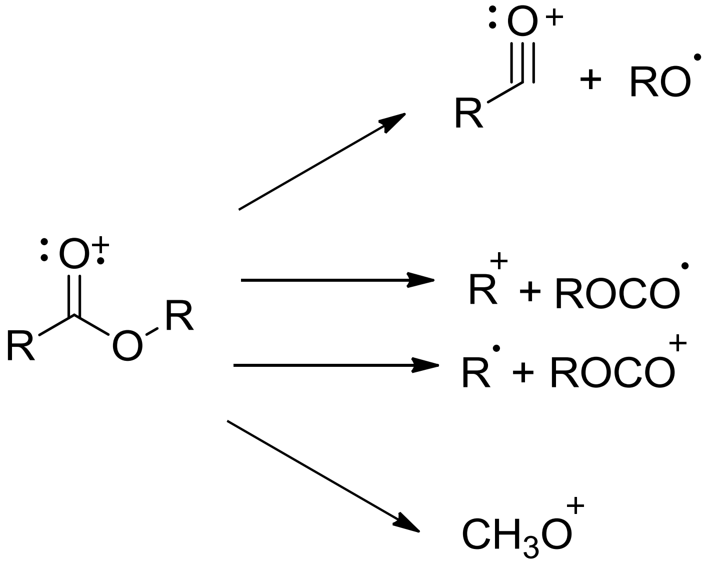

m/z 60



# Example

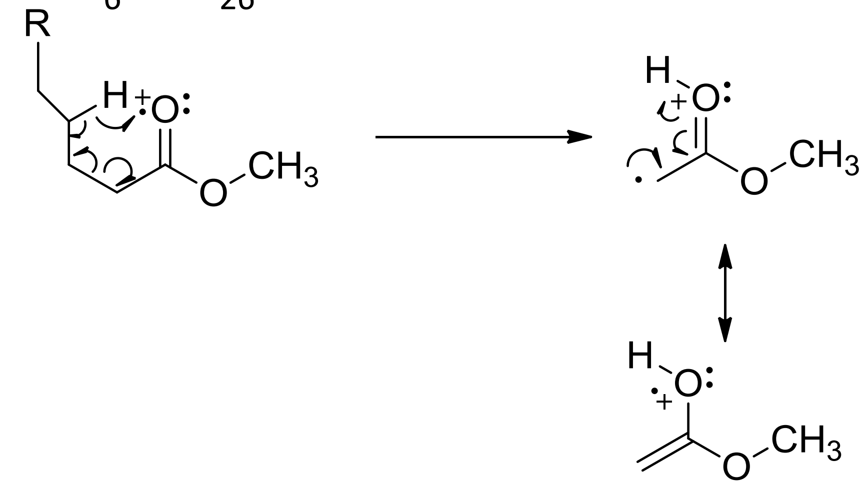

29
43
57
71
85
99
113
128
O
CH3 CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH2 C OH
143
129
115
101
87
73
59
45
H
O
HO
CH2
60
73
Decanoic acid
C10H20O2
Mol. Wt.: 172
43
129
M+
112
143
172
% of Base Peak
100
50
0
50
100
150
m/z

