1. Methodology

http://www.chemguide.co.uk/analysis/masspec/howitworks.html

http://en.wikipedia.org/wiki/Mass_spectrometry

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/MassSpec/masspec1.htm

   1.1. Ionize sample in gas phase
   1.2. Depending on ionization method and sample- mixture of ions with different mass
   1.3. determine mass – molecular weight, formula, structure
   1.4. separation of ions by mass
      1.4.1. m/z = measure mass-to-charge ratio
      1.4.2. 200 Mass with +2 charge has same m/z as 100 mass of +1 charge
   1.5. path in magnetic and electric field depends on mass and charge particle
   1.6. acceleration to specific velocity
      1.6.1. **F** = m**a**, **a** = **F**/m
      1.6.2. **F** = z**E, E** = magnitude and direction of electric field
      1.6.3. **a** = z**E**/m
   1.7. Most mass spectrometers have a method to separate mixtures of compounds
      1.7.1. Sample does not have to be pure to determine MW, formula, structure
      1.7.2. MS interfaced to chromatograph- gas or liquid
2. Mass Spectrum EI, electron impact causes ionization and fragmentation
      2.1.1. Ionization forms molecular ion and fragments
      2.1.2. $CH_3OH + e^- \rightarrow CH_3OH^{\bullet +} (m/z\ 32) + 2e^-$ (orbitals?)
      2.1.3. Fragments to radical and cation
         2.1.3.1. $CH_3OH^{\bullet +} \rightarrow CH_2OH^+ (m/z\ 31) + H^\bullet$ (orbitals?)
         2.1.3.2. $CH_3OH^{\bullet +} \rightarrow CH_3^+ (m/z\ 15) + OH^\bullet$
      2.1.4. Fragmentation depends on bond energies and fragment stabilities
      2.1.5. base peak – largest peak set to 100%
      2.1.6. molecular ion peak, M+
      2.1.7. Figure 1.1
3. Ionization methods
   3.1. EI, electron impact

3.1.1. Electron beam, velocity = 70 ev kinetic energy
3.1.2. 15 ev ionization potential typical most molecules
3.1.3. Excess energy, 2 electrons plus disruption of bonds (3-10 ev)
3.1.4. Fragmentation pattern: intensity/masses reproducible at standard condition
3.1.5. Molecular ion often not detected, why? Fix?
3.1.6. EI-MS, >400,000 published spectra, computer searchable

3.2. CI, chemical ionization
3.2.1. Reagent gas like CH_4 is ionized
3.2.2. CH_5^+ is formed (and other cations)
3.2.3. CH_5^+, transfers a proton during low energy collision (5 ev)
3.2.4. $[M+1]^+$ often is base peak, fragmentation less likely
3.2.5. $[M-1]^+$ by hydride abstraction, what is abstraction product?
3.2.6. $[M+15]^+$ CH_3^+ addition
3.2.7. other ion additions $[M+29]^+$, $[M+41]^+$ how?
3.2.8. Compare EI and CI for 3,4-dimethoxyacetophenone, Figure 1.3

3.3. Desorption methods – non-volatile samples
3.3.1. Sample diluted in solid or non-volatile liquid
3.3.2. FAB – fast atom bombardment- collision with argon or xenon (6-10 ev)
3.3.3. LSIMS- liquid secondary ionization mass spectrometry- Cs^+ (10-30 ev)
3.3.4. MALDI – matrix assisted laser desorption ionization – matrix molecules like nicotinic acid vaporize absorb pulsed laser radiation to protonate anylate -600,000 MW

3.4. ESI – Electrospray Ionization Figure 1.7
3.4.1. Sample in solution introduced via fine capillary directly into vacuum
3.4.2. Capillary has high voltage
3.4.3. Droplets form with excess charge
3.4.4. Evaporation creates high charge density
3.4.5. Coulombic repulsion – liberates charged analyte
3.4.6. Useful for proteins that have multiple protonation
3.4.7. Single fragment can have several charges and M/z ratios
3.4.8. 40 charges on a single peptide are common

3.4.9. Two ions for same M_s fragment, differ by single charge: $[M_s+zH^+]$ and $[M_s+(z+1)H^+]$

3.4.10. The two peaks appears, $m_1 = (M_s+zH)/z$ and $m_2 = (M_s+(z+1)H)/(z+1)$

3.4.11. Eliminate M_s and solving for z

3.4.11.1. : $zm_1 - zH = M_s$

3.4.11.2. $(z+1)m_2 - (z+1)H = M_s$

3.4.11.3. $zm_1 – zH = (z+1)m_2 - (z+1)H$

3.4.11.4. $z(m_1-m_2) = m_2 –H \Rightarrow z = \frac{m_2 - H}{m_1 - m_2}$

3.4.11.5. computer programs can compare peaks to see which can originate from the same fragment with different number of protons

