

# **Organic Spectral Analysis**

**College of Pharmacy**

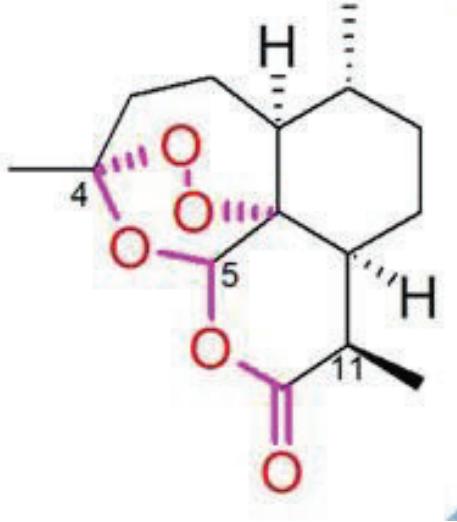
**Yong Chen**



# Artemisinin (青蒿素)



## How do we know:



- how atoms are connected together?
- Which bonds are single, double, or triple?
- What functional groups exist in the molecule?
- If we have a specific stereoisomer?

The field of organic structure determination attempts to answer these questions.

# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

Ionization Method

Ion Separation Methods

**Determination of Molecular Mass**

**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

# INSTRUMENTAL METHODS OF STRUCTURE DETERMINATION

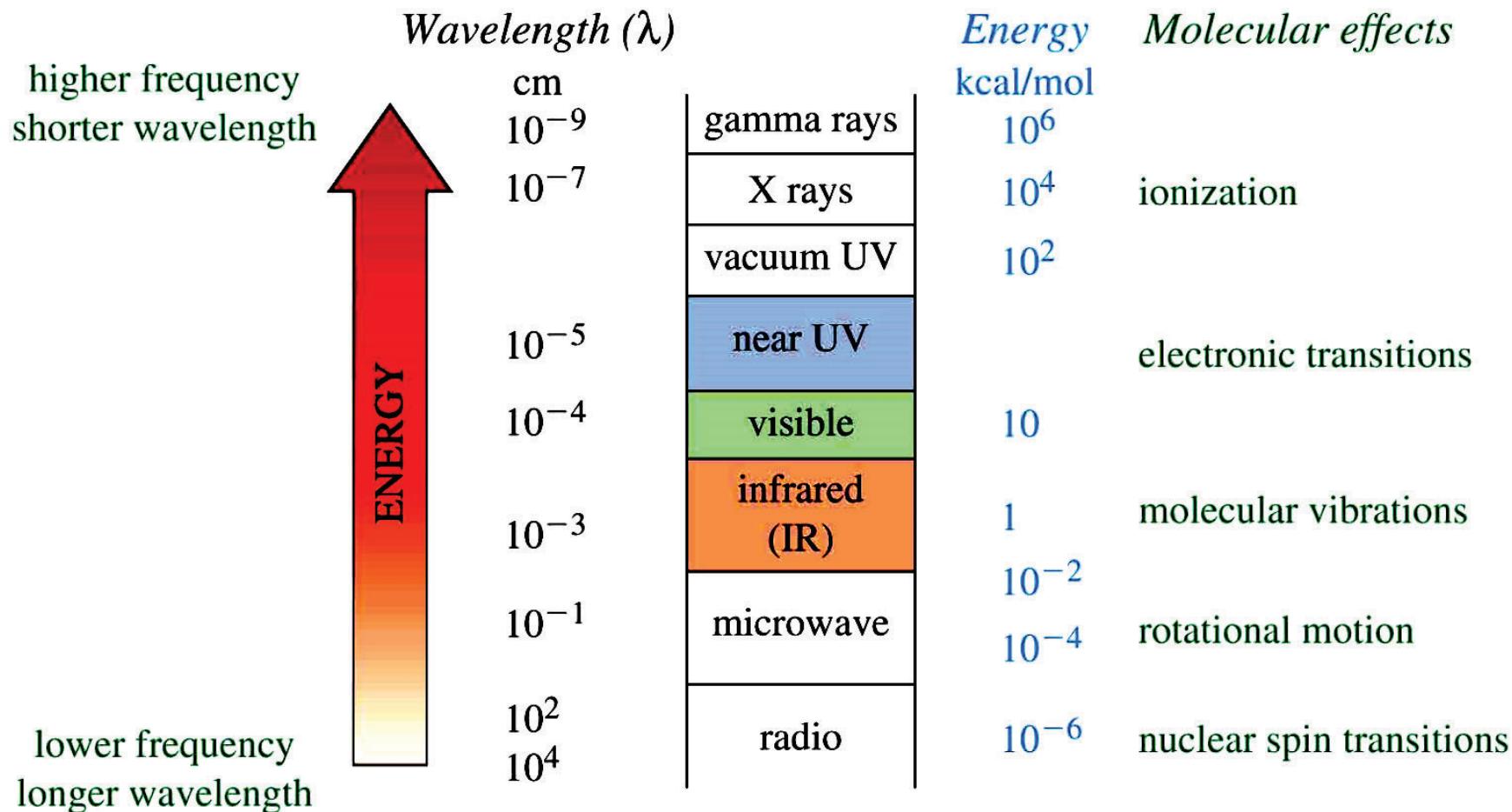
**1. Nuclear Magnetic Resonance (NMR)** – Excitation of the nucleus of atoms through radiofrequency irradiation. Provides extensive information about molecular structure and atom connectivity.

**2. Infrared Spectroscopy (IR)** – Triggering molecular vibrations through irradiation with infrared light. Provides mostly information about the presence or absence of certain functional groups.

**3. Mass spectrometry** – Bombardment of the sample with electrons and detection of resulting molecular fragments. Provides information about molecular mass and atom connectivity.

**4. Ultraviolet spectroscopy (UV)** – Promotion of electrons to higher energy levels through irradiation of the molecule with ultraviolet light. Provides mostly information about the presence of conjugated  $\pi$  systems and the presence of double and triple bonds.

# EFFECT OF ELECTROMAGNETIC RADIATION ON MOLECULES

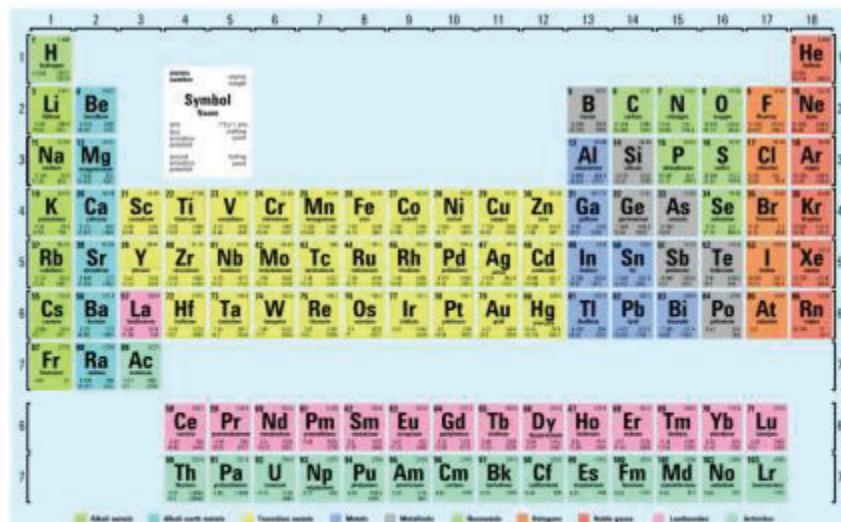


Graphics source: Wade, Jr., L.G. *Organic Chemistry*, 5th ed. Pearson Education Inc., 2003

# Mass Spectrometry

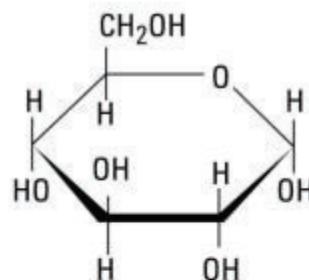
## Basic Considerations

Elements can be uniquely identified by their mass. Mass Spectrometry is an analytical method to measure molecular or atomic weight.

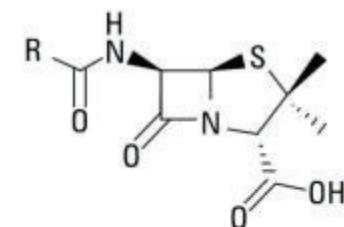


Source: Periodic table, [poster SI-0186](#)

Compounds, consisting of different elements, can be distinguished by their mass:



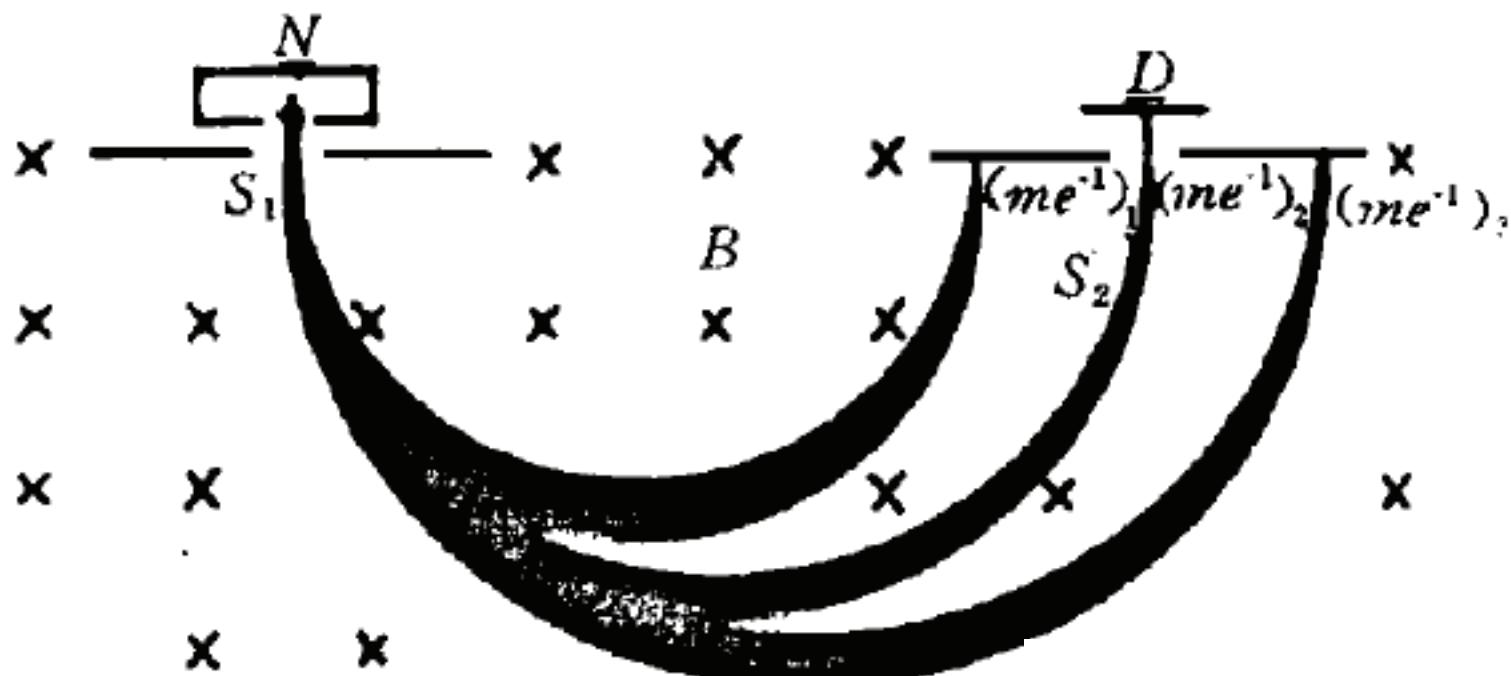
Glucose  $C_6H_{12}O_6$   
MW: 180,1559 g/mol



Penicillin  $C_{16}H_{18}N_2O_4S$   
MW: 334,39 g/mol

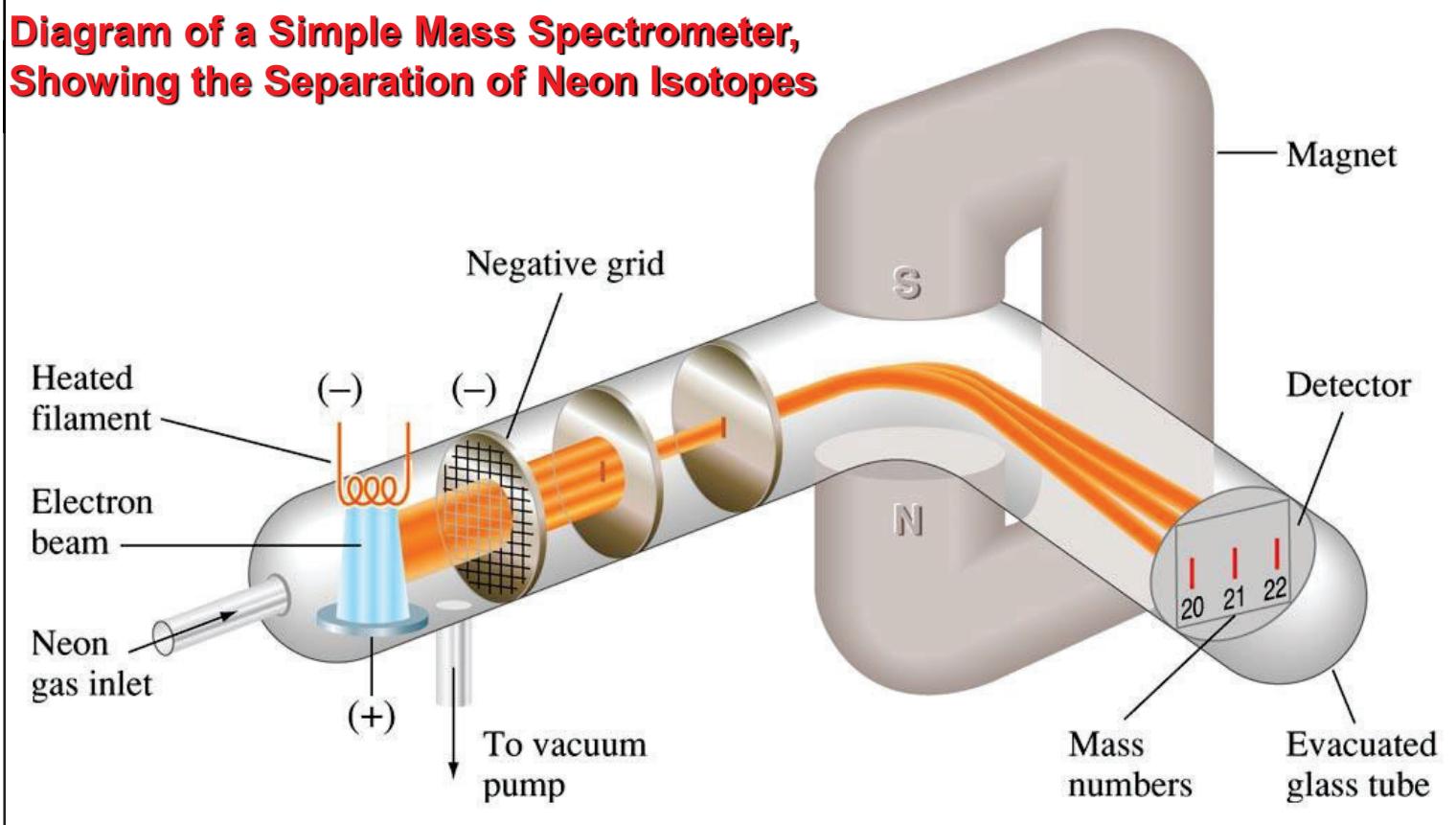
# Mass Spectrometry

Mass spectrometry involves ionizing a compound, separating the ions that result on the basis of mass to charge ratio ( $m/z$ ) and obtaining a plot of number of ions (abundance) versus  $m/z$ .



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# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

    Ionization Method

    Ion Separation Methods

**Determination of Molecular Mass**

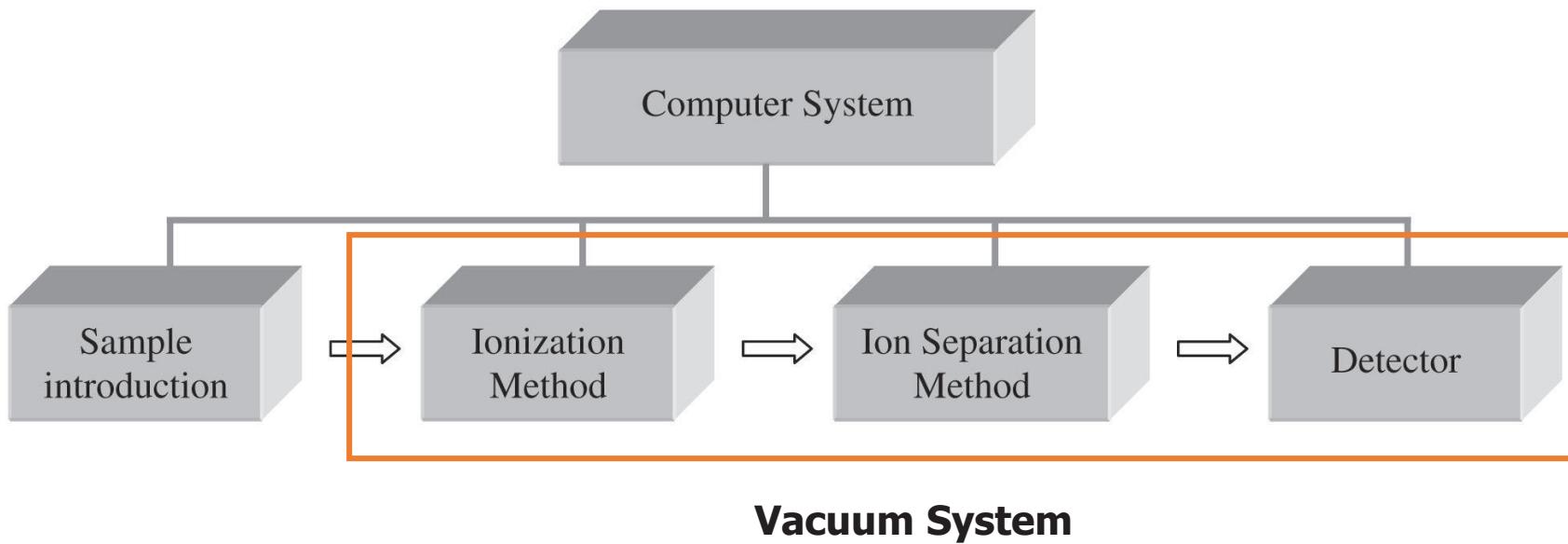
**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

# Mass Spectrometry

Mass spectrometry involves ionizing a compound, separating the ions that result on the basis of mass to charge ratio ( $m/z$ ) and obtaining a plot of number of ions (abundance) versus  $m/z$ .



# Mass spectrometer



**AB SCIEX TripleTOF® 5600+**



**Thermo Scientific LTQ-Orbitrap**



**Agilent 6546 LC/Q-TOF**

# **Ionization Method**

Before the sample can be mass analyzed, it must be ionized in the ion source.

## **Gaseous Sample Introduction:**

- Electron Ionization (**EI**) 电子轰击电离
- Chemical Ionization (**CI**) 化学电离

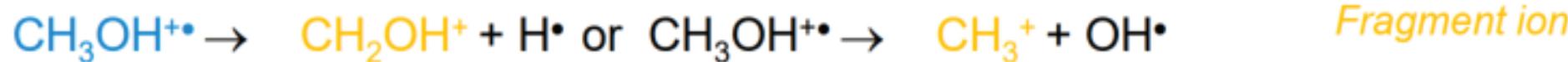
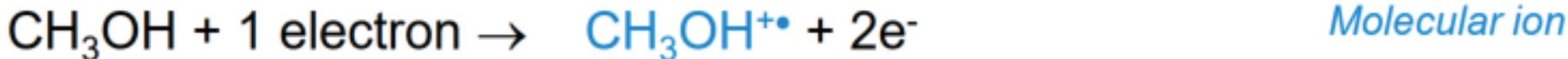
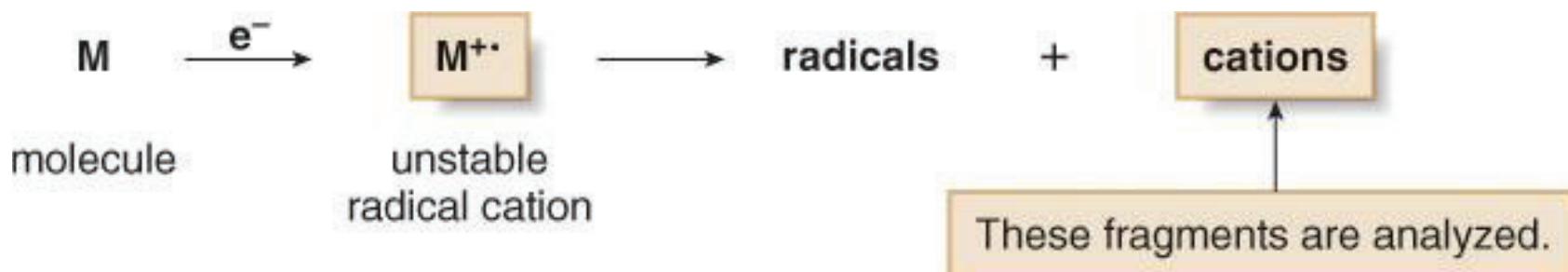
## **Liquid Sample Introduction:**

- Electrospray Ionization (**ESI**) 电喷雾电离
- Atmospheric Pressure Chemical Ionization (**APCI**)  
大气压化学电离
- Fast atom bombardment (FAB) 快原子轰击
- Matrix Assisted Laser Desorption Ionization (**MALDI**)  
基质辅助激光解吸电离

# Electron Impact (EI)

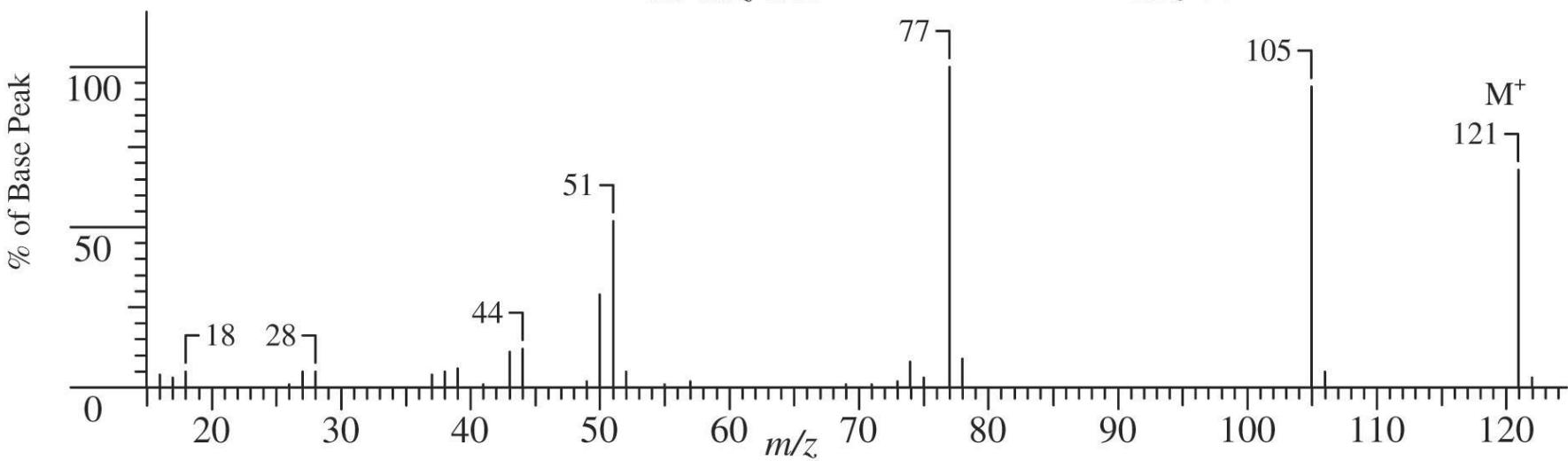
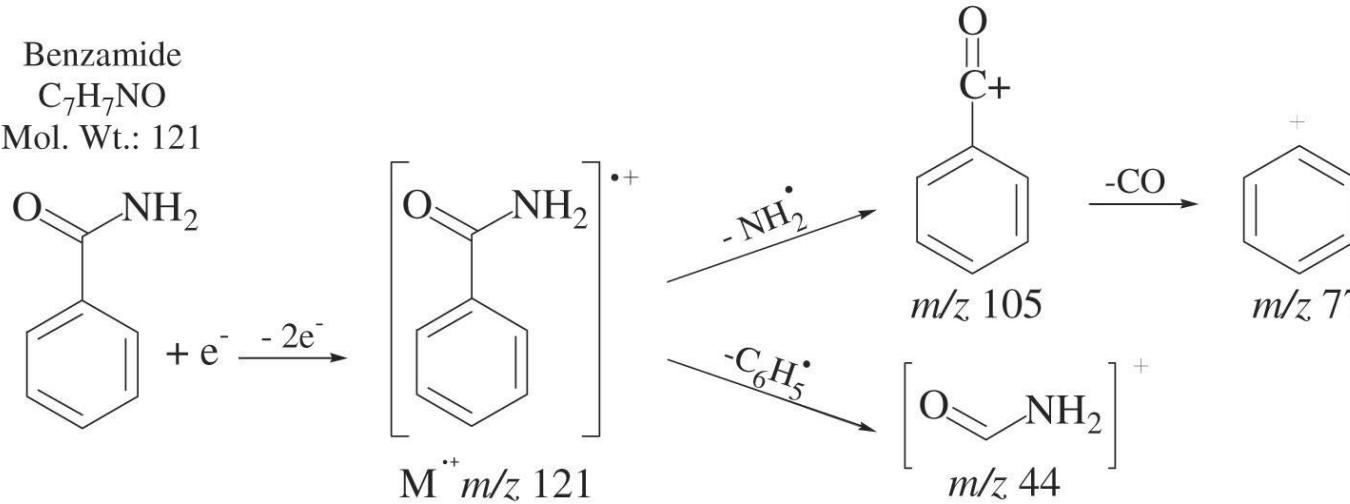
Vapor-phase sample molecules are bombarded with high energy electrons (70 eV). This causes the sample molecules to eject an electron forming a radical cation.

Ionization potential of typical compound < 15 eV. Therefore, extensive bond breaking occurs resulting in smaller cations and radicals.

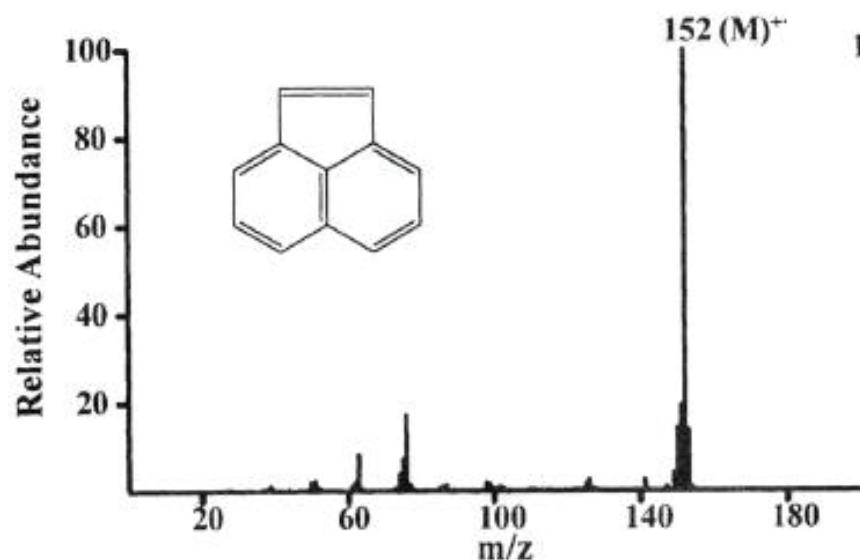


# Example of EI MS

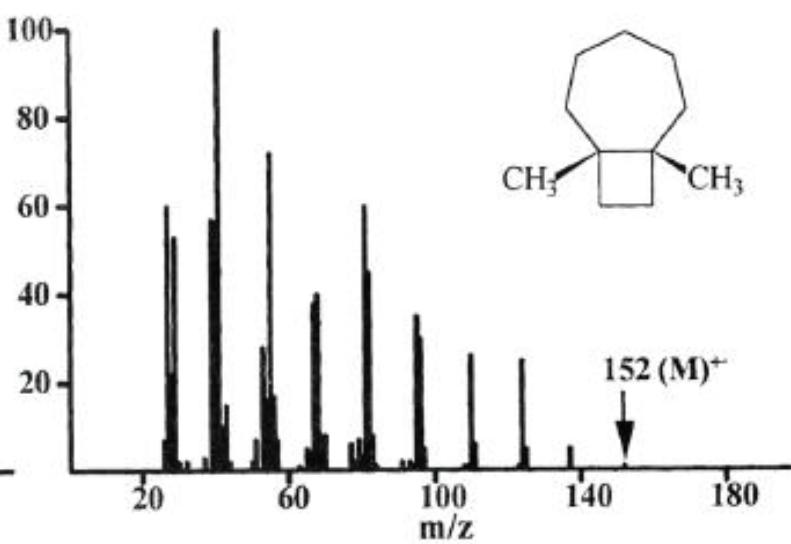
Benzamide  
 $C_7H_7NO$   
Mol. Wt.: 121



# EI MS



*Little fragmentation,  
parent strong*

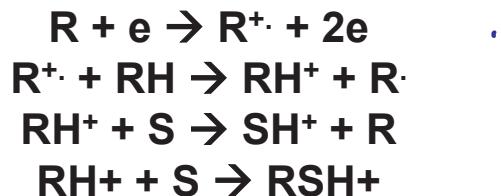


*Lots of fragmentation,  
parent nearly invisible*

# Chemical ionization (CI)

Chemical ionization is a “soft” ionization method. Much less bond breaking occurs than in EI.

Vapor phase sample molecules are mixed with a reagent gas such as CH<sub>4</sub>, or NH<sub>3</sub>. The reagent gas molecules are ionized by electron impact and then the resulting molecular ions of the reagent gas molecules react with the sample molecules.

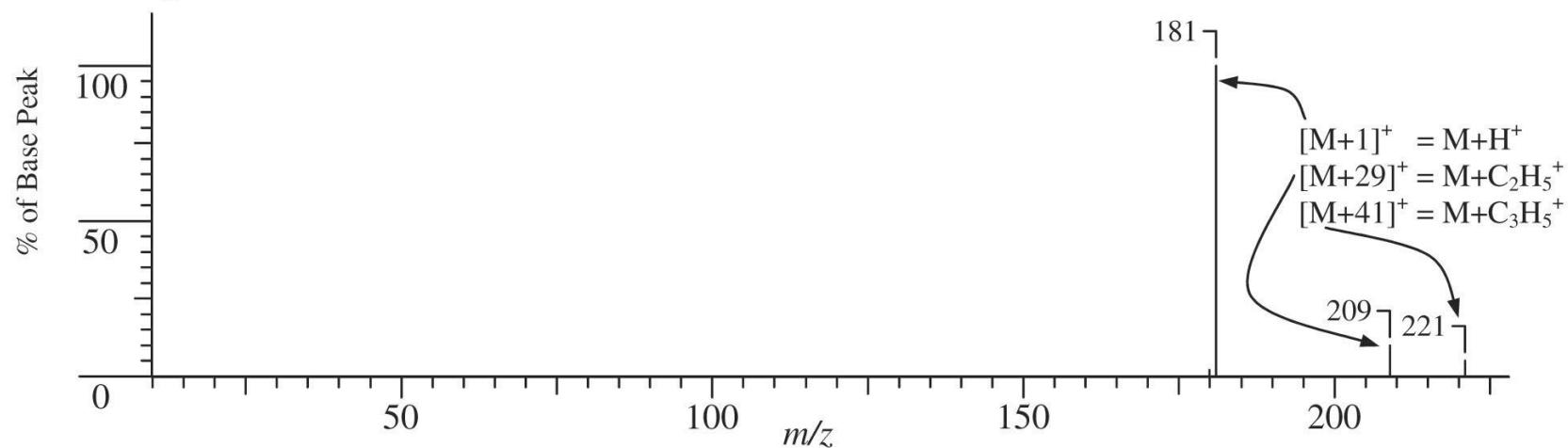
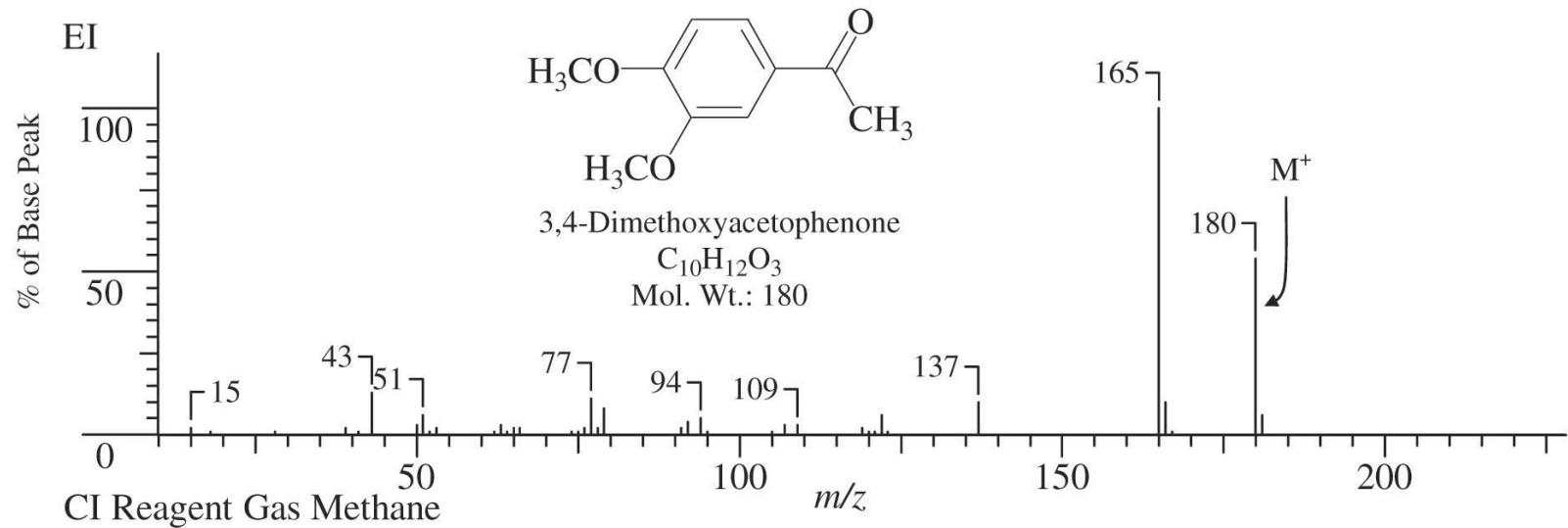


(R = reagent, S = sample, e = electron, . = radical electron , H = hydrogen):

SH<sup>+</sup> is the sample molecule plus one proton m/z = M+1

It is not a radical-cation (it is an even-electron species) and was not produced by excess ionizing voltage. It remains relatively intact – does not fragment.

# Example of CI MS



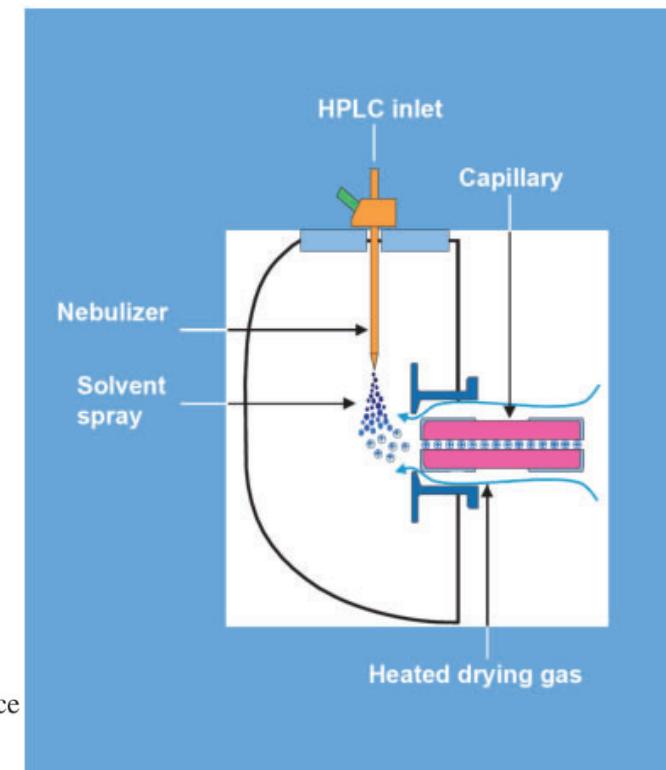
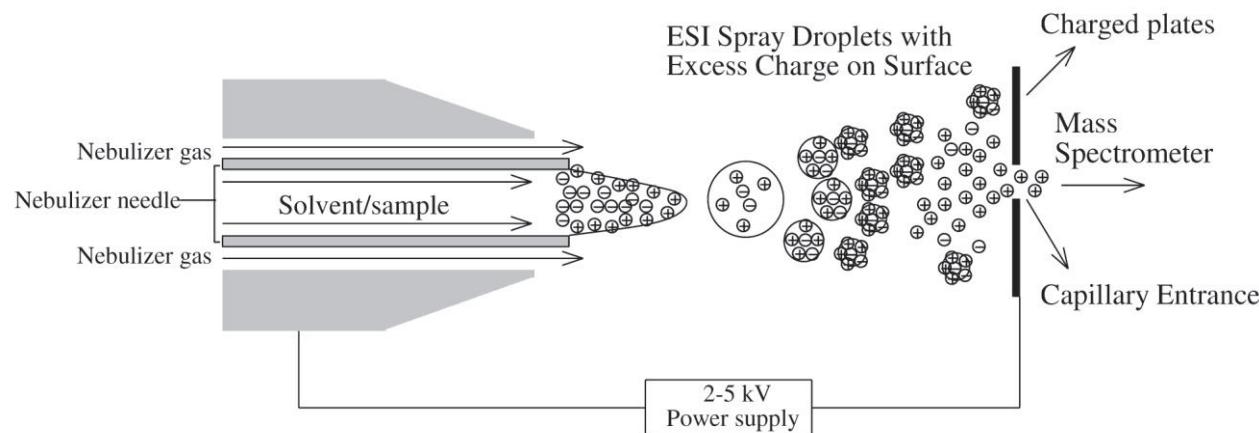
# Electrospray ionization (ESI)

Electrospray ionization (ESI) is a soft ionization technique.

LC eluent is sprayed (nebulized) into a spray chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field occurs between the nebulizer, which is at ground in this design, and the capillary, which is at high voltage.

Suitable molecules:

- Small molecules (glucose) and large biomolecules (proteins, oligonucleotides)



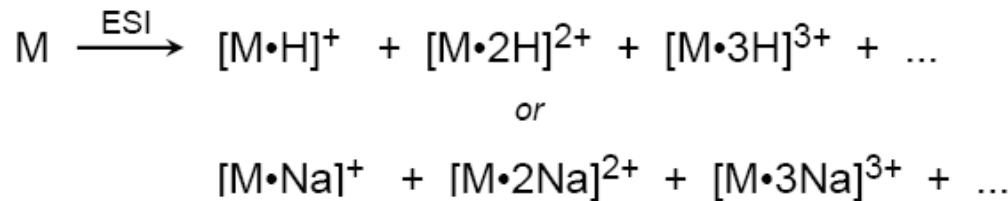
From Agilent\_MS\_Theory\_EN

# Electrospray Ionization (ESI)

Multiple charging is the phenomena in ESI that allows analysis of larger molecules

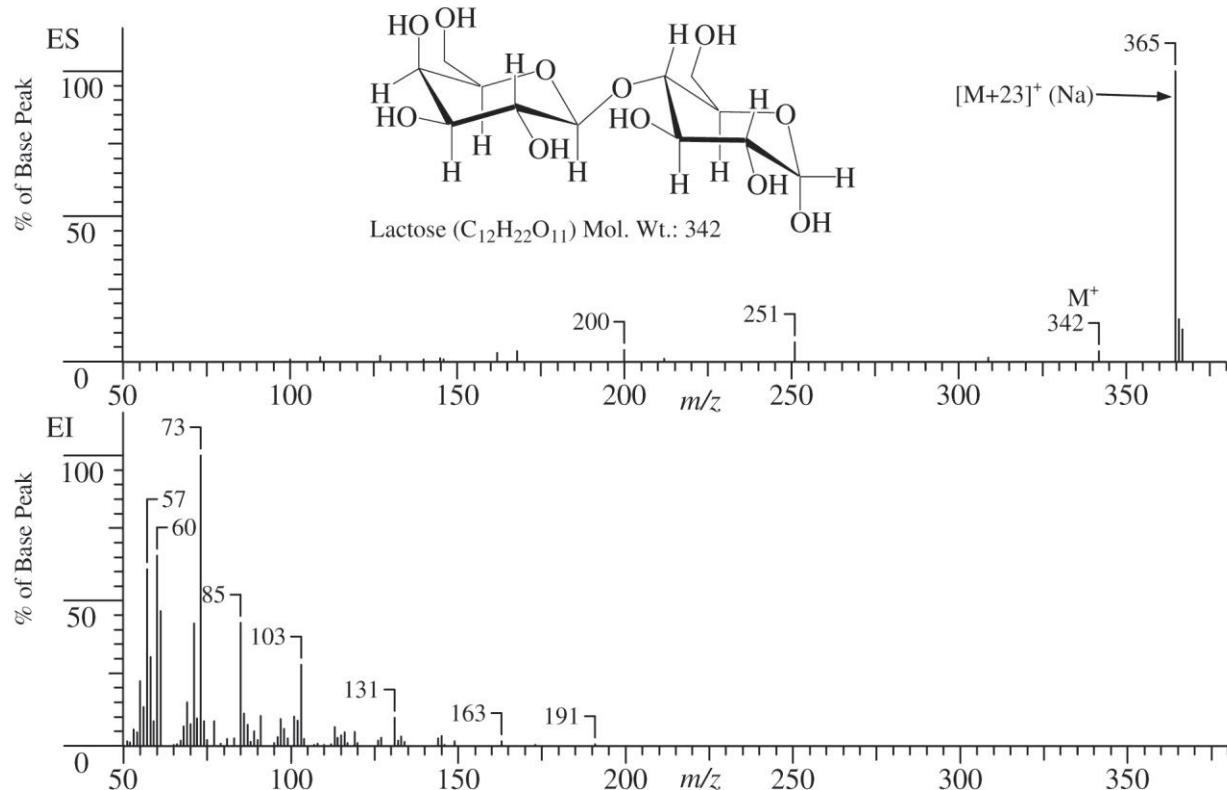
Electrospray Ionization (ESI)

*Multiply charged ions frequently observed.*

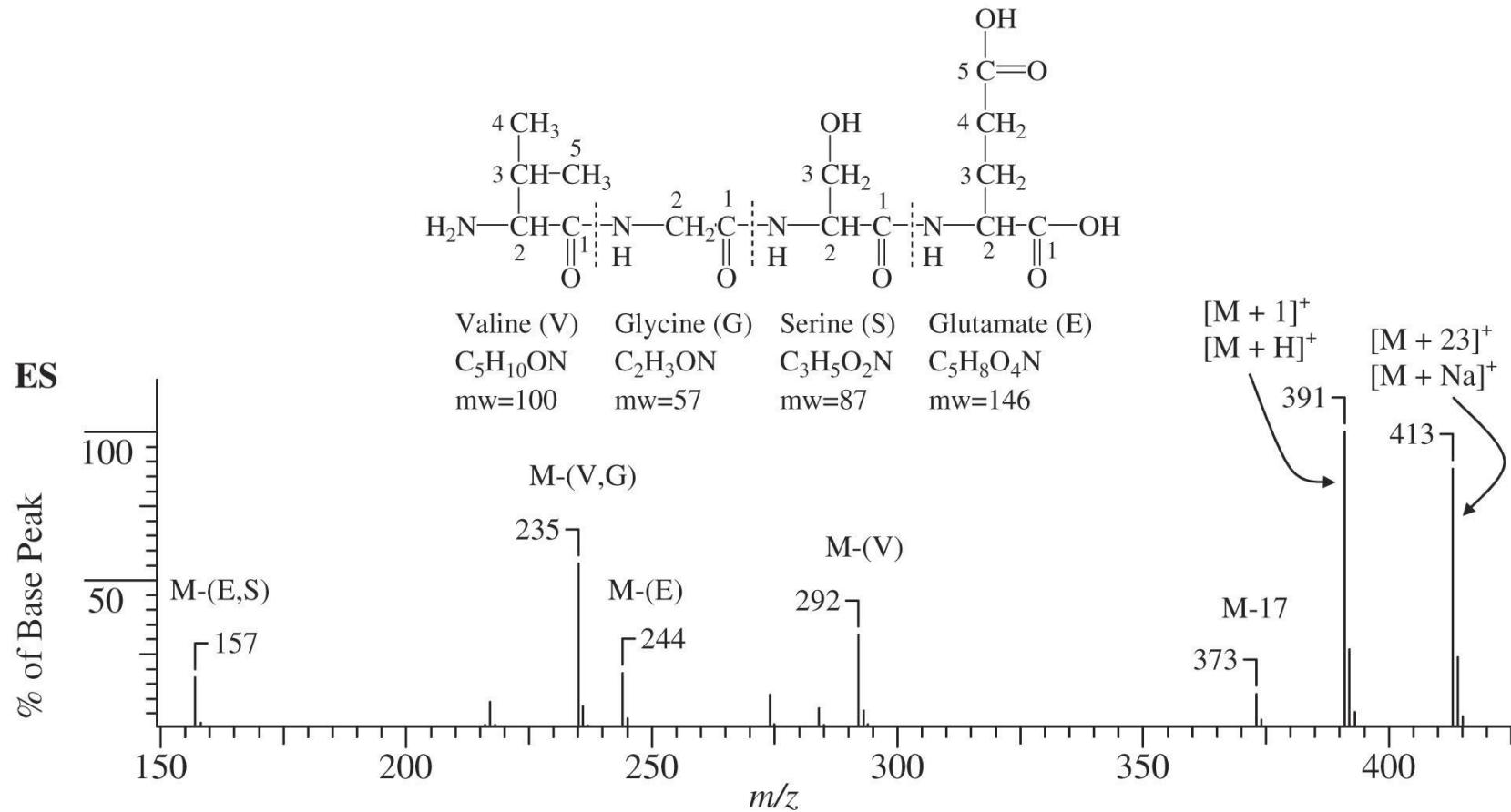


ESI是目前应用最多的电离方式，具有极为广泛的应用领域。ESI 属于浓度敏感型离子化技术，样品浓度越高，灵敏度越高。**适合分析中等极性到极性的分子；**另外，由于其可以产生多电荷离子的特性和相对较低的离子化温度，因而**也适合分析生物大分子，比如蛋白质和多肽的分析。**

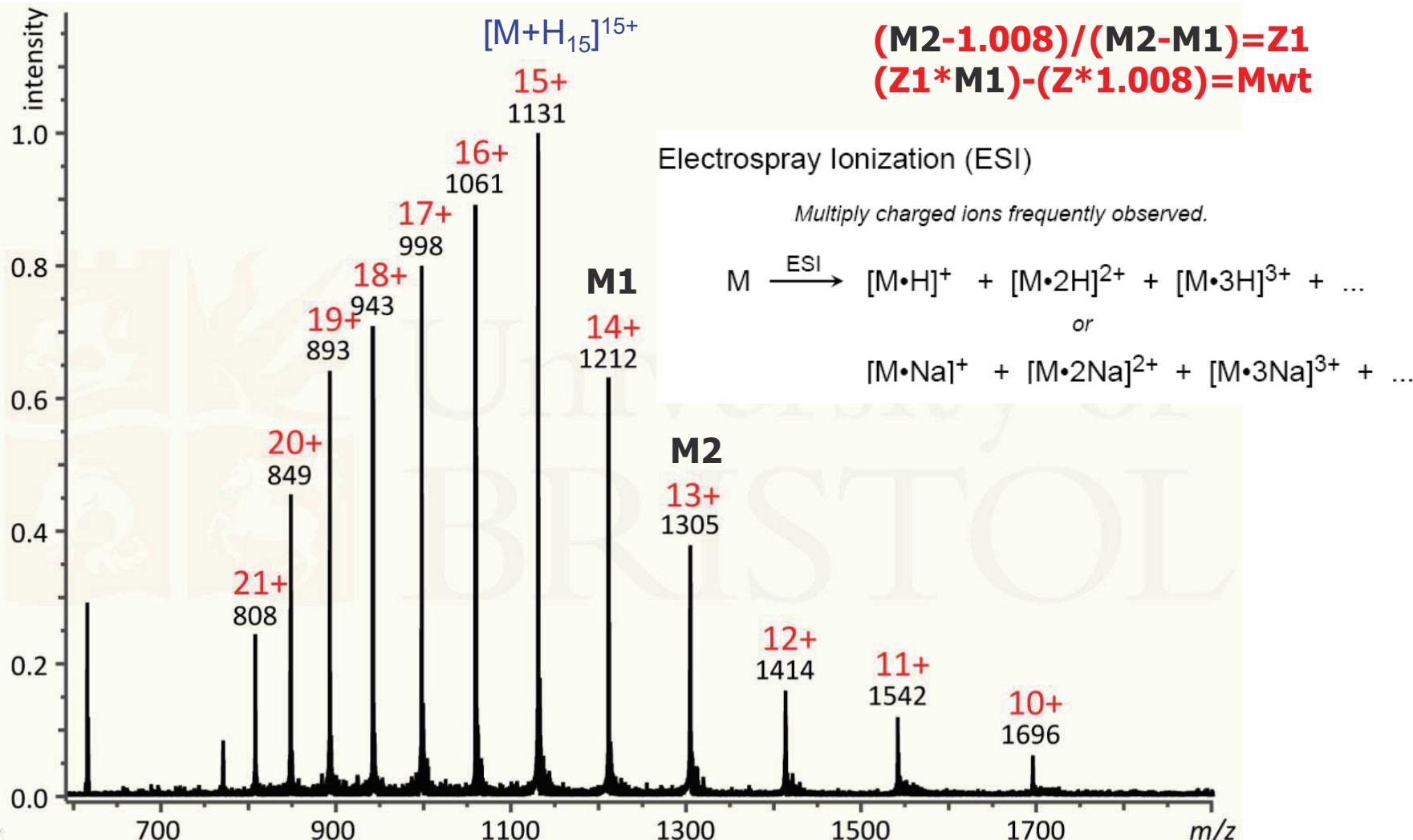
# ESI MS of lactone



# ESI MS of a Protein



# ESI MS of multiply charged myoglobin (肌红蛋白, molecular weight of 16,951 ) ions



# Atmospheric Pressure Chemical Ionization (APCI)

APCI is a gas-phase chemical ionization process.

Therefore, the analyte needs to be in the **gas phase** for ionization.

LC eluent passes a nebulizing needle, which creates a fine spray.

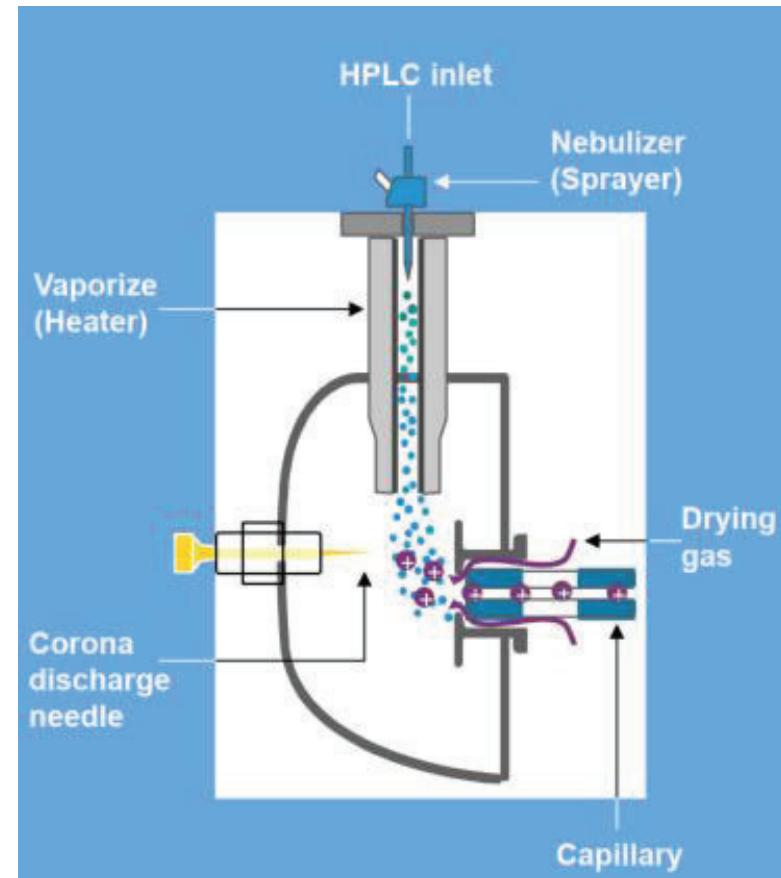
The droplets are fully vaporized in a heated ceramic

tube (~ 400 to 500° C).

Suitable molecules:

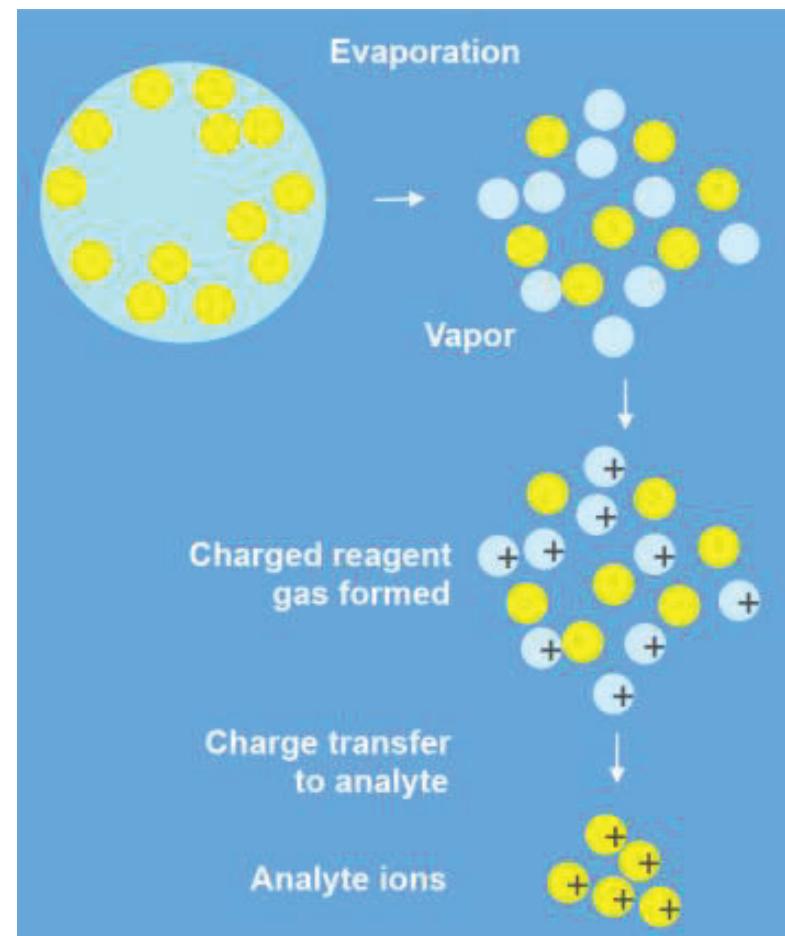
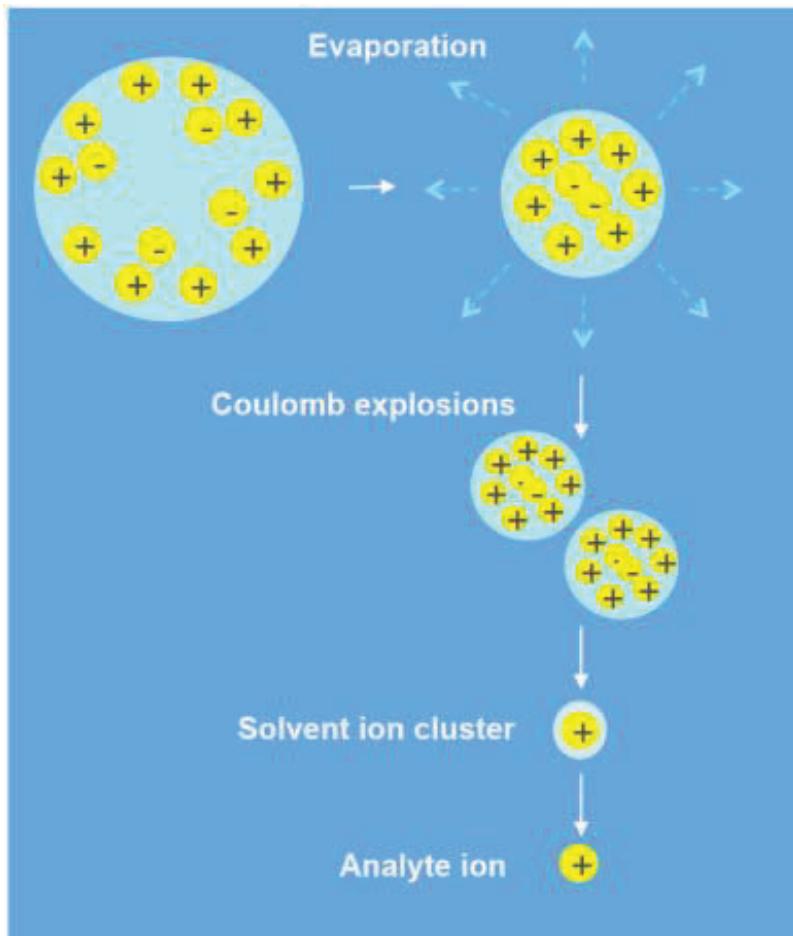
- Molecules < 1,500 u
- Less polar and non-polar compounds

(typically  
analyzed by normal-phase chromatography)



From Agilent\_MS\_Theory\_EN

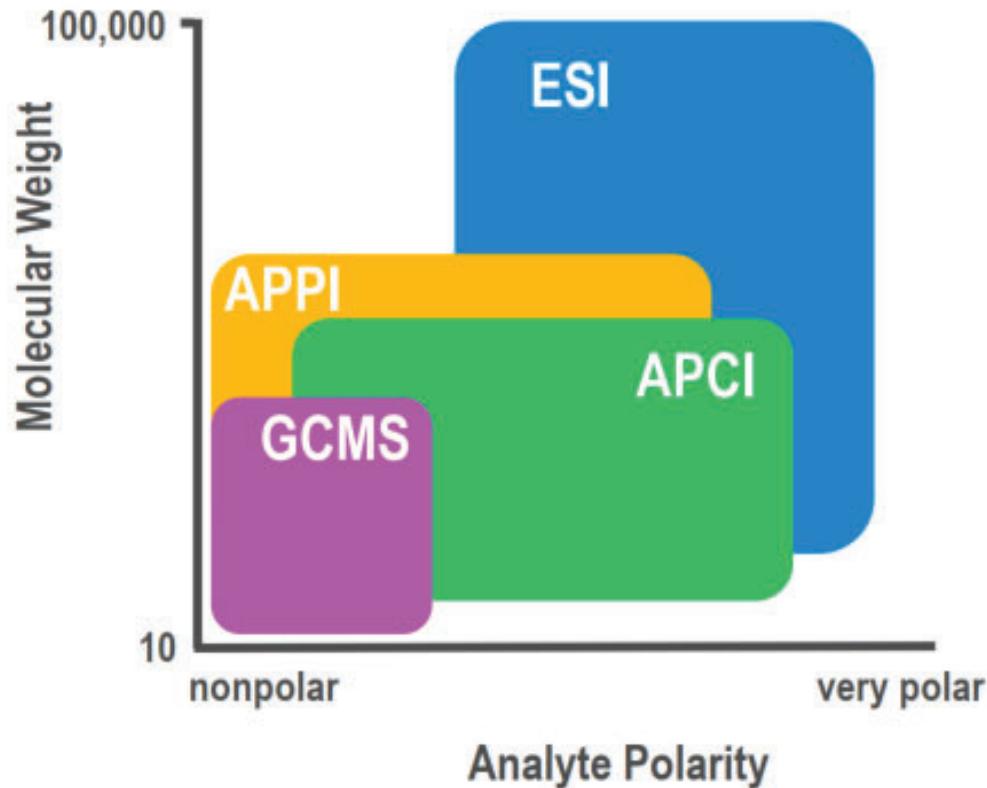
# ESI vs APCI



From Agilent\_MS\_Theory\_EN

# Ionization Method

Polarity of analytes determines the ionization source.



