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MODERN SPECTROSCOPIC METHODS IN ORGANIC CHEMISTRY

Tutorial

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This tutorial is designed for students and master students of chemical specialties. The tutorial covers such topics as ultraviolet, infrared, nuclear magnetic resonance spectroscopy and mass spectrometry. This tutorial will provide students and master students with a strong foundation in spectroscopic methods of organic molecules structure elucidation.

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PREFACE

This tutorial is an introductory guide to the interpretation of the ultraviolet, infrared, nuclear magnetic resonance and mass spectra of organic compounds. These four spectroscopic methods have been used routinely to determine the structure of organic compounds, both natural and synthetic ones. Every organic chemist needs to be skilled how to apply these four spectroscopic methods for structure determination. The ultraviolet spectrum identifies conjugated systems, the infrared spectrum identifies functional groups, the nuclear magnetic resonance spectra identify how the atoms are connected, and the mass spectrum gives the molecular formula.

Since application of the spectroscopic methods is possible without a detailed theory behind them, discussion of the theoretical background is kept to a minimum. It has been described how the techniques work, and how to read each of the four kinds of spectra, including important 2D NMR spectra. In Chapter 6, we work through examples, in which all four spectroscopic methods can be brought together to solve structural problems, and there are a lot of problem sets for students to work through for practice.

The chapters are written in the same general order as found in most textbooks. Key terms and concepts are italicized. Each chapter ends with questions, covering the material presented. Use each question to check your comprehension and progress.

This tutorial can be used for such disciplines as “Modern Spectroscopic Methods in Organic Chemistry”, “Organic Chemistry”, “Theoretical Fundamentals of Organic Chemistry”, and it is designed for such specialties as 6M072100 – “Chemical technology of organic substances”, 5B072100 – “Chemical technology of organic substances”, 5B074800 – “Pharmaceutical manufacturing engineering”, 5B011200 – “Chemistry”, 5B060600 – “Chemistry”, 5B072000 – “Chemical technology of inorganic substances”.

INTRODUCTION

Structure determination is highly important for an organic chemist. Diverse physical methods are used to study structure of organic molecules. The greatest information can be obtained by studying the interaction of matter with electromagnetic radiation in a wide frequency range, beginning with radio waves and ending with γ-rays, that is, throughout the entire electromagnetic spectrum. In this case, the energy of the molecules changes, which is determined by the relation:

$$E = h\nu,$$

where h is the Planck's constant, and ν is a radiation frequency.

If the energy of the final state is higher than the energy of the initial state, energy is absorbed, and conversely, the energy is radiated. The first case corresponds to the absorption spectra; the second one corresponds to the emission spectra.

When substance reacts with radiation this energy is distributed on all types of motion that are in the substance, namely electron excitation, the amplitude variation of longitudinal and transverse vibrations of each bond, change in the rate and direction of rotation of particulate matter, etc. Each type of motion absorbs a certain quantum of energy that can be registered.

The ability to absorb electromagnetic radiation is a common property of all molecules. *Spectrophotometry* is a measurement of how much a chemical substance absorbs or transmits and a spectrophotometer is an instrument that measures the amount of the intensity of light absorbed after it passes through sample solution. The region of absorption is called *the absorption band*. The complex of the absorption bands of the molecule is called *the absorption spectrum*. It is characteristic for the molecule and can't be reproduced by any other molecules, even of a very similar structure. At the same time individual bands, corresponding to absorptions of individual bonds or groups of atoms, can be repeated in the spectra of different molecules. This fact allows detecting them.

Electromagnetic spectrum. Electromagnetic radiation can be characterized by wave and energy parameters. The wave parameter is expressed by wavelength λ or frequency v, which are related to each

other by the equation $\lambda = c/\nu$, where c is the velocity of light. There is often used wavenumber (cm^{-1}), $\nu = 1/\lambda$.

Wavelength is defined below as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10^{-9} meters).

Frequency is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz).

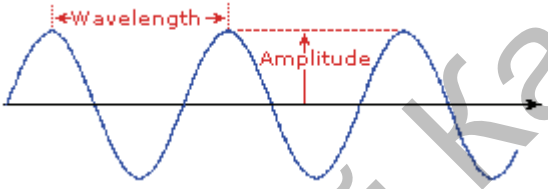


Figure 1 shows the electromagnetic spectrum. There are different types of electromagnetic waves that make up the electromagnetic spectrum (EMS).

- *Gamma rays* are produced primarily by four different nuclear reactions. Gamma-rays are sometimes used to treat cancerous tumors in the body by damaging the DNA of the tumor cells. However, gamma-rays can also damage the DNA of surrounding healthy tissue cells.
- *X-rays* are less harmful than gamma except in high doses. One of the most common and beneficial uses of X-rays is for medical imaging. X-rays are also used in treating cancer and in exploring the cosmos.
- *UV light* has enough energy to break chemical bonds. Most of the natural UV light people encounter comes from the sun.
- *Visible light.* The most important characteristic of visible light is colour. Colour is both an inherent property of light and an artifact of the human eye.
- *Infrared radiation* is felt as heat.
- *Radio waves* have the lowest energy (frequency). Radio waves are also used in NMR and in magnetic resonance imaging MRI.

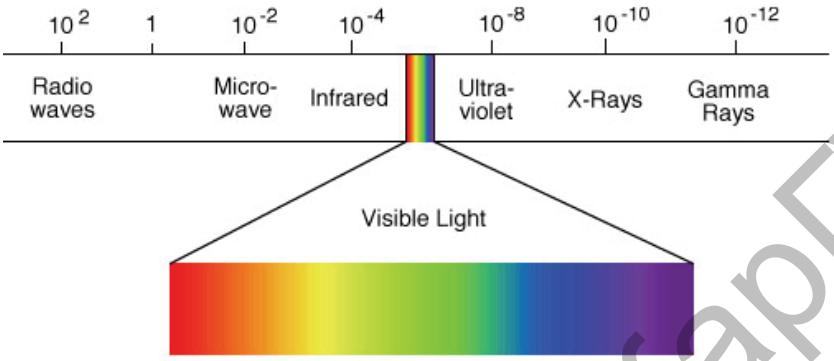


Figure 1. The electromagnetic spectrum

Absorption spectra in the ultraviolet, visible, infrared and nuclear magnetic resonance spectroscopy are most widely used in the study of organic compounds. At present, mass spectrometry based on the transformations of a substance under the action of an electron impact has become widely used along with the listed spectral methods for studying the structure of organic compounds.

CHAPTER 1.

UV-Visible Spectroscopy

1.1 UV-Visible Absorption Spectra

UV-Visible Spectroscopy is a general technique because most molecules will absorb in the UV-Vis wavelength range. The UV extends from 100–400 nm. The 100–200 nm range is called the deep UV. Light sources are more difficult to find for this range, so it is not routinely used for UV-Vis measurements. Visible wavelengths cover a range from approximately 400 to 800 nm. The longest visible wavelength is red and the shortest is violet. The wavelengths of what we perceive as particular colours in the visible portion of the spectrum are shown in Figure 2. The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole [1-3].

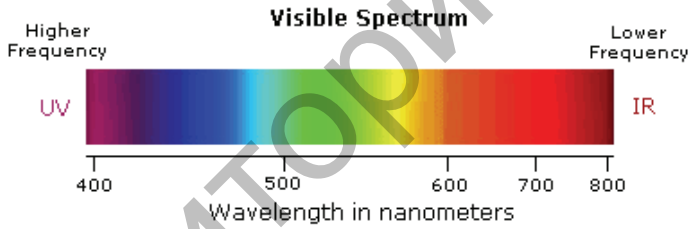


Figure 2. Visible spectrum

When a photon hits a molecule and is absorbed, the molecule is promoted into a more excited energetic state. UV-visible light has enough energy to promote electrons to a higher electronic state, from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The energy difference between the HOMO and the LUMO is called the band gap. Typically, these orbitals are called bonding and anti-bonding. A diagram, showing the various kinds of electronic excitation that may occur in organic molecules, is shown in the Figure 3. The most common transitions that fall in the UV-Vis range are π-π^* and n-π^* (coloured blue). Pi orbitals arise due to double bonds, and n orbitals are for non-bonding electrons. Thus, the best UV-Vis absorption is by molecules that contain double bonds.

Groups in a molecule, which absorb light, are known as chromophores.

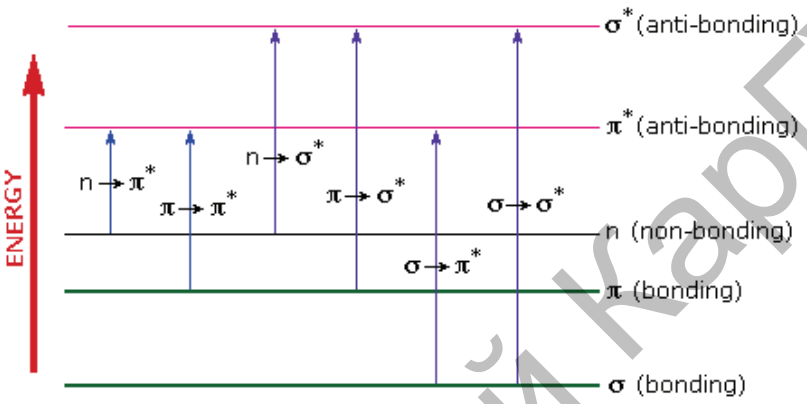


Figure 3. A diagram, showing the various kinds of electronic excitation that may occur in organic molecules

Figure 4 shows a simple UV-visible absorption spectrum for buta-1,3-diene. Absorbance is a measure of the amount of light absorbed. The higher the value, the more of a particular wavelength is being absorbed.

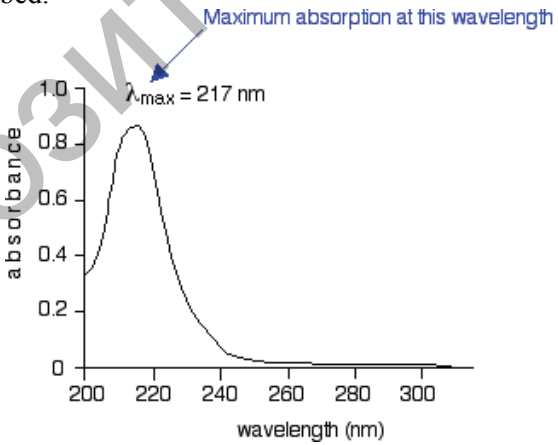


Figure 4. UV-Visible spectrum of buta-1,3-diene

The absorption peak appears at a value of 217 nm. This is in the ultra-violet, and buta-1,3-diene is colourless. There are no non-bonding electrons in buta-1,3-diene, CH_2=CH-CH=CH_2. That means that the only electron jumps, taking place (within the range that the spectrometer can measure), are from pi bonding to pi anti-bonding orbitals.

Figure 5 shows UV-Vis spectrum of acetone. Acetone has π and non-bonded electrons. Therefore there are 2 possible transitions. *Lower the energy is longer the* λ is. One important distinguishing characteristic of n to π* transitions results from the fact that the lone-pair (n) electrons are concentrated in a different region of space from the π electrons. This makes the n to π* transition less probable than the π to π* (Figure 6) [4].

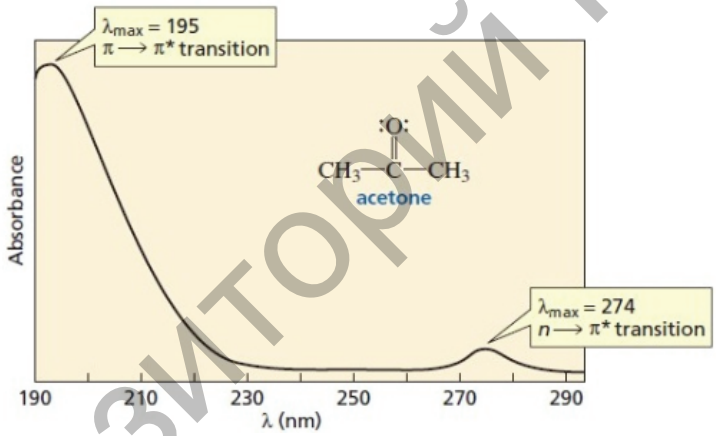


Figure 5. UV-Vis spectrum of acetone

The compounds will often appear coloured for molecules with absorption in the visible region. However, a common misconception is that the wavelength of peak absorption (λ_{max}) for a compound is the colour it appears. A compound that appears red does not have much absorption in the red region of the spectrum. Instead, the λ_{max} for a compound that looks red is green. The colour of a compound arises because those wavelengths of light are selectively transmitted through the sample, and thus they are not absorbed. A colour wheel is helpful in determining what colour a compound will absorb and what range

the λ_{max} will be, as the colour directly across the wheel from the observed colour is the colour that is most absorbed (Figure 7).

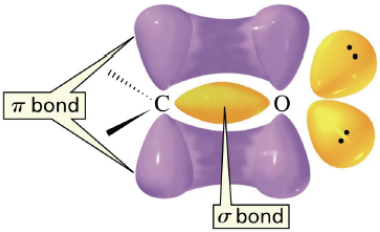


Figure 6. Structure of a carbonyl group

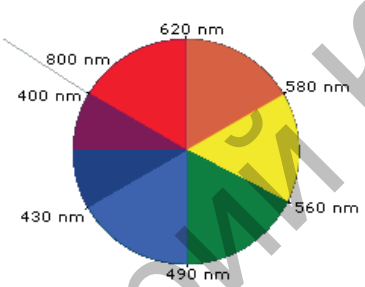


Figure 7. The colour wheel

Effect of conjugation on wavelength

Pi orbitals adjacent to each other that are connected, called conjugation, typically increases absorption. Let's compare ethene with buta-1,3-diene. In ethene, there is one pi bonding orbital and one pi anti-bonding orbital. There are two pi bonding orbitals and two pi anti-bonding orbitals in buta-1,3-diene (Figure 8).

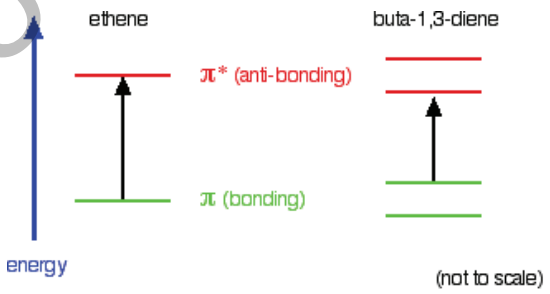
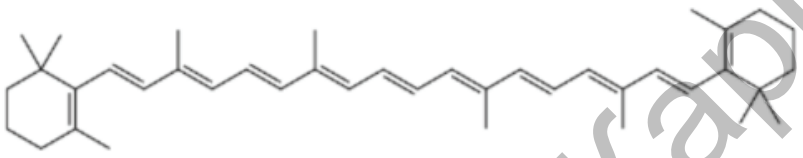


Figure 8. A diagram, showing one pi bonding orbital and one pi anti-bonding orbital in ethene and buta-1,3-diene.

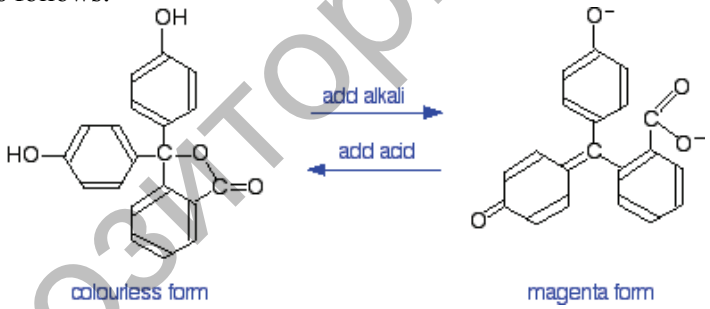
Conjugation decreases the energy gap between HOMO and LUMO and less energy is required for electronic transitions. Therefore transitions occur at longer wavelengths. If a compound has enough double bonds, it will absorb visible light and the compound will be coloured, e.g. β-carotene which is orange and is found in carrots and tomatoes has λ_{max}= 455nm.



β-carotene

Colour changes of indicators

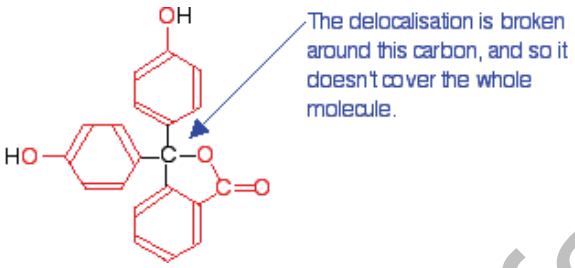
Phenolphthalein. Phenolphthalein is an acid-base indicator, and it is colourless in acidic conditions and magenta (bright pink) in an alkaline solution. The structures of the two differently coloured forms are as follows:



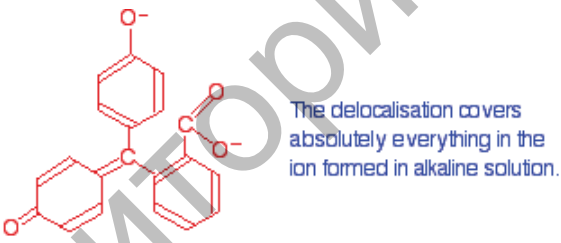
Both of these forms absorb light in the ultra-violet region, but the one on the right also absorbs in the visible with a peak at 553 nm, which is in the green region of the spectrum. According to the colour wheel the complementary colour of green is magenta, since phenolphthalein appears bright pink in alkali solution.

The amount of delocalisation shifts the absorption peak to a higher wavelength. The delocalisation doesn't extend over the whole molecule in the case of colourless form (the extent of the delocalisation is shown in red). The carbon atom in the centre of the molecule

with its four single bonds prevents the three delocalised regions interacting with each other.



The rearrangement lets the delocalisation extend over the entire ion in alkali solution. This greater delocalisation lowers the energy gap between the highest occupied molecular orbital and the lowest unoccupied pi anti-bonding orbital. It needs less energy to make the jump and so a longer wavelength of light is absorbed [5].



1.2 The Beer-Lambert Law

The greater the number of molecules that absorb light of a given wavelength, the greater the extent of light absorption and higher the peak intensity in absorption spectrum. If there are only a few molecules that absorb radiation, the total absorption of energy is less and consequently lower intensity peak is observed. This makes the basis of Beer-Lambert Law, which states that the fraction of incident radiation absorbed, is proportional to the number of absorbing molecules in its path.

When the radiation passes through a solution (Figure 9), the amount of light absorbed or transmitted is an exponential function of

the molecular concentration of the solute and also a function of length of the path of radiation through the sample. Therefore,

log I_o / I = ε c l,

where I_o = Intensity of the incident light (or the light intensity, passing through a reference cell)

I = Intensity of light transmitted through the sample solution

c = concentration of the solute in mol L^{-1}

l = path length of the sample in cm

ε = molar absorptivity or the molar extinction coefficient of the substance whose light absorption is under investigation. It is a constant and is a characteristic of a given absorbing species (molecule or ion) in a particular solvent at a particular wavelength. ε is numerically equal to the absorbance of a solution of unit molar concentration (c = 1) in a cell of unit length (l = 1) and its units are L mol^{-1} cm^{-1}.

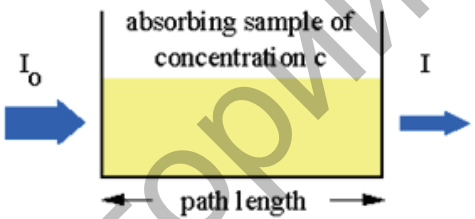


Figure 9. The Beer-Lambert Law

The ratio I / I_o is known as transmittance T and the logarithm of the inverse ratio I_o / I is known as the absorbance A.

Therefore,

- log I / I_o = - log T = ε c l

and log I_o / I = A = ε c l

or A = ε c l

The positions of peaks are reported as λ_{max} (in nm) values and the absorptivity is expressed in parenthesis for presenting the absorption characteristics of a spectrum [6].

1.3 UV-Vis Spectrometers

A basic UV visible spectrophotometer consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette, sample solution, photoelectric detector, and a digital meter (Figure 10).

Spectrometer produces the light beam of desire wavelength that transmits through collimator (lens) to make a straight beam of light. Further it passes through a monochromator that splits it into several component wavelengths. One can select particular wavelength with the help of a wavelength selector (slit). The light beam of certain wavelength is passed through the sample solution that is placed in cuvette. Photometer is used to detect the amount of absorbed light and forward a signal to digital display. The photoelectric detector measures the intensity of light and one can record that data to calculate the absorbance. Absorbance of a sample is proportional to the length of the sample and the number of the molecules in sample solution. The curve between the absorbance and transmittance with wavelength of radiation provide information about concentration of sample solution.

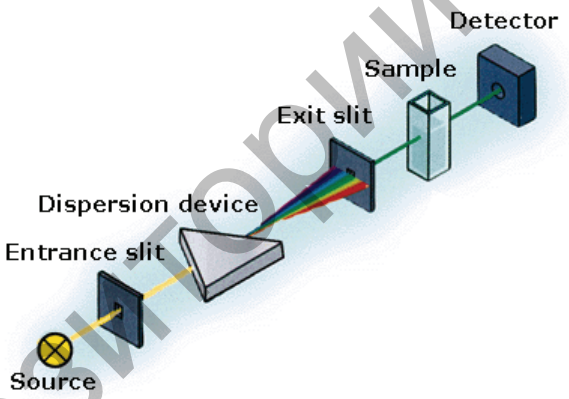


Figure 10. Schematic of a UV-Vis spectrophotometer

A diode-array instrument (Figure 11) allows all colours of light to be transmitted through the sample, and then the light is separated into different wavelengths spatially and detected using photodiodes. Diode-array instruments collect full spectra faster, but are more complicated and more expensive [7].

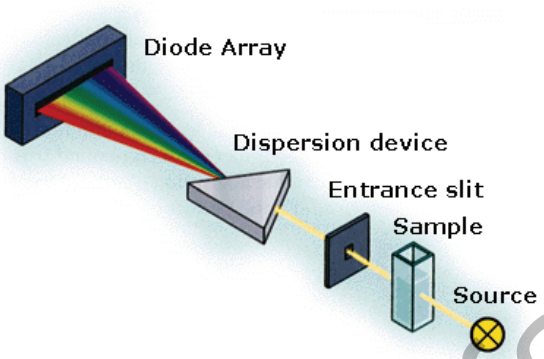


Figure 11. A diode-array instrument

The UV-Vis spectra are usually measured in very dilute solutions and the most important criterion in the choice of solvent is that the solvent must be transparent within the wavelength range being examined. Table 1 lists some common solvents with their lower wavelength cut off limits. Below these limits, the solvents show excessive absorbance and should not be used to determine UV spectrum of a sample [8-9].

Table 1. Common solvents with their cut-off limits

No.	Solvent	Cut-off wavelength (nm)
1	Acetonitrile	190
2	Water	191
3	Cyclohexane	195
4	Hexane	201
5	Methanol	203
6	95% ethanol	304
7	1,4-dioxane	215
8	Ether	215
9	Dichloromethane	220
10	Chloroform	237
11	Carbon tetrachloride	257
12	Benzene	280

1.4 Important Terms and Definitions. Application of Electronic Spectroscopy for Prediction of Organic Molecules Absorption Maxima

Chromophore is the group of atoms, containing electrons, responsible for the absorption.

Auxochromes are the substituents that do not absorb ultraviolet radiation but shift the absorption maximum to longer wavelength. Examples of auxochromes are methyl, hydroxyl, alkoxy, halogen, amino group etc.

Hypsochromic shift or Blue shift is a shift of an absorption maximum towards shorter wavelength or higher energy.

Bathochromic shift or Red shift is a shift of an absorption maximum towards longer wavelength or lower energy.

Hypochromic effect is an effect that results in decreased absorption intensity.

Hyperchromic effect is an effect that results in increased absorption intensity [10].

Conjugated dienes and polyenes

The increase in size of the conjugated system gradually shifts the absorption maximum (λ_{max}) to longer wavelength and also increases the absorption. For example, ethylene absorbs at 175 nm (ε = 1000) and the conjugation in butadiene gives a strong absorption at longer wavelength at 230 nm and with higher intensity (ε = >1000). The presence of alkyl substituents on double bond also produces bathochromic shift and hyperchromic effect. These effects are additive in dienes and up to some extent in trienes.

Carbonyl compounds

Carbonyl compounds have two principal UV radiations, the allowed $\pi \rightarrow \pi^*$ transitions and the forbidden $n \rightarrow \pi^*$ transitions. The heteroatom withdraws electrons from carbonyl carbon in amides, acids, esters or acid halides and makes carbonyl oxygen lone pair of electrons more stabilized due to its involvement in increasing C=O bond order. As a result, the $n \rightarrow \pi^*$ transition of these compounds is shifted to 200-215 nm range relative to 270 nm in aldehydes and ketones. Conjugation of the carbonyl group with double bond shifts both $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions to longer wavelengths.

Aromatic compounds

The simplest aromatic compound is benzene. It shows two primary bands at 184 (ε = 47,000) and 202 (ε = 7400) nm and a secondary fine structure band at 255 nm (ε = 230 in cyclohexane). Substituents on the benzene ring also cause bathochromic and hypsochromic shifts of various peaks. Unlike dienes and unsaturated ketones, the effects of various substituents on the benzene ring are not predictable. However, qualitative understanding of the effects of substituents on the characteristics of UV-Vis spectrum can be considered by classifying the substituents into electron-donating and electron-withdrawing groups. In case of polycyclic aromatic hydrocarbons, both primary and secondary bands are shifted to longer wavelength due to extended conjugation. These spectra are usually complicated but are characteristic of parent compound.

1.5 Application of UV-Vis Spectroscopic Data

UV-Vis spectroscopic data can give qualitative and quantitative information of a given compound or molecule. In practice, a calibration curve is constructed by plotting absorbance vs. molar concentration and the concentration of unknown with 'X' absorbance is determined by finding the concentration, corresponding to the measured absorbance on the calibration curve. At least three concentrations of the compound will be needed to make a calibration curve. The concentrations should start at just above the estimated concentration of the unknown sample and should go down to about an order of magnitude lower than the highest concentration. The calibration solutions should be spaced relatively equally apart, and they should be made as accurately as possible, using digital pipettes and volumetric flasks instead of graduated cylinders and beakers.

An example of absorbance spectra of calibration solutions of Rose Bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) can be seen in Figure 12. The value for the absorbances of each of the spectral curves at the highest absorbing wavelength is plotted in a graph similar to that in Figure 13 of absorbance versus concentration to make a calibration curve. The correlation coefficient of an acceptable calibration is 0.9 or better [8, 11].

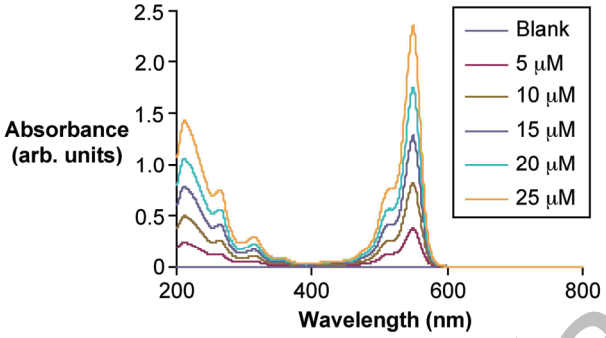


Figure 12. UV-Vis spectra of different concentrations of Rose Bengal

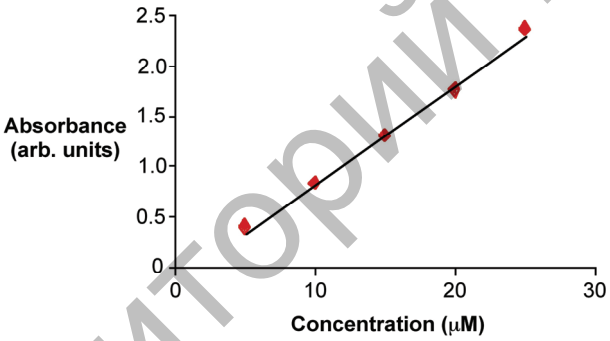


Figure 13. Calibration curve of Rose Bengal
Equation of line: y = 0.0977x – 0.1492 (R2 = 0.996)

Questions and Assignments for Self-Study:

1. What is UV-Vis Spectroscopy?
2. What are the wavelength ranges for the ultraviolet and visible regions of the spectrum?
3. What molecular or structural features give rise to absorption of ultraviolet/visible (UV/VIS) radiation in organic species? Give an example of an organic compound that would not absorb UV/VIS radiation.

4. Choose a suitable solvent for the following analyses dimethylbenzene (250-300 nm), sodium benzoate (250-320 nm), and aspirin (280-320 nm).

5. What colour of light is absorbed by (a) the blue jeans? (b) the leaves?

6. Lycopene (λ_{max}=469 nm) is present in tomatoes. What colour of light does lycopene absorb?

7. Benzoic acid has an absorption maximum at 230 nm. Where do you expect to see the absorption maximum in cinnamic acid?

8. Look up the structure of aspirin. It absorbs at around 275 nm. What structural components cause it to absorb radiation?

9. What solvent would be appropriate to record as much of the UV/VIS spectrum for (a) caffeine and (b) naphthalene. You should look up the structures of each of these to help with your answer.

10. For the following pigments, namely tartrazine, malvidin, carminic acid, Sudan II, and rosinidine

a) draw the chemical structure and determine the main chromophore
b) identify the class of chromophore
c) predict the observed colour.

11. What does the Beer-Lambert law state?
12. What is molar absorptivity?
13. Which electronic transitions is UV-Vis Spectroscopy of organic compounds concerned with?
14. What is UV-Vis spectroscopy used for?
15. What is an effect of conjugation on wavelength?
16. What is a schematic of UV-Vis spectrometer?
17. What is a chromophore?
18. What is a red shift?
19. What is a hypsochromic shift?
20. What is an auxochrome called?
21. What is a hypochromic effect?
22. What is a hyperchromic effect called?

CHAPTER 2.

IR Spectroscopy

2.1 Introduction to IR Spectroscopy

Atoms in a molecule do not maintain fixed positions and vibrate back and forth. The two atoms joined together by a chemical bond macroscopically can be composed as two balls joined by a spring (Figure 14). The application of a force for stretching the balls (atoms) away from each other or closer to each other or bending one of the atoms either vertically or horizontally and then release of the force results in the vibrations on the two balls (atoms). These vibrations depend on the strength of the spring and also the mode (stretching or bending), in which the force is being applied.