3.4.12. ESI is useful for determining amino acid composition and sequence

3.4.13. Peptides tend to fragment at amide backbone Figure 1.7

4. Mass analyzer

4.1. acceleration of ions in electric field to a velocity depends on mass and charge

4.2. ion trajectory deflected by electric or magnetic field

4.3. extent of deflection depends on velocity

4.4. fragments with same charge and mass have same velocity

4.5. fragments with same charge have velocities inversely dependent on mass: **a** = z**E**/m

4.6. magnetic sector analyzer

4.6.1. kinetic energy of particle accelerated through a voltage drop V: $KE = zV = mv^2/2$

4.6.2. deflecting force on charged particle moving through a magnetic field: **F** = **v**x**B**

4.6.3. the radius of the path in the magnetic field is related to the velocity and field (Introduction to spectroscopy By Pavia, Lampman, Kriz)

4.6.4. $r = mv/zB \Rightarrow v = rzB/m$: rearrange and substitute for v in the KE expression

4.6.5. $zV = m(r^2z^2B^2/m^2)/2 \Rightarrow m/z = r^2B^2/2V$

4.6.6. for a fixed radius in a spectrometer, this equation indicates the field or voltage can be scanned to select for different m/z.

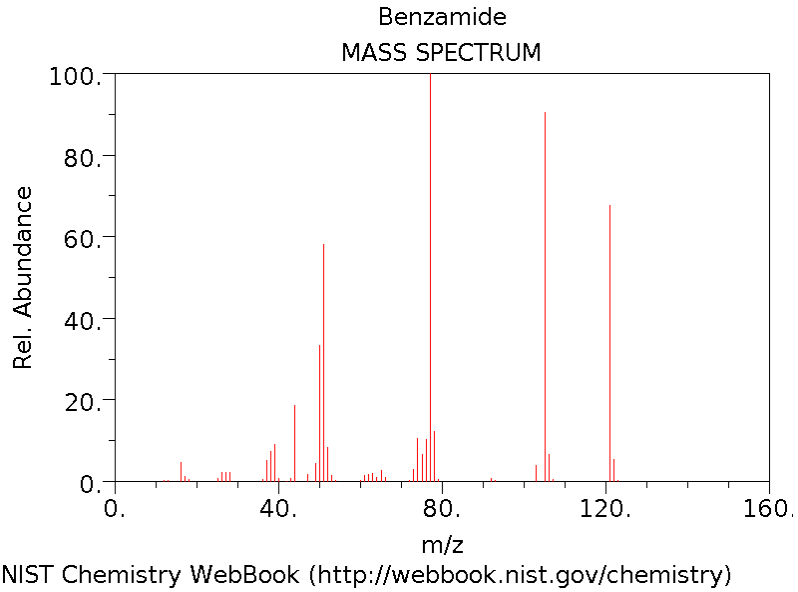
4.7. quadrapole analyzer – inexpensive

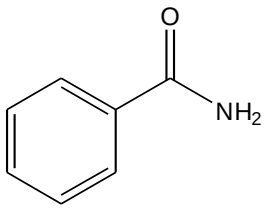
4.7.1. oscillating fields makes ion spiral through the analyzer

4.7.2. incorrect m/z spiral out of the channel and do not get detected

4.8. ion trap analyzer
    4.8.1. all ions can be trapped in cavity and then ions are ejected for specific m/z
4.9. Time of flight
    4.9.1. Ions accelerated in electric field have velocities dependent on mass
    4.9.2. Analyzer based on measuring time ion travels through predetermined distance
4.10. Fourier Transform MS
    4.10.1. All ions are trapped in magnetic field and accelerating the ions alternately with broadband rf electric field makes the ions move in larger and larger circles
    4.10.2. Different m/z absorb different frequencies
    4.10.3. When rf field is off, ions continue to oscillate at frequencies based on m/z
    4.10.4. Ions induce current in detectors at different frequencies

5. identification of M+
    5.1. may not be present or small if unstable
    5.2. “nitrogen rule” even mass for even number of N, odd mass for odd # of N
    5.3. cation and neutral fragments traced to M+ (77 + 44: C_6H_5 and $CONH_2$) Figure 1.1
    5.4. Chemical Ionization (CI) avoids high energy of electron impact (EI)
        5.4.1. M+1 peak is very large due to proton addition (CH_4: M+29 for $C_2H_5^+$ addition)
    5.5. M+ stability
        5.5.1. aromatics > alkenes > cyclic > sulfide > short alkanes
        5.5.2. Ketones > amines > esters > ethers > carboxylic acids > aldehydes ~ amides ~halides
        5.5.3. Bond energies: http://web.chem.ucsb.edu/~zakariangroup/11---bonddissociationenergy.pdf
        5.5.4. M-15 (CH_3) M-18 (H_2O) M – 31 (OCH_3) suggest M+ is molecular ion
        5.5.5. Not often observed for alcohols, nitrates, nitro, nitriles, highly branched
6. Molecular formula
    6.1. obtain from molecular ion peak
    6.2. add masses of most abundant isotopes





