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Mass Spectrometry—Finding the Molecular Ion and What It Can Tell You: An Undergraduate Organic Laboratory Experiment

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Abstract: Mass spectrometry is a widely used method to obtain information about the structures of molecules. This technique was introduced to beginning first-year organic chemistry students as an experiment that focused on the identification of the molecular ion from experimental data. After students had correctly identified the molecular ion in their samples, they learn other information that can be deduced from the molecular ion.

Introduction

Electron ionization-mass spectrometry (EIMS) is one of a number of techniques used by organic chemists to determine the structure of organic molecules. The technique is introduced in most first-year texts on organic chemistry and is usually illustrated by idealized spectra where the molecular ion is clearly marked. Our students had little trouble working the spectral interpretation problems in these texts; however, this was not the case when they analyzed experimental spectra generated during analyses of organic "unknowns." Most of our students initially failed because their experimental spectra contained background peaks, a feature that was excluded from textbook examples. Invariably, these students incorrectly identified the background peak with the highest mass number peak as the molecular ion. Furthermore, when they had learned to recognize background peaks, they frequently assigned a large $M^+ - 1$ peak as the molecular ion. It was evident they needed instruction on how to correctly identify a molecular ion.

To this end, we developed a laboratory experiment using experimental spectra so our first-year organic students would learn how to correctly identify the molecular ion. This experiment was done at the beginning of the course soon after students had learned to generate structural formulas and degree of unsaturation from molecular formulas. A laboratory lecture on mass spectrometry and a demonstration on the use of the mass spectrometer was presented before the student exercise. Unfortunately, with 40 students in the class it was not practical for each student to prepare a sample and be instructed in the use of the GC–MS. Instead, spectra were generated by the instructor and downloaded to a second computer where students could analyze the data. In this exercise, our beginning students learned the use of the data analysis software that was installed on our gas chromatograph-mass spectrometer (GC-MS). Student data collection using the GC–MS was done in more advanced classes where they could receive individual instruction on the use of the instrument.

In addition to learning to recognize molecular ions in experimental data, our students learned what other information can be deduced from analysis of the molecular ion, specifically, when the molecular ion occurs at an odd mass number the compound contains an odd number of nitrogen atoms. The isotope peaks for compounds that contain bromine, chlorine, and/or sulfur are diagnostic for these elements. Finally, candidate empirical formulas can be calculated for unknown compounds using the "rule of 13."

Electron Ionization Mass Spectrometry—Molecular Ion Generation

The process of obtaining an electron ionization mass spectrum first requires an organic molecule to be vaporized and then bombarded with a stream of high-energy electrons. Knocking one or more electrons out of the molecule creates positively charged particles and at the same time may fragment the molecule into smaller pieces. Removal of a single electron from the molecule results in a positively charged particle called the molecular ion (M^+). The mass of the molecular ion is the same as the molecular weight of the original molecule because the mass of the expelled electron is negligible. The molecular ion is called a radical cation because it contains an odd number of electrons (radical) and a positive charge (cation). The formation of such a molecular ion is illustrated for ethane (Figure 1). The mass of these charged particles is written as $m/z = 30$ amu, where m/z is the mass (amu) divided by the charge, which in most cases is +1. It should be noted that EIMS will fragment many compounds so the molecular ion may be small or nonexistent. In our experiment, we choose compounds that had readily observable molecular ions, and we did let students know that not all compounds produce molecular ions with EIMS.

Recognition of the Molecular Ion (M^+)

Identifying the molecular ion in experimentally generated spectra is not as easy as might be expected. Spectra in organic textbooks show only those peaks generated from a given compound, but experimental spectra contain "background" peaks due to minor impurities that are usually present in the mass spectrometer. When first presented with experimental spectra, students frequently will think the highest mass peak in the spectrum is the molecular ion. In reality, this is usually part of the background. After being taught how to examine a spectrum for the molecular ions, our students could find it consistently.

The first place to start in the identification of a molecular ion (M^+) is to find the highest even-mass-number (even

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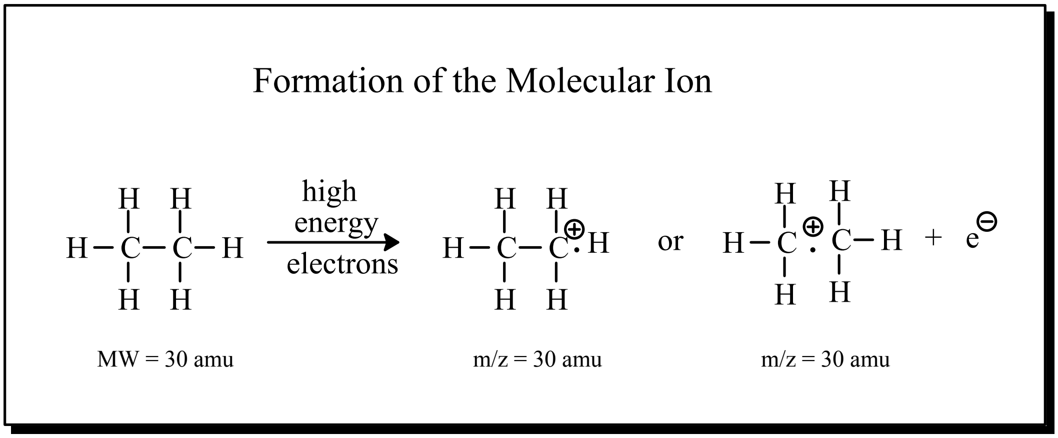