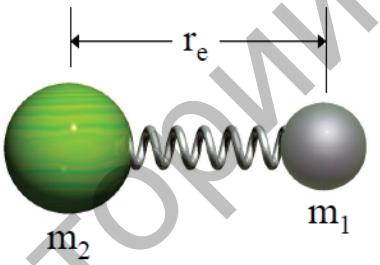


Figure 14. Representation of a chemical bond as two balls joined by a spring

Range of electromagnetic waves 10^{-4} - 10^{-1} cm is referred to the infra-red region. The absorption of the energy by the molecule in this range results in the change of the vibrational states of the nuclei of the atoms that make up molecules, and rotational states of the molecules. A non-linear molecule containing N atoms has 3N-6 fundamental vibrations (linear molecule 3N-5). It is shown in the form of bands set in the spectrogram, the position of which can be characterized by the values of the wave numbers, wavelengths or frequencies. Wavenumbers (cm^{-1}) are often used in the graphical illustration of the IR spectra. *How much of a particular frequency gets through the compound is measured as percentage transmittance* [12-13].

$$\text{wavenumber} = \frac{1}{\text{wavelength in cm}} \quad \text{cm}^{-1}$$

Hooke's law and absorption of radiations

The analogy of a chemical bond with two atoms linked through a spring can be used to rationalize several features of the infrared spectroscopy. The approximation to vibration frequency of a bond can be made by the application of Hooke's law. In Hooke's law, two atoms and their connecting bond are treated as a simple harmonic oscillator composed of two masses joined by a spring and frequency of vibration is stated as

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\left(\frac{K}{(m_1 m_2)/(m_1 + m_2)}\right)}$$

Where $\bar{\nu}$ is the vibrational frequency (cm^{-1})
c is velocity of light (cm/s)
K is force constant of the bond (dyne/cm)
m_1 and m_2 are masses of the two atoms

The quantity $(m_1 m_2) / (m_1 + m_2)$ is often expressed as μ, the reduced mass of the system.

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

Therefore, the vibrational frequency of a bond would increase with the increase in bond strength. Consequently, one can expect that C=C and C=O stretching will have higher frequencies than C-C and C-O stretching, respectively. Therefore, the vibrational frequency of a bond would increase with the decrease in reduced mass of the system. It implies that C-H and O-H stretching absorptions should appear at higher frequencies than C-C and C-O stretching frequencies. Further, in parallel with the general knowledge that the stretching of the spring requires more energy than to bend it, the stretching absorption of a band always appear at higher energy than the bending absorption of the same band.

The Hooke's law can be used to theoretically calculate the approximate stretching frequency of a bond. The value of K is approximately 5×10^5 dyne/cm for single bonds and approximately two and three times this value for the double and triple bonds, respectively.

Modes of molecular vibrations

The atoms in molecules make continuous oscillatory movements, which are divided into two groups. Fluctuations, in which a change in the bond lengths occurs, are *stretching vibrations,* and vibrations accompanied by changes in the value of bond angles are *bending vibrations*. Stretching vibrations are possible only for diatomic molecules. Stretching and bending vibrations are characteristic for complex polyatomic molecules. Stretching and bending vibrations come in several types. Some of these are shown in Figure 15 [14-15].

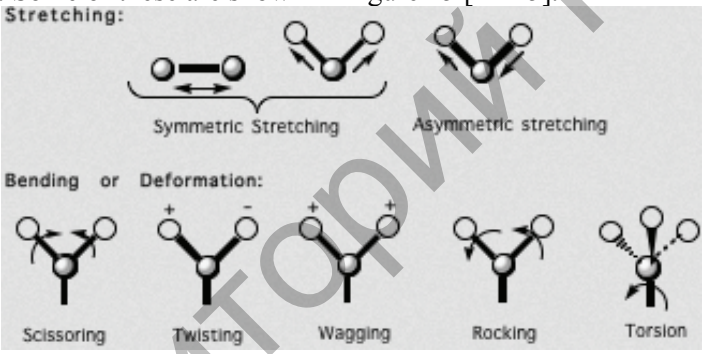


Figure 15. Types of stretching and bending vibrations

2.2 IR Spectra of Organic Compounds

Infrared spectra can be measured for gaseous, liquid, and solid substances. Special gas cells are used to measure the spectra of gaseous substances. Sample preparation for recording infrared spectra is carried out in accordance with the special techniques. Discs with KBr are prepared for solids. A sample of the solid compound (1.3 mg) is thoroughly mixed in a vibrating mill or a mortar with spectroscopically pure potassium bromide (150-200 mg) and the mixture is compressed at a pressure of 7.5-10 tons/sq. cm for 2-5 minutes under a vacuum. Spectrum of the sample is recorded relatively to the

disk prepared from the pure KBr placed in the second channel of the spectrometer.

IR spectra recorded by the IR spectrometer consist of more or less broad absorption bands. This happens because the vibrations are accompanied by the rotation and thus the spectrum is rotational-vibrational one. Assigning each band to a particular mode of vibration is practically impossible but two non-identical molecules generally have different IR spectra. An IR spectrum, therefore, is a fingerprint of the molecule. The IR region of electromagnetic radiation (4000-1300 cm^{-1}) is the most useful for identification of functional groups. The region to the right-hand side of the spectrum (from about 1300 to 650 cm^{-1}) is called *the fingerprint region*. The importance of the fingerprint region is that each different compound produces a different pattern of troughs in this part of the spectrum. The basic information about the vibrational modes in basic functional groups has been discussed in the following sections.

C-H and C-C stretching and bending vibrations of hydrocarbons

Alkanes: Only two types of atoms - C and H and only two types of bonds – C-C and C-H are present in simple hydrocarbons. The C-H stretching vibrations usually occur in the general region between 3300 cm^{-1} (in alkynes) and 2700 cm^{-1} (in aldehydes).

Alkenes: The carbon-carbon double bond has a higher force constant than a C-C single bond and C=C stretching vibrations appear at higher frequency (1680-1620 cm^{-1} than that of the C-C stretching vibrations (1200-800 cm^{-1}) in a non-conjugated olefin.

Alkynes: All alkynes both terminal or non-terminal contain carbon – carbon triple bond but the non-terminal alkynes also contain a CH bond. The force constant for a triple bond is greater than that for a double bond. Consequently, whereas a C-C stretching vibrations occur between 1300-800 cm^{-1} and the C=C stretching vibration occur in the region 1700-1500 cm^{-1} the C≡C vibrations are observed at significantly higher frequencies in the region of 2300 to 2050 cm^{-1}.

Aromatic Hydrocarbons: The most prominent bands are due to out-of-plane bending of ring C-H bonds in the region of 900-650 cm^{-1} in the aromatic compounds. These bands can be used to assign the ring substitution pattern in mono substituted benzenes and 1,2-, 1,3-, and 1,4-substituted benzene derivatives. The spectra of aromatic com-

pounds typically exhibit many weak or medium intensity C-H stretching vibrations in the region 3100-3030 cm^{-1}, which is the region of olefinic compounds. Figure 16 shows the IR spectrum of toluene [16-17].

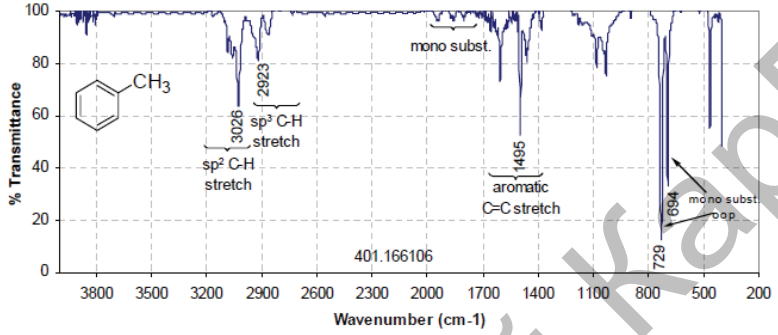


Figure 16. The IR spectrum of toluene (neat liquid)

Alcohols and Phenols: Absorption band from 3700 to 3400 cm^{-1} (see IR spectrum of 1-butanol in Figure 17) is a strong indication that the sample is an alcohol or phenol. The exact position and shape of this band depends largely on the degree of H-bonding. A strong, sharp peak in the region as higher 3700 cm^{-1} in gaseous or extremely dilute solutions represents unbounded or free OH group(s).

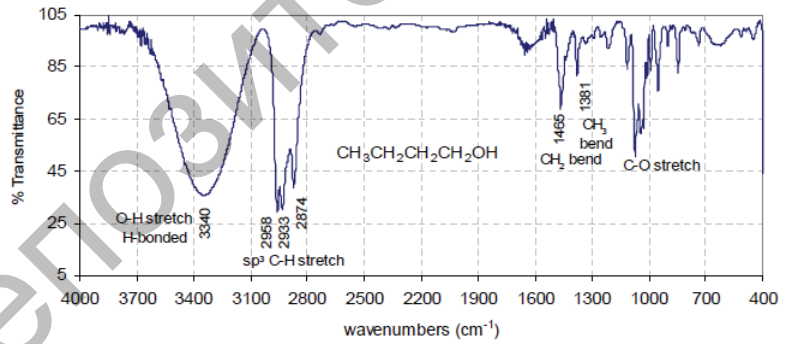


Figure 17. The IR spectrum of 1-butanol (neat liquid)

Carbonyl Compounds: The absorption peak for C=O stretching in the region 1870 to 1600 cm^{-1} is perhaps the easiest band to recognize in IR spectrum and is extremely useful in analysis of carbonyl

compounds. Aliphatic aldehydes show strong C=O stretching in the region of 1740 – 1725 cm^{-1}. The conjugation of an aldehyde to a C=C or a phenyl group lowers C=O stretching by ~ 30 cm^{-1}. Figure 18 shows the IR spectrum of benzaldehyde). Aldehyde C-H stretching vibrations appear as a pair of weak bands between 2860-2800 and 2760-2700 cm^{-1}. The higher C-H stretching band (2860-2800 cm^{-1}) of aldehyde is often buried under aliphatic C-H band. But the lower C-H band at 2760-2700 cm^{-1} is usually used to distinguish aldehydes from ketones. The C-H bending vibrations appear between 945-780 cm^{-1}.

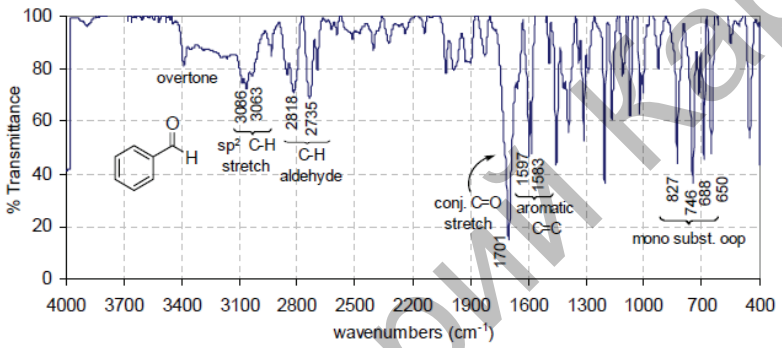


Figure 18. The IR spectrum of benzaldehyde

Carboxylic Acids, Esters and Carboxylates: In case of carboxylic acids, in solid state or pure liquid state, the intermolecular hydrogen bonding weakens the C=O bond and thus lower the stretching frequency to ~1720 cm^{-1}. The O-H stretch appears as a very broad band between 3400 – 2500 cm^{-1} (see IR spectrum of benzoic acid, Figure 19).

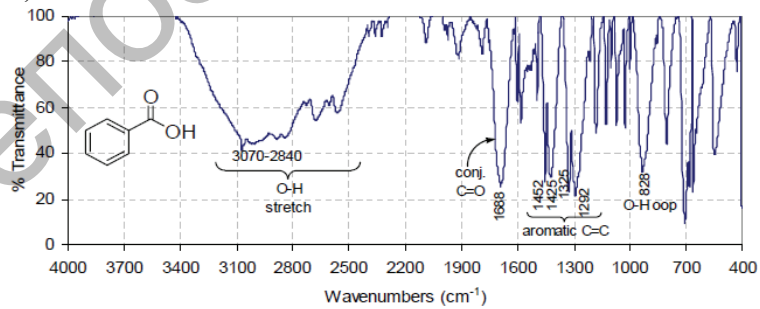


Figure 19. The IR spectrum of benzoic acid

Acid Chlorides and Anhydrides: Both carboxylic acid halides and anhydrides show strong C=O absorptions at characteristically high frequencies > 1800 cm^{-1} and are easily differentiated from other carbonyl compounds. The acid anhydrides show two absorption bands in carbonyl region at 1820 cm^{-1} due to symmetric and at 1760 cm^{-1} due to asymmetric stretching vibrations. In case of anhydrides of conjugated carboxylic acids, the frequencies due to these bands are shifted to 1775 and 1720 cm^{-1}. Figure 20 shows the infrared spectrum of benzoyl anhydride.

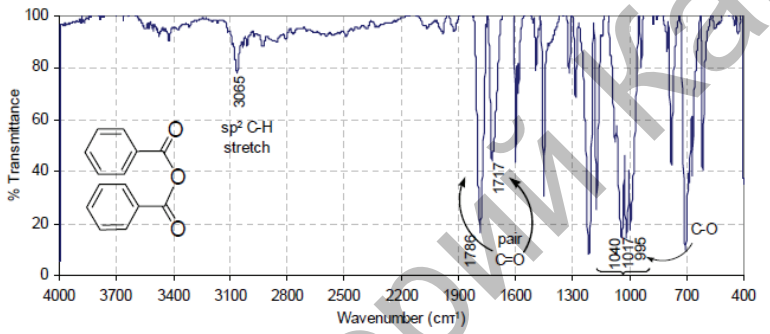
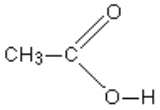


Figure 20. The infrared spectrum of benzoyl anhydride (nujol mull)

Amides: In case of amides strong resonance participation of lone pair of electrons by amide nitrogen weakens the carbonyl bond. Consequently, the C=O stretching frequency in amides appears in the range 1680-1630 cm^{-1} i.e. 20-50 cm^{-1} lower than that of C=O stretching of ketones. The C=O stretching band in IR spectra of amide is called amide I band. NH deformation band appears in the region 1655-1595 cm^{-1} in primary and secondary amides and is called amide II band.

2.3 Interpretation of IR Spectra of Some Organic Molecules

Ethanoic acid contains the following bonds, namely carbon-oxygen double, C=O, carbon-oxygen single, C-O, oxygen-hydrogen, O-H, carbon-hydrogen, C-H, carbon-carbon single, C-C.



The carbon-carbon bond has absorptions, which occur over a wide range of wavenumbers in the fingerprint region; that makes it very difficult to pick out on an infra-red spectrum. The carbon-oxygen single bond also has an absorption in the fingerprint region, varying between 1000 and 1300 cm^{-1}, depending on the molecule it is in. The other bonds in ethanoic acid have easily recognized absorptions outside the fingerprint region.

The C-H bond absorbs in the range from 2853-2962 cm^{-1}. The carbon-oxygen double bond, C=O, is one of the really useful absorptions, found in the range 1680-1750 cm^{-1}. Its position varies slightly, depending on what sort of compound it is in. The other really useful bond is the O-H bond. This absorbs differently, depending on its environment. It is easily recognized in an acid because it produces a very broad trough in the range 2500-3300 cm^{-1}. Figure 21 shows the IR spectrum of ethanoic acid [18-19].

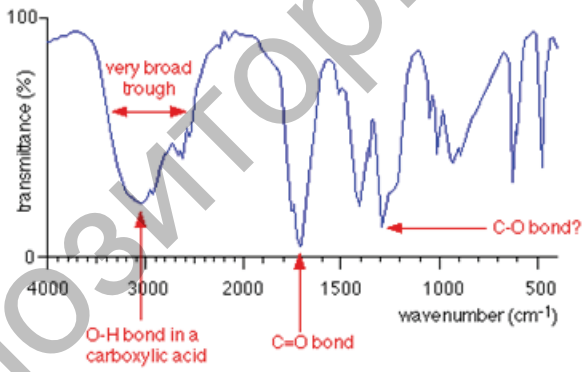


Figure 21. The infra-red spectrum of ethanoic acid

Questions and Assignments for Self-Study:

1. Point out the region of the IR spectrum, in which one can reveal the absorption bands, due to the double bond of monoalkenes.
2. Point out the region of absorption in the vibrational spectra of hydrocarbon molecules with the triple bond.

3. In what region of the IR spectrum (cm^{-1}) can one reveal the stretching vibrations due to the OH-group?

4. The IR-spectra of all types of carbonyl compounds are characterized by intense absorption. Characterize the region (cm^{-1}).

5. What is the range for the absorption due to the aliphatic aldehydes carbonyl group?

6. Point out the region of the IR spectrum (cm^{-1}), in which troughs due to the benzene ring C-H, can be found.

7. Point out the region (cm^{-1}) where the nitro group absorbs.

8. In what region of the IR spectrum the stretching vibrations due to the $N-H$ bond can be found?

9. Point out the region, in which bending vibrations due to the amino-group can be revealed.

10. In what region do nitriles absorb?

CHAPTER 3.

Nuclear Magnetic Resonance Spectroscopy

3.1 Introduction to Nuclear Magnetic Resonance Spectroscopy

The nuclear magnetic resonance method is more efficient than ultraviolet or infrared spectroscopy in the study of the structure of organic compounds. However, this method does not replace the older techniques; they complement each other.

Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance spectroscopy is spectroscopy, which records the transitions between magnetic energy levels of atomic nuclei caused by radio frequency radiation. Only nuclei with spin quantum number *I*, other than "0" can cause the NMR signal, or be active in NMR.

The spin quantum number of the nucleus is determined by the number of protons and neutrons. There is an empirical rule:

1) *I* is equal to 0 for nuclei with an even number of protons and neutrons;

2) *I* is equal to integers (1, 2, 3 ...) for nuclei with odd number of protons and neutrons;

3) *I* is equal to half-integers (1/2, 3/2, 5/2, etc.) for nuclei with even number of protons and odd number of neutrons and vice versa.

The nucleus with spin number *I* can take $2I + 1$ orientations (or hold $2I + 1$ energy levels) in the applied magnetic field with strength B_0. The energy difference between the levels increases with increasing B_0, but energy difference between two adjacent levels is a constant at the given value of B_0 (Figure 22).

The energy difference between two adjacent levels ΔE is given by:

$$\mathbf{\Delta E = B_0 \gamma\ h/2\pi,}$$

where γ is gyromagnetic ratio, which is constant for a given isotope; B_0 is strength of the external magnetic field; h is the Planck's constant.

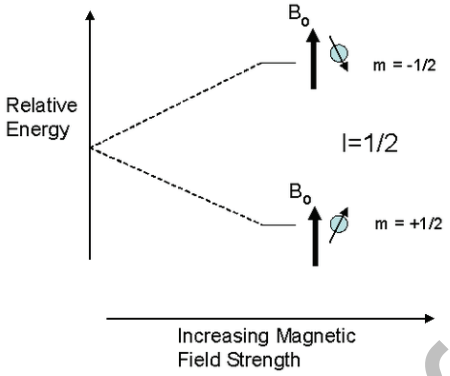


Figure 22. Energy levels for a nucleus with spin quantum number ½

The NMR experiment is to deliver energy to the nucleus and transfer it from one energy level to a higher level. The exact value of ΔE is dependent on the molecular environment of the excited nucleus, it is possible to bind the value of ΔE with the structure of the molecule, and determine the structure of the molecule [20].

The nucleus of atoms with odd spin number can give signals in the NMR spectra. Isotopes of particular interest and use to organic chemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have I = 1/2. ^{1}H NMR and ^{13}C NMR techniques are the most widely used in the study of organic compounds and polymers.

3.2 ^{1}H NMR Spectroscopy

Substance is dissolved in a suitable solvent for studies, using NMR spectroscopy (however, NMR analysis can be carried out in the solid phase). Analysis requires ~ 10-20 mg of sample. The solution prepared is placed in ~ 0.5 ml ampoule with a diameter of 5 mm. The choice of a solvent is determined by the solubility of the compound analyzed and the most complete separation of resonance signals of the compound and the solvent. Deuterated solvents are used for this purpose as deuterium does not give a signal in the NMR spectrum. However, these solvents contain residual amounts of protons, which give low intensity signals.

The ampoule with the sample is placed between the poles of a strong magnet in the NMR experiment. Hydrogen nuclei behave as

little magnets and a hydrogen nucleus can be aligned with an external magnetic field or opposed to it. The alignment where it is opposed to the field is less stable (at a higher energy). It is possible to make nucleus flip from more stable alignment to the less stable one by supplying exactly the right amount of energy as $\Delta E = h\nu$ (Figure 23) [21].

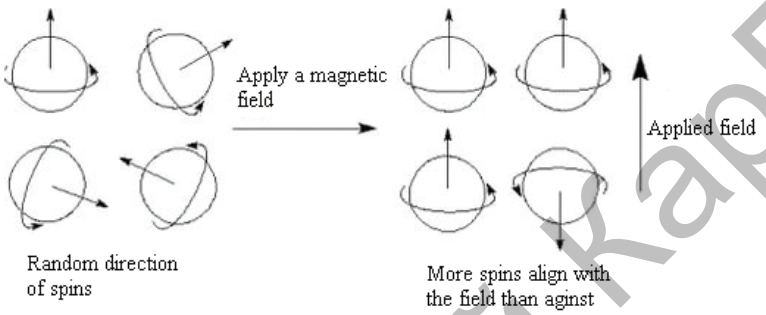


Figure 23. Alignment of spins in a magnetic field

As the lower energy level contains the excess of nuclei, a larger number of nuclei will move into the higher energy state. Absorption of electromagnetic radiation will occur as the result of the interaction of nuclei with radiation of a particular frequency. This absorption gives rise to the NMR signal. The exact value of frequency that causes transitions between energy levels of the nucleus is called *a resonant frequency of the nucleus.*

Resonance can be also achieved by changing the magnetic field strength and leaving the frequency constant. Generators of fixed frequencies 200, 300, 400, 500 or even 800 MHz are used in NMR spectrometers.

The transfer of energy of molecular motion on the nuclear spins is necessary for the existence of differences in the populations of the energy levels. The difference in population occurs when some time passes after a magnetic field is applied. This time is called *the spin-lattice relaxation time.*

The magnitude of the spin-lattice relaxation time is important. If the relaxation time is small (nuclei have fast energy transfer), the NMR signal is broadened. A great relaxation time, for example, for ^{13}C nuclei, also makes the observation of absorption signals difficult.

The latter fact is one of the reasons for the lower sensitivity of ^{13}C NMR compared with ^{1}H NMR.

The main characteristics of the NMR spectra are as follows:

- *chemical shift*
- *multiplicity*
- *spin - spin coupling constant*
- *resonance signal area*

These characteristics depend on the chemical environment of the nucleus or nuclei group, the number of neighboring nuclei, having a magnetic moment and their relative location, and the number of nuclei analyzed in various structural fragments of molecules.

Chemical shift

The difference of the proton signal position and the position of the standard signal is called the chemical shift of the proton.

Tetramethylsilane (TMS) $Si(CH_3)_4$ is the most commonly used standard. Recording NMR spectra is carried out in such a way that B_0 is increased from the left to the right. At the same time the chemical shift of the TMS signal is taken as zero, and is registered in the strongest field (right-hand side of the spectrum). In practice, chemical shift is expressed in parts per million (ppm) and is denoted by the «δ» symbol. Chemical shifts do not depend on the spectrometer working frequency.

The proton chemical shifts of organic compounds of different classes can be found in different areas, and thus, the structure of the substance can be determined by position of the NMR signals (Figure 24).

Factors affecting chemical shift

The magnetic field applied to a particular proton is rarely equals to B_0. Instead, the proton is affected by a magnetic field, which is somewhat different from B_0. The field applied B_0 causes the electrons to circulate around the nucleus, inducing a magnetic field directed against B_0. As a result, the nucleus is shielded from the full strength of the magnetic field applied. Thus, the external magnetic field needed to bring the hydrogen into resonance will be smaller if it is attached to a more electronegative element, because the hydrogen nucleus feels more of the field. Even small differences in the electronegativities of the attached atom or groups of atoms will make a difference to the magnetic field needed to achieve resonance.

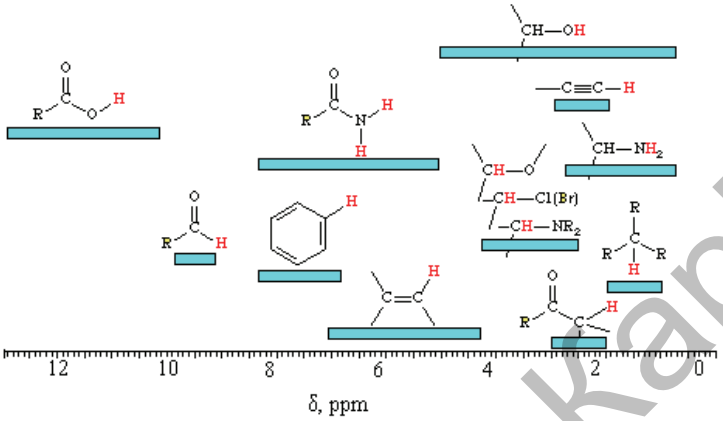
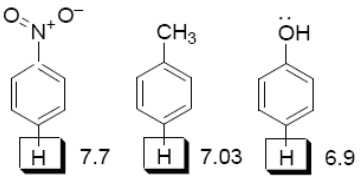


Figure 24. Generalized fields of proton chemical shifts

For a given radio frequency each hydrogen atom will need a slightly different magnetic field applied to it to bring it into the resonance condition, depending on what exactly it is attached to.

Thus, the signal of *para*-protons in monosubstituted benzenes shifts into the weak field under the effect of electron-withdrawing substituents, and into the strong field under the influence of strong electron donors. The position of *ortho*- and *meta*-protons signals depends not only on the electron density on the adjacent carbon atom and varies in less regular manner.

The value of the chemical shift cannot be always explained by the increase or decrease in the electron density. Thus, the electronegativity of carbon atoms varies as follows: C sp^3 < C sp^2 < C sp.



It is expected that acetylene protons will be deshielded to a higher extent compared to ethane and ethylene. However, it is not so. Acetylene protons are shielded. The signal is observed at 1.8 ppm.

This fact is explained by the effect of the diamagnetic anisotropy. Shielding or deshilding of the nucleus depends on the orientation of the molecule toward the external magnetic field.

Areas of shielding and deshielding for groups with magnetic anisotropy is usually depicted with the help of so-called “anisotropy cones”. Let’s consider the phenomenon of the diamagnetic anisotropy.

Acetylene molecule is linear, and the triple bond is symmetrical about an axis. If this bond is aligned with the external magnetic field, the π-bond electrons can circulate at right angles to the external magnetic field, creating their own magnetic field, directed opposite to the external magnetic field. Since acetylenic protons are aligned with the external magnetic field, the induced magnetic field will shield them (Figure 25).

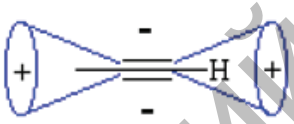


Figure 25. Areas of shielding (+) and deshielding (-) in the acetylene molecule

On the other hand, the magnetic anisotropy of the C = O groups leads to strong deshielding of aldehyde protons (δ ~ 10 ppm), as these protons are in the deshielding part of the magnetic field induced (Figure 26).

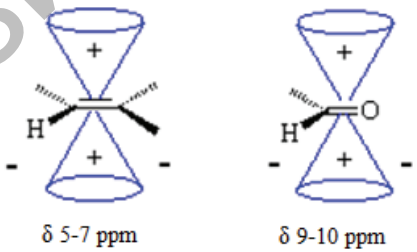


Figure 26. Areas of shielding (+) and deshielding (-) in alkenes and aldehydes

Another example of diamagnetic anisotropy is the “ring current effect” (Figure 27, the benzene anisotropy cone is depicted), which is responsible for strong deshielding of protons of the benzene ring.

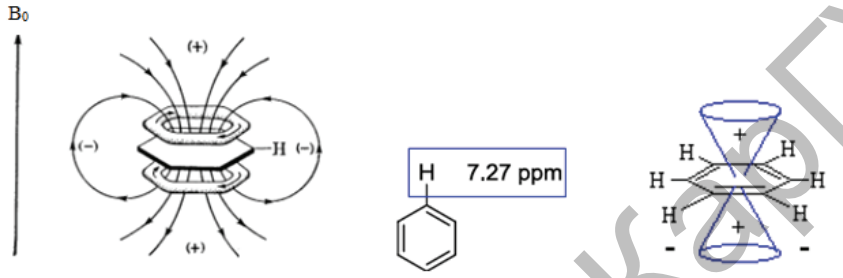
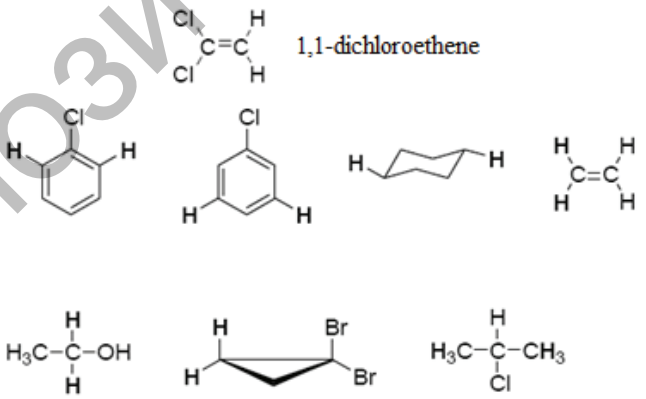


Figure 27. Benzene ring current. Benzene anisotropy cone

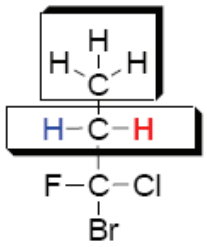
Thus, the resonance signal position is determined by the electron density around the proton considered and the anisotropic effects of adjacent groups.

Magnetic non-equivalence by chemical shift

In general, the protons can be “magnetically equivalent” or “isochronous” and give rise to one signal, and they can be “magnetically non-equivalent” or “anisochronous”. Examples of magnetically equivalent protons are shown in bold:



A classic example of anisochronous protons (magnetically non-equivalent) are protons of the methylene group attached to the chiral center, for example, 1-bromo-1-chloro-fluoropropane.

