Mass Spectrometry

Information from Mass Spectrometry

The molecular mass

The molecular formula

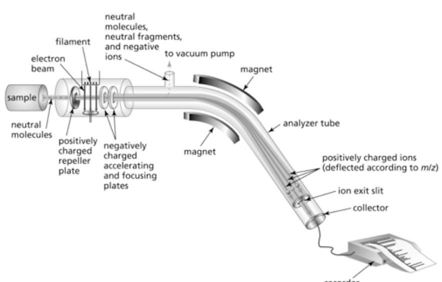
Structural features of a compound

The exact structure when compared to known structures (digital libraries).

A Mass Spectrometer

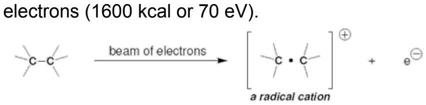
http://my.rsc.org/video/52

The mass spectrometer records a mass spectrum



Mass Spectrometry

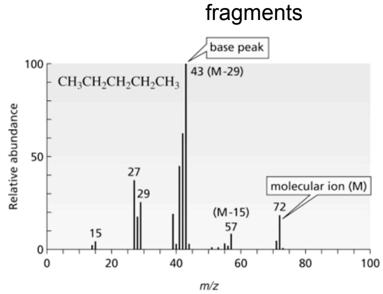
The sample is bombarded with a beam of high energy electrons (1600 kcal or 70 eV).



If the radical cation remains intact, it is known as the molecular ion ($M^{+\bullet}$) or parent ion.

The resulting fragments may undergo further fragmentation.

A mass spectrum records only positively charged fragments



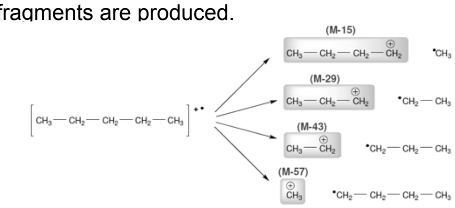
m/z	Relative abundance
73	0.52
72	18.56
71	4.32
57	11.20
43	100.00
42	55.27
41	37.93
39	12.44
29	26.65
28	17.75
27	31.22
15	4.22
14	2.56

m/z = mass to charge ratio of the fragment

The molecular ion (or parent ion) peak is a radical cation M+· as a result of removing one electron from the molecule.

Fragmentation

- Fragmentation is more prevalent when more stable fragments are produced.

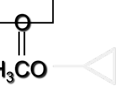


- *Nominal molecular mass*: the molecular mass to the nearest whole number
- Each *m/z* value is the nominal molecular mass of the fragment
- The peak with the highest *m/z* value usually represents the molecular ion (M)
- Peaks with smaller *m/z* values—called fragment ion peaks—represent positively charged fragments of the molecule

High Resolution Mass Spectrometry (MS)

- High resolution MS allows m/z to be measured with up to 4 decimal places.
- Masses are generally not whole number integers:
  1 proton = 1.0073 amu and 1 neutron = 1.0086 amu
- One ^{12}C atom = exactly 12.0000 amu, because the amu scale is based on the mass of ^{12}C.
- All atoms other than ^{12}C will have a mass in amu that can be measured to four decimal places by a high-res MS instrument.

Exact Molecular Weights
High Resolution MS

$CH_3(CH_2)_5CH_3$ — CH_3CO 

	Heptane	Cyclopropyl acetate
Molecular formula	C_7H_{16}	$C_5H_8O_2$
Molecular weight	100	100
Exact mass	100.1253	100.0524

High resolution mass spectrometry can provide exact masses and molecular formulas. See: *http://www.chm.davidson.edu/java/beynon/beynon.html*

Exact Molecular Weights
High Resolution MS

http://www.chm.davidson.edu/java/beynon/beynon.html

Exact mass 170.0134

Molecular weight 170

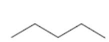
Molecular formula $C_8H_7ClO_2$

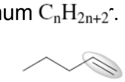
Degrees of Unsaturation

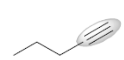
- Using high resolution MS a molecular formula of an organic compound can be determined.
- A molecular formula will suggest the possible number of double bonds, triple bonds and rings in the structure. Alkanes are *saturated*, having a maximum number of hydrogen atoms. C_nH_{2n+2}

Degrees of Unsaturation

- Each π bond or ring removes 2 hydrogen atoms from the maximum C_nH_{2n+2}.