# Esters

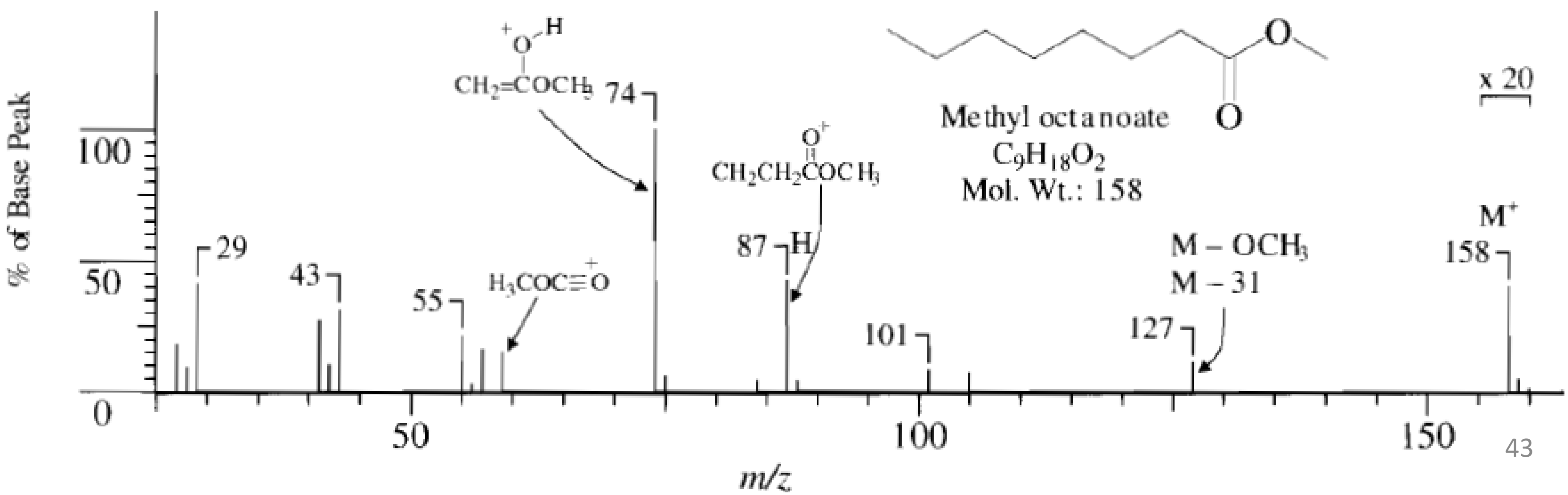



(in case of methyl esters)

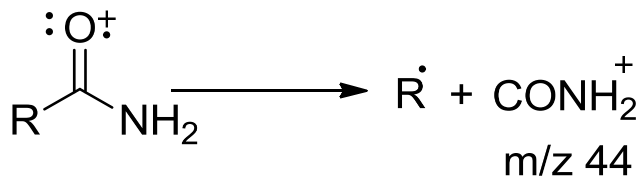
For saturated straight-chain methyl esters from $C_6$ to $C_{26}$