Figure 1. The formation of a radical cation molecular ion for ethane.

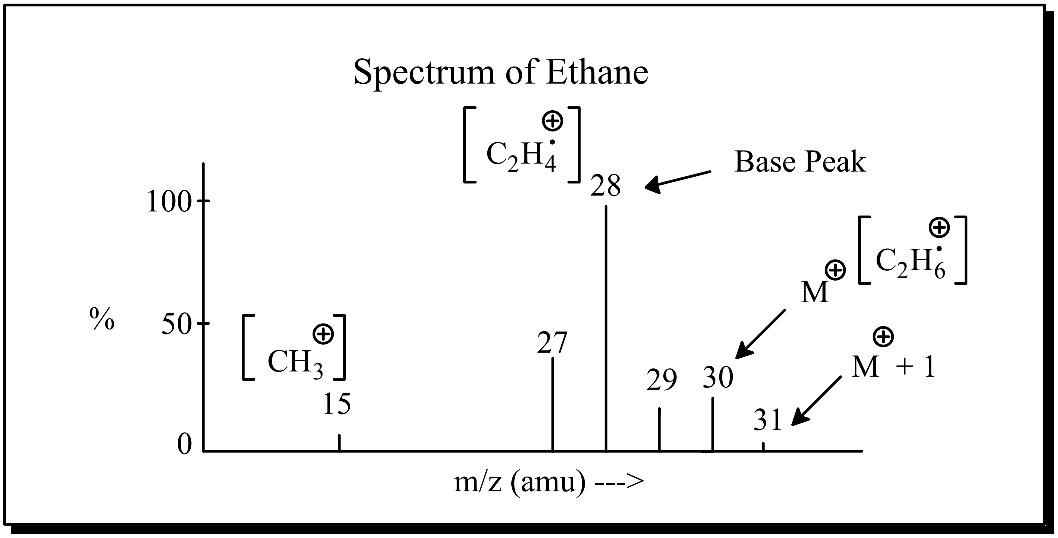

Figure 2. The mass spectrum of ethane. The molecular ion (M^+) is at 30 amu and the base peak at 28 amu is due to the loss of two hydrogen atoms.

numbers) peak in the spectrum. Generally, odd-mass components (odd numbers) of the spectrum are fragments of the original molecule. This is illustrated in the mass spectrum of ethane (Figure 2). The exceptions to this general rule are compounds that contain an odd number of nitrogen atoms: These compounds have a molecular ion at odd mass numbers. Compounds with an even number of nitrogen atoms have molecular ions that are at an even mass number. The mass spectra of ammonia (NH_3) and hydrazine (N_2H_4) show this difference (Figures 3 and 4).

Another feature that helps in the identification of the molecular ion (M^+) are the peaks that are 1 and 2 amu above it. The sizes of these peaks are directly related to the number of carbons in the molecule, the $M^+ + 1$ and + 2 peaks increase in size with increasing numbers of carbon atoms. Molecular ion recognition can depend on ascertaining that it has the proper ratio of M^+:$M^+ + 1$ ions. Table 1 gives the relative size of $M^+ +$ 1 and + 2 for selected hydrocarbons. The reason that the molecular ion and the $M^+ + 1$ and $M^+ + 2$ peaks are in fixed ratios is that natural carbon contains 1.1% of ^{13}C. The $M^+ + 1$ and + 2 peak size becomes larger as compounds contain more carbons because the probability any one carbon can be a ^{13}C is greater. Each carbon in a molecule increases the size of the $M^+ + 1$ peak by 1.1%. (On high resolution mass spectrometers the ratio of the M^+:$M^+ + 1$:$M^+ + 2$ intensity can be used to determine the number carbon atoms present. Unfortunately, conventional low-resolution mass spectrometers usually cannot make this measurement with the accuracy needed to determine the number of carbon atoms that are present.)