C_5H_{12}


C_5H_{10}


C_5H_8

- Each removal adds a “degree of unsaturation” and decreases the number of H atoms by two.

Hydrogen Deficiency Index (Degree of Unsaturation)

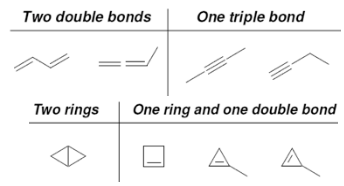
Relates to multiple bonds and rings

hydrogen deficiency Index (HDI) =

$$\frac{1}{2}\text{(molecular formula of alkane – molecular formula of compound)}$$

Degrees of Unsaturation

- Consider C_4H_6.
- How many degrees of unsaturation are there?
- 1 degree of unsaturation = 1 unit on the hydrogen deficiency index (HDI); *Possibilities:*



Degrees of Unsaturation

- NOTE: a halogen is treated as if it were a hydrogen atom.

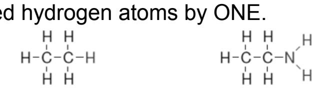
ethane **chloroethane**

- How many degrees of unsaturation are there in C_5H_7Br?
- An oxygen atom does not affect the degree of unsaturation

ethane **ethanol**

Degrees of Unsaturation

- NOTE: a nitrogen increases the number of expected hydrogen atoms by ONE.



ethane **ethyl amine**

- How many degrees of unsaturation are there in C_5H_8BrN?
- The formula below can be used:

$$\text{HDI} = (2C + 2 + N - H - X) / 2$$

Question

Calculate the degrees of unsaturation (HDI: hydrogen deficiency index) for each of the following.

A. $C_{20}H_{40}$

B. $C_{16}H_{24}O$

C. $C_8H_{10}Br_2Cl_4$

D. $C_9H_{14}Br_3O_2N$

A. A = 1; B = 4; C = 2; D = 2
B. A = 1; B = 5; C = 1; D = 2
C. A = 2; B = 4; C = 1; D = 2
D. A = 2; B = 5; C = 2; D = 1
E. A = 2; B = 5; C = 1; D = 2

Examples

Knowing that the molecular formula of a substance is C_7H_{16} tells us immediately that is an alkane because it corresponds to C_nH_{2n+2}.

C_7H_{14} lacks two hydrogens of an alkane, therefore contains either a ring or a double bond.

Example 1

C_7H_{14}

Index of hydrogen deficiency

$= \frac{1}{2}$ (molecular formula of alkane – molecular formula of compound)

$= \frac{1}{2} (C_7H_{16} – C_7H_{14})$

$= \frac{1}{2} (2) = 1$

Therefore, one ring or one double bond.

Example 2

C_7H_{12}

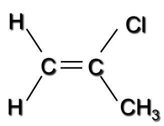
$= \frac{1}{2} (C_7H_{16} – C_7H_{12})$

$= \frac{1}{2} (4) = 2$

Therefore, two rings, one triple bond,
two double bonds, or one double bond + one ring.

If Halogen is Present

Treat a halogen as if it were hydrogen.



C_3H_5Cl

Same index of hydrogen deficiency as for C_3H_6.

Oxygen Has No Effect

$CH_3(CH_2)_5CH_2OH$ (1-heptanol, $C_7H_{16}O$) has same number of H atoms as heptane.

Index of hydrogen deficiency =

$\frac{1}{2} (C_7H_{16} – C_7H_{16}O) = 0$

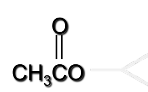
No rings or double bonds.

Question

What is the hydrogen deficiency in a compound with a molecular formula $C_7H_5Cl_3O$?

A) 2
B) 4
C) 6
D) 8
E) 10

Oxygen Has No Effect



Cyclopropyl acetate

Index of hydrogen deficiency =

$\frac{1}{2} (C_5H_{12} – C_5H_8O_2) = 2$

One ring plus one double bond.

Question

How many rings plus multiple bonds are there in a compound with a molecular formula $C_7H_5Cl_3O$?

A) 1
B) 2
C) 3
D) 4
E) 5

Rings versus Multiple Bonds

Index of hydrogen deficiency tells us the sum of rings plus multiple bonds.

Catalytic hydrogenation tells us how many multiple bonds there are.