# Matrix-Assisted Laser Desorption/Ionization(MALDI)

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique.

Sample is mixed with matrix and applied to a metal plate.

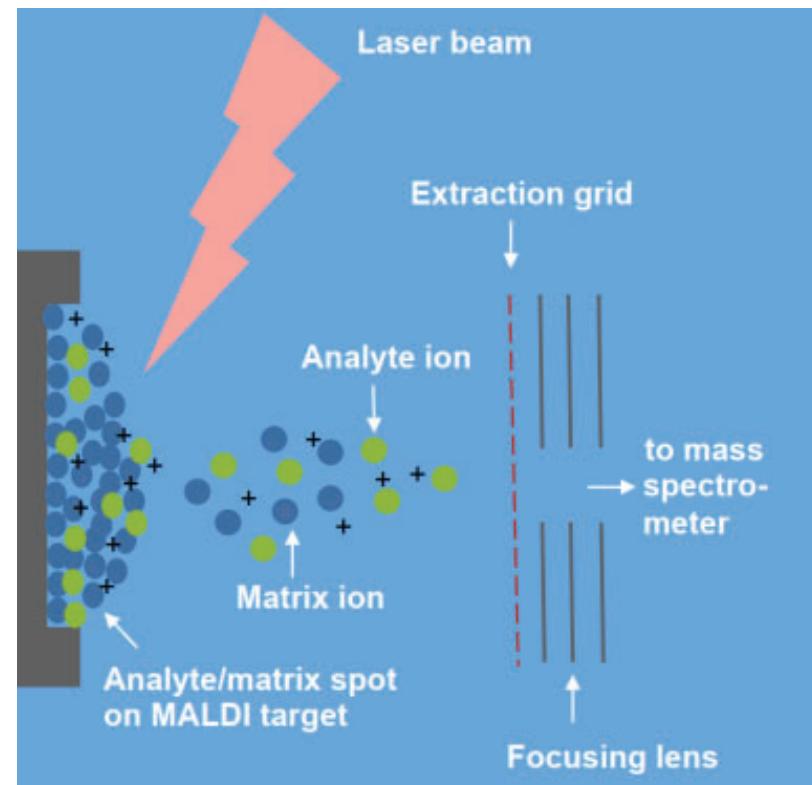
A pulsed laser irradiates the sample, triggering ablation and desorption.

The analyte molecules are ionized in the hot plume of ablated gases.

Ions are accelerated into the mass spectrometer.

## Suitable molecules:

- Biomolecules (DNA, proteins, sugars)
- Large organic molecules (polymers)



# Ionization Methods Summary

**TABLE 1.1** Summary of Ionization Methods.

Ionization Method	Ions Formed	Sensitivity	Advantage	Disadvantage
Electron impact	$M^+$	ng–pg	Data base searchable Structural information	$M^+$ occasionally absent
Chemical ionization	$M + 1, M + 18$ , etc	ng–pg	$M^+$ usually present	Little structural information
Field desorption	$M^+$	$\mu g$ –ng	Non volatile compounds	Specialized equipment
Fast atom bombardment	$M + 1, M +$ cation	$\mu g$ –ng	Non volatile compounds	Matrix interference
	M + matrix		Sequencing information	Difficult to interpret
Plasma desorption	$M^+$	$\mu g$ –ng	Non volatile compounds	Matrix interference
Laser desorption	$M + 1, M +$ matrix	$\mu g$ –ng	Non volatile compounds Burst of ions	Matrix interference
Thermospray	$M^+$	$\mu g$ –ng	Non volatile compounds	Outdated
Electrospray	$M^+, M^{++}, M^{+++}$ , etc.	ng–pg	Non volatile compounds interfaces w/ LC Forms multiply charged ions	Limited classes of compounds Little structural information

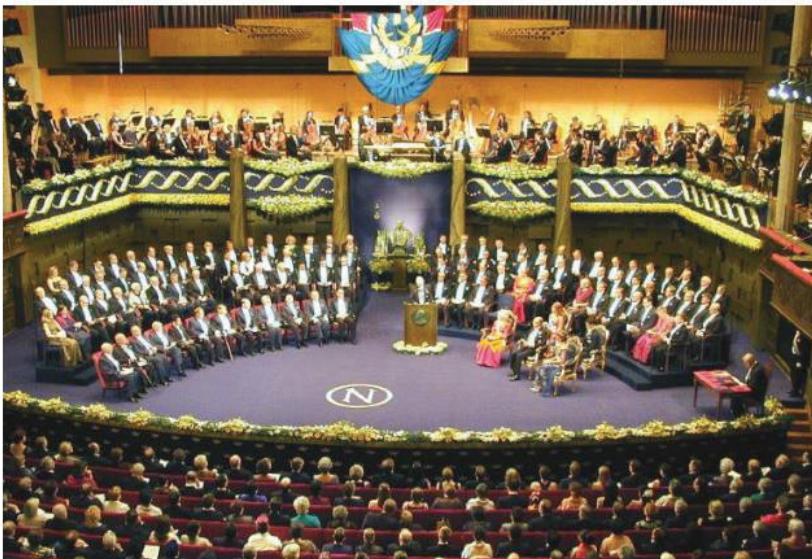
Q: Advantage and disadvantage of EI and ESI?

# Ionization Method

## Nobel Prize Winning Technology

John Fenn and Koichi Tanaka won the Nobel Prize in Chemistry in 2002 for the development of two soft ionization technologies:

- Electrospray technology, Dr. Fenn
- Soft laser desorption, Dr. Tanaka



Concert Hall, Stockholm Sweden, Dec 2002



Dr. Fenn getting his Nobel Prize from the King of Sweden

# **Ion Separation Methods**

**After ionization and ion transport, analytes enter the mass analyzer. The mass spectrometer measures the ion signals resulting in a mass spectra, which can provide valuable information about the molecular weight, structure, identity, and quantity of a compound.**

**磁质量分析器 (Magnetic Sector Analyzer)**

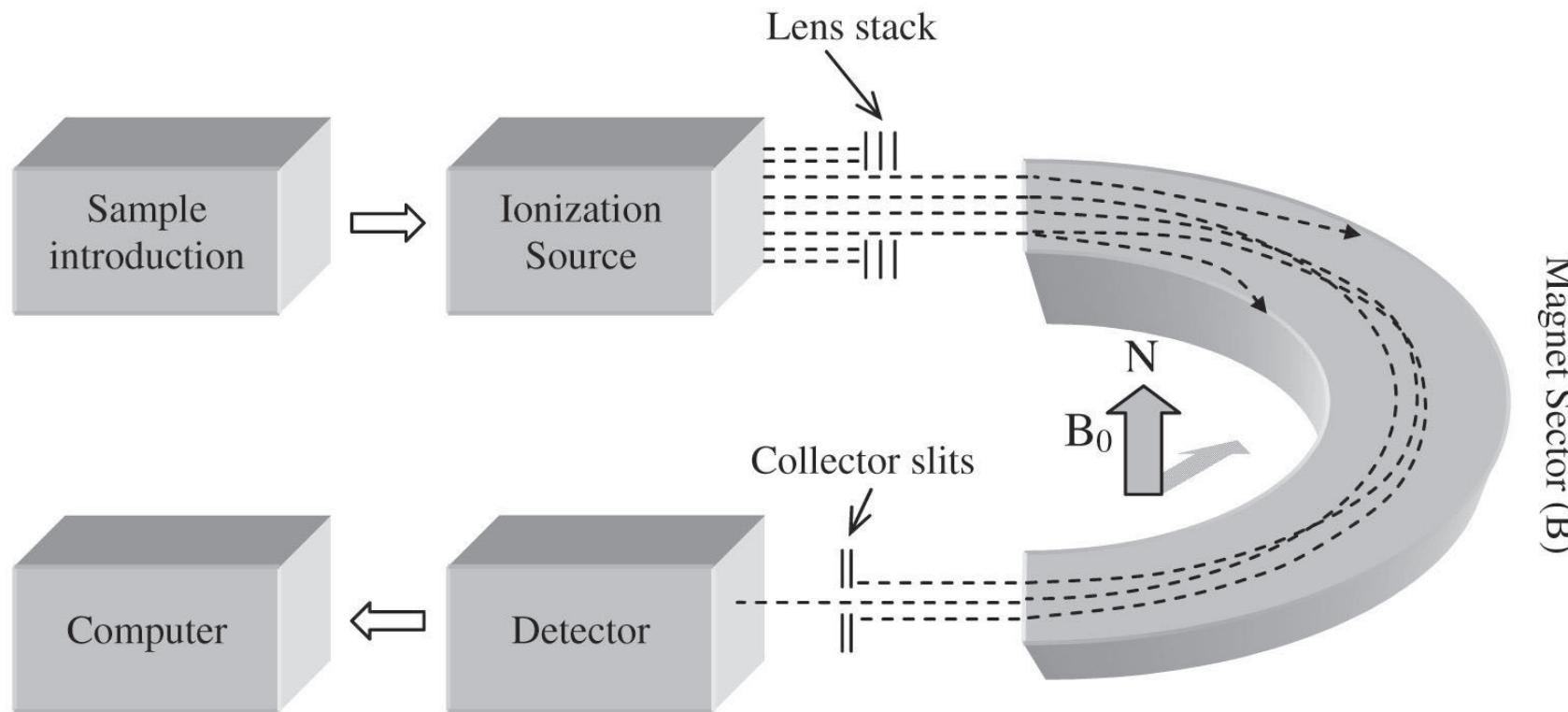
**飞行时间质谱计 (time of flight, TOF )**

**四极质量分析器 (quadrupole mass analyzer)**

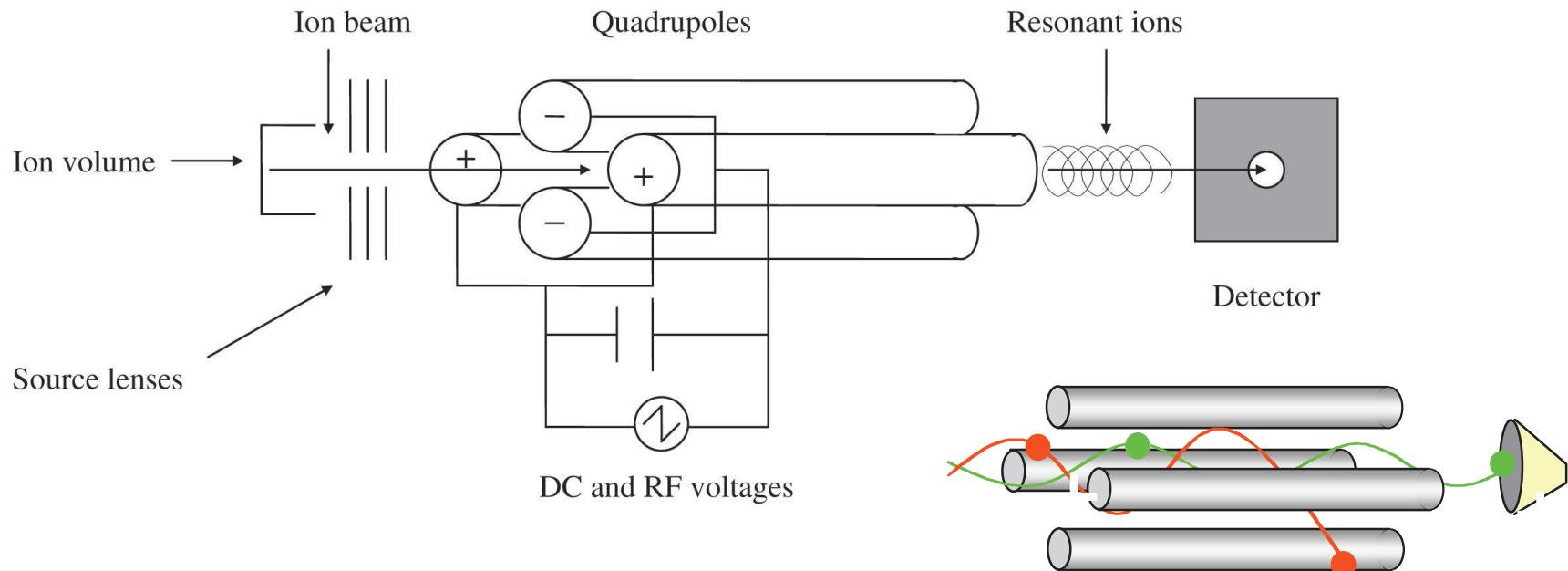
**离子阱 (ion trap)**

**傅立叶变换离子回旋共振质谱计 (Fourier transform ion cyclotron resonance, FT-ICR)**

# Magnetic Sector Analyzer



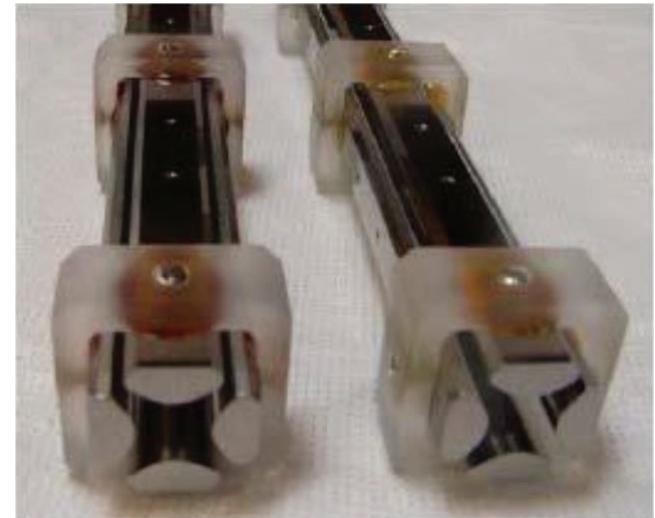
# Quadrupole



Charged ions generated in the ion source enter the mass analyzer.

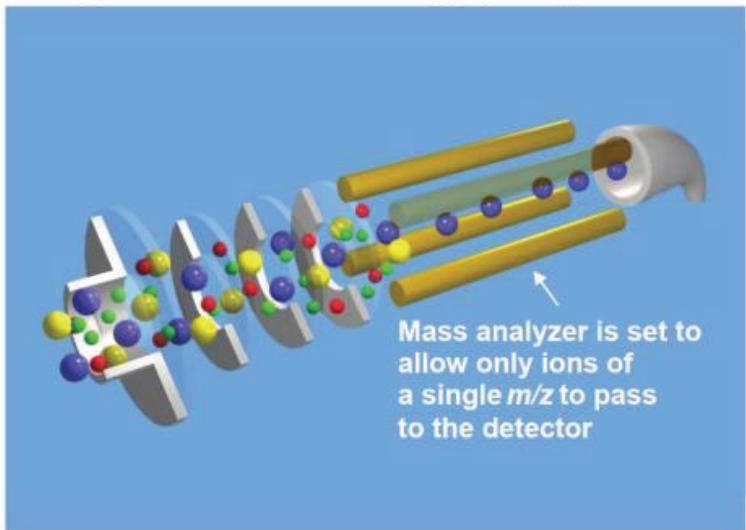
The quadrupole mass analyzer is scanned sequentially such that only a single ion  $m/z$  may be passed at one time. All other ions are lost.

Information received: **MS only**

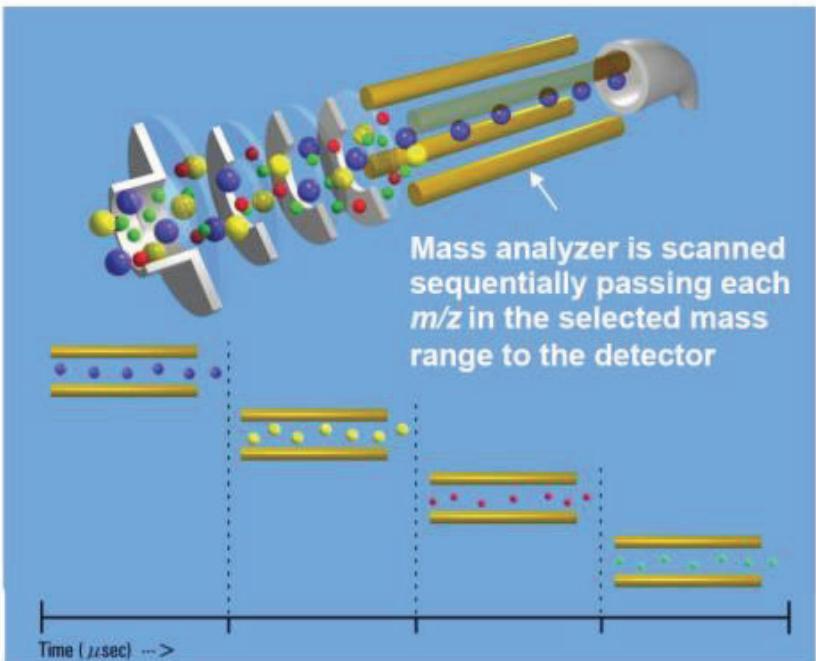


# Single Quadrupole (SQ)

## Single Ion Monitoring (SIM)



## Scan Mode

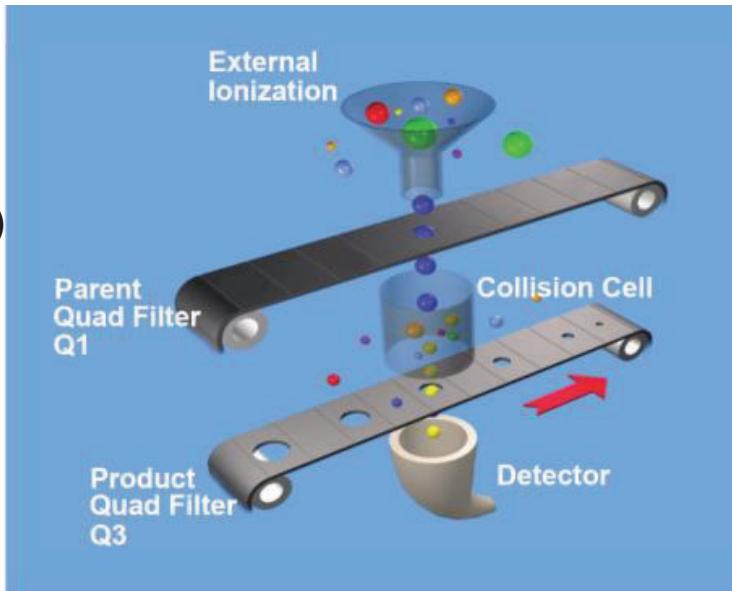


# Triple Quadrupole (QQQ)

A common set is the following:

- Q1: used as a filter for specific  $m/z$  (precursor ion)
- Q2: used as collision cell to fragment the precursor ion and generate product ions
- Q3: set to specific  $m/z$  (SRM or MRM) or scan mode (product ion scan)

Information received: **MS** and **MS/MS**



# Time of flight, TOF

Charged ions generated in the ion source enter the mass analyzer.

Analyzer components:

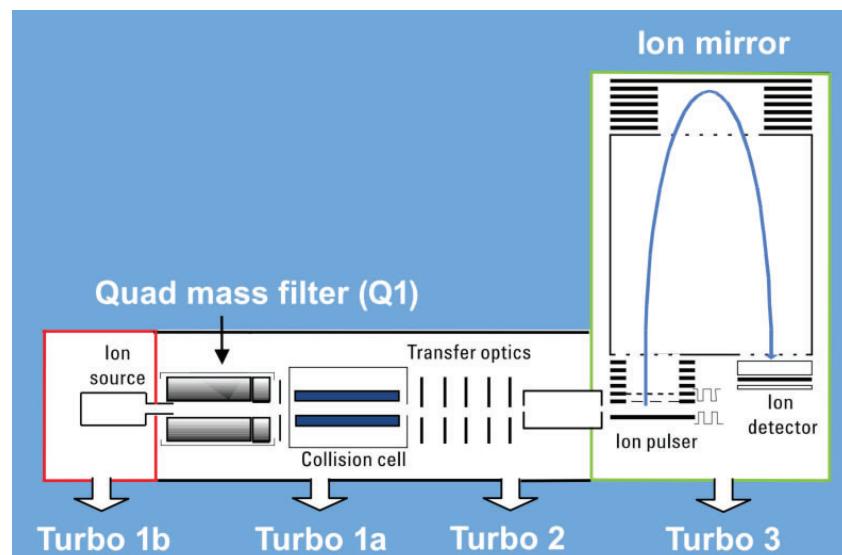
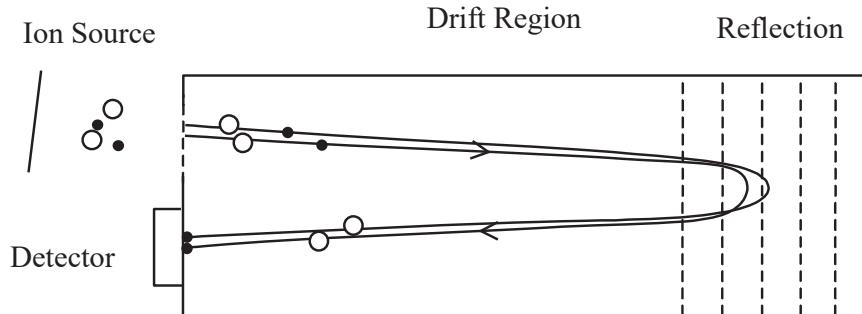
- Mass filter (Q1), optional
- Flight tube
- Collision cell (Q-TOF)

After ions have passed the quadrupole or collision cell they arrive at the ion pulser. A high voltage pulse is applied which accelerates the ions into the flight tube. An ion mirror at the end of the tube reflects the ions and sends them to the detector that records their time of arrival.

Information received:

TOF: **MS only**

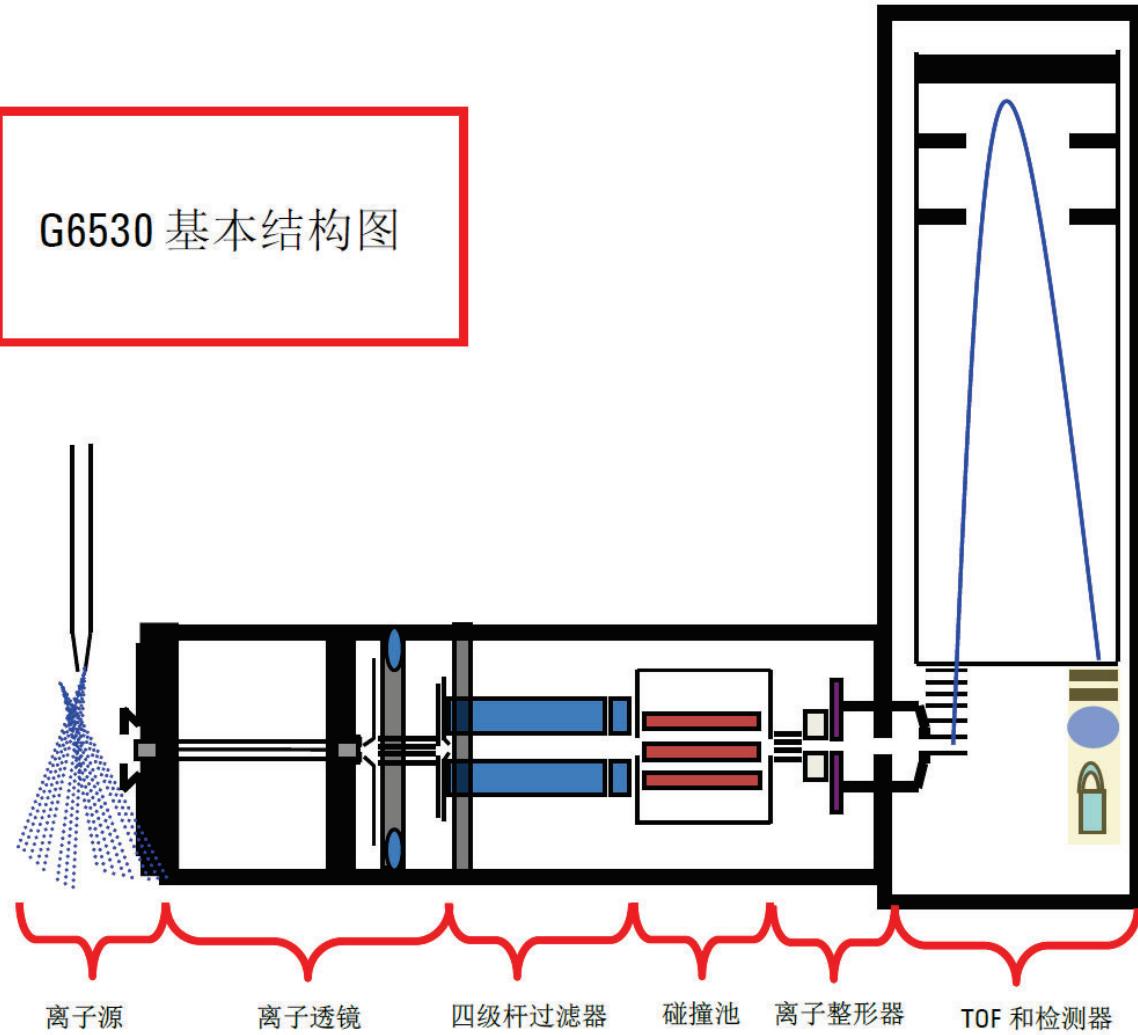
Q-TOF: **MS and MS/MS**



# Time of flight, TOF



G6530 基本结构图



$$E = 1 / 2mv^2$$

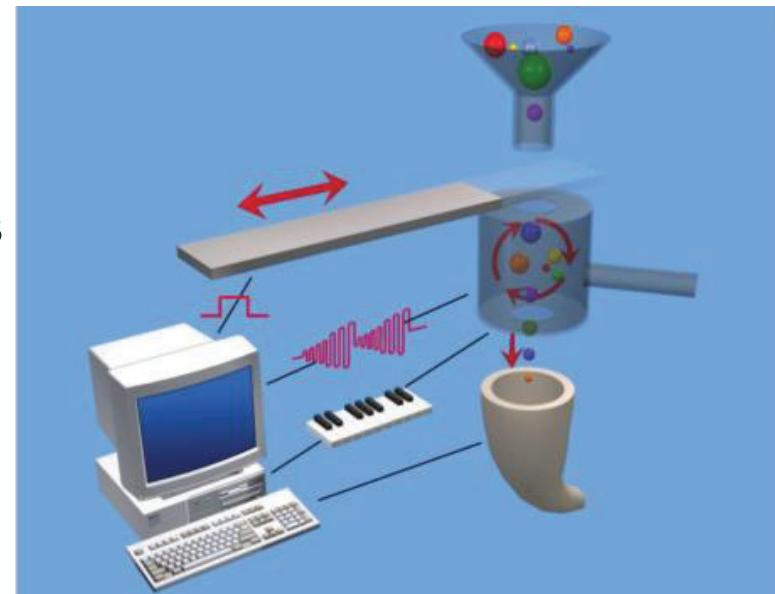
$$m = 2E / v^2$$

$$v = \sqrt{(2E / m)}$$

# Ion Trap, IT

Charged ions generated in the ion source enter the mass analyzer. All ions of the selected polarity over the selected mass range can be stored at once in the trap. The ions can be manipulated in the ion trap mass analyzer – performing multiple isolation and fragmentation stages – until time to detect.

Instead of four parallel rods, the ion trap consists of a circular ring electrode plus two end caps that form a “trap”.



From Agilent\_MS\_Theory\_EN

Information received: **MS** and **MS/MS**

# Ion Separation Methods

**TABLE 1.2** Summary of Mass Analyzers.

Mass Analyzer	Mass Range	Resolution	Sensitivity	Advantage	Disadvantage
Magnetic Sector	1–15,000 $m/z$	0.0001	Low	High res.	Low sensitivity Very expensive High technical expertise
Quadrupole	1–5000 $m/z$	unit	High	Easy to use Inexpensive High sensitivity	Low res. Low mass range
Ion trap	1–5000 $m/z$	unit	High	Easy to use Inexpensive High sensitivity Tandem MS ( $MS''$ )	Low res. Low mass range
Time of flight	Unlimited	0.0001	High	High mass range Simple design	Very high res.
Fourier transform	up to 70 kDa	0.0001	High	Very High res. and mass range	Very expensive High technical expertise

File View Run Help



Tools

## Instrument



Inlet Method



Solvent Monitor



Edit Shutdown or Startup



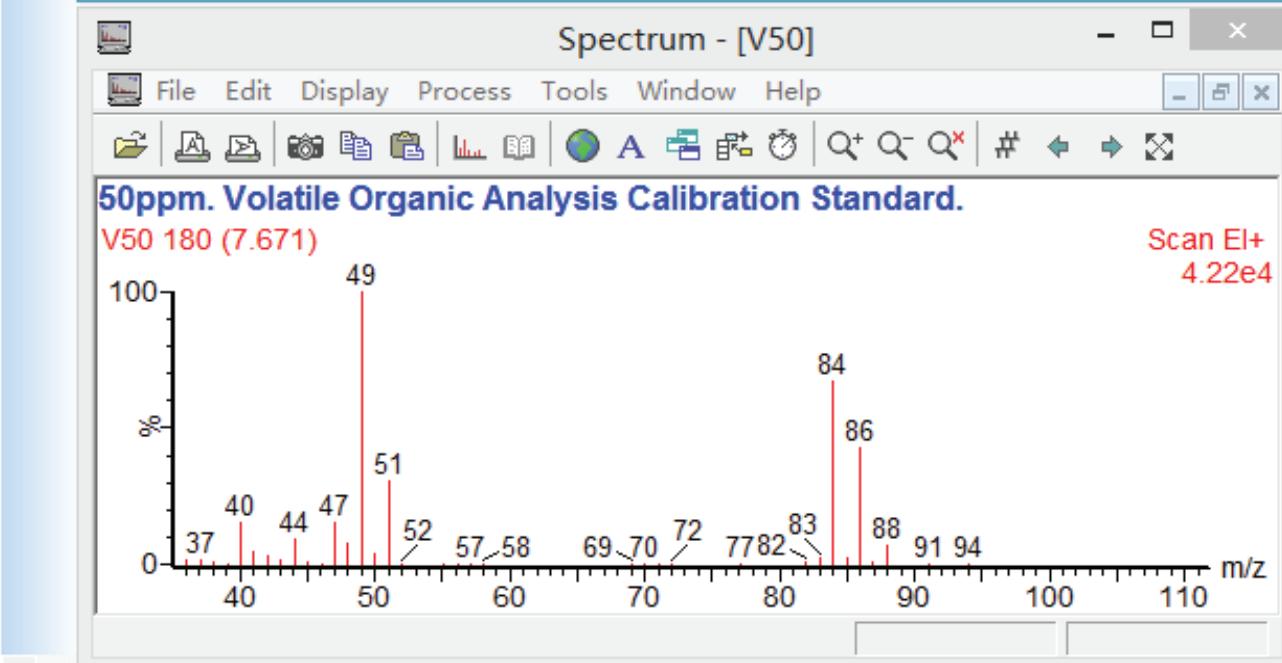
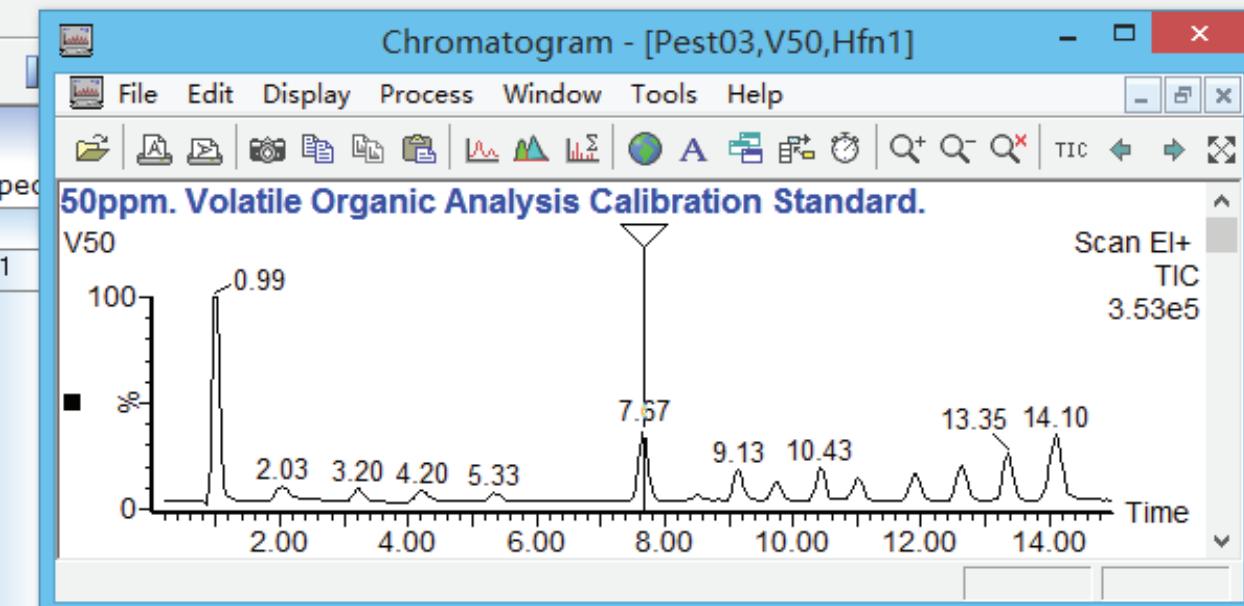
Shutdown



Startup



Options



# MassLynx 4.1

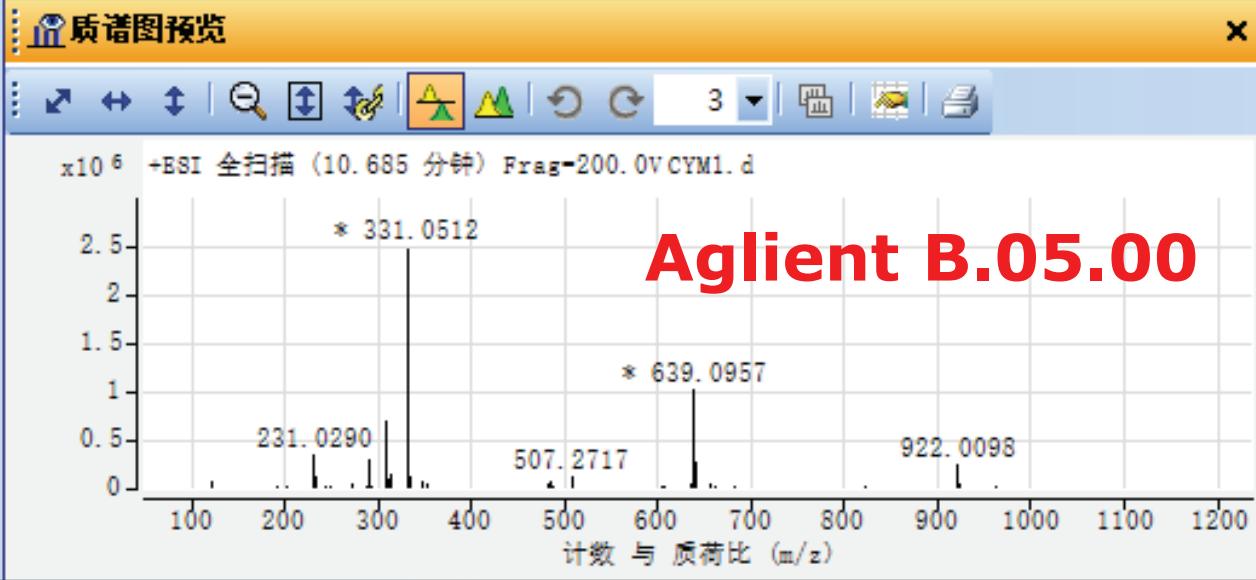
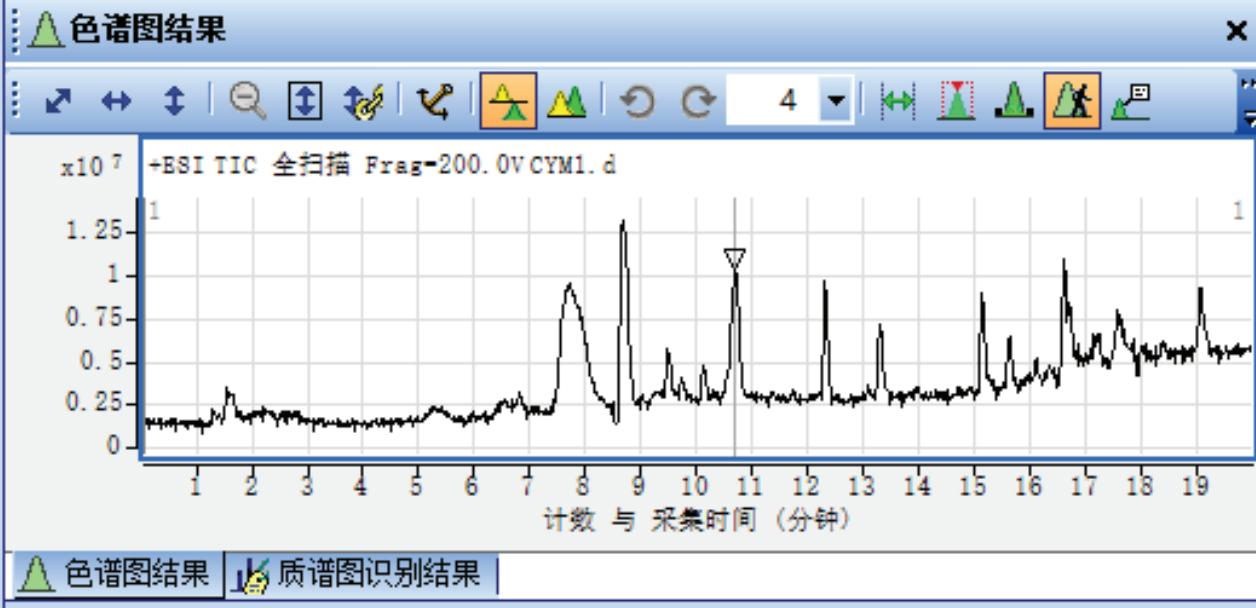
文件(F) 编辑(E) 视图(V) 查找(D) 识别(I) 色谱图(C) 质谱图(S) 方法(M) 向导(W) 操作(A) 配置(G) 工具(T)  
帮助(H)



**数据浏览器**

按数据文件排序

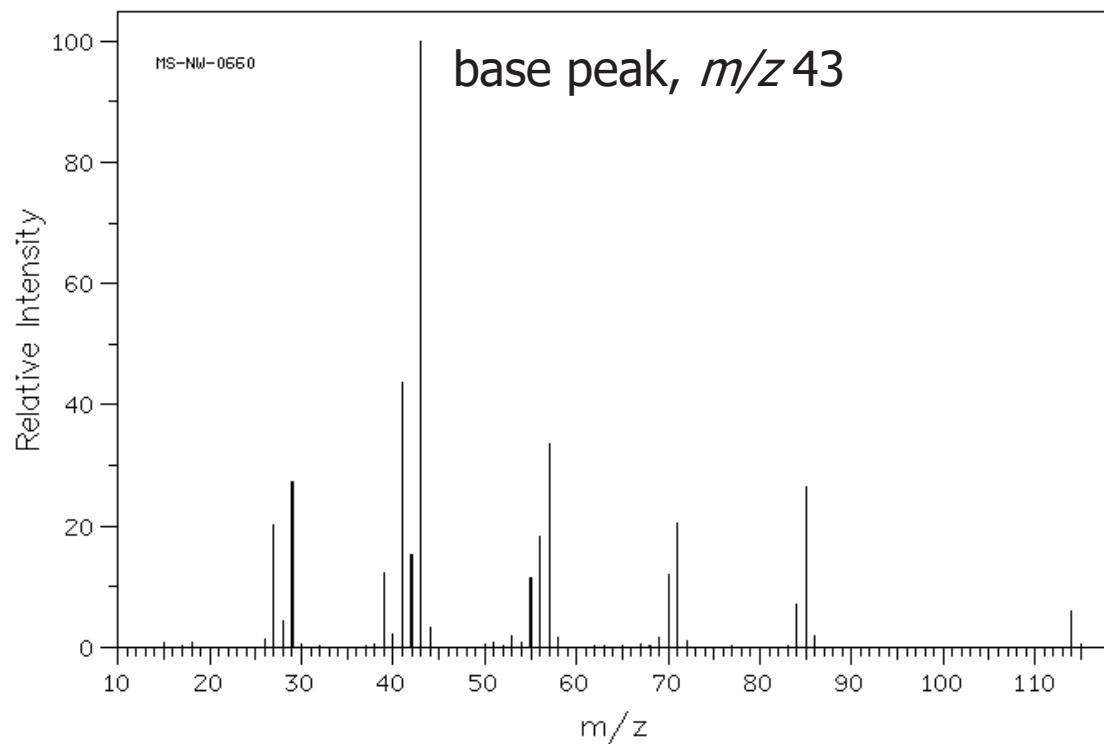
- CYM1.d
  - 用户色谱图
    - + TIC 全
    - DAD1 - G:
    - DAD1 - E:
    - + EIC(599)
    - + EIC(621)
    - + EIC(291)
    - + EIC(309)
    - + EIC(331)
  - 用户质谱图
  - 背景质谱图
  - 化合物



# EI-MS

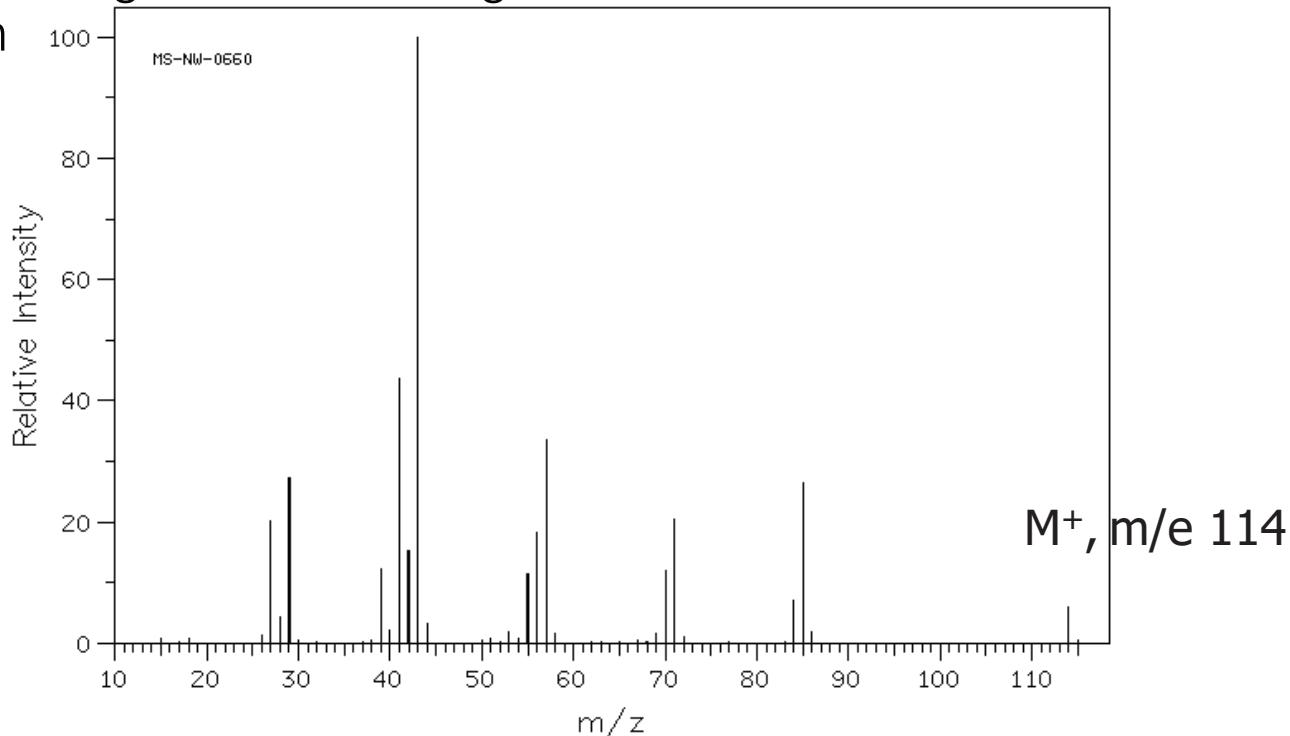
- Presentation of data

1. The mass spectrum is presented in terms of ion abundance vs.  $m/z$  ratio (mass)
2. The most abundant ion formed in ionization gives rise to the tallest peak on the mass spectrum – this is the *base peak*



# EI-MS

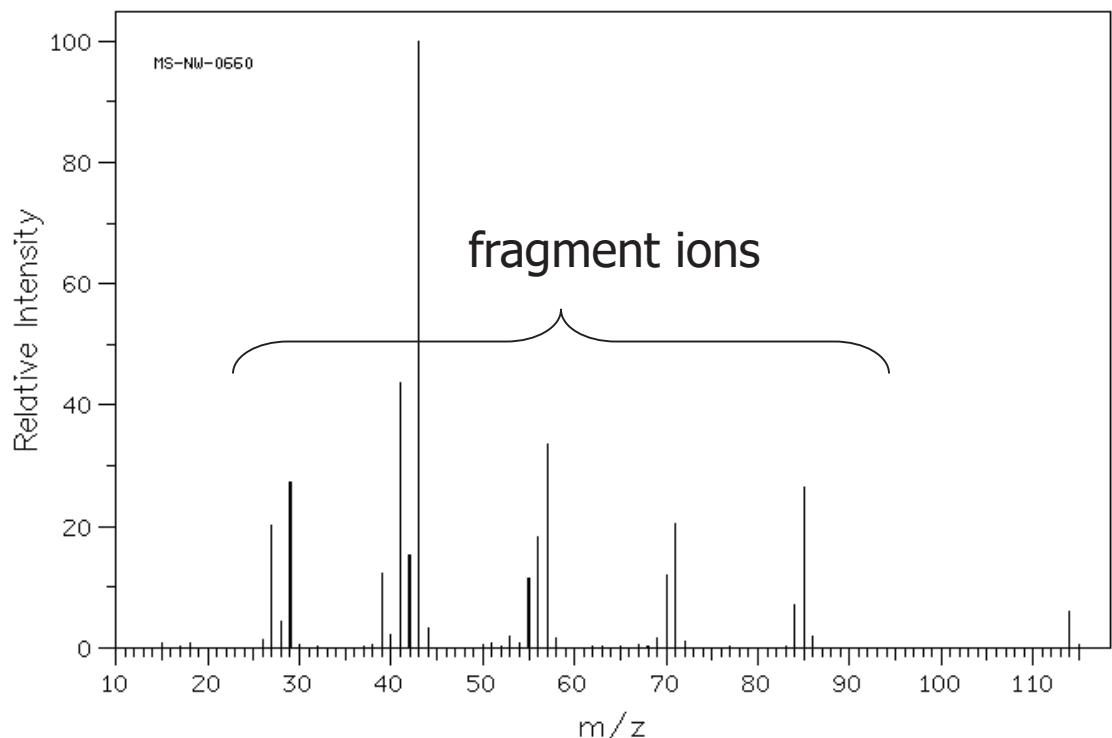
- Presentation of data
  3. All other peak intensities are relative to the base peak as a percentage
  4. If a molecule loses only one electron in the ionization process, a **molecular ion** is observed that gives its molecular weight – this is designated as  **$M^+$**  on the spectrum



# EI-MS

- Presentation of data

5. In most cases, when a molecule loses a valence electron, bonds are broken, or the ion formed quickly fragment to lower energy ions
6. The masses of charged ions are recorded as **fragment ions** by the spectrometer – ***neutral fragments are not recorded!***



# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

Ionization Method

Ion Separation Methods

**Determination of Molecular Mass**

**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

# Determination of Molecular Mass

Three facts must apply for a molecular ion peak:

- 1) The peak must correspond to the **highest mass ion** on the spectrum excluding the isotopic peaks
- 2) The ion must have an odd number of electrons – usually a radical cation
- 3) The ion must be able to form the other fragments on the spectrum by loss of logical neutral fragments (No M-3, M-13, M-20 to M-25)

# Determination of Molecular Mass

The **Nitrogen Rule** is another means of confirming the observance of a molecular ion peak:

- 1) If a molecule contains an even number of nitrogen atoms (only “common” organic atom with an odd valence) or no nitrogen atoms the molecular ion will have an even mass value
  
- 2) If a molecule contains an odd number of nitrogen atoms, the molecular ion will have an odd mass value

# Determination of Molecular Formula

## Exact Masses and Molecular Formulae

Element	Atomic Weight	Nuclide	Mass	Relative Abundance
Hydrogen	1.00797	<sup>1</sup> H	1.00783	100.0
		D( <sup>2</sup> H)	2.01410	0.015
Carbon	12.01115	<sup>12</sup> C	12.00000 <sup>b</sup>	100.0
		<sup>13</sup> C	13.00336	1.11
Nitrogen	14.0067	<sup>14</sup> N	14.0031	100.0
		<sup>15</sup> N	15.0001	0.37
Oxygen	15.9994	<sup>16</sup> O	15.9949	100.0
		<sup>17</sup> O	16.9991	0.04
		<sup>18</sup> O	17.9992	0.20
Fluorine	18.9984	<sup>19</sup> F	18.9984	100.0
Silicon	28.086	<sup>28</sup> Si	27.9769	100.0
		<sup>29</sup> Si	28.9765	5.06
Phosphorus	30.974	<sup>30</sup> Si	29.9738	3.36
		<sup>31</sup> P	30.9738	100.0
Sulfur	32.064	<sup>32</sup> S	31.9721	100.0
		<sup>33</sup> S	32.9715	0.79
		<sup>34</sup> S	33.9679	4.43

<sup>a</sup><sup>12</sup>C mass set to 12 amu, exactly.

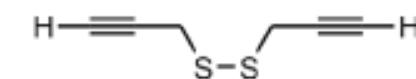
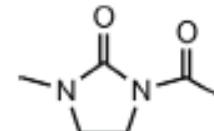
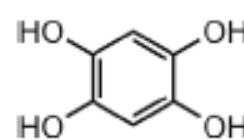
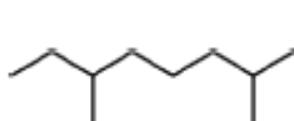
As a result, <sup>1</sup>H mass is actually higher than 1 amu.

And <sup>16</sup>O mass is lower than 16 amu.

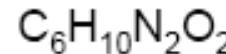
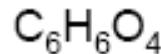
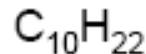
# Determination of Molecular Formula

## Exact Masses and Molecular Formulae

*So, molecules with different molecular formulae have different exact masses.*



molecular  
formula



$m/z$   
(unit)

142

142

142

142

$m/z$   
(exact mass)

142.1723

142.0264

142.0743

141.9911

# Determination of Molecular Formula

## High Resolution Mass Spectrometry

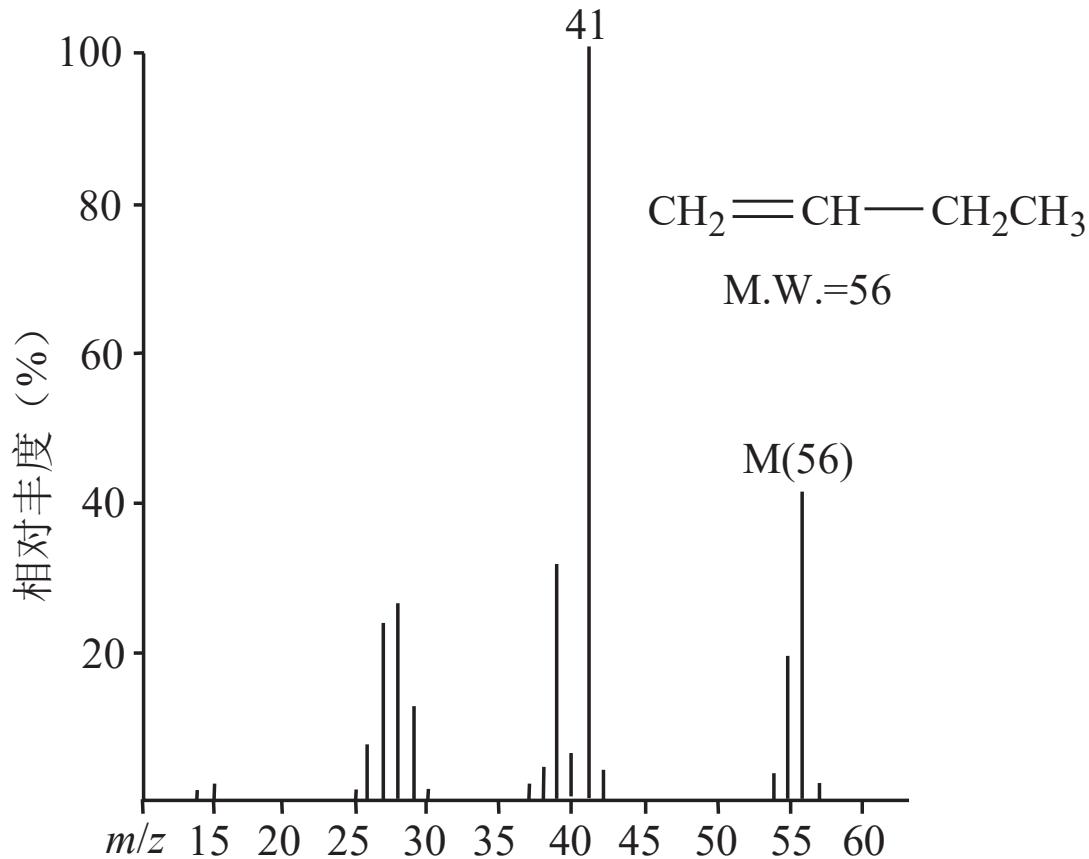
- If sufficient resolution ( $R > 5000$ ) exists, mass numbers can be recorded to precise values (6 to 8 significant figures)
- From tables of combinations of formula masses with the natural isotopic weights of each element, it is often possible to find an ***exact*** molecular formula from HRMS

Example: HRMS gives you a molecular ion of 98.0372; from mass 98 data:

$C_3H_6N_4$	98.0594
$C_4H_4NO_2$	98.0242
$C_4H_6N_2O$	98.0480
$C_4H_8N_3$	98.0719
$C_5H_6O_2$	98.0368 ← gives us the exact formula
$C_5H_8NO$	98.0606
$C_5H_{10}N_2$	98.0845
$C_7H_{14}$	98.1096

# Inferences from Isotopic Ratios

M+1、M+2...