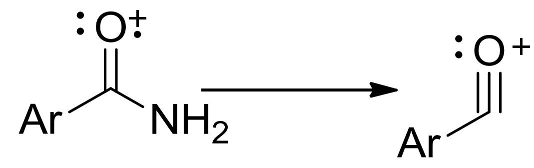



m/z 74

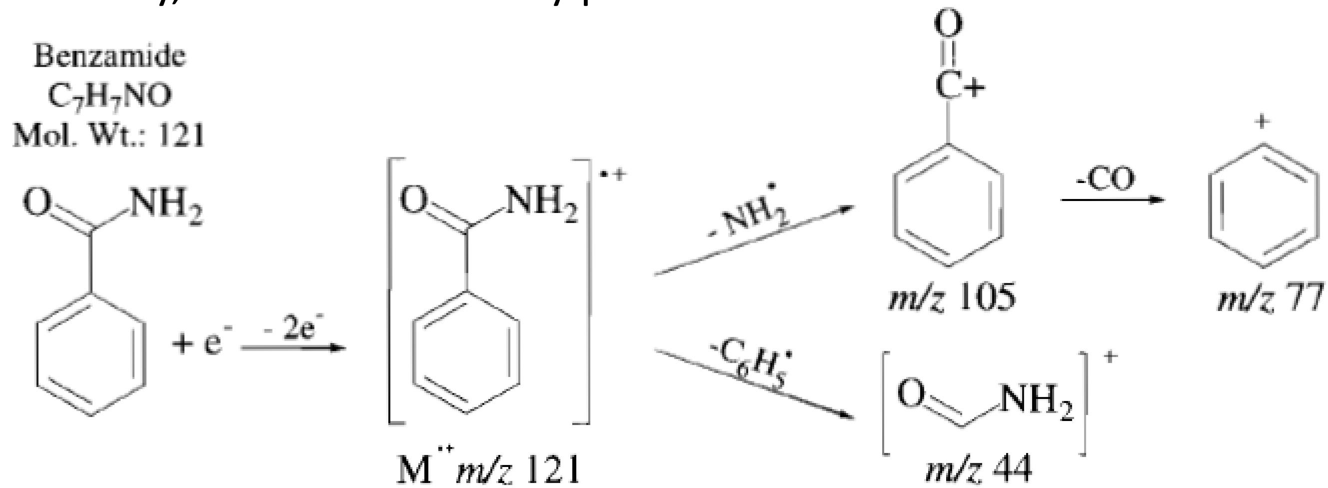


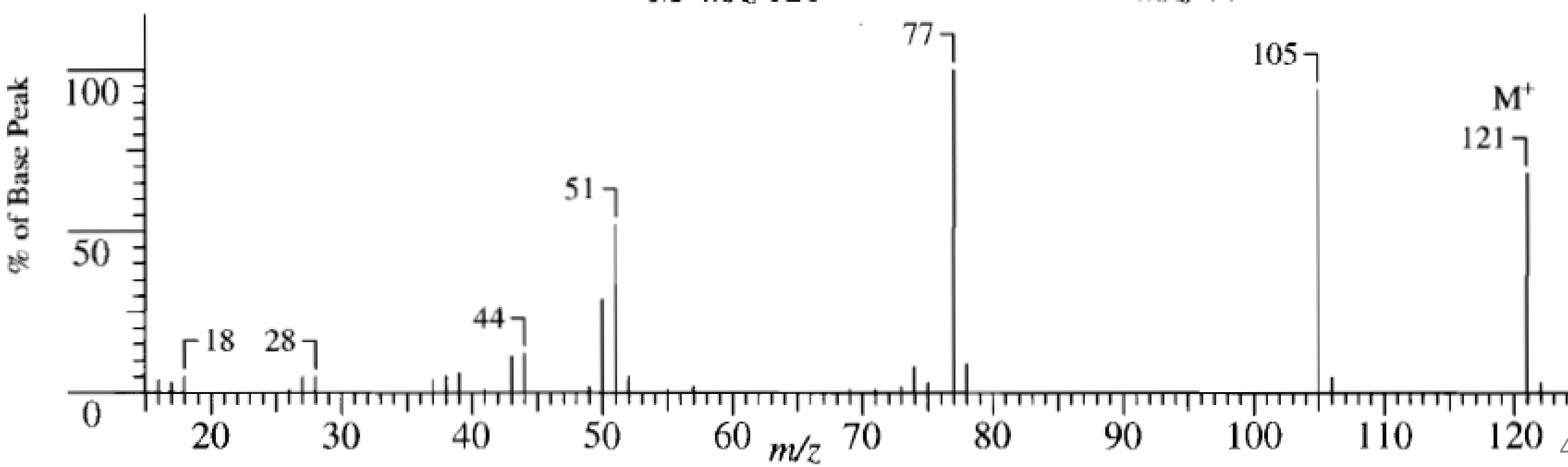
# Amides



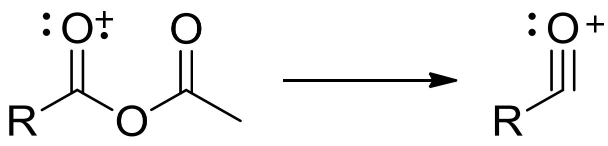



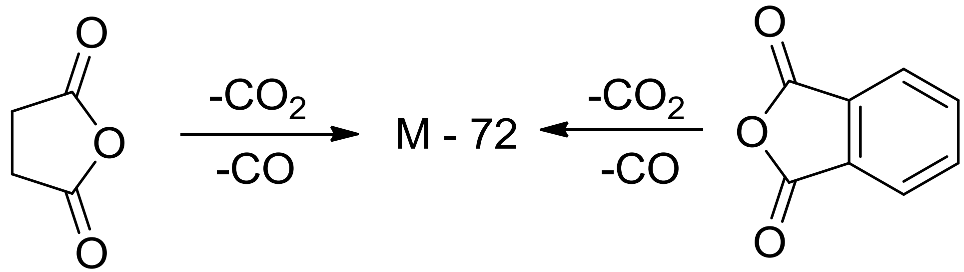

For secondary and tertiary, there are too many possibilities



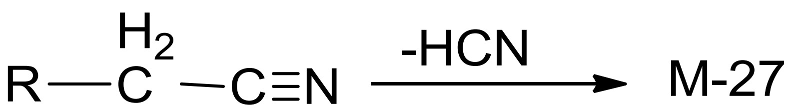



# Anhydrides



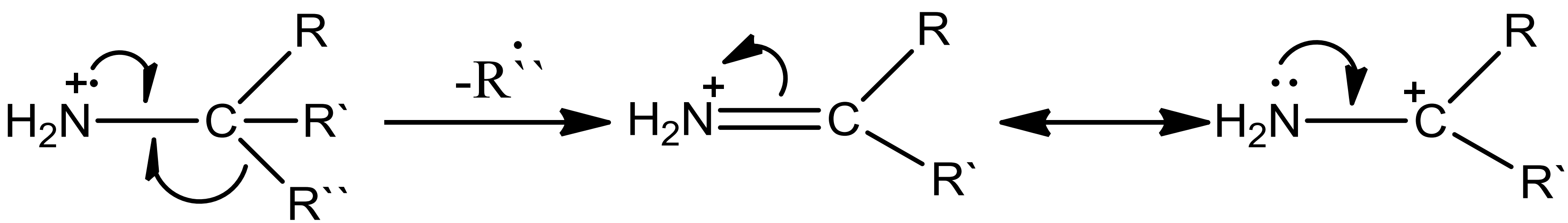




# Nitriles

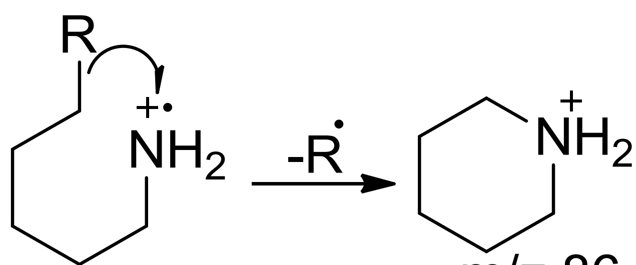



McLafferty ion is usually the base peak from C4 on

# Amines




Cyclic fragments occur during the fragmentation of longer chain amines



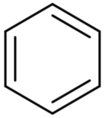
# Rule of 13

Rule of 13: assumes a $C_nH_n$ structure

Mass of a CH unit = 13

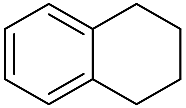
So divide *m/z by 13 and remainder is additional hydrogens*

For *m/z = 78, then 78/13 = 6*

MF = $C_6H_6$ 

For *m/z = 132, then 132/13 = 10 and 2 remain*