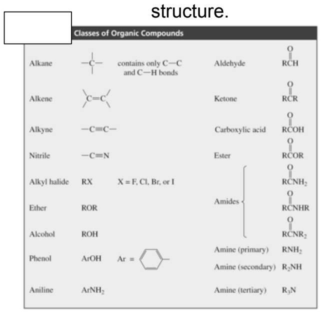
Question

A compound with a molecular formula $C_7H_5Cl_3O$ is catalytically hydrogenated. It consumed 3 moles of hydrogen. How many rings and double bonds does the molecule respectively have?

A) 3 rings; no double bonds
B) 3 rings; 1 double bond
C) 1 rings; 3 double bonds
D) no rings; 3 double bonds
E) None of the above

•IR is used to determine functional groups.

•A molecular formula plus IR data can produce a possible structure.



Classes of Organic Compounds

Alkane	—C—	contains only C—C and C—H bonds	Aldehyde	RCH (C=O)
Alkene	C=C		Ketone	RCR (C=O)
Alkyne	—C≡C—		Carboxylic acid	RCOH (C=O)
Nitrile	—C≡N		Ester	RCOR (C=O)
Alkyl halide	RX	X = F, Cl, Br, or I	Amides	$RCNH_2$ (C=O)
Ether	ROR		Amides	RCNHR (C=O)
Alcohol	ROH		Amides	$RCNR_2$ (C=O)
Phenol	ArOH	Ar = (phenyl)	Amine (primary)	RNH_2
			Amine (secondary)	R_2NH
Aniline	$ArNH_2$		Amine (tertiary)	R_3N

Question

A molecule has a molecular ion m/z = 112.0888, intensity = 100%; M+1 = 8.075%; M+2 = 0.482%, and the following distinctive IR peaks:

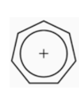
- A strong peak at 1687 cm^{-1}
- NO IR peaks above 3000 cm^{-1}

Which of the compounds below best fits the data?

A. 3-heptyn-1-ol
B. trans-3-hepten-2-one
C. cyclohexanone
D. 2-octanone
E. 3-methyl-2-cyclohexen-1-ol

MS Common Fragmentation Behavior

1. Weak bonds break in preference to strong bonds
2. A bond between carbon and an atom of similar electronegativity break homolytically
2. The bonds most likely to break are those which lead to formation of the most stable cation
3. Rearrangements occur.



Double bonds and arene fragments tend to resist fragmentation.

Allylic cations are stable and resist fragmentation.

The tropylium ion is frequently encountered in the form of a signal at m/z = 91 (see: mass spectrum analysis). This fragment is often found for aromatic compounds containing a benzyl unit. On ionization, the benzyl fragment is cleaved off. ($PhCH_2$+) rearranges to the highly stable tropylium cation (C_7H_7+).

Isotopes in Mass Spectrometry

- peaks that are attributable to isotopes can help identify the compound's molecular formula

Natural abundance of some elements [3]					
Isotope	% nat. abundance	atomic mass	isotope	% nat. abundance	atomic mass
^{1}H	99.985	1.007825	^{12}C	98.89	12 (definition)
^{2}H	0.015	2.0140	^{13}C	1.11	13.00335
^{16}O	99.76	15.99491	14N	99.64	14.00307
^{17}O	0.04		15N	0.36	15.00011
^{18}O	0.2				
^{28}Si	92.23	27.97693	^{32}S	95.0	31.97207
^{29}Si	4.67	28.97649	^{33}S	0.76	32.97146
^{30}Si	3.10	29.97376	^{34}S	4.22	33.96786
^{35}Cl	75.77	34.96885	^{79}Br	50.69	78.9183
^{37}Cl	24.23		^{81}Br	49.31	80.9163

- the height of the M+1 peak as a percentage of the height of the M+ peak relates to the number of atoms

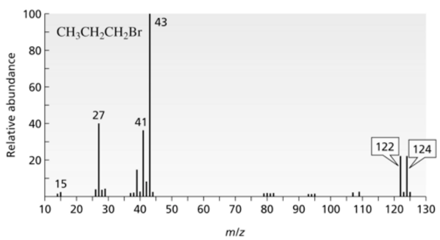
Isotopic Effects

Relative Percent Abundances for –Cl and –Br halogens as percent of Molecular Ion peak

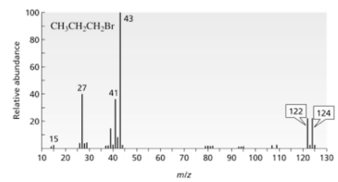
Halogen	% M + 2	% M + 4	% M + 6
Br	97.9		
Br_2	195	95.5	
Br_3	293	286	93.4
Cl	32.6		
Cl_2	65.3	10.6	
Cl_3	97.8	31.9	3.47