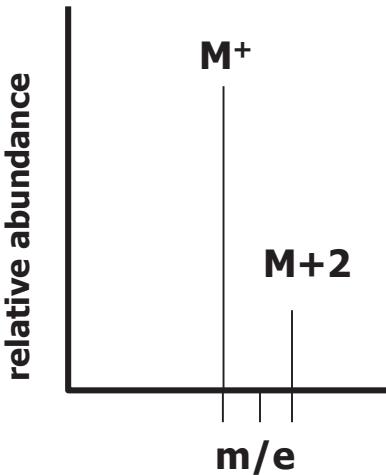
# Inferences from Isotopic Ratios

Natural abundances of common elements and their isotopes – (relative abundance vs. a value of 100 for the most common isotope)

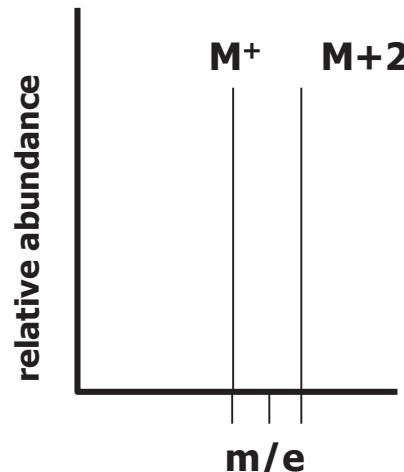
Element	Atomic Weight	Nuclide	Mass	Relative Abundance	
Hydrogen	1.00797	$^1\text{H}$	1.00783	100.0	<i>For some, isotope abundance is low.</i>
		D( $^2\text{H}$ )	2.01410	0.015	
Carbon	12.01115	$^{12}\text{C}$	12.00000 <sup>b</sup>	100.0	<i>For others, isotope abundance is high.</i>
		$^{13}\text{C}$	13.00336	1.11	
Nitrogen	14.0067	$^{14}\text{N}$	14.0031	100.0	<i>For others, isotope abundance is high.</i>
		$^{15}\text{N}$	15.0001	0.37	
Oxygen	15.9994	$^{16}\text{O}$	15.9949	100.0	<i>For others, isotope abundance is high.</i>
		$^{17}\text{O}$	16.9991	0.04	
		$^{18}\text{O}$	17.9992	0.20	
Chlorine	35.453	$^{35}\text{Cl}$	34.9689	100.0	<i>For others, isotope abundance is high.</i>
		$^{37}\text{Cl}$	36.9659	31.98	
Bromine	79.909	$^{79}\text{Br}$	78.9183	100.0	<i>For others, isotope abundance is high.</i>
		$^{81}\text{Br}$	80.9163	97.3	
Iodine	126.904	$^{127}\text{I}$	126.9045	100.0	

*These differences are exhibited in peak intensities in mass spec.*

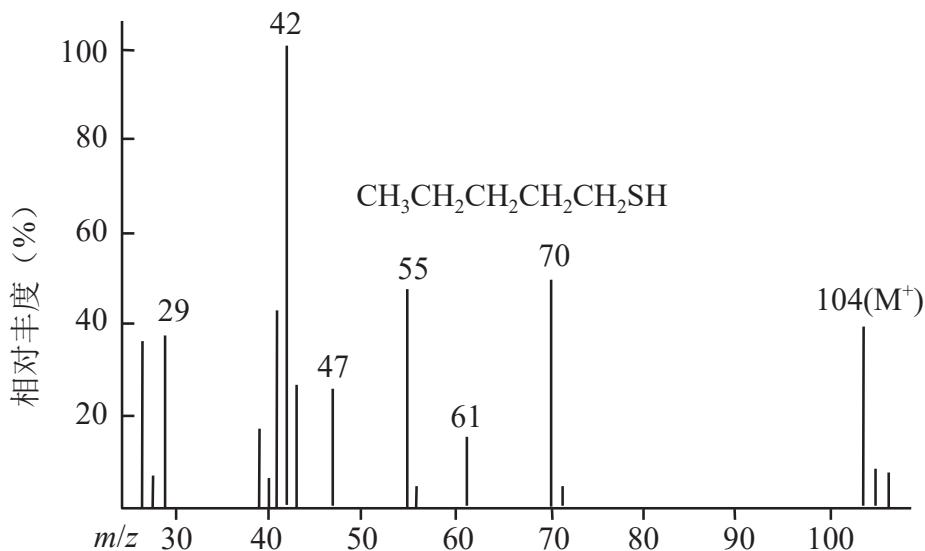
# Inferences from Isotopic Ratios



The  $M+2$  peak would be 24% the size of the  $M^+$  if one Cl is present



The  $M+2$  peak would be about the size of the  $M^+$  if one Br is present



Sulfur will give a  $M+2$  peak of 4% relative intensity and silicon 3%

# Summary

If  $M^+$  is visible be sure to test for its validity:

- 1) The peak must correspond to the highest mass ion on the spectrum excluding the isotopic peaks
- 2) The ion must be able to form the other fragments on the spectrum by loss of logical neutral fragments
- 3) Using the  $M+1$  peak (if visible) make some inference as to the number of carbon atoms (for small molecules this works as H, N and O give very low contributions to  $M+1$ )
- 4) If  $M+2$  becomes apparent, analyze for the presence of one or more Cl or Br atoms (sulfur and silicon can also give prominent  $M+2$ )

# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

Ionization Method

Ion Separation Methods

**Determination of Molecular Mass**

**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

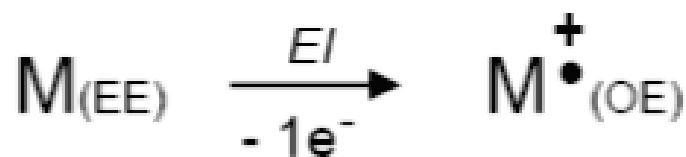
# Fragmentation - General

1. The collision of a high energy electron with a molecule not only causes the loss of a valence electron, it imparts some of the kinetic energy of collision into the remaining ion
2. This energy typically resides in an increased vibrational energy state for the molecule – this energy may be lost by the molecule breaking into *fragments*
3. The time between ionization and detection in most mass spectrometer is  $10^{-5}$  sec.
  - If a particular ionized molecule can “hold together” for greater than  $10^{-5}$  sec. a  $M^+$  ion is observed
  - If a particular ionized molecule fragments in less than this time, the fragments will be observed

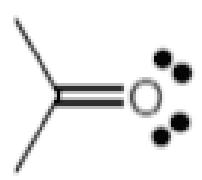
# Fragmentation - General

4. Due to the low concentration of molecules in the ionization chamber, all fragmentation processes are unimolecular
5. Fragmentation of a molecule that is missing one electron in most cases results in a covalent bond breaking homolytically – one fragment is then missing a full pair of electrons and has a + charge and the other fragment is a neutral radical
6. **Only the + charged ions will be observed; but the loss of a neutral fragment is inferred by the difference of the M+ and the m/e of the fragment**
7. Fragmentation will follow the trends you have learned in organic chemistry – fragmentation processes that lead to the most stable cations and radicals will occur with higher relative abundances

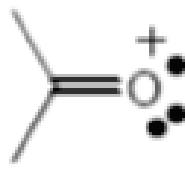
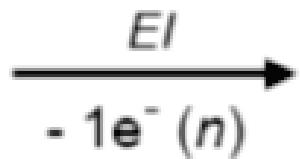
# Fragmentation - Chemistry of Ions



Electron can come from anywhere.



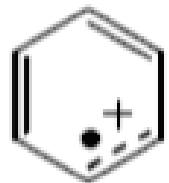
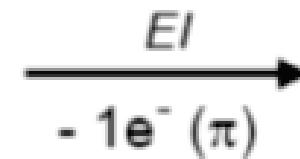
even  
electron



odd  
electron



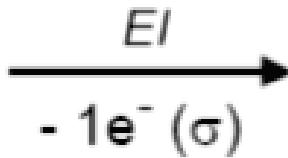
even  
electron



odd  
electron

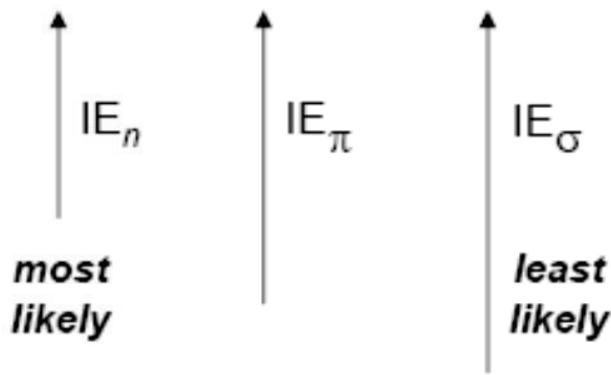
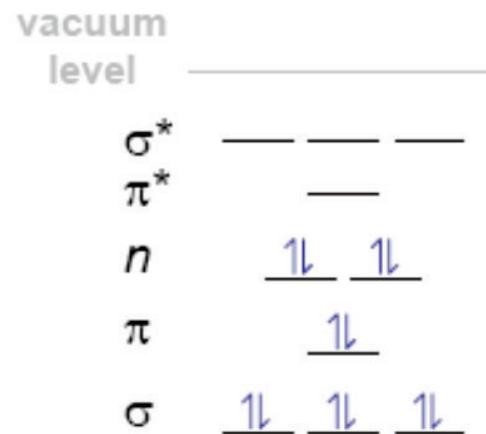
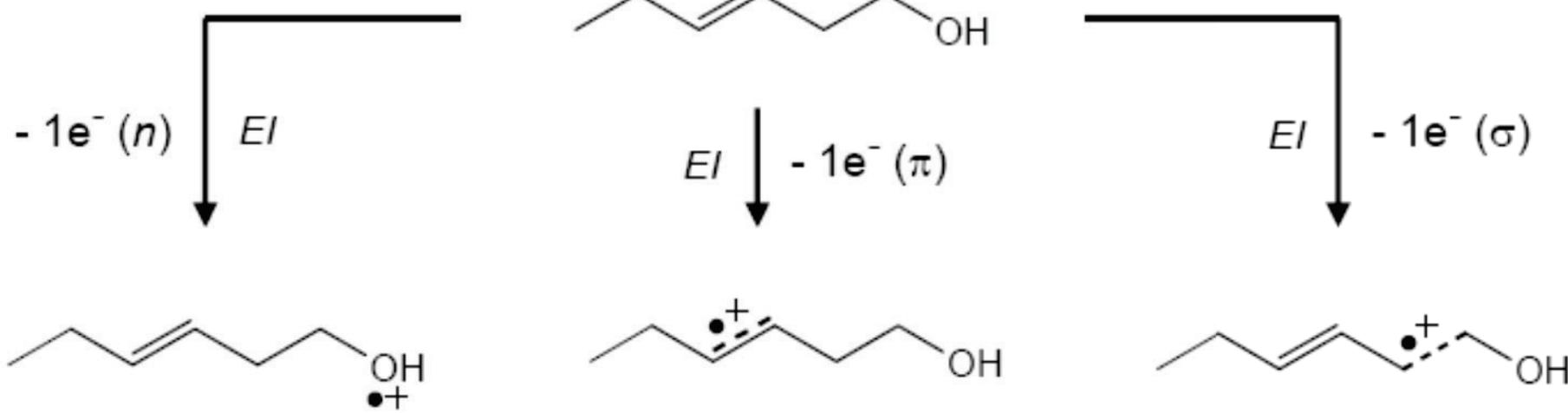


even  
electron



odd  
electron

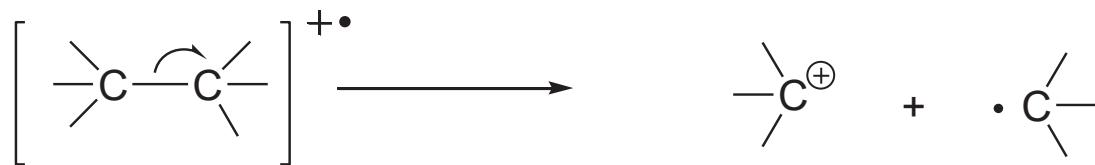
# Fragmentation - Chemistry of Ions



Likelihood of each of these depends on energy levels in molecular orbitals:

# Fragmentation - Chemistry of Ions

- One bond  $\sigma$ -cleavages:
  - cleavage of C-C

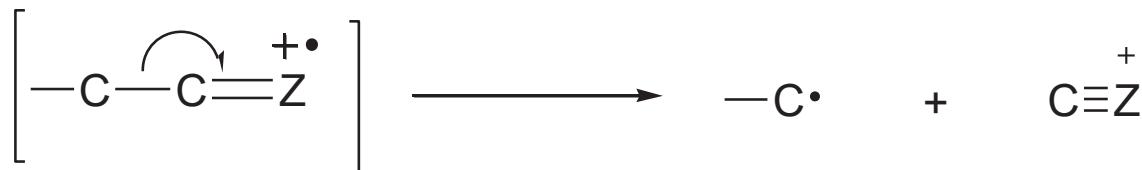
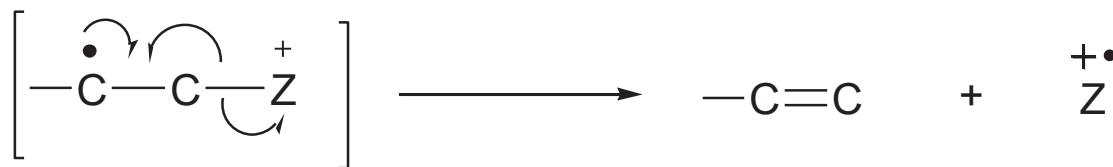
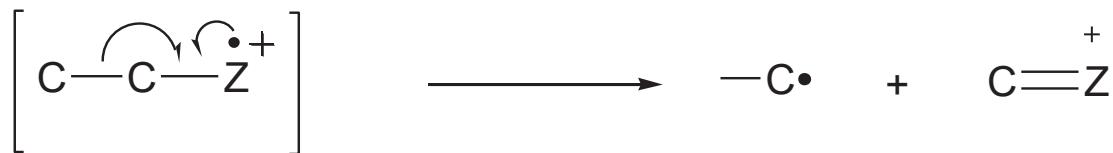


- cleavage of C-heteroatom



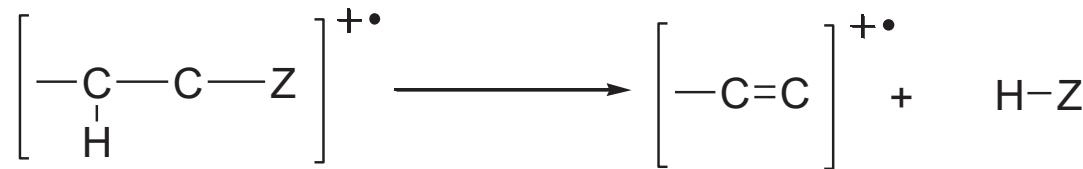
# Fragmentation - Chemistry of Ions

- One bond  $\sigma$ -cleavages:
  - c.  $\alpha$ -cleavage of C-heteroatom



# Fragmentation - Chemistry of Ions

2. Two bond  $\sigma$ -cleavages/rearrangements:
  - a. Elimination of a vicinal H and heteroatom:



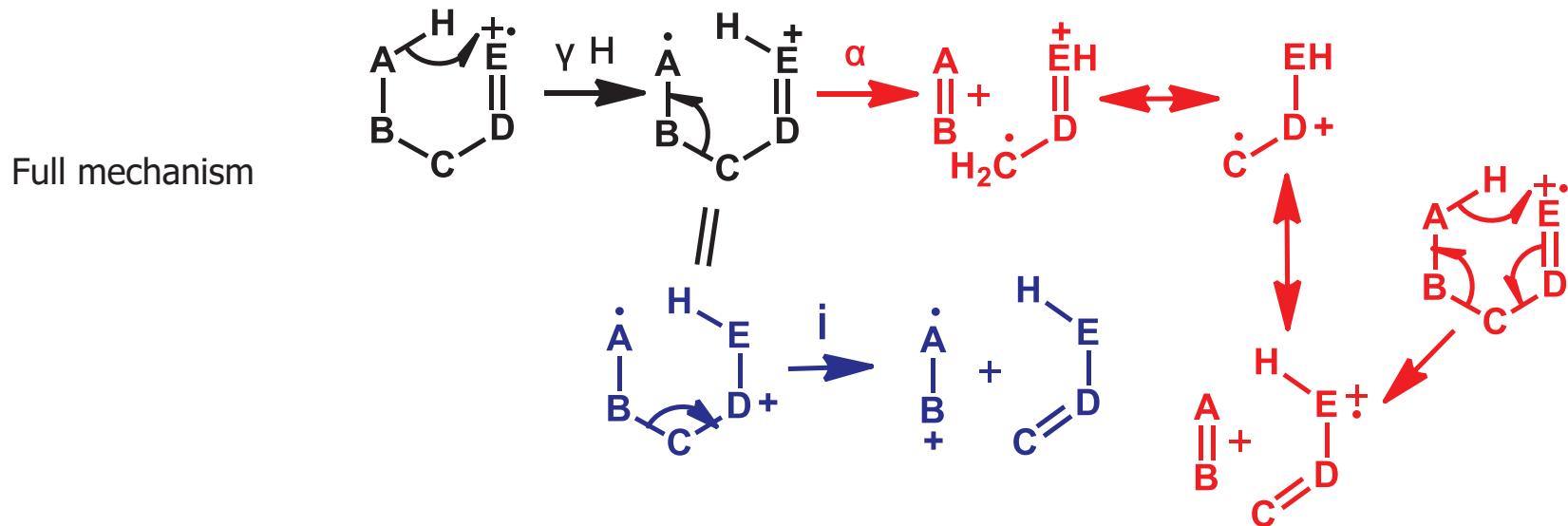
- b. Retro-Diels-Alder



# Fragmentation - Chemistry of Ions

2. Two bond  $\sigma$ -cleavages/rearrangements:

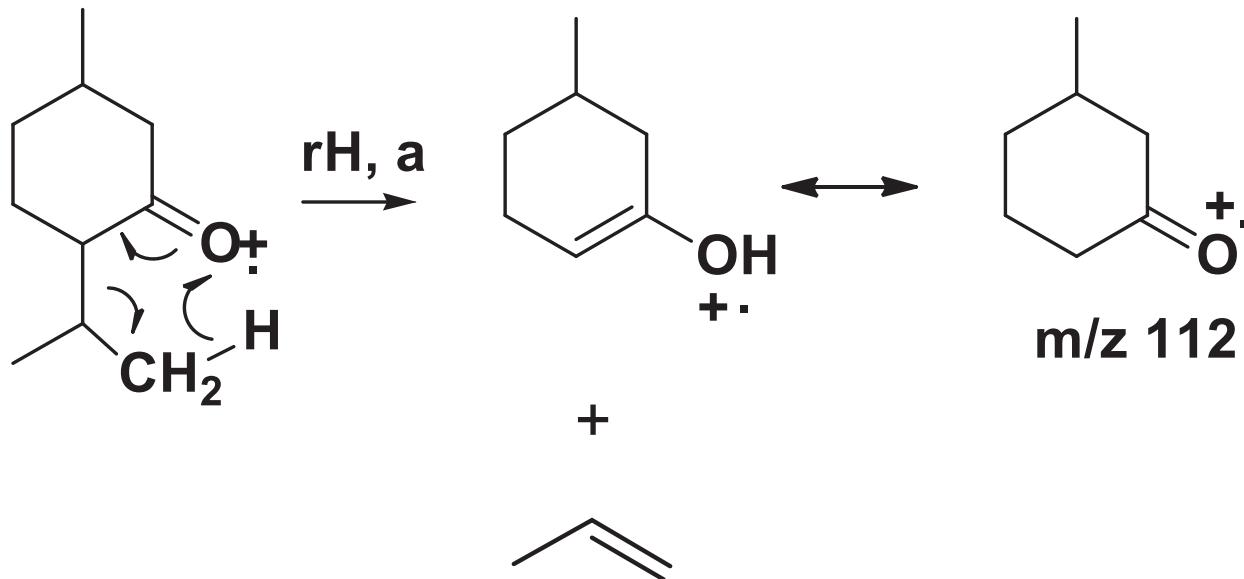
c. McLafferty Rearrangement ( $\gamma$ -H Rearrangement)



说明：D=E代表一个双键（或叁键）基团；  
C可以是碳原子也可以是杂原子；  
H是相对于不饱和键 $\gamma$ 位置碳原子A上的氢原子。

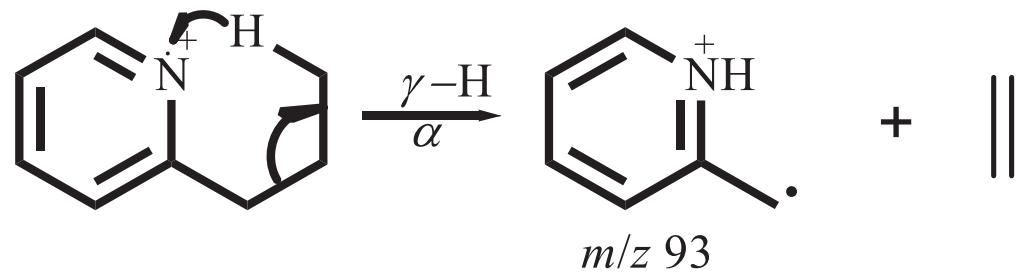
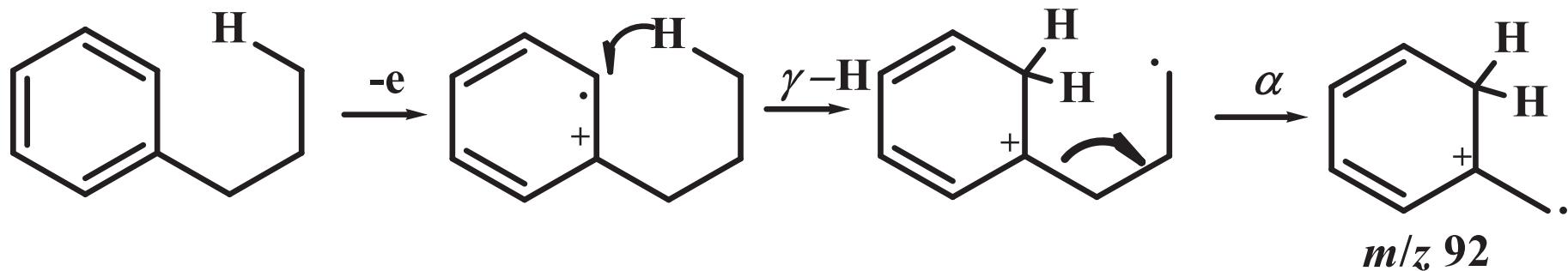
# Fragmentation - Chemistry of Ions

## 薄荷酮的麦氏重排



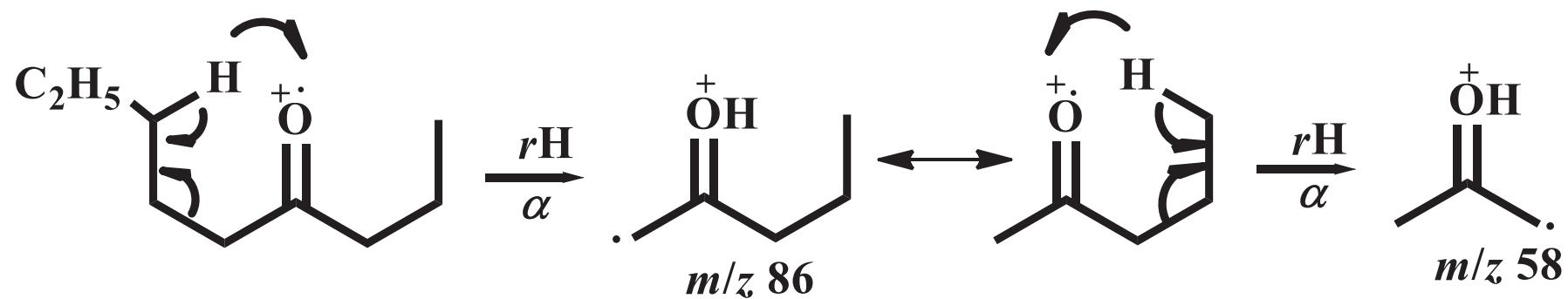
# Fragmentation - Chemistry of Ions

## 芳香环的麦氏重排

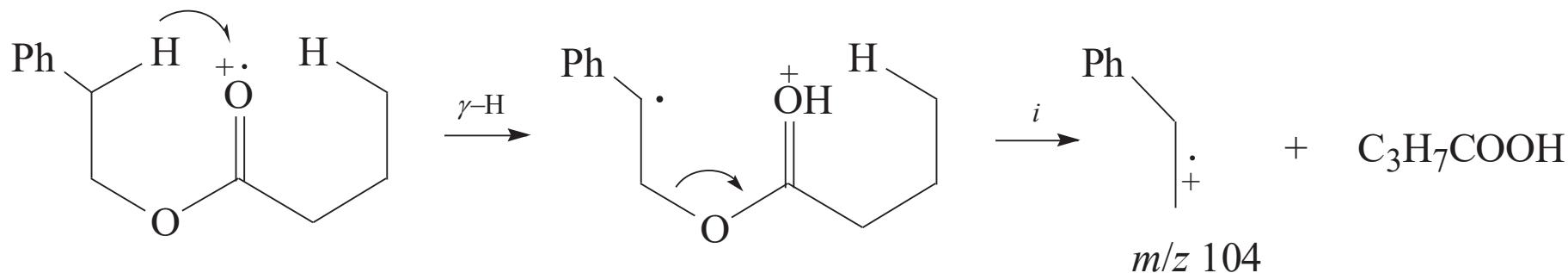
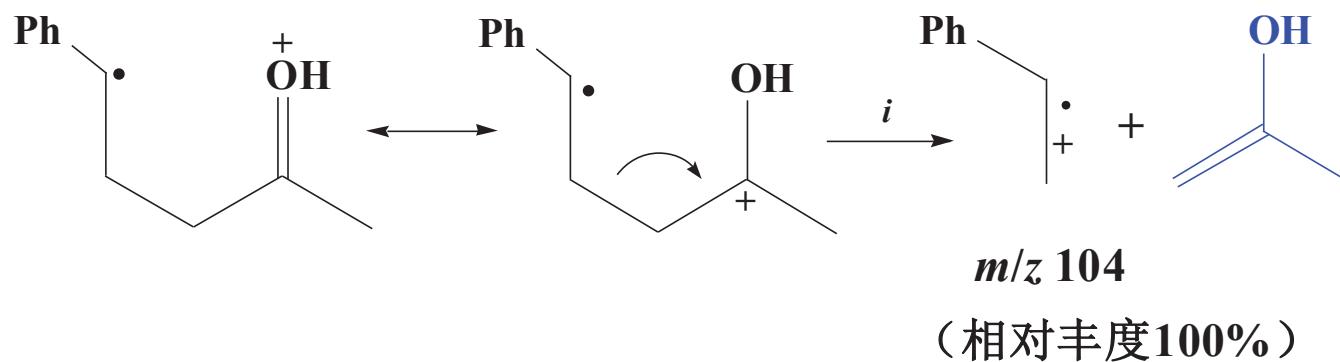
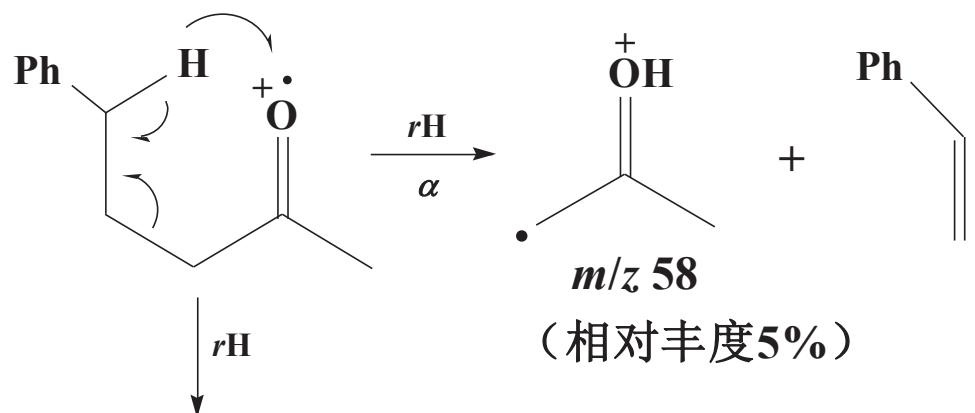


# Fragmentation - Chemistry of Ions

## 两次麦氏重排



# Fragmentation - Chemistry of Ions



# Fragmentation - Chemistry of Ions

常见的麦氏重排离子（最低质量数）

化合物类型	最小重排离子	$m/z$	化合物类型	最小重排离子	$m/z$
醛	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{C}=\text{OH} \\    \\ \text{H} \end{array}$	44	甲酯	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{C}=\text{O} \\    \\ \text{OCH}_3 \end{array}$	74
酮	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{C}=\text{OH} \\    \\ \text{CH}_3 \end{array}$	58	腈	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{C}\equiv\text{N}^+ \end{array}$	41
羧酸	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{C}=\text{OH} \\    \\ \text{OH} \end{array}$	60	硝基化合物	$\begin{array}{c} \cdot \\   \\ \text{H}_2\dot{\text{C}}-\text{N}=\text{O} \\    \\ \text{OH} \end{array}$	61

# Fragmentation - Chemistry of Ions

When deducing any fragmentation scheme:

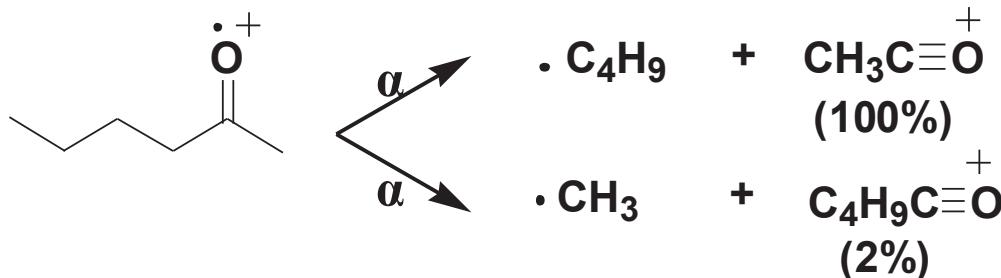
- The *even-odd electron rule* applies: “thermodynamics dictates that even electron ions cannot cleave to a pair of odd electron fragments” (偶电子规律)



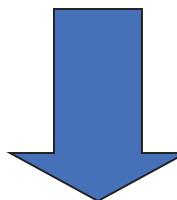
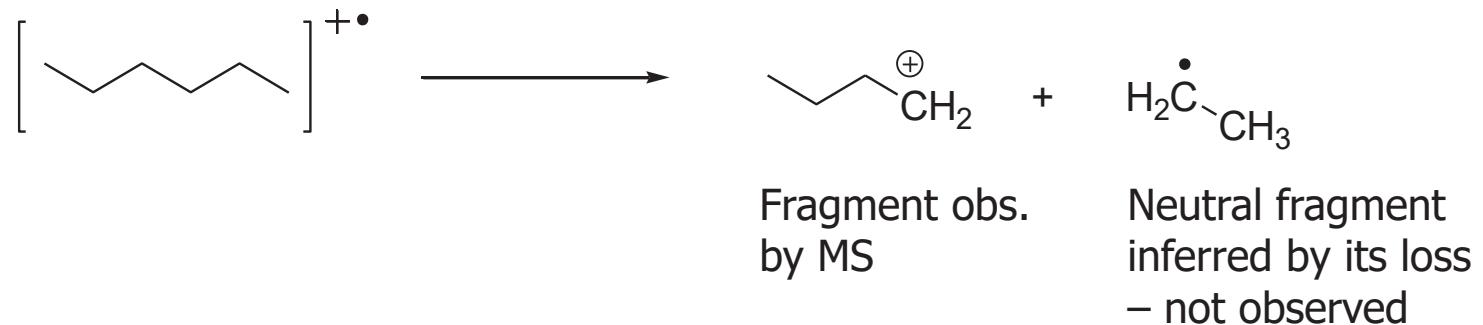
- The order of carbocation/radical stability is



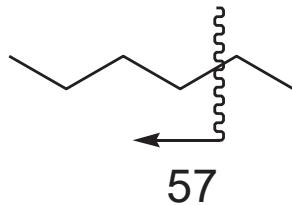
\* the loss of the longest carbon chain is preferred



# Fragmentation Patterns of Groups



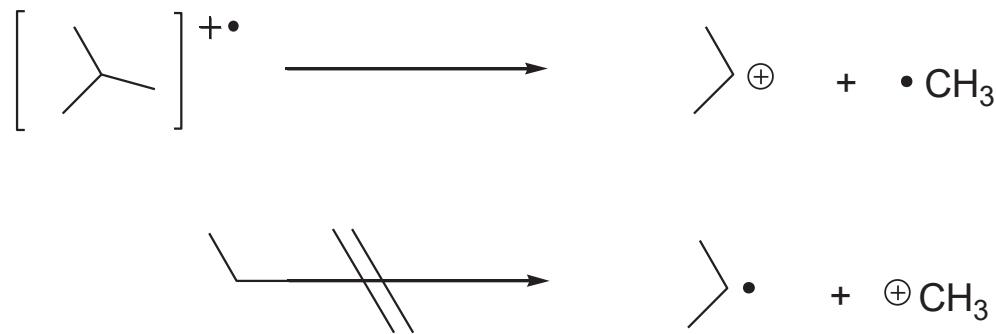
Is written as:



# Fragmentation Patterns of Groups

- Alkanes
  - Very predictable – apply the lessons of the stability of carbocations (or radicals) to predict or explain the observation of the fragments
  - Method of fragmentation is single bond cleavage in most cases
  - This is governed by **Stevenson's Rule** – the fragment with the lowest ionization energy will take on the + charge – the other fragment will still have an unpaired electron

Example: *iso*-butane



# Fragmentation Patterns of Groups

- Alkanes

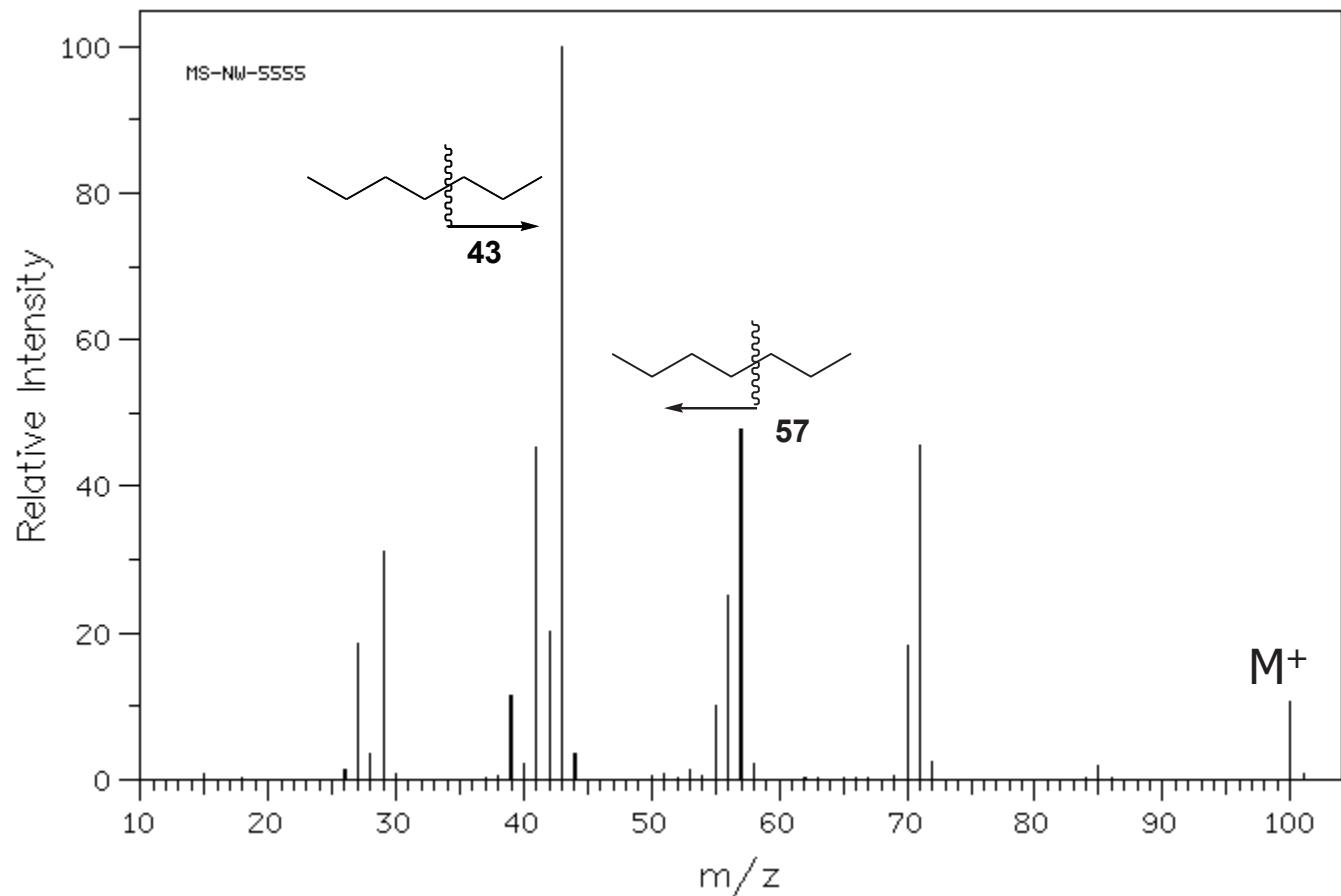
## Fragment Ions : *n*-alkanes

- For straight chain alkanes, a  $M^+$  is often observed
- Ions observed: clusters of peaks  $C_nH_{2n+1}$  apart from the loss of  $-CH_3$ ,  $-C_2H_5$ ,  $-C_3H_7$ , etc.
- Fragments lost:  $\cdot CH_3$ ,  $\cdot C_2H_5$ ,  $\cdot C_3H_7$ , etc.
- In longer chains – peaks at 43 and 57 are the most common

# Fragmentation Patterns of Groups

- Alkanes

**Example MS:** *n*-alkanes – *n*-heptane



# Fragmentation Patterns of Groups

- Alkanes

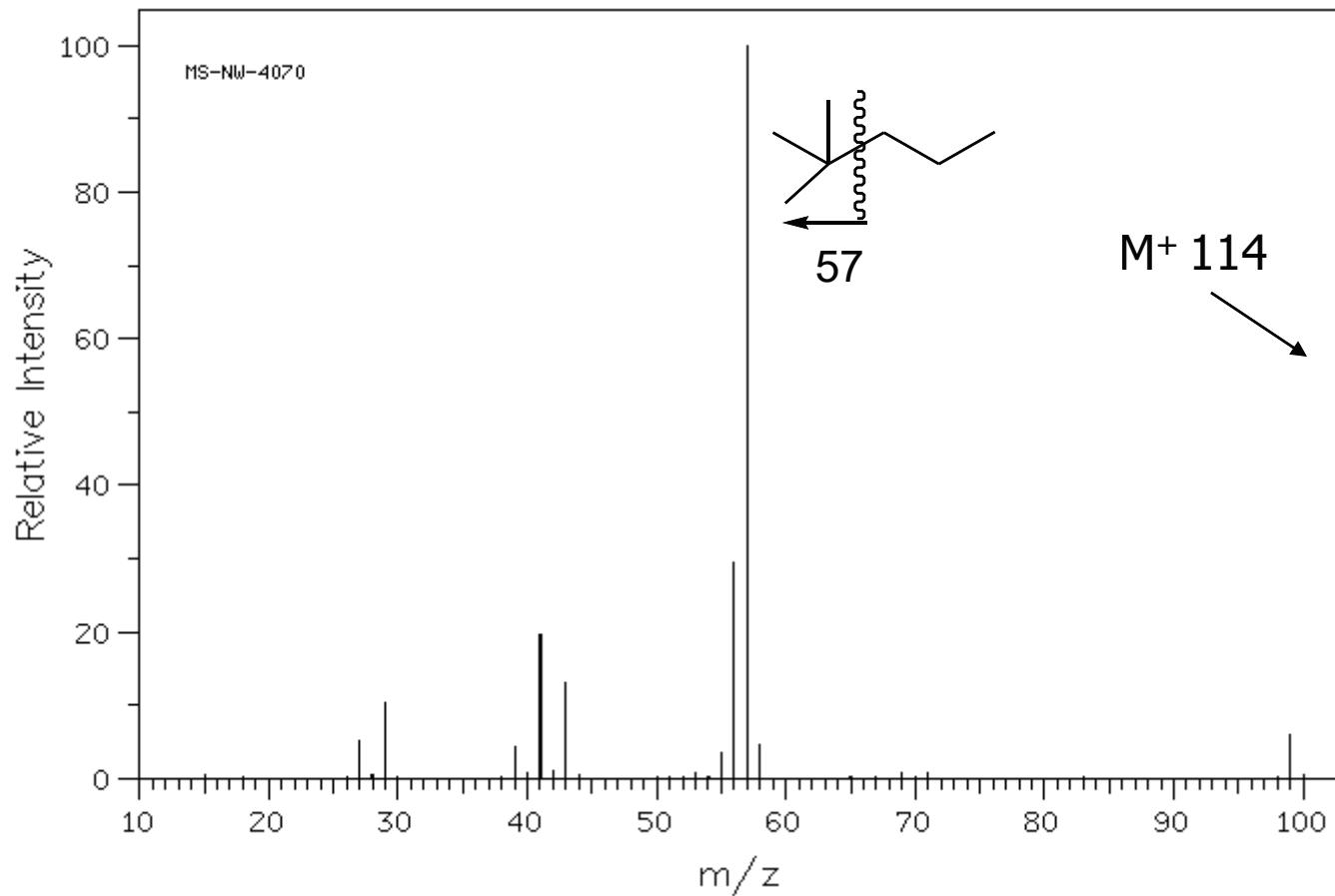
## **Fragment Ions : branched alkanes**

- Where the possibility of forming 2° and 3° carbocations is high, the molecule is susceptible to fragmentation
- Whereas in straight chain alkanes, a 1° carbocation is always formed, its appearance is of lowered intensity with branched structures
- M+ peaks become weak to non-existent as the size and branching of the molecule increase
- Peaks at 43 and 57 are the most common as these are the *iso*-propyl and *tert*-butyl cations

# Fragmentation Patterns of Groups

- Alkanes

**Example MS:** branched alkanes – 2,2-dimethylhexane

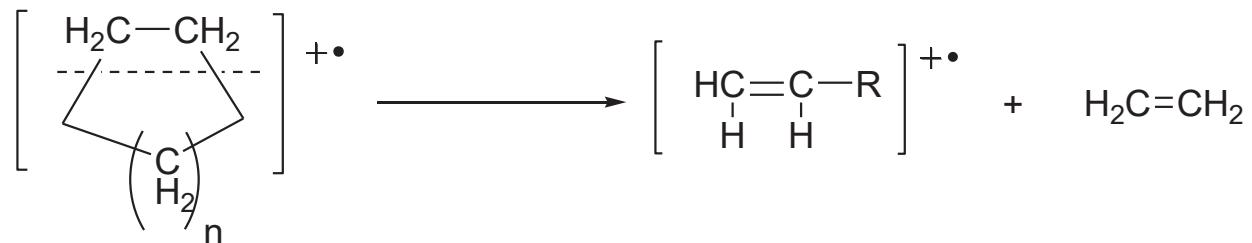


# Fragmentation Patterns of Groups

- Alkanes

## Fragment Ions : cycloalkanes

- Molecular ions strong and commonly observed – cleavage of the ring still gives same mass value
- A two-bond cleavage to form ethene ( $C_2H_4$ ) is common – loss of 28

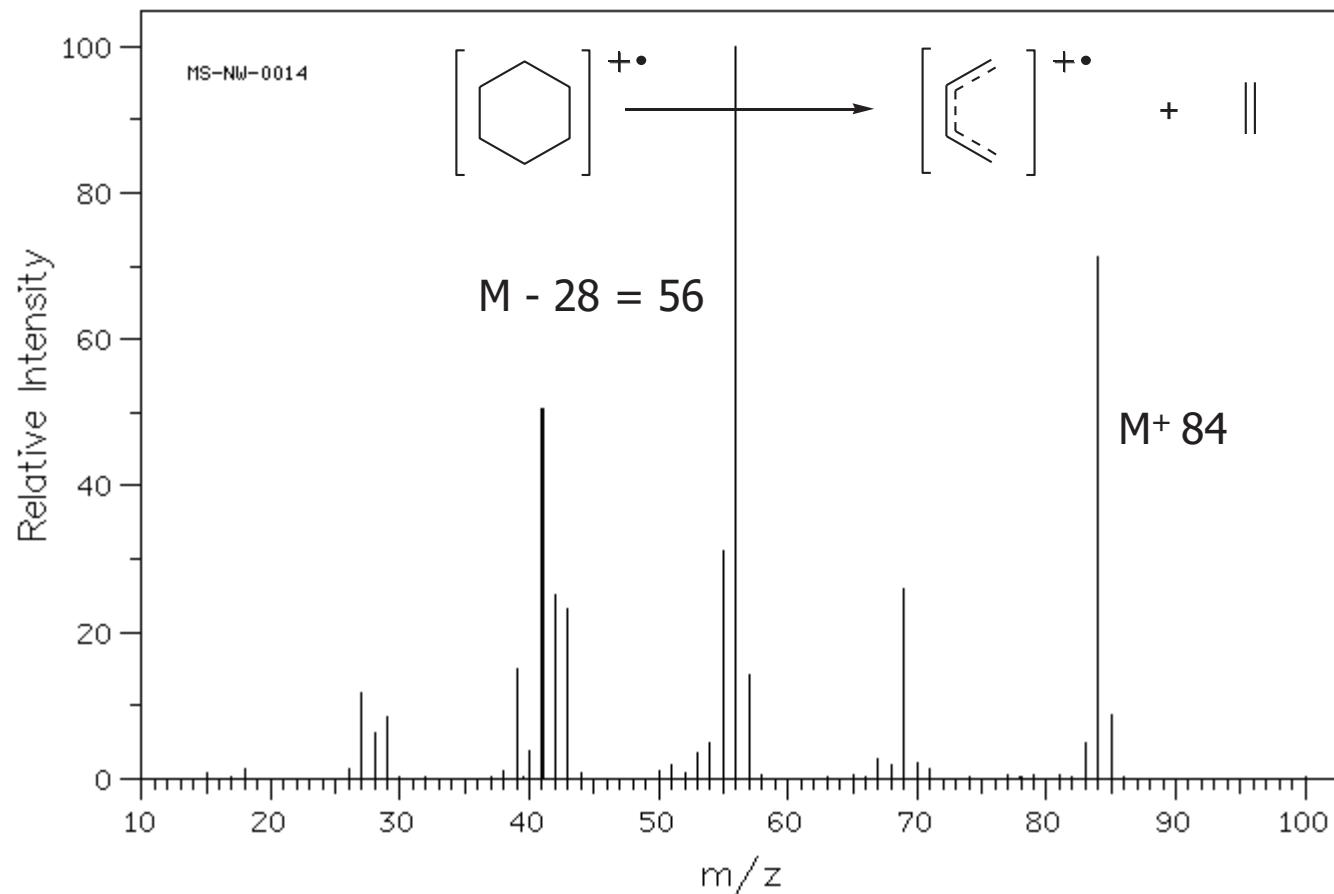


- Side chains are easily fragmented

# Fragmentation Patterns of Groups

- Alkanes

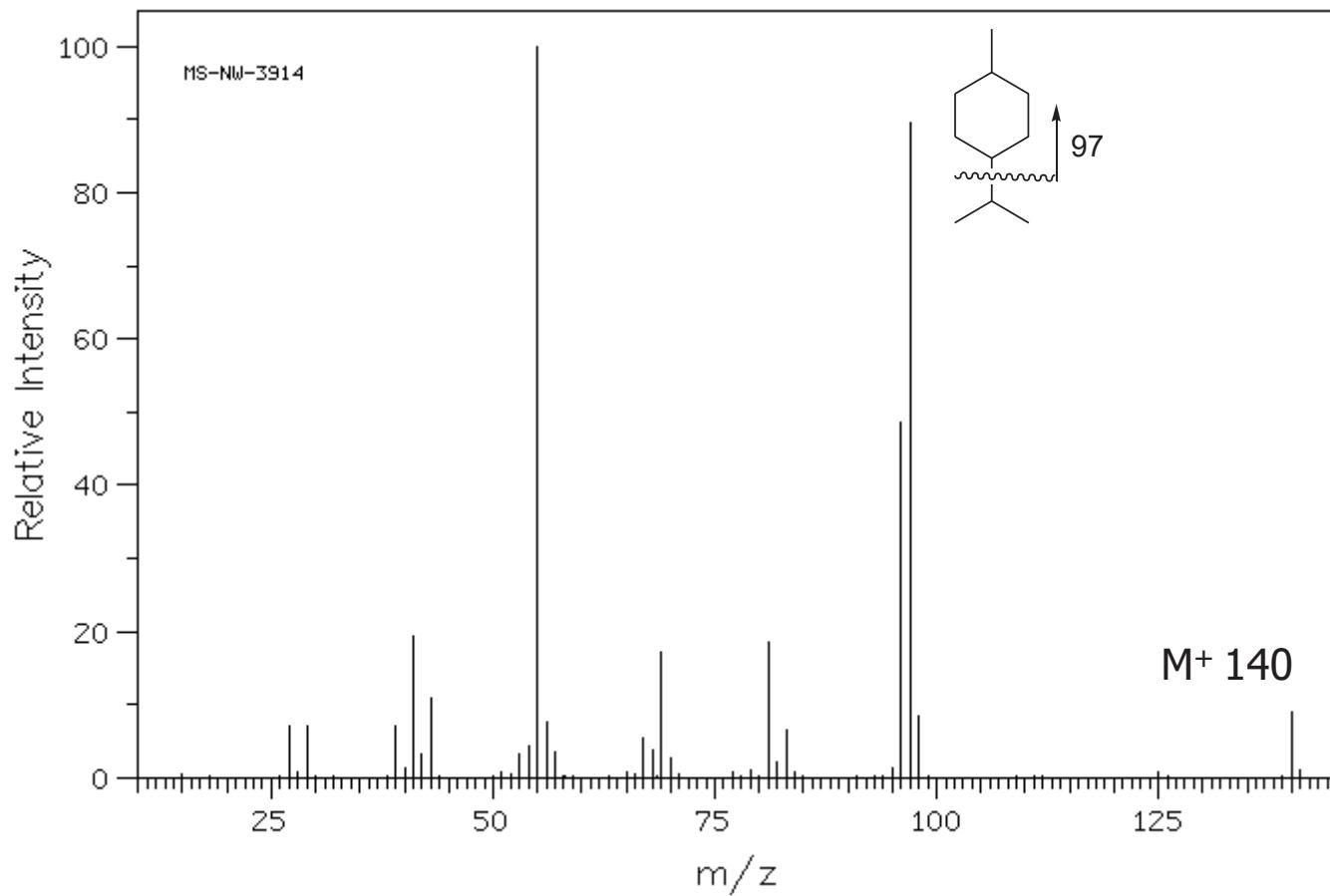
**Example MS:** cycloalkanes – cyclohexane



# Fragmentation Patterns of Groups

- Alkanes

**Example MS:** cycloalkanes – *trans-p*-menthane



# Fragmentation Patterns of Groups

## 2. Alkenes

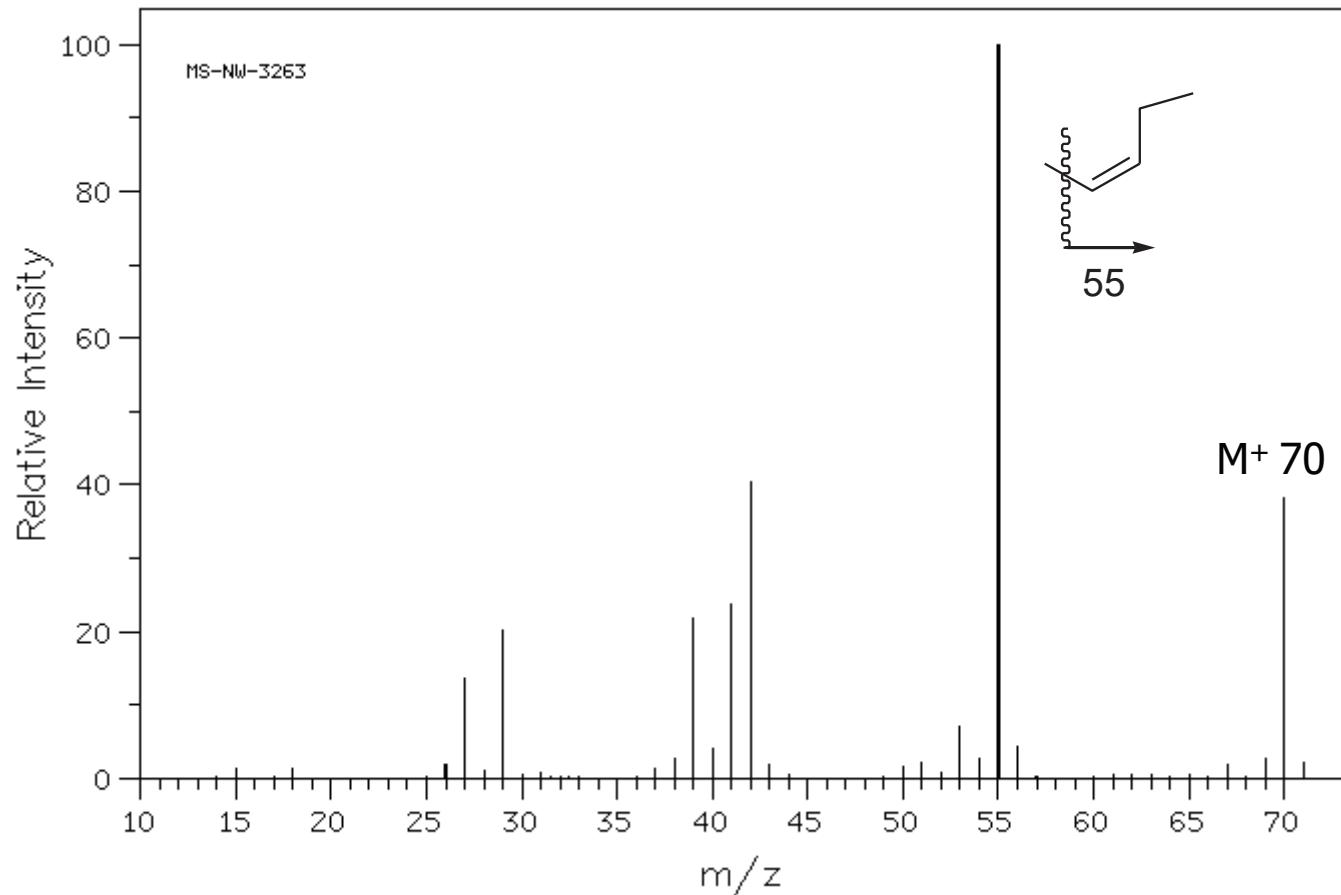
- The  $\pi$ -bond of an alkene can absorb substantial energy – molecular ions are commonly observed
- After ionization, double bonds can migrate readily – determination of isomers is often not possible
- Ions observed: clusters of peaks  $C_nH_{2n-1}$  apart from  $-C_3H_5$ ,  $-C_4H_7$ ,  $-C_5H_9$  etc. at 41, 55, 69, etc.
- Terminal alkenes readily form the allyl carbocation, m/z 41



# Fragmentation Patterns of Groups

## 2. Alkenes

**Example MS:** alkenes – *cis*- 2-pentene

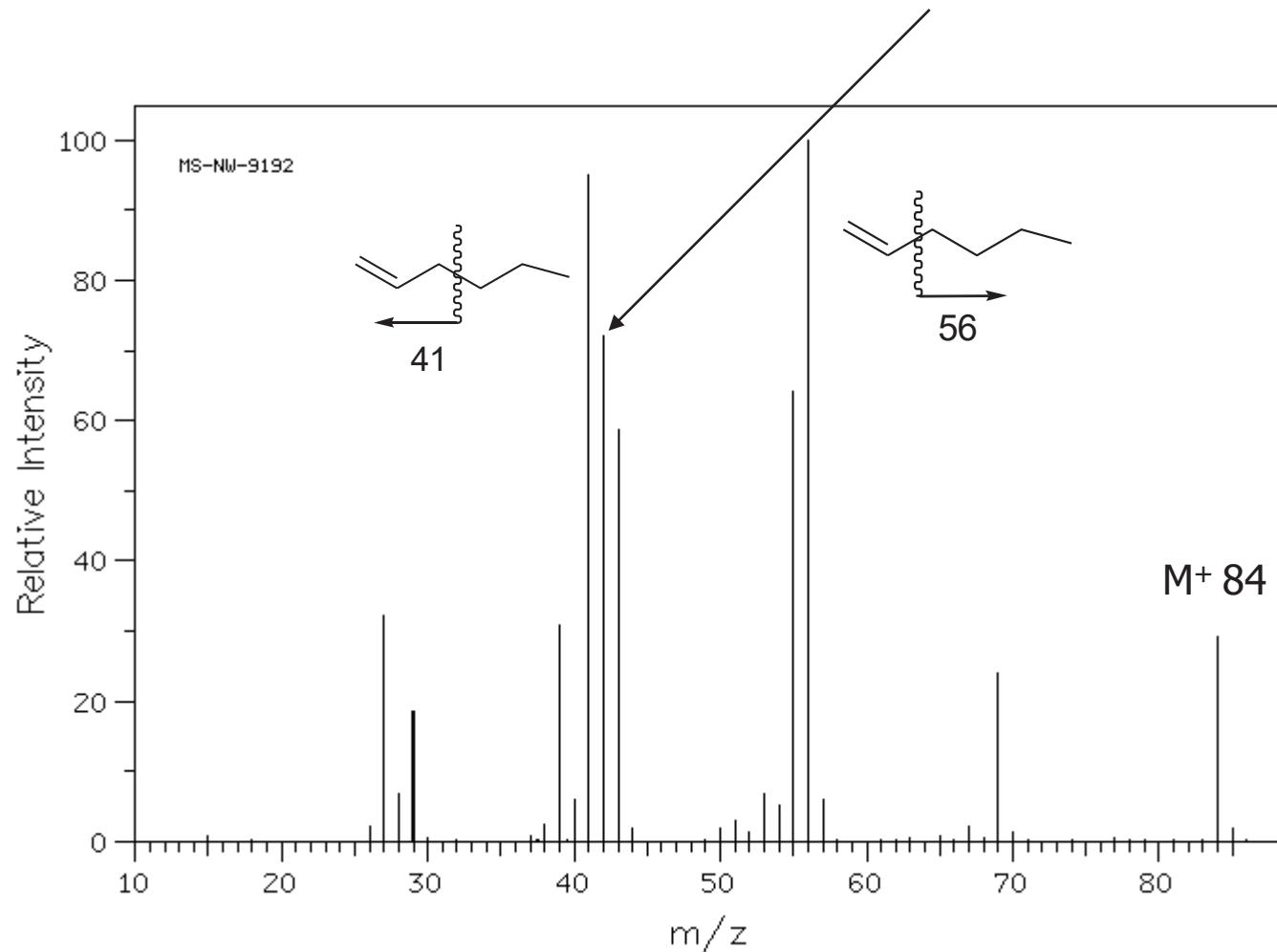


# Fragmentation Patterns of Groups

## 2. Alkenes

Example MS: alkenes –1-hexene

What is M-42 and  $m/z$  42?