$C_{10}H_{10}$ + two additional Hs → $C_{10}H_{12}$ or isomers



In this model, heteroatoms are viewed as CH equivalents:

$^{16}O = CH_4$

$^{14}N = CH_2$

$^{19}F = CH_7$

$^{32}S = C_2H_8$ ,etc.

so *m/z = 112*      *112/13 = 8 and 8 remain*

$$C_8H_8 + 8Hs \longrightarrow C_8H_{16}$$

If one O: replace $CH_4$ with O

$$C_8H_{16} \longrightarrow C_7H_{12}O$$

If two O: replace $C_2H_8$ with $O_2$

$$C_8H_{16} \longrightarrow C_7H_{12}O \longrightarrow C_6H_8O_2$$

## When Nitrogen is Present:

Nitrogen Rule: A compound with an even numbered MW contains zero or an even number of nitrogen atoms; if MW is odd, compound has an odd number of nitrogens.

*m/z = 93 use rule of 13*

93/13 = 7 (2 H remaining)

$C_7H_7$ and additional 2 Hs: $C_7H_9$

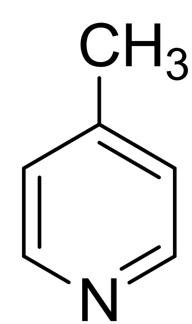
If you try to determine UN

UN = 7 + 1 –(9/2) = 3 ½ (?)

Suspect N because of odd molecular formula

Make substitution N = $CH_2$

$C_7H_9 \longrightarrow C_6H_7N$ (UN = 4)




Or Isomers