In contrast to the $M^+ + 1$ and + 2 peaks, those at $M^+ - 1$ and $M^+ - 2$ are fragments due to loss of a hydrogen atoms and their sizes are variable. In some cases a peak due to loss of a hydrogen ($M^+ - 1$) may be larger than the molecular ion, but will be recognized as a fragment as it generally occurs at an odd mass number. Alternately, it can be recognized as a fragment because the size of the M^+:$M^+ + 1$ is not correct (Table 1). A typical molecular ion and peaks surrounding it are shown in Figure 5.

Structural Information from the Molecular Iion

The molecular ion indicates the molecular weight of a compound and gives important information about the number and kind of atoms that make up the molecule. The following steps can be used to gain information about the identity of an unknown molecule and in some cases can lead to an unambiguous identification.

1. Search the spectrum for the highest even-mass peak with characteristic $M^+ + 1$ and $M^+ + 2$ peaks. This is generally the molecular ion. In a few cases, the molecular ion is an odd mass

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Table 1. Relative Size of the Peaks for $M^+ + 1$ and $M^+ + 2$ Compared to M^+ for Selected Hydrocarbons. Each Carbon Increases the Size of the $M^+ +$ 1 peak by 1.1%

Hydrocarbon	Molecular Ion (amu)	M^++1	M^++2
CH_4	16	1.15	
C_2H_6	30	2.26	0.01
C_3H_8	44	3.37	0.04
C_4H_{10}	58	4.48	0.08
C_5H_{12}	72	5.60	0.13
C_7H_{16}	100	7.82	0.26
$C_{10}H_{22}$	142	11.16	0.56
$C_{13}H_{28}$	184	14.50	0.97
$C_{15}H_{32}$	212	16.72	1.31

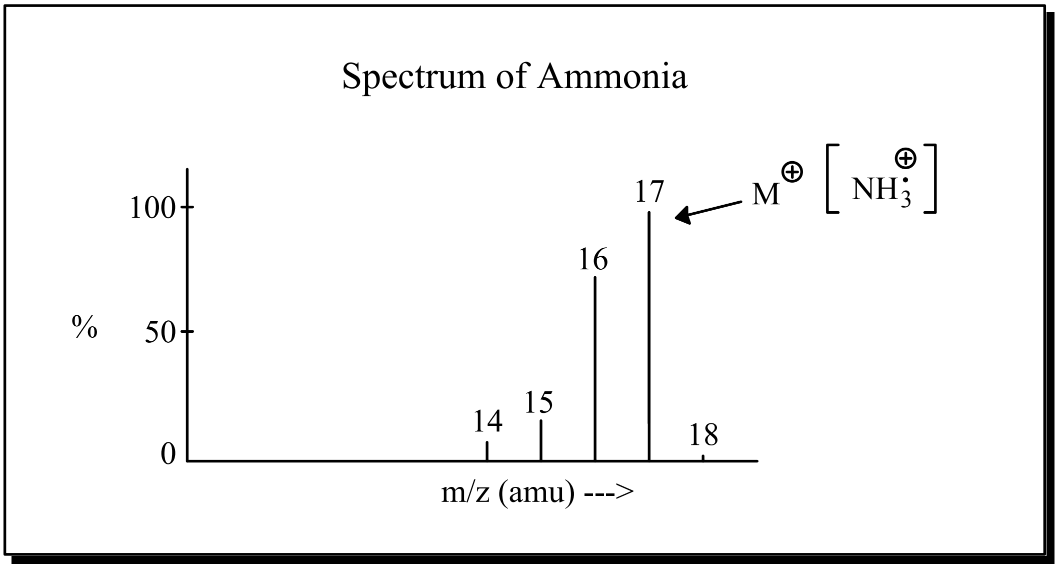

Figure 3. The mass spectrum of ammonia (NH_3). The molecular ion is at an odd mass number.