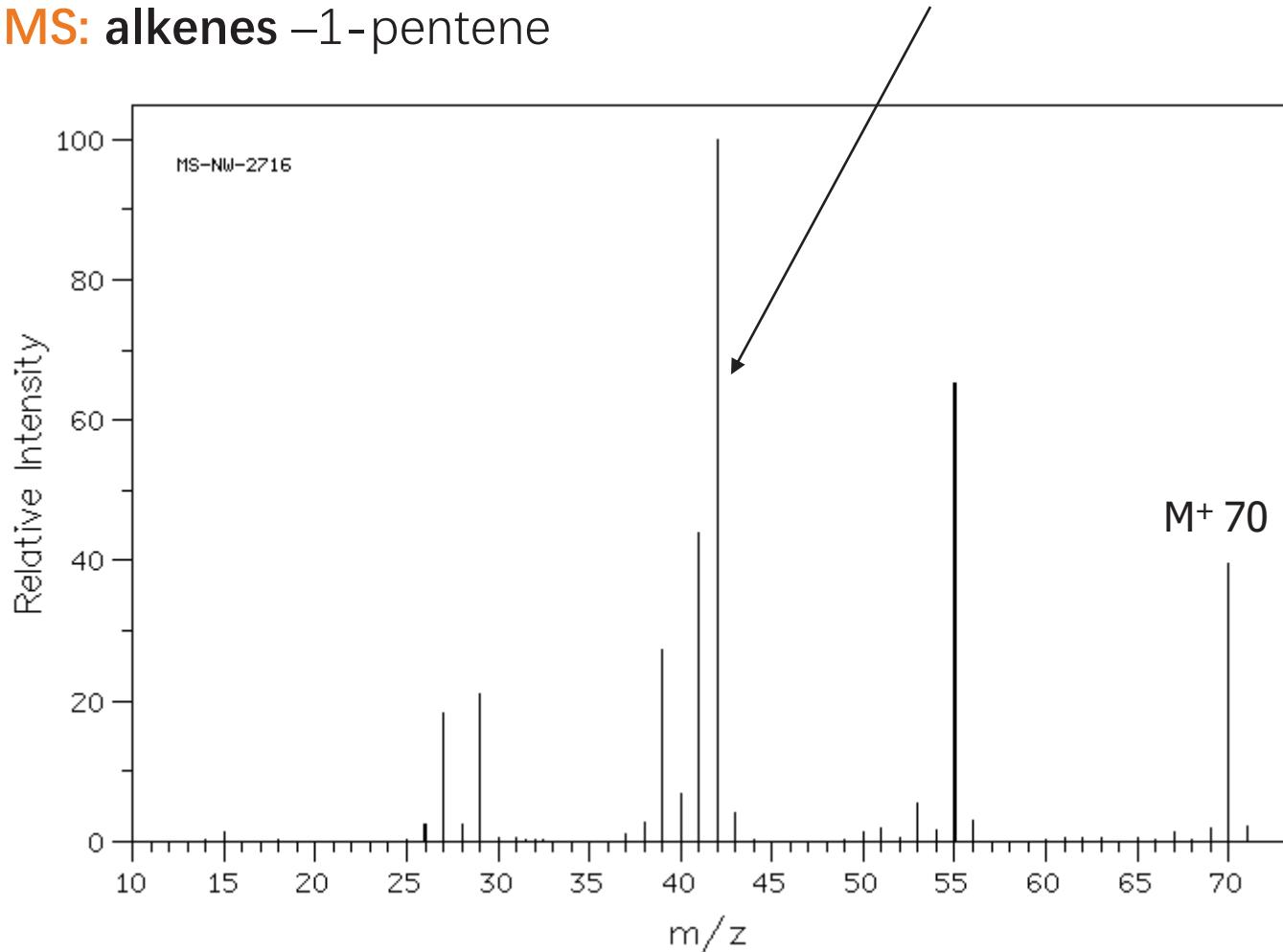


# Fragmentation Patterns of Groups

## 2. Alkenes

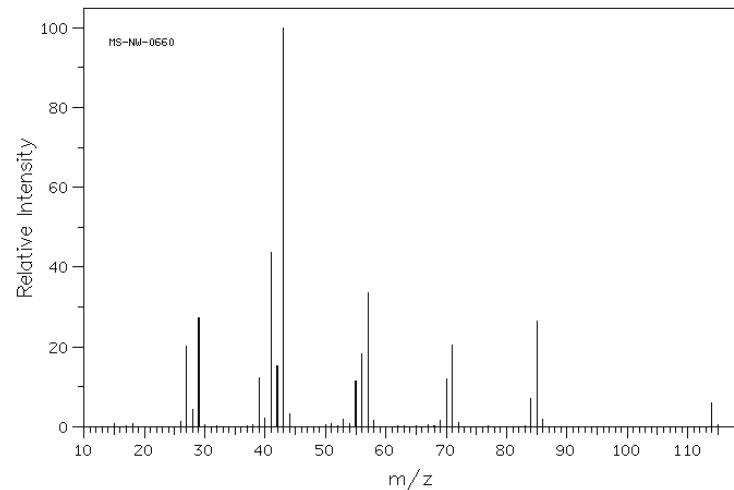
Take home assignment 2:  
What is  $m/z$  42?

Example MS: alkenes –1-pentene

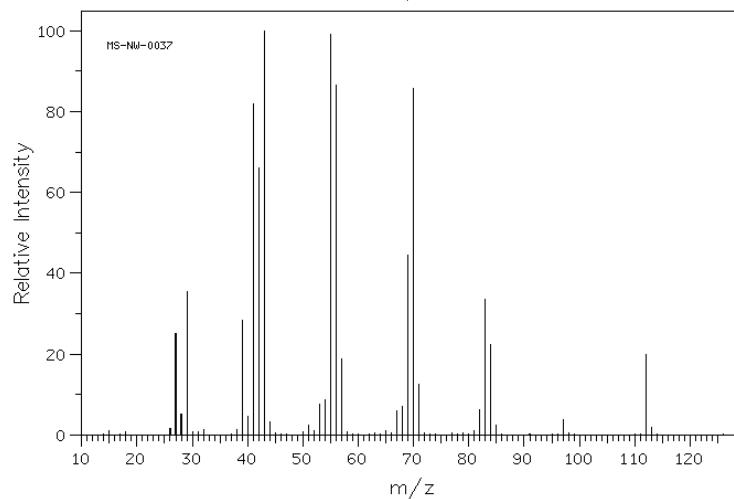


# Fragmentation Patterns of Groups

## Comparison: Alkanes vs. alkenes



Octane (75 eV)  
 $M^+$  114  
m/z 85, 71, 57, 43 (base), 29



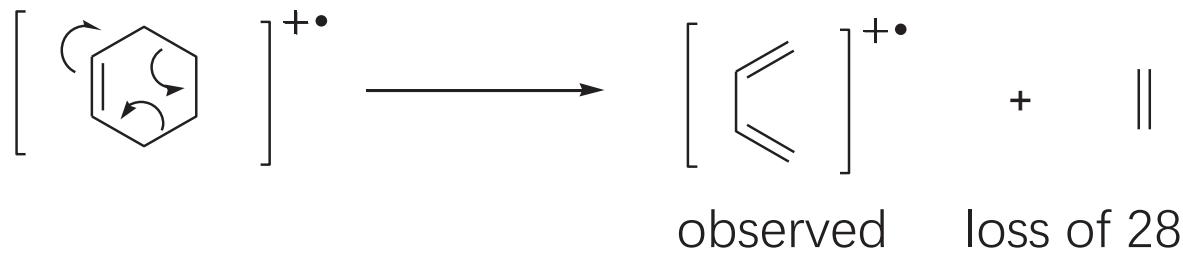
Octene (75 eV)  
 $M^+$  112 (stronger @ 75eV than octane)  
m/z 83, 69, 55, 41, 29

# Fragmentation Patterns of Groups

## 2. Alkenes

### Fragment Ions : cycloalkenes

- Molecular ions strong and commonly observed – cleavage of the ring still gives same mass value
- Retro-Diels-Alder is significant

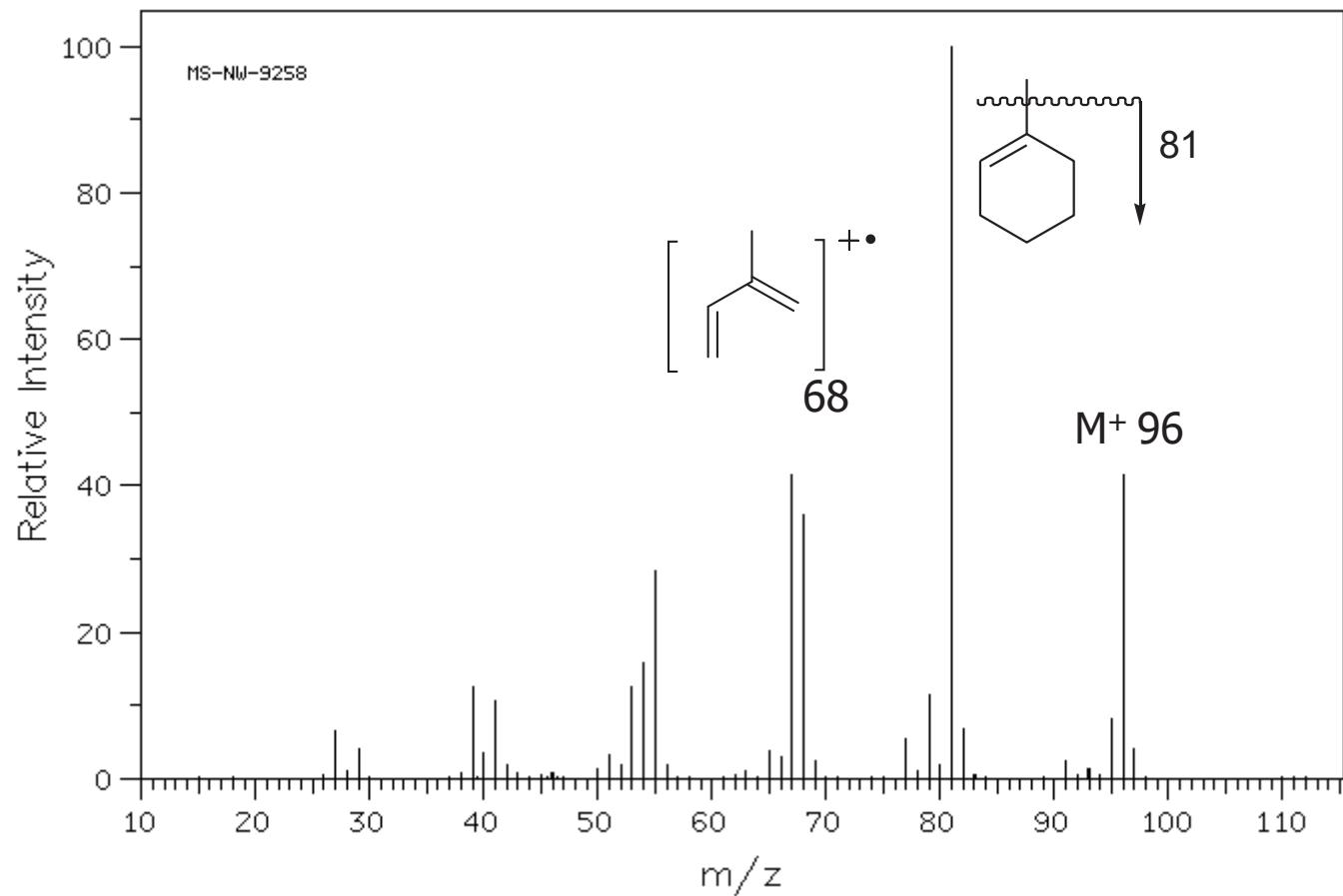


- Side chains are easily fragmented

# Fragmentation Patterns of Groups

## 2. Alkenes

**Example MS:** cycloalkenes –1-methyl-1-cyclohexene



# Fragmentation Patterns of Groups

## 3. Alkynes – Fragment Ions

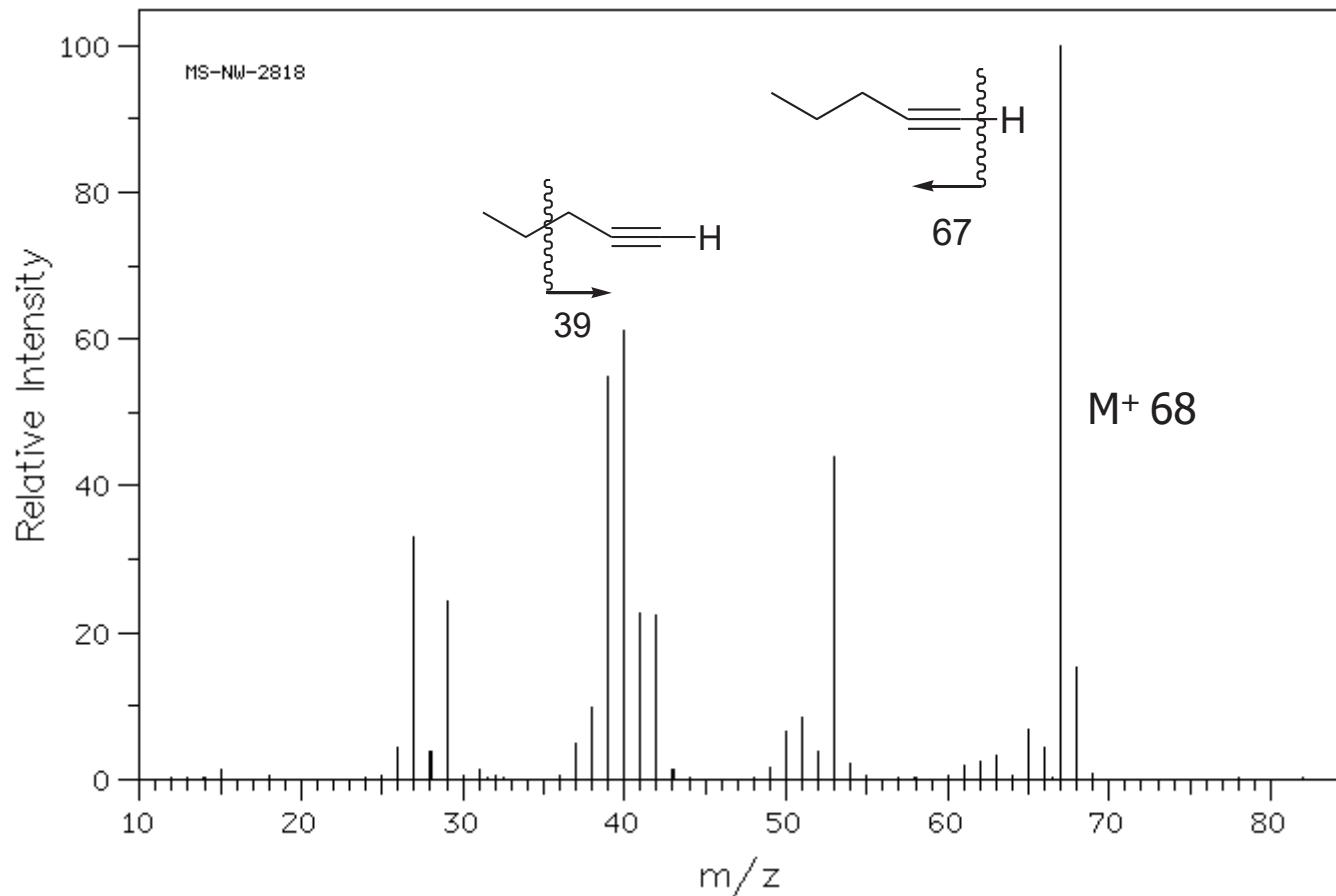
- The  $\pi$ -bond of an alkyne can also absorb substantial energy – molecular ions are commonly observed
- For terminal alkynes, the loss of terminal hydrogen is observed (**M-1**) – this may occur at such intensity to be the base peak or eliminate the presence of  $M^+$
- Terminal alkynes form the propargyl cation, m/z 39 (lower intensity than the allyl cation)



# Fragmentation Patterns of Groups

## 3. Alkynes

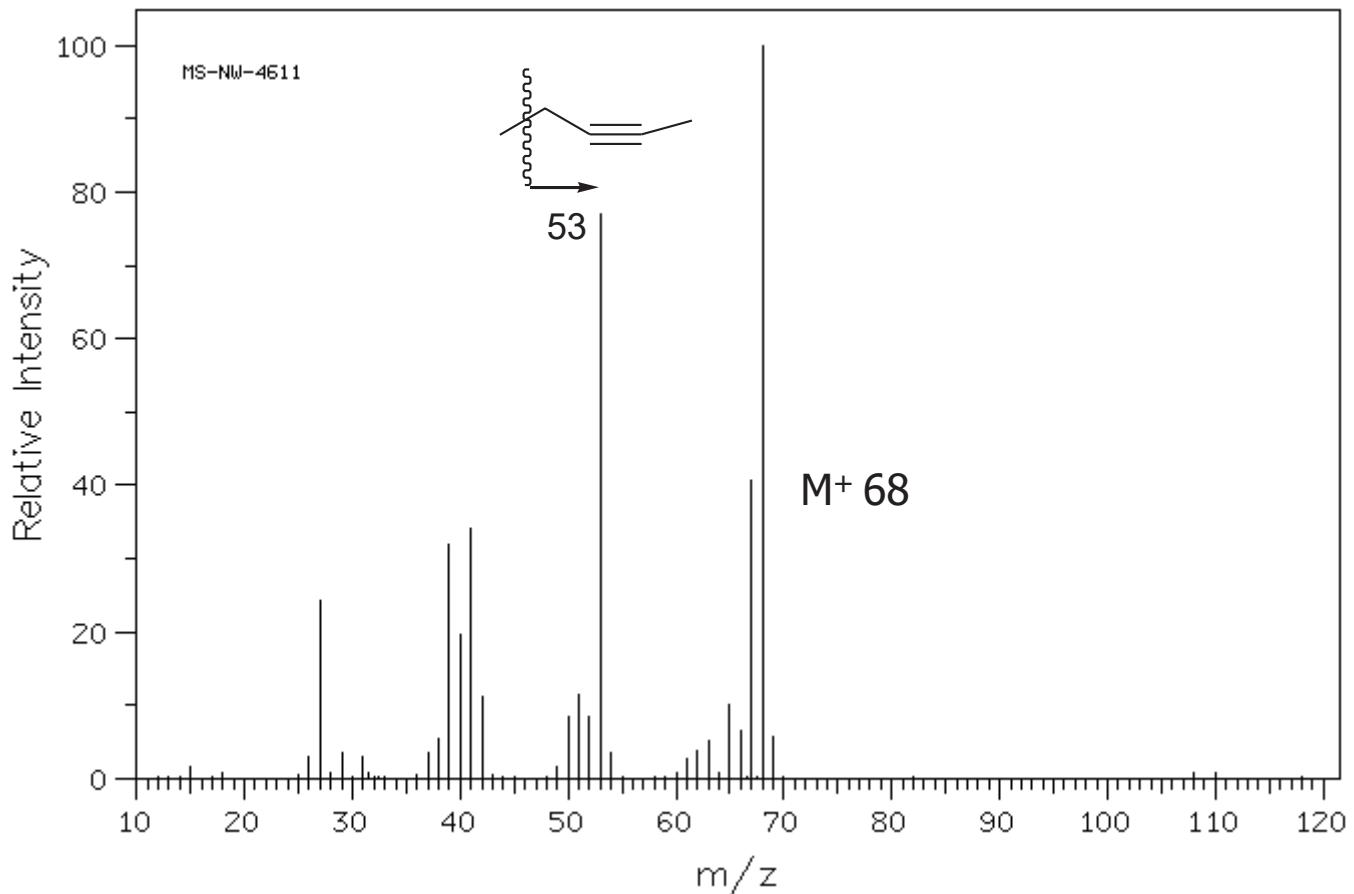
**Example MS: alkynes – 1-pentyne**



# Fragmentation Patterns of Groups

## 3. Alkynes

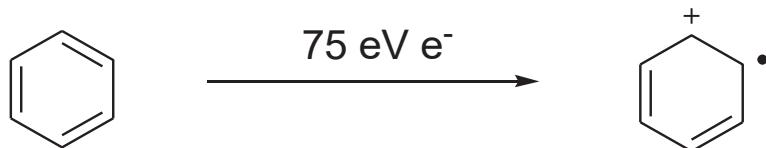
Example MS: alkynes – 2-pentyne



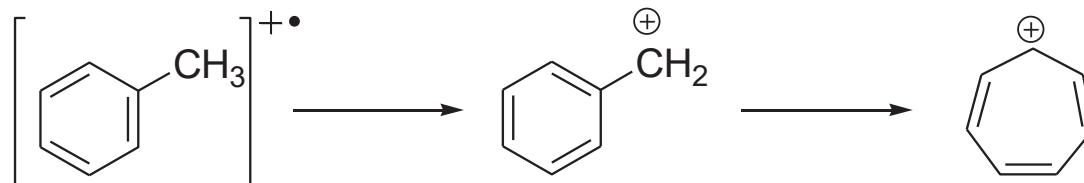
# Fragmentation Patterns of Groups

## 4. Aromatic Hydrocarbons – Fragment Ions

- Very intense molecular ion peaks and little fragmentation of the ring system are observed



- Where alkyl groups are attached to the ring, a favorable mode of cleavage is to lose a H-radical to form the C<sub>7</sub>H<sub>7</sub><sup>+</sup> ion (m/z 91)
- This ion is believed to be the tropylum ion; formed from rearrangement of the benzyl cation



# Fragmentation Patterns of Groups

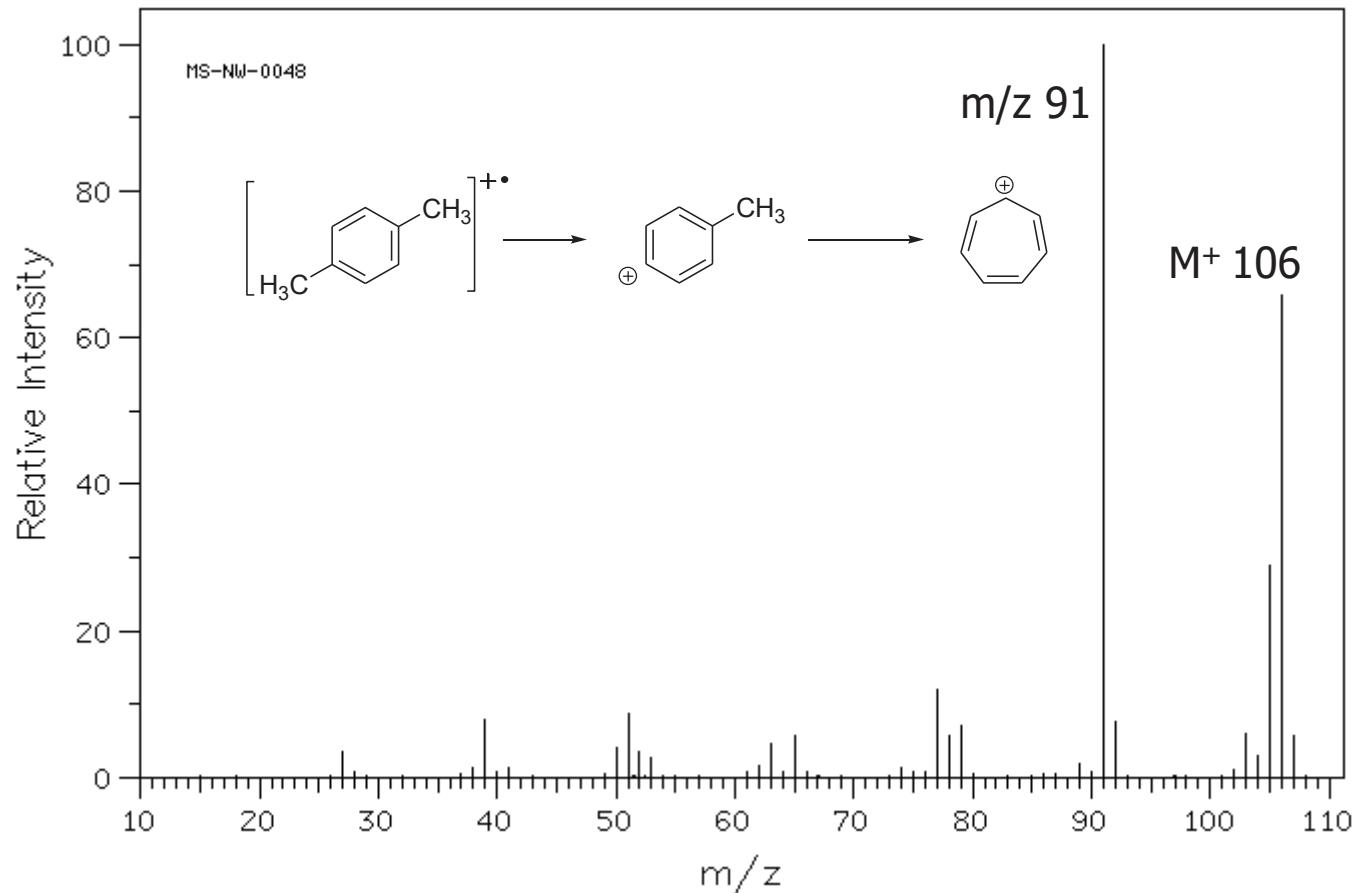
## 4. Aromatic Hydrocarbons – Fragment Ions

- d) If a chain from the aromatic ring is sufficiently long, a McLafferty rearrangement is possible
- e) Substitution patterns for aromatic rings are able to be determined by MS – with the exception of groups that have other ion chemistry

# Fragmentation Patterns of Groups

## 4. Aromatic Hydrocarbons

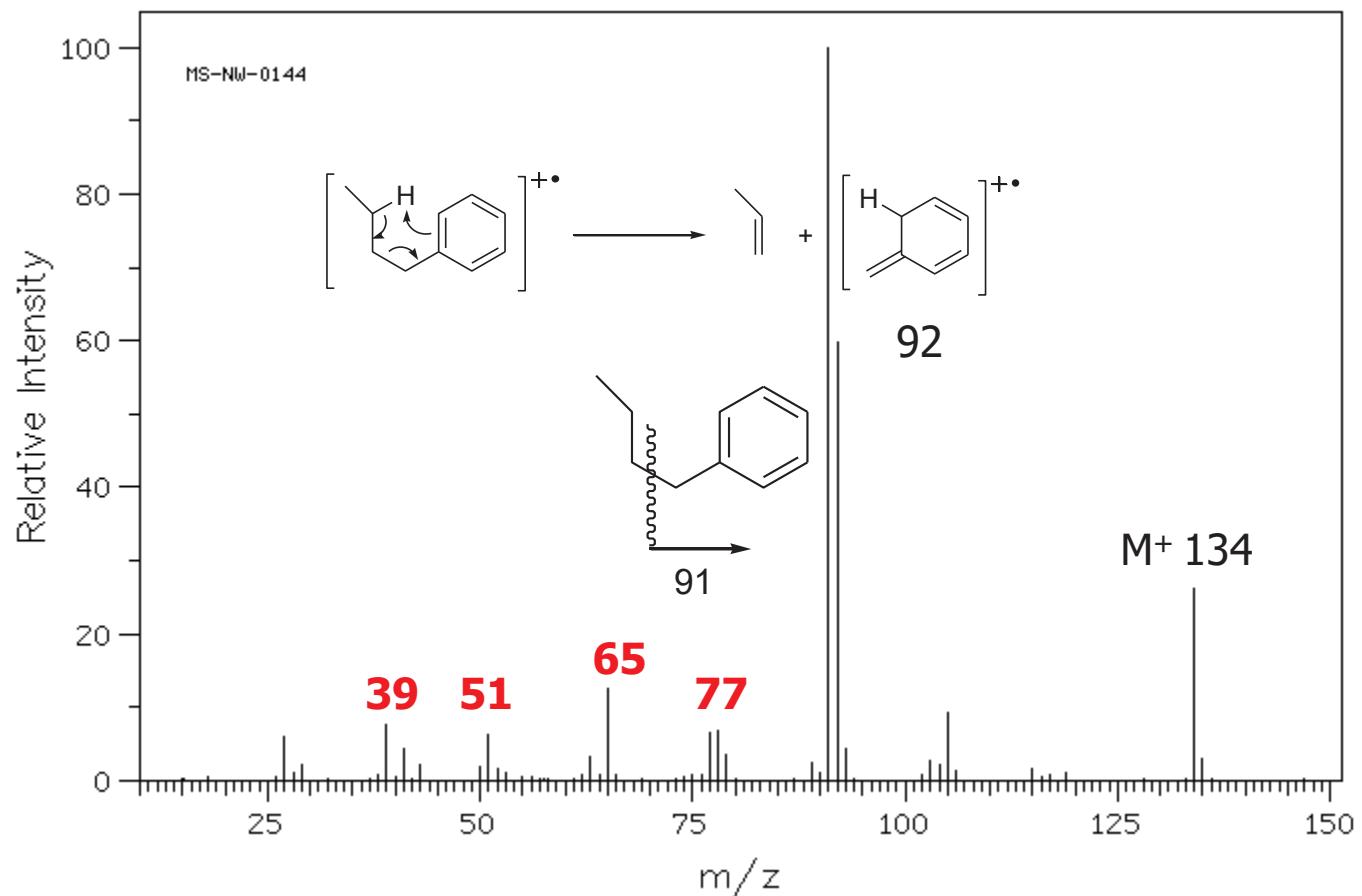
**Example MS:** aromatic hydrocarbons – *p*-xylene

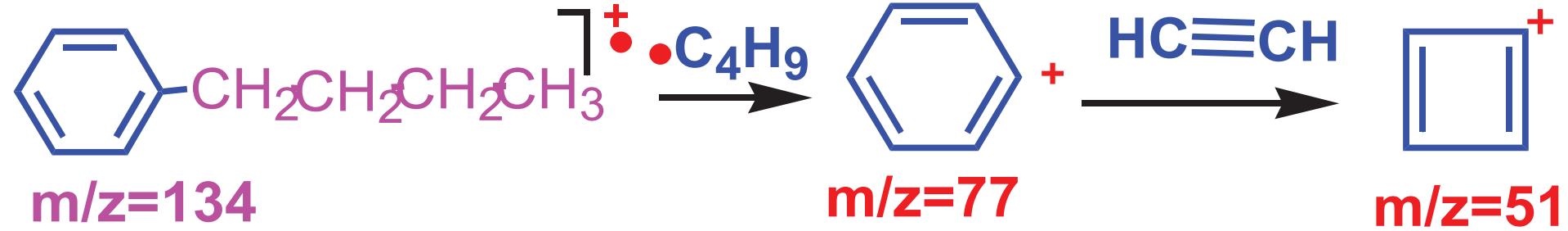
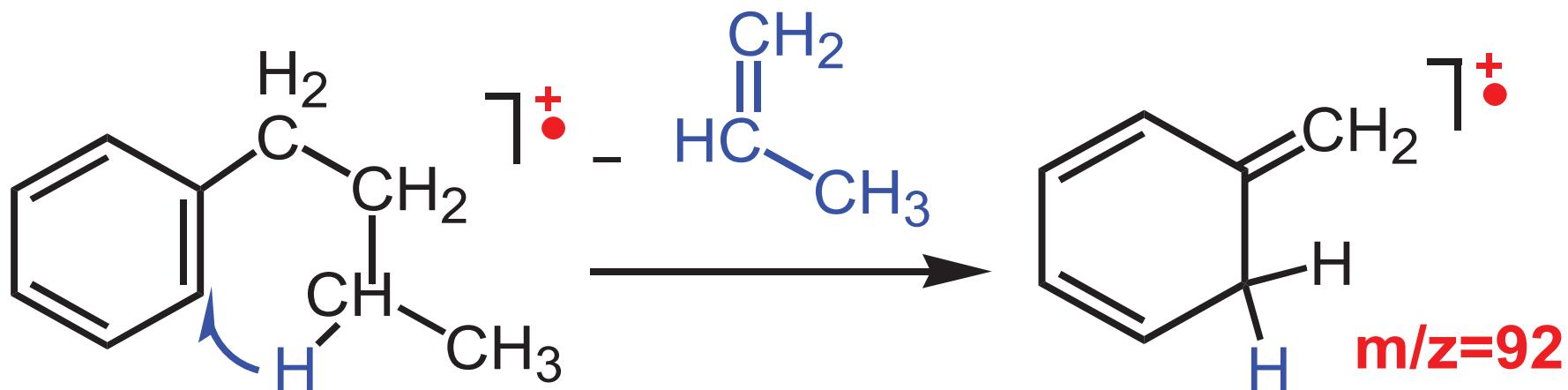
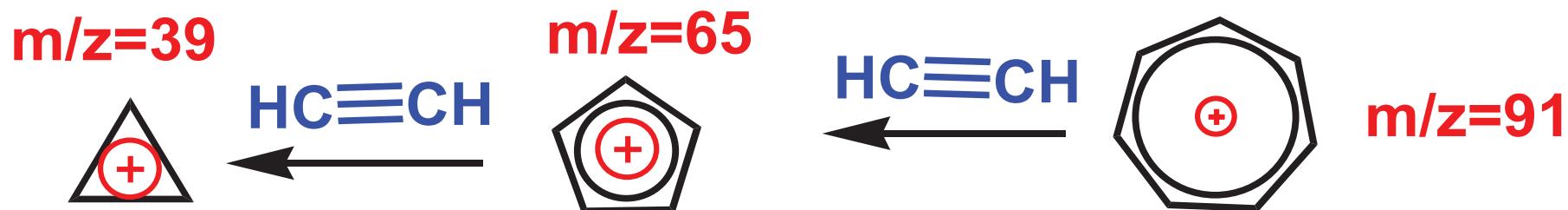
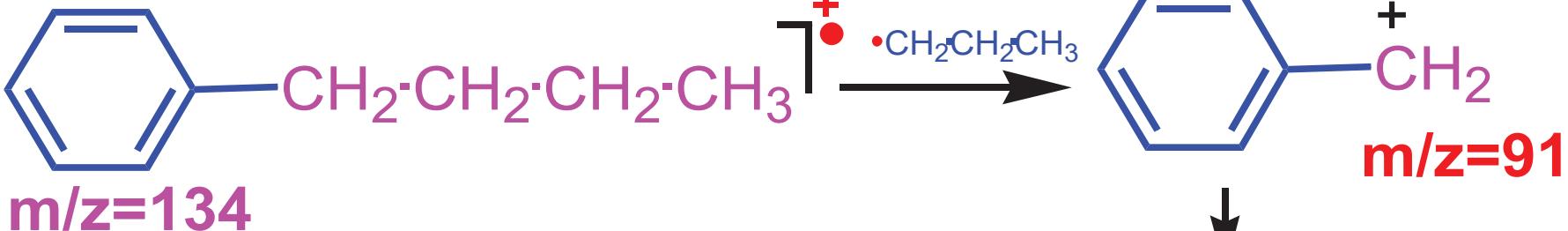


# Fragmentation Patterns of Groups

## 4. Aromatic Hydrocarbons

**Example MS:** aromatic hydrocarbons – *n*-butylbenzene



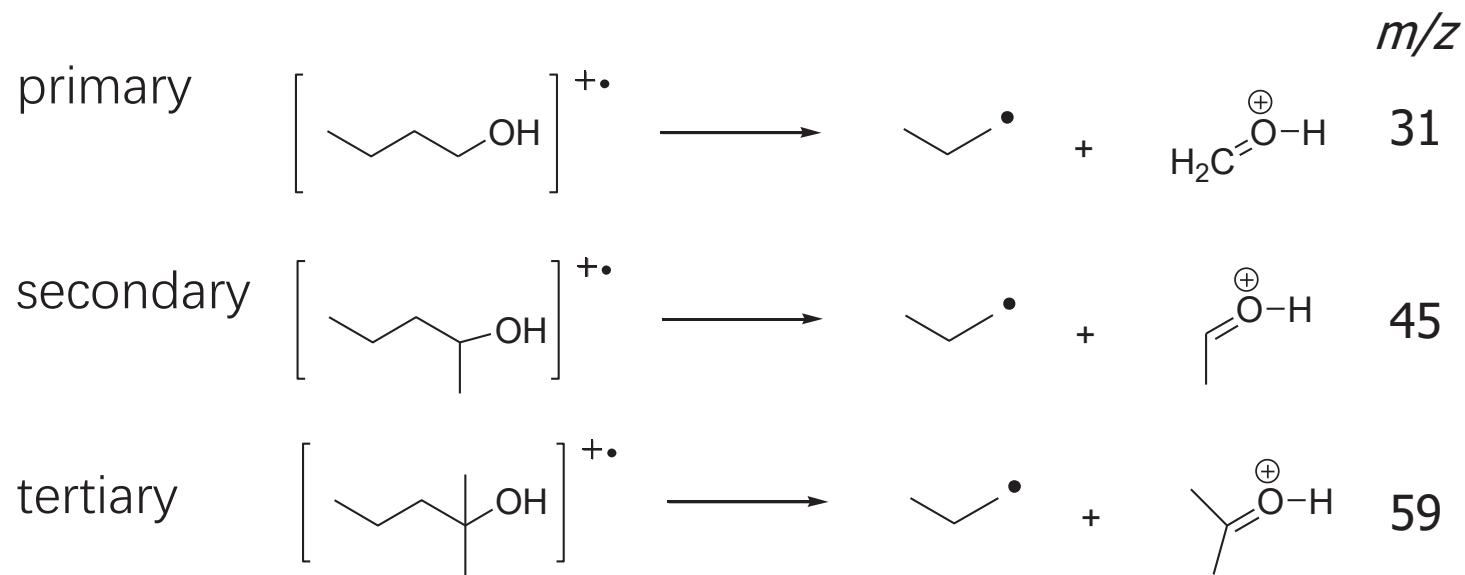


# Fragmentation Patterns of Groups

## 5. Alcohols– Fragment Ions

- Additional modes of fragmentation will cause lower  $M^+$  than for the corresponding alkanes
  - $1^\circ$  and  $2^\circ$  alcohols have a low  $M^+$ ,  $3^\circ$  may be absent

- b) The **largest alkyl group is usually lost**; the mode of cleavage typically is similar for all alcohols:



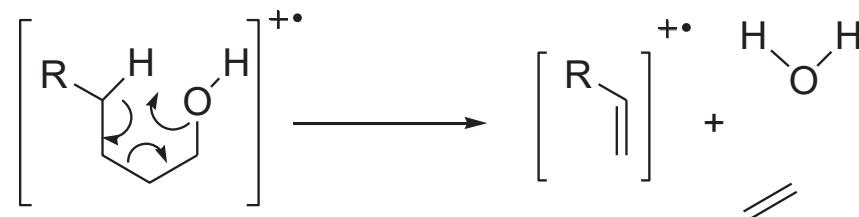
# Fragmentation Patterns of Groups

## 5. Alcohols– Fragment Ions

c) Dehydration (**M - 18**) is a common mode of fragmentation – importance increases with alkyl chain length (>4 carbons)

- 1,2-elimination – occurs from hot surface of ionization chamber
- 1,4-elimination – occurs from ionization
- both modes give M - 18, with the appearance and possible subsequent fragmentation of the remaining alkene

d) For longer chain alcohols, a McLafferty type rearrangement can produce water and ethylene (**M - 18 - 28**)



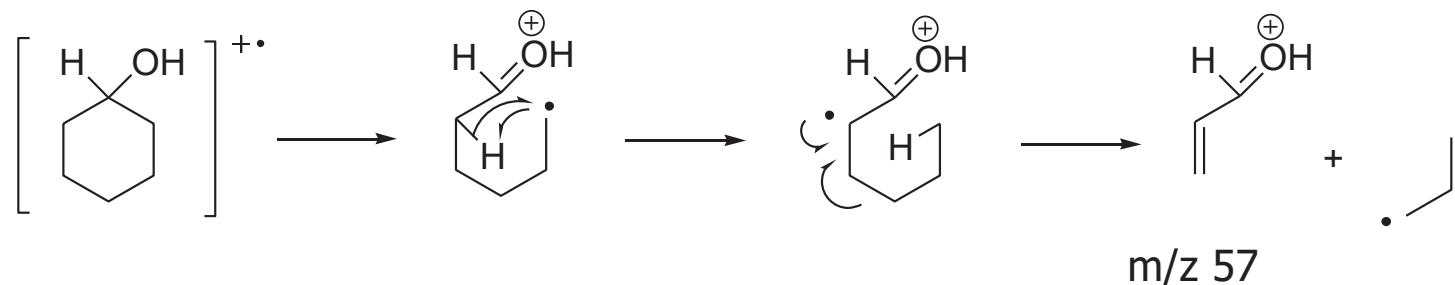
# Fragmentation Patterns of Groups

## 5. Alcohols— Fragment Ions

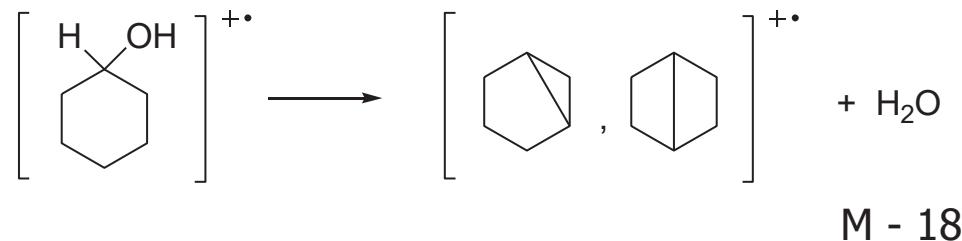
e) Loss of H is *not* favored for alkanols (**M – 1**)

f) Cyclic alcohols fragment by similar pathways

- $\alpha$ -cleavage



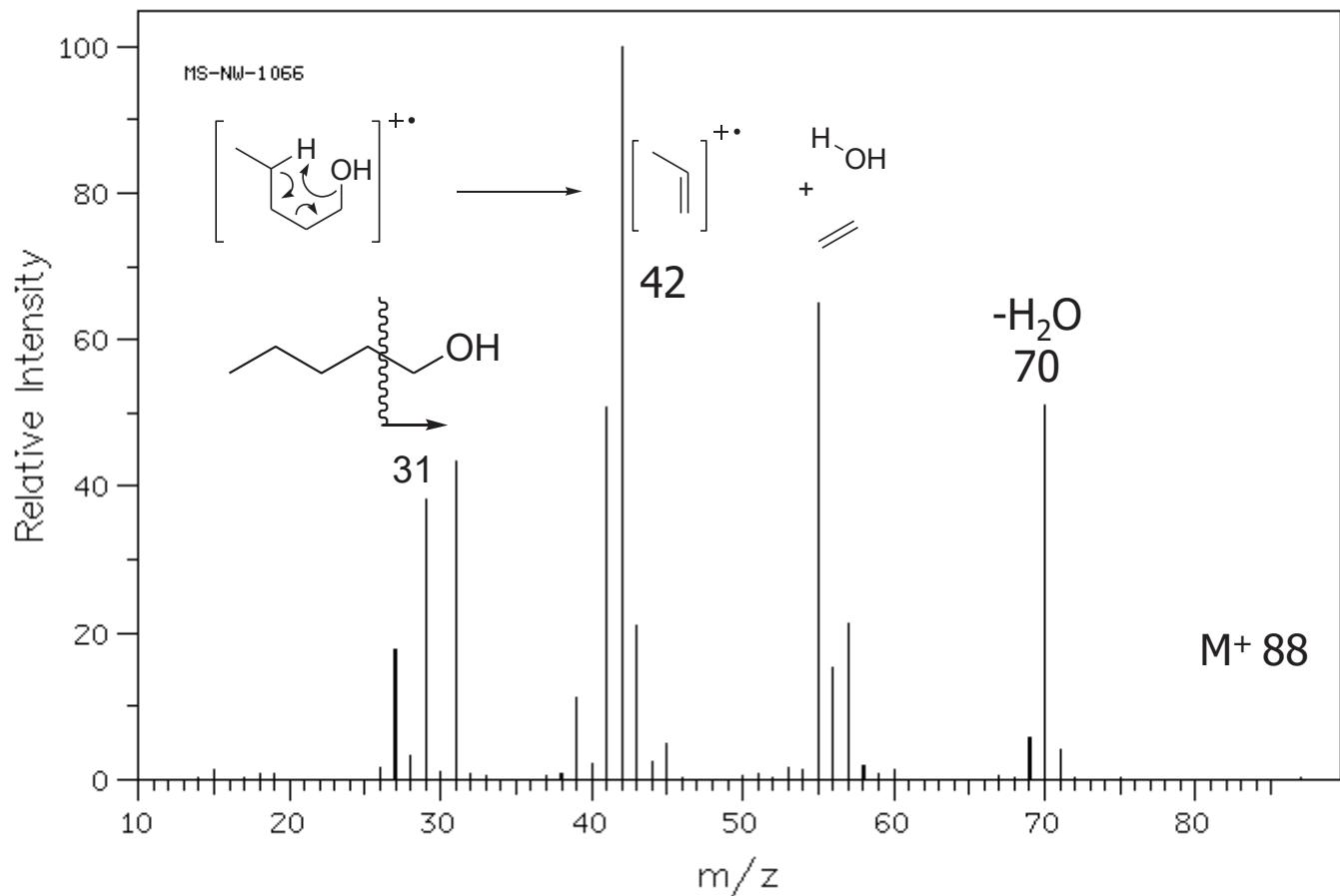
- dehydration



# Fragmentation Patterns of Groups

## 5. Alcohols

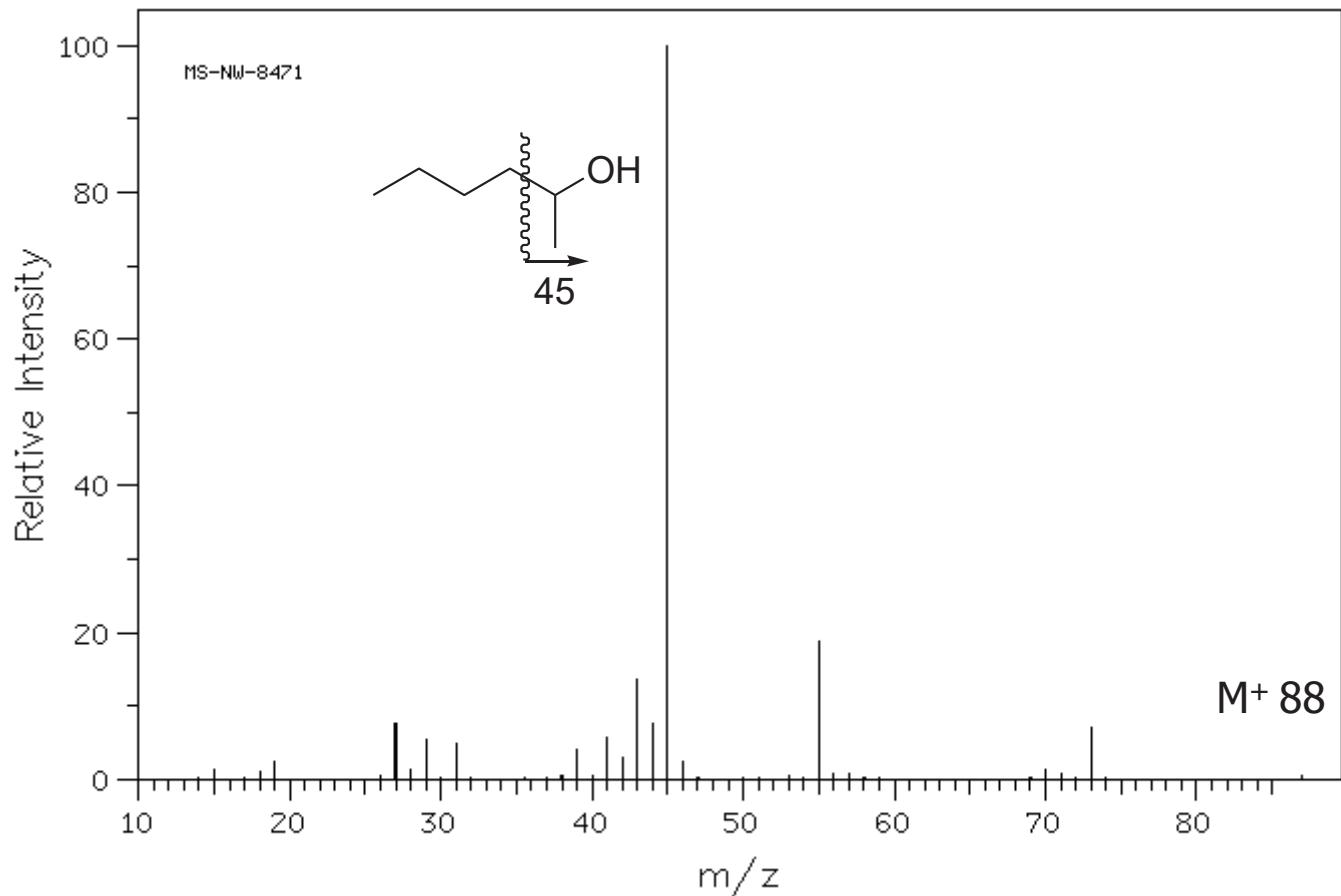
Example MS: alcohols – *n* -pentanol



# Fragmentation Patterns of Groups

## 5. Alcohols

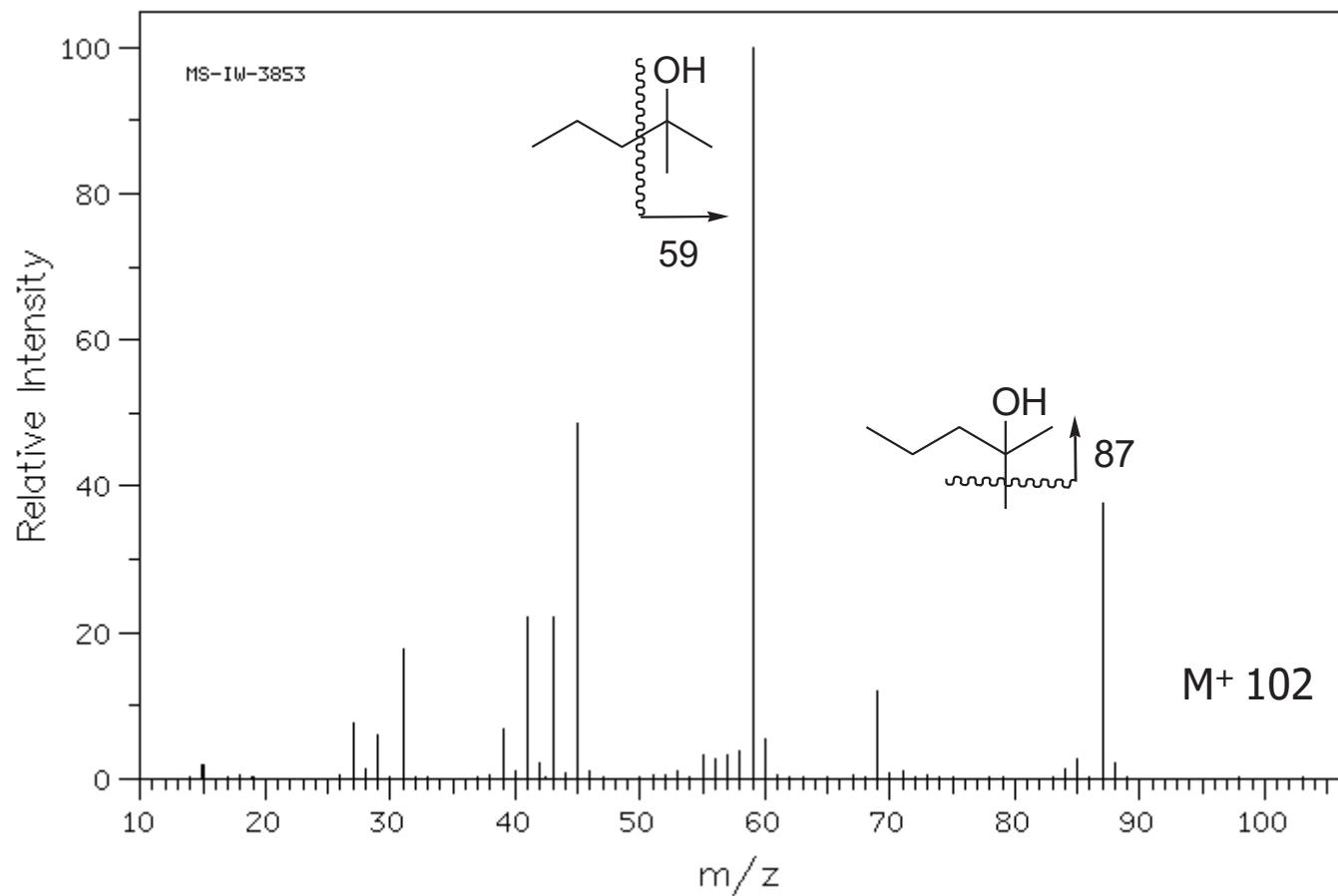
Example MS: alcohols – 2-pentanol



# Fragmentation Patterns of Groups

## 5. Alcohols

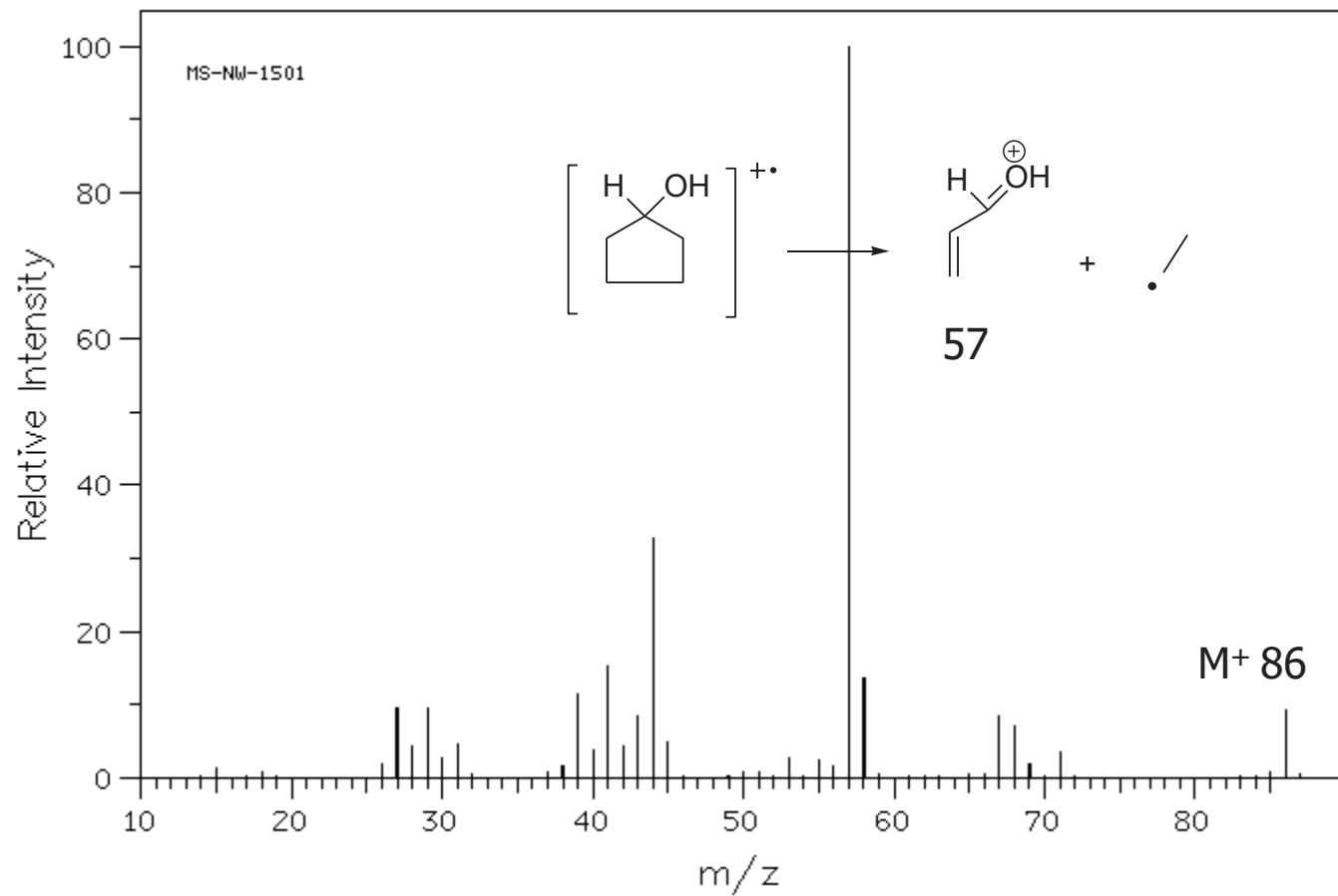
**Example MS:** alcohols – 2-methyl-2-pentanol

