THE STRUCTURE DETERMINATION OF ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

INTRODUCTION

Mass spectrometry (MS) is an analytical technique that measures the mass of individual molecules and atoms. The first essential step in mass spectrometry analysis is to convert the neutral analyte molecules into gas-phase ionic species. The excess energy transferred to the molecule during ionization leads to fragmentation. Next, a mass analyzer separates these molecular ions and their charged fragments according to their *m/z* ratio. The ion current due to these mass-separated ions is detected by a suitable detector, and displayed in the form of a mass spectrum. Each of these steps is carried out under high vacuum (10^{-4} to 10^{-8} torr).

Thus, a mass spectrometer consists of several essential functional units, which are: inlet system, vacuum system, ion source, mass analyzer, detector, electronics, and data acquisition software.

A sample is introduced into a mass spectrometer by any one of the following techniques: reservoir inlet, direct insertion probe, gas chromatography, or liquid chromatography. You will use gas chromatography (GC).

Electron Ionization (EI): You will ionize your sample via EI. This typically involves bombardment of the sample molecules with a beam of 70 eV of electrons.

$$M + e \rightarrow M^{+\cdot} + 2e \quad \text{(odd-electron ions)}$$

It provides a fingerprint spectrum, not amenable to thermally labile and nonvolatile compounds, and yields extensive fragmentation. Therefore, softer modes of ionization are used for those compounds. These are chemical ionization (CI), ^{252}Cf-plasma desorption ionization, fast atom bombardment (FAB) ionization, matrix-assisted laser desorption ionization (MALDI), field desorption ionization, electrospray ionization (ESI), and thermospray ionization.

Several mass analyzers are used to mass-analyze the ions. These include magnetic sector mass spectrometer, quadrupole mass spectrometer, ion trap mass spectrometer, time-of-flight mass spectrometer, and Fourier-transform ion cyclotron resonance mass spectrometer. You will use a quadrupole mass analyze.

EXPERIMENTAL PROCEDURE

Eye protection is required throughout this laboratory. Nitrile gloves are recommended.

Sample Preparation

Dissolve ~ 30 mg (~1-drop) of your sample (whether liquid or solid) in 1-2 mL of acetone or appropriate solvent. Make sure you have enough sample to get good M+1 and M+2 data for molecular formula calculations.

Data collection by GC/MS

1) Check the regulator level of carrier gas. If it needs to be imminently replaced, inform Dr. Brewster IMMEDIATELY via email.
2) Log in to the system with the proper password and open ChemStation
3) If you are the first user of the day, check the tune of the MS. We are using the stune file on this instrument. If the tune is ineffective, contact Dr. Brewster immediately.
4) Load the method you wish to use
    a. Edit any method parameters as needed and save under a NEW method name
    b. MAKE SURE THE METHOD IS APPROPRIATE FOR THE COLUMN INSTALLED!!!!! IF YOU DO NOT KNOW WHAT COLUMN IS INSTALLED, OPEN THE OVEN DOOR AND CHECK!!!!!
5) Create your "sequence"
    a. Go to sequence → Edit sequence and fill in the needed parameters
        i. Make sure to create a new data path each day
        ii. Make sure the method entered matches EXACTLY with your desired method
6) Run Sequence
    a. Injection volume should ALWAYS be 1 μL or less.
    (fill syringe and gently pump to expel air then expel to 1 uL mark)
    b. Press "Pre-run" on the instrument and WAIT for the light to hold steady. NEVER override the GC ready indication
    c. Once ready, insert the syringe and inject the sample. Immediately press "Start" on the GC – this sets t = 0.
7) You can monitor chromatogram progress in real time on this instrument via the data analysis software. Use retention times to modify your method. There is no reason to run a 60 minute method when 30 is sufficient!
8) Open your file in the data analysis software to analyze peak areas. These should ALWAYS be calibrated and integrated relative to an internal standard for quantitative analysis!
9) Get files containing tabulated ion data, plots of gas chromatogram the mass spectrum.
10) Repeat your GC-MS experiments until they are reproducible. Students often have problems with "overrun" from a previous GC-MS experiment, too small, or to large injections. GC intensity should be $>10^{?}$. More concentrated or larger injection or lower threshold may be needed to observe M+1 and M+2 peaks. Send me a copy (email me to confirm an M+ peak).
11) Make sure the gas saver mode has initiated BEFORE leaving the GC area.