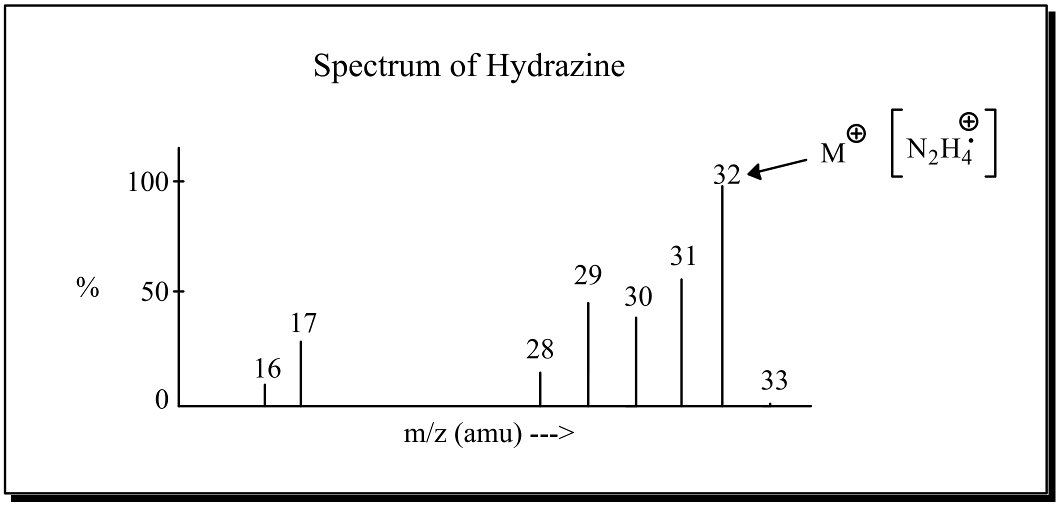

Figure 4. The mass spectrum of hydrazine (N_2H_4). The molecular ion is at an even mass number.

number, which indicates that it contains an odd number of nitrogen atoms (1, 3, 5, etc.). Because most fragments occur at an odd mass number, care must be taken not to assign a fragment as the molecular ion.

2. Examine the spectrum for any $M^+ + 2$ peaks (molecular ion + 2 amu) that are larger than the $M^+ + 1$ peak. This indicates the presence of bromine, chlorine, or sulfur atoms in the molecule. Compounds with a single chlorine have an $M^+ +$ 2 peak that is 1/3 the size of the molecular ion. Compounds containing a single bromine atom have an $M^+ + 2$ peak that is approximately the same size as the molecular ion. Compounds with a single sulfur atom have an $M^+ +2$ peak that is about 5% of the molecular ion. These are illustrated in Figure 6. If there are large $M^+ + 2$ and $M^+ + 4$ peaks in the spectrum, the molecular ion contains multiple chlorine or bromine atoms. Figure 7 contains examples of molecular ions for compounds

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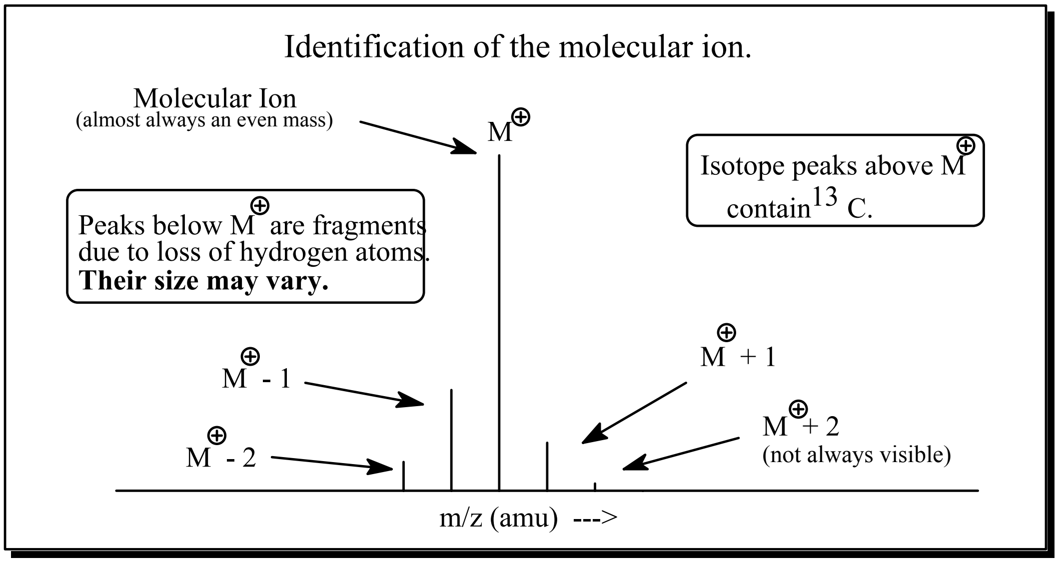

Figure 5. The molecular ion is usually an even-mass peak surrounded by ^{13}C peaks at higher mass units and peaks due to loss of hydrogen at lower mass units.