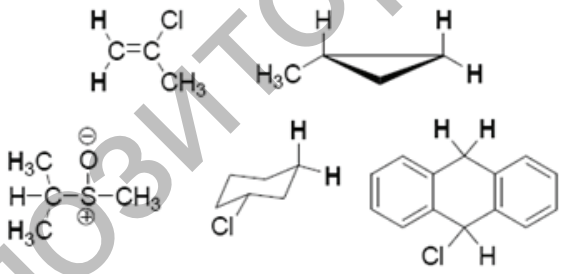


isochronous protons
three protons give rise to one signal

anisochronous protons
each of two protons gives rise to a signal

1-bromo-1-chloro-fluoropropane

Protons of the methylene group in the 1-bromo-1-chloro-fluoropropane are diastereotopic ones. Diastereotopic groups are groups in a diastereomeric environment. Replacement of one of them to another achiral group leads to the formation of diastereomers. The chemical shifts of diastereotopic protons are different. Diastereotopic protons can be identified by the substitution them for deuterium; diastereomers are to be obtained in the result. There are examples of anisochronous nuclei:



Spin-spin coupling

Neighboring magnetic nuclei have an important effect on the signal to be detected. If a nearby nucleus has a spin, that spin affects the magnetic environment of the nucleus observed, and the signal is not a single peak, but a group of peaks, the complexity of which depends upon the nature and number of the nearby atoms.

Signals of a proton or a group of protons in the spectrum can be represented as a single line (the signal is called a singlet) or in the

form of a group of lines. If the signal is represented as two lines of certain intensity the signal is called a doublet; the signal in the form of three lines is a triplet; the signal in the form of four lines is a quartet or quadruplet. The signal can be represented by a group of six or more lines. It is called a multiplet in this case (Figure 28) [22].

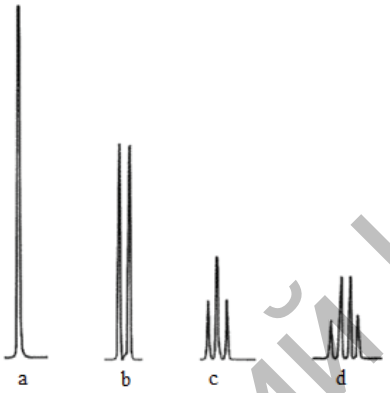


Figure 28. ^{1}H NMR signals: a – singlet (s), b – doublet (d), c – triplet (t), d – quartet (q)

The intensity of each multiplet line can be obtained from the Pascal's triangle (Figure 29).

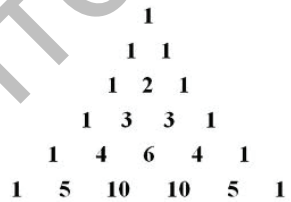


Figure 29. The Pascal's triangle

The chemical shift of the doublet is determined as follows. There is a "center of gravity" of the doublet and the corresponding value of ppm is taken. The chemical shift of a triplet, quadruplet and other multiplets is indicated by two values: the left value is the position of the leftmost component of the multiplet, the right value is the position of the extreme right component of the multiplet. Chemical shifts are usually given with two decimal places.

Example: Figure 30 shows fragments of the ^{1}H NMR spectrum: a) doublet b) multiplet.

Calculation of the chemical shift of the doublet: δ ppm = (7.776 + 7.768) / 2 = 15.544 / 2 = 7.77 ppm.

The chemical shift of the multiplet: δ ppm = 7.17 - 7.21.

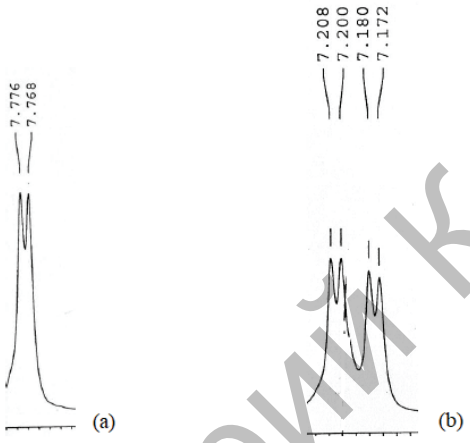


Figure 30. The fragment of the ^{1}H NMR spectrum: a) doublet, b) multiplet

The two lines are separated by the difference in the resonance frequency, which is the measured in Hz and is called *the coupling constant, J*.

The spin-spin coupling through two bonds is called geminal coupling. The spin-spin coupling through three bonds is called vicinal coupling.

The n+1 rule

If ***n*** protons of one group (denoted as *A*) react with ***n'*** protons of another group (denoted as *B*), the signal of the protons A will consist of ***n' + 1*** lines, and the signal of the protons B will consist of ***n +1*** lines. The general rule is 2nI + 1, as I = ½, the multiplicity is equal to n + 1.

Thus, a particular splitting of the signal in the ^{1}H NMR spectrum allows determining the compound structure.

The spin-spin coupling constant

If the signal is represented as a multiplet (doublet, triplet, quadruplet, etc.), the line of any multiplet will be spaced from the neighboring lines of the same multiplet by the same number of hertz. The numerical value of this distance is called the spin-spin coupling constant and is denoted by "J" (Figure 31).

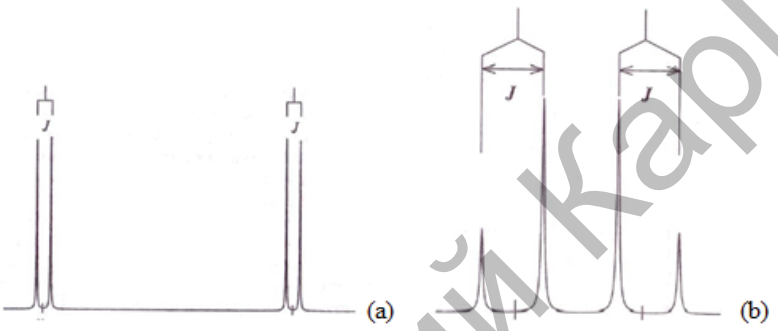


Figure 31. a: The spin-spin coupling constant (J) in the case of signal splitting into a doublet; b: The spin-spin coupling constant (J) in the case of splitting the signal into a quadruplet.

The values of the spin-spin coupling constants of all those present in the spectrum of doublets and triplets are given in the description of the spectrum.

How is the spin-spin coupling constant calculated on the basis of experimental data? First of all, for these purposes it is necessary to know the operating frequency of the instrument on which the processed spectrum is taken. Usually this information is printed directly on the spectrum. The next step is the determination of the difference in chemical shifts between the components of the multiplet under consideration in ppm. After this value is determined, it is multiplied by the value of the operating frequency of the device.

Example. Calculate the spin-spin coupling constant of the doublet shown in Figure 30a (spectrum is recorded at 300 MHz).

J = (7.776 - 7.768) x 300 = 0.008 x 300 = 2.4 Hz

The spin-spin coupling constant characterizes the degree of interaction between nuclei and does not depend on B_0. This is very im-

portant, since it makes it possible to distinguish, for example, two singlets from a doublet, recording the spectrum at two different radio frequencies. If the interval in Hertz between the two lines has not changed, then the signal is a doublet.

The value of J depends on several factors, among which is the relative arrangement of the interacting nuclei and the number of the bonds separating them. Spin-spin coupling is usually not observed between protons separated by more than three simple bonds. In the presence of multiple bonds, the total number of bonds, through which the spin-spin coupling can be observed, increases.

The spin-spin coupling constant depends on geometric factors. Thus, ^{1}H NMR is the most informative method for identifying cis- and trans- isomeric alkenes: the spin-spin trans-coupling constants are always larger than the corresponding cis constants.

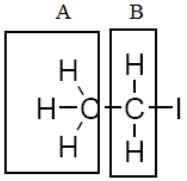
The intensity of the signal

The relative amount of protons, which have the same chemical shifts, can be determined in the NMR experiment. In other words, the NMR experiment can determine how many protons are "responsible" for the signal.

The signal intensity is proportional to the number of each proton type and the peak area measured. The relative intensities of the various signals are shown in the spectrum by an integral trace. An integrator trace measures the relative areas under the various peaks in the spectrum. When the integrator trace crosses a peak or group of peaks, it gains height. The height gained is proportional to the area under the peak or group of peaks. For example, if the heights were 0.7 cm, 1.4 cm and 2.1 cm, the ratio of the peak areas would be 1:2:3. That in turn shows that the ratio of the hydrogen atoms in the three different environments is 1:2:3.

Interpreting the ^{1}H NMR spectrum

Iodoethane molecule has two non-equivalent groups of protons, namely protons of the methyl group (denoted as A) and the protons of the methylene group (denoted as B).



Protons within each group are magnetically equivalent with each other in chemical shift. Thus, two signals can be expected in the spectrum, which is observed in reality. They are a triplet and a quartet (Figure 32). Protons of the methylene group are deshielded by an electron-accepting impact of iodine, the signal is observed in the lower fields (2.9 - 3.4 ppm) than the methyl proton signal (1.7 - 2.0 ppm).

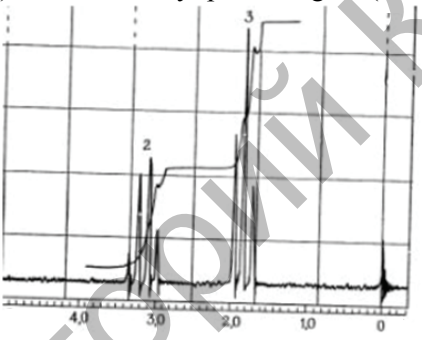


Figure 32. ^{1}H NMR spectrum of iodoethane

The signal of the methylene group is observed as a quartet at the 2.9 - 3.4 ppm in the ^{1}H NMR spectrum considered. Methyl protons are observed as a triplet at the 1.7 - 2.0 ppm.

Figures 33-35 show more examples of ^{1}H NMR spectra of organic compounds [23].

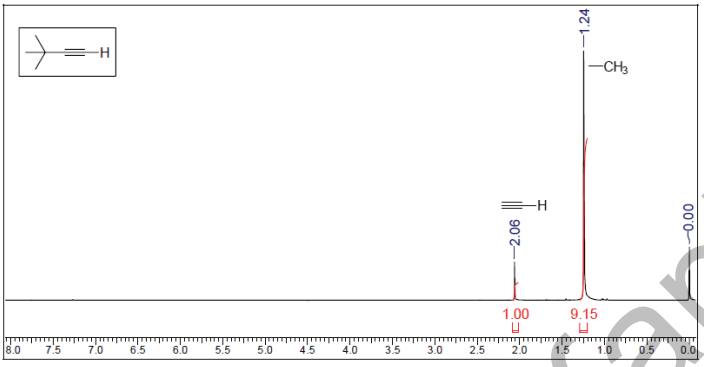


Figure 33. The ^{1}H NMR spectrum of 3,3-dimethyl-1-butyne

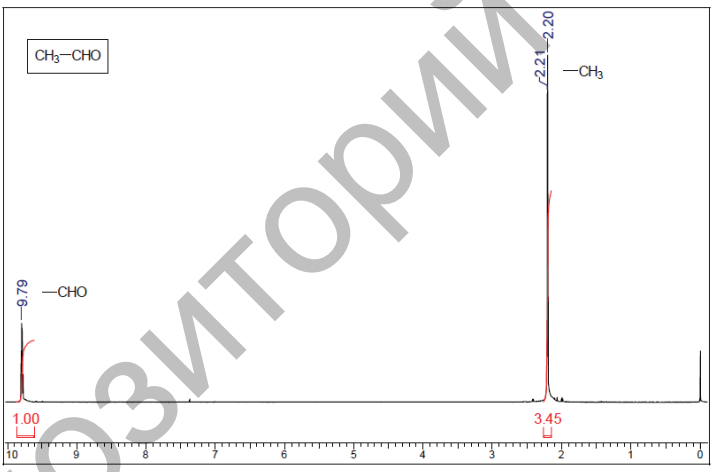


Figure 34. The ^{1}H NMR spectrum of acetaldehyde

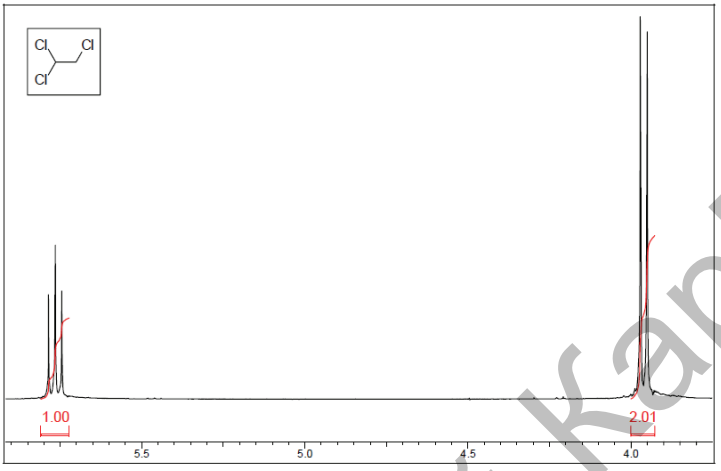
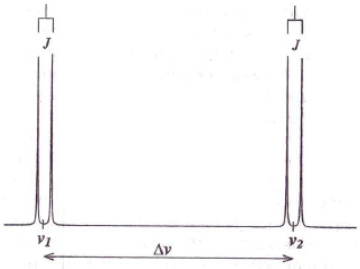


Figure 35. The ^{1}H NMR spectrum of 1,1,2-trichloroethane

Thus, we obtain three main parameters from the NMR spectrum that allow determining the structure of molecules, namely the chemical shift, its multiplicity, and the integrated intensity. Measurement of the integrated intensities of signals allows using NMR spectroscopy for the quantitative determination of the composition of mixtures of organic compounds.

Classification of spin systems

A spin system is a system of two or more interacting protons. The molecule of an organic compound can contain several spin systems. The nuclei in the system are denoted by uppercase letters of the alphabet, and the number of equivalent protons is indicated by the numbers below. If the difference in the chemical shifts of interacting protons (δH_A - δH_B), expressed in hertz, is six or more times greater than their spin-spin coupling constant, then such protons are designated by far distant letters of the alphabet (AX, A_2X, AMX, etc.):



The most weakly polar proton is denoted by the letter A, following it in the order of decreasing chemical shift - by the letter B, etc. When all weakly-polarized protons are designated, the strong-field protons are likewise called. The last letters of the Latin alphabet, starting with X (i.e, X, Y, Z), are used. This system does not cover all the protons in the molecule and is used to characterize the interacting protons at a given moment.

For example, protons in the spectra of ethyl bromide (60 MHz operating frequency) refer to the A_3X_2 ppm system: 1.63 t (3H, CH_3), 2.65 q (2H, CH_2), and in diisopropyl ether to the system AX_6 ppm: 1.12 d (1H CH), 3.52 m (6H, $2CH_3$).

Protons are denoted by neighboring letters of the alphabet with a decrease in the ratio $(\delta H_A - \delta H_B) / J_{AB}$. So, olefinic protons belong to the ABC system in the spectrum of styrene (Figure 36) [24].

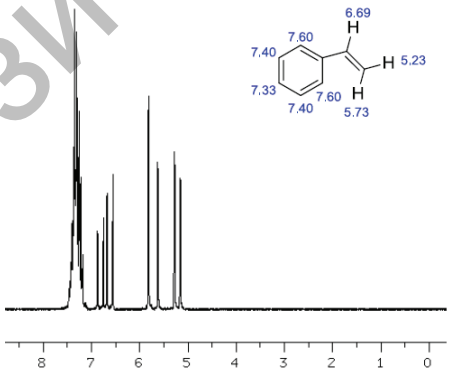


Figure 36. ^{1}H NMR spectrum of styrene

The system is denoted by AA', BB' with the same chemical shifts and different spin-spin coupling constants (for example, the above case with substituted benzene). If the spectrum consists of multiplets, in which the number of components and the ratio of their intensities correspond to rules of spin-spin coupling, then they are called spectra of the first order, for which $(\delta H_A - \delta H_B) / J_{AB} > 6$. There is a strong change in the ratio of the intensities of the multiplet components and sometimes additional signals appear in the spectra of systems with a smaller ratio $(\delta H_A - \delta H_B) / J_{AB}$. In practice, not all lines of complex multiplets can be visualized due to the insufficient resolution of the NMR spectrometer.

Suppression of the spin-spin coupling (simplification of complex spectra)

The transformation of the complex spectrum into a first-order spectrum can be achieved by increasing the ratio $(\delta H_A - \delta H_B) / J$. Increasing the operating frequency of the instrument increases the distance between the signals, keeping the spin-spin coupling constant, and all spectra can become first-order spectra at a sufficiently high operating frequency.

Paramagnetic shifting reagents (PSR) are used to increase the ratio $(\delta H_A - \delta H_B) / J$ for some compounds. They are most often intracomplex compounds of lanthanides (europium, praseodymium) with β-diketones. These PSRs are capable of giving complexes with many organic compounds, containing heteroatoms with unshared electron pairs. Amines, alcohols, aldehydes, thioethers, nitriles, epoxides are investigated with the help of paramagnetic shifting agents.

Selective suppression of the spin-spin coupling

The sample is irradiated with a radio frequency, corresponding to the resonant frequency of one of the nuclei, to suppress the spin-spin coupling between two interacting nuclei. When the spectrum is recorded under conditions of double resonance, the signal of the irradiated proton is not observed, other signals are simplified but due to the disappearance of the spin-spin interaction with this proton.

Chemical exchange

The phenomenon of migration of a proton from an atom to an atom is called *chemical exchange*.

The rate of chemical exchange of protons of the OH group, for example, for pure ethanol, is comparatively small, but it increases

sharply in the presence of acids or bases. If the rate of chemical exchange is small, then the OH signal of the proton in the ethanol spectrum is seen as a triplet. If the exchange rate is large, then the signal of the hydroxyl proton degenerates into a singlet. The signal can take the form of a wide peak at intermediate velocities.

A rapid chemical exchange leads to the fact that in a given time interval each proton has time to enter into the composition of many molecules of alcohol, so that the spin orientations of the protons, interacting with it, are averaged to a certain value. This explains the appearance of the singlet. For the same reason, resonance signals of methylene protons are not cleaved by rapidly exchanging hydroxyl protons. Thus, *a rapid chemical exchange leads to suppression of the spin-spin coupling*. The rate of chemical exchange increases with increasing temperature.

There is a rapid exchange of the proton NH in aliphatic amines, and therefore the spin-spin splitting is almost never observed. Therefore, an acute singlet of the NH proton is characteristic for such compounds. The exchange rate is intermediate in some amines, which leads to broadening of the signal [25].

3.3 ^{13}C Nuclear Magnetic Resonance Spectroscopy

The ^{12}C nucleus is magnetically inactive (I is 0). But the ^{13}C nucleus, like the proton, has a spin of ½. Because the natural content of the ^{13}C isotope is only 1.1%, and the sensitivity of the ^{13}C nucleus (a large value of the relaxation time) is only 1.6% of the sensitivity of the proton, the overall sensitivity of the ^{13}C NMR method is ~ 1/5700 from the sensitivity of the ^{1}H NMR. Figure 37 shows generalized areas of chemical shifts of carbon ^{13}C.

The main features of ^{13}C spectroscopy

1. When ^{13}C NMR spectra are recorded with complete suppression of the spin-spin coupling with protons, all the signals are singlets, unless there are other magnetically active nuclei in the molecule (for example, ^{2}H, ^{31}P, ^{19}F).

The natural content of ^{1}H is more than 99%; therefore, each ^{13}C nucleus is connected by a spin-spin coupling with some number of protons. Because of this, ^{13}C NMR spectra without suppression of spin-spin coupling with protons reveal complex overlapping multiplets, which are difficult to interpret. Figure 38 shows ^{13}C NMR

spectra of cholesterol in $CDCl_3$ at 150.9 MHz without suppression (a) and complete suppression (b) of the spin-spin coupling with protons.

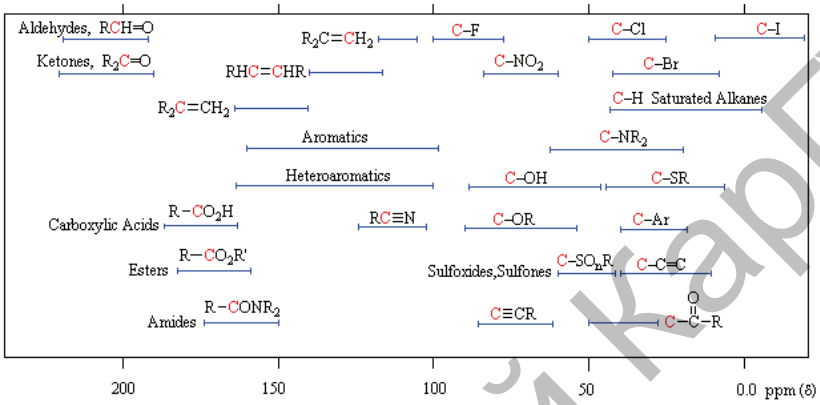


Figure 37. Generalized areas of chemical shifts of carbon ^{13}C

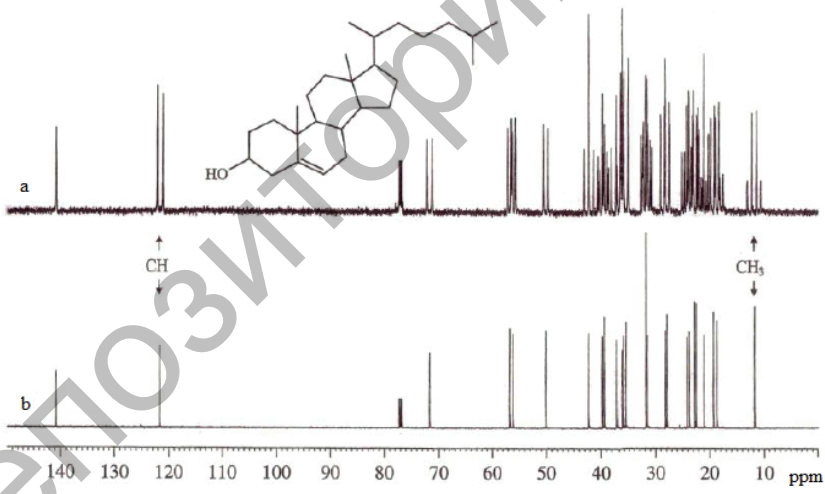


Figure 38. ^{13}C NMR spectra of cholesterol in $CDCl_3$ at 150.9 MHz without suppression (a) and complete suppression (b) of the spin-spin coupling with protons

2. The ^{13}C signals are distributed over a much larger range of chemical shifts than the range for ^{1}H nuclei.

As in the case of ^{1}H NMR, chemical shifts are expressed in units of ppm in ^{13}C NMR spectra. The range of chemical shifts is usually 220 ppm in a weak field from the TMS signal. Due to this, as well as the narrowness of the spectral lines, the coincidence of chemical shifts from nonequivalent carbon atoms is less likely than in the ^{1}H NMR spectra.

3. The intensity of the ^{13}C signals does not correlate with the number of carbon nuclei.

4. Larger amount of sample is required for analysis (~ 20 mg) due to the lower sensitivity of the ^{13}C NMR method compared with the ^{1}H NMR.

5. ^{1}H and ^{13}C NMR signals have a different multiplicity for a given deuterated solvent.

A small amount of residual $CHCl_3$ in deuterated commercial chloroform ($CDCl_3$) gives a small singlet about 7.26 ppm. The solvent $CDCl_3$ gives an intense triplet of the form 1: 1: 1 at 77 ppm in the ^{13}C NMR spectrum, which is caused by the spin-spin coupling of the ^{13}C nucleus with one deuterium nucleus.

Rules for interpreting the ^{13}C NMR spectra

1. ^{13}C Chemical shifts mainly depend on the type of hybridization of the carbon atom, on the electronegativity of the substituents and, to a lesser extent, on the diamagnetic anisotropy.

2. The order of arrangement of chemical shifts of classes of compounds in ^{13}C and ^{1}H NMR spectroscopy is similar.

3. The intensity of the signals of the quaternary carbon atom is low.

4. Additivity equations for substituted compounds are useful for the ^{13}C spectra, as for ^{1}H.

5. The form of the spectrum is affected by the solvent.

Features of ^{13}C NMR spectra of the main classes of organic compounds

Alkanes

Alkane hydrocarbon signals are observed in the range from 0 to 60 ppm. Alkylation usually shifts the carbon signal to a weak field. This is true both for sp^3-hybridized carbon (in alkanes) and for sp^2-hybridized carbon (in alkenes).

Alkenes

sp^2-Hybridized carbon atoms of alkenes give signals in the region of ~ 110-150 ppm, if alkyl groups are substituents. Double bonds have a rather weak effect on the chemical shifts of sp^3-hybridized carbon atoms in the molecule. As a rule, end $=CH_2$-groups give signals in more strong fields in comparison with internal = CH-groups, and signals fragments (Z)-CH=CH- are in stronger fields compared with their (E)-analogues. The central carbon atom (=C=) in alkyl-substituted allenes gives a signal in the range of 200-215 ppm, while the terminal atoms carbon in the group -C = C = C- is in the range of 75-79 ppm.

Alkynes

sp-Hybridized carbon atoms of alkynes, having only alkyl substituents, give a signal in the region of ~ 65-90 ppm. The acetylene group shifts the signal of the sp^3-hybridized carbon in weak fields by 5-15 ppm. The end group ≡CH gives a signal strongly shifted to strong field compared with the non-terminal group ≡CR.

Aromatic compounds

^{13}C NMR spectra can be used for recognition of substitution type in aromatic compounds. If there are two identical substituents, then there will be two peaks in the spectrum for the *para*-isomer in aromatic area, for *ortho*-isomer - three, and for *meta*-isomer - four; this is due to the symmetry of the molecule.

Alcohols

The replacement of a proton in an alkane by an OH group shifts the signal into weak fields by 35-52 ppm for the C-1 atom, by 5-12 ppm for carbon C-2 and for the C-3 atom to strong fields.

Halogen-containing compounds

Effects caused by the presence of a halogen atom in the molecule are rather complex. The presence of one fluorine atom with CH_3F (75.4 ppm) leads to a significant signal bias in comparison with CH_4 (2.3 ppm), which is explained by the electronegativity of fluorine. The chlorine atom leads to a similar effect (shifts the signal to weak fields), which can also be explained by electronegativity.

However, the “heavy atom effect” appears for bromine and iodine. Thus, the ^{13}C signal first is shifted to the left (weak fields) as the number of bromine atoms increases, and then to the right (strong fields): CH_4 (2.3 ppm), CH_3Br (10 ppm), CH_2Br_2 (24.1 ppm), $CHBr_3$ (12.1 ppm), CBr_4 (28.5 ppm).

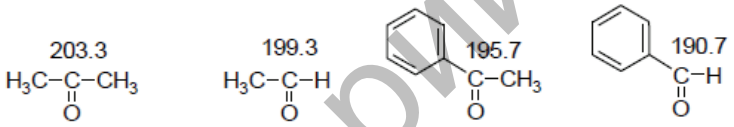
A strong shift to the right for the carbon atom CH_3I (139.9 ppm) is observed in comparison with the carbon signal CH_4 (2.3 ppm). ^{13}C NMR spectroscopy is a reliable method of establishing presence of an iodine atom in an aromatic compound molecule: signal of carbon C-I is located approximately in the region of 90 ppm, where practically no signals of carbon atoms associated with other heteroatoms are observed.

Amines

The terminal NH_2-group bonded to the alkyl chain displaces the shift of the C-1 atom to the left by about 30 ppm, the C-2 atom to the left by 11 ppm and the C-3 atom to the right by 4 ppm in comparison with similar signals alkanes. N-alkylation leads to an increase in the shift of C-1 to weak field.

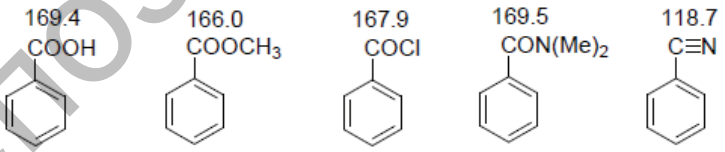
Ketones and aldehydes

Carbonyl carbon atoms in the compounds $R_2C = O$ $RCH = O$ have characteristic shifts in the region of weak fields.



Carboxylic acids and their derivatives

Carboxylic carbon atom in carboxylic acids and their derivatives give a signal in the range of 150-185 ppm. Nitriles give a signal in the range of 115-125 ppm. N-alkylation causes an insignificant (several ppm) shift of the carboxyl carbon signal to the right in the amides [26].



Interpreting the ^{13}C NMR spectrum

The results of ^{13}C NMR analysis of the solution of *para*-anisidine in $CDCl_3$ is as follows (ppm): 57, 115, 116, 140, 153. Fulfill a correspondence between the ^{13}C NMR spectrum (Figure 39) data and structure of *para*-anisidine.

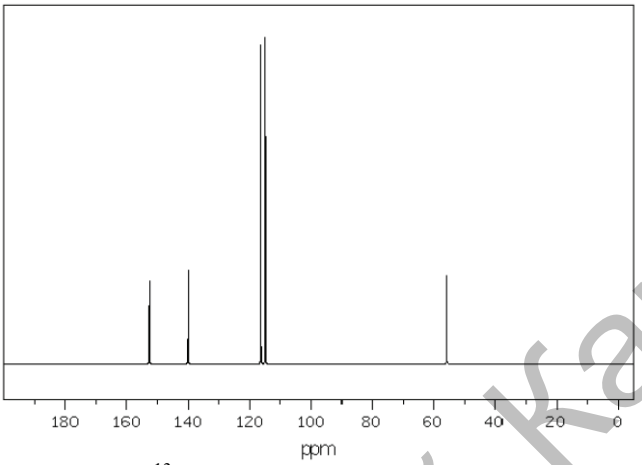
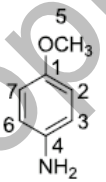


Figure 39. The ^{13}C NMR spectrum of *para*-anisidine

Solution: 1. Write the formula of the aniside and number the carbon atoms (in any sequence).



2. Determine the number of magnetically nonequivalent atoms (groups of atoms) of carbon. There are 5 groups of magnetically nonequivalent C-atoms, namely C-1; C-2,7; C-3,6; C-4; C-5 in the *para*-anisidine molecule. Therefore, there are five signals in the spectrum that are actually detected.

3. Determine, which group of magnetically nonequivalent carbon atoms correspond to signals, whose chemical shifts are given in the the problem. The strongest signal (57 ppm) refers to the carbon atom of a methoxy group (C sp^3, bound to an electron-withdrawing agent). Two low-field signals (140, 163 ppm) refer to carbon atoms directly related to the groups OCH_3, NH_2 (C sp^2, bound to substituent).

The two remaining signals (115, 116 ppm) refer to C-3,7 and C-4,6.