- a large M + 2 peak suggests a compound containing either chlorine or bromine: a Cl if M + 2 is 1/3 the height of M; a Br if M + 2 is the same height as M

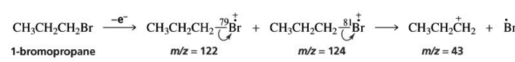
The Mass Spectrum of Bromopropane



The weakest bond is the C–Br bond

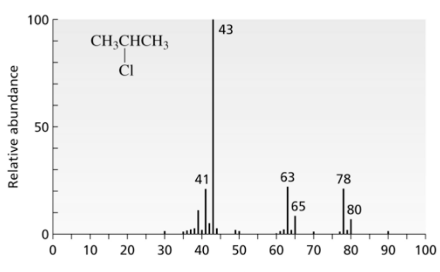


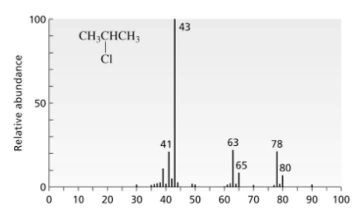
The base peak is at *m/z* = 43 [M – 79, or (M + 2) – 81]



The propyl cation has the same fragmentation pattern it exhibited as noted earlier

The Mass Spectrum of 2-Chloropropane



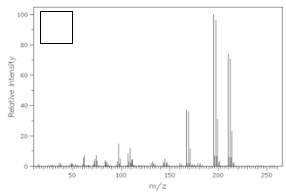


The M + 2 peak is 1/3 the height of the molecular ion peak, which confirms chlorine.

The base peak at *m/z* = 43 results from heterolytic cleavage of the C–Cl bond

The peaks at *m/z* = 63 and *m/z* = 65 have a 3:1 ratio, indicating the presence of a chlorine atom

Question



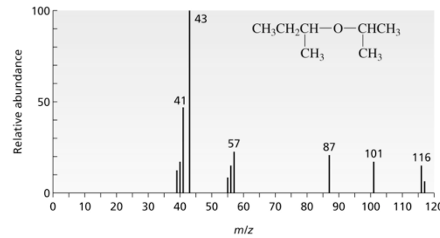
210.0	73.9
211.0	6.1
212.0	70.8
213.0	5.7
214.0	22.8
215.0	1.8
216.0	2.5

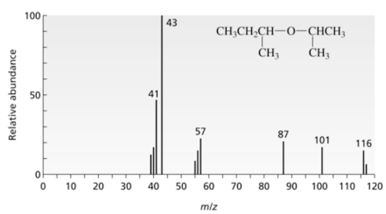
Which molecular formula is reasonable for the compound?

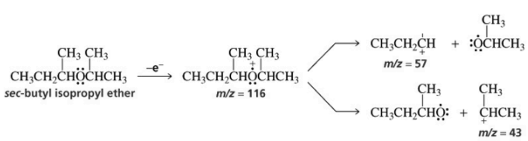
1) $C_9H_3ClO_4$ 2) $C_6H_4Cl_2O_4$ 3) $C_7H_5Cl_3O$ 4) $C_7H_5BrN_3$

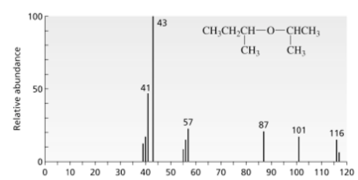
A) only #1 B) only #2 C) only #3 D) only #4 E) more than one