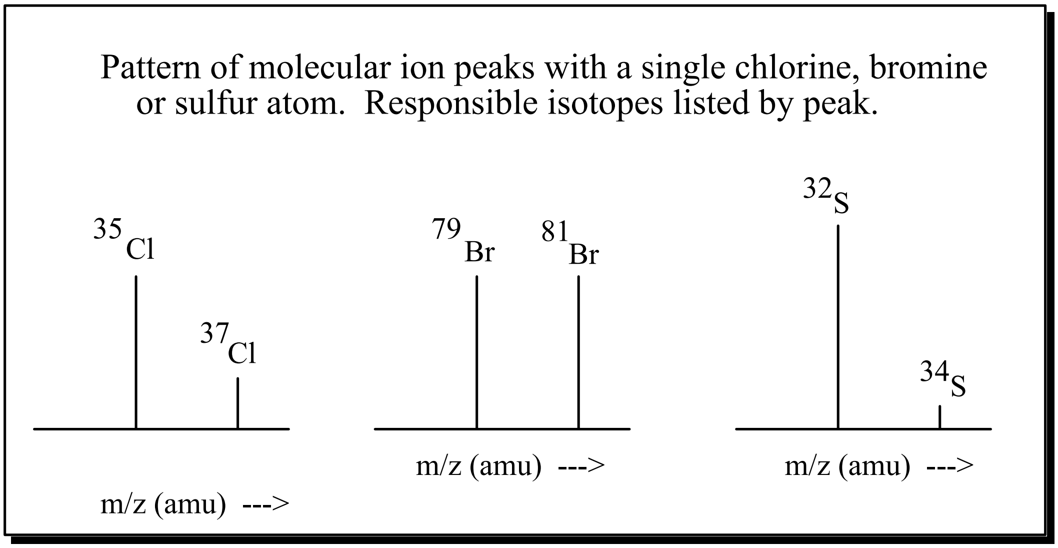

Figure 6. The characteristic shapes of molecular ions that contain a single chlorine bromine or sulfur atom.

that have multiple chlorine or bromine atoms. Examination of the patterns in Figures 6 and 7 can be used to identify the number and type of halogens present in the molecule.

3. Once the molecular ion has been identified, possible molecular formulas (candidate formulas) can easily be calculated by the "rule of 13" [1]. The first step in this procedure is to divide the molecular weight (mass of molecular ion) by 13. The mathematical description for this is,

$$M^{+}/13 = n + r/13,$$

where n is the number of times 13 can be divided into the molecular weight and r/13 is the remainder. If the compound is a hydrocarbon with a molecular weight below 156, it has the molecular formula, C_nH_{n+r}. An example for this algorithm using a compound with a molecular ion at 92 would give:

$$92/13 = 7 + 1/13 \text{ so } C_nH_{n+r} \text{ becomes } C_7H_{7+1} \text{ or } C_7H_8$$

At or above the molecular weight of 156, the formula changes depending on the type of hydrocarbon. Aliphatic or alkane-like compounds use the formula $C_{n-1}H_{n+12+r}$, changing a C atom for 12 H atoms. Compounds that are a mixture of similar sized aromatic and aliphatic components use the original molecular formula, C_nH_{n+r}. Highly aromatic compounds use the formula $C_{n+1}H_{n-12+r}$, an exchange of 12 H for one C atom. (See the next section on size of the molecular ions in relation to fragments to determine if a compound is aliphatic, aromatic, or a mixture of types.)

4. If the compound contains atoms other than carbon and hydrogen (heteroatoms), possible molecular formulas can be found by subtracting an appropriate number of carbons and hydrogens from the "hydrocarbon formula, C_nH_{n+r}" and then adding the heteroatom to the formula. These substitutions are listed in Table 2. An example of this type of substitution follows; a compound with a molecular ion at m/z = 112 amu would generate the formula C_8H_{16}. If it contains one oxygen