6.2.1. M+ = 121: page 50 in SWK (61 in pdf)

7 x ^{12}C = 84, 7 x ^{1}H = 7, 1 x ^{14}N = 14, 1 x ^{16}O = 16

6.3. M+1, M+2, caused by other isotopes

6.3.1. M+1 is about 8% of M+ reproducible?

6.3.2. ^{13}C has relative abundance of 1.1% of ^{12}C

6.3.3. M+1 peak should be 7 x 1.1 = 7.7% due to presence of ^{13}C

6.3.4. ^{15}N has relative abundance of 0.38% of ^{14}N

6.3.5. contribution of N and C is 8.1%, reproducible?

6.4. isotopic peaks can often be used to determine molecular formula

6.5. how would you know silicon is not contributing to M+1?

element	isotope	Relative abundance	isotope	Relative abundance	isotope	Relative abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	0.016		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100			^{18}O	0.2
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
^{31}phosphorous	^{31}P					

Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0
iodine	^{127}I	100				

6.6. high resolution MS see Table 1.4, page 15 SWK (pdf page 26)

6.6.1. masses are not exact whole numbers, ^{14}N = 14.0031

6.6.2. $^{12}CH_2$ = 12.0000 + 2 x 1.0078= 14.0156

6.6.3. so molecular formulas can often be determined by high resolution MS

6.6.4. look up mass 121 page 50 in SWK (61 in pdf): 15 entries with different formulas and mass

6.6.5. $C_2H_5N_2O_4$ = 121.0249, $C_2H_7N_3O_3$ =121.0488 … C_9H_{13} = 121.1018

6.6.6. $$$$$ 600k

6.6.7. Determine molecular formulas for 114.0651, 141.081, 201.2104

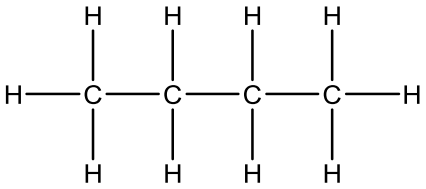
7. Degree of unsaturation = index of hydrogen deficiency = # of double bonds and rings

7.1. $IHD = \#C - \frac{\#H}{2} - \frac{\#X}{2} + \frac{\#N}{2} + 1$. X = halogens (monovalent)