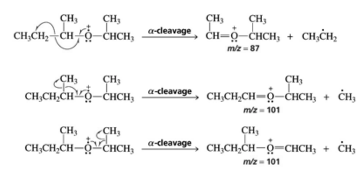
The Fragmentation Pattern of Ethers



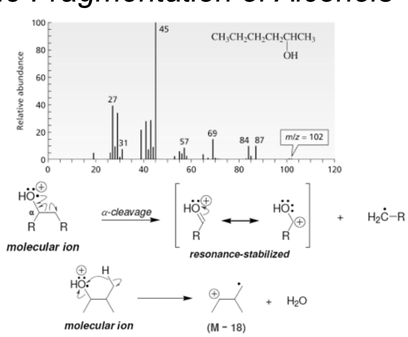


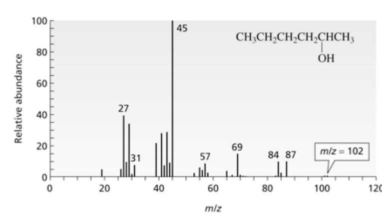


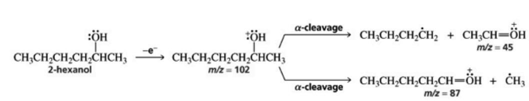


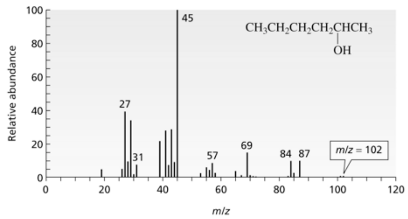


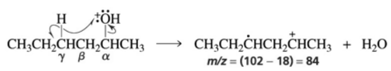
The Fragmentation of Alcohols



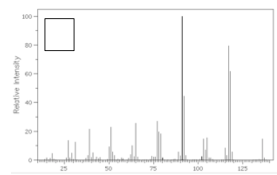








Question

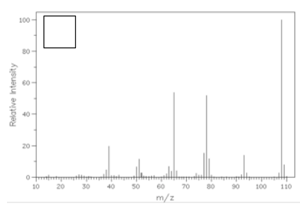


63.0	10.2	93.0	3.3
64.0	2.4	102.0	2.3
65.0	25.7	103.0	14.7
66.0	2.2	104.0	7.0
74.0	2.8	105.0	15.4
75.0	2.2	106.0	1.5
76.0	2.2	107.0	1.5
77.0	26.9	115.0	8.3
78.0	19.6	116.0	3.2
79.0	18.3	117.0	79.6
80.0	1.5	118.0	61.8
89.0	5.6	119.0	5.8
90.0	3.0	136.0	14.6
91.0	100.0	137.0	1.5
92.0	44.4		

Which compound is the most reasonable for the MS spectrum above (M = 136)?

A) m-isopropylphenol
B) (2-methoxyethyl)benzene
C) 3-phenyl-1-propanol
D) m-ethylanisole

Question



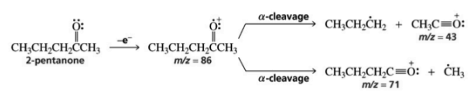
65.0	53.9
66.0	4.0
74.0	2.4
75.0	1.2
76.0	1.2
77.0	15.2
78.0	52.0
79.0	11.8
80.0	1.4
92.0	1.5
93.0	13.9
94.0	2.8
107.0	2.7
108.0	100.0
109.0	7.9

Which compound is most reasonable for the MS spectrum above?

A) benzyl alcohol B) m-cresol C) p-cresol D) o-cresol E) anisole

Fragmentation Pattern of Ketones & Aldehydes

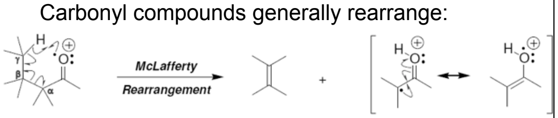
An intense molecular ion peak



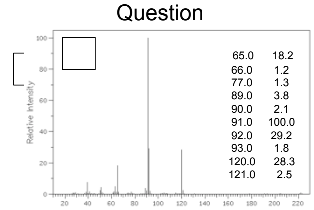
Aldehydes have M-1 and an M-29 peaks

McLafferty rearrangement may occur

Carbonyl compounds generally rearrange:



Question

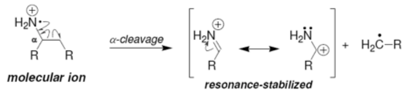


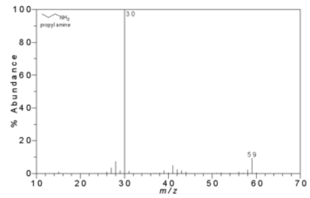
65.0	18.2
66.0	1.2
77.0	1.3
89.0	3.8
90.0	2.1
91.0	100.0
92.0	29.2
93.0	1.8
120.0	28.3
121.0	2.5

Which compound is the most reasonable for the MS spectrum above (M = 120)?