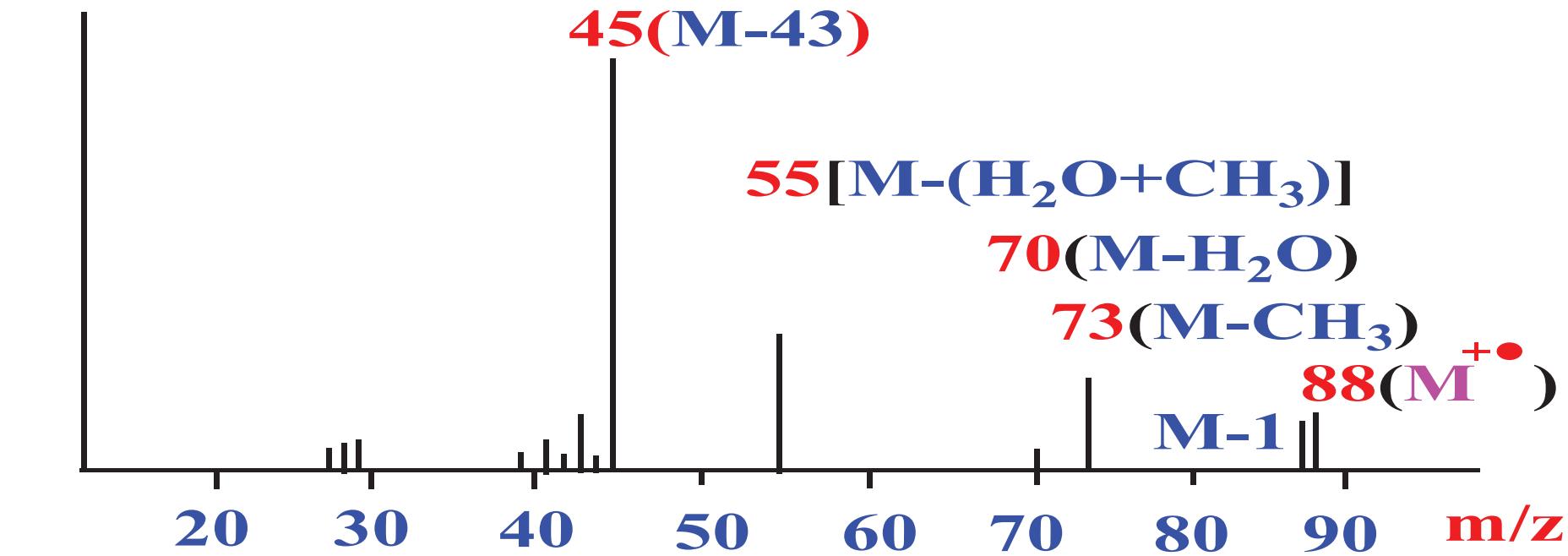
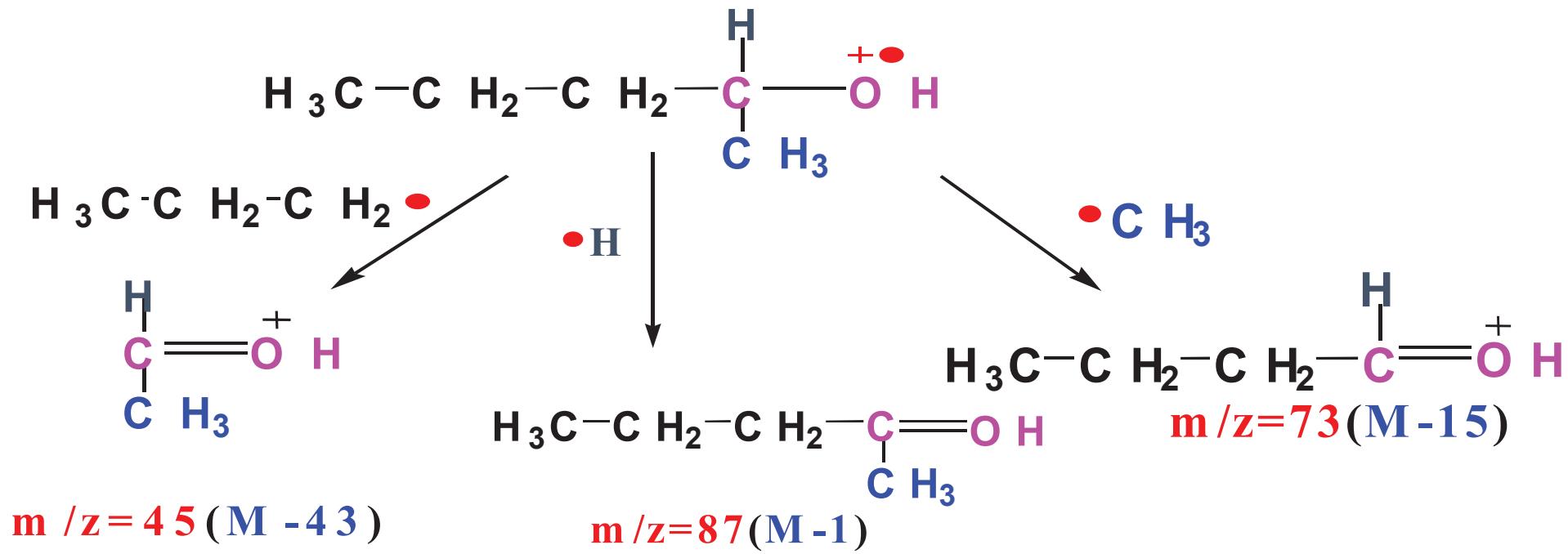


# Fragmentation Patterns of Groups

## 5. Alcohols

Example MS: alcohols – cyclopentanol

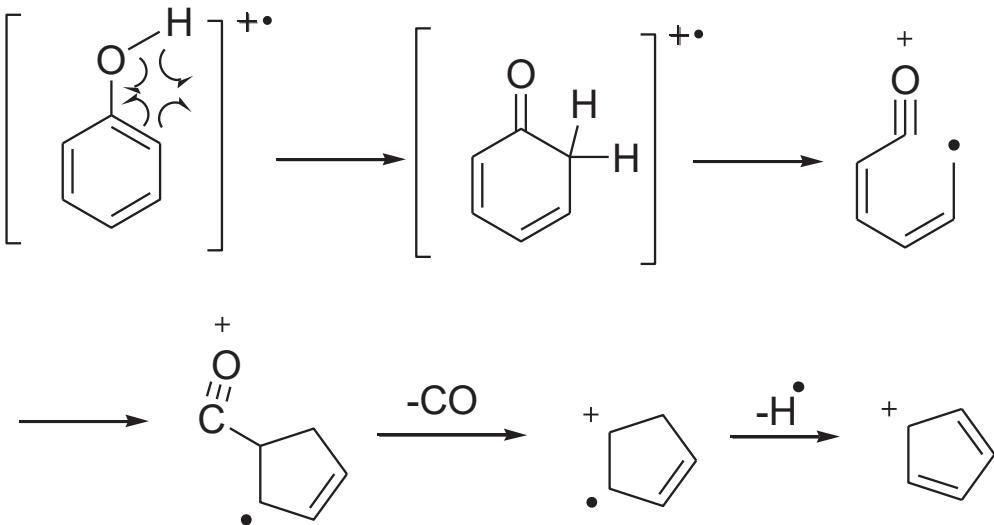




# Fragmentation Patterns of Groups

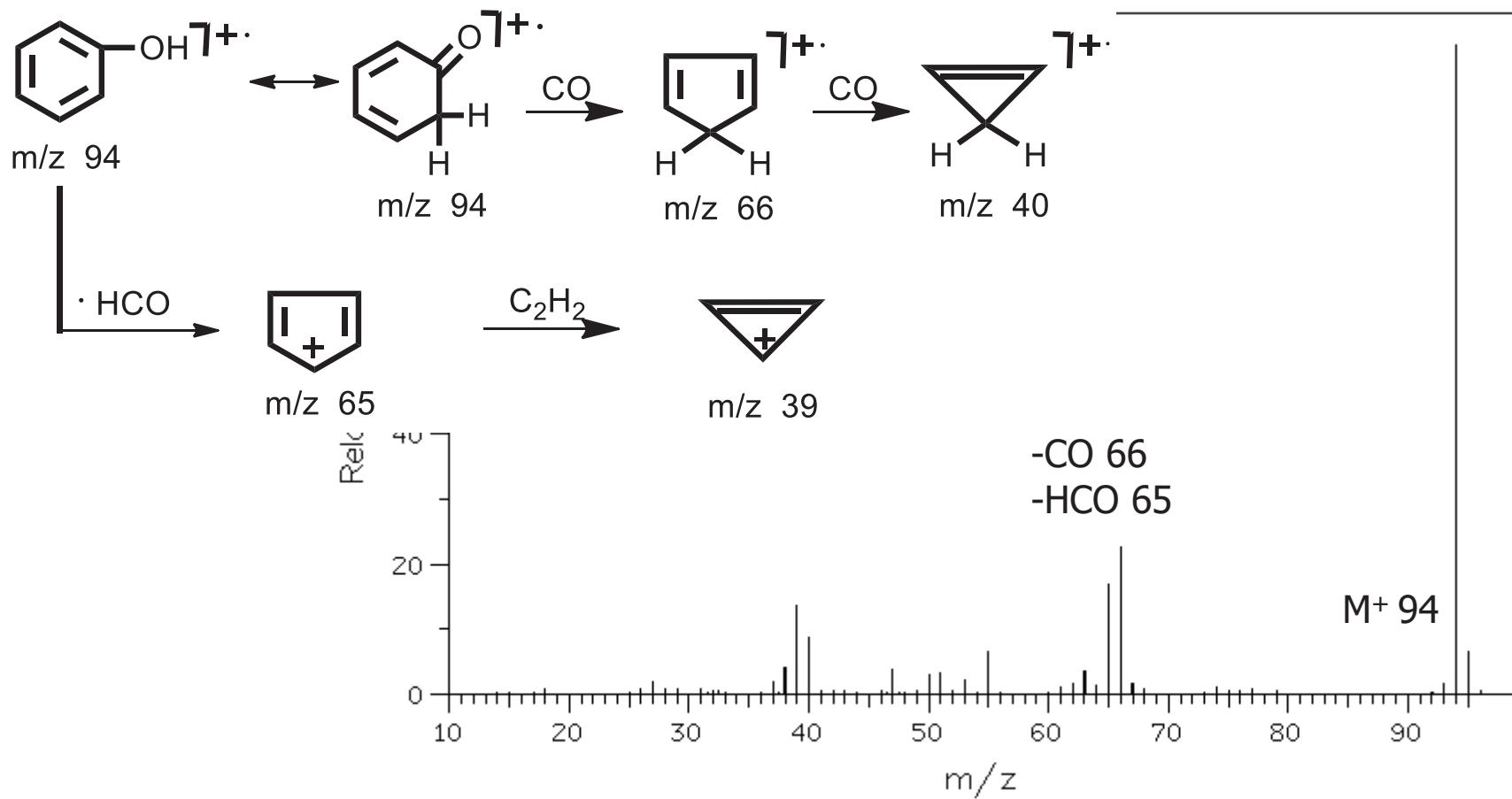
## 6. Phenols– Fragment Ions

- Do not fully combine observations for aromatic + alcohol; treat as a unique group
- b) For example, loss of  $\text{H}\cdot$  is observed ( $\text{M} - 1$ ) – charge can be delocalized by ring – most important for rings with EDGs
- c) Loss of CO (extrusion) is commonly observed ( $\text{M} - 28$ ); Net loss of the formyl radical ( $\text{HCO}\cdot$ ,  $\text{M} - 29$ ) is also observed from this process



# Fragmentation Patterns of Groups

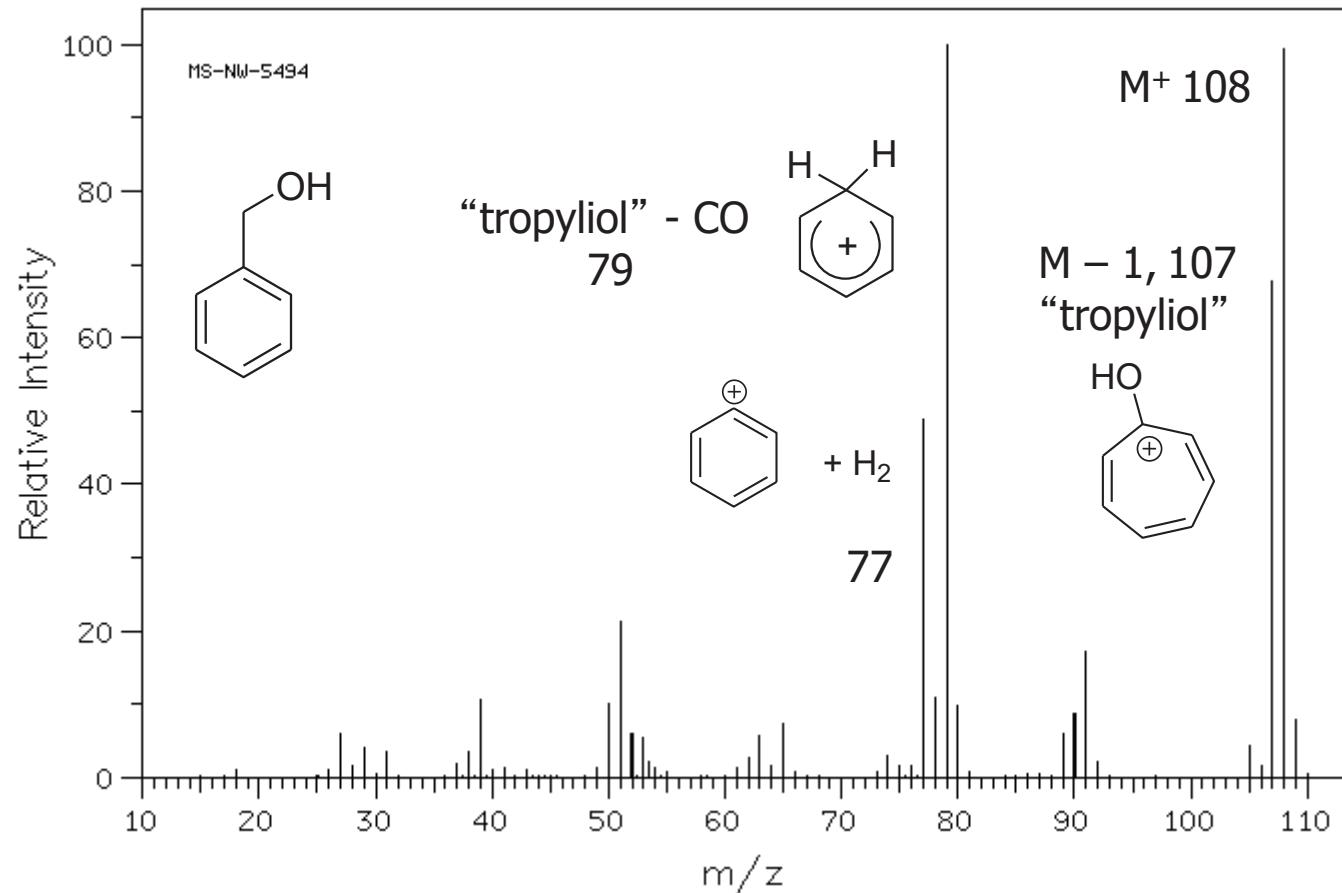
## 5. Example MS: phenols – phenol



# Fragmentation Patterns of Groups

An interesting combination of functionalities: benzyl alcohols

Upon ring expansion to tropylum ions, they become phenols!



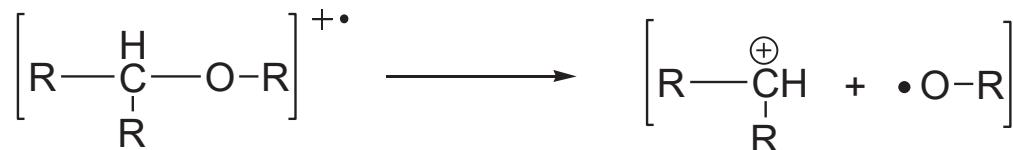
# Fragmentation Patterns of Groups

## 7. Ethers– Fragment Ions

- Slightly more intense  $M^+$  than for the corresponding alcohols or alkanes
- b) The largest alkyl group is usually lost to  $\alpha$ -cleavage; the mode of cleavage typically is similar to alcohols:



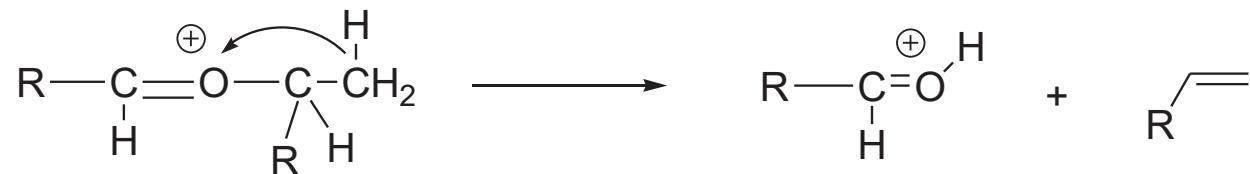
- c) Cleavage of the C-O bond to give carbocations is observed where favorable



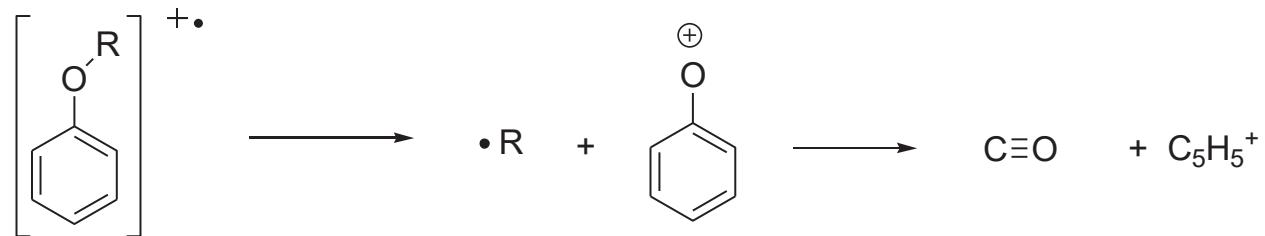
# Fragmentation Patterns of Groups

## 7. Ethers– Fragment Ions

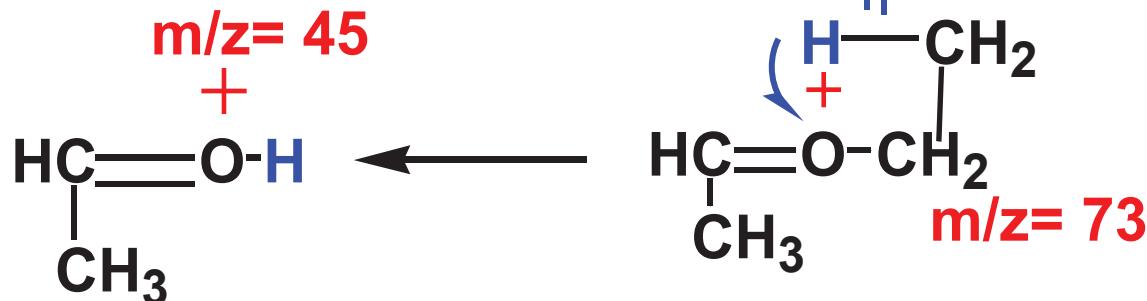
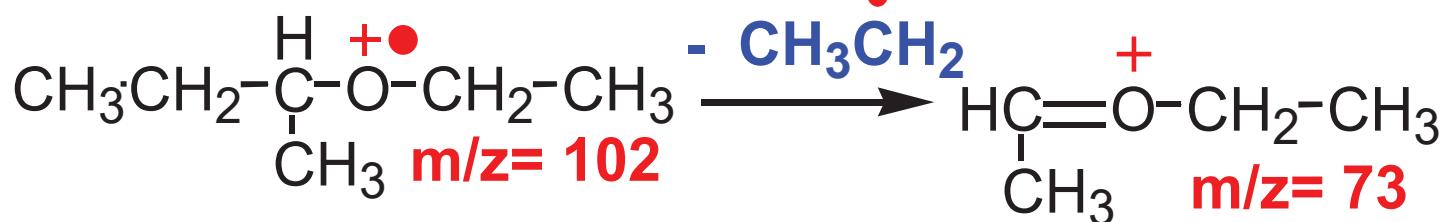
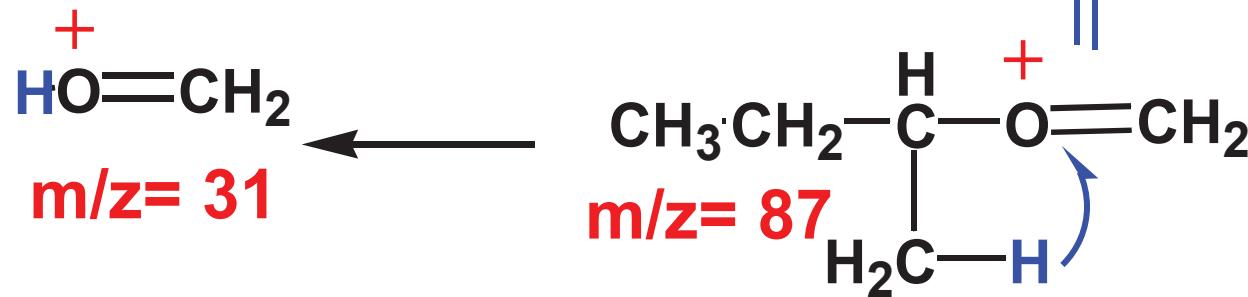
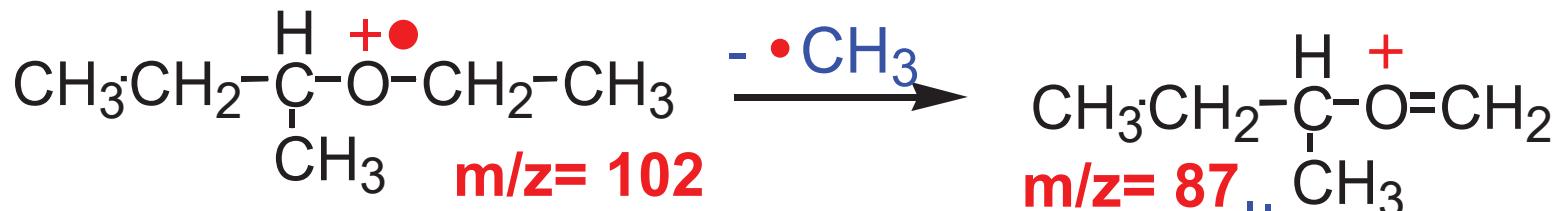
- d) Rearrangement can occur of the following type, if  $\alpha$ -carbon is branched:



- e) Aromatic ethers, similar to phenols can generate the  $\text{C}_6\text{H}_5\text{O}^+$  ion by loss of the alkyl group rather than H; this can expel  $\text{C}\equiv\text{O}$  as in the phenolic degradation

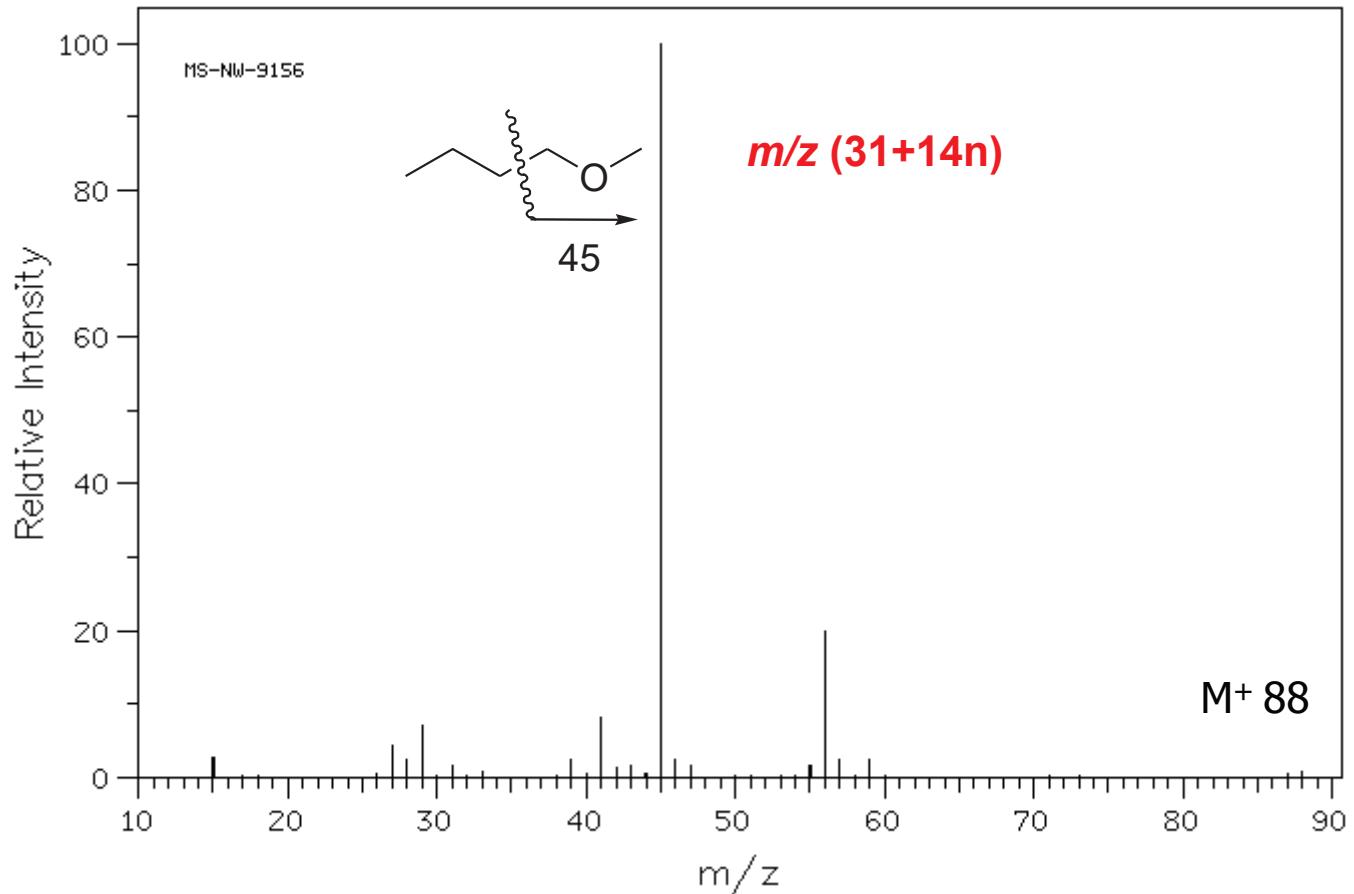


# Fragmentation Patterns of Groups



# Fragmentation Patterns of Groups

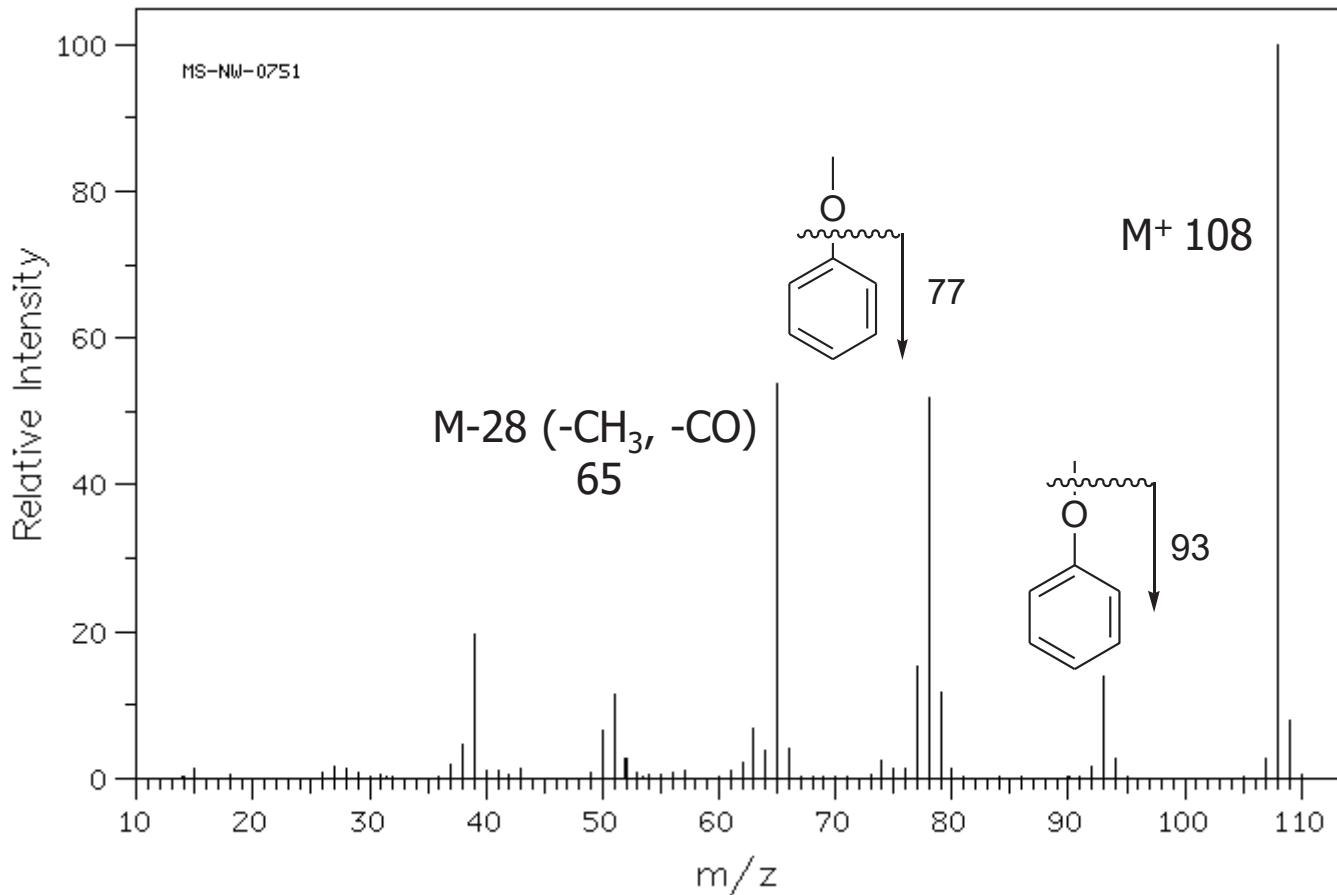
## 7. Example MS: ethers – butyl methyl ether



# Fragmentation Patterns of Groups

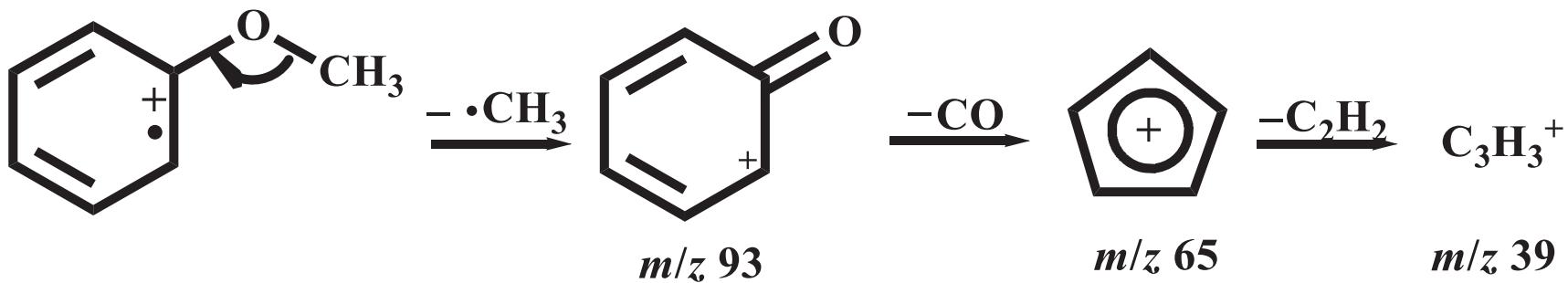
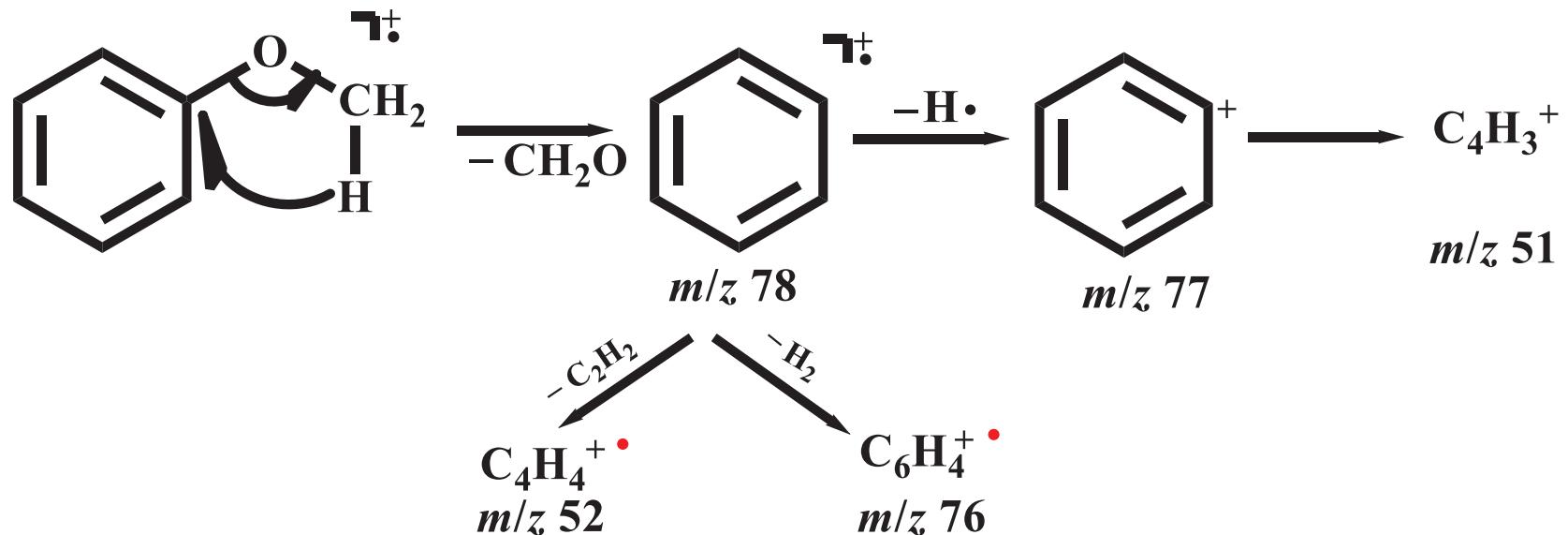
## 7. Example MS: ethers – anisole

Take home – what is m/z 78?



# Fragmentation Patterns of Groups

$\text{C}_7\text{H}_8\text{O}$  ( $M=108$ )

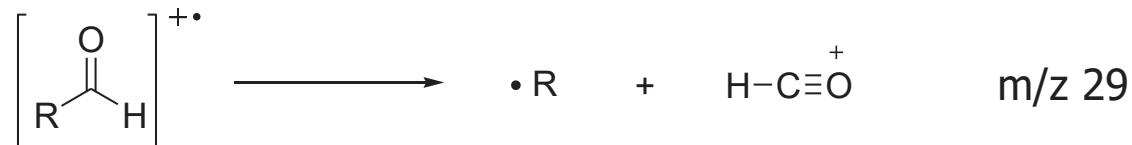
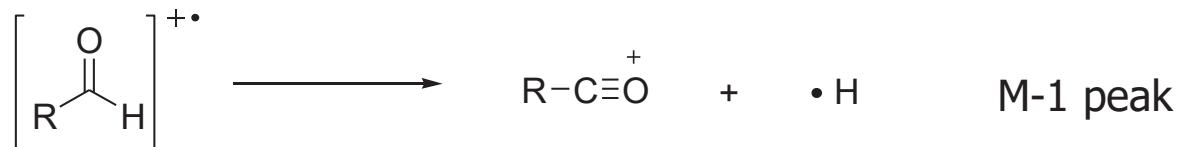


# Fragmentation Patterns of Groups

## 8. Aldehydes - Fragment Ions

- Weak  $M^+$  for aliphatic, strong  $M^+$  for aromatic aldehydes

- b)  $\alpha$ -cleavage is characteristic and often diagnostic for aldehydes – can occur on either side of the carbonyl



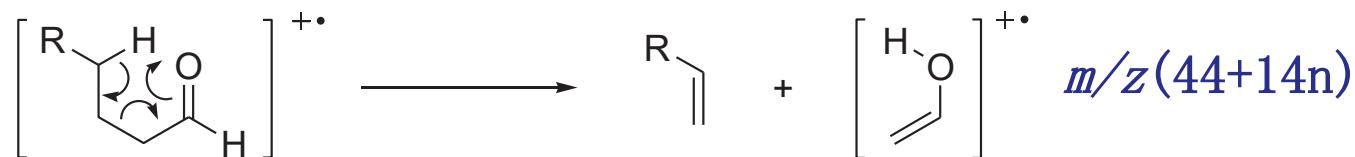
- c)  $\beta$ -cleavage is an additional mode of fragmentation



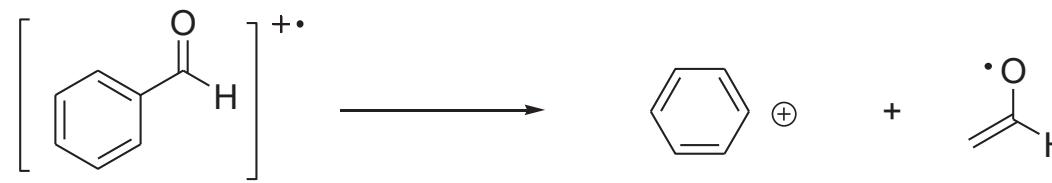
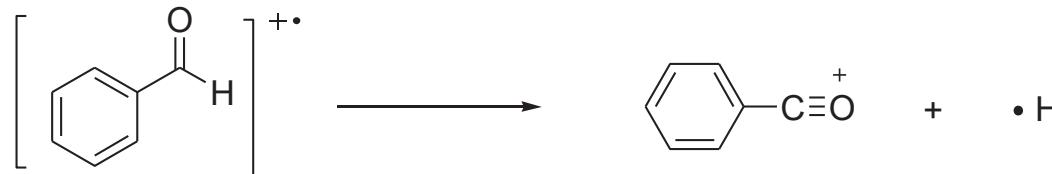
# Fragmentation Patterns of Groups

## 8. Aldehydes - Fragment Ions

- d) McLafferty rearrangement observed if  $\gamma$ -Hs present

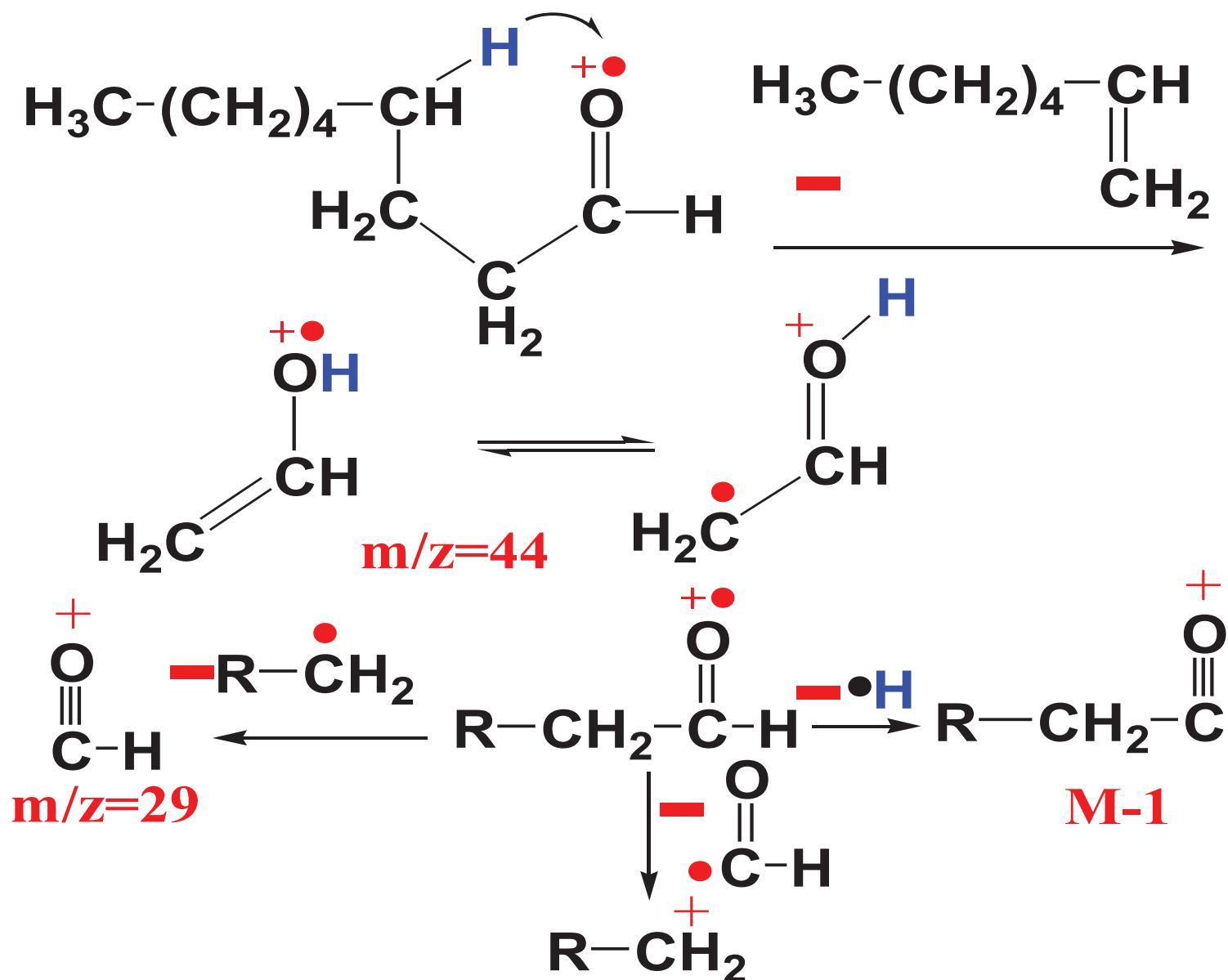


- e) Aromatic aldehydes –  $\alpha$ -cleavages are more favorable, both to lose  $H\cdot$  ( $M - 1$ ) and  $HCO\cdot$  ( $M - 29$ )



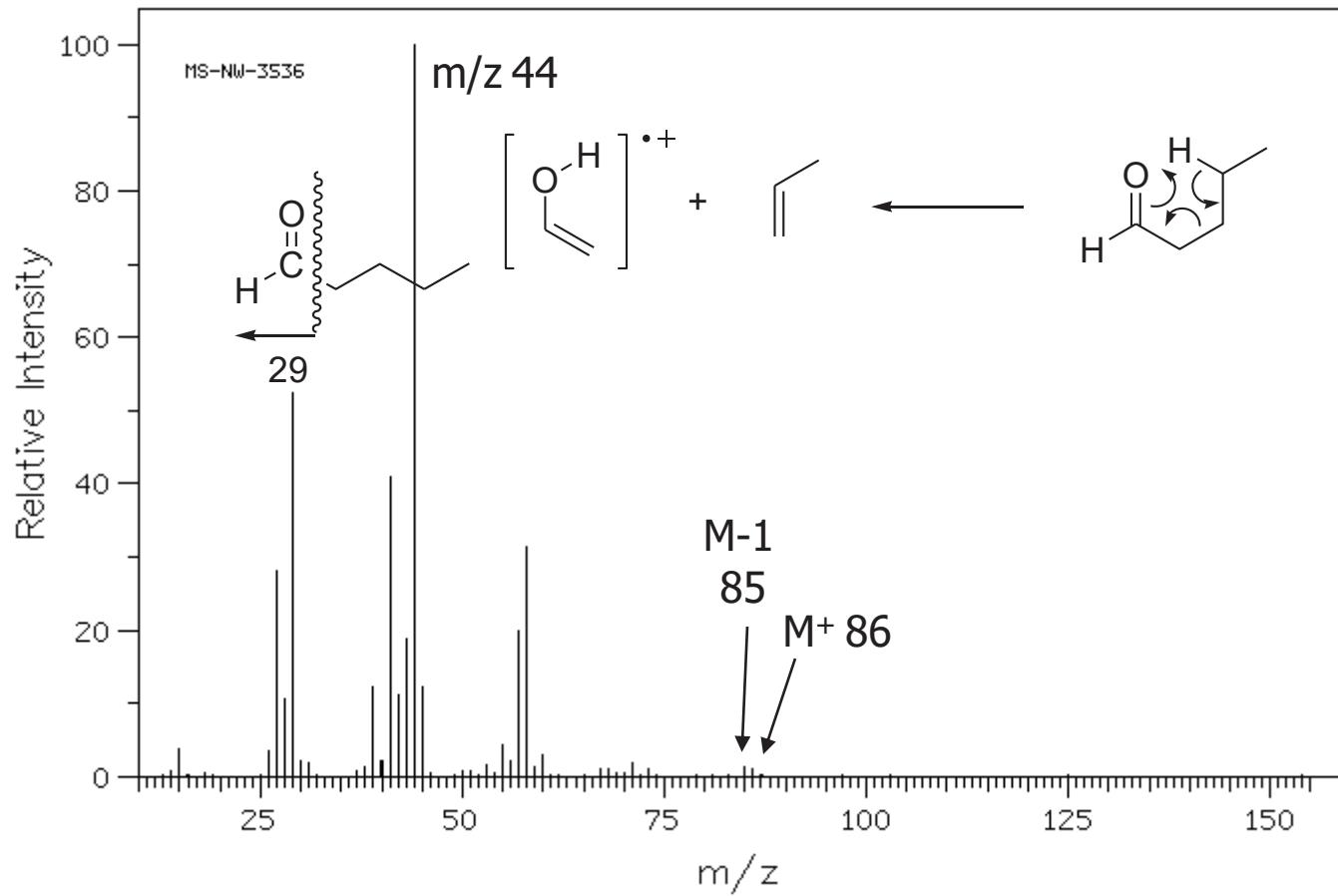
$m/z R^+$   
Remember:  
aromatic ring can  
be subs.

# Fragmentation Patterns of Groups



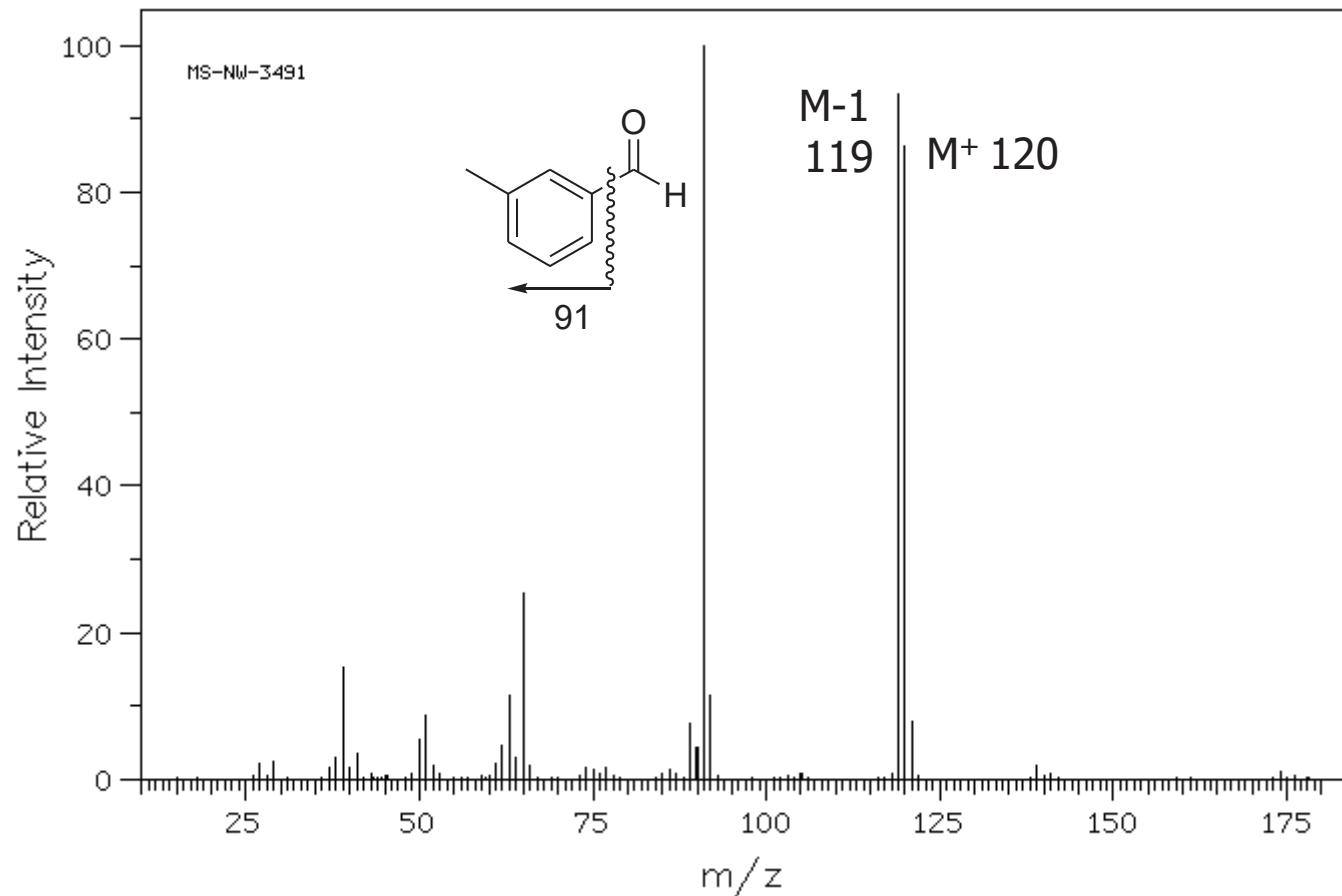
# Fragmentation Patterns of Groups

## 8. Example MS: aldehydes (aliphatic) – pentanal



# Fragmentation Patterns of Groups

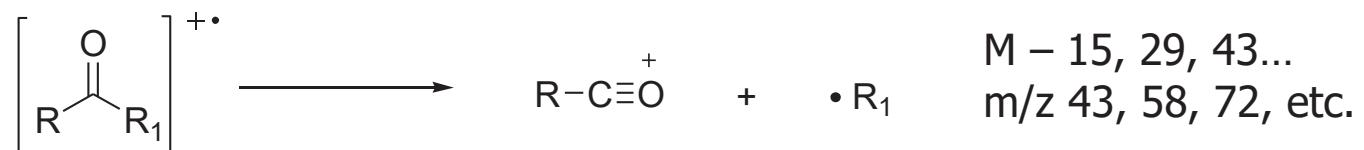
## 8. Example MS: aldehydes (aromatic) – *m*-tolualdehyde



# Fragmentation Patterns of Groups

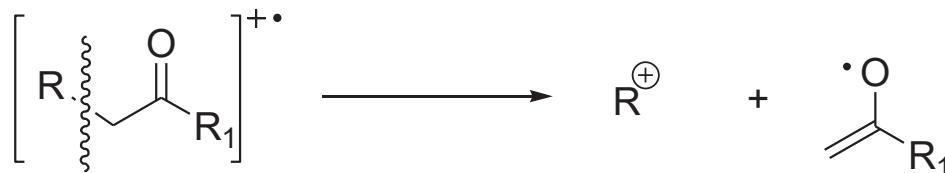
## 9. Ketones - Fragment Ions

- Strong  $M^+$  for aliphatic and aromatic ketones
- b)  $\alpha$ -cleavage can occur on either side of the carbonyl – the larger alkyl group is lost more often



$\text{R}_1$  is larger than  $\text{R}$

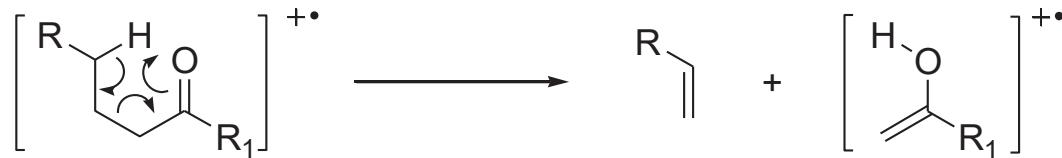
- c)  $\beta$ -cleavage is not as important of a fragmentation mode for ketones compared to aldehydes – but sometimes observed



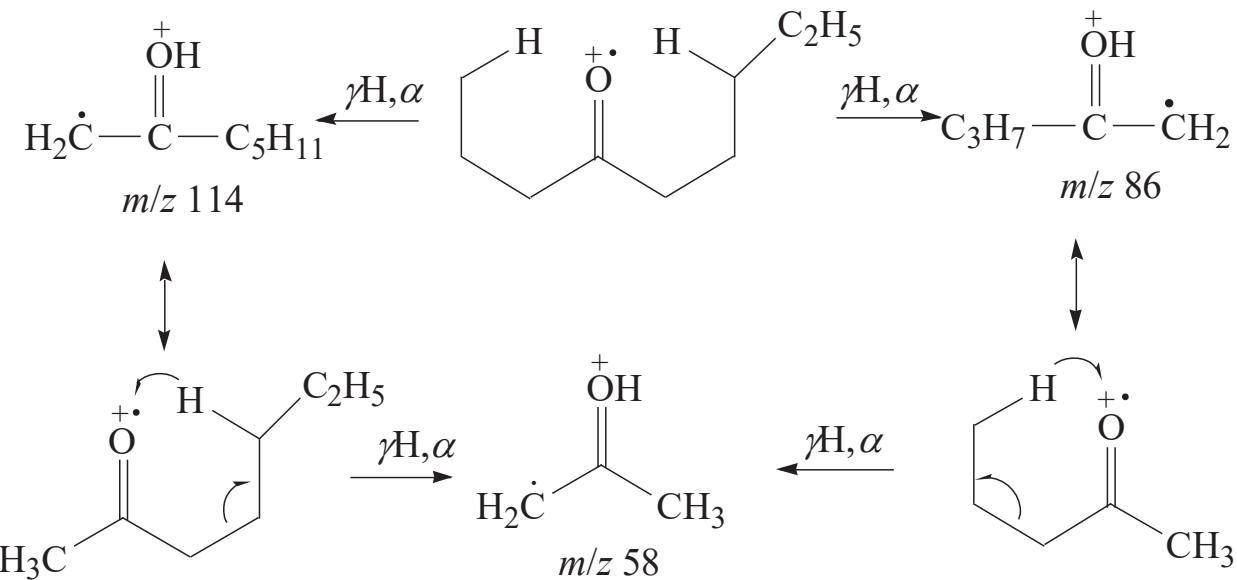
# Fragmentation Patterns of Groups

## 9. Ketones - Fragment Ions

- d) McLafferty rearrangement observed if  $\gamma$ -H's present – if both alkyl chains are sufficiently long – both can be observed



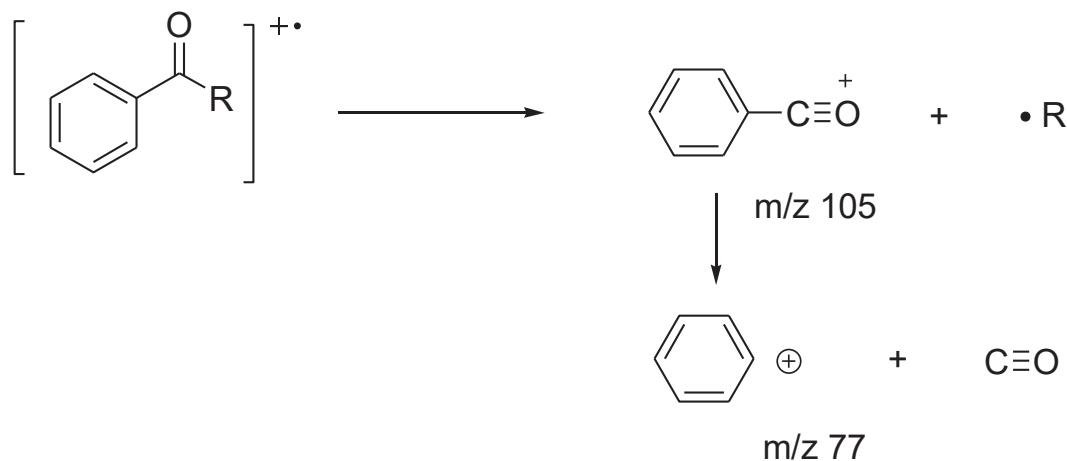
4-壬酮  $\text{C}_9\text{H}_{18}\text{O}$  ( $M=142$ ) 的裂解：



# Fragmentation Patterns of Groups

## 9. Ketones - Fragment Ions

- e) Aromatic ketones –  $\alpha$ -cleavages are favorable primarily to lose  $R\cdot$  ( $M - 15, 29\cdots$ ) to form the  $C_6H_5CO^+$  ion, which can lose  $C\equiv O$

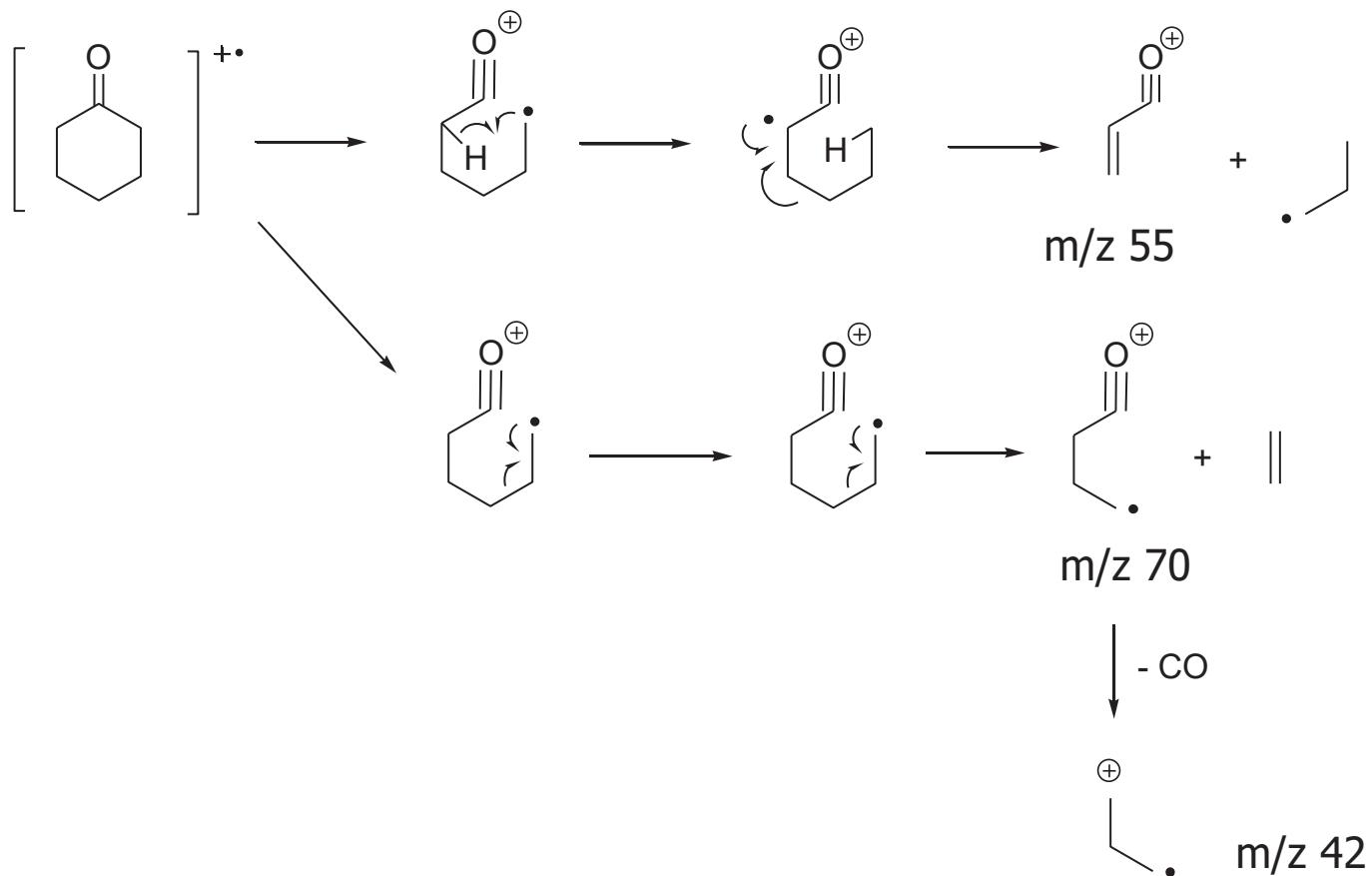


Remember:  
aromatic ring can  
be subs.