7.2. Eliminate possible structures

7.3. could indicate olefin, aromatic, C=Y, confirm by IR or NMR, hydrogenation

7.4. Butane, C_4H_{10}, IHD = 4- 10/2 +1 = 0

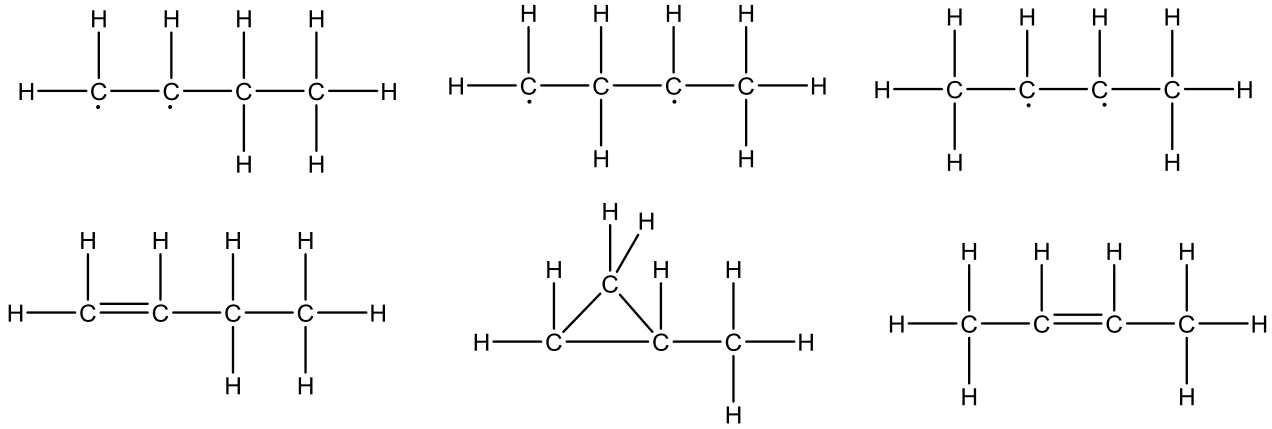


7.5. Each interior carbon has 2H

7.6. Each terminal carbon has 2H + 1

7.7. Total #H = 2#C + 2 = 2*4 +2 = 10, alkane formula = C_nH_{2n+2}

7.8. Remove two hydrogen atoms



7.8.1. Formally make a diradical

7.8.2. Join radicals to make alkene or ring: one double bond or ring

7.8.3. Hydrogen deficiency of 2H (relative to alkane) leads to one ring or double bond

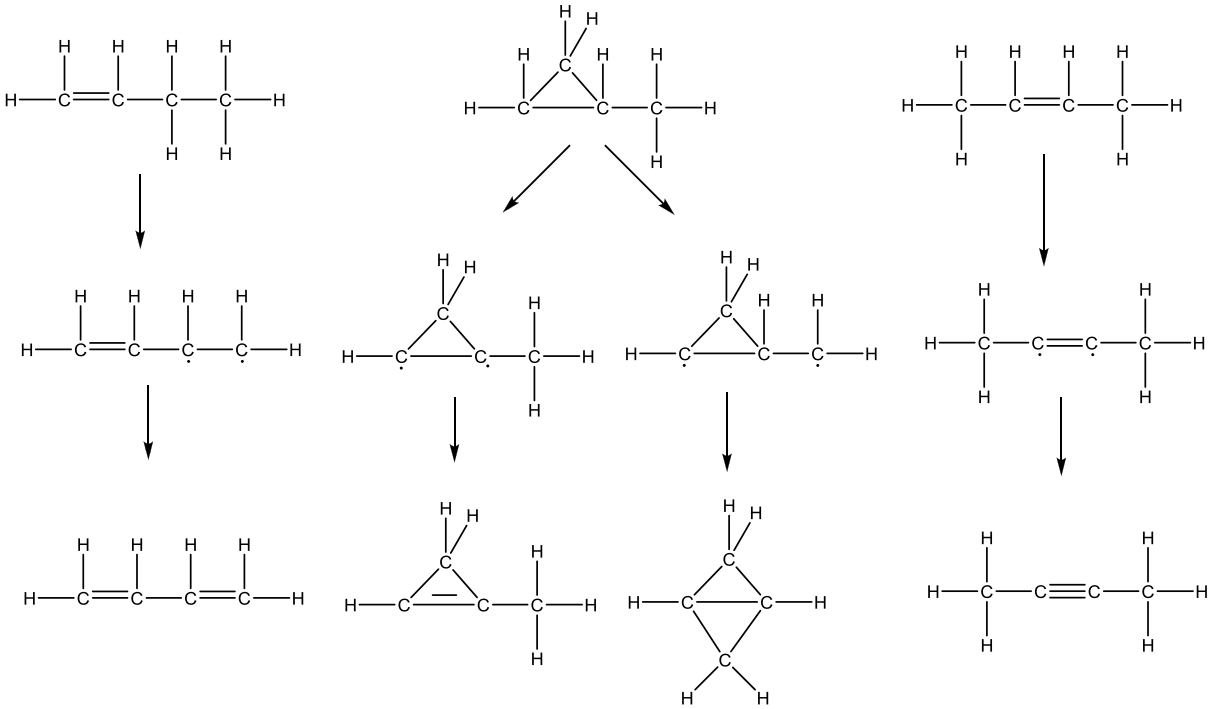
7.8.4. IHD = 1 for every double bond or ring or for every 2H missing

7.9. IDH = #H missing/2

7.10. Missing #H = 2#C+2 - #H

7.11. Divide equation by 2: $IHD = \#C - \frac{\#H}{2} + 1$

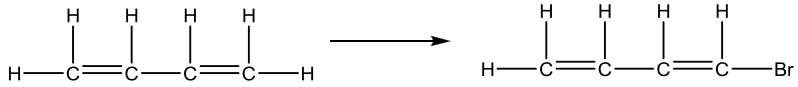
7.12. Remove 4H: 2 double bonds and rings, other possibilities



7.13. Effect of halogens: replaces H

7.13.1. $IHD = \#C - \frac{\#H}{2} - \frac{\#X}{2} + 1$

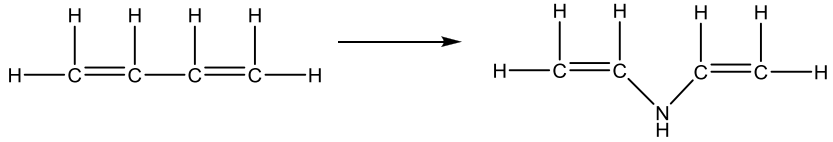
7.13.2. IHD = 4 -5/2 – ½ +1 = 2 same as non halogenated