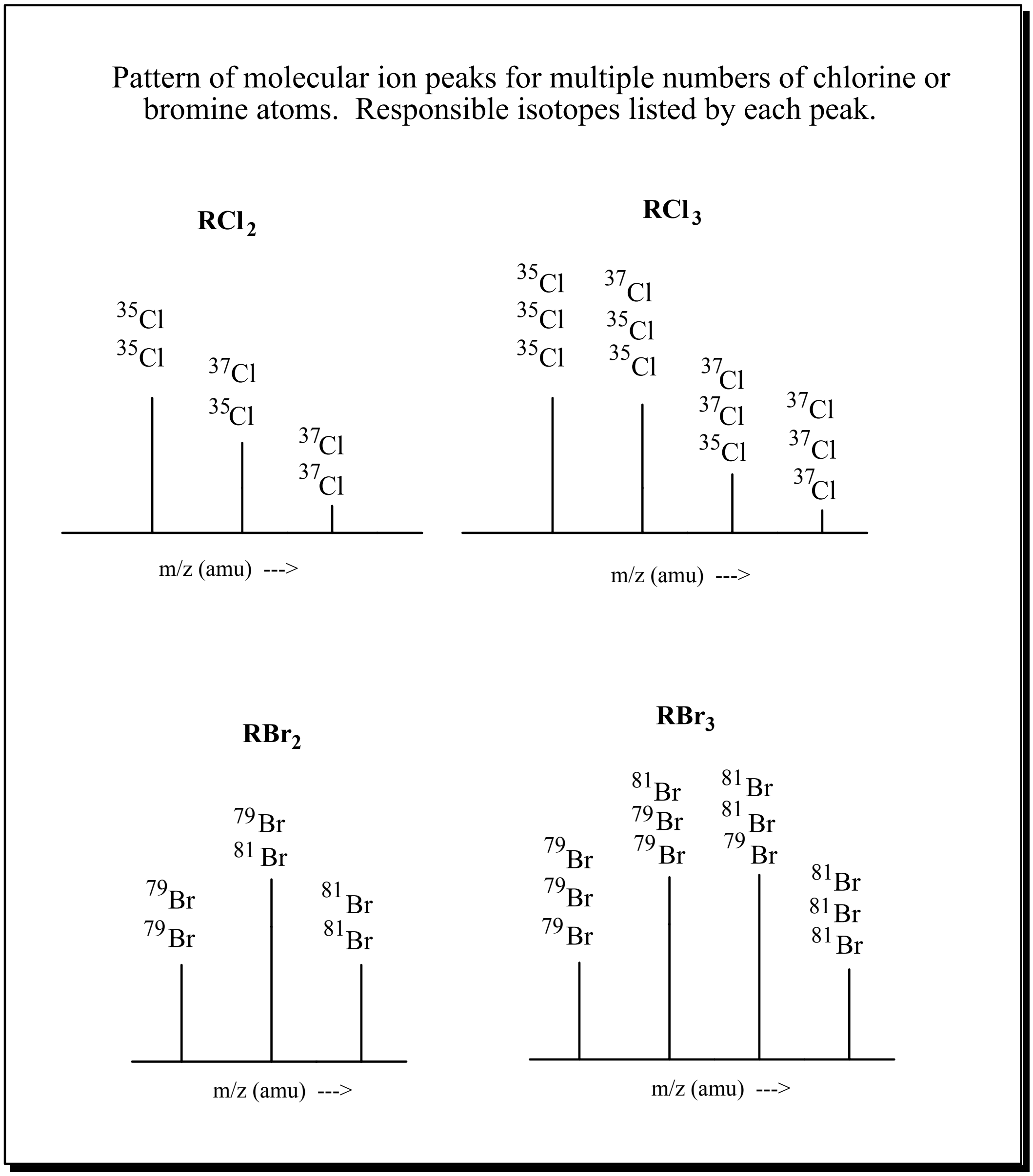

Figure 7. Patterns of the molecular ion with multiple chlorine or bromine atoms. The isotopes of each atom are above the peak to which they contribute.

Table 2. Heteroatom Substitutions for Carbon and Hydrogen

for 1 O, subtract CH_4 and add O
for 2 O, subtract C_2H_8 and add O_2
for 1 N, subtract CH_2 and add N
for 2 N, subtract C_2H_4 and add N_2
for 1 S, subtract C_2H_8 and add S
for 1 Cl, subtract C_2H_{11} and add Cl
for 1 Br, subtract C_6H_7 or C_5H_{19} and add Br
for 1 N and 1 O subtract C_2H_6 and add NO

atom, subtract CH_4 and add O, to give $C_7H_{12}O$. If it contains one chlorine atom, subtract C_2H_{11} and add Cl, to give C_6H_5Cl.

Size of the Molecular Ion Compared To Fragments—General Compound Types

The size of the molecular ion in relation to the fragment peaks in a spectrum can give valuable information about the compound. The following correlations of general structure type and fragmentation patterns are correct most of the time, but there are exceptions to the following. Spectra with small or weak molecular ions that have an increasing abundance of fragments at low m/z values are usually aliphatic or alkane-like compounds (See Figure 8A). In contrast, the spectra of aromatic compounds have a molecular ion that is much larger than the lower mass fragments. For aromatic compounds the molecular ion is frequently the base peak (see Figure 8B). Cycloalkane-like compounds frequently have the molecular ion and the fragments of equal or similar intensity (see Figure 8C). Compounds that are a mixture of aromatic and alkane moieties can have small molecular ions because the alkane part is easily lost and large fragments of stable aromatic fragments (see Figure 8D).