Assignment of CH_3, CH_2, CH and quaternary carbons in ^{13}C NMR

The pulse and acquisition technique used in FT spectroscopy makes it possible to carry out diverse experiments. It is possible to give a second or third pulse on the same or a different axis, and it is possible to wait for various lengths of time between pulses. In this paragraph we will discuss only the output of these experiments, illustrate them and give a brief overview in order for you to know what kind of information can be obtained from them.

In structure determination, it is extraordinary helpful to identify the number of CH_3, CH_2, CH and C atoms with no attached hydrogen atoms (quaternary carbon). Although this can be done by off-resonance decoupling in a ^{13}C spectrum, it is more effectively achieved, using a multi-pulse sequence. A multi-pulse sequence, which is commonly used, is called Distortionless Enhancement through Polarisation Transfer) DEPT. In this sequence, three spectra are taken in which pulses at three different angles are applied to the protons, influencing the way the protons transfer polarization to the carbons. The angles are 45°, 90° and 135°, giving the three spectra in Figure 40, from the ester 3,5-dimethylbenzyl methoxyacetate [27].

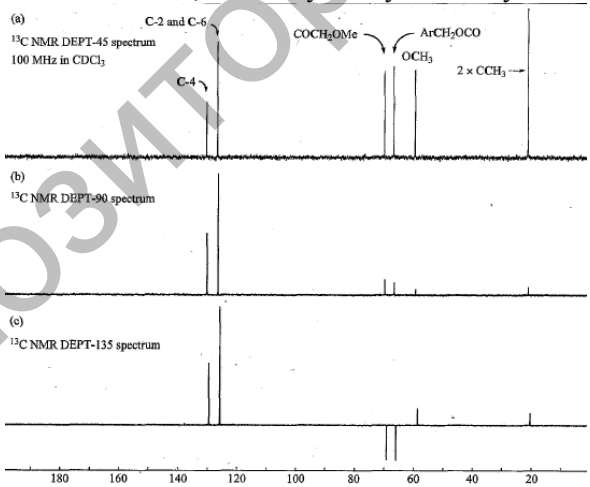


Figure 40. The DEPT spectra of 3,5-dimethylbenzyl methoxyacetate

The DEPT-45 spectrum (Figure 40a) shows positive CH_3, CH_2 and CH signal; in other words everything except the fully substituted carbons: the C=O carbon, C-1, C-3 and C-5. The DEPT-90 spectrum (Figure 40b) shows only the CH signals, or rather shows only those signals strongly, since they are small signals from other carbons, stemming from a not uncommon but imperfect choice of parameters in the pulse sequence. And the DEPT-135 spectrum (Figure 40c) shows the CH and CH_3 signals positive, but the CH_2 signals negative. The spectrum (Figure 40b) allows us to identify unambiguously the signals derived from CH groups, and the spectrum (Figure 40c) allows us to identify unambiguously the signals derived from CH_2 groups. Both spectra (Figure 40b and c) reveal that those signals still left unaccounted for in Figure 40a are derived from the CH_3 groups, and those signals still left unaccounted for in the full spectrum, not appearing in any of the three spectra in Figure 40, are derived from the fully substituted carbons. It is not necessary to process these spectra further, since we have already learned all that we want to know. However, it is possible, by weighted additions and substractions of one spectrum from another to generate three spectra showing a separate ^{13}C spectrum for each of the CH_3, CH_2 and CH signals, and DEPT spectra are sometimes shown in this form.

3.4 The Nuclear Overhauser Effect

The interaction of one magnetic nucleus with another, leading to spin-spin coupling, takes place through the bonds of the molecule. The information is relayed by electronic interactions, as one can see from the dependence of the coupling constant on the geometrical arrangement of the intervening bonds.

Magnetic nuclei can also interact through space, but the interaction does not lead to coupling. The interaction is revealed when one of the nuclei is irradiated at its resonance frequency and the other is detected as a more intense or weaker signal than usual. This is called the nuclear Overhauser effect (NOE or nOe). The NOE is only noticeable over short distances, generally 2-4 Å, falling off rapidly as the intense sixth power of the distance apart of the nuclei. This is because the interaction is dependent upon the relaxation of the observed nucleus by the irradiated nucleus.

NOE Difference Spectra

NOEs are much more easily detected by subtracting, in the computer, the normal spectrum from a spectrum taken with the irradiating signal on, and printing only the difference between the two spectra. All the unaffected signals simply disappear, and all that shows the enhancement itself, together with an intense signal at the irradiating frequency. The lower trace in Figure 41 shows the complex ^{1}H spectrum of the oxindole, and the upper trace is the difference spectrum created after irradiating the sample at the frequency of the heavy downward-pointing arrow. This frequency is that of proton H_{7a}, which is close in space both to its neighbor H_{7b} and to $H_{5'}$ on the benzene ring. Only signals from these two protons appear in the difference spectrum, and demonstrate that the stereochemistry of the molecule is that shown, and not the isomer with the spirooxindole ring the other way up. When a similar experiment is carried out on that isomer, no signal appears in the aromatic region of the difference spectrum. The signal from H_{7b} in the difference spectrum (Figure 41) still shows the coupling to H_{7a}; this is because the signal used to create the NOE is applied before the acquisition pulse, but is turned off during acquisition. Coupling is therefore unaffected [28].

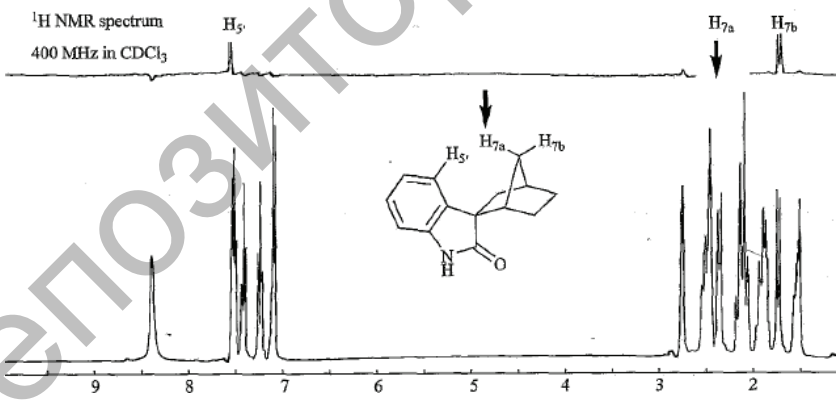


Figure 41. The complex ^{1}H spectrum of the oxindole

Questions and Assignments for Self-Study:

1. What phenomenon is method of NMR spectroscopy based on?
2. What nuclei of atoms can cause the signal in the NMR spectra?
3. What is the resonant frequency of the nucleus?
4. What is the spin-lattice relaxation time?
5. What is the ^{1}H NMR spectrum?
6. What is the chemical shift of the NMR signal?
7. What is the basis of the anisotropy phenomenon?
8. What is the splitting of the NMR signal caused by?
9. What does the multiplicity of the signal show?
10. What does the intensity of the multiplet show?
11. What does the spin-spin coupling show? What factors does it depend on?
12. What are magnetic equivalent protons?
13. What is called a spin system?
14. What methods are known to simplify complex spectra?

CHAPTER 4.

Two-Dimensional NMR Techniques

4.1 Introduction to Two-Dimensional Spectroscopic Methods

The methods that have been described to this point are examples of one-dimensional experiments. The signal is presented as a function of a single parameter, usually the chemical shift, in a one-dimensional experiment. There are two coordinate axes in a two-dimensional experiment. Generally these axes also represent ranges of chemical shifts. The signal is presented as a function of each of these chemical shift ranges. The data are plotted as a grid; one axis represents one chemical shift range, the second axis represents the second chemical shift range, and the third dimension constitutes the magnitude (intensity) of the observed signal. The result is a form of contour plot where contour lines correspond to signal intensity.

Types of 2D NMR include correlation spectroscopy (COSY), J-spectroscopy, exchange spectroscopy (EXSY), and Nuclear Overhauser effect spectroscopy (NOESY). Two-dimensional NMR spectra provide more information about a molecule than one-dimensional NMR spectra and are especially useful in determining the structure of a molecule, particularly for molecules that are too complicated to work with, using one-dimensional NMR.

A more useful type of 2D NMR spectroscopy is shift-correlated spectroscopy (COSY), in which both axes describe the chemical shifts of the coupled nuclei, and the cross-peaks obtained tell us which nuclei are coupled to which other nuclei. The coupled nuclei may be of the same type, protons coupled to protons, as in homonuclear 2D shift-correlated experiments or of different types, protons coupled to carbon nuclei, as in heteronuclear 2D shift-correlated spectroscopy.

^{1}H-^{1}H NOESY (Nuclear Overhauser Effect Spectroscopy) and EXSY (Exchange Spectroscopy) are the same experiment used for different purposes. NOESY is useful for determining which signals arise from protons that are close to each other in space even if they are not bonded. A NOESY spectrum yields through space correlations via spin-lattice relaxation. NOESY also detects chemical and conformational exchange. It is called EXSY when used for this purpose.

4.2 Homonuclear and Heteronuclear Shift-Correlation Spectroscopy

Homonuclear Shift-Correlation Spectroscopy (COSY)

2D NMR uses a sequence of two pulses with a series of different evolution times to determine which nuclear spins are coupled to one another. COSY spectra indicate through-bond coupling, and can be used to gain structural information about molecules of a wide range of sizes.

Figure 42 shows the COSY spectrum of 2-nitropropane. The proton NMR spectrum of 2-nitropropane is plotted along both the horizontal and vertical axes, and each axis is calibrated according to the chemical shift values (in ppm). The COSY spectrum shows distinct spots on a diagonal, extending from the upper right corner of the spectrum down to the lower left corner. You can easily see that each spot on the diagonal corresponds with the same peak on each coordinate axis, by extending vertical and horizontal lines from each spot on the diagonal.

The diagonal peaks serve only as reference points. The off-diagonal peaks are the important peaks in the spectrum. You can extend a horizontal line from the spot at 1.56 ppm in the spectrum of 2-nitropropane (which is labeled A and corresponds to the methyl protons). This horizontal line eventually encounters an off-diagonal spot (al the upper left of the COSY spectrum) that corresponds to the methine proton peak at 4.66 ppm (labeled B). A vertical line drawn from this off-diagonal spot intersects the spot on the diagonal that corresponds to the methine proton (B). The presence of this off-diagonal spot, which correlates the methyl proton spot and the methine proton spot, confirms that the methyl protons are coupled to the methine protons. A similar result would have been obtained by drawing a vertical line from the 1.56-ppm spot (A) and at horizontal line from the 4.66-ppm spot (B). The two lines would have intersected at the second off-diagonal spot (at the lower right of the COSY spectrum). The vertical and horizontal lines described in this analysis are drawn on the COSY spectrum in Figure 42.

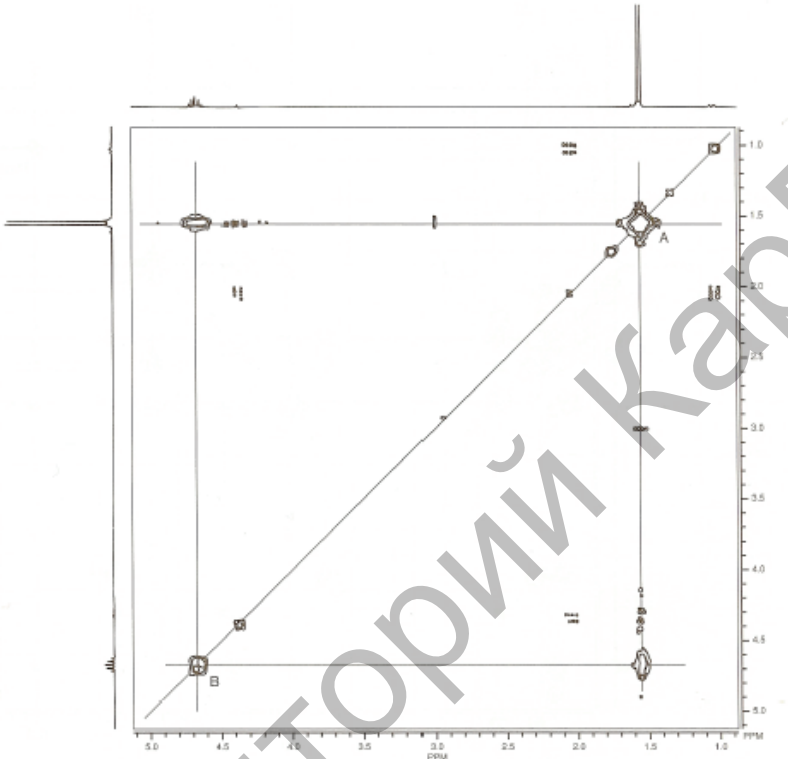


Figure 42. COSY spectrum of 2-nitropropane

The COSY spectrum for isopentyl acetate is given in Figure 43. The proton spectrum of isopentyl acetate is plotted along each axis. The COSY spectrum shows a distinct set of spots on a diagonal, with each spot corresponding to the same peak on each coordinate axis. Lines have been drawn to help identifying the correlations. The protons of the two equivalent methyl groups (1) correlate with the methine proton (2) in the COSY spectrum of isopentyl acetate. There is also correlation between the two methylene groups (3 and 4) and between the methine proton (2) and the neighboring methylene (3). The methyl group of the acetate moiety (6) does not show off-diagonal peaks, because the acetyl methyl protons are not coupled to other protons in the molecule [29-31].

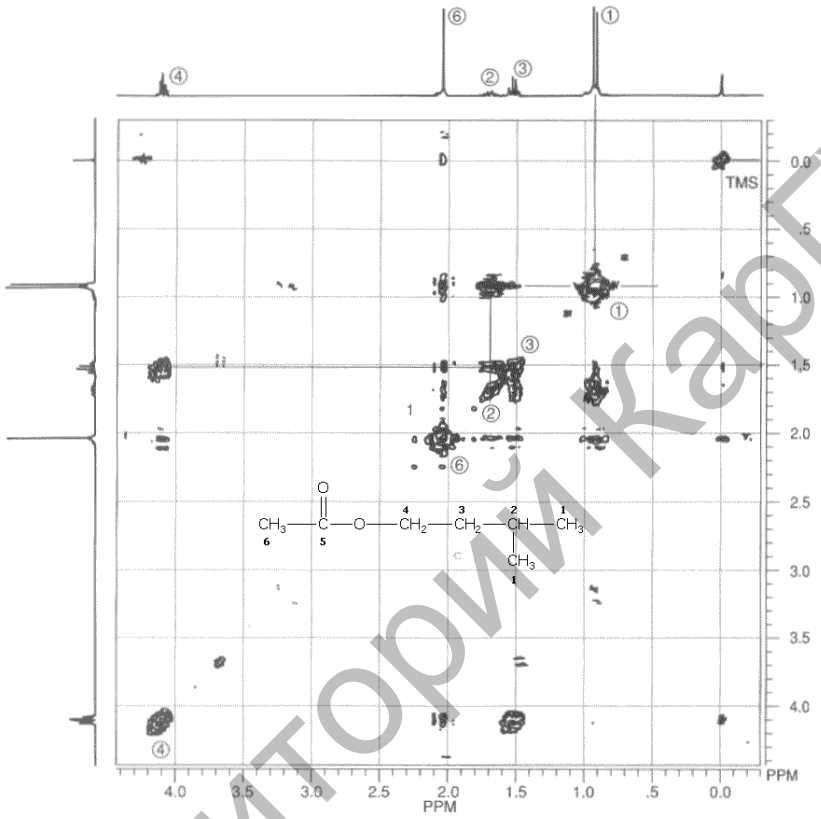
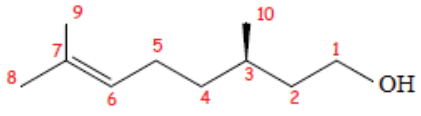


Figure 43. The COSY spectrum for isopentyl acetate

The COSY spectrum of citronellol is a third example. The spectrum (Figure 44) is rather complex in appearance. Nevertheless, one can identify certain important coupling interactions. Again, lines have been drawn to help identifying the correlations. The proton on C6 is clearly coupled to the protons on C5. Closer examination of the spectrum also reveals that the proton on C6 is coupled through allylic (four-bond) coupling to the two methyl groups at C8 and C9. The protons on C1 are coupled to two nonequivalent protons on C2 (at 1.4 and 1.6 ppm).

They are nonequivalent, owing to the presence of a stereocenter in the molecule at C3. The splitting of the methyl protons at C10 by

the methine proton at C3 can also be seen although the C3 spot on the diagonal line is obscured by other spots that are superimposed on it. However, from the COSY spectrum one can determine that the methine proton at C3 must occur at the same chemical shift as one of the C8 or C9 methyl groups (1.6 ppm). Thus, a great deal of useful information can be obtained even from a complicated COSY pattern.



citronellol

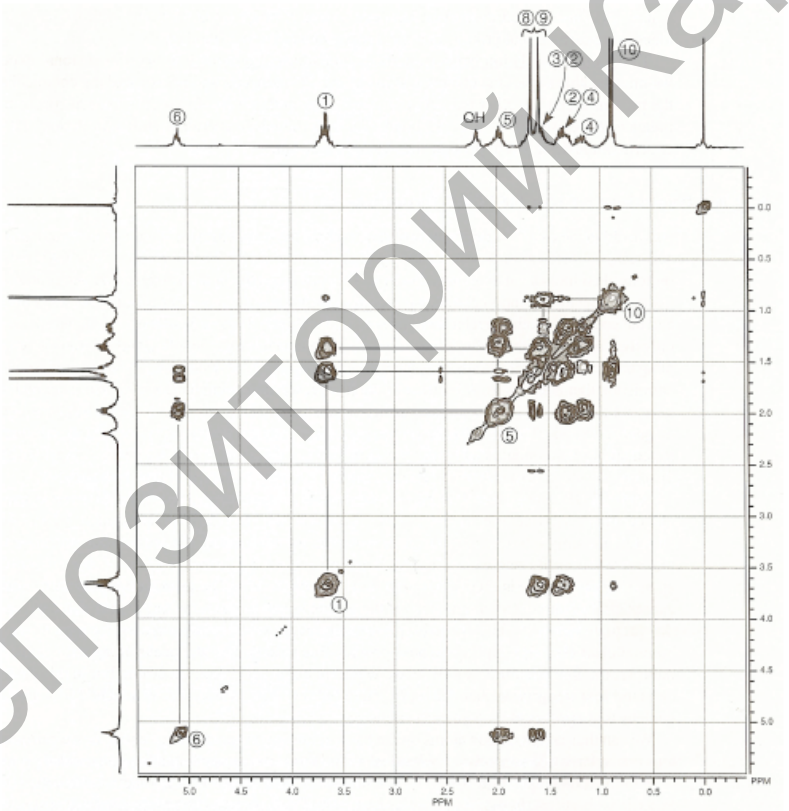


Figure 44. COSY spectrum of citronellol

The COSY spectra shown contain additional spots besides the ones examined. Often these “extra” spots have much lower intensities than the principal spots on the plot. The COSY method can sometimes detect interactions between nuclei over ranges that extend beyond three bonds. Besides this long-range coupling, nuclei that are several atoms apart but that are close together spatially also may produce off-diagonal peaks. In some variations of the method, however, spectroscopists make use of such long-range interactions to produce two-dimensional NMR spectra that specifically record this type of information.

HETCOR Spectra

Figure 45 is an example of a simple HETCOR plot of 2-nitropropane. It is common practice to plot the proton spectrum of the compound being studied along one axis and the carbon spectrum along the other axis. Each spot of intensity on the two-dimensional plot indicates a carbon atom that bears the corresponding protons. One should be able to see a peak, corresponding to the methyl carbons in Figure 45, which appear at 21 ppm in the carbon spectrum (horizontal axis), and a peak at 79 ppm, corresponding to the methine carbon.

On the vertical axis, one should also be able to find the doublet for the methyl protons at 1.56 ppm (proton spectrum) and a septet for the methine proton at 4.66 ppm. If you draw a vertical line from the methyl peak of the carbon spectrum (21 ppm) and a horizontal line from the methyl peak of the proton spectrum (1.56 ppm), the two lines would intersect at the exact point on the two-dimensional plot where a spot is marked. This spot indicates that the protons at 1.56 ppm and the carbons at 21 ppm represent the same position of the molecule. That is, the hydrogens are attached to the indicated carbon. In the same way, the spot in the lower left corner of the HETCOR plot correlates with the carbon peak at 79 ppm and the proton septet at 4.66 ppm, indicating that these two absorptions represent the same position in the molecule [32-35].

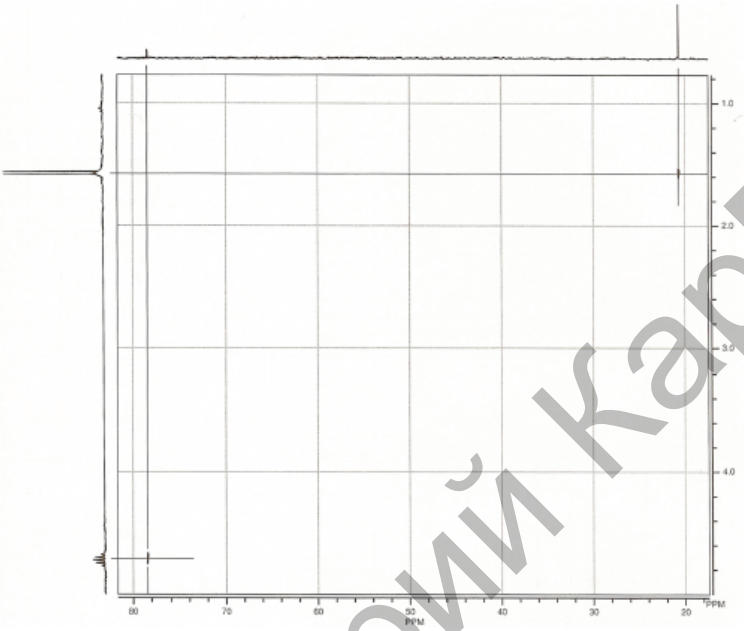


Figure 45. HETCOR spectrum of 2-nitropropane

A second, more complex example is isopentyl acetate. Figure 46 is the HETCOR plot for this substance. Each spot on the HETCOR plot has been labeled with a number and lines have been drawn to help you see the correlations between proton peaks and carbon peaks. The carbon peak at 23 ppm and the proton doublet at 0.92 ppm correspond to the methyl groups (1); the carbon peak at 25 ppm and the proton multiplet at 1.69 ppm correspond to the methine position (2); and the carbon peak at 37 ppm and the proton quartet at 1.52 ppm correspond to the methylene group (3). The other methylene group (4) is deshielded by the nearby oxygen atom. Therefore, a spot on the HETCOR plot for this group appears at 63 ppm on the carbon axis and 4.10 ppm on the proton axis. It is interesting that the methyl group of the acetyl function (6) appears down field of the methyl groups of the isopentyl group (1) in the proton spectrum (2.04 ppm). We expect this chemical shift, since the methyl protons should be deshielded by the anisotropic nature of the carbonyl group. In the carbon spectrum, however, the carbon peak appears upfield of the methyl carbons of the

isopentyl group. A spot on the HETCOR plot that correlates these two peaks confirms the assignment.

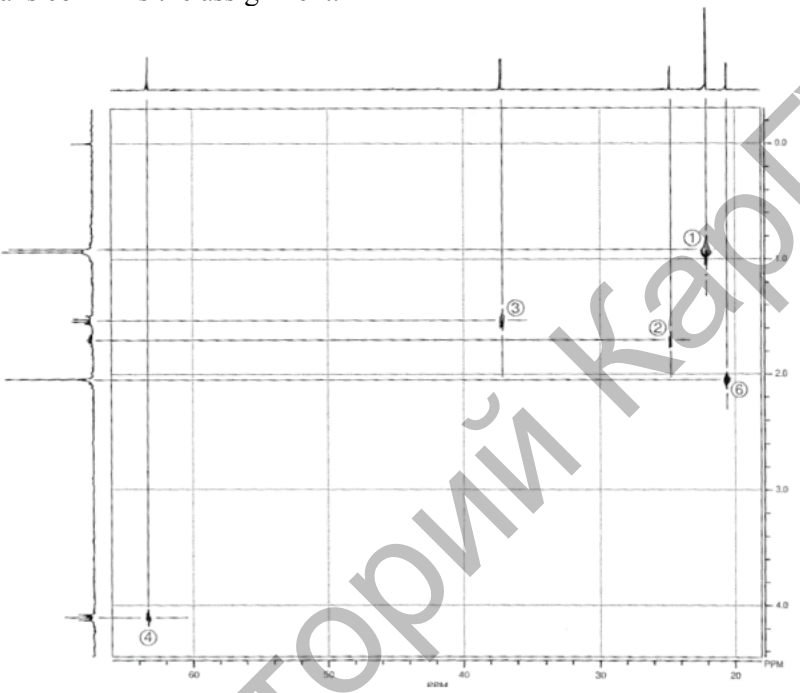


Figure 46. HETCOR spectrum of isopentyl acetate

Figure 47 shows the final example that illustrates some of the power of the HETCOR technique for 4-methyl-2-pentanol. Lines have been drawn on the spectrum to help you find the correlations. This molecule has a stereocenter at carbon 2. An examination of the HETCOR plot for 4-methyl-2-pentanol reveals two spots that correspond to the two methylene protons on carbon 3. Two contour spots appear at 48 ppm on the carbon axis, one at about 1.20 ppm on the proton axis and the other at about 1.40 ppm. The HETCOR plot shows that there are two nonequivalent protons attached to carbon 3. If we examine a Newman projection of this molecule, we find that the presence of the stereocenter makes the two methylene protons (a and b) nonequivalent. As a result, they appear at different values of chemical shift.

The carbon spectrum also reveals the effect of a stereocenter in the molecule. In the proton spectrum, the apparent doublet (actually it is a pair of doublets) at 0.91 ppm arises from the six protons on the methyl groups, which are labeled 5 and 6 in the preceding structure. Looking across to the right on the HETCOR plot, you will find two contour spots, one corresponding to 22 ppm and the other corresponding to 23 ppm. These two carbon peaks arise because the two methyl groups are also not quite equivalent; the distance from one methyl group to the oxygen atom is not quite the same as that from the other methyl group, when we consider the most likely conformation for the molecule.

A great many advanced techniques can be applied to complex molecules. We have introduced only a few of the most important ones here. As computers become faster and more powerful, as chemists evolve their understanding of what different pulse sequences can achieve, and as scientists write more sophisticated computer programs to control those pulse sequences and treat data, it will become possible to apply NMR spectroscopy to increasingly complex systems.

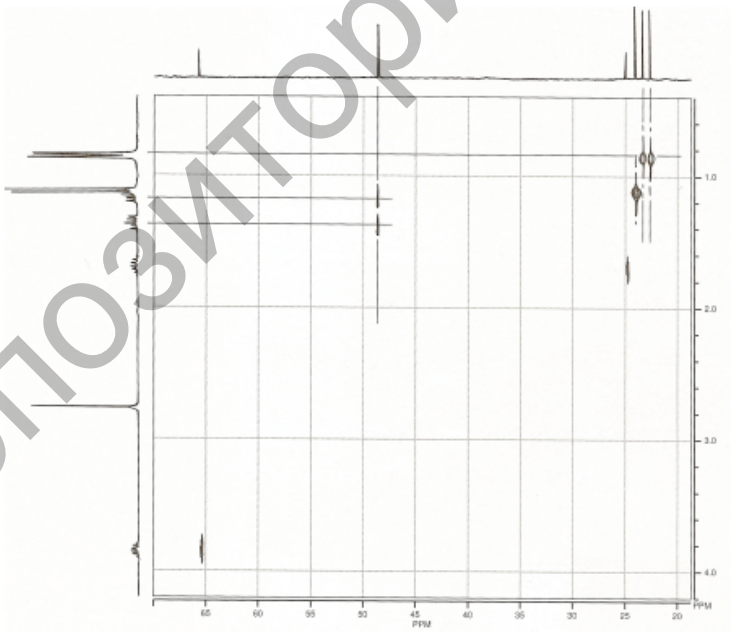


Figure 47. HETCOR spectrum of 4-methyl-2-pentanol

Questions and Assignments for Self-Study:

1. What is the major difference between 1D and 2D NMR experiments?
2. What are the main 2D NMR techniques?
3. What are the major benefits of 2D NMR spectra?
4. What are homonuclear-shift-correlation 2D NMR techniques?
5. What is COSY?
6. What is NOESY?
7. What is EXSY?

CHAPTER 5.

Mass Spectrometry

5.1 Introduction to Mass Spectrometry

Mass spectrometry is fundamentally different from spectroscopic methods discussed above. Structural mass spectrometry is based on the destruction of organic molecule as a result of ionization. The ions formed are sorted by the values of their mass / charge ratio (m/z), then the number of ions for each the values of this ratio in the form of a spectrum is registered. General scheme of a typical mass spectrometer is presented in Figure 48.

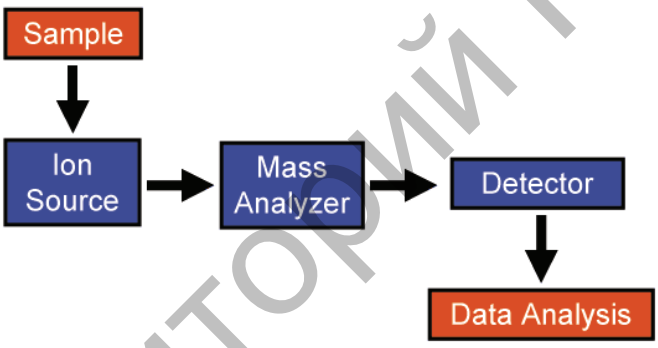


Figure 48. Block diagram of a typical mass spectrometer

A gas (GC-MS) or liquid (LC-MS) chromatography is used to inject a sample into a mass spectrometer. However, many devices have the ability for direct injection of the sample into the ionization chamber. There are devices for ionizing the sample and dividing ions according to the m/z ratio in all mass-spectrometers. After separation, it is necessary to detect ions and measure their number. A typical ion collector consists of collimating slots that direct only one type of ion to the collector, where they are detected, and the detection of signal is amplified by an electronic multiplier. Modern mass spectrometers are equipped with specialized software. Computers control the accumulation, storage and visualization of data. All mass spectrometers are divided into two classes: low (single) and high resolution (R) devices.

5.2 Electron Impact Mass Spectra

Electron impact (EI) is the most common method of ionization in mass spectrometry. The advantage of this method is the ability to use search databases.