A) acetophenone
B) m-tolualdehyde
C) phenylacetaldehyde
D) 2,3-dihydrobenzofuran

- Amines generally undergo alpha cleavage:

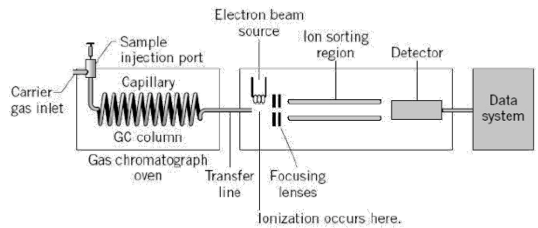




The presence of nitrogen in an amine can be detected by its odd molecular weight and the even fragments that it produces

GC/Mass Spectrometry

- MS instruments are commonly linked to a gas chromatograph (GC) so mixtures can be analyzed.

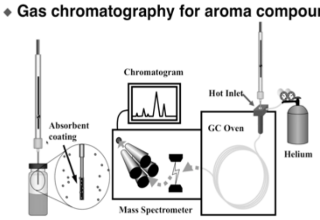


GC/MS Applications

- Many organic compounds have been identified using large libraries of data:
  - **Pharmaceuticals**: drug discovery and drug metabolism, reaction monitoring
  - **Biotech**: amino acid sequencing, analysis of macromolecules
  - **Clinical**: neonatal screening, hemoglobin analysis
  - **Environmental**: drug testing, water quality, food contamination testing
  - **Geological**: evaluating oil composition
  - **Forensic**: explosives detection
  - **Viticulture / Enology:** volatile molecules & sensory perceptions

Chemical Analysis

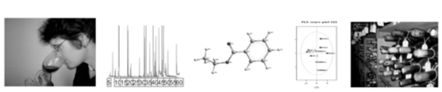
- **Gas chromatography for aroma compounds**



N. Lloyd, 2010

A Toast: To the Chemistry Of Noble Grapes

Susan E. Ebeler
Department of Viticulture and Enology
University of California, Davis, CA

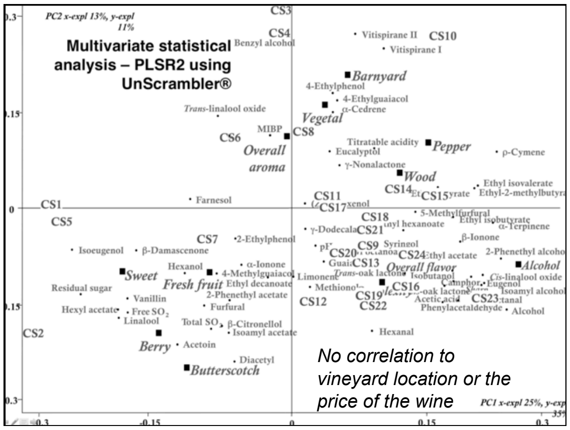


Cabernet Sauvignon

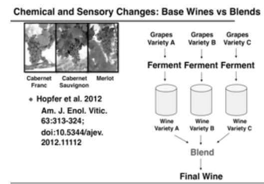
Chemical (GC) Analysis

- **61 compounds**
  - **Esters (isoamyl acetate)**
  - **Alcohols (isobutanol)**
  - **Terpenes (linalool)**
  - **Volatile acids (acetic acid)**
  - **Lactones (γ-nonalactone)**
  - **Norisoprenoids (β-damascenone, β-ionone)**
  - **Carbonyls (hexanal, diacetyl)**
  - **Etc.**

Multivariate statistical analysis – PLSR2 using UnScrambler®



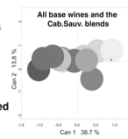
No correlation to vineyard location or the price of the wine



- Hopfer et al. 2012 Am. J. Enol. Vitic. 63:313-324; doi:10.5344/ajev.2012.11112

Base Wines vs Blends

- Similar sensory properties possible with different blends
- Sensory properties did not change consistently—attribute intensities were enhanced, suppressed, averaged—depending on blending ratio



Studying Viticulture & Enology at UCDavis

- http://wineserver.ucdavis.edu
- B.S. in Viticulture and Enology
- M.S. in Viticulture and Enology
- PhD in Various Disciplines (Agricultural Chemistry, Food Science, Microbiology, Plant Biology, Horticulture, Genetics, Engineering, etc…..)
- Certificate in Winemaking for Distance Learners
  http://extension.ucdavis.edu/unit/winemaking/certificate/winemaking/
- University Extension 1- and 2-Day Shortcourses
  http://extension.ucdavis.edu/index.asp