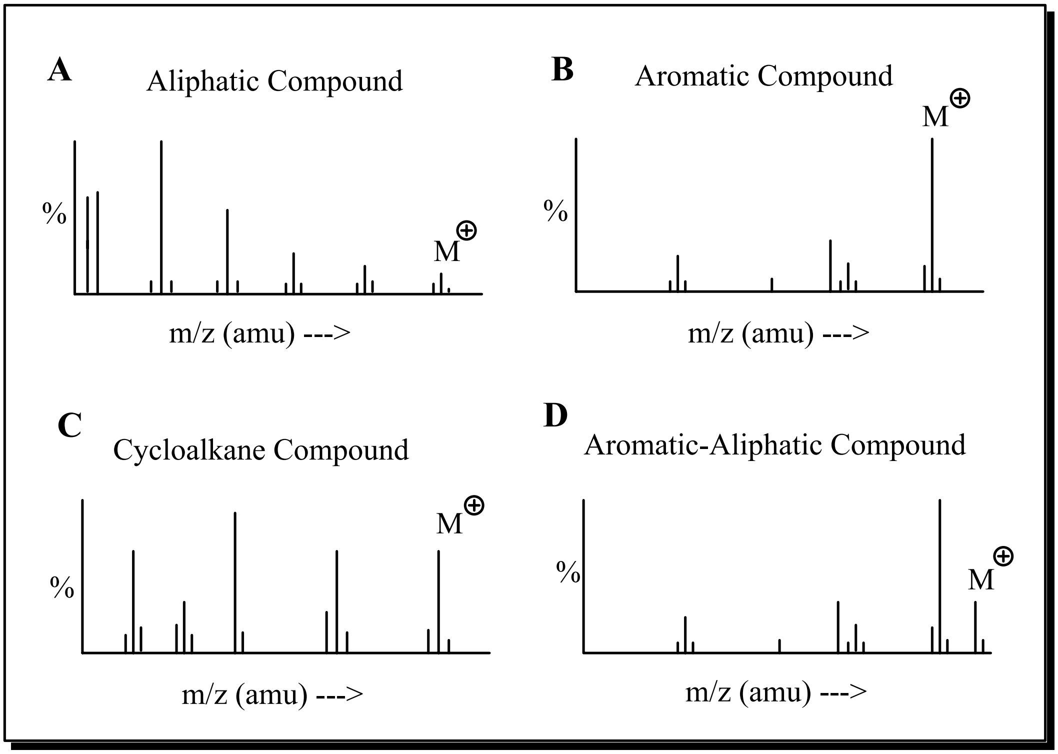

Figure 8. Types of molecules generally associated with the generalized spectra: aliphatic (A), aromatic (B), cycloalkane (C) and aromatic-aliphatic (D). These correlations are correct most of the time, but there are exceptions to these general guidelines.

The Laboratory Experiment

Students were given a prelaboratory lecture on mass spectrometry relevant to this experiment. The instructor then did a demonstration of how samples were prepared and spectra obtained. Due to the large number of students in the beginning organic chemistry class, it was not possible to teach each of them how to prepare samples and operate the gas chromatograph–mass spectrometer. Before the experiment, the instructor had previously done 40 individual GC–MS runs, a different one for each student. Each sample contained one aliphatic compound and one aromatic compound, which had been chosen from compounds that had discernable molecular ions.

Student then analyzed these GC–MS runs using HP Analytical MSD Productivity ChemStation software (B.01.00) at a computer isolated from the one that was used to operate the GC–MS. Becausee all 40 students could not use this computer at the same time, it was made available for their use over a one-week period. Students were instructed to examine their assigned GC–MS run and to obtain spectra and tabulations for each of the compounds in the unknown. They also took background spectra before and after the compound had eluted from the GC column. Even though they had not run the samples, they were presented with experimental spectra and became expert at using the data analysis software. (Note: a searchable mass spectra database was excluded from this computer.)

Students were given a report form that listed the steps they should follow in analysis of their unknowns (see next section). First, students had to determine if their unknowns contained nitrogen, chlorine, and/or bromine atoms. Next, using the rule of 13, they determined a molecular formula for a hydrocarbon that corresponded to mass of their molecular ion. After determining a molecular formula for a hydrocarbon, they made substitutions for any heteroatoms they had previously identified. Next, "CH_4" units were substituted for "O" to generate molecular formulas for compounds that contain oxygen. Finally, after examination of the possible molecular formulas students were asked to draw possible structural formulas. Unknowns were graded on the correct use of these steps to finding a final structure, rather than a correct identification of the unknown. Not surprisingly, many times students did derive correct structures or close isomers of those structures. Reports were returned to students with correct structures.

Chemistry instructors that do not have experimental spectra available can still use the following exercise. Mass spectra are available from a number of print sources like the "Registry of Mass Spectral Data" [2] and the "Mass Spectral and GC Data of Drugs, Poisons, and their Metabolites" [3]. Spectra are also available from online sources like the National Institute of Standards and Technology (NIST) Web Book [4] and the SDBS Integrated Spectral Data Base System for Organic Compounds [5].

Free data analysis programs (Wsearch16 and Wsearch32) to process chromatograms and mass spectra obtained on most mass spectrometers are available via the Internet [6]. This free software will allow students to work simultaneously on GC–MS data rather than the one station our student used. (Several mass spectral libraries are also available at this site. The largest is a drug library of 1800 spectra library, many of which are replicate entries.)