The molecule of the sample in the gas phase undergoes bombardment of electrons with high energy (usually 70 eV) and ejects the electron, forming a radical cation, called *the molecular ion*:

$M + e \rightarrow M^{+}\bullet$ (molecular ion) + 2e

The lowest energy of bombarding (ionizing) electrons, at which the formation of an ion from a given molecule is possible, is called the energy (or, less successfully, the "potential") of the ionization of matter (Ue). *The ionization energy is a measure of the strength with which the molecule holds the electron that is least strongly bound to it.*

For organic molecules, the ionization energy is 9-12 eV, so bombardment by electrons with energy of 50 eV and higher gives the excess internal energy to the emerging molecular ion. This energy is partially dissipated due to the rupture of covalent bonds. As a result of this disruption, the molecular ion decomposes into smaller particles (fragments). This process is called *fragmentation*.

Fragmentation occurs selectively, is highly reproducible and characteristic for a given compound. Moreover, the processes of fragmentation are predictable, and they are responsible for the broad possibilities of mass spectrometry for structural analysis. In fact, structural analysis by mass spectrometry is to identify fragment ions and retrospectively restore the structure of the original molecule, starting from the direction of fragmentation of the molecular ion. Each of the fragments formed can then itself decompose into even smaller fragments (secondary fragmentation).

If some of the molecular ions have enough long lifetimes, they reach the detector and register as a peak of a molecular ion. Since the charge of the original ion is equal to 1, the ratio m / z for this peak gives the molecular weight of the test substance. *Thus, the mass spectrum is a representation of relative concentrations of positively charged fragments (including the molecular ion), depending on their masses.*

The height of the most intense peak in the spectrum is taken as 100%, and the intensities of the other peaks, including the peak of the molecular ion, expressed as a percentage of the maximum peak. In

certain cases, the most intense may be a peak of a molecular ion. In the general case: the intensity of the peak depends on stability of the ion formed.

In mass spectra, a series of peaks of fragment ions, which differ by the homological difference (CH_2), i.e. 14 amu are present. Homological series of ions are characteristic for each class of organic substances, and therefore they carry important information about the structure of the substance under study [36-37].

Table 2. Homological series of ions of some classes of organic compounds

Compound class	Formula	m/z
Alkanes	$C_nH^+_{2n+1}$	15, 29, 43, 57, 71, 85…
Alkenes, naphthenes	$C_nH^+_{2n-1}$	27, 41, 55, 69, 83…
Alkynes, dienes	$C_nH^+_{2n-3}$	25, 39, 53, 67, 81…
Alcohols, ethers	$C_nH_{2n+1}O^+$	31, 45, 59, 73, 87…
Aldehydes, ketones	$C_nH_{2n-1}O^+$	29, 43, 57, 71, 85…
Acids, esters	$C_nH_{2n-1}O^{2+}$	45, 59, 73, 87, 101…
Amine	$C_nH_{2n+2}N^+$	30, 44, 58, 72, 86, 100…
Nitriles	$C_nH_{2n-2}N^+$	40, 54, 68, 82, 96…
Alkylbenzenes		38, 39, 50-52, 63-65, 75-78, 91, 105, 119…

Usually, peaks with masses M + 1 and M + 2 appear in the mass spectrum of any organic compound, which is due to the isotopic composition of the elements entering into the organic compound. For convenience, the elements are called A, A + 1, A + 2, depending on which isotope they have in addition to the main one.

The ratio of the intensities of the peaks M, M + 1 and M + 2 depends on the elemental composition, on the number of atoms of a given element in the molecule, and on the natural content of the heavier isotope of this element. Thus, for hydrocarbons, the isotope ^{13}C gives the most significant contribution to isotopic peaks. For methane, the intensity of the peak M + 1 is 1.1% of the molecular ion peak, for the

hydrocarbon with fourteen carbon atoms, the probability of inclusion of the ^{13}C isotope is increased, so the intensity of M + 1 = 14 • 1.1 = 15.4% of the molecular peak.

It is necessary to separate the intensity of the M + 1 peak as a percentage of M by 1.1 to determine the number of carbon atoms in a molecule by mass spectrum. For example, a molecular ion is observed in the spectrum, the peak intensity M is 66.5%, and the M + 1 intensity is 2.29%. We find the intensity of the peak M + 1 in relation to M in percent:

66.5 – 100%

2.29 – x%

x = 3.44 %

We find the maximum number of carbon atoms: 3.44: 1.11 = 3.

It should be taken into account that intense peaks M + 4 and M + 6, etc. can appear in the spectrum in the case of the presence of several atoms of (A + 2)-elements in the molecule.

There is a simple rule: "If the intensity of the M + 2 peak is less than 3% of the intensity of the M peak, the compound does not contain chlorine, bromine, sulfur and silicon atoms."

Chlorine, bromine, sulfur and silicon are well identified by mass spectrometry due to the signal multiplicity characteristic of each element. A reliable interpretation of the multiplicity of the signal is possible only when the mass spectrum is obtained on a high-resolution instrument. Figures 49-51 show examples of mass spectra [20, 38].

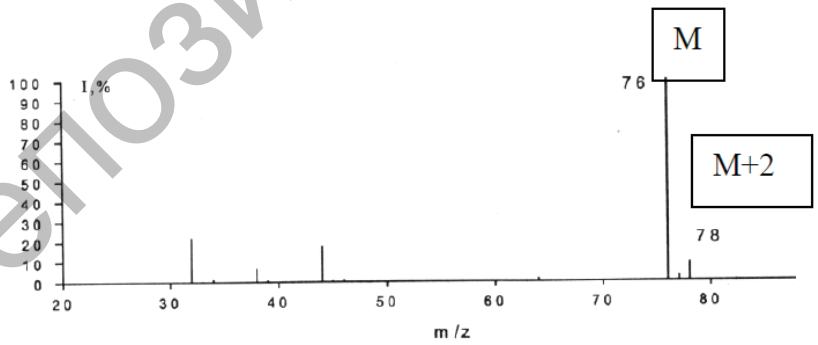


Figure 49. Carbon disulfide mass spectrum

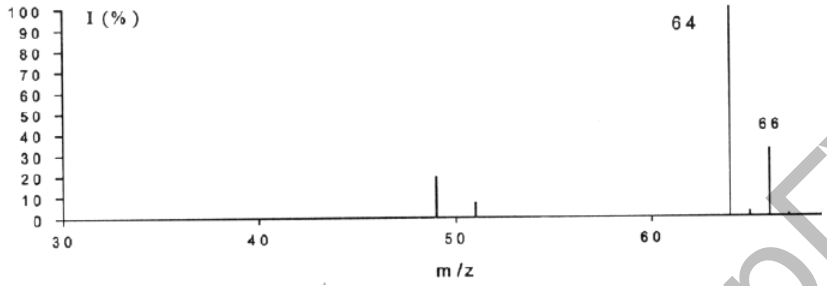


Figure 50. Ethyl chloride mass spectrum

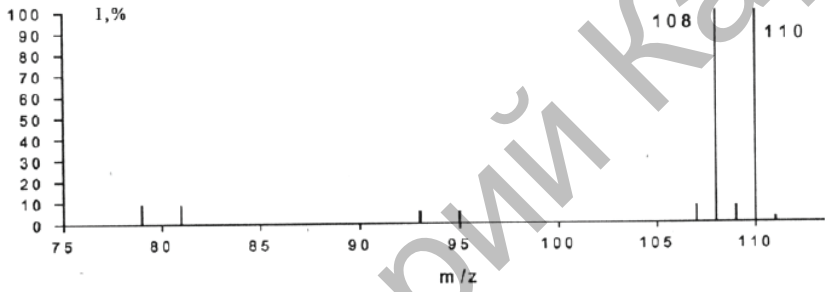


Figure 51. Ethyl bromide mass spectrum

5.3 Interpretation of EI mass spectra

Interpretation of the mass spectrum involves the identification of a molecular ion, fragment ions and the writing a fragmentation scheme. There is no general methodology for learning how to decode mass spectra, as is the case in other spectral methods (IR, NMR spectroscopy). Nevertheless, one can formulate the main steps in solving the problem of interpretation of mass spectrometry data.

1. It is necessary to have reference literature, where the main regularities of fragmentation of classes of organic compounds are described, the most frequently encountered fragment ions (structural formula, m / z value) are characterized. Often, the structural formula of the sample is initially assumed, which means that it is possible to determine the class of organic compound, to look in the reference literature for the main regularities of fragmentation and from this standpoint to analyze the available experimental results.

2. It is necessary to have as much information as possible on the test sample:

- the reaction scheme, which results in the preparation of the compound;
- reaction conditions (reagents, solvent);
- starting products, by-products;
- method of isolation and purification (reagents, solvent);
- the results of the study of this compound by other methods (IR-, NMR-Spectroscopy).

3. It is necessary to work with the available databases.
4. To specify parameters, at which mass spectrum was recorded (mass spectrometer brand, detector type, ionizing radiation energy).
5. Proceed to the analysis of the general view of the spectrum.

- To determine if there is a peak of a molecular ion in the spectrum;
- To select peaks in the region of large masses (they carry essential information about the direction of fragmentation);
- To determine the most intense peaks in the spectrum (they are characteristic for the fragmentation of certain classes of organic compounds);
- To separate homological series in the spectrum (they are also characteristic for the fragmentation of a certain class of organic compounds).

Further, the task of interpreting the mass spectrum has several options for solving, depending on whether a structure is originally intended or not, whether a molecular ion is fixed, whether "recognizable" fragment ions or clusters of homologous series are detected. It is obvious that it is impossible to describe the solutions of all possible variants of tasks, and it is not advisable.

Identification of the molecular ion peak

The identification of a molecular ion in the spectra with ionization by electron impact is often problematic: peak may not be very intensive or completely absent. If there is a possibility spectrum with chemical ionization should be recorded; in this case you will get a spectrum with intensive molecular ion and little fragmentation.

Otherwise, we will have to resort to some empirical rules.

1. Nitrogen rule: “A molecule with an even molecular weight either should not contain nitrogen, or the number of nitrogen atoms should be even.”

2. Usually, the molecular ion easily cleaves CO molecules, CO_2, H_2O, C_2H_4, Hal; radicals Alk·, H·, Hal·, OH·.

Losses from the molecular ion from 5 to 14 or from 21 to 25 amu, leading to the emergence of intense peaks of ions, are extremely unlikely.

For example, in the mass spectrum, the heaviest ion is 120 following 112. Conclusion: ion 120 is not molecular, but fragmentary.

3. As already noted, the intensity of a molecular ion is determined by its stability. The ability of organic compounds give a peak molecular ion decreases in the series: aromatic compounds > conjugated alkenes > saturated cyclic compounds > organic sulphides > unbranched alkanes with short chains > thiols.

4. The following compounds, namely ketones, amines, esters, ethers, carboxylic acids, aldehydes, amides, halogenated derivatives form noticeable peaks of molecular ions.

5. The peak of a molecular ion is often not detected for aliphatic alcohols, nitriles, nitrates, nitro compounds, nitrites, and for highly branched compounds.

6. Molecular ion should:

- Have the largest molecular mass in the spectrum;
- Be odd-electronic (determination of unsaturation R);
- Be able to form the most important ions with a large mass due to the release of neutral particles;
- Include all the elements whose presence in the sample can be seen by fragment ions.

The degree of unsaturation (the number of multiple bonds and cycles in the ion) can be calculated in several ways. Here is a method based on the replacement of heteroatoms by hydrocarbon fragments. The essence of the method is as follows:

1. All monovalent elements (with the exception of hydrogen) are replaced by CH_3, divalent - CH_2, trivalent - CH, tetravalent (with the exception of carbon itself) - C;

2. The formula obtained is compared with the formula of an alkane with the same content of carbon atoms;

3. The difference between the number of hydrogen atoms in the alkane (2n + 2) and in the sample divided by "2" gives the value of R.

Example: calculate the degree of unsaturation of the ion with composition $C_5H_9N_3O_2ClBr$.

$C_5H_9N_3O_2ClBr$: $C_5H_9 + 3CH + 2CH_2 + 2CH_3 = C_{12}H_{22}$

The corresponding alkane is $C_{12}H_{26}$ (dodecane).

R = (26 - 22)/2 = 2

Conclusion: The unsaturation is equal to 2, an odd electron, can be a molecular ion.

Fragmentation and rearrangements

General rules for predicting the most intense peaks in the mass spectrum obtained using an electron impact (EI)

1. The relative intensity of the peak of the molecular ion is maximal for unbranched compounds and decreases with increasing branching (Figures 52 and 53).

2. The cleavage occurs mainly along the alkyl-substituted carbon atoms [39-41].

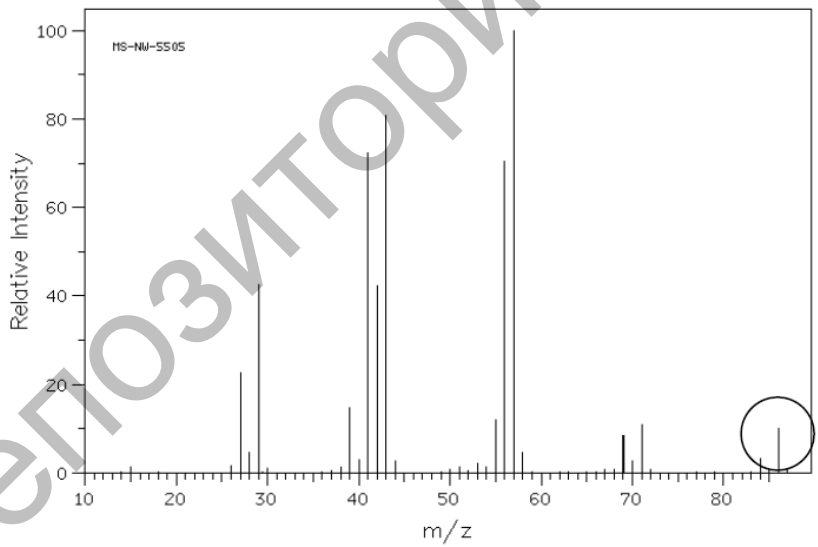


Figure 52. The mass spectrum (EI) of hexane (molecular ion 86)

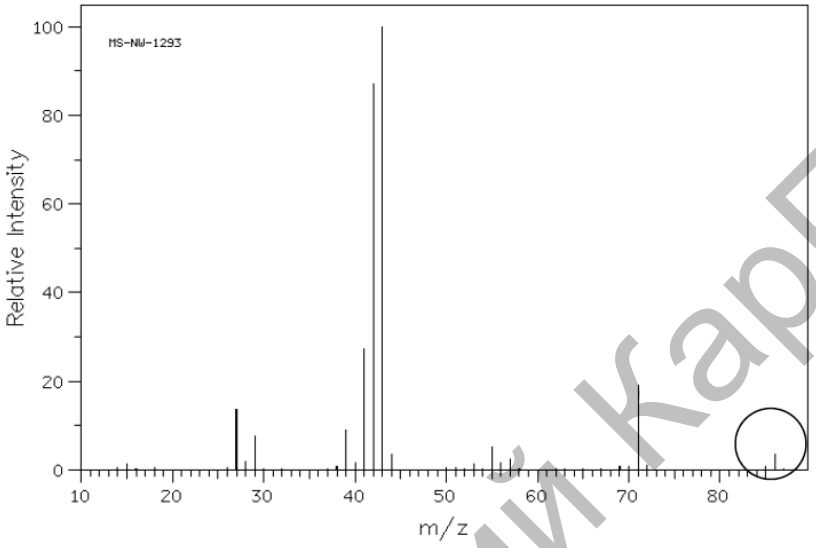
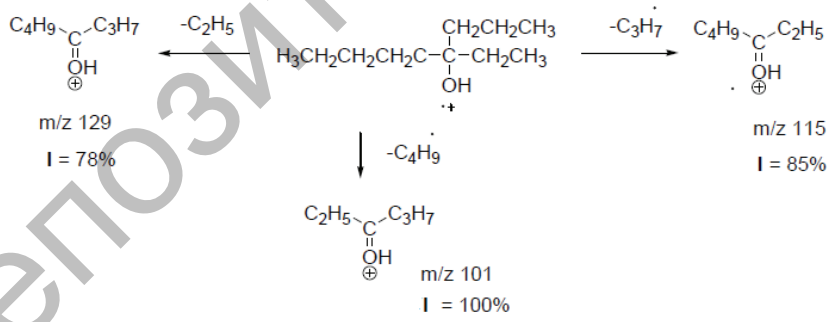


Figure 53. The EI mass spectrum of 2,3-dimethylbutane (molecular ion 86)

The intensity of the peak of the ions formed upon release maximum radical is the highest, with the release of the minimum radical is the lowest.



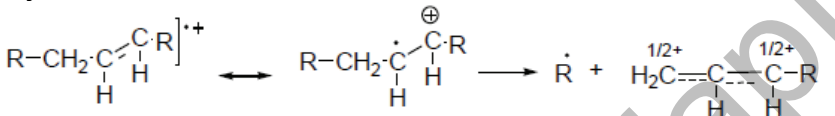
3. Saturated cycles are able to lose side chains in the α-bond. The positive charge remains on the cyclic fragment.

4. The decomposition of aromatic alkyl-substituted compounds occurs to yield the benzyl ion or tropylium ion.

5. The relative intensity of the peak of a molecular ion is usually decreases with an increase in the molecular weight in the homology series. The exception is esters of fatty acids.

6. Double bonds, cyclic structures and, especially, aromatic (heteroaromatic) cycles stabilize the molecular ion.

7. Double bonds promote allyl decomposition with formation of allylic carbocation.



8. The C-C bonds following the heteroatom are often broken leaving charge on the heteroatom-containing moiety.

9. Decomposition is often accompanied by the elimination of small stable neutral molecules (CO, CH_2CH_2, H_2O, NH_3, etc.)

10. It is necessary to know some specific ions characterized by intense peaks:

m/z 77 – phenyl C_6H_5

m/z 91 – tropylium C_7H_7 (benzyl)

m/z 30 – amino group CH_2NH_2

m/z 105 – benzoyl PhC = O

11. If the spectrum is characterized by a large number of fragments, peaks, which have an increasing intensity, when moving down the scale of mass, most likely it is an aliphatic compound.

12. Rare intense peaks are characteristic of aromatic structures.

13. Peaks with mass numbers 73, 147, 207, 281, 355, etc. do not apply to the spectrum of the investigated compound, but are a consequence of the ejection of fragments of the most common polydimethylsilicon phases of chromatographic column into the source of the mass spectrometer.

Rearrangements

In the mass spectra there are ions formed in the result of intermolecular rearrangement of atoms in the process fragmentation. Rearrangements are particularly widespread, including the migration of hydrogen atoms in molecules containing heteroatom. One of the most important rearrangements is the McLufferty rearrangement (Figure 54).

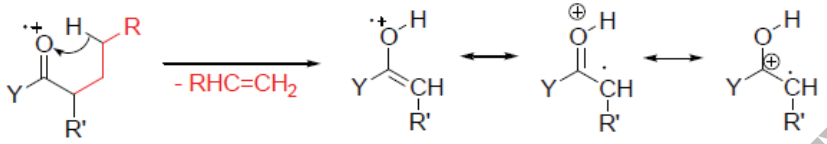


Figure 54. The general scheme of the McLufferty rearrangement

A molecule should have suitably located heteroatom (for example, O), a π-system (usually a double bond) and the hydrogen atom in the γ-position relatively to the C=O group capable of elimination to undergo this rearrangement. Rearrangements accompanied by elimination of stable neutral molecules are widespread. Such rearrangements often lead to the emergence of intensive characteristic peaks that greatly facilitate interpretation of spectra.

For example, an intense peak with m/z 31 is observed in the spectrum of diethyl (medical) ether (Figure 55).

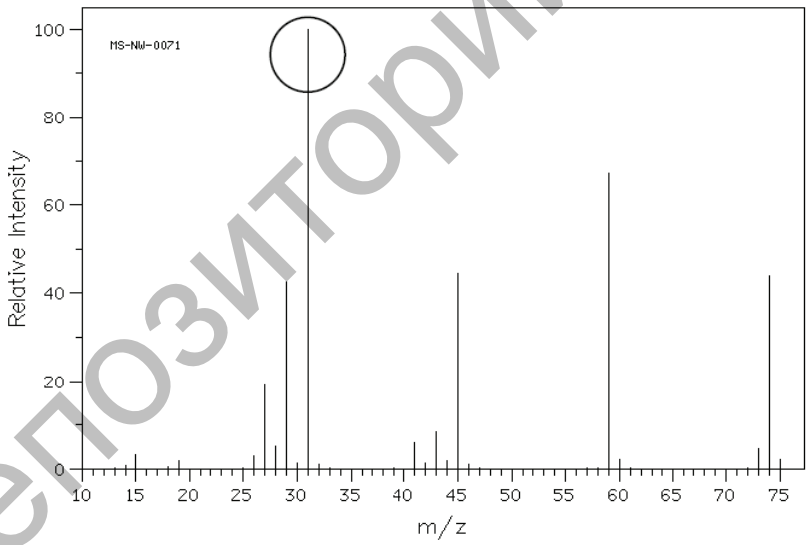


Figure 55. The EI mass spectrum of diethyl ether

Its origin is explained by the McLufferty rearrangement of fragment ion with m/z 59 (Figures 55 and 56), resulting in elimination of the ethylene molecule.

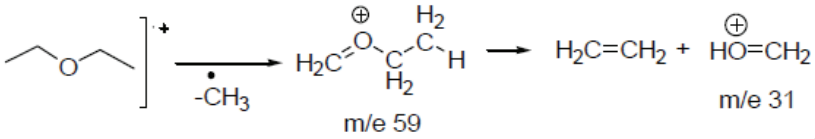


Figure 56. Scheme of fragmentation of a molecular ion of diethyl ether

The McLufferty rearrangement is observed during fragmentation of molecular ions of alcohols, ketones, esters, amides, alkylheterocycles, aromatic ethers, vinyl ethers, and olefins.

Peaks of rearrangement ions can be identified, considering the mass numbers (m/z) of the fragment ions and corresponding molecular ions.

A simple (without rearrangement) decomposition of a molecular ion with an even mass number gives a fragment ion with an odd mass number.

A simple decomposition of a molecular ion with an odd mass number gives a fragment with an even mass number.

A peak with an even mass number, arising from a molecular ion with an even mass number, is obtained as a result of two decompositions, which are accompanied by a rearrangement.

5.4 General Characteristics of Mass Spectra of Classes of Organic Compounds

Unbranched alkanes (Figure 52)

Molecular ion is of the average intensity, the signal strength decreases with increasing degree of branching (rule 1).

Fragmentation. Alkyl fragments of large size (with C>4) are formed, mainly, in direct decomposition. Their dehydrogenation is accompanied by a rearrangement, involving the H atoms of the skeleton. Alkyl fragments of smaller size (from C2 to C4) are formed during the secondary decomposition of larger alkyl fragments. At the same time, groups from the middle of the chain (and recombination of its ends) are eliminated.

A series of ions. The alkane series C_nH_{2n+1} is (m/z 29, 43, 57, 71, 85 ...). The intensity maximum is at m/z 43 or 57. Peaks of C_nH_{2n+1}

are accompanied by the signals C_nH_{2n-1} (m/z 27, 41, 55, 69 ...) and C_nH_{2n} (m/z 28, 42, 56, 70 ...) of lesser intensity.

Unbranched alkenes

Molecular ion is identified.

Fragmentation. The elimination of alkyl residues and neutral alkenes predominates. The determination of the position of the C=C bond in acyclic alkenes is difficult. It is also impossible to determine belonging to the *cis*- or *trans*-isomer. However, that the intensity of the molecular ion of the *trans* isomer is higher than that of *cis*-isomer.

A series of ions. The peak sequences corresponding to C_nH_{2n-1} (27, 41, 55, 69, 83 ...) are accompanied by signals of alkyl C_nH_{2n+1} (m/z 29, 43, 57, 71, 85 ...) and alkene ions C_nH_{2n} (m/z 28, 42, 56, 70 ...) usually of low intensity. Formation of alkene series is due to allylic decomposition (rule 7):

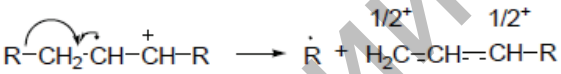


Figure 57 shows the EI mass spectrum of β-myrcene.

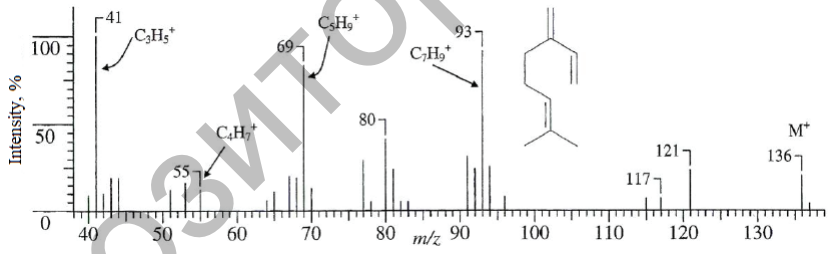


Figure 57. The EI mass spectrum of β-myrcene

Arenes

Molecular ion gives the intensive peak due to stabilization (rule 6).

Fragmentation. Compounds show a weak propensity to fragmentation. There is elimination of H· with subsequent loss of H_2, which leads to the peaks [M-1] +, [M-3] +, [M-5]+ with decreasing intensity.

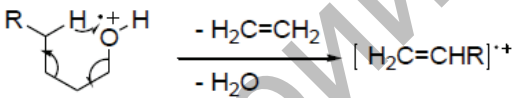
The subsequent typical fragmentation is cleavage of acetylene (Δm 26) and C_3H_3 (Δm 39).

Aliphatic alcohols

Molecular ion is usually weak. It is often indistinguishable for tertiary alcohol.

Fragmentation. The cleavage of the C-C bond with the oxygen atom is regularity (rule 8). There is a characteristic peak due to the ion $CH_2{=}^{+}OH$ (m/z 31) in the mass spectra of primary alcohols; secondary and tertiary alcohols give rise characteristic peaks due to $RCH{=}^{+}OH$ ions (m/z 45, 59, 73, etc.) and $RR'C{=}^{+}OH$ (m/z, 59, 73.87, etc.). Often a significant peak is observed at M-18 due to elimination of water.

The elimination of water and alkene is characteristic for all alcohols, which causes the appearance of a peak at M – (alkene + H_2O), i.e. [M-46], [M-74], [M-102]:



A series of ions. The sequences of alkene ions predominate C_nH_{2n-1} (41, 55, 69 ...), C_nH_{2n} (m/z 42, 56, 70 ...) accompanied by weaker peaks $C_nH_{2n+1}O$ (m/z 31 (predominant in primary alcohols), 45, 59 ...).

Figures 58-60 show EI mass spectra of isomeric pentanols [42-43].

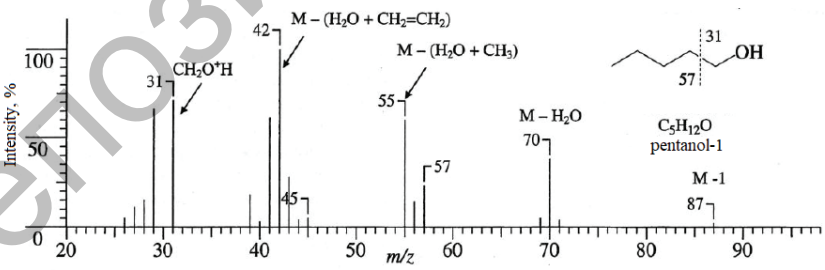


Figure 58. The EI mass spectrum of pentanol-1

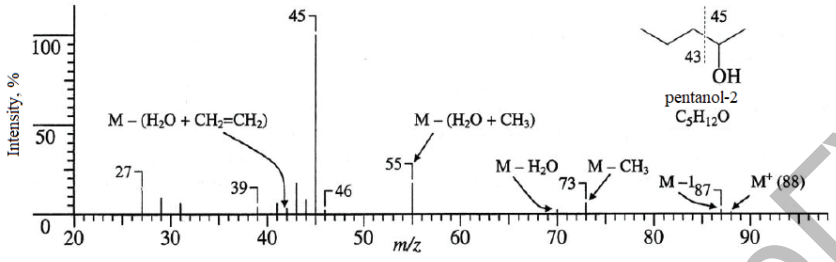


Figure 59. The EI mass spectrum of pentanol-2

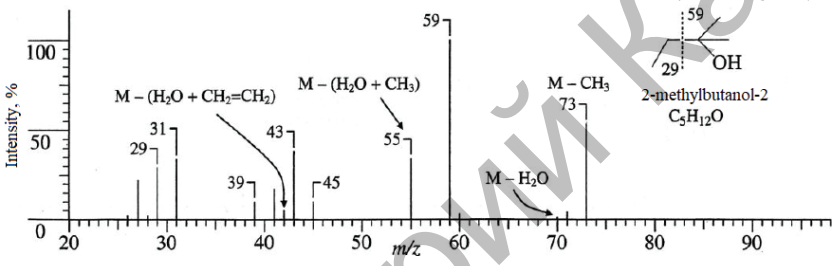
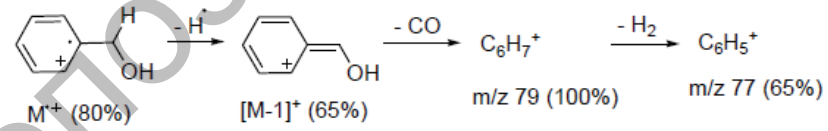


Figure 60. The EI mass spectrum of 2-methylbutanol-2

Benzyl alcohols

Molecular ion gives rise to an intense peak (Figure 61).

Fragmentation. Loss of H· and subsequent elimination of CO (Δm 28) leads to a protonated benzene molecule, from which H_2 further eliminates.



The second important direction of fragmentation is the elimination of OH (Δm 17) with the formation of a tropylium cation:

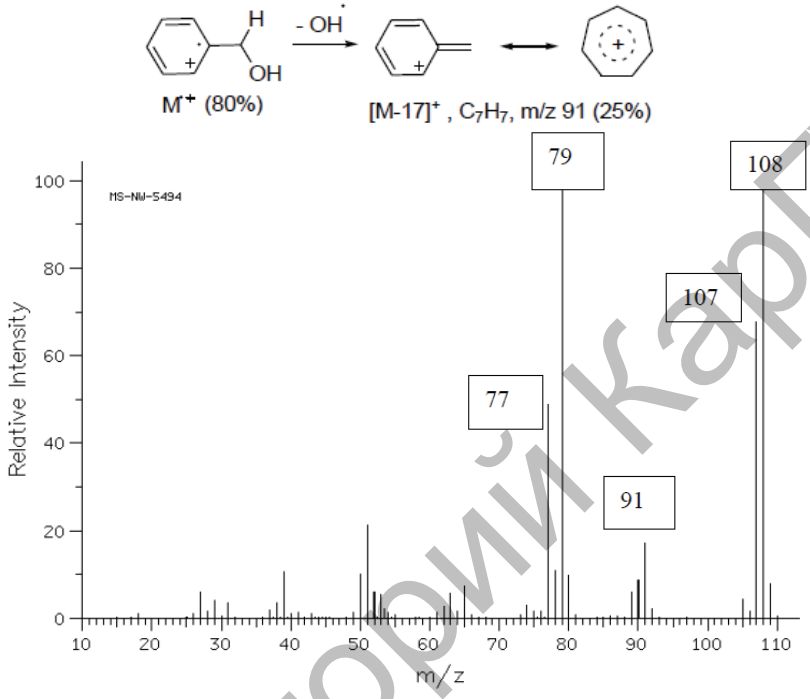


Figure 61. The EI mass spectrum of benzyl alcohol (molecular ion 108).