# Fragmentation Patterns of Groups

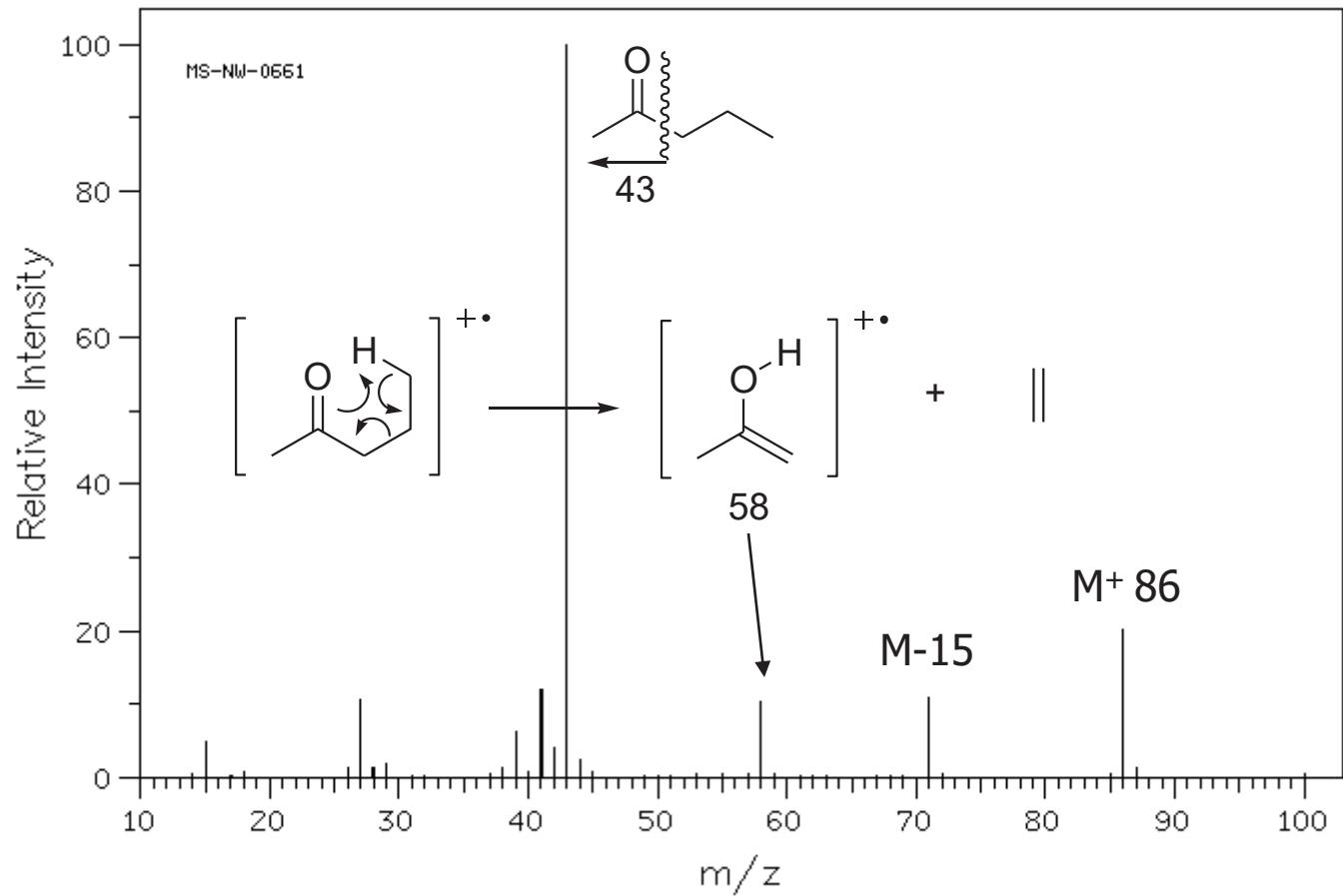
## 9. Ketones - Fragment Ions

f) cyclic ketones degrade in a similar fashion to cycloalkanes and cycloalkanols:



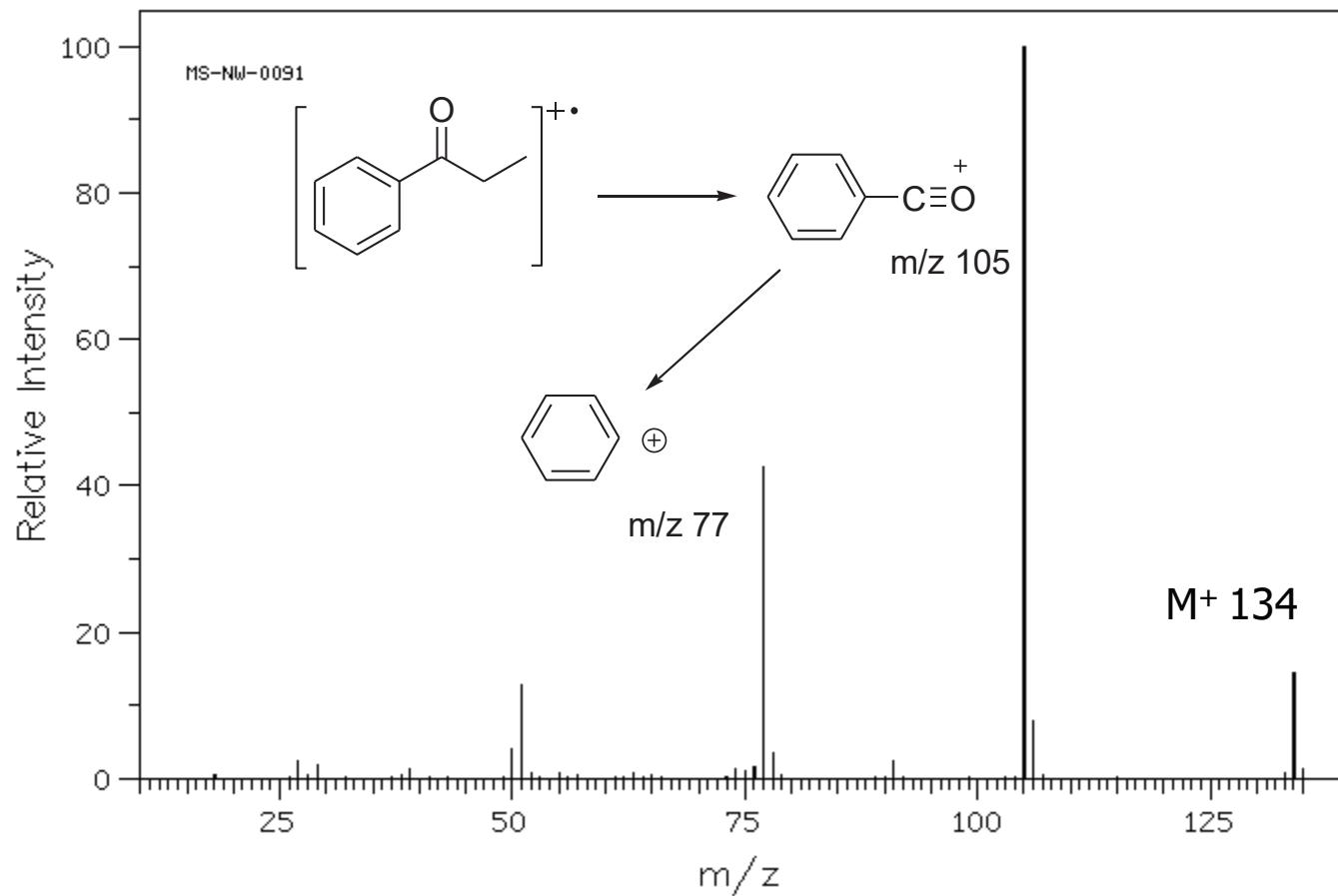
# Fragmentation Patterns of Groups

## 9. Example MS: ketones (aliphatic) – 2-pentanone



# Fragmentation Patterns of Groups

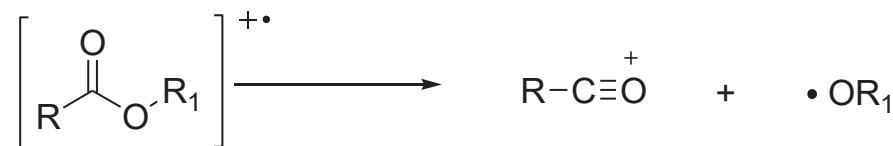
## 9. Example MS: ketones (aromatic) – propiophenone



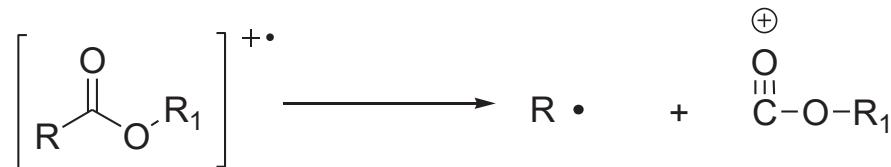
# Fragmentation Patterns of Groups

## 10. Esters - Fragment Ions

- $M^+$  weak in most cases, aromatic esters give a stronger peak
- b) Most important  $\alpha$ -cleavage reactions involve loss of the alkoxy-radical to leave the acylium ion



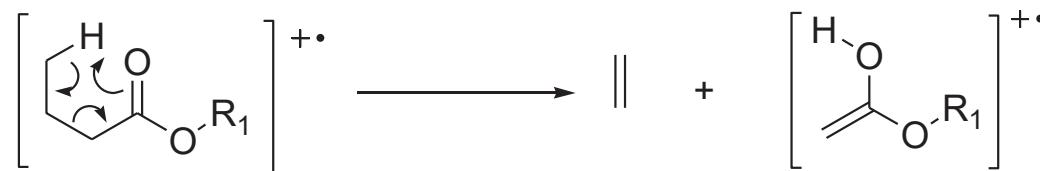
- c) The other  $\alpha$ -cleavage (most common with methyl esters, m/z 59) involves the loss of the alkyl group



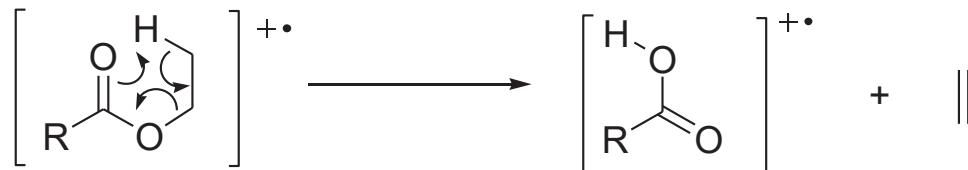
# Fragmentation Patterns of Groups

## 10. Esters - Fragment Ions

- d) McLafferty occurs with sufficiently long esters



- e) Ethyl and longer (alkoxy chain) esters can undergo the McLafferty rearrangement

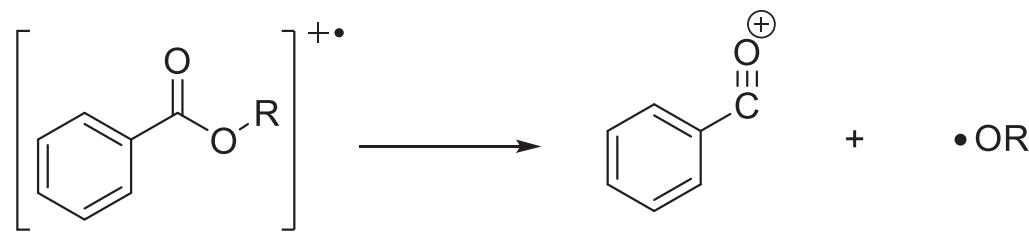


***m/z 60+14n***

# Fragmentation Patterns of Groups

## 10. Esters - Fragment Ions

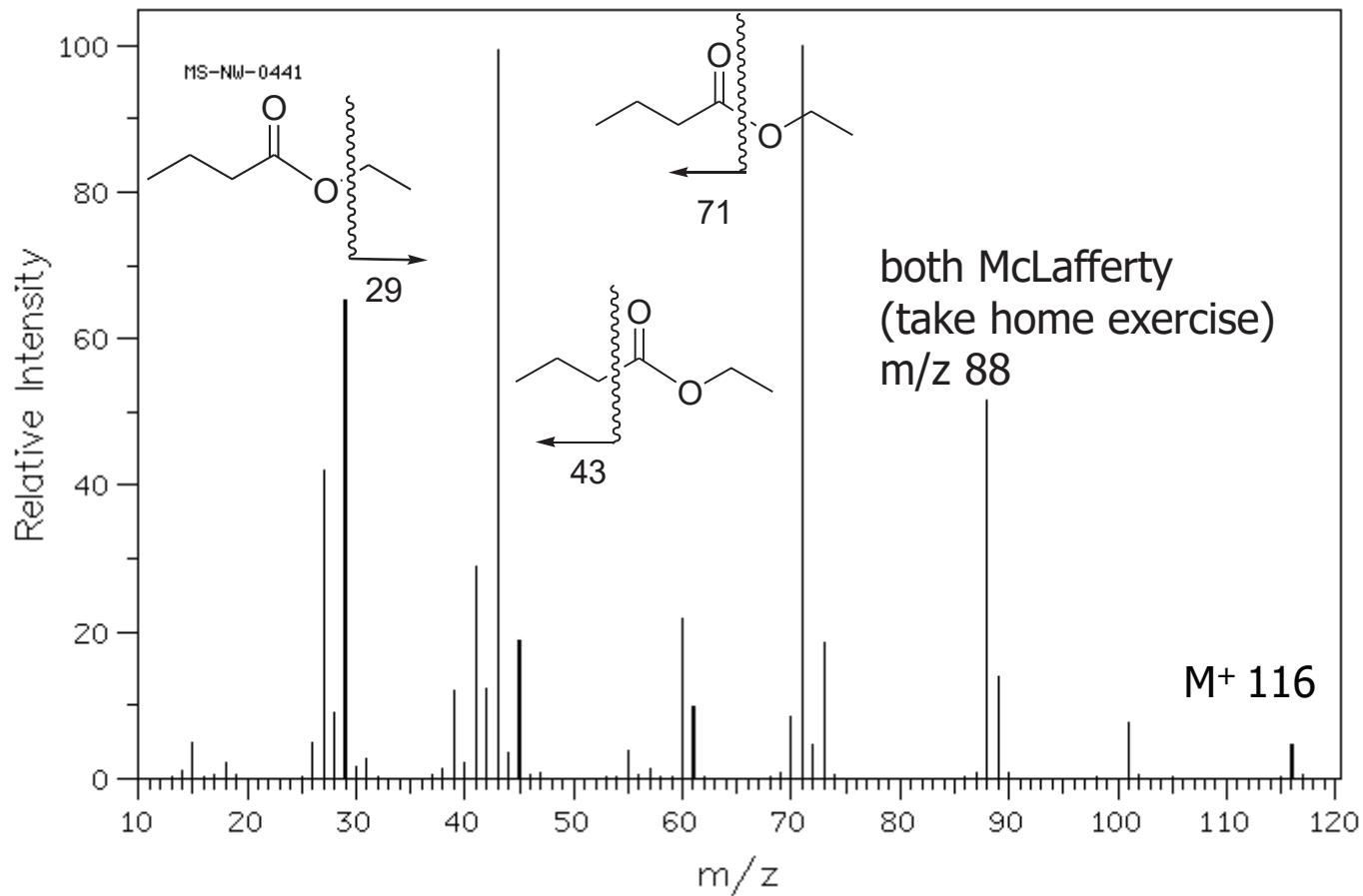
- f) The most common fragmentation route is to lose the alkyl group by  $\alpha$ -cleavage, to form the  $C_6H_5CO^+$  ion ( $m/z$  105)



Can lose CO to  
give  $m/z$  77

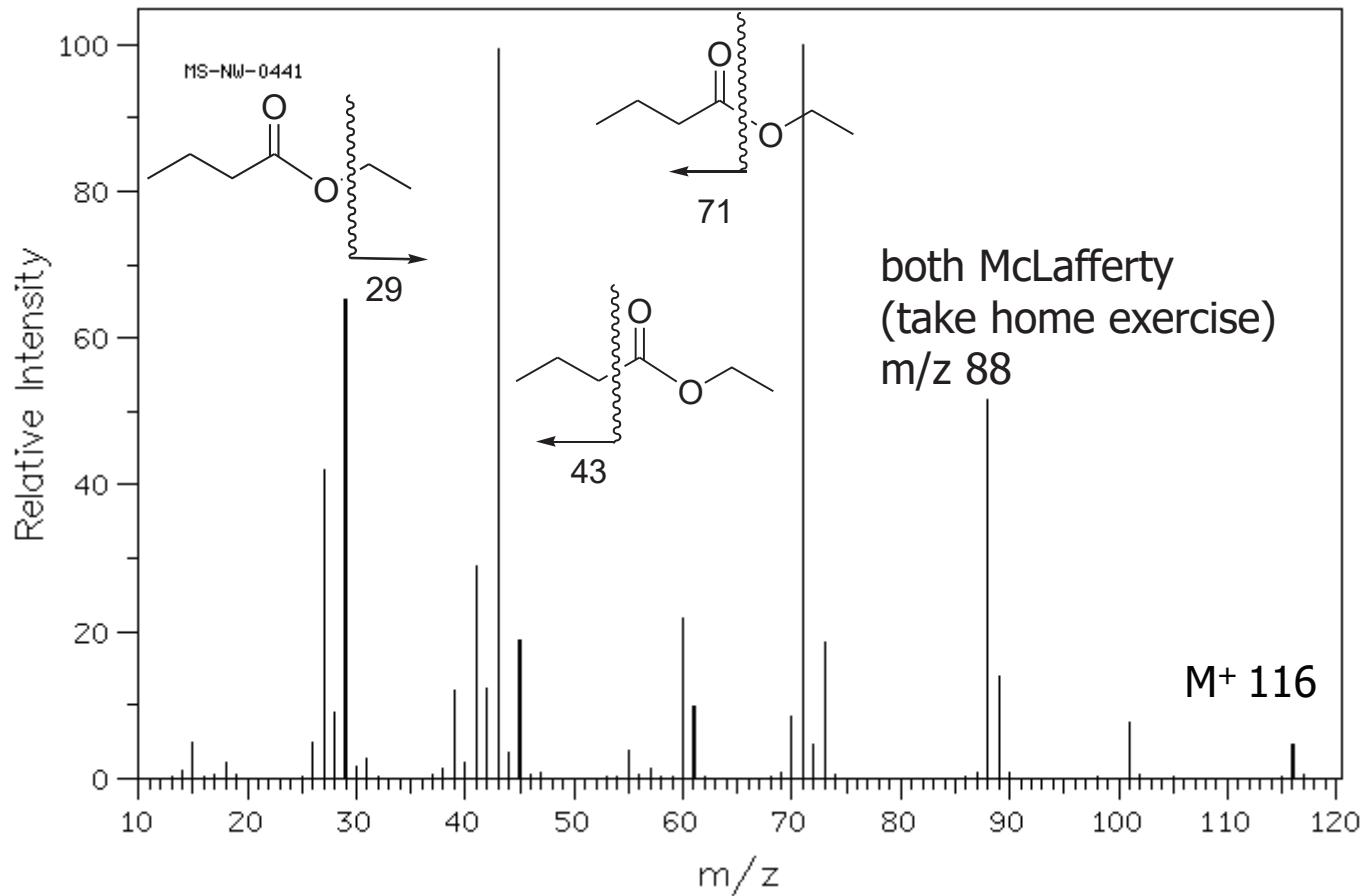
# Fragmentation Patterns of Groups

## 10. Example MS: esters (aliphatic) – ethyl butyrate



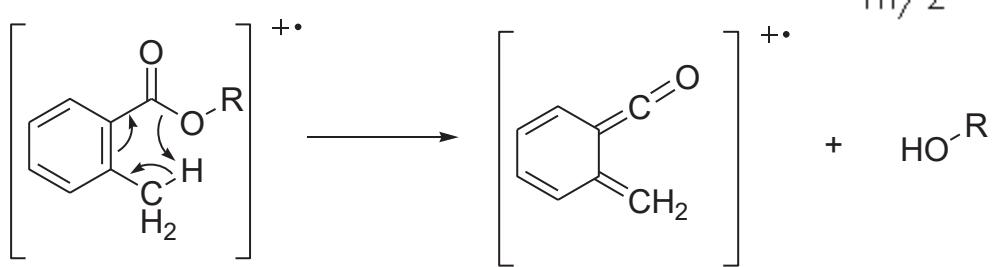
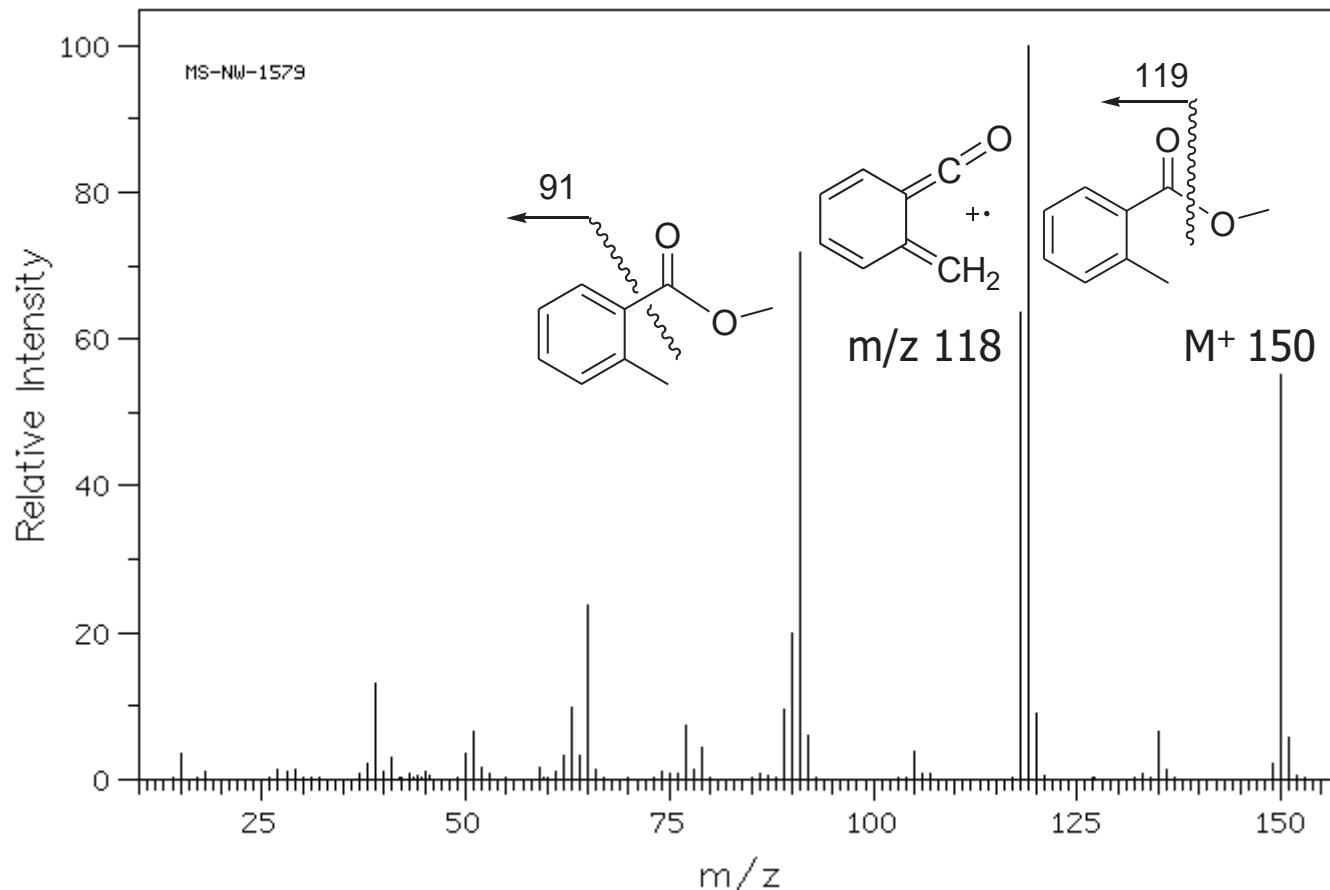
# Fragmentation Patterns of Groups

## 10. Example MS: esters (aliphatic) – ethyl butyrate



# Fragmentation Patterns of Groups

## 10. Example MS: esters (benzoic) – methyl *ortho*-toluate

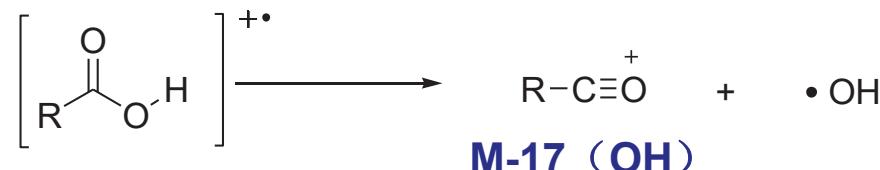


# Fragmentation Patterns of Groups

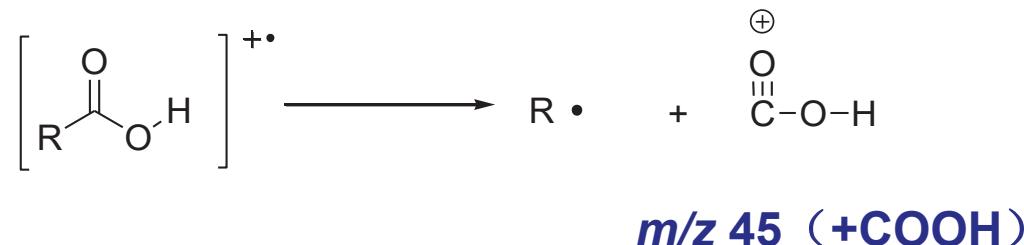
## 11. Carboxylic Acids - Fragment Ions

- As with esters,  $M^+$  weak in most cases, aromatic acids give a stronger peak

- b) Most important  $\alpha$ -cleavage reactions involve loss of the alkoxy-radical to leave the acylium ion



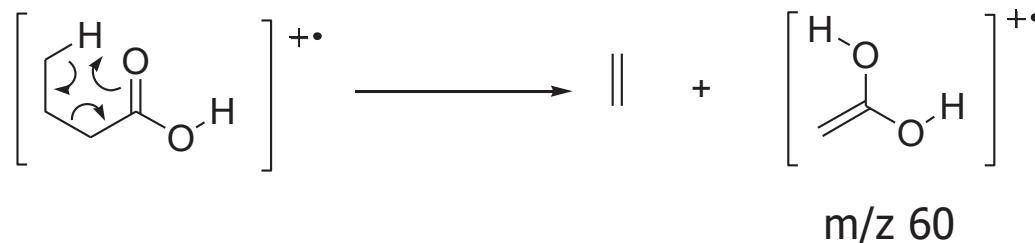
- c) The other  $\alpha$ -cleavage (less common) involves the loss of the alkyl radical. Although less common, the  $m/z$  45 peak is somewhat diagnostic for acids.



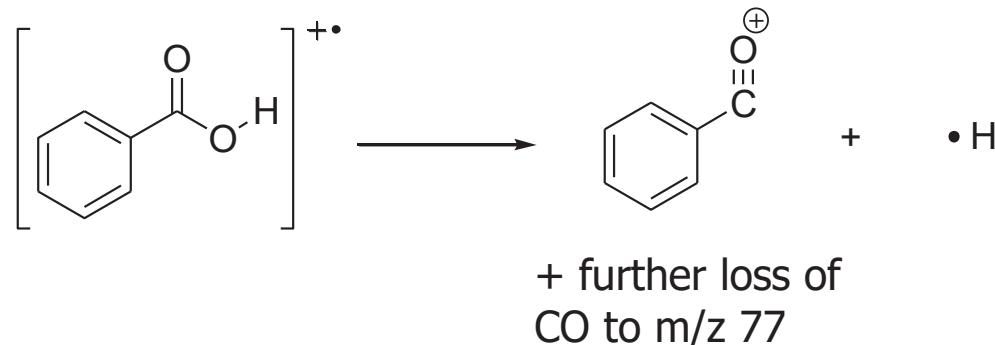
# Fragmentation Patterns of Groups

## 11. Carboxylic Acids - Fragment Ions

- d) McLafferty occurs with sufficiently long acids



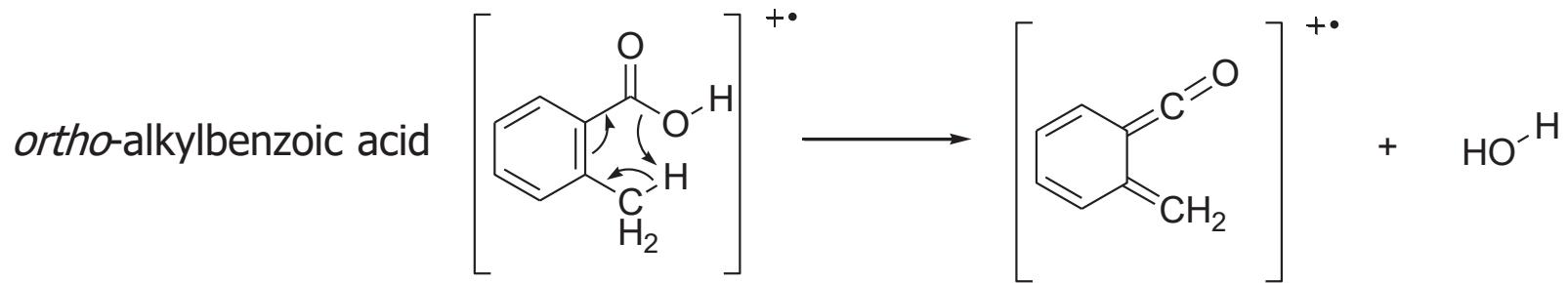
- e) aromatic acids degrade by a process similar to esters, loss of the  $\text{HO}^\bullet$  gives the acylium ion which can lose  $\text{C}\equiv\text{O}$ :



# Fragmentation Patterns of Groups

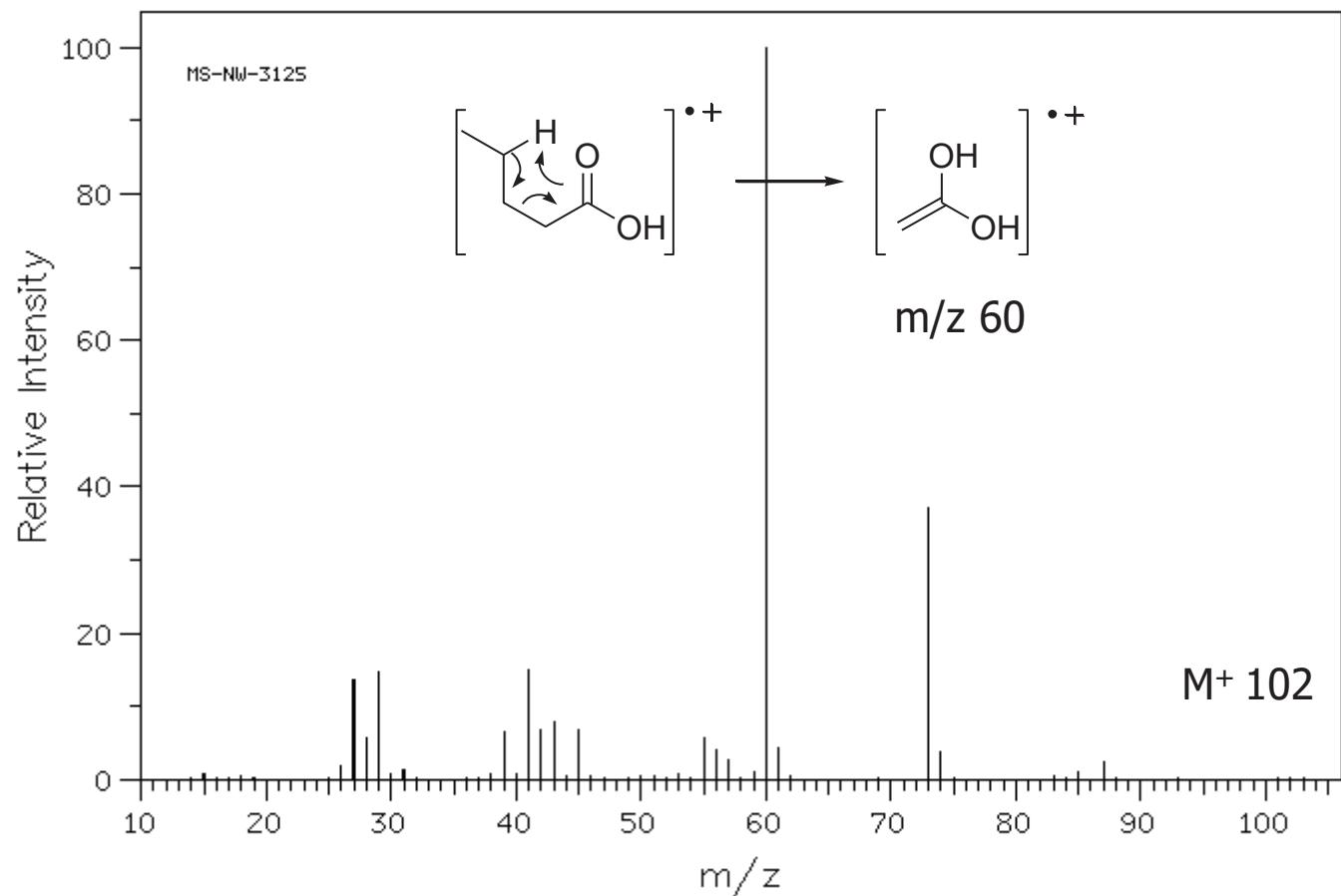
## 11. Carboxylic Acids - Fragment Ions

- f) As with esters, those benzoic acids with an *ortho*-alkyl group will lose water to give a ketene radical cation



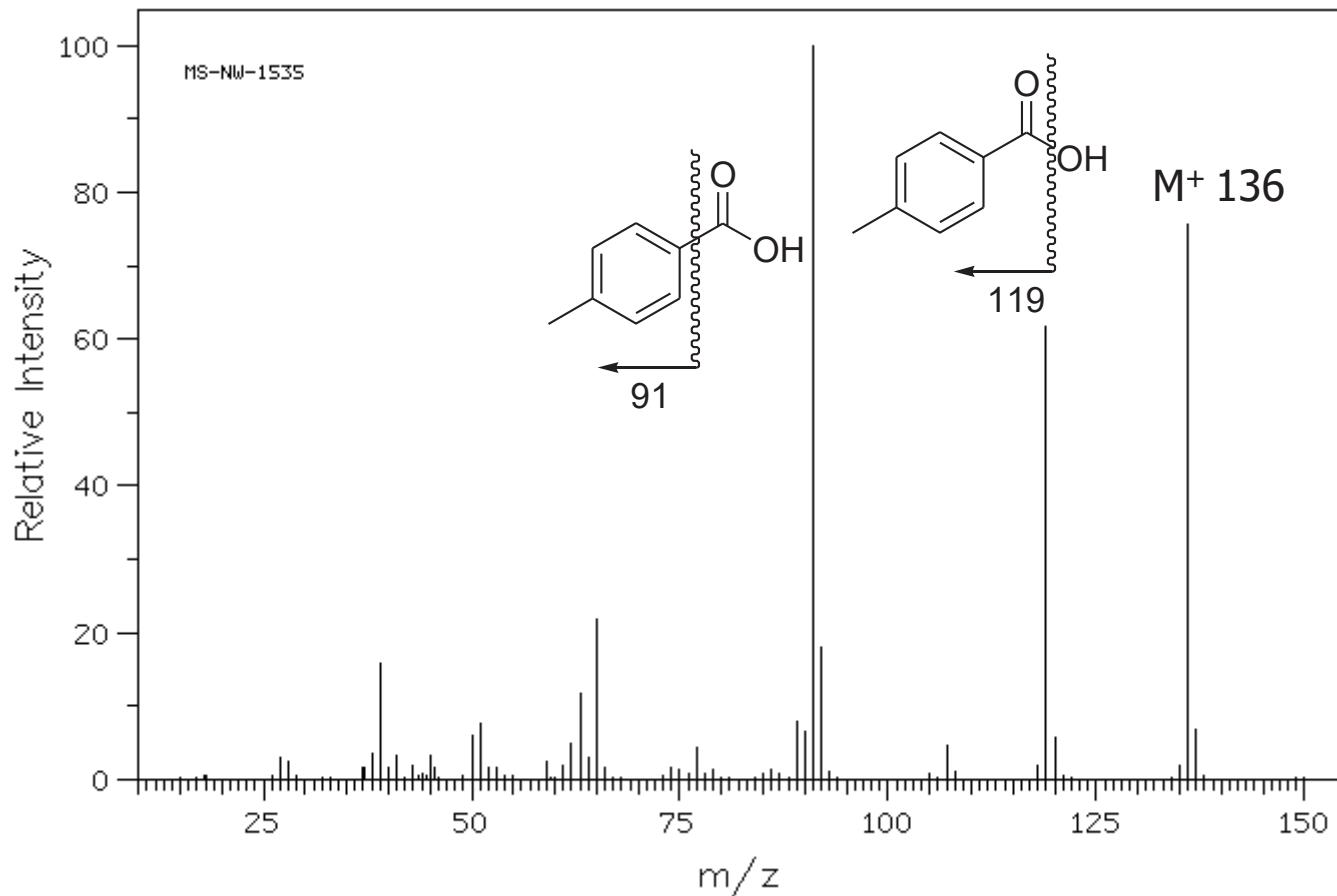
# Fragmentation Patterns of Groups

## 11. Example MS: carboxylic acids (aliphatic) – pentanoic acid



# Fragmentation Patterns of Groups

## 11. Example MS: carboxylic acids (aromatic) – *p*-toluic acid

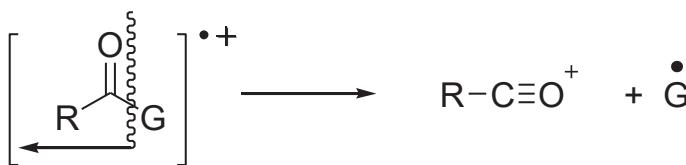
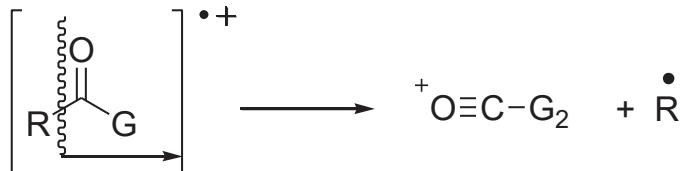


# Fragmentation Patterns of Groups

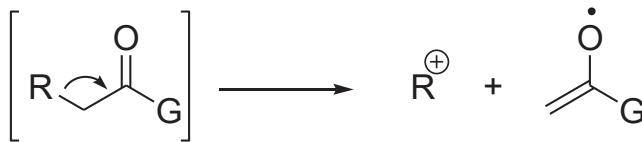
## Summary – Carbonyl Compounds

For carbonyl compounds – there are 4 common modes of fragmentation:

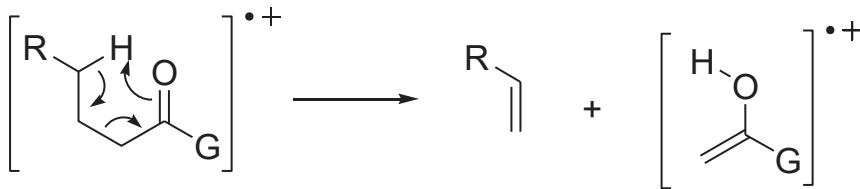
- A<sub>1</sub> & A<sub>2</sub> -- two  $\alpha$ -cleavages



- B --  $\beta$ -cleavage



- C – McLafferty Rearrangement



# Fragmentation Patterns of Groups

## Summary – Carbonyl Compounds

In tabular format:

Fragmentation	Path	m/z of ion observed				
		Aldehydes G = H	Ketones G = R	Esters G = OR'	Acids G = OH	Amides G = NH <sub>2</sub>
<b>A<sub>1</sub></b> $\alpha$ -cleavage	- R	<b>29</b>	43 <sup>b</sup>	59 <sup>b</sup>	<b>45</b>	44 <sup>d</sup>
<b>A<sub>2</sub></b> $\alpha$ -cleavage	- G	43 <sup>b</sup>	43 <sup>b</sup>	43 <sup>b</sup>	43 <sup>b</sup>	43 <sup>b</sup>
<b>B</b> $\beta$ -cleavage	- G	43 <sup>a</sup>	57 <sup>b</sup>	73 <sup>b</sup>	59 <sup>a</sup>	58 <sup>a</sup>
<b>C</b> McLafferty		44 <sup>a</sup>	58 <sup>b,c</sup>	74 <sup>b,c</sup>	60 <sup>a</sup>	59 <sup>a</sup>

<sup>b</sup> = *base*, add other mass attached to this chain

<sup>a</sup> = *base*, if  $\alpha$ -carbon branched, add appropriate mass

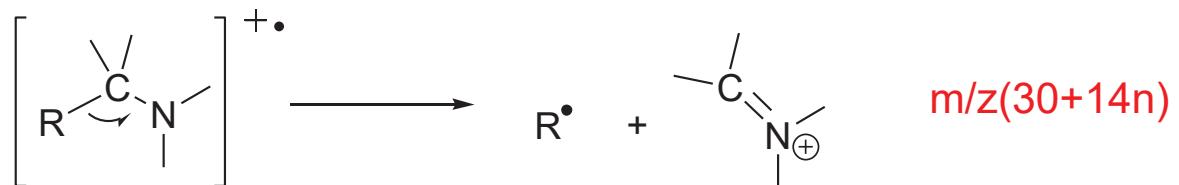
<sup>c</sup> = sufficiently long structures can undergo on either side of C=O

<sup>d</sup> = if *N*-substituted, add appropriate mass

# Fragmentation Patterns of Groups

## 12. Amines - Fragment Ions

- Follow nitrogen rule – odd  $M^+$ , odd # of nitrogens; nonetheless,  $M^+$  weak in aliphatic amines
- b)  $\alpha$ -cleavage reactions are the most important fragmentations for amines; **for 1° *n*-aliphatic amines  $m/z$  30 is diagnostic**

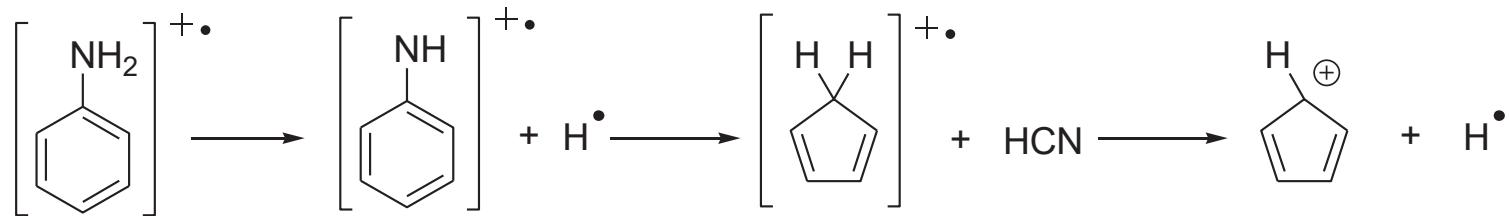


- c) **McLafferty not often observed with amines**, even with sufficiently long alkyl chains
- d) Loss of ammonia ( $M - 17$ ) is not typically observed

# Fragmentation Patterns of Groups

## 12. Amines - Fragment Ions

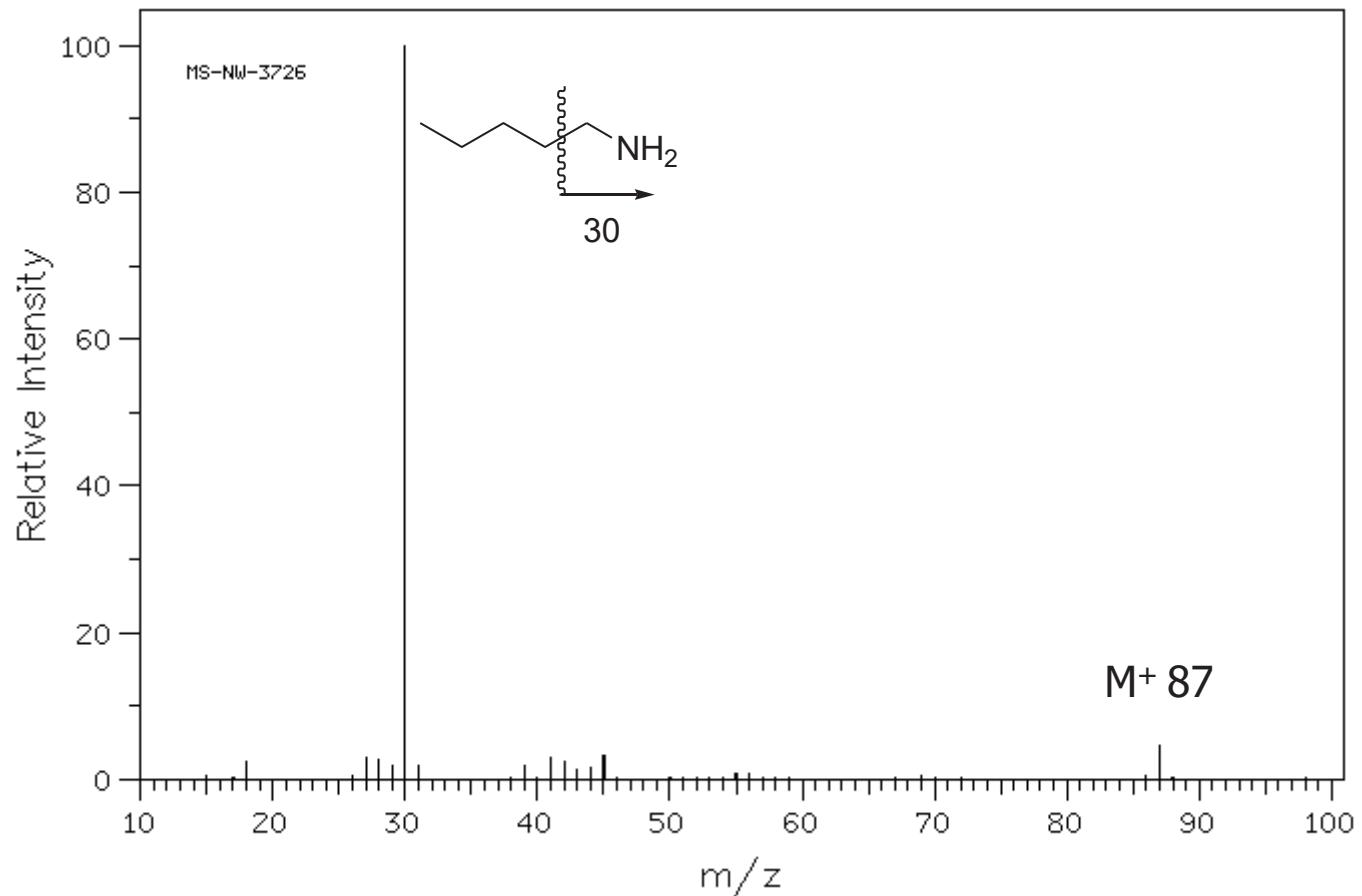
- e) Mass spectra of cyclic amines is complex and varies with ring size
- f) Aromatic amines have intense  $M^+$
- g) Loss of a hydrogen atom, followed by the expulsion of HCN is typical for anilines (苯胺类)



- h) Pyridines (吡啶类) have similar stability (strong  $M^+$ , simple MS) to aromatics, expulsion of HCN is similar to anilines

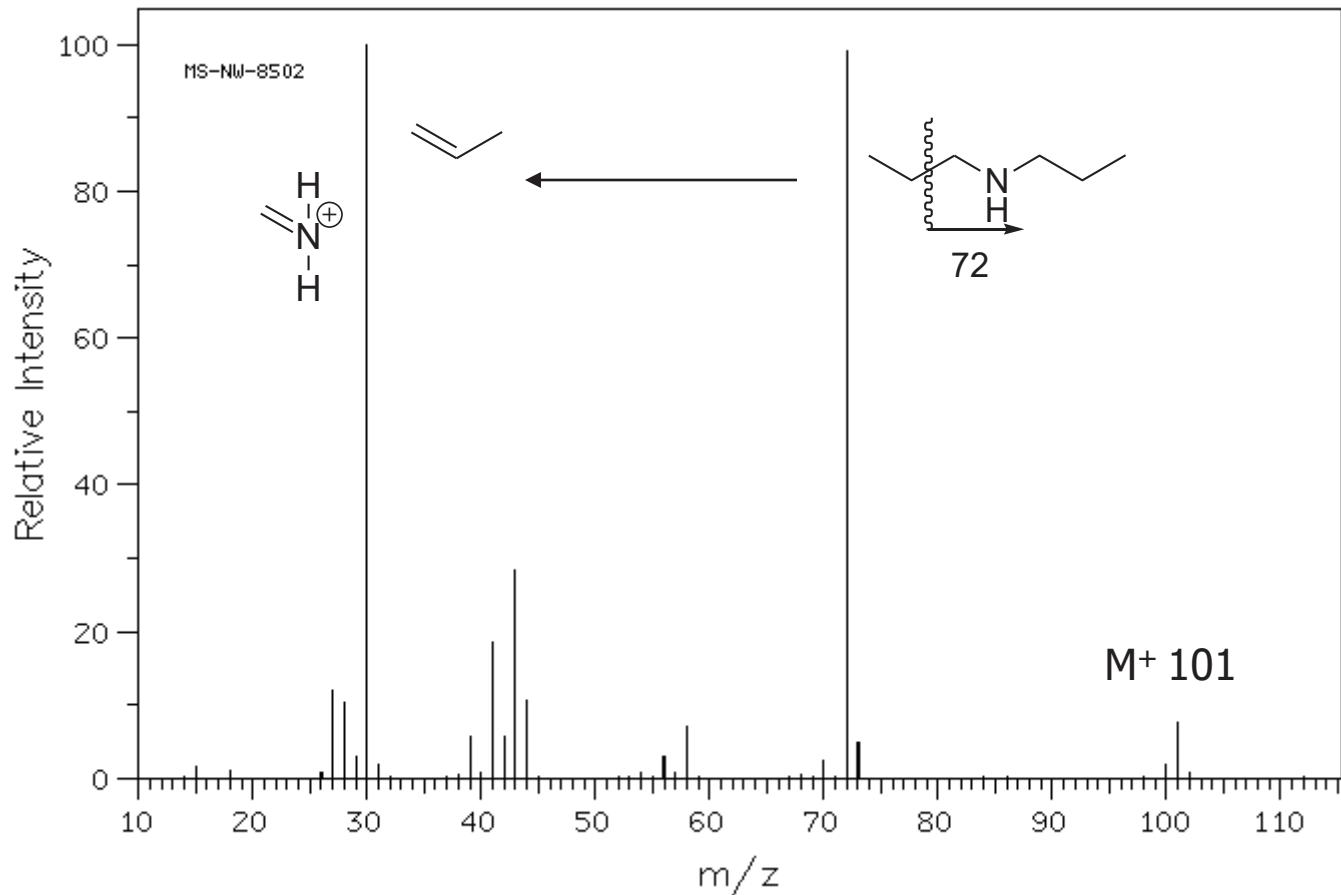
# Fragmentation Patterns of Groups

## 12. Example MS: amines, $1^\circ$ – pentylamine



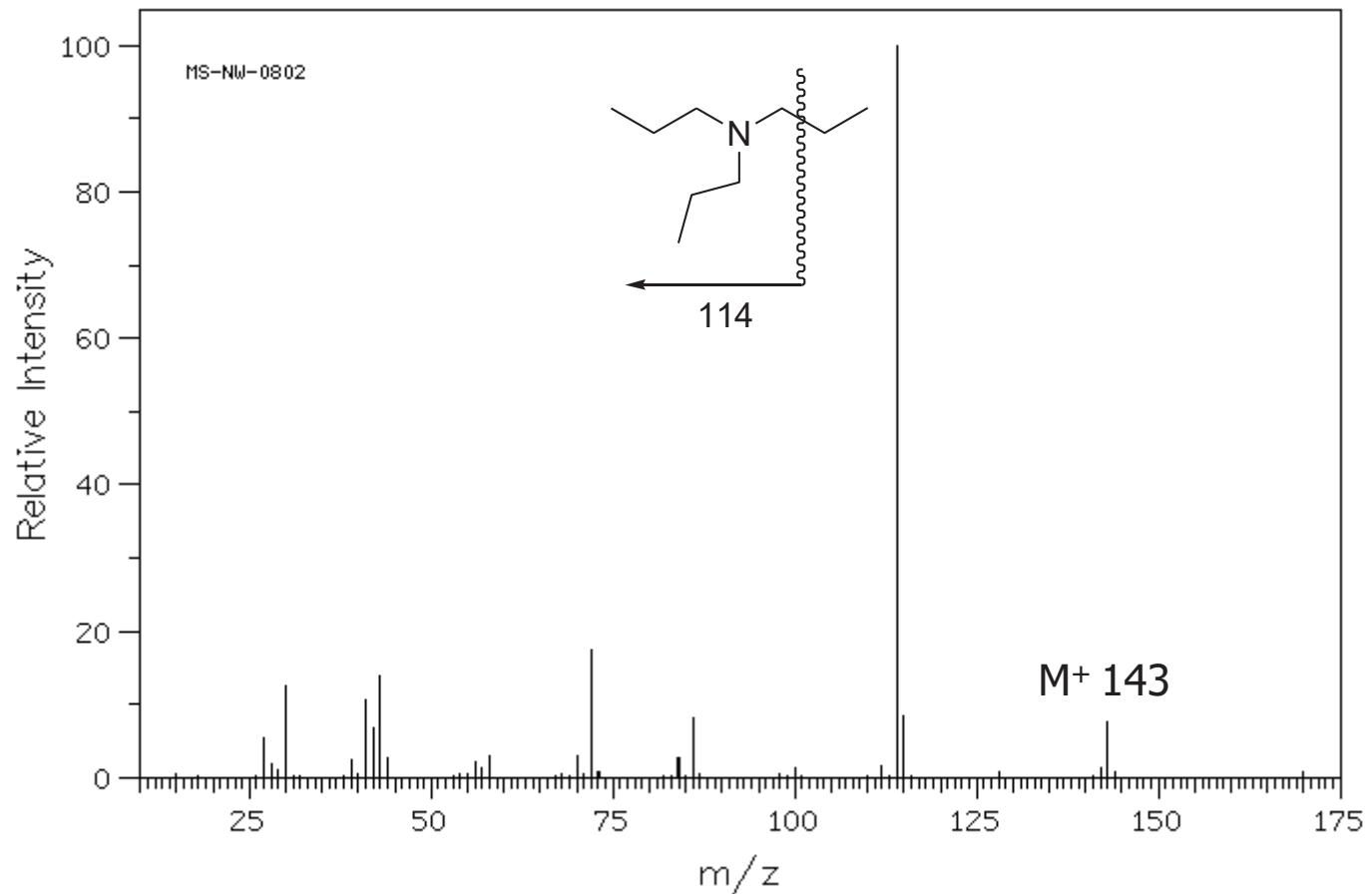
# Fragmentation Patterns of Groups

## 12. Example MS: amines, 2° – dipropylamine



# Fragmentation Patterns of Groups

## 12. Example MS: amines, 3° – tripropylamine

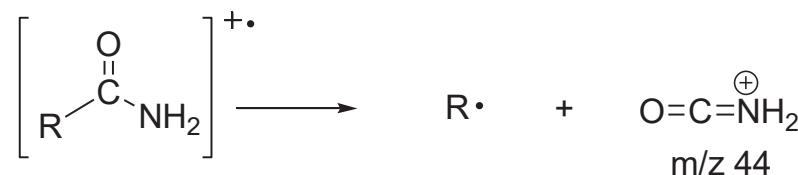


# Fragmentation Patterns of Groups

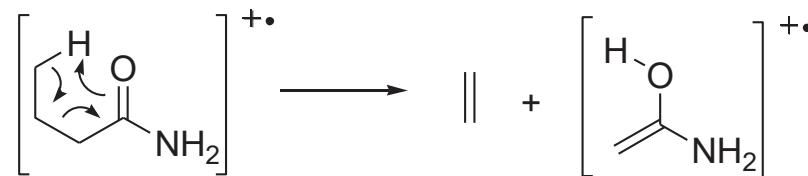
## 13. Amides - Fragment Ions

- Follow nitrogen rule – odd  $M^+$ , odd # of nitrogens; observable  $M^+$

- b)  $\alpha$ -cleavage reactions afford a specific fragment of  $m/z$  44 for primary amides