Experiments for known sample

1. Run first chromatogram at 60 ^{o}C initial temperature.
2. second chromatogram at initial temperature 40 ^{o}C greater than the first chromatogram.

3. For a third chromatogram push syringe plunger to 0 μL before injecting needle into GC inlet. Then immediately inject needle and start chromatograph.
4. These three experiments do not need to repeated if the results are consistent with each other.

INTERPETATION OF MASS SPECTRUM

To interpret your mass spectrum, use the following steps:

A. Recognize the molecular ion ($M^{+\cdot}$) first; it provides the molecular mass information
It is usually the most abundant ion in the high-mass cluster. It must be an odd-electron ion (i.e., at even mass, except when the compound contains odd numbered nitrogens (see the nitrogen rule).

Look for the logical and unusual losses; losses of 4-14 and 21-25 amu are unlikely.

High abundance of $M^{+\cdot}$ shows stability of the molecule, $M^{+\cdot}$ increases with increased unsaturation and number of rings, decreases with chain branching and increase in the molecular size up to a certain value; aromatic compounds give prominent $M^{+\cdot}$

B. Apply the Nitrogen Rule: An odd-electron ion has even mass if it contains even number of N.

C. Calculate the Elemental Composition

Use the relative abundances of M, M + 1, and M + 2, etc. peaks. For example, the number of O, S, Si, Cl, and Br atoms can be calculated from the relative ratio of the M and M + 2 peaks, and the number of C and N atoms can be found from the relative ratio of the M and M + 1 peaks. You can also use the table (Appendix a) given in the Silverstein's book.

If $M^{+\cdot}$ is not present or ambiguous, look for the next lower-mass cluster.

The elemental composition of the compound can also be calculated if we know its exact molecular mass (up to the 4th decimal place). (Note-for calculating the molecular mass, use the atomic masses of the most abundant isotopes).

D. Calculate Ring + Double Bonds (or degree of unsaturation)
For $C_xH_yN_zO_n$, the $R+DB = x - y/2 + z/2 + 1$

E. Common Fragmentation
(i) σ-e ionization and cleavage of the bond
(ii) Radical site initiated fragmentation (α-cleavage)
(iii) Charge site initiated fragmentation (inductive effect)
(iv) Rearrangement reactions,
e.g., McLafferty rearrangement and ortho effect; produce even-electron ions

Note: look for the odd-electron (i.e., even mass) ions in the spectrum; they have special meaning.

Also, pay attention to the general appearance of the spectrum, and the low-mass ion series, eg., 29, 43, 57, etc. due to alkanes and aliphatic ketones; 30, 44, 58,...and 31, 45, 59, ..., respectively, due to long chain amines and alcohols; 39, 51, 63, 77..due to the aromatic ring; 39, 51, 65, 91,...due to the benzylic group. Look for characteristic ions, e.g., m/z 74, 77, 91, 92, 93, 105, 127, etc.
Some useful hints for the compounds are as follows:

Straight-chain alkanes: Their mass spectrum is characterized by weak $M^{+\cdot}$; low abundant ions in the high-mass region of the spectrum, cluster of ions; each cluster separated by 14 Da; the $C_nH_{2n+1}^+$ ion-series is the most abundant (i.e., ions 29, 43, 57, 71...); also, a lesser abundant $C_nH_{2n-1}^+$ ion-series is present; and a smooth curve of clusters at the low-mass end, with C3 and C4 ions being the most abundant.

Branched-chain alkanes: branching reduces the $M^{+\cdot}$ intensity compared to the straight-chain alkanes; smooth curve is broken by the preferred cleavage at the branch point; the largest group at the branch point is lost preferentially; the branch group + H loss, i.e., produces an even-electron ion.

Cycloalkanes: a somewhat larger $M^{+\cdot}$ ion; loss of the side chain and olefin moiety; and the $C_nH_{2n-1}^+$ ion-series (27, 41, 55,..) is now prominent.

Alkenes: the $M^{+\cdot}$ is observed; allylic cleavage due to the radical site initiation; the McLafferty rearrangement; and the $C_nH_{2n-1}^+$ ion-series is prominent.

Cyloalkenes: distinct $M^{+\cdot}$ ion and retro-Diels-Alder reaction.

Polycyclic aromatic hydrocarbons: the $M^{+\cdot}$ ion is the most abundant and other ions are of low abundance.