Phenols

Molecular ion is quite intense. It is basic one in the phenol itself.

Fragmentation. Usually, peaks of the $C_6H_5^+$ ion with m/z 77 and peaks, arising from the loss of CO [M-28], CHO [M-29], are observed. An important role is played by the loss of H_2O_2 (Δm 34), H_2O (Δm 18), HO·(Δm 17), and O (Δm 16).

A series of ions. Mainly, the aromatic series C_nH_n and $C_nH_{n \pm 1}$ (m/z 29, 51-53, 63-65, 75-77 ...) is found. Usually there is an insignificant peak m/z 55 (C_3H_3O). The peak m/z 69 (O≡CCH═C═O) is characteristic of the 1,3-dihydroxyl substituted.

Figure 62 shows the EI mass spectrum of *o*-ethylphenol.

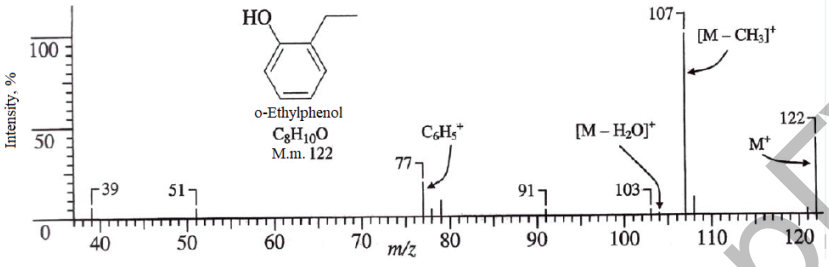


Figure 62. The EI mass spectrum of *o*-ethylphenol

Ethers

Aliphatic ethers

Molecular ion is a noticeable or weak peak, the intensity decreases with increasing chain length and branching.

Fragmentation.

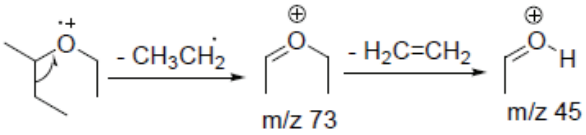
1. Homolytic cleavage of the C-C bond adjacent to the oxygen atom. The bond between the most substituted C-atom is broken with the separation of the largest alkyl group (rule 2). This homolysis leads to the elimination of alkenes, carbonyl groups, and water.

2. Heterolytic bond, breaking at O-atom, leading to strong signals of alkyl ions.

3. Migration of hydrogen with simultaneous elimination of the alcohol molecule.

A series of ions. Alkyl fragments C_nH_{2n+1} (m/z 29, 43, 57 ...); alkenyl series C_nH_{2n} (m/z 28, 42, 56 ...); oxygen-containing fragments $C_nH_{2n+1}O$ (m/z 31, 45, 59 ...).

As an example, the mass spectrum of *sec*-butyl ethyl ether, as well as a scheme, explaining the formation of fragment ions with mass numbers 73 and 45, is shown in the Figure 63.



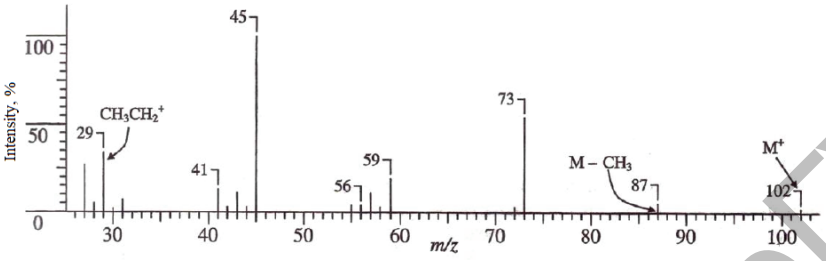


Figure 63. The EI mass spectrum of *sec*-butylethyl ether

Aromatic ethers

Molecular ion gives rise to an intensive signal.

Fragmentation. H· (1), CO (Δm 28), CHO (Δm 29) are eliminated from $M^{+\bullet}$. The cleavage of the C-O bond and the decarbonylation of the decomposition products with subsequent dehydrogenation are occurred.

A series of ions. Aromatic series C_nH_n and $C_nH_{n \pm 1}$ (m/z 39, 51-53, 63-65, 75-77 ...) is found.

An anisole mass spectrum and a fragmentation scheme, explaining the formation of basic ions, are shown in Figures 64 and 65.

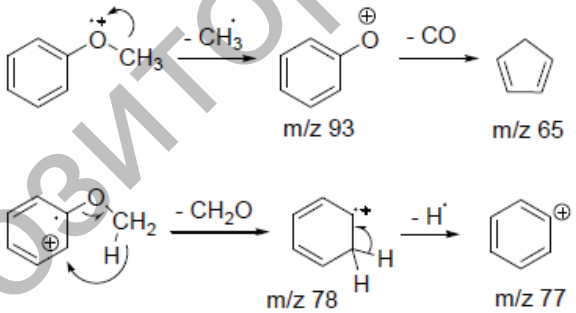


Figure 64. Fragmentation scheme of molecular ion

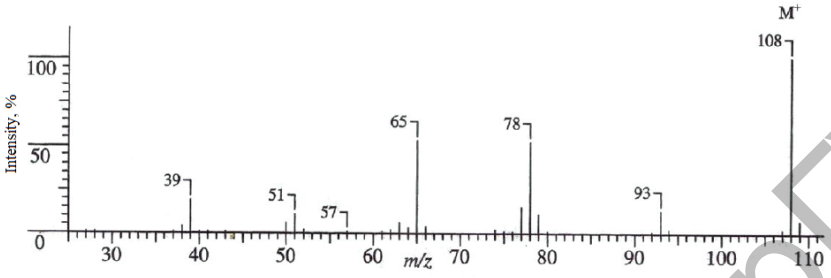


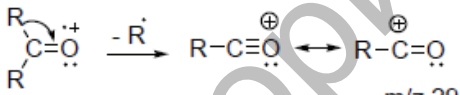
Figure 65. The EI mass spectrum of the anisole

Ketones

Aliphatic ketones

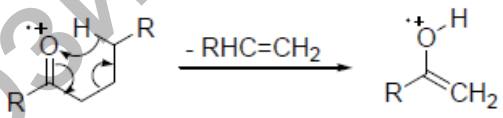
Molecular ion gives rise to a relatively intense signal.

Fragmentation. The cleavage following the C=O bond is the most important primary process, the charge remains on the resonance-stabilized acyl cation:



m/z 29, 43, 57, 71...

Then the acyl ions eliminate CO. The McLufferty rearrangement leads to the formation of ions $C_nH_{2n}O^{+\bullet}$ (m/z 58, 72, 86 ...).



Fragmentation of the hydrocarbon chain is similar to fragmentation in corresponding alkanes.

A series of ions. Sequences of fragmentation ions C_nH_{2n+1} and $C_nH_{2n-1}O^+$ (in both cases m/z 29, 43, 57 ...) predominate; maxima with even masses $C_nH_{2n}O^{+\bullet}$ (57, 72, 86 ...).

Aromatic ketones

Molecular ion gives rise to an intensive signal.

Fragmentation. α-Cleavage predominates with formation of benzoyl ion PhC=O (m/z 105) (often the most intensive) followed by decarbonylation leading to a phenyl ion with a peak of lower intensity. As an example a mass spectrum of an asymmetric ketone and a fragmentation scheme, explaining the formation of basic ions, are shown below (Figures 66 and 67).

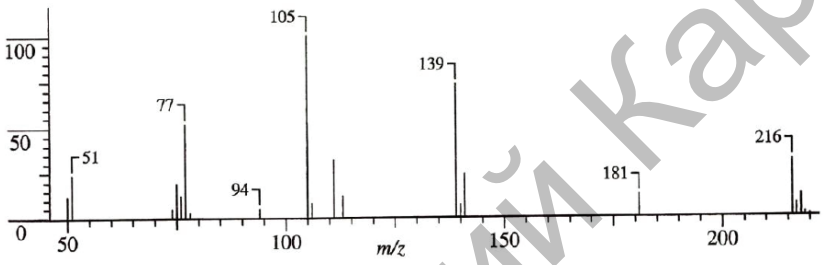


Figure 66. The EI mass spectrum *p*-chlorobenzophenone

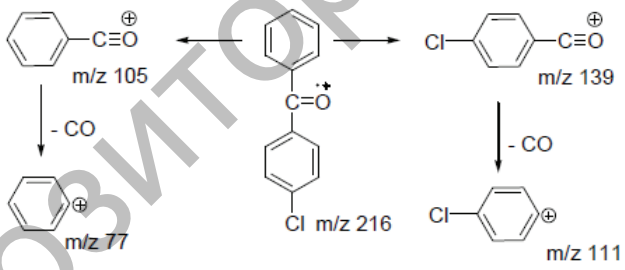


Figure 67. An asymmetric ketone fragmentation scheme

If the alkyl chain contains three or more carbon atoms, the McLufferty rearrangement with elimination of an alkene occurs; at the same time maxima with even masses appear.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...).

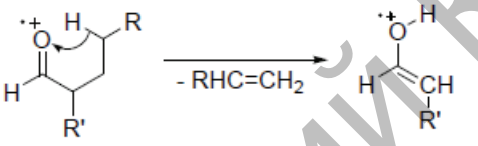
Aldehydes

Aliphatic aldehydes

Molecular ion. The signal is intense only for molecules with low molecular weight; it is very weak for $C_n > 9$. The peak $[M-1]^+$ can be more significant than $M^{+\bullet}$ (Figure 68).

Fragmentation. The cleavage of the C-H and C-C bonds following the oxygen atom, leads to the M-1 and M-R peaks (m/z 29, CHO^+).

The McLafferty rearrangement is observed in aldehydes, containing 4 or more carbon atoms, leading to the formation of $C_nH_{2n}^{+\bullet}$ (m/z 28, 42, 56 ...) and $C_nH_{2n}O^{+\bullet}$ (m/z 44, 58, 72 ...).



The following characteristic peaks are recognized in the normal chain aldehydes: [M-18] (water elimination), [M-28] (elimination of ethylene), [M-43] (elimination of $CH_2 = CH - O\bullet$) and [M-44] (elimination of CH_2=CH-OH).

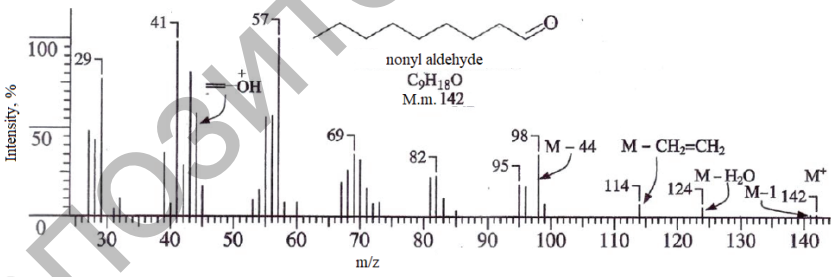


Figure 68. The EI mass spectrum of nonyl aldehyde

A series of ions. The sequences of the fragment ions C_nH_{2n+1} and $C_nH_{2n-1}O$ predominate (in both cases m/z 29, 43, 57 ...).

Aromatic aldehydes

The signal of a molecular ion is relatively intense. Usually the value has the peak $[M-1]^+$.

Fragmentation. The characteristic loss of $H\bullet$ leads to the formation of the corresponding benzoyl ion (Ph-C≡O^+), which is always intense. Subsequent decarbonylation gives a phenyl ion (m/z 77), which in turn eliminates acetylene to form the $C_4H_3^+$ ion (m/z 51).

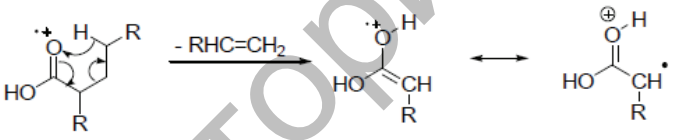
A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...).

Carboxylic acids

Aliphatic acids

Molecular ion is usually registered.

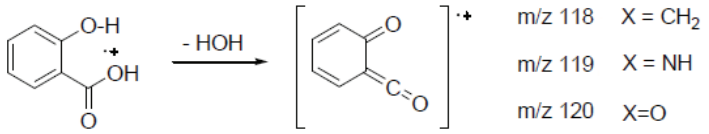
Fragmentation. The cleavage of the C-CO bond leads to particles with conservation of charge on oxygen-containing fragments (m/z 45, 59, 73, 87 ...), or on alkyl fragments (m/z 29, 43, 57, 71, 85). The most characteristic (often the maximum) are the peaks with m/z 60, 74, 88 ... caused by the Mc-Lafferty rearrangement:



Aromatic carboxylic acids

Molecular ion is intensive.

Fragmentation. The loss of $OH\bullet$ leads to the appearance of an ion $[M-17]^+$ (characteristic peak), subsequent decarbonylation (Δm 28) provides the formation of a phenyl ion (less intense). A loss of CO_2H also leads to the appearance of a characteristic ion [M-45]. If there is an *ortho*-substituent, containing H atom capable of transferring, then there is elimination of water (one of the McLafferty rearrangements) and the formation of $\left[M-18\right]^{+\bullet}$:



The described fragmentation is characteristic only of *ortho*-isomers, which allows them to be distinguished from *meta*- and *para*-isomers.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are observed. Figures 69 and 70 show examples of EI mass spectra of carboxylic acids.

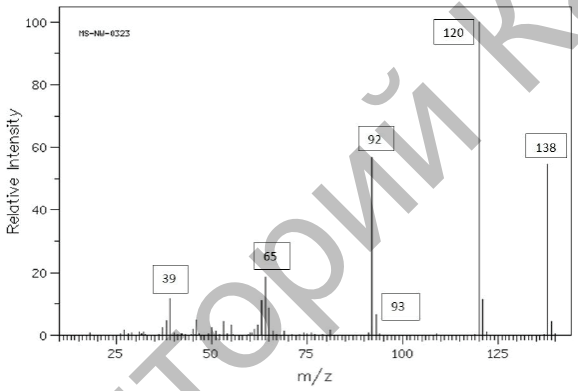


Figure 69. The EI mass spectrum of salicylic acid

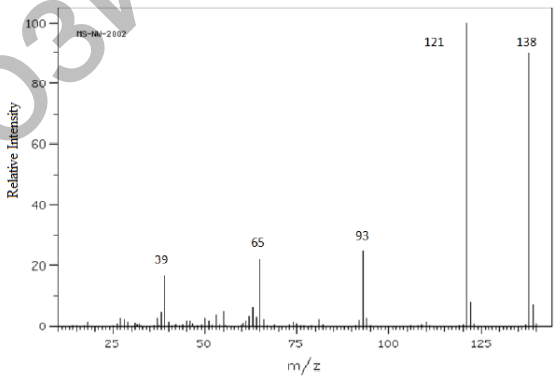


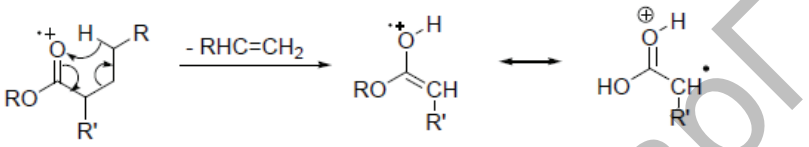
Figure 70. The EI mass spectrum of *p*-hydroxybenzoic acid

Esters of carboxylic acids

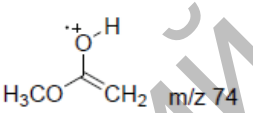
Esters of aliphatic acids

Molecular ion is usually distinguishable.

Fragmentation. The most characteristic peak is due to the McLafferty rearrangement t:



An intense peak with m/z 74 caused by an ion, which is shown below, is observed in the spectra of methyl esters that do not have branching for α-carbon atom.



In general, one can expect appearance of the following ions in the spectrum of the ester R-COOR':

- R^+ is found in the mass spectra of esters with a short chain;
- The ion $[C(=O)OR']^+$ is not very important, but are easily recognized in methyl ethers (m/z 59);
- $R\text{-}C{\equiv}O^+$ is easily recognized. In methyl esters it is observed at [M-31] ($M\text{-}OCH_3$), while the $[OR']^+$ ion, as a rule, does not have great importance.

Further, the fragmentation pattern depends on which part of the molecule (acidic or alcoholic) contains a larger alkyl radical.

Esters of aromatic acids

Molecular ion gives an intensive signal.

Fragmentation. The loss of $RO^{\bullet}$ with the formation of benzoyl ion (main peak) predominates, and then decarbonylation (Δm 28) and loss of acetylene (Δm 26) occur. Cleavage of ˙COOR leads to the appearance of another intense peak; it is the peak [M-59] in methyl esters.

ROH eliminates from *ortho*-substituted benzoates according to the mechanism described above. The maximum peak with m/z 120 is in the mass spectrum of methyl salicylate (Figure 71), and then this ion loses CO, giving an intense peak with m/z 92.

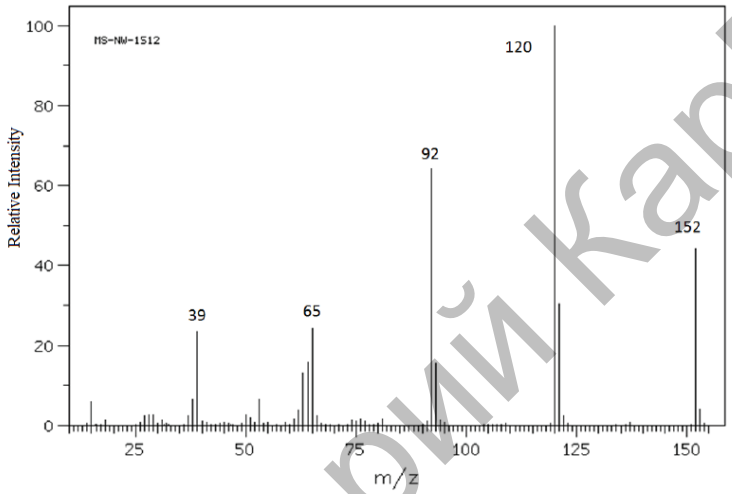


Figure 71. The EI mass spectrum of methyl salicylate

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are observed.

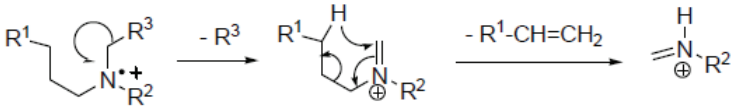
Amines

Saturated aliphatic amines

Molecular ion. The peak of the molecular ion of the primary aliphatic amine has an odd mass number value ("Nitrogen rule"), usually of low intensity or none at all absent.

Fragmentation. The loss of alkyl residues is predominant cleavage of the C-C bond adjacent to the nitrogen atom (rule 8). This is the peak with m/z 30 ($CH_2NH_2^+$) for primary amines unbranched at the α-carbon atom.

The larger substituents are cleaved first (rule 1). If γ-H is present, alkenes elimination occurs as a result of the McLafferty rearrangement:



Elimination of NH_3, RNH_2, RR'NH from primary, secondary and tertiary amines are accordingly negligibly small.

A series of ions. Fragments of the type $C_nH_{2n+2}N$ with even masses (m/z 30, 44, 58, 72, 86 ...) are observed.

Aromatic amines (anilines)

Molecular ion is intensive if there are no aliphatic substituents with more than one carbon atom; otherwise it is medium or weak peak (odd mass number).

Fragmentation. The detachment of one hydrogen atom gives the peak [M-1] of moderate intensity. Separation of the neutral HCN molecule and the subsequent detachment of the hydrogen atom produce noticeable characteristic peaks with m/z 66 and 65, respectively.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are detected.

Figure 72 shows the EI mass spectrum of aniline as an example.

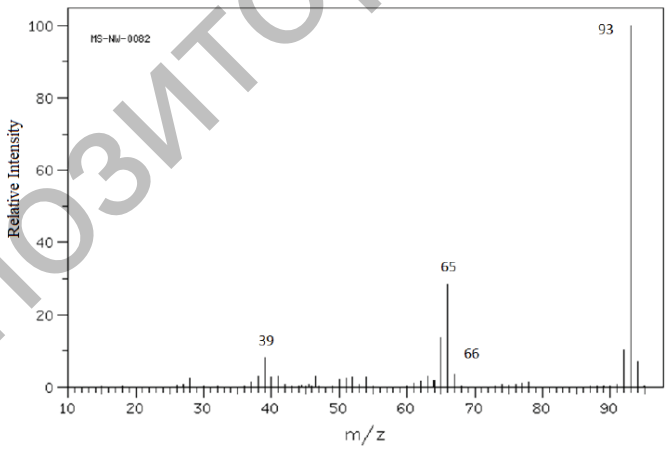


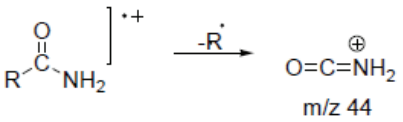
Figure 72. The EI mass spectrum of aniline

Amides

Aliphatic amides

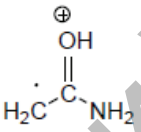
Intensity of a *molecular ion* is considerable.

The predominant type of *fragmentation* depends on length of the acyl moiety, as well as the degree of substitution of amide nitrogen and the value of alkyl substituents attached to it. Primary amides with C_1-C_3 and isobutyric acid amide give maximum peak with m/z 44:

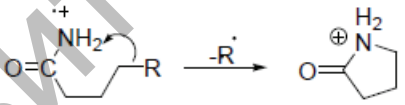


m/z 44

In primary amides with C > 3 (unbranched chain) maximum peak with m/z 59 is observed:

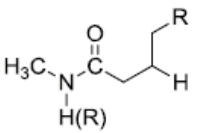


It is formed as a result of the McLafferty rearrangement. There are homological peaks of ions with m/z 73, 87 ... upon branching at the α-carbon atom. A peak of moderate intensity with m/z 86 appears due to cleavage of γ, δ-bonds and possible cyclization:



m/z 86

Secondary and tertiary amides of the type shown below give the dominant peak due to the McLafferty rearrangement. In other cases, fragmentation proceeds in a different way with the formation of an ion $^{+}NH_2{=}CH_2$ (m/z 30).



Amides of aromatic acids

Molecular ion produces an intensive signal.

Fragmentation. The maximum peaks result from the cleavage of the amide bond to form a benzoyl cation and subsequent decarbonylation. The diagram of benzamide fragmentation is in Figure 73. Figure 74 shows its mass spectrum.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are observed.

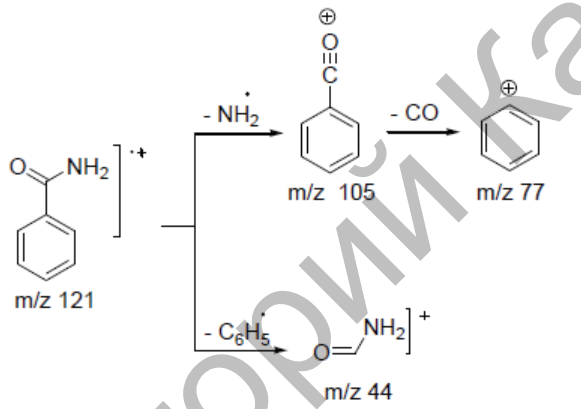


Figure 73. The diagram of benzamide fragmentation

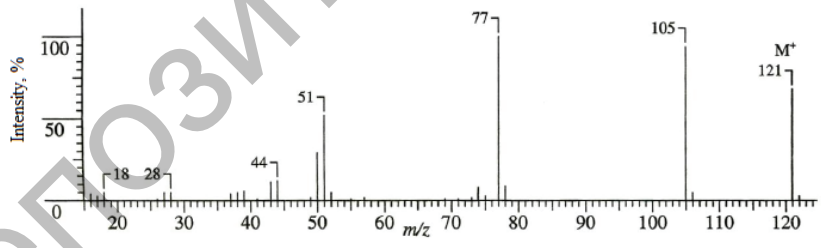


Figure 74. The EI mass spectrum of benzamide

Nitriles

Aliphatic nitriles.

Molecular ion is weak or missing.

Fragmentation. Elimination of alkyl radicals with the formation of ions $(CH_2)_nCN^+$ (m/z 40, 54, 68 ...). The McLafferty rearrangement leads to $CR_2 = C = NH^{+\bullet}$ (m/z 41 for R = H).

A series of ions. Peaks of saturated and unsaturated ions mainly in the region of small masses (C_nH_{2n+1} m/z 29, 43, 57 ... and C_nH_{2n-1} 27, 41, 55 ...) are detected.

Aromatic Nitriles

Molecular ion is often of the maximum intensity.

Fragmentation. Successive elimination of HCN and acetylene.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are observed.

Nitro compounds

Aliphatic nitro compounds

The molecular ion peak is weak or absent.

Fragmentation. The main peaks are due to hydrocarbon fragments up to $[M-NO_2]$. The nitro group is found from the intense peak of the ion with m/z 30 (NO^+) and the less intense peak of the ion with m/z 46 (NO_2^+).

A series of ions. (C_nH_{2n+1} and C_nH_{2n-1}, m/z 43, 57, 71 ... and 41, 55, 69 ...).

Aromatic nitro compounds

Molecular ion is an intense peak.

Fragmentation. Significant peaks for identification (Figure 75):

- [M-46] (maximum in nitrobenzene) is due to elimination of NO_2. Cleavage of the acetylene molecule from [M-46] leads to the appearance of an intense peak [M-72];
- [M-30] is due to phenoxy-cation formation as a result of elimination of NO with further rearrangement. When the CO is cleaved from the ion [M-30], a peak [M-58] appears;
- peak with m/z 30 is due to NO^+ ion.

A series of ions. The aromatic series C_nH_n and $C_nH_{n\pm1}$ (m/z 39, 51-53, 63-65, 75-77 ...) are detected.

Figure 75 shows the EI mass spectrum of nitrobenzene (molecular ion 123).

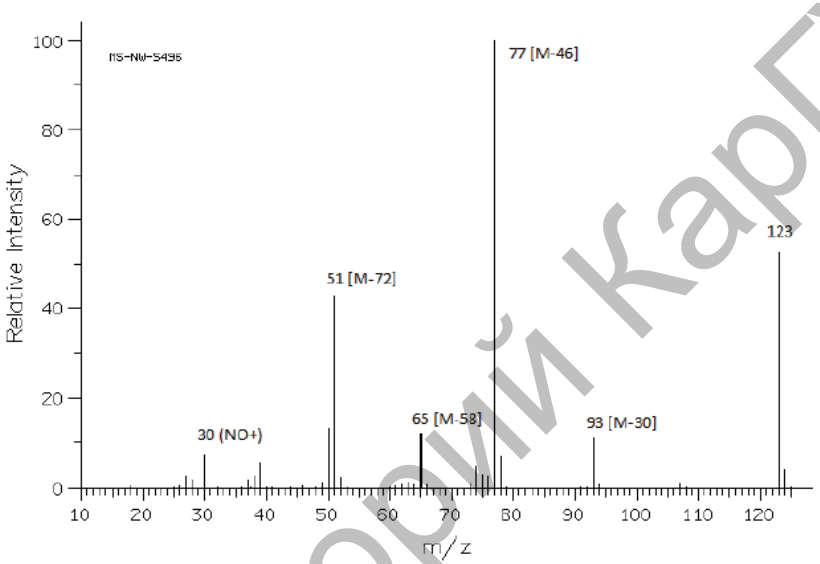


Figure 75. The EI mass spectrum of nitrobenzene

Halogen-containing compounds

Mass spectrometry is a reliable method for establishing the presence of chlorine and bromine in a molecule of an organic compound. In the mass spectra of compounds with one chlorine atom M+2 peak whose intensity is approximately one-third the intensity of the molecular ion appears due to the presence of an ion with an isotope of ^{37}Cl. The compound with one bromine atom demonstrates a peak with M+2, almost equal in intensity to the peak of the molecular ion and related to a molecular ion with the ^{81}Br isotope. The number of chlorine and (or) bromine atoms in the molecule can be determined from the number of peaks larger in mass than in the molecular ion. Thus, three chlorine atoms in the molecule give peaks M+2, M+4 and M+6. Figure 76 shows the sets of peaks M, M+2, M+4 ... for compounds with a different combination of chlorine and bromine atoms.

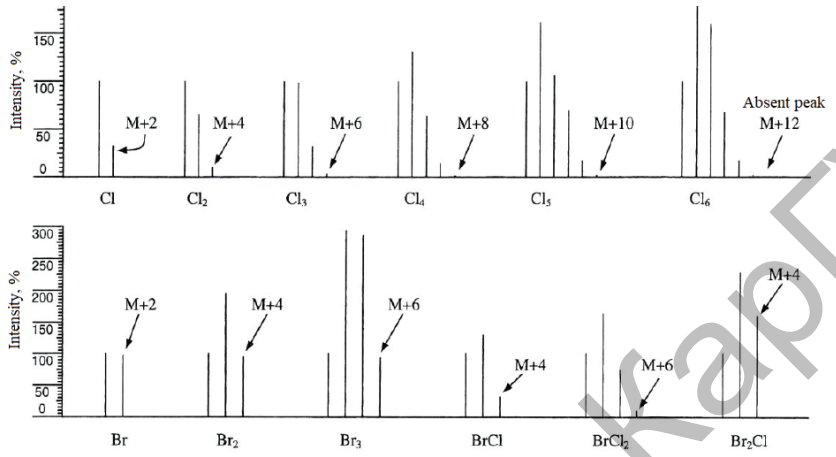


Figure 76. Calculated sets of peaks M, M+2, M+4 ... for compounds with different combination of chlorine and bromine atoms (intensity of peaks in % relative to molecular ion peak).

Aliphatic halides

Molecular ion is an intense peak for small alkanes with increasing intensity in the series F, Cl, Br, I. The intensity decreases with increasing mass and branching of the hydrocarbon radical.