7.14. Effect of nitrogen, adds one hydrogen

7.14.1. $IHD = \#C - \frac{\#H}{2} - \frac{\#X}{2} + \frac{\#N}{2} + 1$

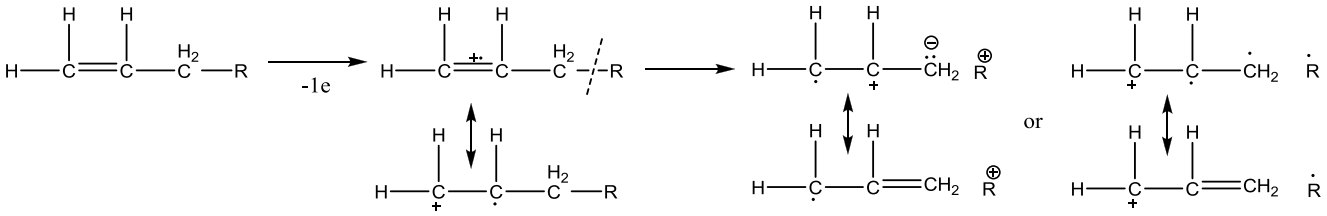
7.15. IHD = 4 – 7/2+1/2 +1



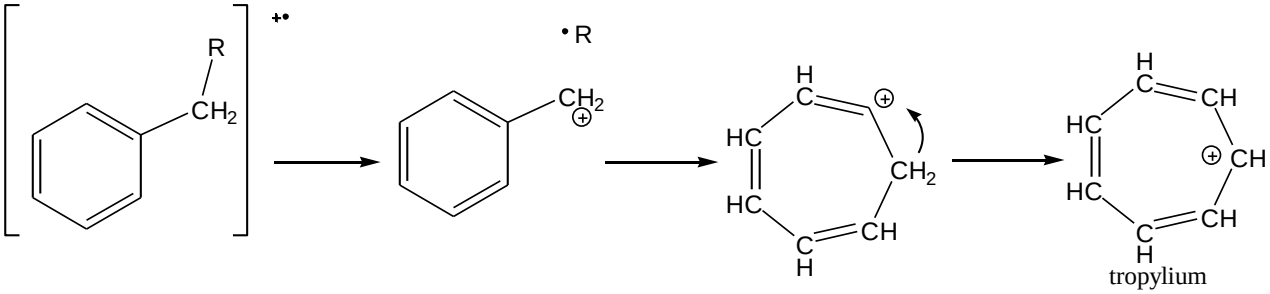
7.16. Affect of oxygen? sulfur?, phosphorous? Silicon?

7.17. Calculate IHD and show two structures: $C_{10}H_{17}Br$ (1b), $C_{13}H_{18}O$ (1c), $C_{13}H_9NO_3$ (1i)

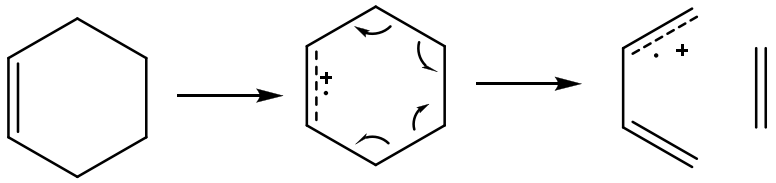
8. Ion formation – general observations for electron impact (EI)
   - 8.1. form even M+ if #N is even, heptane
   - 8.2. M+ are odd masses if #N is odd
   - 8.3. M+ intensity decreases with branching: cleavage favored by branching, examples?
   - 8.4. M+ intensity decreases with molecular weight
   - 8.5. double bonds and rings stabilize M+
9. Fragment ions
   - 9.1. Electron impact can remove most any valence electron
     - 9.1.1. Electrons appear to rearrange to form most stable ions
     - 9.1.2. Highest occupied orbitals will have a vacancy
     - 9.1.3. Butane? Butene? Pentanol? Phenol?
   - 9.2. Structure of initial radical cation suggests possible fragments
   - 9.3. Cleavage favored at branching points
     - 9.3.1. Form most stable cation
     - 9.3.2. $CH_3^+ < CH_2R^+ < CHR_2^+ < CR_3^+$
   - 9.4. double bonds promote allylic cation formation.
     - 9.4.1. sometimes both cation and radical paths occur, 2 peaks add up to M+
     - 9.4.2. never see radicals