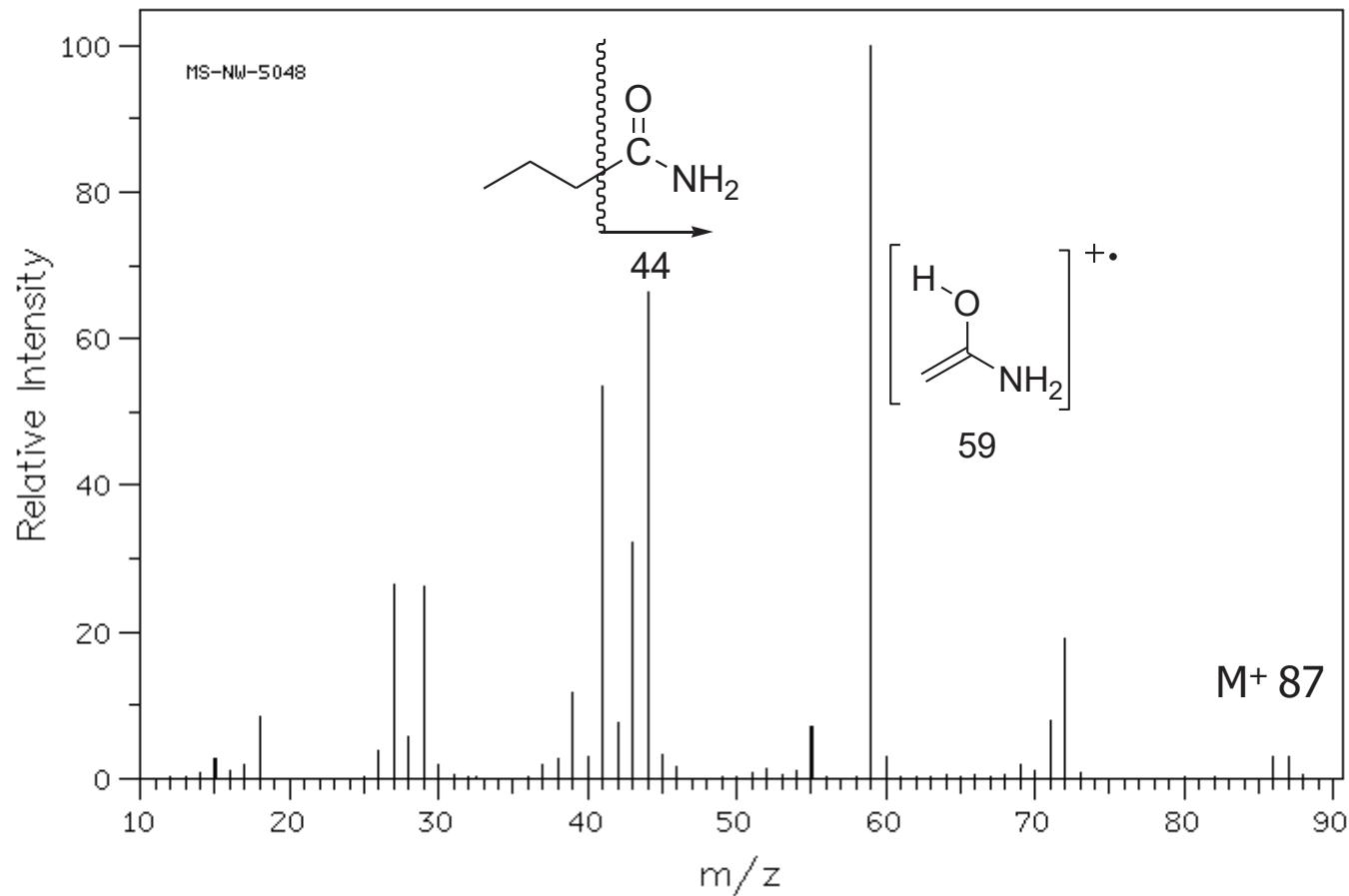


- c) McLafferty observed where  $\gamma$ -hydrogens are present



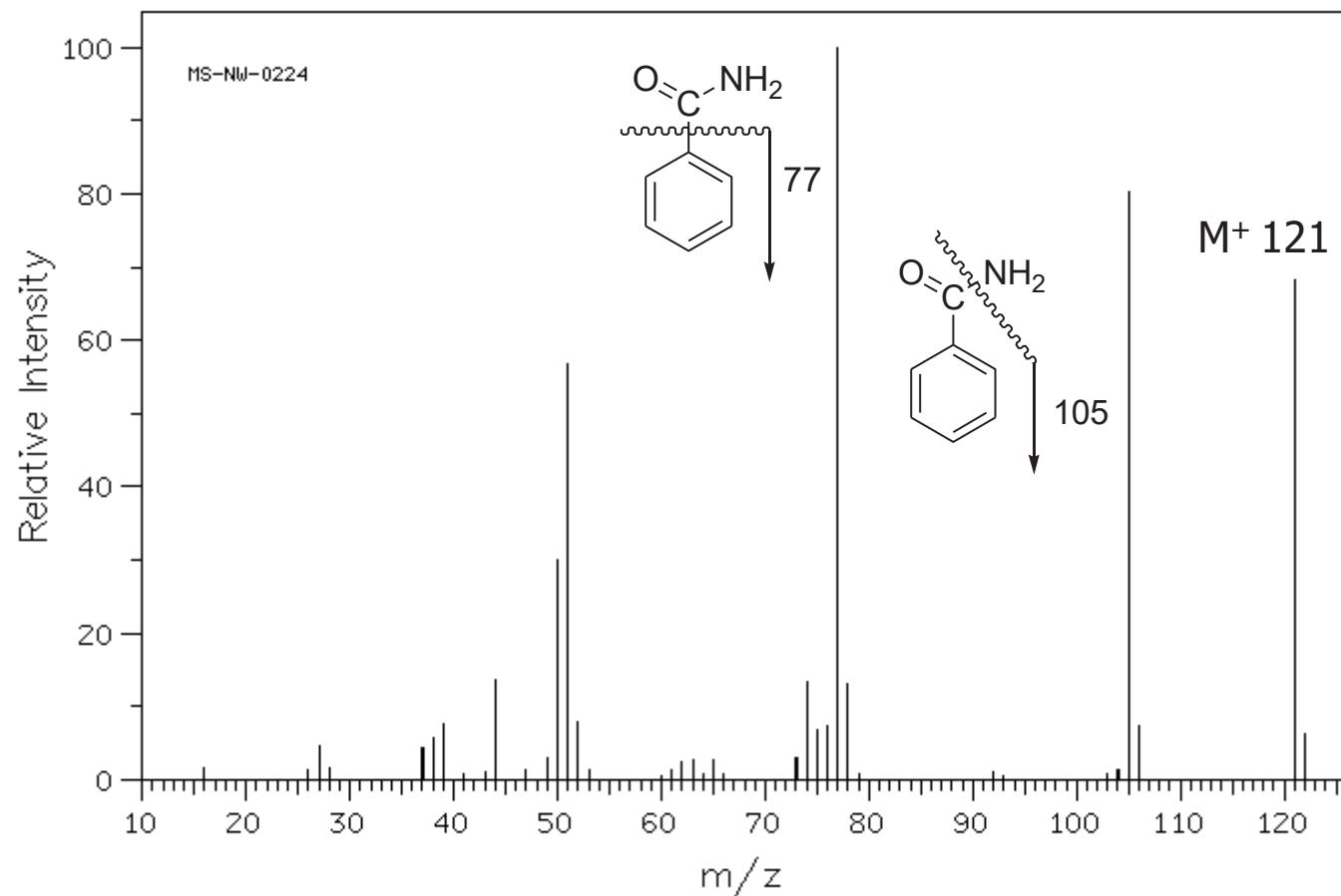
# Fragmentation Patterns of Groups

## 13. Example MS: amides – butyramide



# Fragmentation Patterns of Groups

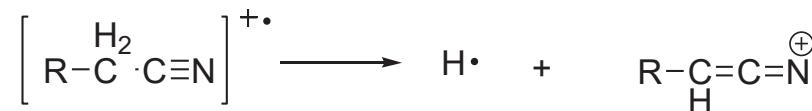
## 13. Example MS: amides (aromatic) – benzamide



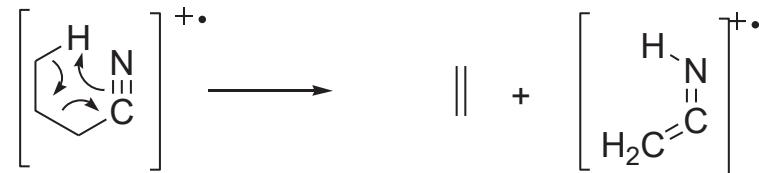
# Fragmentation Patterns of Groups

## 14. Nitriles - Fragment Ions

- Follow nitrogen rule – odd  $M^+$ , odd # of nitrogens; weak  $M^+$
- b) Principle degradation is the loss of an H-atom ( $M - 1$ ) from  $\alpha$ -carbon:



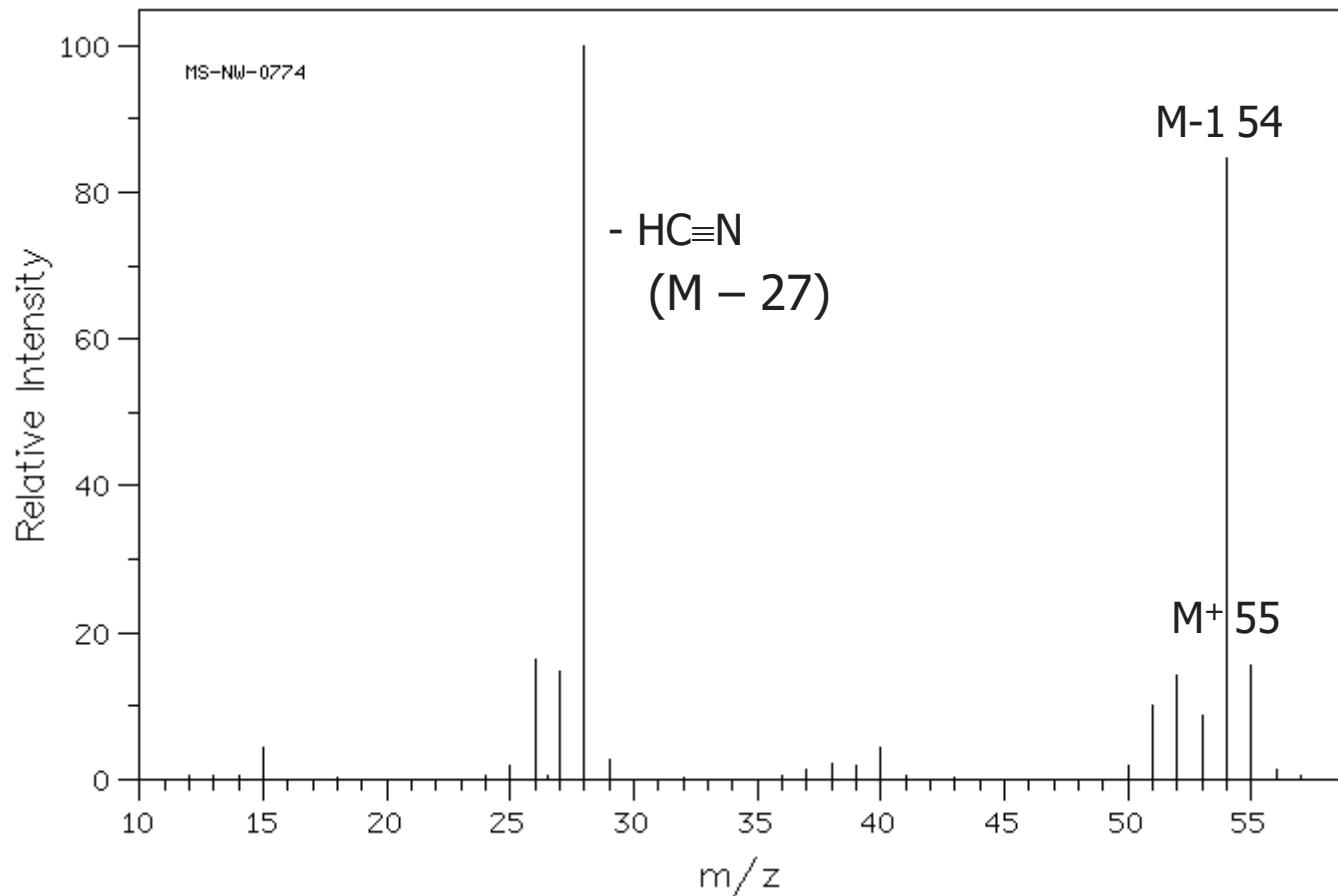
- c) Loss of HCN observed ( $M - 27$ )
- d) McLafferty observed where  $\gamma$ -H are present



- e) Aromatic nitriles give a strong  $M^+$  as the strongest peak, loss of HCN is common ( $m/z$  76) as opposed to loss of CN ( $m/z$  77)

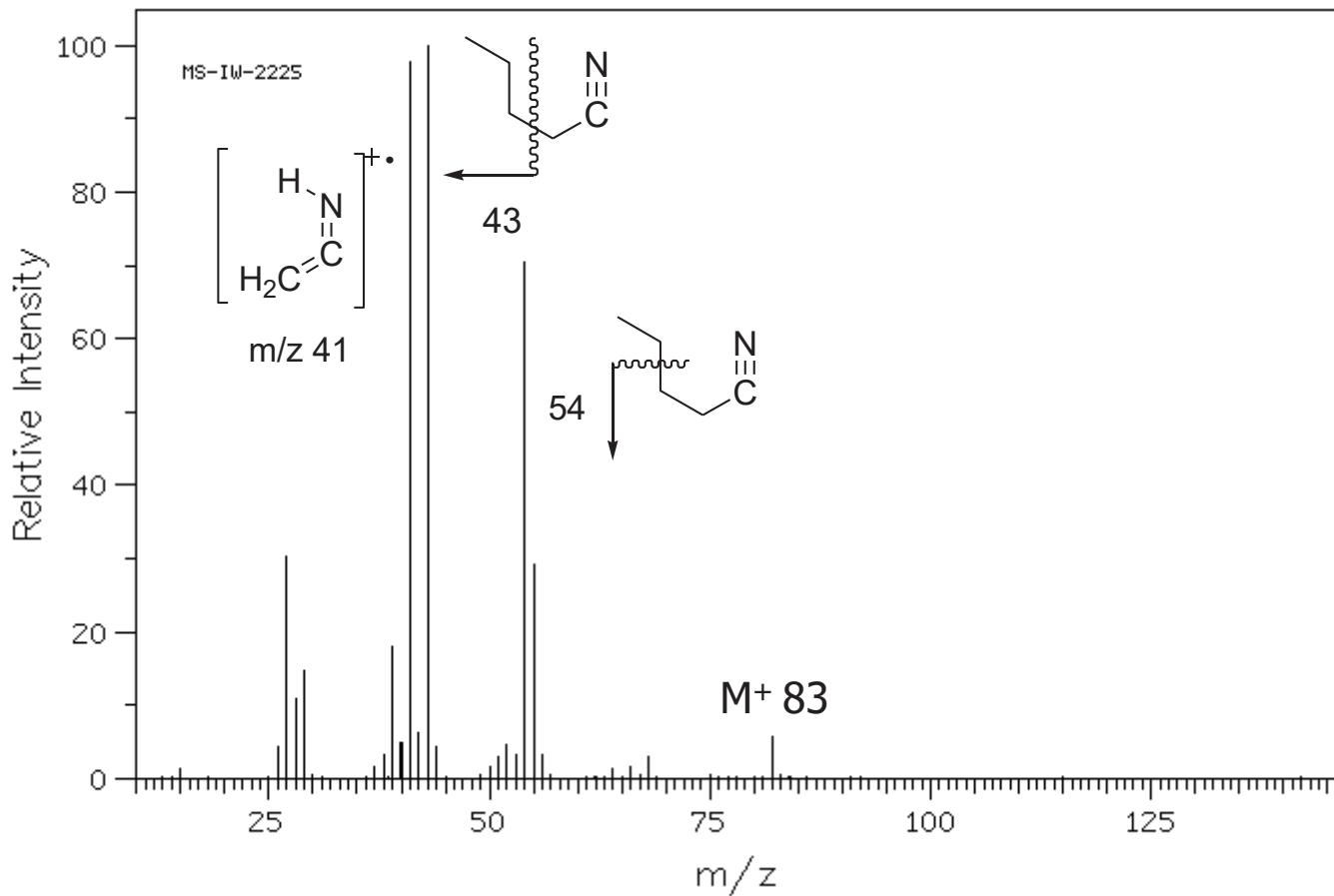
# Fragmentation Patterns of Groups

## 14. Example MS: nitriles – propionitrile



# Fragmentation Patterns of Groups

## 14. Example MS: nitriles – valeronitrile (pentanenitrile) (戊腈)

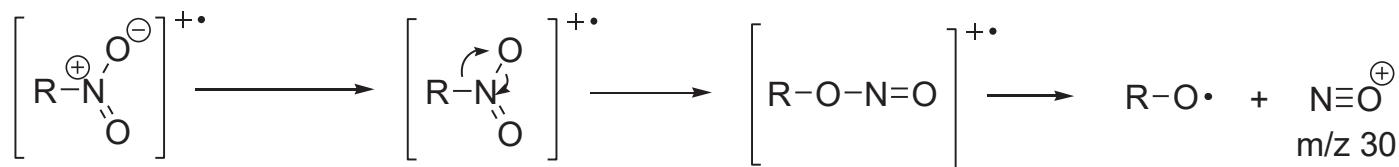
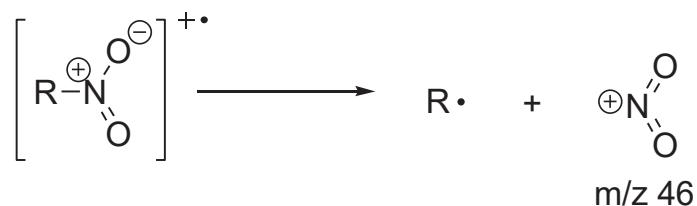


# Fragmentation Patterns of Groups

## 15. Nitro - Fragment Ions

- Follow nitrogen rule – odd  $M^+$ , odd # of nitrogens;  $M^+$  almost never observed, unless aromatic

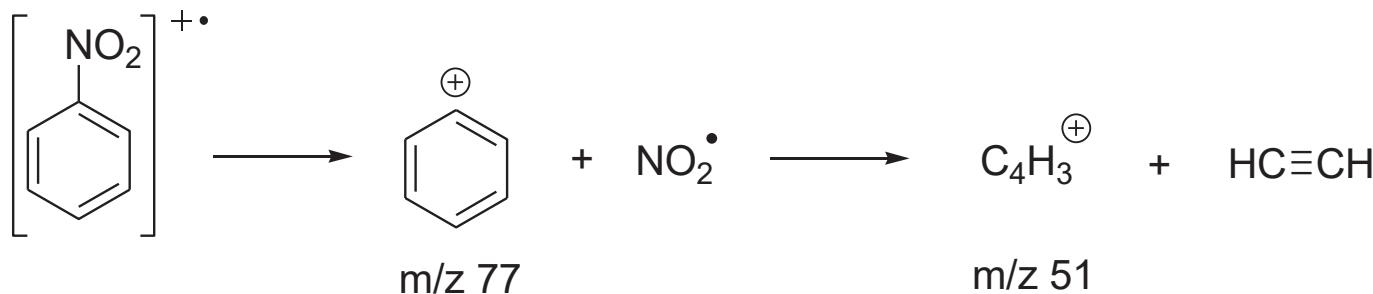
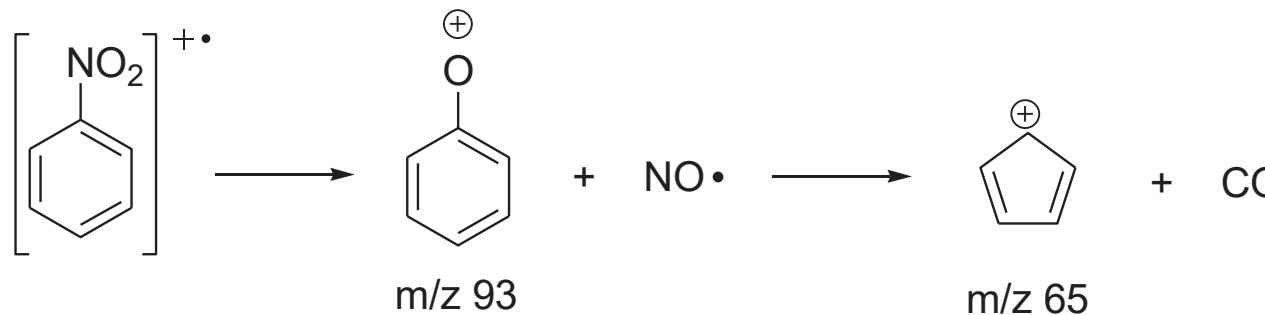
- b) Principle degradation is loss of  $\text{NO}^+$  ( $m/z$  30) and  $\text{NO}_2^+$  ( $m/z$  46)



# Fragmentation Patterns of Groups

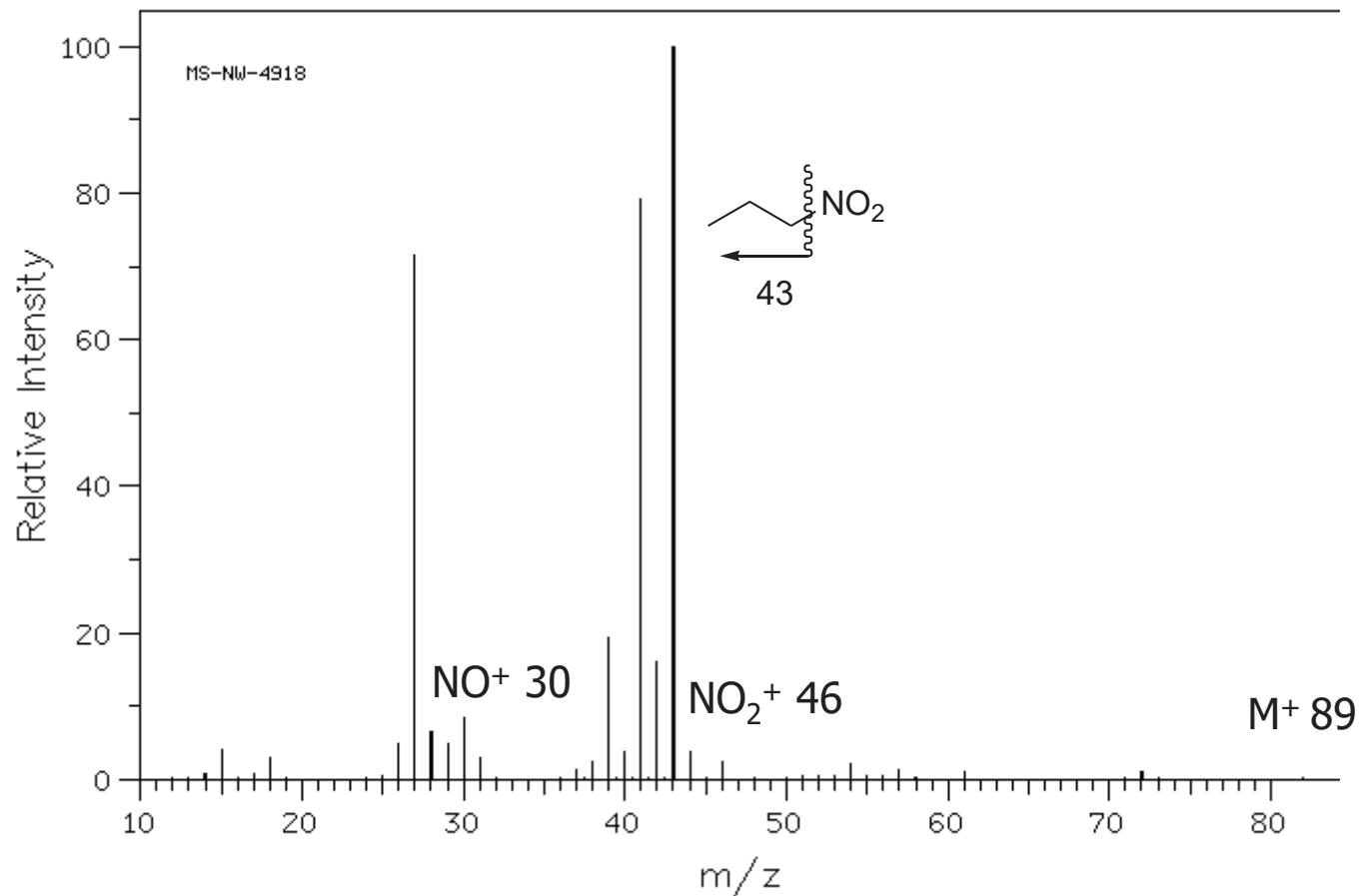
## 15. Nitro - Fragment Ions

- c) Aromatic nitro groups show these peaks as well as the fragments of the loss of all or parts of the nitro group



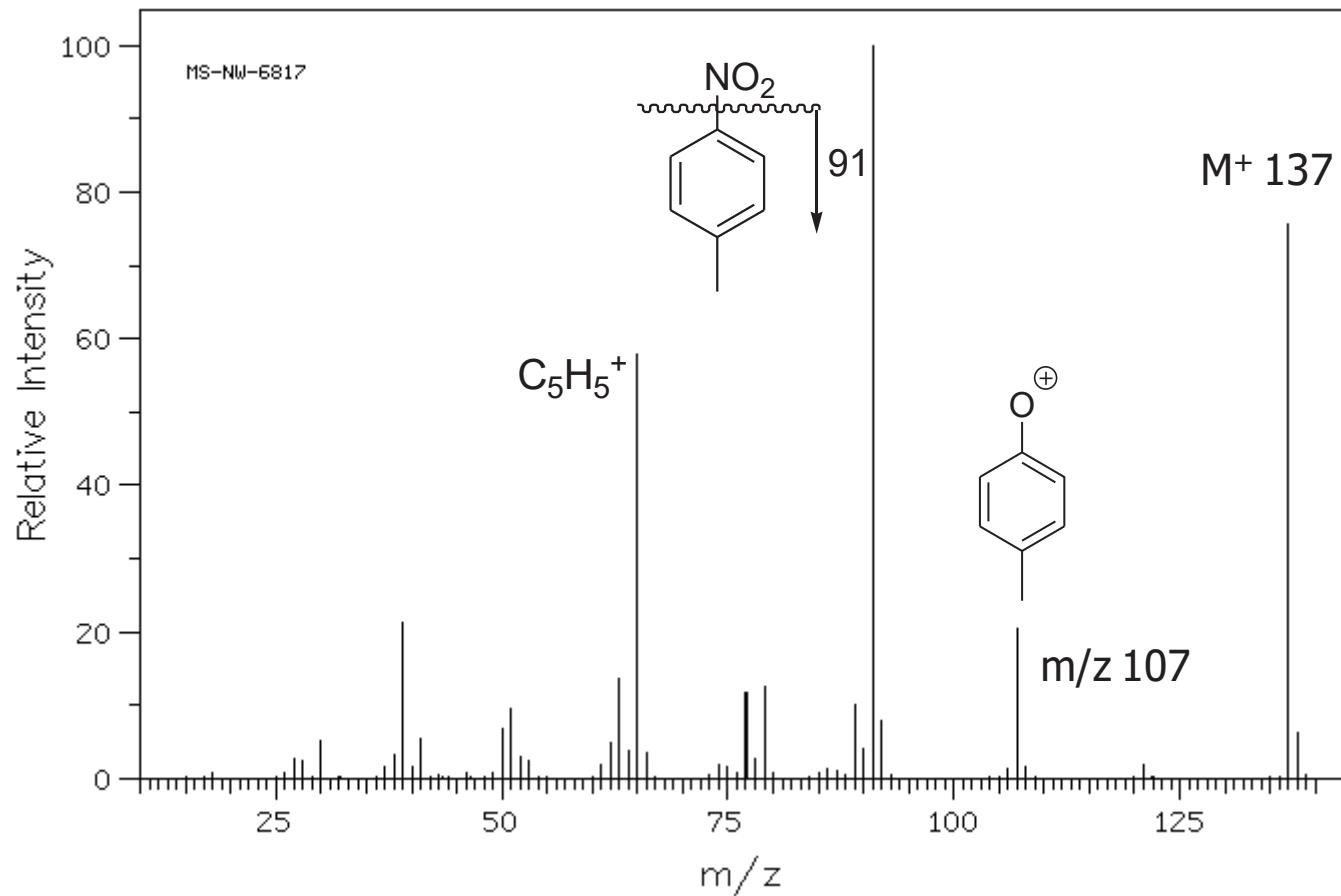
# Fragmentation Patterns of Groups

## 15. Example MS: nitro – 1-nitropropane



# Fragmentation Patterns of Groups

## 15. Example MS: nitro (aromatic) – *p*-nitrotoluene



# Fragmentation Patterns of Groups

## 16. Halogens - Fragment Ions

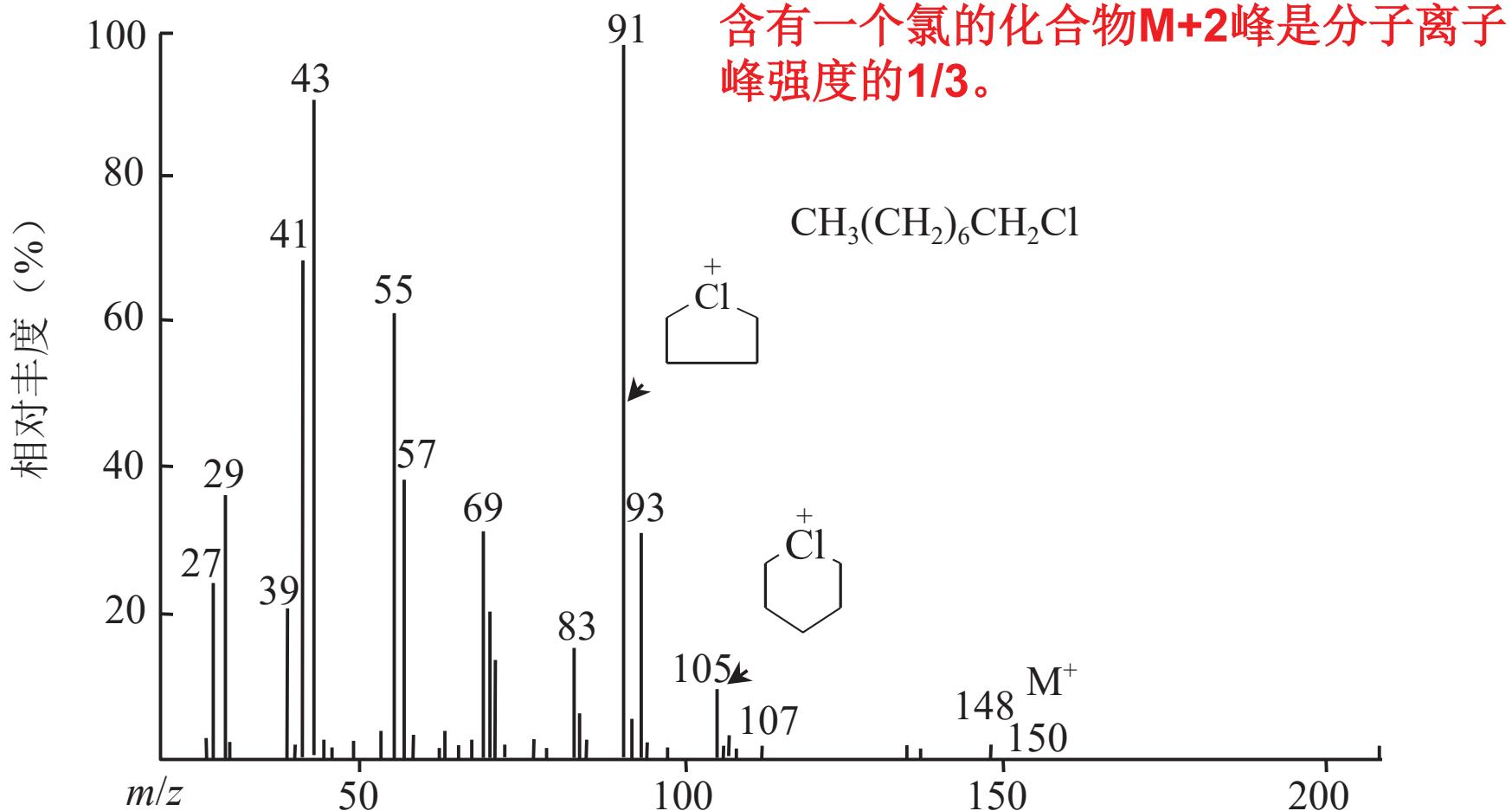
- Halogenated compounds often give good  $M^+$
- Fluoro- and iodo-compounds do not have appreciable contribution from isotopes
- Chloro- and bromo-compounds are unique in that they will show strong  $M+2$  peaks for the contribution of higher isotopes

For chlorinated compounds, the ratio of  $M^+$  to  $M+2$  is about 3:1

For brominated compounds, the ratio of  $M^+$  to  $M+2$  is 1:1

- An appreciable  $M+4, 6, \dots$  peak is indicative of a combination of these two halogens – use appropriate guide to discern number of each

# Fragmentation Patterns of Groups



1-氯辛烷的质谱图

# Fragmentation Patterns of Groups

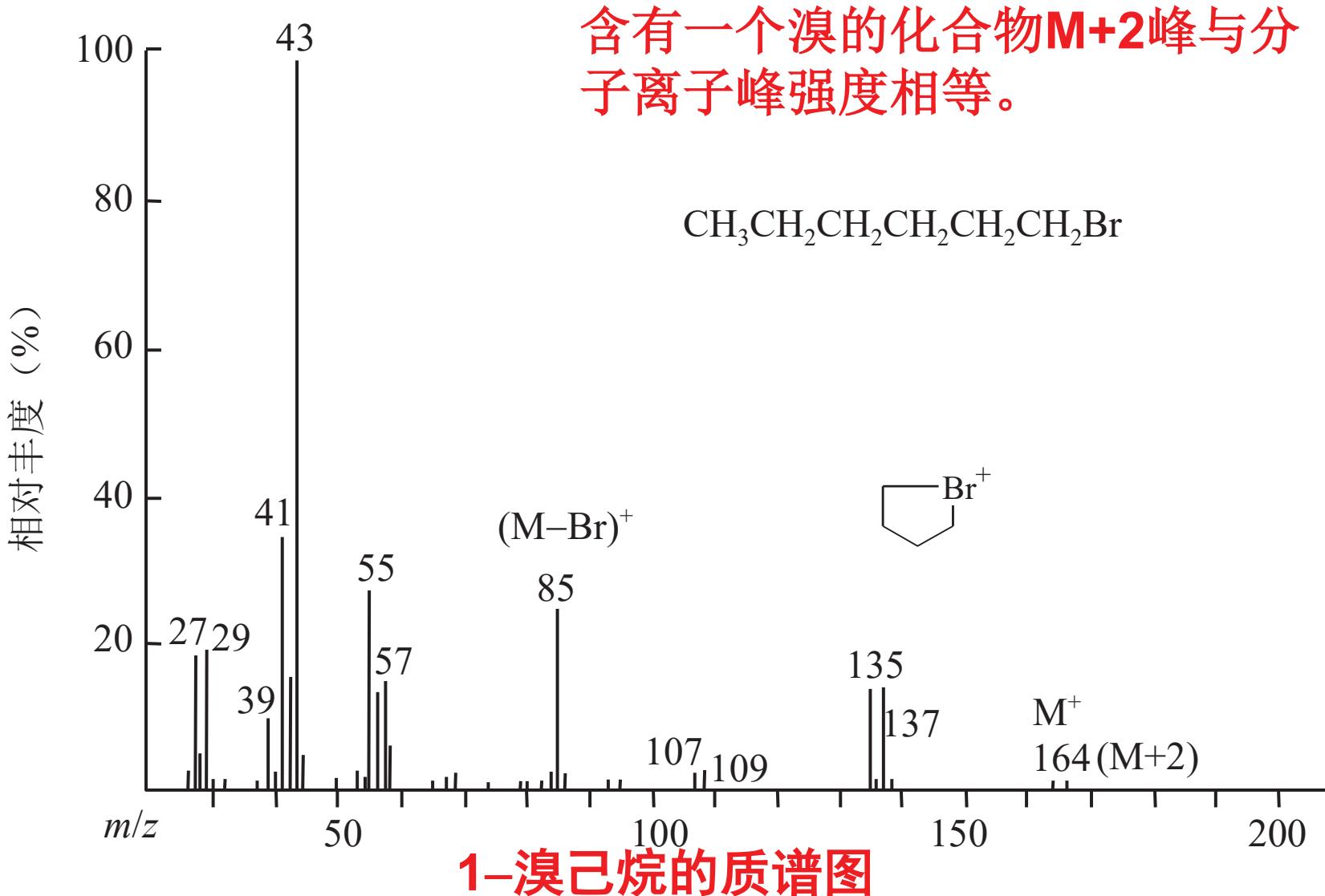


表 1.5 氯和溴组合时同位素峰的强度(相对于分子离子峰)

# Fragmentation Patterns of Groups

含有多个氯或溴化合物的质谱图

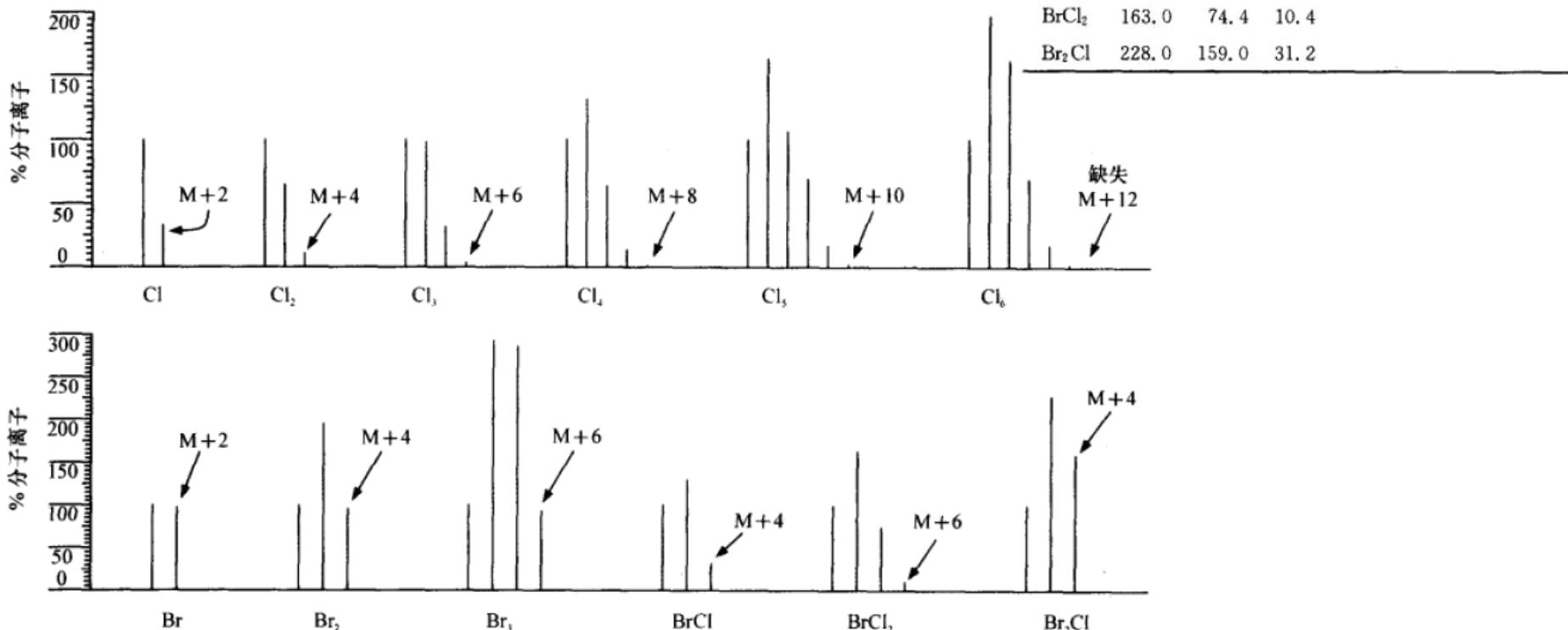


图 1.29 各种氯和溴组合的化合物的 M, M+2, M+4, … 图形

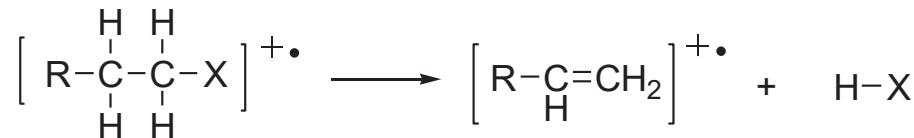
# Fragmentation Patterns of Groups

## 16. Halogens - Fragment Ions

- g) Principle fragmentation mode is to lose halogen atom, leaving a carbocation – the intensity of the peak will increase with cation stability



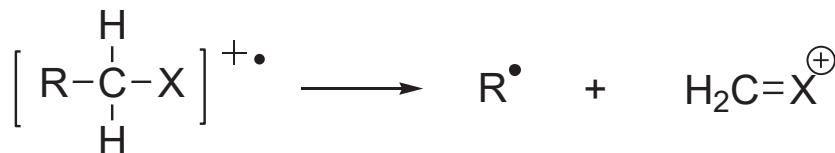
- h) Leaving group ability contributes to the loss of halogen most strongly for -I and -Br less so for -Cl, and least for -F
- i) Loss of HX is the second most common mode of fragmentation – here the conjugate basicity of the halogen contributes ( $\text{HF} > \text{HCl} > \text{HBr} > \text{HI}$ )



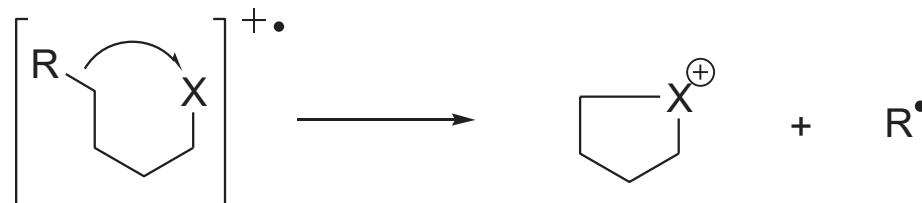
# Fragmentation Patterns of Groups

## 16. Halogens - Fragment Ions

- j) Less often,  $\alpha$ -cleavage will occur:



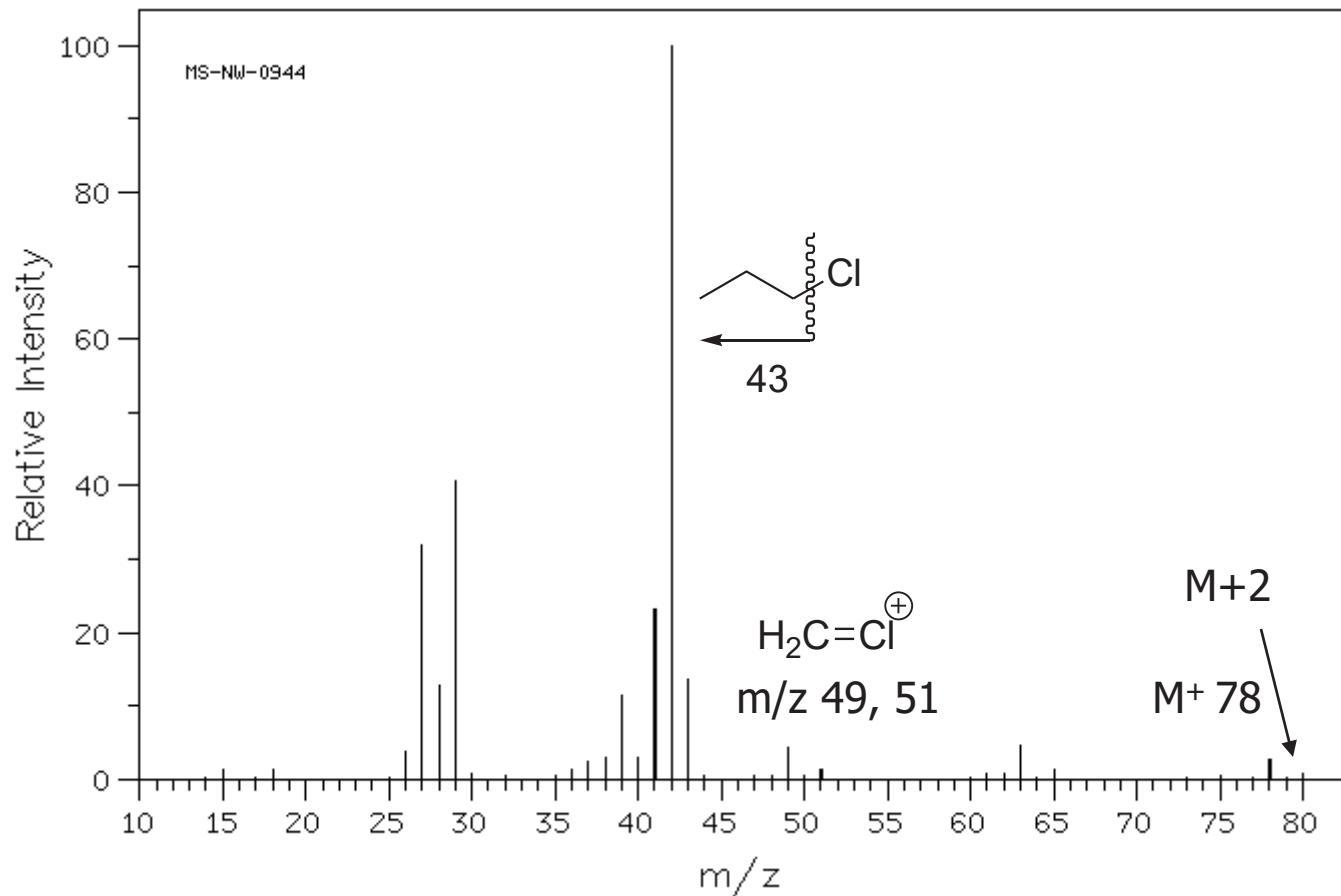
- k) For longer chain halides, the expulsion of a  $>\delta$  carbon chain as the radical is observed



- l) Aromatic halides give stronger  $\text{M}^+$ , and typically lose the halogen atom to form  $\text{C}_6\text{H}_5^+$