Alkyl benzenes: cleavage of the benzylic bond to give <u>*m/z* 91</u>; subsequent expulsion of C_2H_2 from the tropylium cation; and when the alkyl chain is propyl or larger, the McLafferty rearrangement is observed to give <u>*m/z* 92</u>.

Aliphatic Alcohols: the $M^{+\cdot}$ is very weak for primary and secondary alcohols, and is absent for tertiary; the primary alcohols produce ($M^{+\cdot}$ - 18) ion; the $C_nH_{2n+1}O^+$ ion-series (31, 45, 59, ..) is observed; the $(M - 1)^+$, $(M - 2)^+$ peaks are also prominent; a characteristic peak at m/z 31 ($CH_2{=}OH^+$) for primary alcohols; secondary and tertiary alcohols show the loss of alkyl radicals.

Aromatic Alcohols: in addition to the above reactions of the side chain alcohol moiety, the ions due to the aromatic ring are seen (e.g., 91, 77, 65, 51, etc.); they undergo the McLafferty rearrangement involving the phenyl ring (m/z 92)

Phenols: give strong $M^{+\cdot}$ ion; exhibit loss of CO and CHO; and undergo ortho effect.

Aliphatic Aldehydes: α-cleavage gives $(M - 1)^+$ and $(M - R)^+$ peaks; the McLafferty rearrangement to give *m/z* 44, 58, or 72

Aromatic Aldehydes: the $M^{+\cdot}$ ion is very large; the (M - 1) ion (C_6H_5CO) is usually larger than the $M^{+\cdot}$ ion; the loss of CO from (C_6H_5CO) to give *m/z* 77

Aliphatic Ketones: thee $M^{+\cdot}$ ion peak is very pronounced; the α-cleavage occurs, the loss of larger R group is favored; the McLafferty rearrangement also occurs.

Cyclic ketones: the $M^{+\cdot}$ is very prominent; cleavage of the ring at the C-C bond near to the CO group

Aromatic ketones: the $M^{+\cdot}$ is very prominent; characteristic ion at *m/z* 105 (C_6H_5CO); further loss of CO from *m/z* 105 to yield *m/z* 77; the McLafferty rearrangement occurs, if the alkyl chain is longer.

Carboxylic Acids; give weak but discernible $M^{+\cdot}$; prominent *m/z* 60 due to the McLafferty rearrangement; the (M - OH) and (M - COOH) peaks

Aliphatic Esters: give weak $M^{+\cdot}$ ion; in methyl esters, the α-Cleavage gives RCO^+ and CH_3O^+, R^+ and CH_3OCO^+; usually the base peak is observed at m/z 74 due to the McLafferty rearrangement; in higher esters, α-cleavage is observed due to the CO and -O- groups; aromatic esters will show additional ions due to the ring.

Nitriles: the $M^{+\cdot}$ is usually negligible or absent for aliphatic nitriles and strong for aromatic compounds. The (M - HCN) peak is often observed. Also, nitriles exhibit the McLafferty rearrangement.

Halides: look for (M + 2) and higher peaks; (M - HX) peaks for F^-, Cl^-, and Br-containing aliphatic halides. Aliphatic iodocompounds show *m/z* 127 and (M - 127) peaks. Aromatic halides show (M - X) peak.

LABORATORY REPORT NOTES
One report for Standard, a second for unknowns

See grade scheme for organization. Include purpose, introduction, (theory, block diagram and description of instrument for first report using an instrument) procedure (including sample preparation and conditions). For Results and Discussion display spectra and assign peaks for your compound you analyzed (show calculations for elemental composition), tabulate data (like peak positons and amplitude) label the important ions in the spectrum such as the M^+, base peak, major or distinctive peaks, show the probable structure of each important ion and propose the mechanism of their formation. Discuss possible formulas and structures of your compound.

Other References

1. Spectrophotometric Identification of Organic Compounds, Silverstein, Bassler, Morrill.
2. Interpretation of Mass Spectra, F.W. McLafferty.
3. Mass Spectrometry of Organic Compounds, Budzikiewicz, Djerassi, and Williams.
4. Mass Spectrometry: Clinical and Biomedical Applications,Vol 2, D.M. Desiderio (Chapter 1 by Dass).
5. Principle and Practice of Biological Mass Spectrometry, Wiley-Interscience, C. Dass.