9.5. tropylium ion <u>91 m/z</u> forms from alkylbenzenes

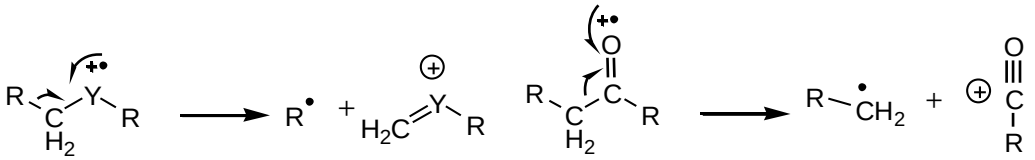


9.6. Unsaturated rings undergo reverse Diels-Alder addition



9.7. β cleavage to heteroatoms

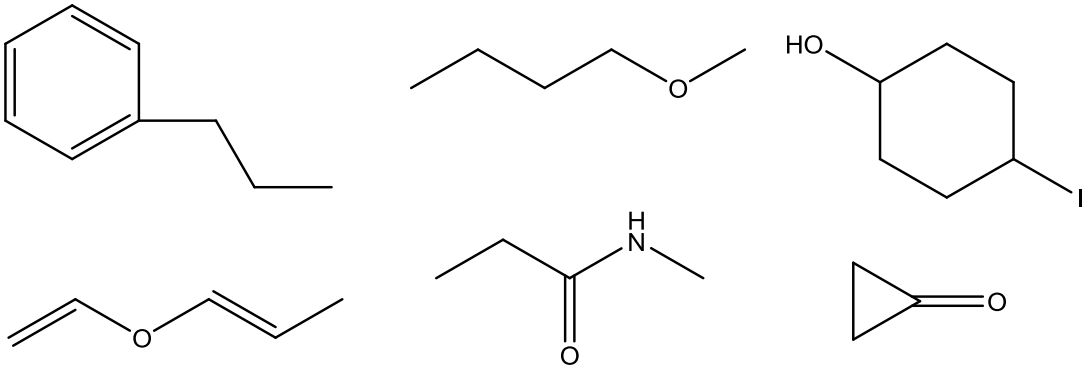
9.7.1. “ionize” lone pair



9.8. elimination of neutral, stable, small molecules

9.8.1. H_2O, H_2S, CO, CO_2, HCN

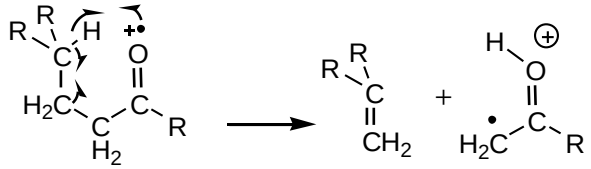
9.8.2. Suggest M+ structures and initial cleavage



9.9. McLafferty rearrangement

9.9.1. Usually occurs when carbonyl and γ hydrogen present

9.9.2. An even mass ion is formed



9.10. Appendix B epage 79– common fragment ions: 43- C_3H_7, $CH_3C{=}O$, C_2H_5N

9.11. Appendix C epage 81 – neutral common fragments lost – not observed

10. Hydrocarbons Figure 1.14

10.1. alkanes clusters of peaks, differ by 14 units

10.1.1. C_nH_{2n+1}, m/z = 14n +1

10.1.1.1. applies to straight chain (*i. e.*, $CH_3(CH_2)_n$) and branched alkyl fragments

10.1.1.2. branching decrease M+, fragmentation at branch point (stable cation)

11. alkenes cleave to give stable fragments Figure 1.16 (double bond migration)

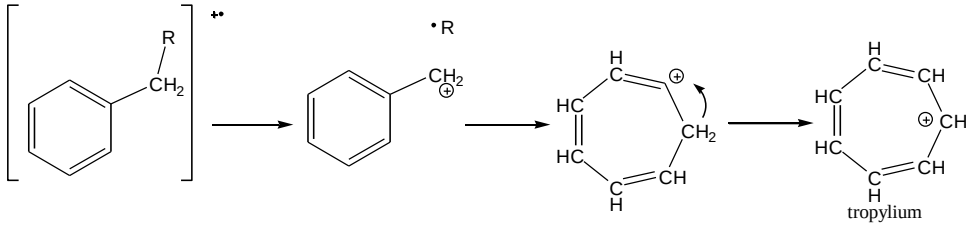
11.1.1. allylic – radicals and cations

11.1.2. identify ion with arrows, radical pair, how they are formed

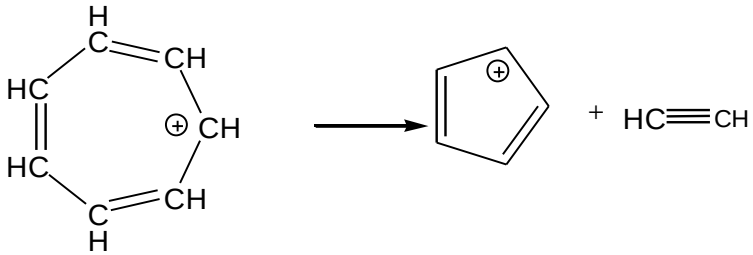
12. aromatics

12.1.1. strong M+, phenyl, C_6H_5: 77 m/z

12.1.2. alkyl arene – 91 m/z for tropylium



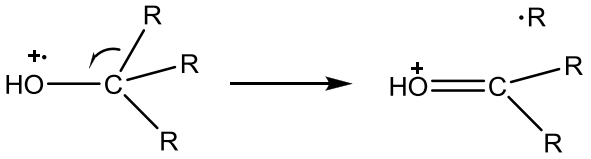
12.1.3. 65 m/z for acetylene elimination from tropylium