Steps in the Student Analyses of the Mass Spectra

1. Identification of the molecular ion. In almost all cases this is an even number peak with characteristic $M^+ + 1$ and $M^+ + 2$ peaks due to ^{13}C and $M^+ - 1$ and $M^+ - 2$ due to loss of hydrogen atoms. Do not arbitrarily pick the highest mass peak in a spectrum—almost all mass spectra contain a few small "background" peaks that are not part of the spectrum for the compound being examined. (Check Table 1 to see if the $M^+ + 1$ peak is the size indicated for molecular ion at that mass.)

   a. If the molecular ion is an odd number, it contains an odd number of nitrogen atoms.

   b If there are major $M^+ + 2$ (molecular ion + 2 amu) peaks present in a 3:1 ratio, the compound contains a single chlorine and if in a 1:1 ratio it contains a bromine atom.

Table 3. Compounds That Have Been Used as Unknowns in This Exercise

Aliphatic compounds	Aromatic compounds
1-bromopentane	acetophenone
cyclooctane	*p* -aminophenol
decane	anethole
3,3-dimethyl-2-butanone	*p*-anisaldehyde
1,2-dimethylcylohexane	*p*-anisidine
dodecane	benzaldehyde
2-heptanone	benzamide
4-heptanone	benzophenone
hexadecane	*p*-bromoacetanilide
2-hexanone	*p*-bromoaniline
hexylamine	*p*-anisole
linonene	1-bromo-4-nitrobenzene
methyl octanoate	*p*-bromophenol
nonane	*o*-bromotoluene
octane	chlorobenzene
	o-chlorobenzonitrile
	4-chloro-3-methylphenol
	coumarin
	p-cyanophenol
	dichlorobenzene
	p-dimethylbenzene
	2,4-dimethylaniline
	diphenylmethane
	o-ethylaniline

c. If there are major M^+ +2, M^+ + 4, and possibly M^+ +6 peaks, the compound contains multiple chlorine or bromine atoms. Check Figure 7 to identify the number and type of halogens present.

2. Using the “rule of 13,” calculate the molecular formula from the molecular weight assuming the compound is a hydrocarbon. Use $M^+/13 = n + r/13$ and C_nH_{n+r} for compounds with a molecular weight less than 156. At or above the molecular weight of 156, the formula changes to $C_{n-1}H_{n+12+r}$ (aliphatic compounds), C_nH_{n+r} (aliphatic-aromatic compounds) and $C_{n+1}H_{n-12+r}$ (highly aromatic compounds). (See Section 6 to determine if a compound is largely aliphatic, aromatic or a mixture of these components.) Draw the structure of a compound that is consistent with the calculated molecular formula. (Hint: if there are four degrees of unsaturation, a compound with benzene ring is a strong possibility.)

3. If the compound contains an odd number of nitrogens and/or halogens, calculate possible molecular formulas (C_xH_yN, $C_xH_yN_3$, $C_xH_yCl_z$, etc.) Ignore this calculation if the compound does not have nitrogens and/or halogens. Draw the structure of a compound that is consistent with the calculated molecular formula. (Hint: if there are four degrees of unsaturation, a compound with benzene ring is a strong possibility.)

4. If the compound does not have an odd number of nitrogens and/or halogens, calculate the molecular formulas for inclusion of one or two oxygen atoms. After each of these calculations, draw the structure of a compound that is consistent with the calculated molecular formula. (Are these possibilities reasonable? Hint: if there are four degrees of unsaturation, a compound with benzene ring is a strong possibility.)

5. If the compound does have an odd number of nitrogens or any halogens, calculate the molecular formulas for inclusion of one or two oxygen atoms. After each of these calculations ,draw the structure of a compound that is consistent with the calculated molecular formula. (Are these possibilities reasonable? Hint: if there are four degrees of unsaturation, a compound with benzene ring is a strong possibility.)

6. Examine the sample spectra in Figure 8. Which compound type is the best match the unknown (aliphatic, aromatic, cycloalkane or aromatic-aliphatic)?

7. Using the information in the preceding sections, what molecular formula best fits this information? Draw a structure.

Gas Chromatographic – Mass Spectral Analysis Conditions

Gas chromatography-mass spectrometry (GC–MS) was performed on the dichloromethane solutions in a splitless mode (0.5 min), using a Hewlett-Packard (Model GCD Plus) fitted with a 30 m × 0.25 mm cross-linked phenyl methyl silicone capillary column (HP-5MS). The gas chromatograph was programmed so the oven temperature was kept at 40°C for 4 min, then increased to a final temperature of 325 °C at a rate of 30 °C/min and kept at this temperature for two minutes. Mass spectral fragments below m/z = 39 were not recorded.

Stock solutions of unknowns were prepared by dissolving 10 mg of each unknown in 5.0 mL of dichloromethane. For analysis one drop of two different stock solutions were added to 1.0 mL of dichloromethane. GC–MS analysis was done using a 1.0-µL sample of this solution. Compounds successfully used as unknowns for this experiment are listed in Table 3.

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