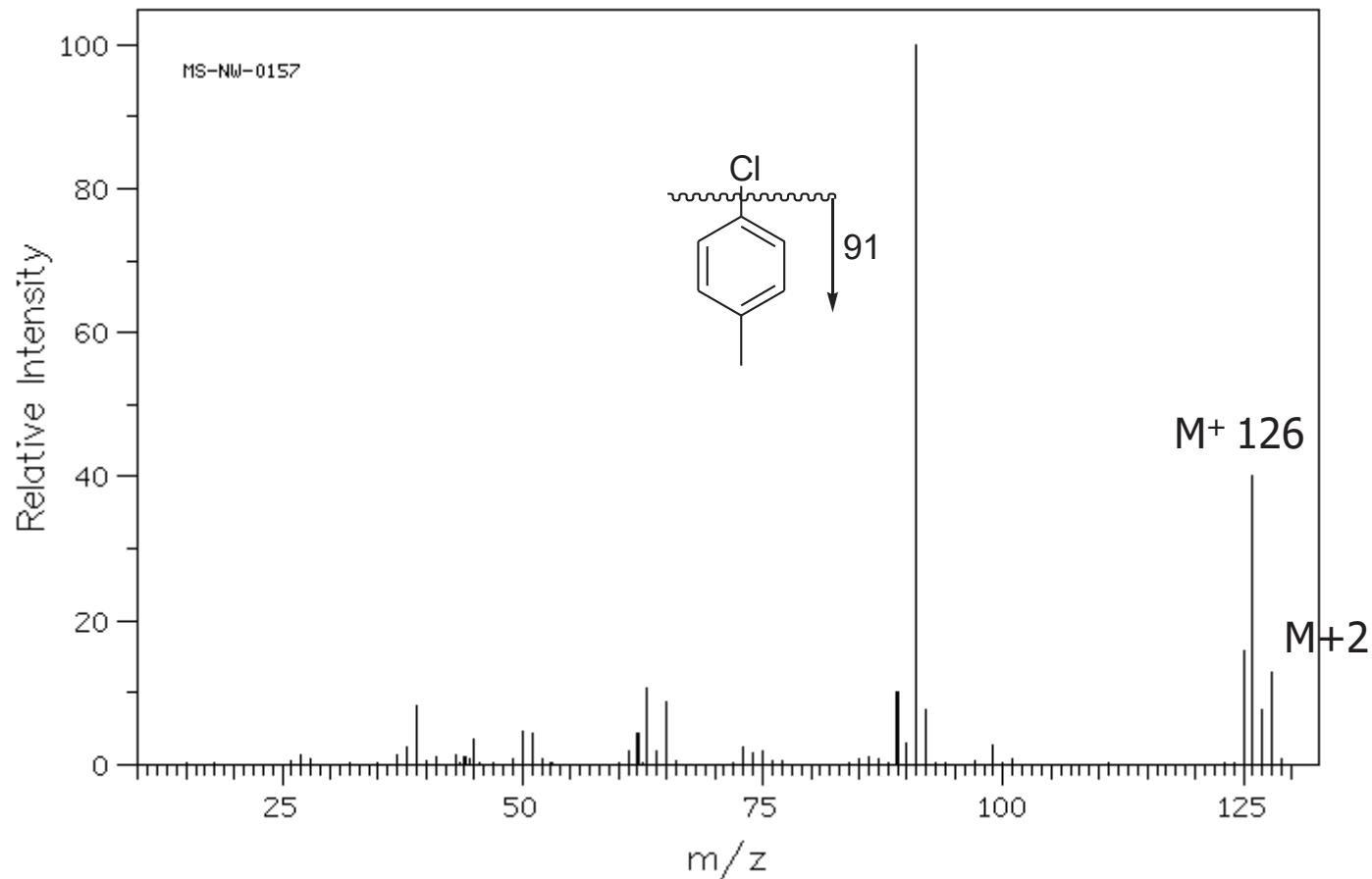
# Fragmentation Patterns of Groups

## 16. Example MS: chlorine – 1-chloropropane



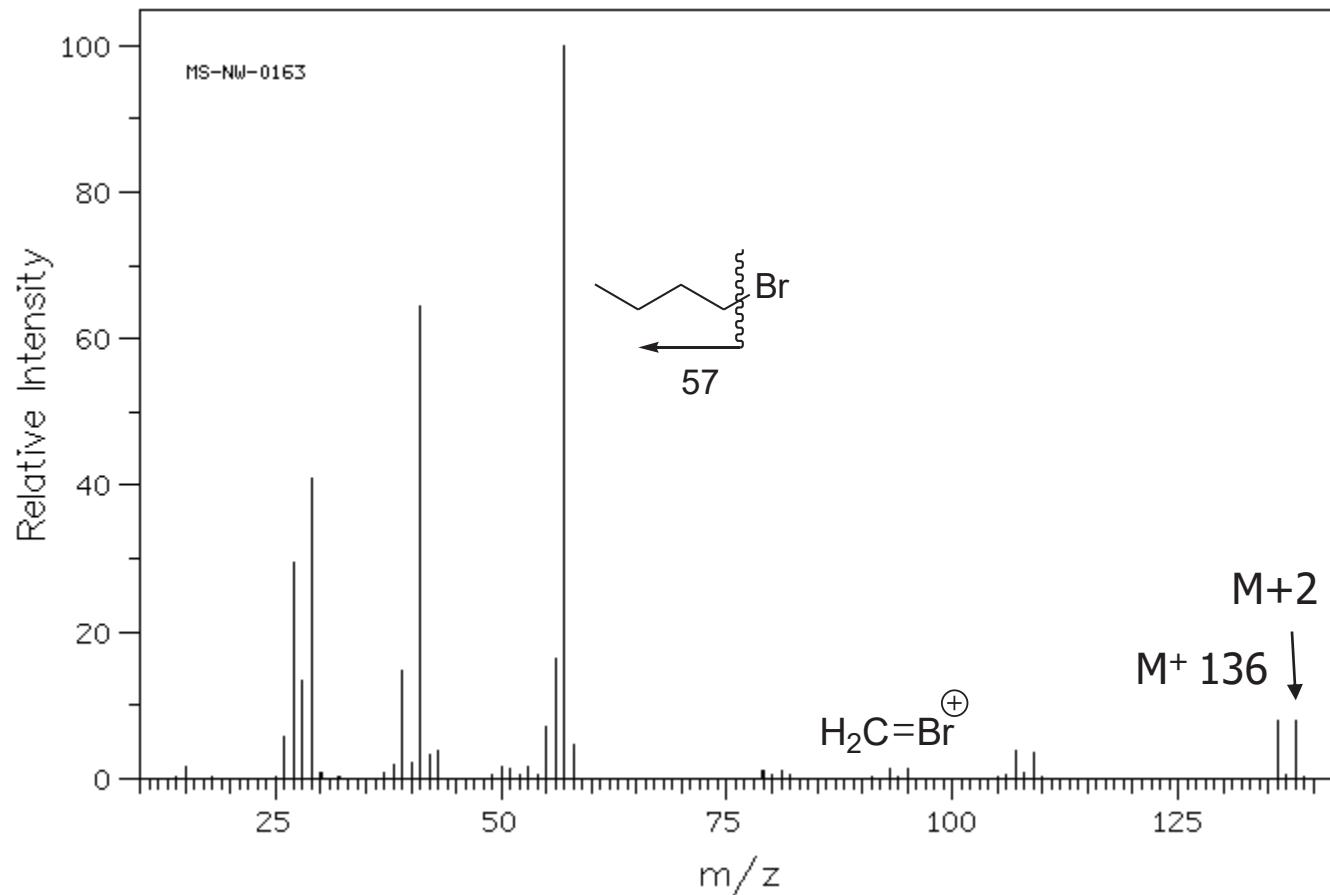
# Fragmentation Patterns of Groups

## 16. Example MS: chlorine – *p*-chlorotoluene



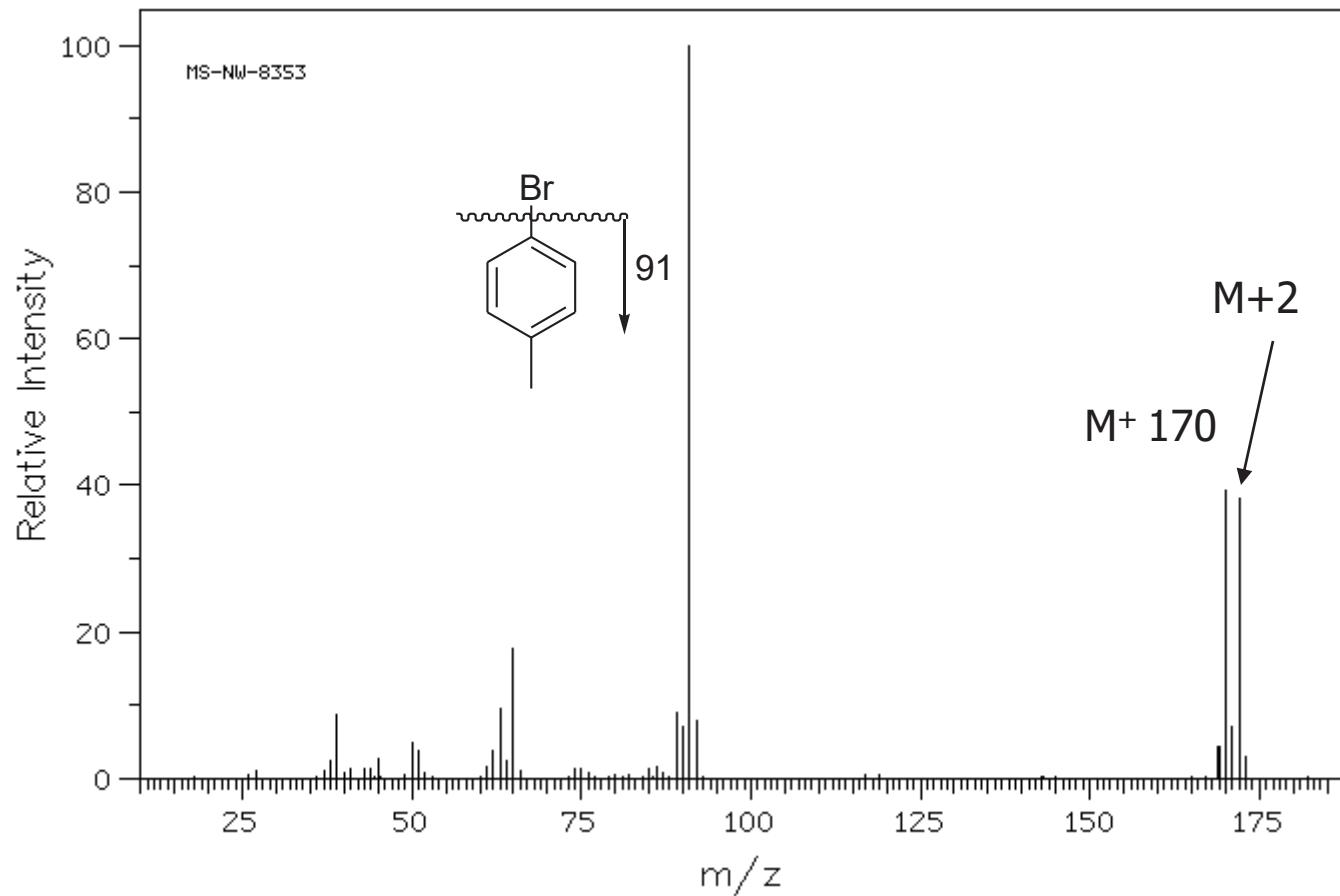
# Fragmentation Patterns of Groups

## 16. Example MS: bromine – 1-bromobutane



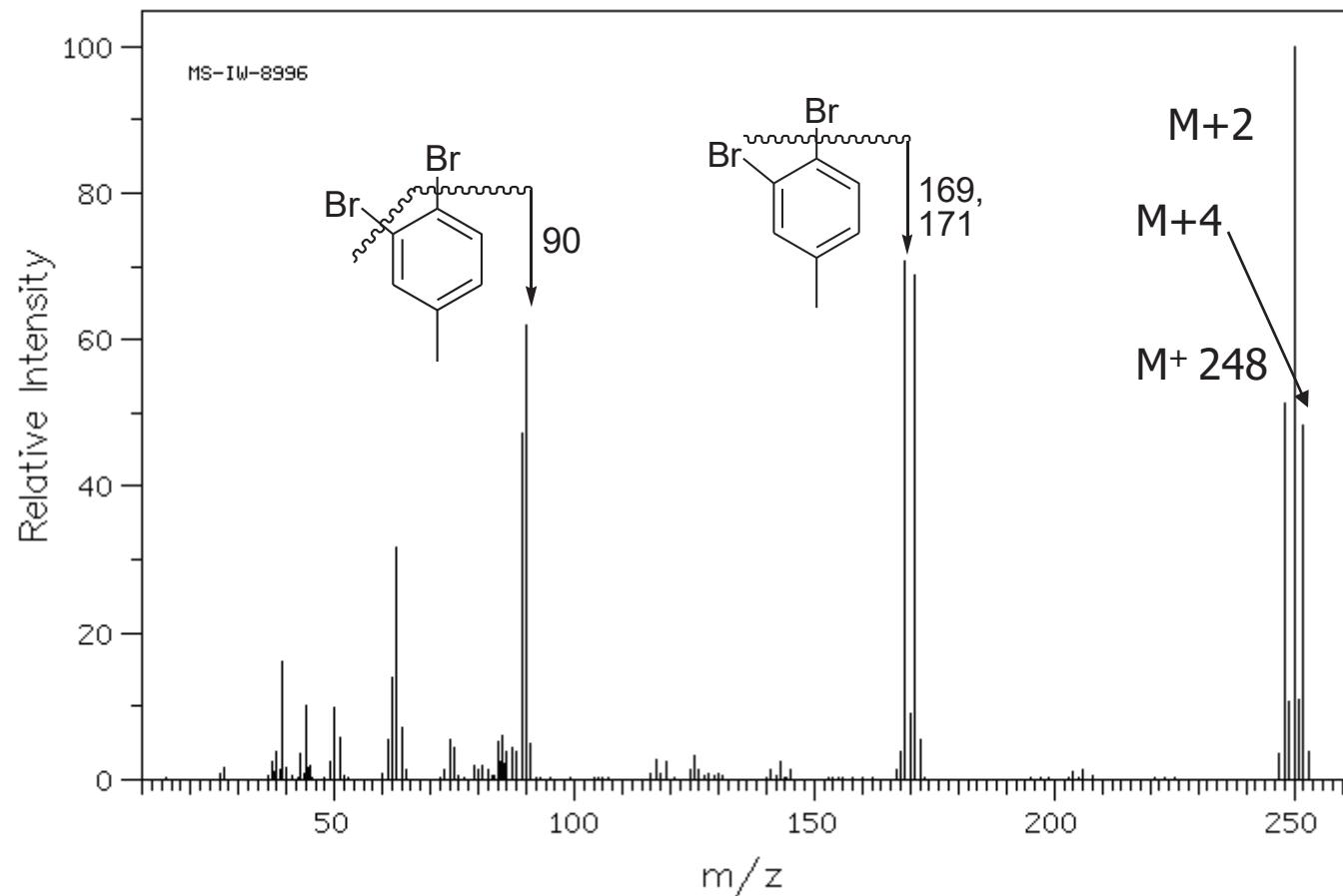
# Fragmentation Patterns of Groups

## 16. Example MS: bromine – *p*-bromotoluene



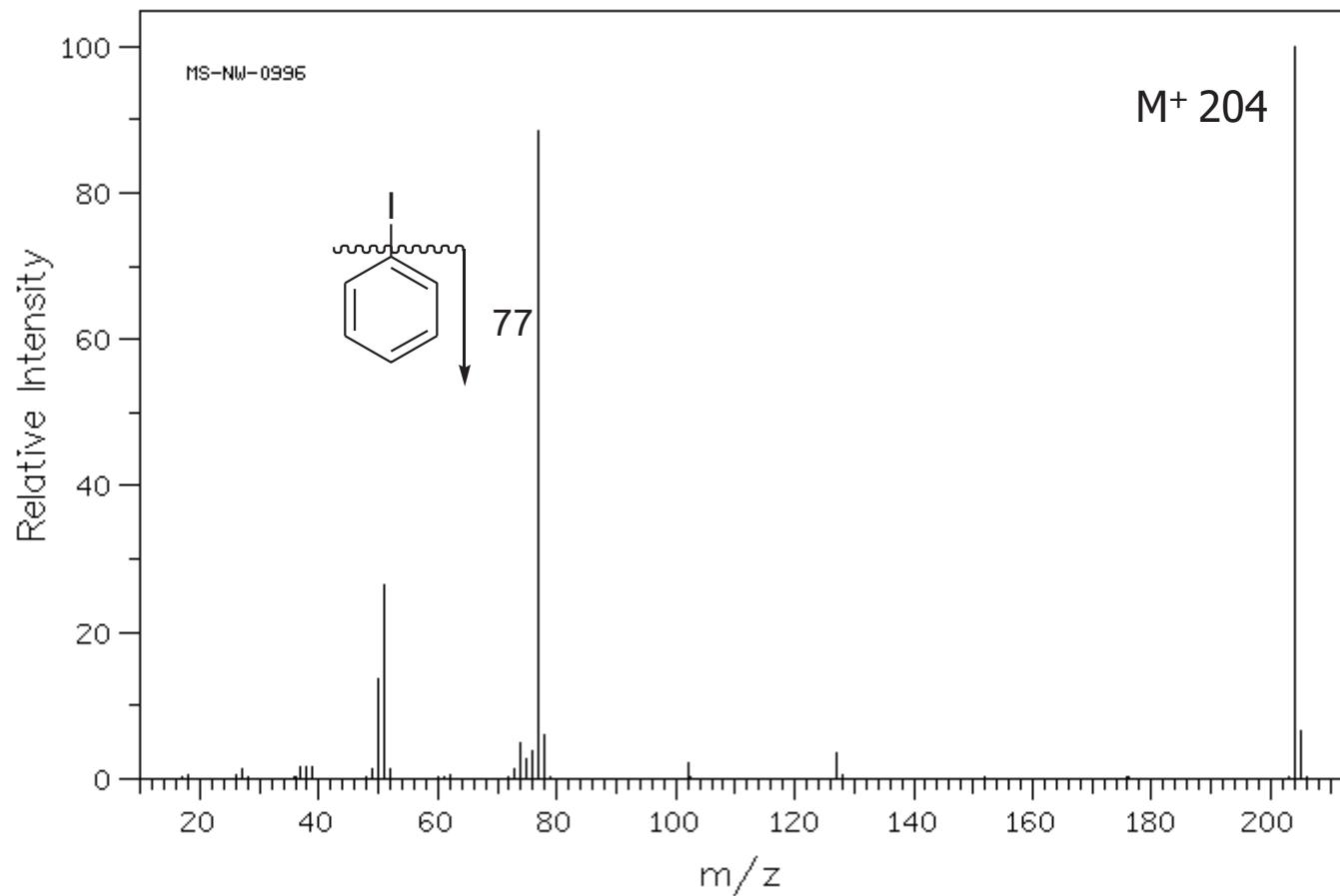
# Fragmentation Patterns of Groups

## 16. Example MS: multiple bromines – 3,4-dibromotoluene



# Fragmentation Patterns of Groups

## 16. Example MS: iodine – iodobenzene



# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

Ionization Method

Ion Separation Methods

**Determination of Molecular Mass**

**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

# Approach to analyzing a mass spectrum

- As with IR, get a general feel for the spectrum before you analyze anything – is it simple, complex, groups of peaks, etc.
- Squeeze everything you can out of the  $M^+$  peak that you can (once you have confirmed it is the  $M^+$ )
  - Strong or Weak?
  - Isotopes?  $M+1$ ?  $M+2, 4, \dots$  (Cl? Br? S?)
  - Apply the Nitrogen rule
  - Is there an  $M-1$  peak?

# Approach to analyzing a mass spectrum

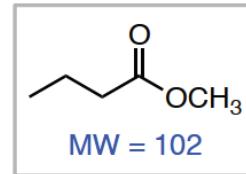
3. Squeeze everything you can out of the base peak
  - What ions could give this peak? ( $m/z$  43 doesn't help much)
  - What was lost from  $M^+$  to give this peak?
  - When considering the base peak initially, only think of the *most common cleavages for each group*
4. Look for the loss of small neutral molecules from  $M^+$ 
  - $H_2C=CH_2$ ,  $HC\equiv CH$ ,  $H_2O$ ,  $HOR$ ,  $HCN$ ,  $HX$
5. Now consider the possible diagnostic peaks on the spectrum (e.g.: 29, 30 (N?), 31 (O?), 45 (-COOH?), 59, 77 (C<sub>6</sub>H<sub>6</sub>?), 91, 105 etc.)
6. Lastly, once you have a hypothetical molecule that explains the data, see if you can verify it by use of other less intense peaks on the spectrum – not 100% necessary (or accurate) but if this step works it can add to the confidence level

# Reporting Mass Spec Data

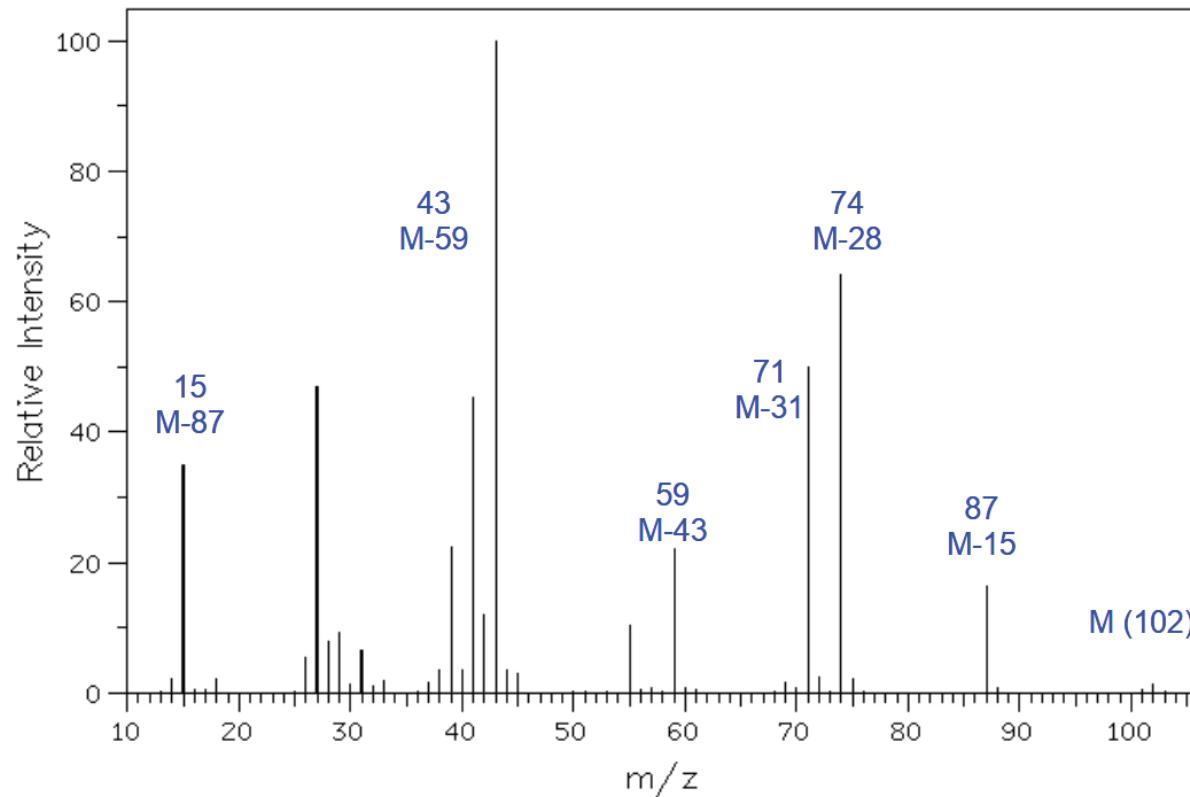
## *Mass Spectrometry: Fragmentation*

### Reporting Mass Spec Data

#### Low Resolution Mass Spec



peak	intensity
14.0	2.1
15.0	35.0
18.0	2.1
26.0	5.4
27.0	47.0
28.0	7.9
29.0	9.2
30.0	1.2
31.0	6.5
32.0	1.1
33.0	1.9
37.0	1.6
38.0	3.5
39.0	22.5
40.0	3.5
41.0	45.3
42.0	12.1
43.0	100.0
44.0	3.6
45.0	3.1
55.0	10.4
59.0	22.2
69.0	1.5
71.0	49.9
72.0	2.3
74.0	64.2
75.0	2.2
87.0	16.4
102.0	1.4



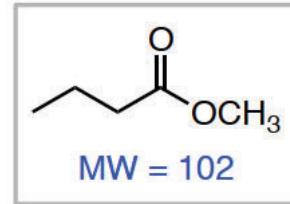
Source Temperature: 240 °C  
Sample Temperature: 180 °C  
RESERVOIR, 75 eV

# Reporting Mass Spec Data

## ***Mass Spectrometry***

### Reporting Mass Spec Data

#### Low Resolution Mass Spec



ionization technique/method



peak assignment



MS (EI, 75 eV): m/z 102 ( $\text{M}^+$ , 1%), 87 (16), 74 (64), 71 (50), 59 (22), 43 (100) ....

mass



height of peak relative to base peak



# Reporting Mass Spec Data

## ***Mass Spectrometry***

### **Reporting Mass Spec Data**

#### **High Resolution Mass Spec**

#### **Mass Spectrum List Report**

---

##### **Analysis Info**

Analysis Name LS-III-156\_pos\_000001.d  
Method XMASS\_Method  
Sample Name: LS-III-156\_pos  
LS-III-156\_pos: in 1:1 THF:MeOH w/ NaCl.

Acquisition Date 7/22/2011 10:54:16 AM  
Operator FTMS\_USER  
Instrument apex-Qe

---

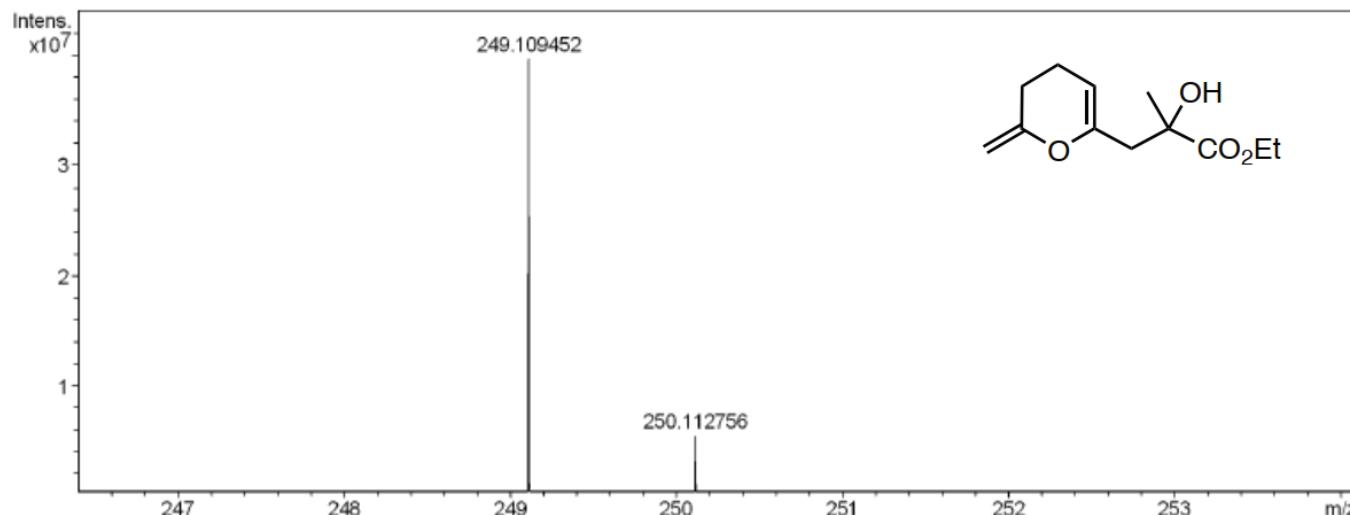
##### **Acquisition Parameter**

**Sample: LS-III-156**

**Exact Mass of (C<sub>12</sub>H<sub>18</sub>O<sub>4</sub>)Na<sup>+</sup> = 249.109730u**

**Observed Mass = 249.109452u**

**Difference = -1.1 ppm**

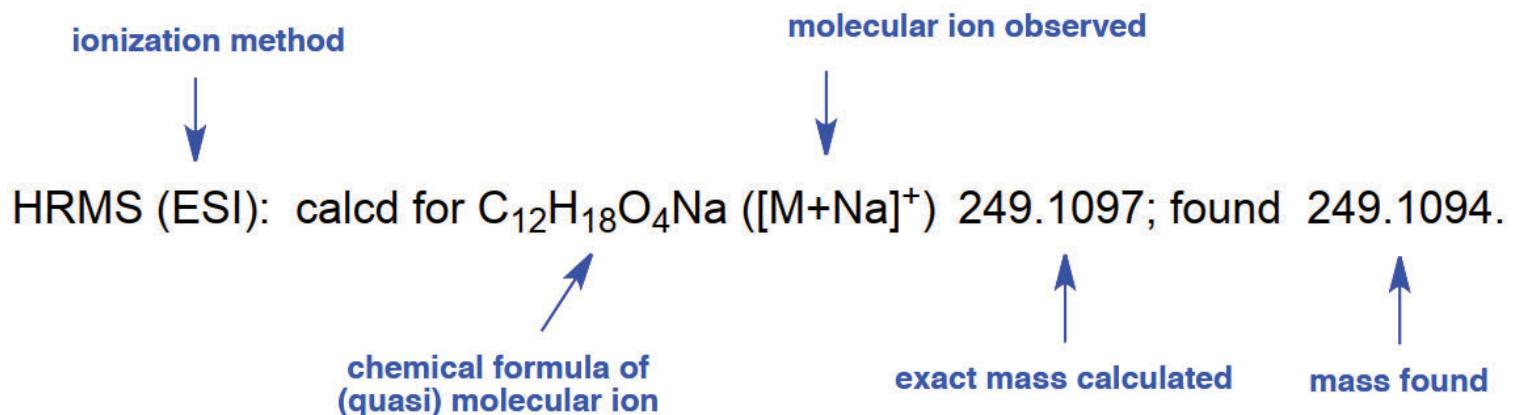


# Reporting Mass Spec Data

## ***Mass Spectrometry***

### Reporting Mass Spec Data

#### High Resolution Mass Spec



# Mass spectrometry (MS)

**Introduction**

**Mass spectrometer**

**Ionization Method**

**Ion Separation Methods**

**Determination of Molecular Mass**

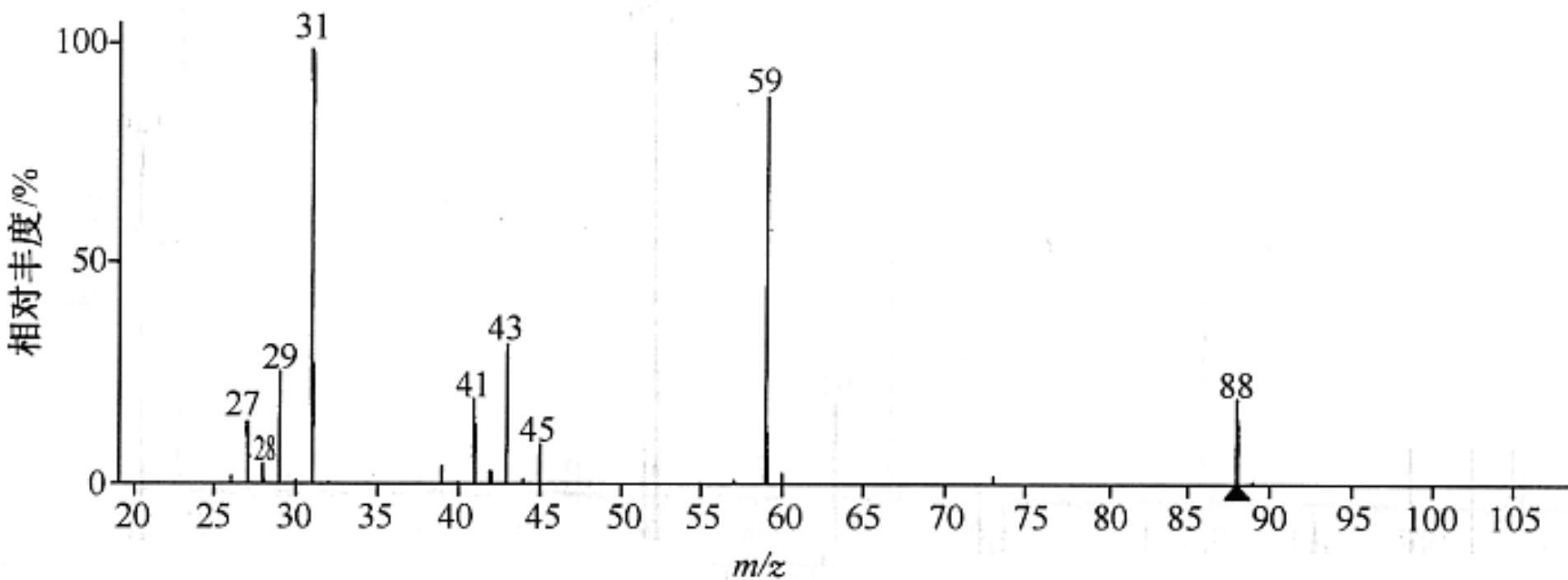
**Fragmentation**

**Approach to analyzing a mass spectrum**

**Practice**

# Practice

例1. 未知物质谱图如下，红外光谱显示该未知物在  $1150\sim1070\text{ cm}^{-1}$  有强吸收，试确定其结构。



# Practice

解：从质谱图中得知以下结构信息：

- ① m/z 88 为分子离子峰；
- ② m/z 88 与 m/z 59 质量差为 29u，为合理丢失。且丢失的可能的是 C<sub>2</sub>H<sub>5</sub> 或 CHO；
- ③ 图谱中有 m/z 29、m/z 43 离子峰，说明可能存在乙基、正丙基或异丙基；
- ④ 基峰 m/z 31 为醇或醚的特征离子峰，表明化合物可能是醇或醚。

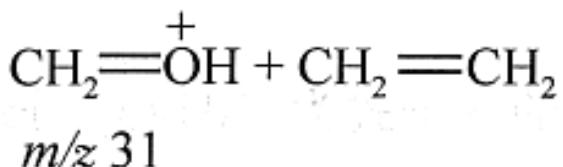
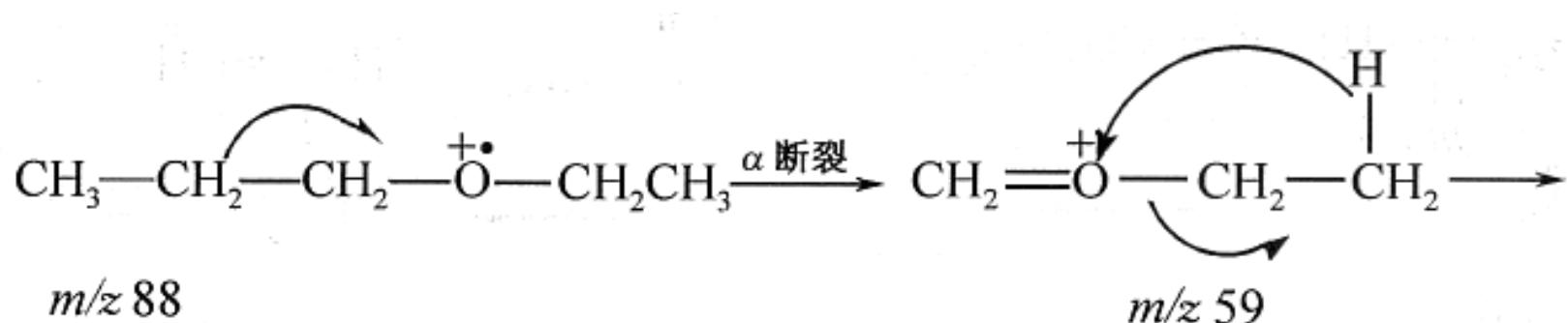
由于红外谱在 1740~1720 cm<sup>-1</sup> 和 3640~3620 cm<sup>-1</sup> 无吸收，可否定化合物为醛和醇。因为醚的 m/z 31 峰可通过以下重排反应产生：

# Practice

据此反应及其它质谱信息，推测未知物可能的结构为：

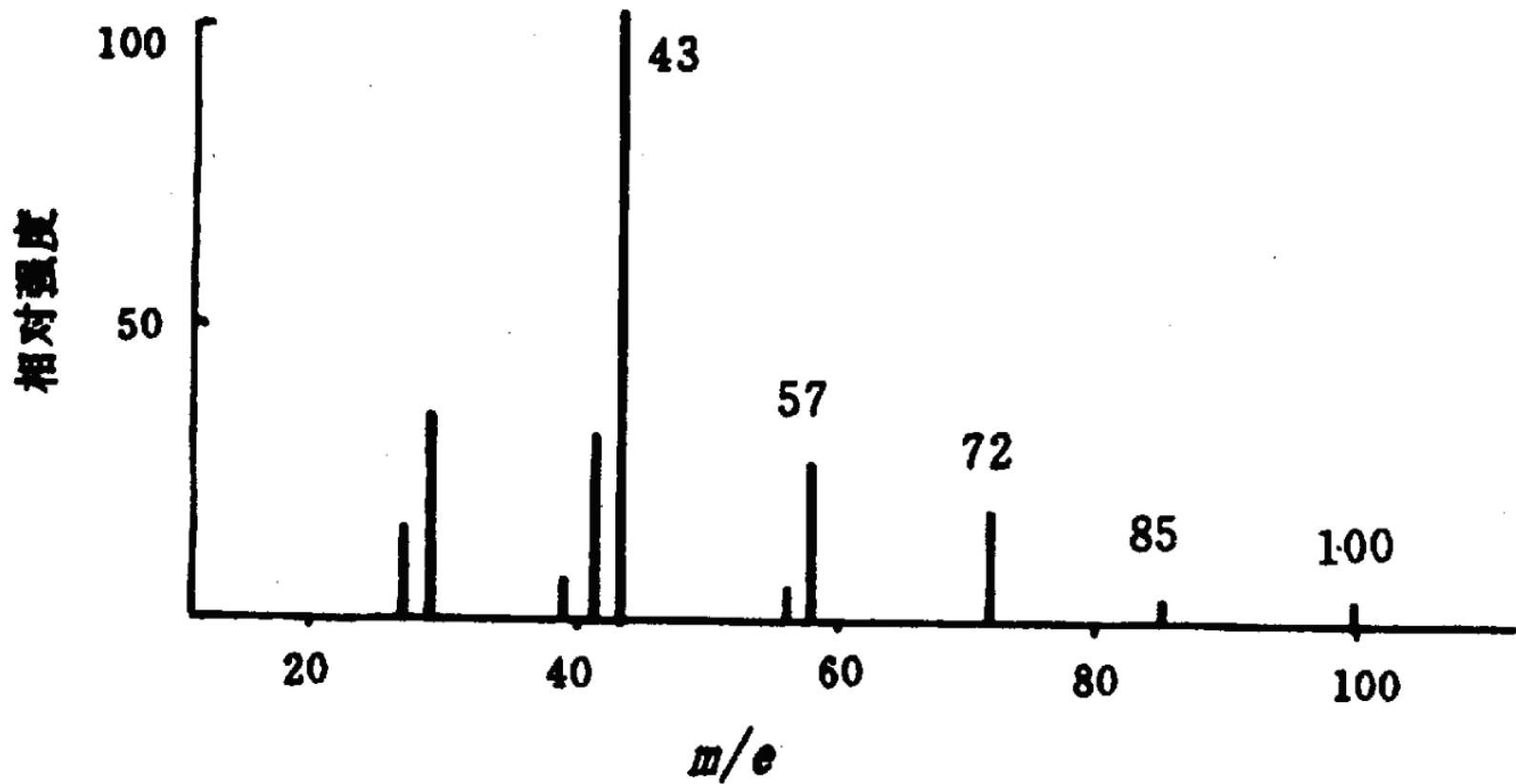


质谱中主要离子的产生过程



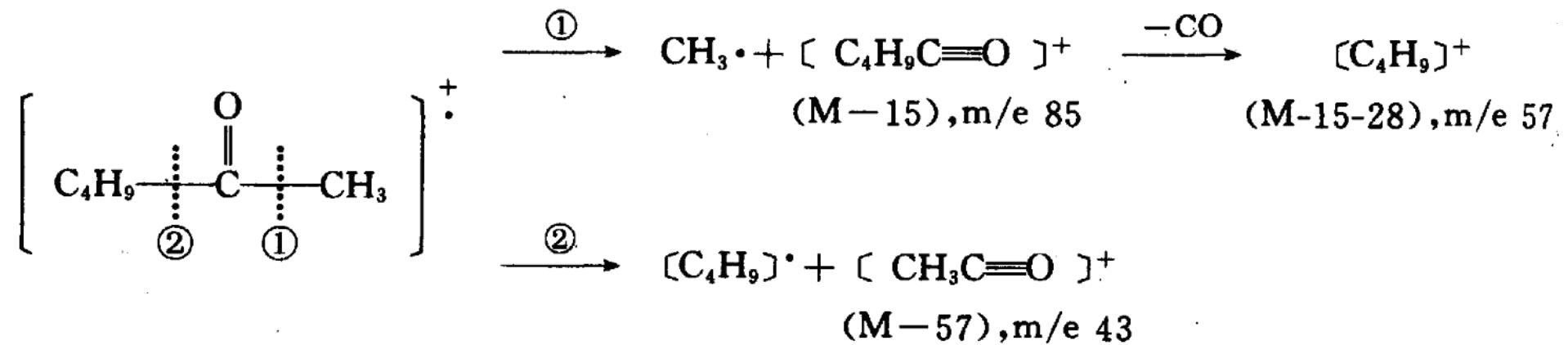
# Practice

例2.一个羰基化合物，经验式为 $C_6H_{12}O$ ，其质谱见下图，判断该化合物是何物。



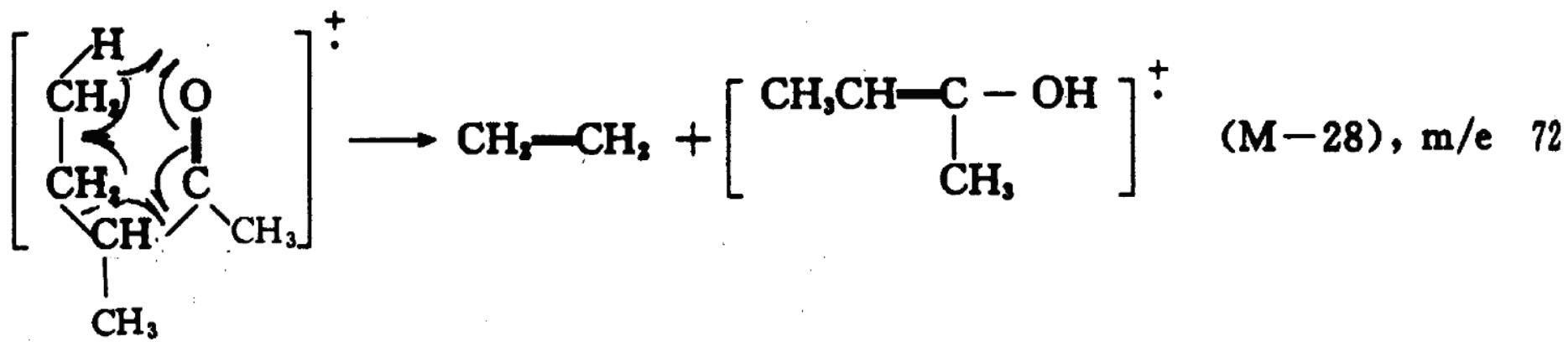
解：图中 $m/z = 100$ 的峰可能为分子离子峰，那么它的分子量则为100。图中其它较强峰有：85，72，57，43等。

•85的峰是分子离子脱掉质量数为15的碎片所得，应为甲基。 $m/z = 43$ 的碎片等于M-57，是分子去掉 $C_4H_9$ 的碎片。 $m/z = 57$ 的碎片是 $C_4H_9^+$ 或者是M-Me-CO。根据酮的裂分规律可初步判断它为甲基丁基酮，裂分方式为：



以上结构中 $C_4H_9$ 可以是伯、仲、叔丁基，能否判断？图中有一 $m/z = 72$ 的峰，它应该是M-28，即分子分裂为乙烯后生成的碎片离子。只有 $C_4H_9$ 为仲丁基，这个酮经麦氏重排后才能得到 $m/z = 72$ 的碎片。若是正丁基也能进行麦氏重排，但此时得不到 $m/z = 72$ 的碎片。

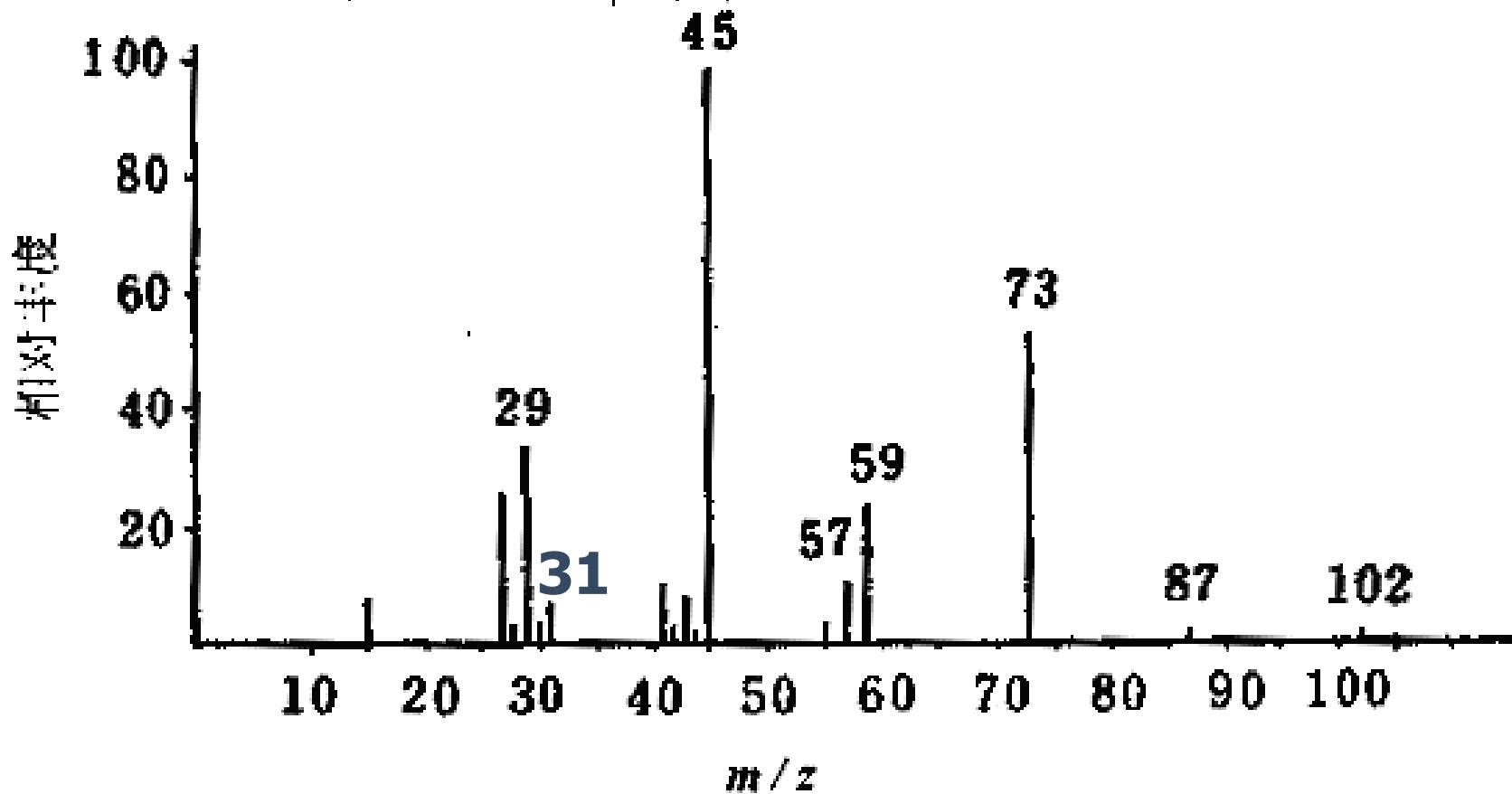
# Practice



- 因此该化合物为3-甲基-2-戊酮。

# Practice

例3. 试由未知物质谱图推出其结构。



# Practice

解：图中最大质荷比的峰为m/z 102，下一个质荷比的峰为 m/z 87，二者相差15u，对应一个甲基，中性碎片的丢失是合理的，可初步确定m/z 102为分子离子峰。

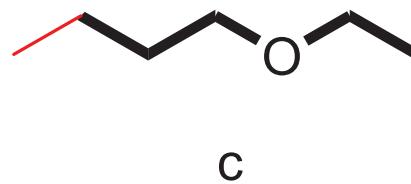
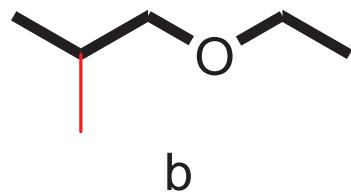
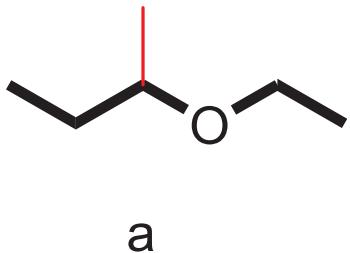
该质谱分子离子峰弱，也未见苯环碎片，由此可知该化合物为脂肪族化合物。

从m/z 31、45、73、87的系列可知该化合物含氧且为醇、醚类型。由于质谱图上无 M-18等有关离子，因此未知物应为脂肪族醚类化合物。结合分子量102可推出未知物分子式为C<sub>6</sub>H<sub>14</sub>O。

从高质量端m/z 87及强峰m/z 73可知化合物碎裂时失去甲基、乙基（剩下的含氧原子的部分为正离子）。

综上所述，未知物的可能结构有下列三种：

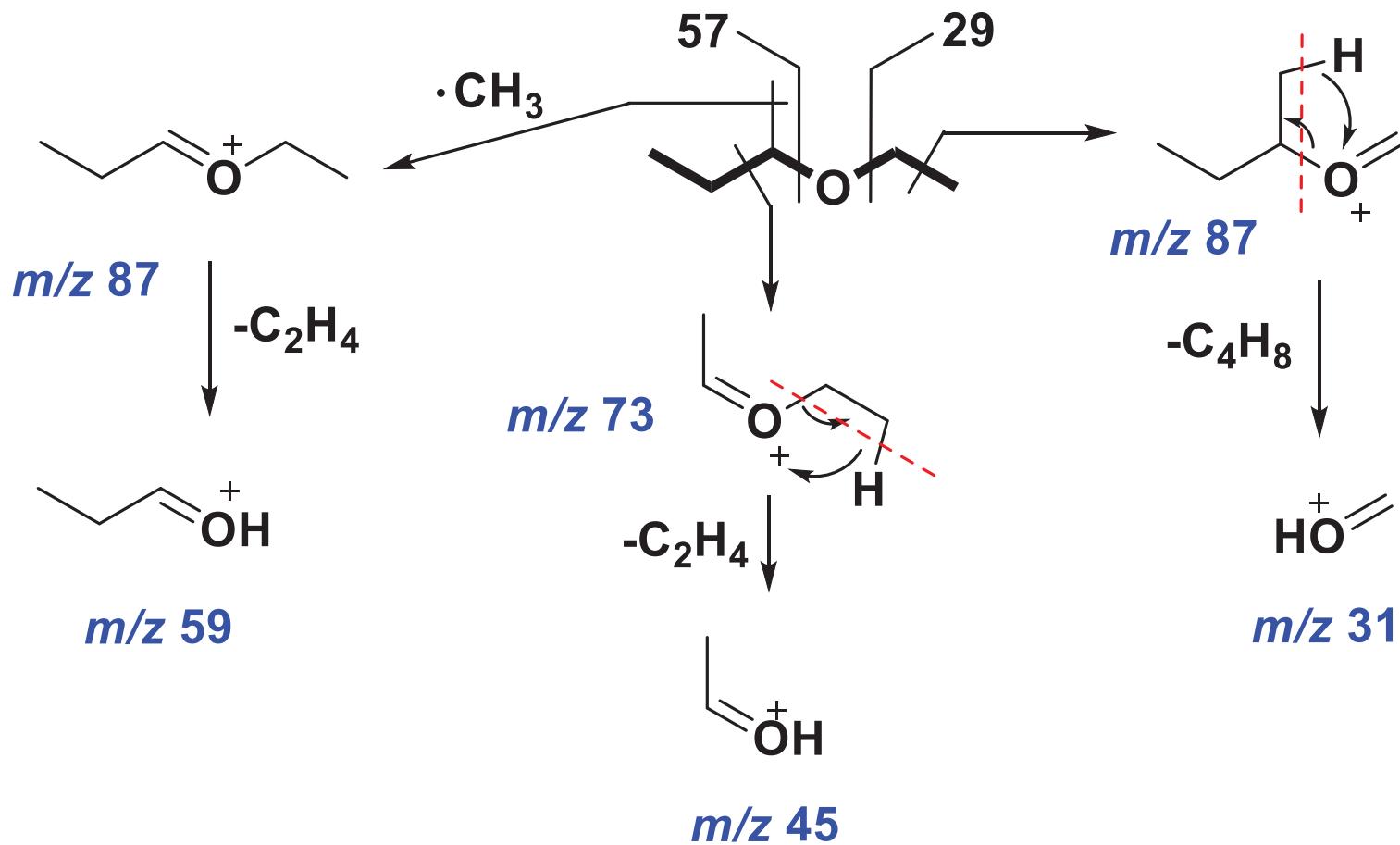
# Practice



$m/z$  59、45分别对应 $m/z$  87、73失28u，可设想这是经四元环氢转移失去 $\text{C}_2\text{H}_4$ 所致。由此可见，未知物结构式为(a)，它产生 $m/z$  59、45的峰，质谱中可见。反之，若结构式为(b)，经四元环氢转移将产生 $m/z$  31的峰，而无 $m/z$  73，45。因此结构式(b)可以排除。而c结构无法产生 $m/z$  73的峰。

# Practice

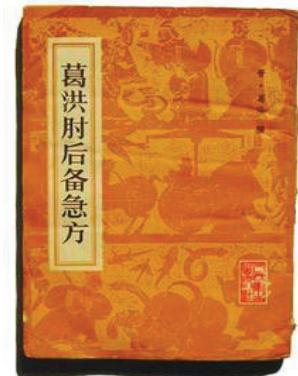
结构式 (a) 的主要碎裂途径:



# MS的应用

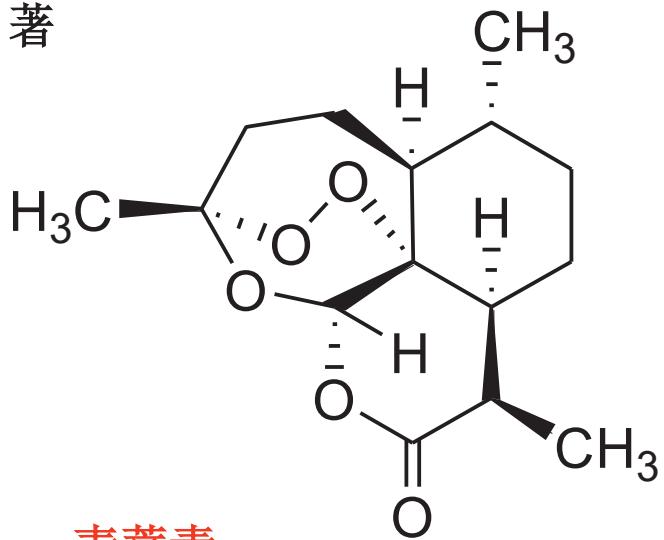


20世纪50年代屠呦呦与老师楼之岑一起研究中药



东晋名医葛洪著

“青蒿一握，以水二升，渍绞取汁，尽服之”可治“久疟”



青蒿素  
单晶结构1976年完成；  
化学学报1979年发表



以身试药的李国桥教授（护士把疟疾病人的血注入李国桥的体内）

# MS的应用

85岁的周维善接受了《科学时报》专访

要了解化合物的结构，首先要测它的分子式和分子量，确定其类型。

“确定分子量需要高分辨率质谱仪，但我们没有，当时只有一个60兆的核磁共振仪和红外光谱。于是，就不得不采用测定分子量的最古老方法，即樟脑冰点降低法，但重复性不好。”周维善说，“后来打听到北京某部有高分辨质谱仪，请该单位做出质谱后，才把分子量定了下来，再结合碳氢分析数据把分子式确定下来，这是一个有15个碳原子、22个氢原子和5个氧原子组成的化合物，我们也证明它是一个倍半萜类化合物。”

下一步就是通过对各种光谱数据的解读，将各个结构单元拼凑起来，也就是说，要凭借大脑将42个原子的结构想象出来。问题是，42个原子有相当多种可能的结构，究竟哪一种是正确的结构呢？这需要靠化学家的尝试、直觉和想象。

在质谱的分析中，他们发现一个特殊的碎片峰M+32，这明显说明分子中存在两个氧原子连在一起的情况，但它们是怎么连在一起的呢？

“为了分析这个M+32峰，我们费了多少脑筋啊！查资料、与同事讨论、与学生讨论。走在路上想，晚上睡觉也在想，有时半夜睡不着，就起来翻书，一直想啊、想啊，头发都想白了。”

# MS的应用

这时，有机所甾体组的吴毓林也对青蒿素产生了兴趣。“他常到隔壁我们的实验室来看看，也提出意见。”吴毓林的妻子李英在中科院上海药物研究所工作，她也参与了“五二三项目”。

1975年4月，李英到成都参加全国“五二三项目”中医中药座谈会。在这个会上，梁晓天报告了另一种抗疟药鹰爪素的结构中也有一个M+32峰值，首次宣布这个峰值是一个过氧基团。李英将这个消息告诉了吴毓林，吴毓林又将它告诉周维善。“这时，我们就知道青蒿素中的这个峰也应该是一个过氧基团，并立即开始做实验来证明。”周维善说。

周维善小组设计了一系列复杂的氧化和还原反应，最终测定出青蒿素的结构。这是一个罕见的含有过氧基团的倍半萜内酯结构，而且，这个药物的分子中不含氮，突破了60多年来西方学者对“抗疟化学结构不含氮(原子)就无效”的医学观念。青蒿素的结构被写进有机化学合成的教科书中，奠定了今后所有青蒿素及其衍生药物合成的基础。

结构测定的工作在1976年就基本结束了，因为卫生部保密的要求，3年后，论文《青蒿素的结构和反应》才发表在1979年5月出版的《化学学报》上，但没有申请专利。

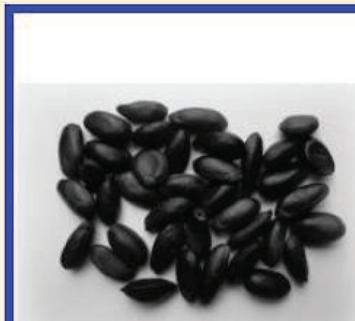
按照国际惯例，如此重要的药物化学结构应该先申请专利、再发表论文。但是，由于历史和体制的种种原因，当时的中国没有专利制度，从政府官员到科学家，基本上都没有知识产权的概念。这是一个遗憾。

## Cytotoxic Bistetrahydrofuran Annonaceous Acetogenins from the Seeds of *Annona squamosa*

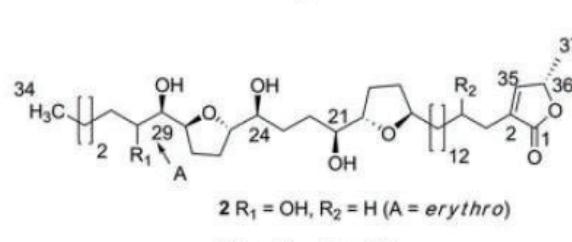
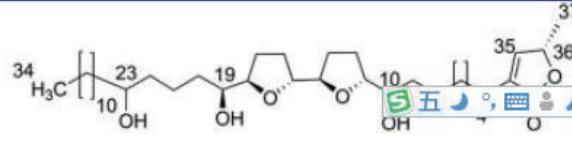
Yong Chen, Jian-wei Chen, and Xiang Li\*

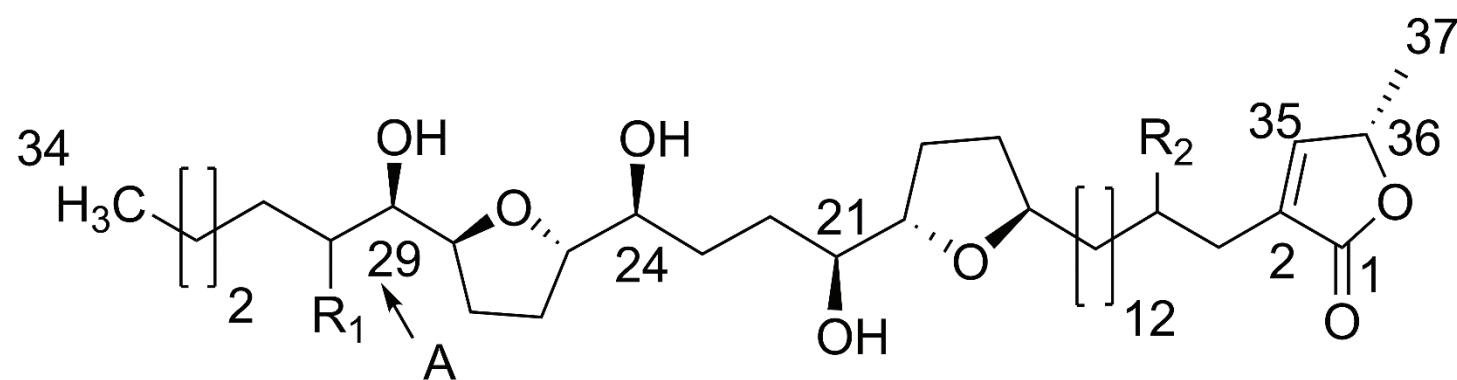
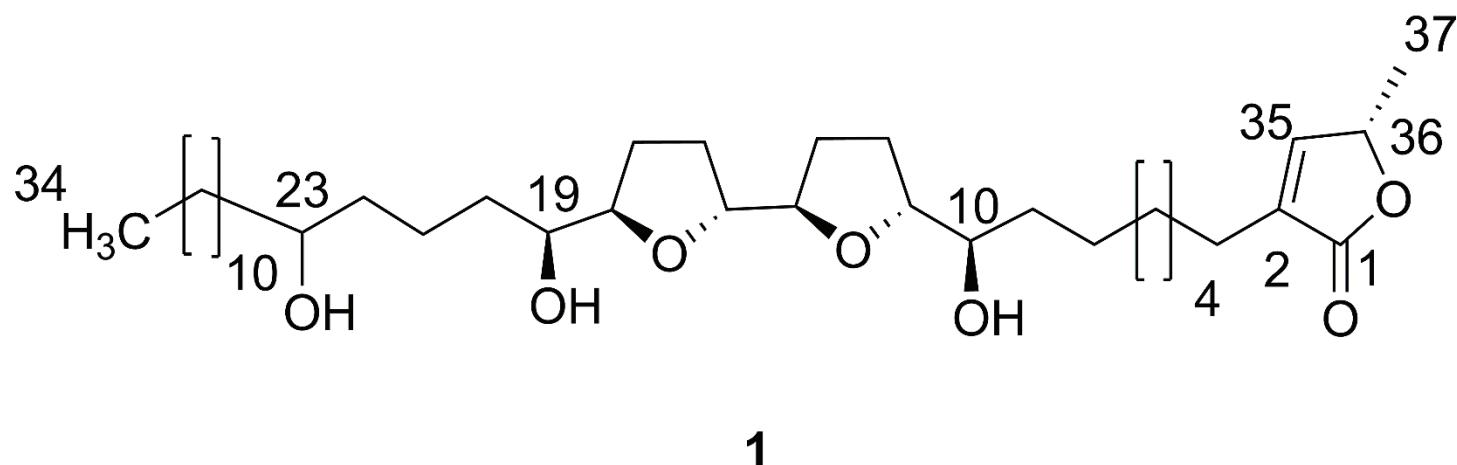
College of Pharmacy, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing, 210046,  
People's Republic of China

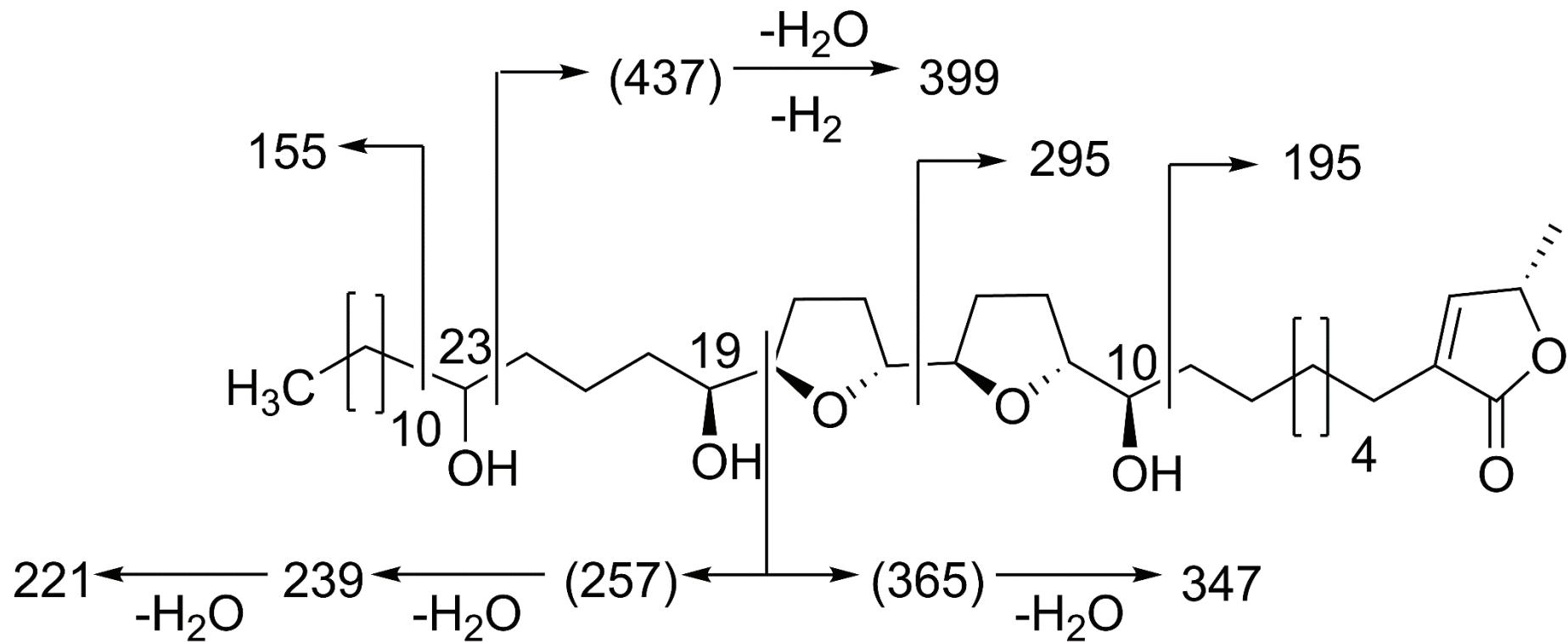
 Supporting Information



*Annona squamosa* seeds







The bis-THF unit with flanking OH groups was placed at C-10 to C-19 according to the EIMS fragment ion peaks at  $m/z$  195, 221, 239, 295, and 347