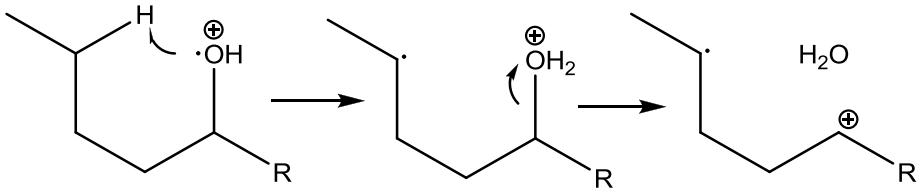
13. alcohols and ethers

13.1. alcohol and ether molecular ions readily cleave, M+ small or undetected

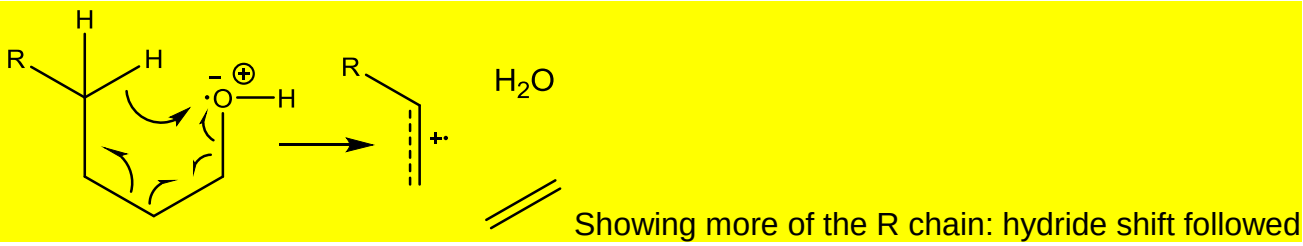
13.2. β cleavage results in m/z = 31 for primary alcohols



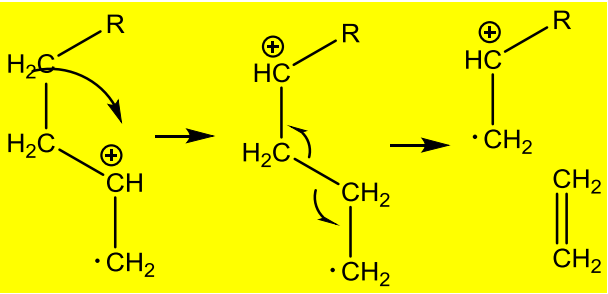
13.3. M-18, water elimination: radical abstraction



13.4. Water and alkene elimination: hydride transfer (abstraction)



Showing more of the R chain: hydride shift followed CC cleavage to make new terminal vinyl cation.



13.5. Compare pentanols in Figure 1.18

13.5.1. Linear versus branched

13.5.2. Base peaks?

13.5.3. β-cleavage, Water, water + alkene? Which is favored? Why?