Fragmentation. The following directions are possible:

- loss of halogen radical (F <Cl <Br <I) followed by elimination of alkenes;
- separation of the alkyl radical and further cleavage of the acid HHal;
- loss of acid to form an alkene radical cation.

A series of ions. The alkenyl series C_nH_{2n-1} predominates (27, 41, 55 ...). Figure 77 shows the EI mass spectrum of *n*-bromobutane.

Aromatic halides

Molecular ion is usually very intense.

Fragmentation. Sequential loss of halogen radicals and / or HHal acids. In the case of alkyl substituents with $C_n > 1$, the main peak occurs as a result of benzyl decomposition.

A series of ions. The aromatic series C_nH_n and $C_nH_n \pm 1$ (m / z 39, 51-53, 63-65, 75-77…).

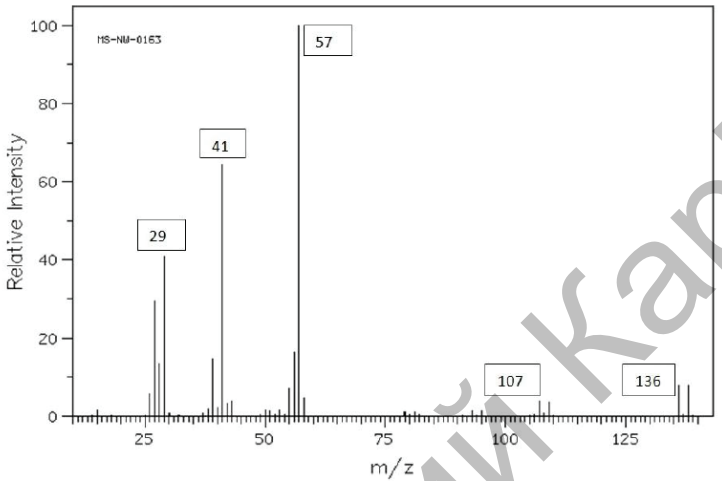


Figure 77. The EI mass spectrum of *n*-bromobutane

Figure 78 shows the EI mass spectrum of bromobenzene.

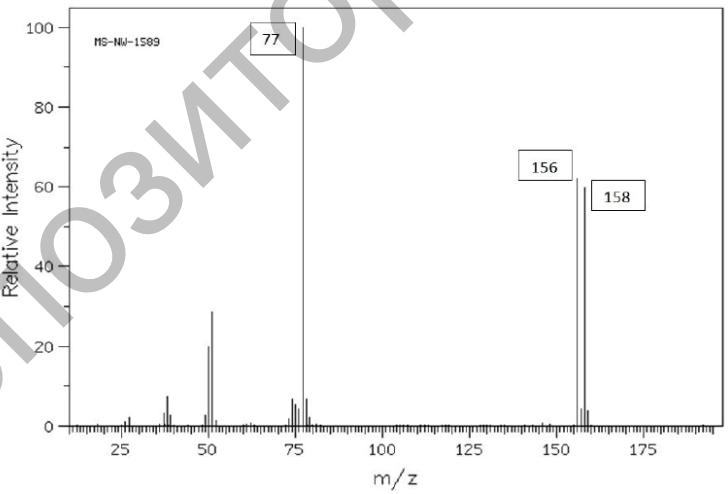
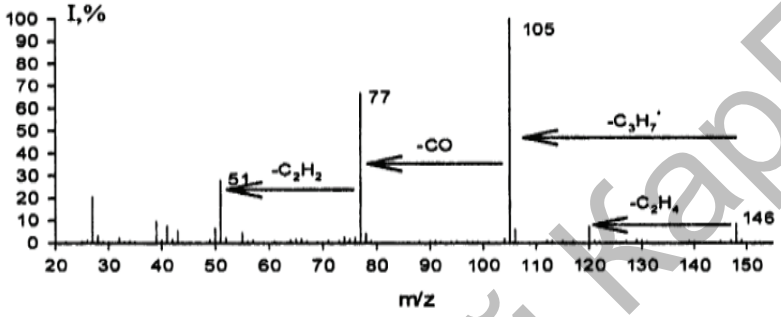


Figure 78. The EI mass spectrum of bromobenzene

Examples of problem solving

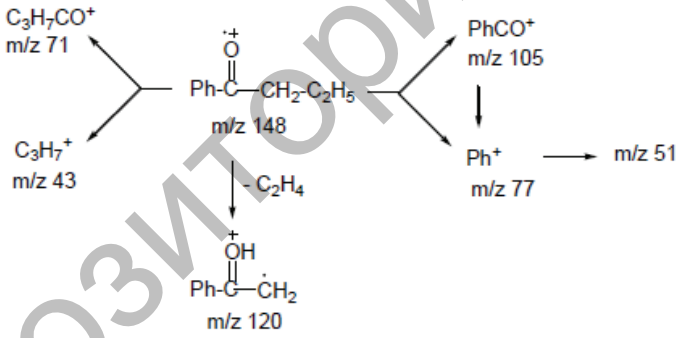
Problem 5.1

Derive the fragmentation scheme of butyrophenone; mass spectrum is presented below.



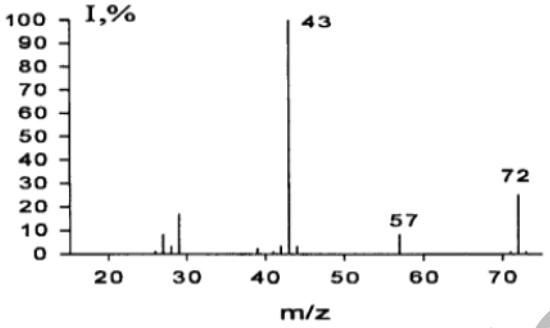
Answer to problem 5.1

Scheme of fragmentation of butyrophenone is as follows:



Problem 5.2

Identify the compound whose mass spectrum is as follows:



m/z	I, %	m/z	I, %
15	4.31	42	3.12
26	1.12	43	100
27	8.34	44	2.23
28	3.25	57	8.87
29	17.6	71	1.29
39	2.78	72	25.00
41	1.03	73	1.11

Answer to problem 5.2

The peak with m/z 72 satisfies the criteria of a molecular one. Using the M +1 peak we set the maximum number of carbon atoms:

25.0 - 100%

1.11 - x%

x = 4.44

The number of carbon atoms is determined to be 4.44: 1.11 = 4.

Further, using the table "Exact Mass Values for Determining Formulas", we assume the formula C_4H_8O.

It can be concluded that the ion with m/z 43 (maximum) has the composition C_2H_3O from the intensity of the m/z 44 peak and is formed as a result of elimination of the ethyl radical from $M^{+\bullet}$, and the ion with m/z 29 is ethyl cation. Therefore, the figure shows the mass spectrum of butanone-2.

Questions and Assignments for Self-Study:

1. What is the ionization energy?
2. How does a molecular ion form?
3. What tasks does mass spectrometry solve?
4. What determines the intensity of the peak in the mass spectrum?
5. How is the isotopic composition of the elements reflected on the mass spectrum of compound?
6. In which case should we expect the McLafferty rearrangement?

CHAPTER 6.

Practice in Structure Determination

There is no fixed way of tackling a problem in structure determination, using the four spectroscopic methods. Each problem has its own unique features, and some knowledge of the provenance makes a big difference to how one starts. It is almost always best to begin with the molecular weight of the unknown derived from its mass spectrum, and ideally to obtain the molecular formula from a high-resolution measurement. The spectra of greatest power in structure determination are ^{13}C and ^{1}H NMR, UV and IR are less important. Sometimes, UV provides critical information on the extent of conjugation, and IR on the presence of a functional group.

If the mass spectrum has given the molecular formula, it is possible to work out the number of "double-bond equivalents" (DBE) in a molecule. It is useful to remember that a benzene ring has a total of four double-bond equivalents: three "double bonds" and one ring. The number of divalent atoms present makes no difference to this calculation, but the number of mono- and trivalent atoms does.

Problem 6.1

Compound A of general formula $C_7H_8O_2$ after treatment with hydroiodic acid forms compound B ($C_6H_6O_2$), which is widely used in photography. Based on the results of the IR, NMR and mass spectrometry analysis (Figures 79-82, respectively), establish the structural formula for compound A [44-46].

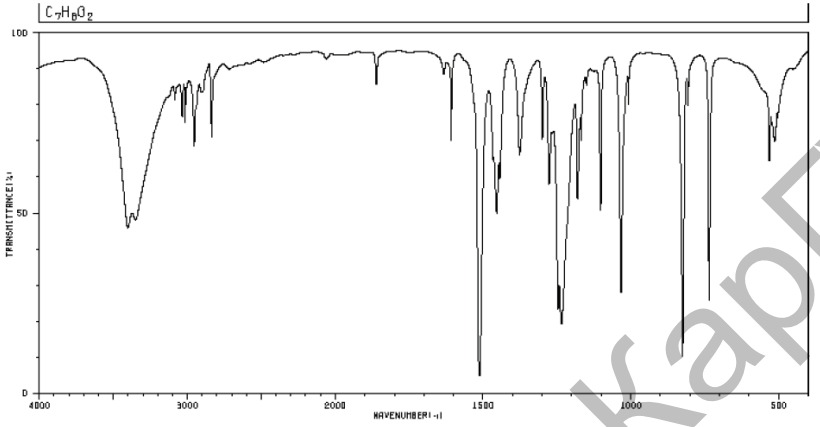


Figure 79. The IR spectrum of compound A recorded in KBr

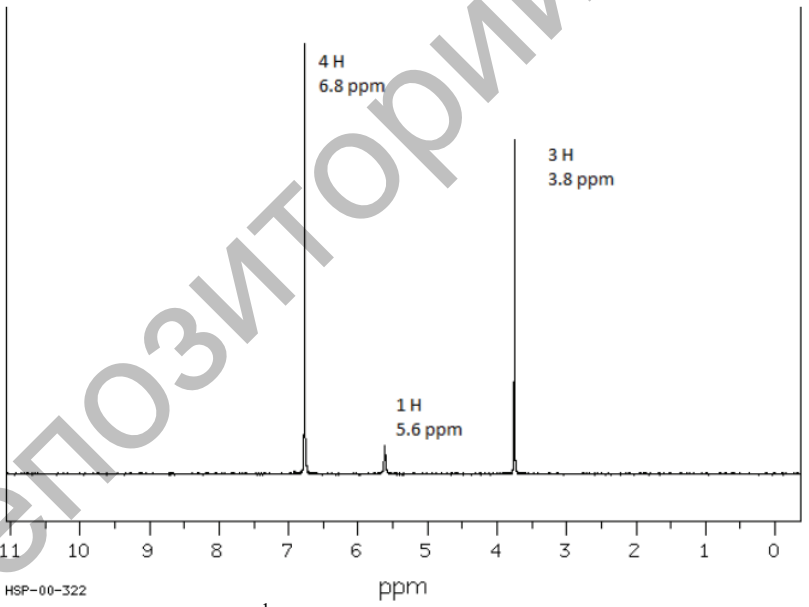


Figure 80. The ^{1}H NMR spectrum of compound A (solvent $CDCl_3$)

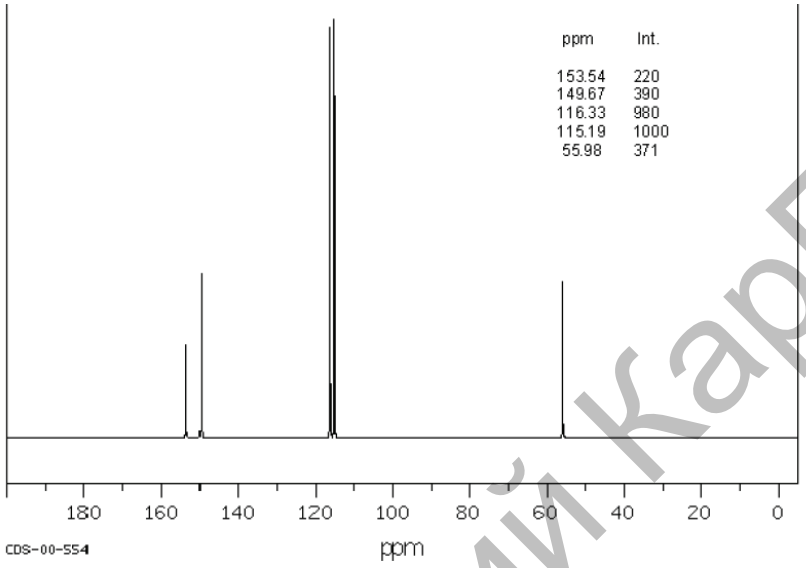


Figure 81. The ^{13}C NMR spectrum of compound A (solvent $CDCl_3$)

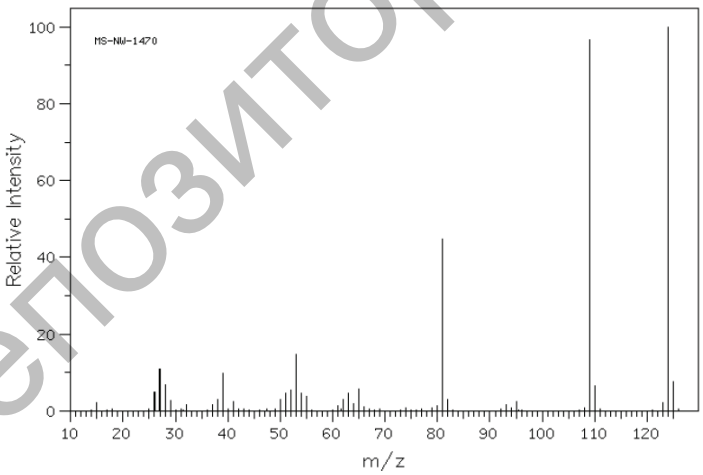


Figure 82. The EI mass spectrum of compound A (molecular ion 124)

Peak table

m/z	I, %	m/z	I, %
15.0	2.2	61.0	1.3
26.0	4.9	62.0	2.9
27.0	11.0	63.0	4.6
28.0	6.8	64.0	1.8
29.0	2.7	65.0	5.6
32.0	1.5	66.0	1.1

Solution:

1. Compound A is an oxygen-containing compound (from the condition of the problem).
2. Compound A is not an aldehyde, ketone, carboxylic acid or carboxylic acid derivative (absence of an absorption band characteristic of the C=O group in the 1650-1700 cm^{-1} region).
3. Compound A is a benzene derivative (absorption bands in the region of 3000 cm^{-1} and 1500 cm^{-1}, a signal in the region of 6.8 ppm (^{1}H NMR spectrum) and signals at 115 and 116 ppm (^{13}C NMR), an aromatic series of m/z 39, 51-53, 63-65 (EI mass spectrum).
4. Compound A has the group OCH_3 in the structure (peak with m/z 109, signal at 3.8 ppm (^{1}H NMR), 56 and 145 ppm (^{13}C NMR).
5. Compound A is a substituted phenol (a broad absorption band in the 3200 cm^{-1} region, the molecular ion is the most intense, the signal at 5.6 ppm (^{1}H NMR), 152 ppm (^{13}C NMR).
6. The compound is *p*-methoxyphenol (singlet at 6.8 ppm (^{1}H NMR), *p*-methoxyphenol is converted to the hydroquinone by HI treatment, which is a developer in photography).

Assignments for Self-Study:

1. Analyze the spectra and solve the structure of the molecule for which data are provided. The following data are provided: EI-MS; IR (thin film on NaCl plates); 500 MHz ^{1}H NMR in $CDCl_3$; 125.8 MHz ^{13}C NMR, DEPT 90, and DEPT 135 in $CDCl_3$ (Figures 83-88, respectively).

Identify any noteworthy heteroatoms present. Determine the molecular formula and unsaturation number. Identify functional groups that are present from the IR and other spectra. Identify key fragments from NMR. Assign the ^{1}H NMR and ^{13}C NMR resonances to the respective atoms in the molecules. Mass spectra are EI-MS [27].

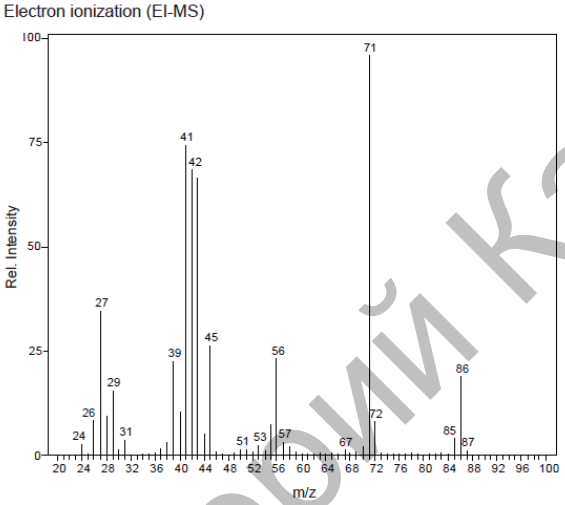


Figure 83. The EI mass spectrum of unknown

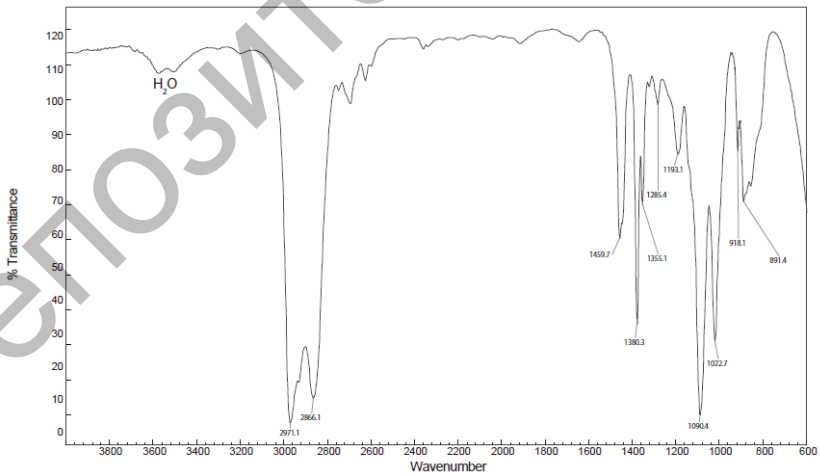


Figure 84. IR spectrum of unknown

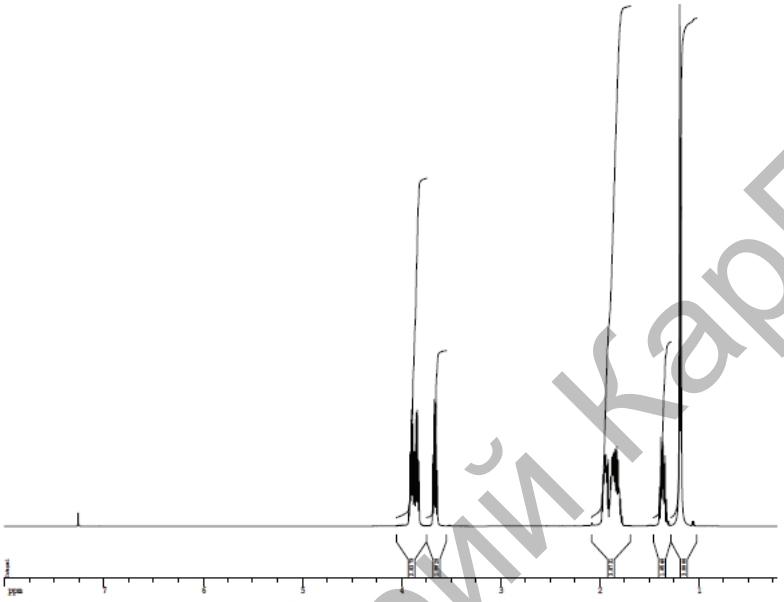


Figure 85. ^{1}H NMR spectrum of unknown

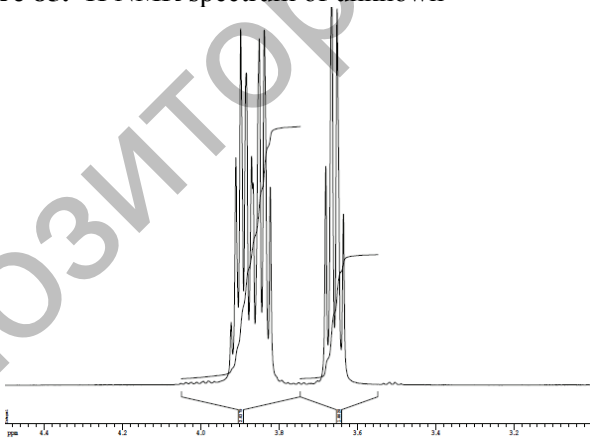


Figure 86. ^{1}H NMR spectrum of unknown

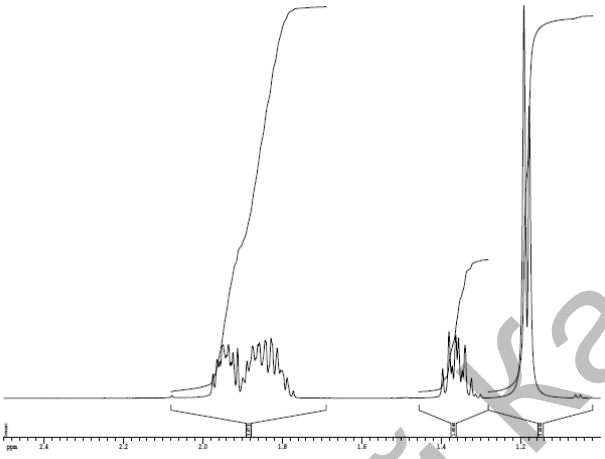


Figure 87. ^{1}H NMR spectrum of unknown

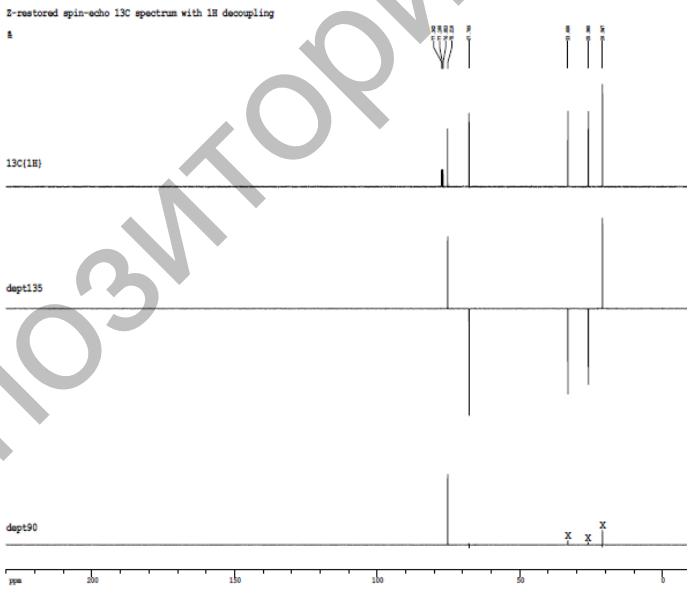


Figure 88. Z-restored spin-echo ^{13}C spectrum with ^{1}H decoupling

2. Analyze the spectra and solve the structure of the molecule for which data are provided. The following data are provided: EI-MS; IR (thin film on NaCl plates); 500 MHz ^{1}H NMR in $CDCl_3$; 125.8 MHz ^{13}C NMR, DEPT 90, and DEPT 135 in $CDCl_3$ (Figures 89-94, respectively).

Identify any noteworthy heteroatoms present. Determine the molecular formula and unsaturation number. Identify functional groups that are present from the IR and other spectra. Identify key fragments from NMR. Assign the ^{1}H NMR and ^{13}C NMR resonances to the respective atoms in the molecules. Mass spectra are EI-MS.