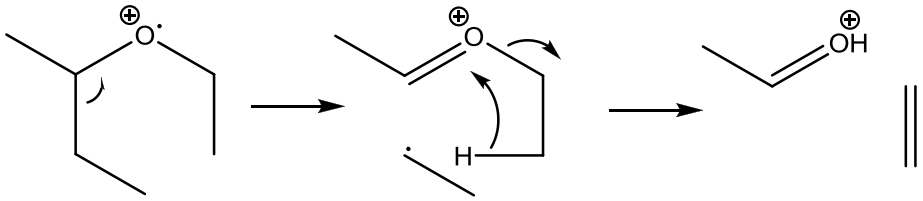
13.5.4. Complimentary peaks

13.5.4.1. Pentanol: 31 and 57

13.5.4.2. 2 pentanol: 45 and 43

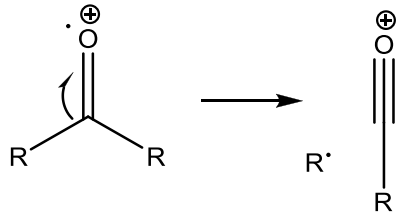
13.5.4.3. 2-methyl-3-butanol: 59 and 29

13.6. ethers are similar β cleavage, then alkene cleavage

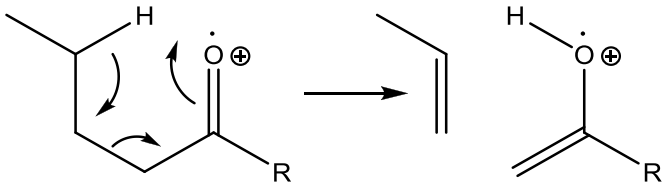


102 29 and 73 45

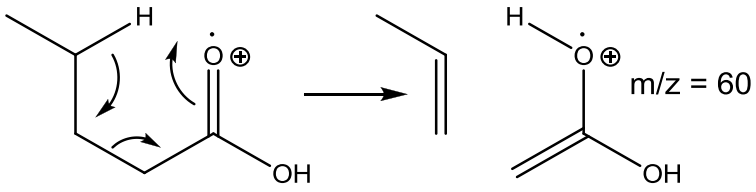
14. Aliphatic ketones
    14.1. M+ normally observed
    14.2. Acyl cleavage - (α to carbonyl)
        14.2.1. M+ – loss of non-bonding electron
        14.2.2. Form radical and acylium ion



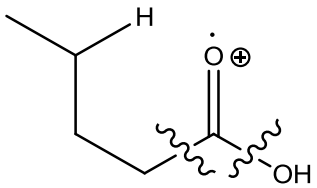
    14.3. McLafferty rearrangement
        14.3.1. γ-CH abstraction followed by reverse Diels Alder
        14.3.2. Neutral olefin and enol cation radical



15. Aldehydes figure 1.24 for nonanal
    15.1. Like ketones – strong M+
    15.2. also M-1 peak
16. Carboxylic acids See figure 1.25
    16.1. Weak M+
    16.2. McLafferty rearrangement



16.3. α-cleavage to CO



M-17 (M - OH)

M-45 (M - CO_2H)

16.4. Aliphatic chains show mass 14 interval clusters, prominent $C_nH_{2n-1}O_2$

16.4.1. Acid formula $C_nH_{2n}O_2$, loose what? Formula?

17. Carboxylic acid esters

17.1. McLafferty rearrangement

17.2. α-carbon cleavage is common

17.3. If the **acid function** dominates, like carboxylic acid aliphatic chains show mass 14 interval clusters, prominent $C_nH_{2n-1}O_2$

17.4. If the **alcohol function** dominates, eliminates acid like alcohols eliminate water.

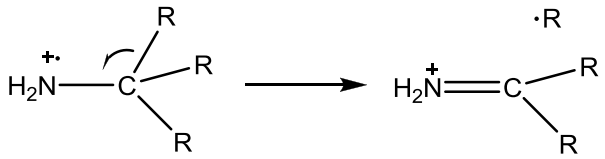
18. Predict fragmentation pattern for Exercise 1.1 (epage 50): a, c, d, e, f, g

19. Amines

19.1. alicylic M+ weak, cyclic is strong

19.2. Like alcohols, amine undergo β-cleavage

19.3. For primary amine, M-1 commonly observed



20. Amides

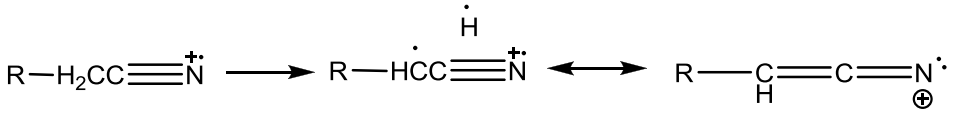
20.1. McLafferty rearrangement- m/z 59, $H_2NC{=}OH^+CH_2$•

20.2. α-C-C cleavage – yields 44 m/z = $CONH_2$

21. Nitriles

21.1. Weak M+

21.2. M-1



22. Nitro compounds

22.1. Aliphatic: weak M+, NO^+ (m/z 30) and NO_2^+ (m/z 46), hydrocarbon fragments

22.2. Aromatic – strong M+, NO_2^+ (m/z 46), phenoxy cation with NO loss (m/z 30)

23. Sulfur compounds

23.1. ^{34}S contribution to M+2 with 4.4%

23.2. Thiols – M+ normally strong, β-cleavage ($CH_2{=}SH^+$, m/z = 47)

23.2.1. M-34: Eliminate H_2S, What is other fragment? $RC^+H_2CH_2\cdot$

23.2.2. Further fragmentation like alcohols

23.3. Sulfides M+ normally strong

23.3.1. Fragmentation similar to ethers

23.3.2. Can be distinguished from thiols by missing M-H_2S or M-SH peaks

24. Halogens

24.1. Chlorinated and brominated compounds have distinctive M+:M+2:M+4:M+6 patterns

Chlorine	^{35}Cl (100)	^{37}Cl (32.5)
Bromine	^{79}Br (100)	^{81}Br (98.0)

24.2. Figure 1.29 and Table 1.5

24.3. Patterns extend to fragment

24.4. Such large M+2 peaks would require a lot of carbons

25. Work 1.6 g, epage – 50, 98.0763