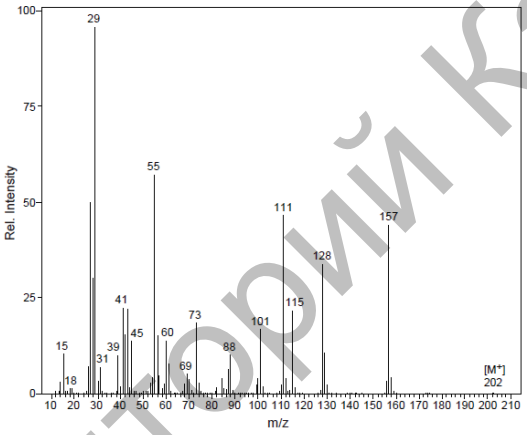


Figure 89. The EI mass spectrum of unknown

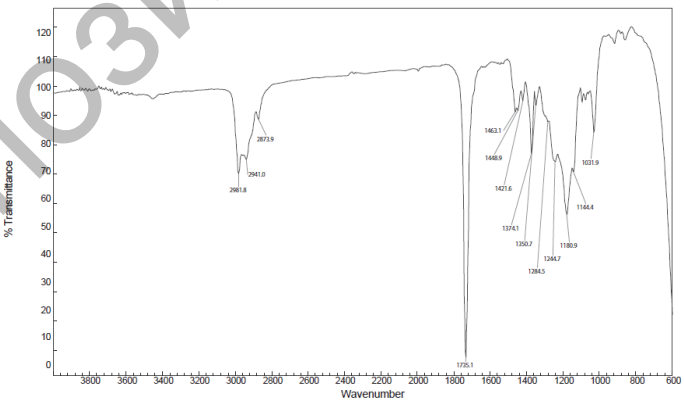


Figure 90. IR spectrum of unknown

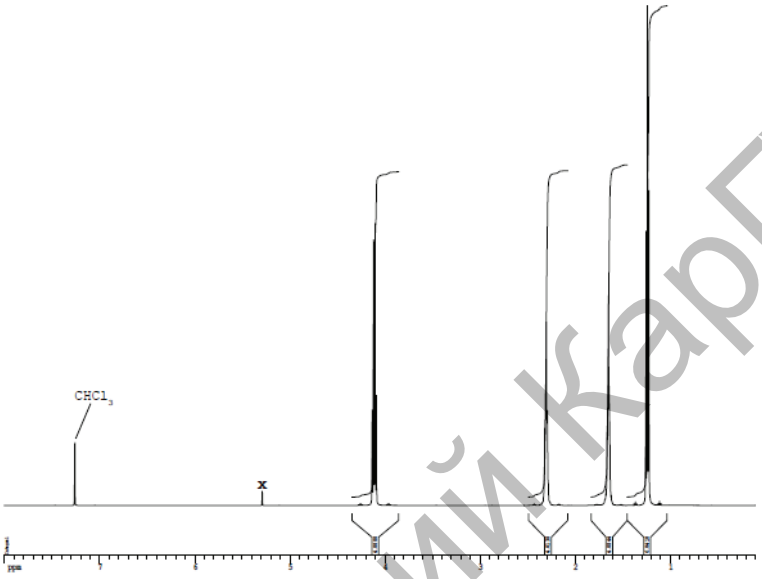


Figure 91. ^{1}H NMR spectrum of unknown

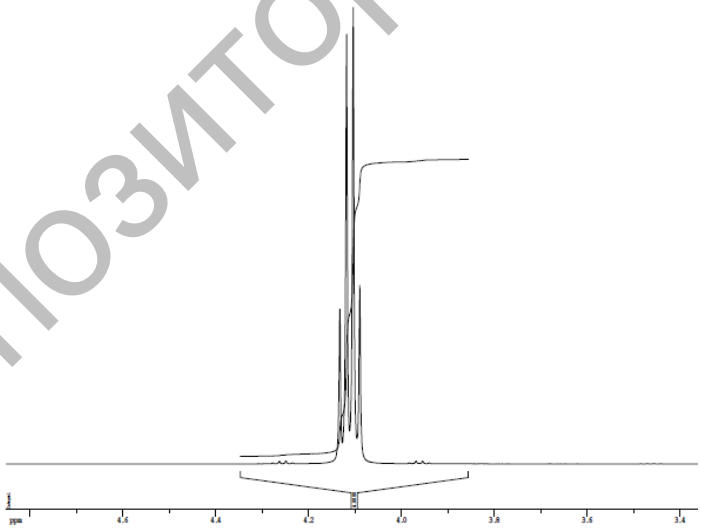


Figure 92. ^{1}H NMR spectrum of unknown

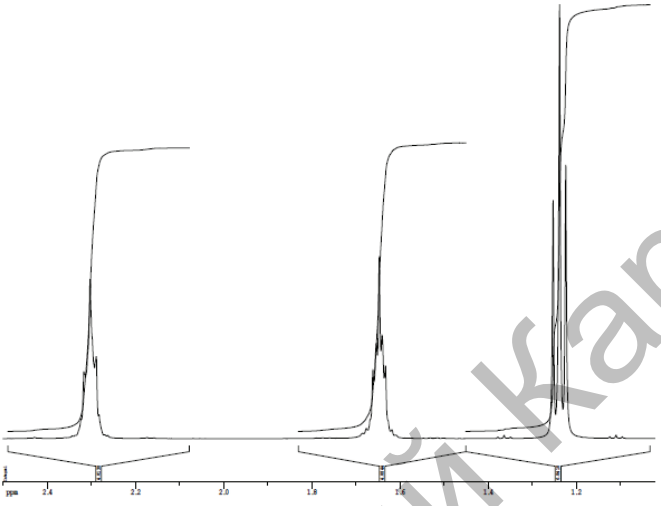


Figure 93. ^{1}H NMR spectrum of unknown

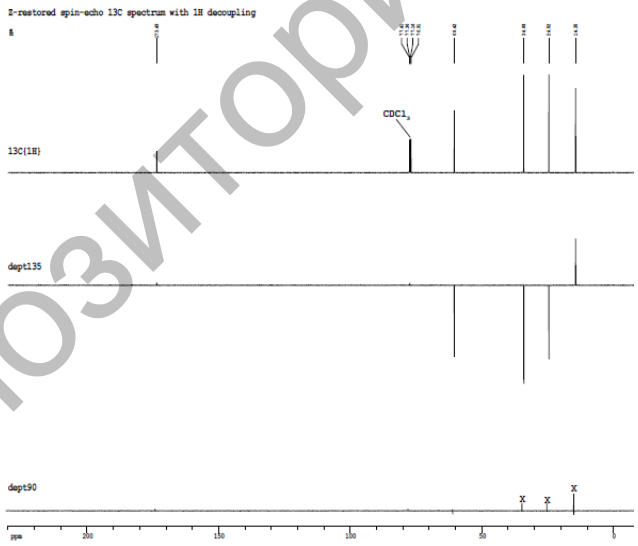
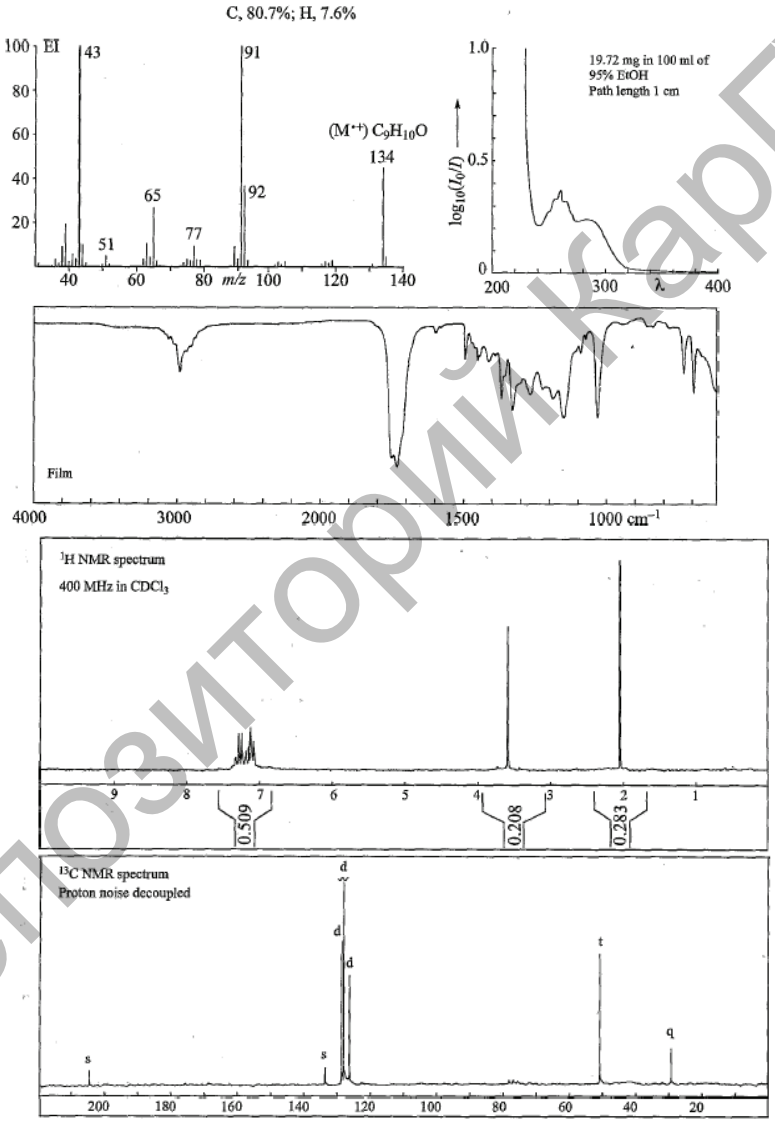
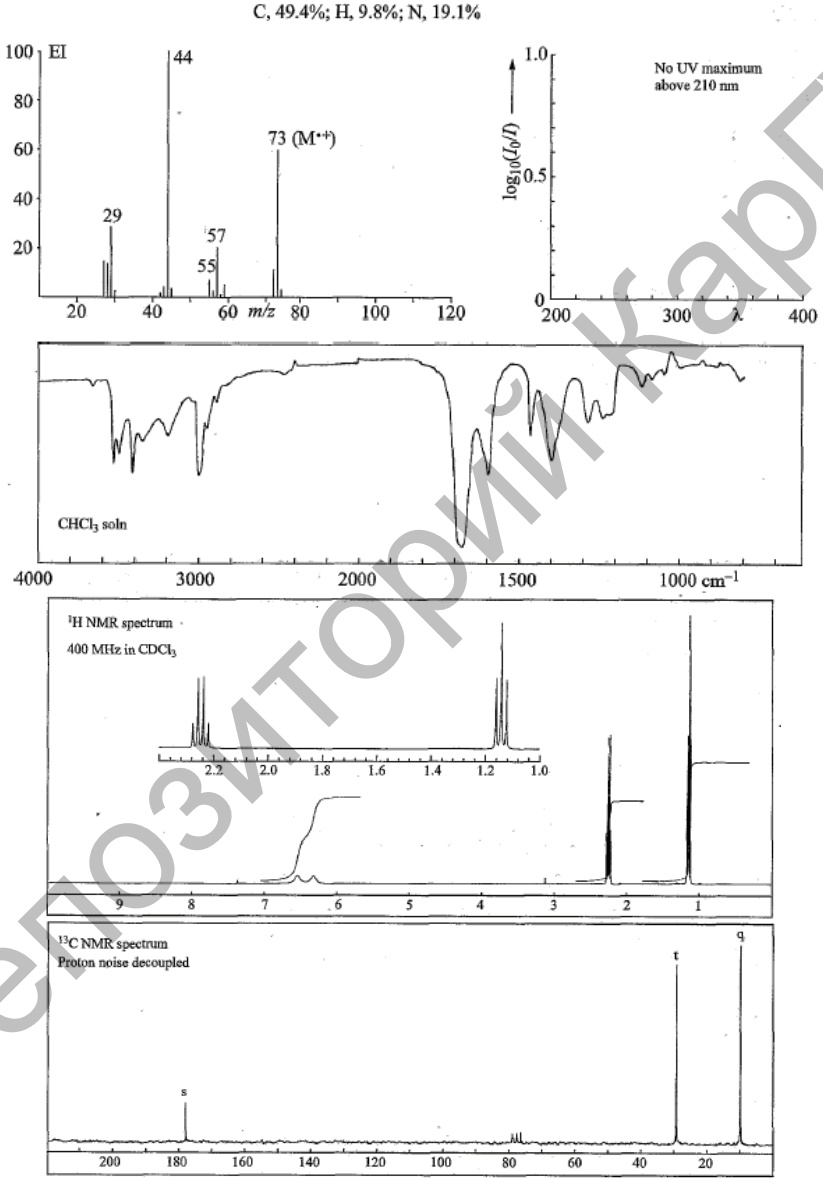


Figure 94. Z-restored spin-echo ^{13}C spectrum with ^{1}H decoupling

3. Analyze the spectra and solve the structure of the molecule for which data are provided below.

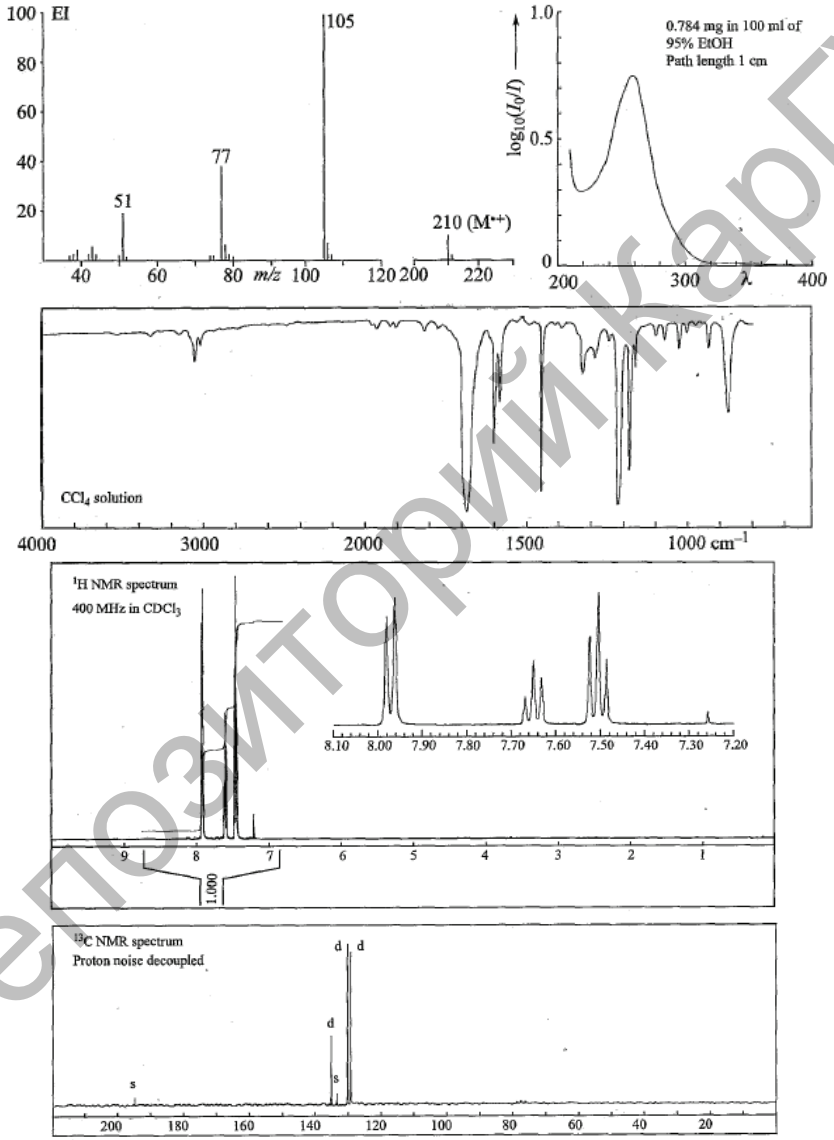


4. Analyze the spectra and solve the structure of the molecule for which data are provided below.

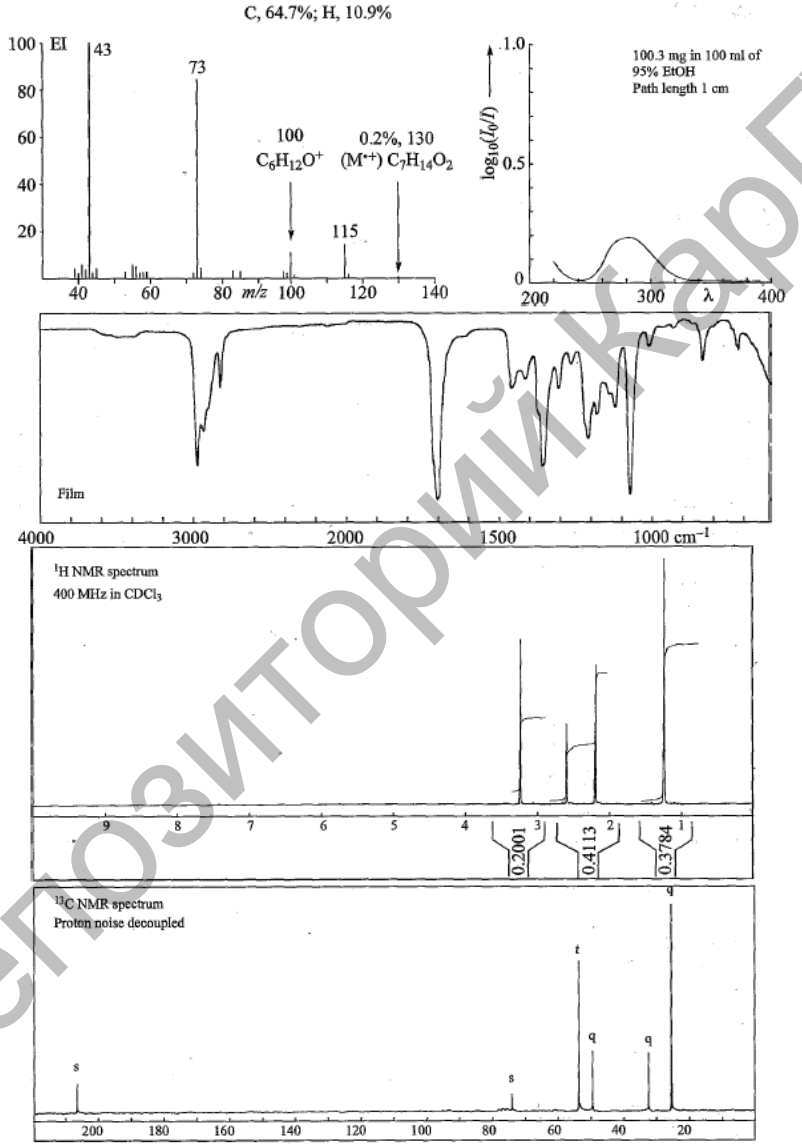


5.Analyze the spectra and solve the structure of the molecule for which data are provided below.

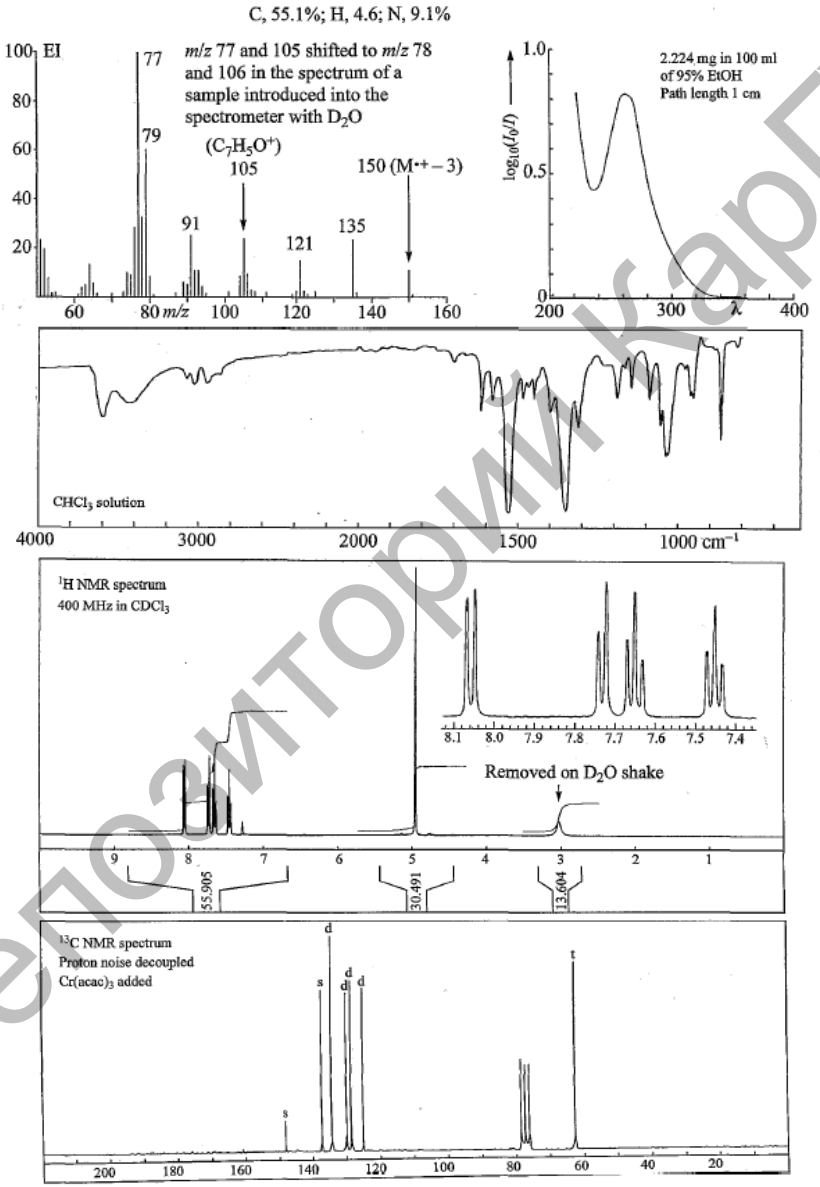
C, 80.0%; H, 4.8%



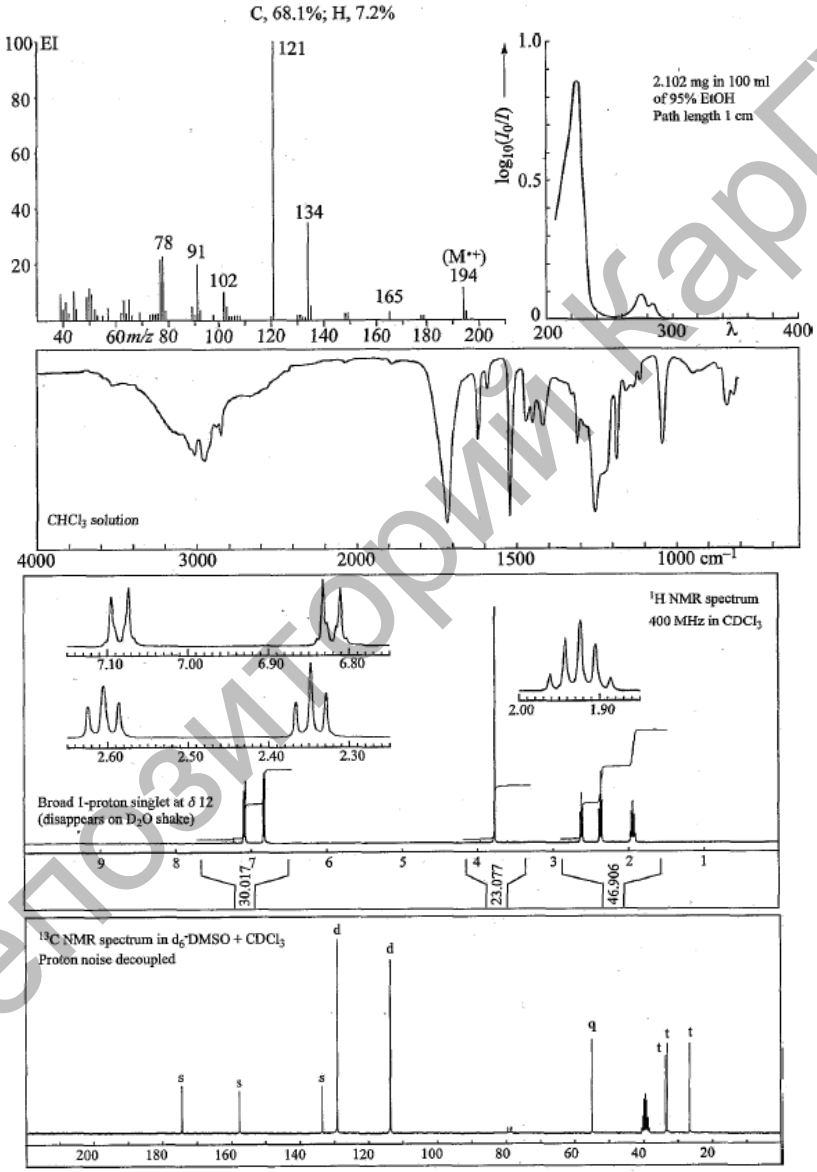
6. Analyze the spectra and solve the structure of the molecule for which data are provided below.

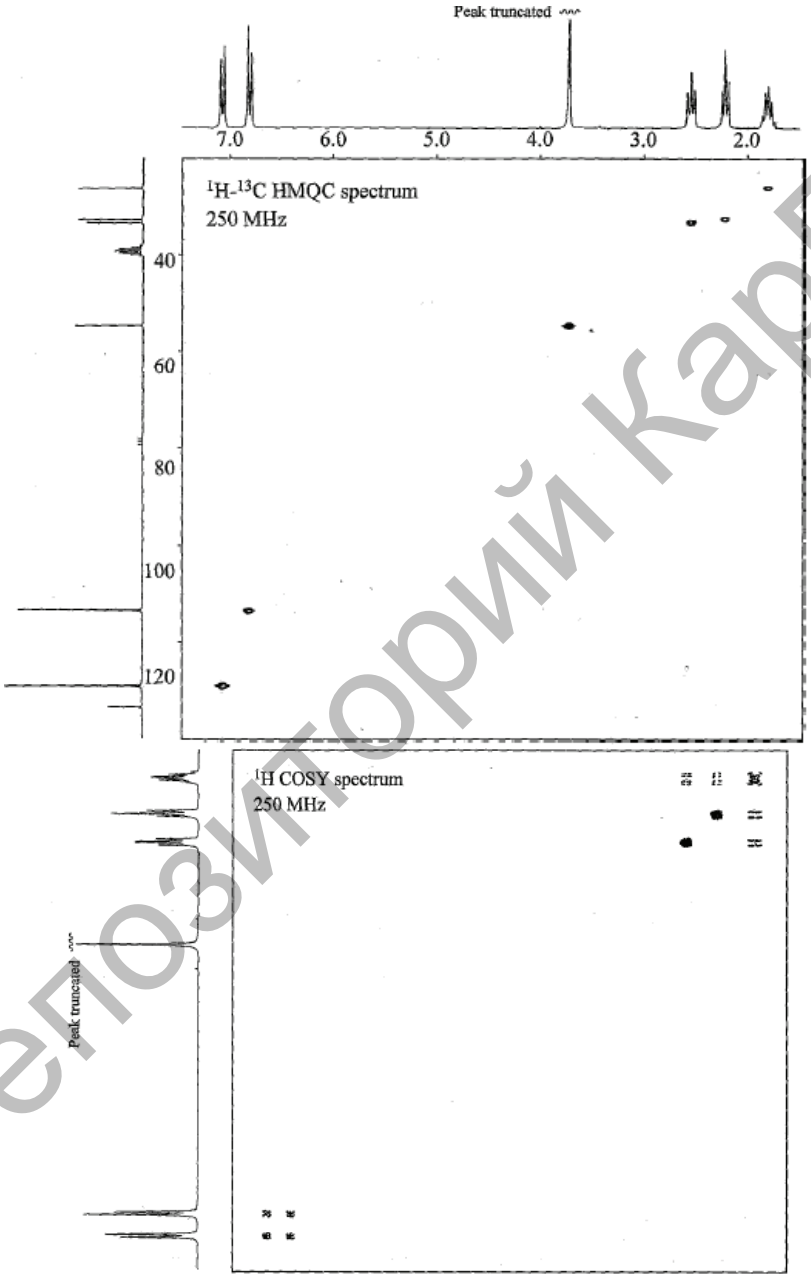


7. Analyze the spectra and solve the structure of the molecule for which data are provided.

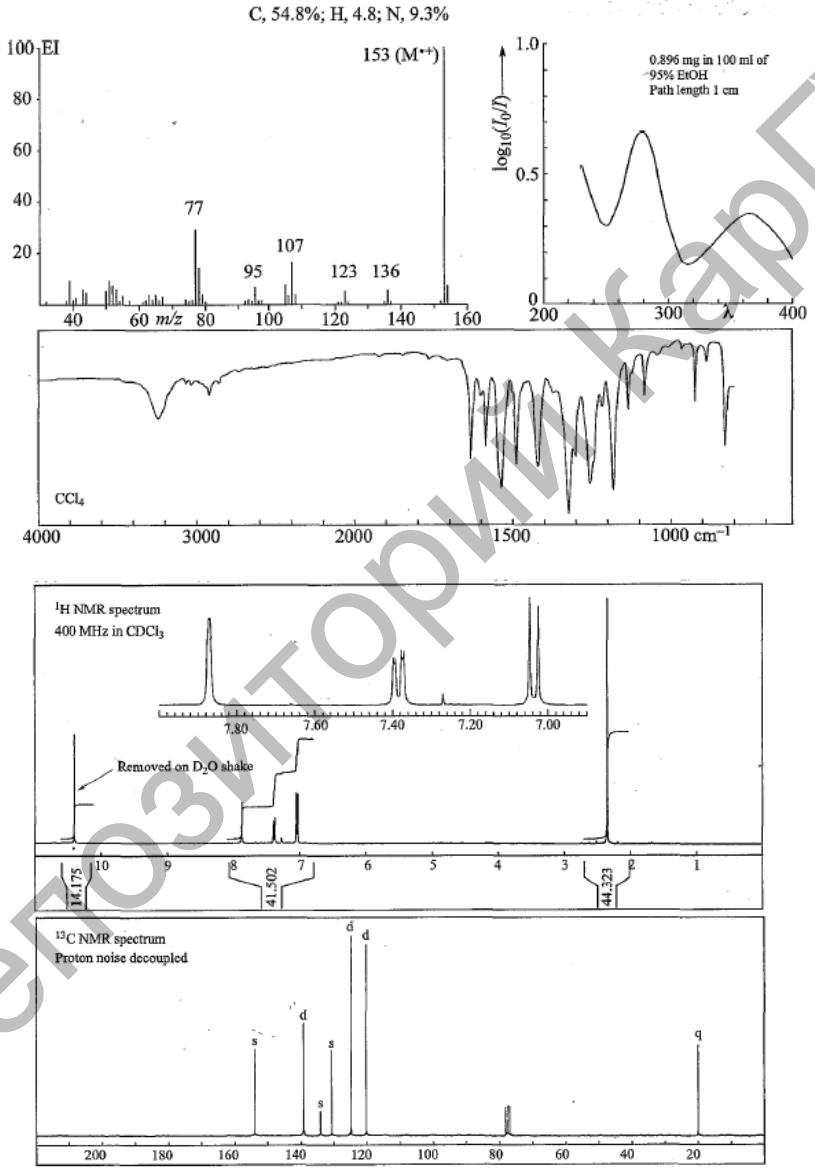


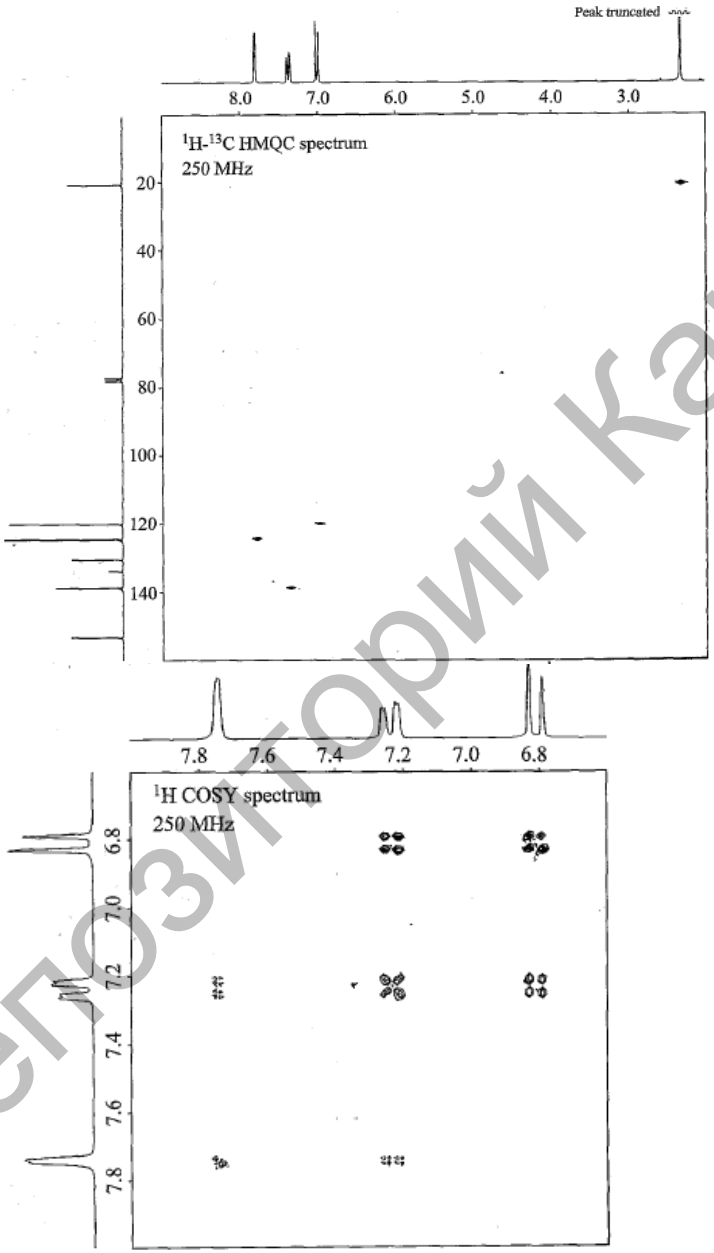
8. Analyze the spectra and solve the structure of the molecule for which data are provided below.



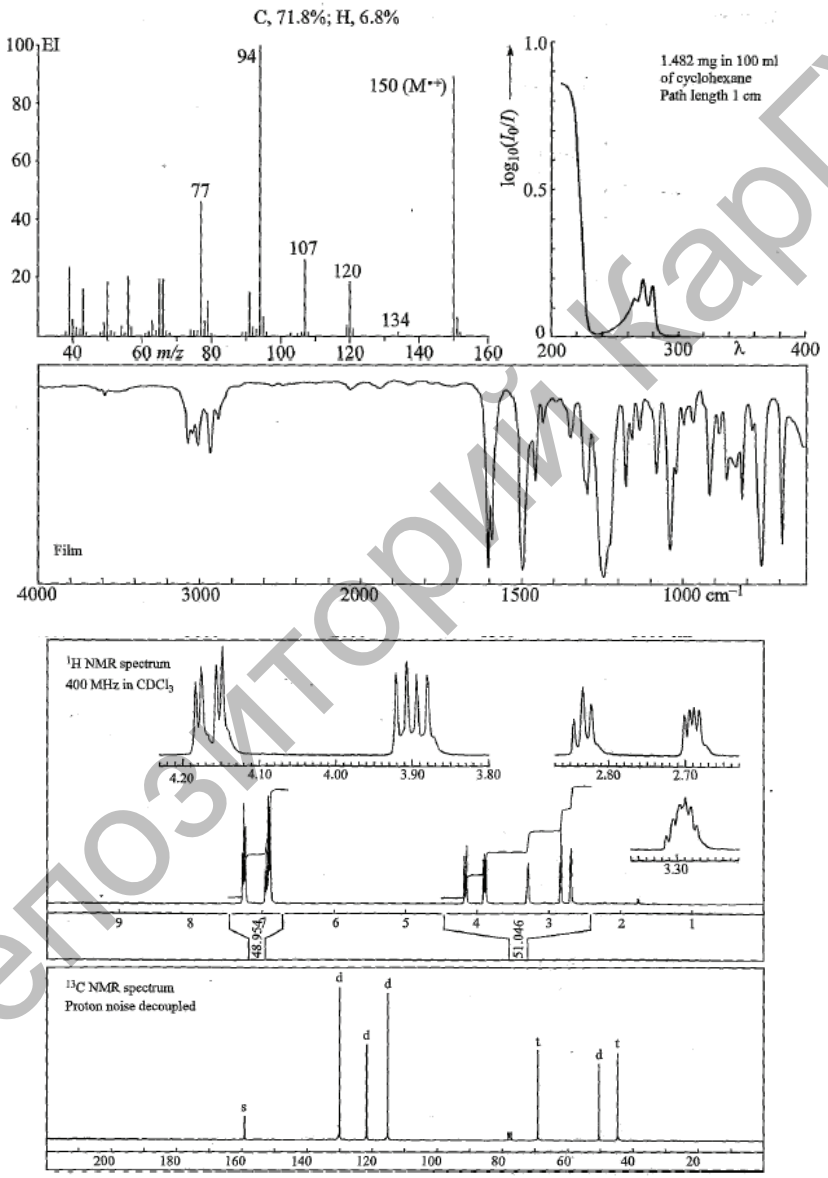


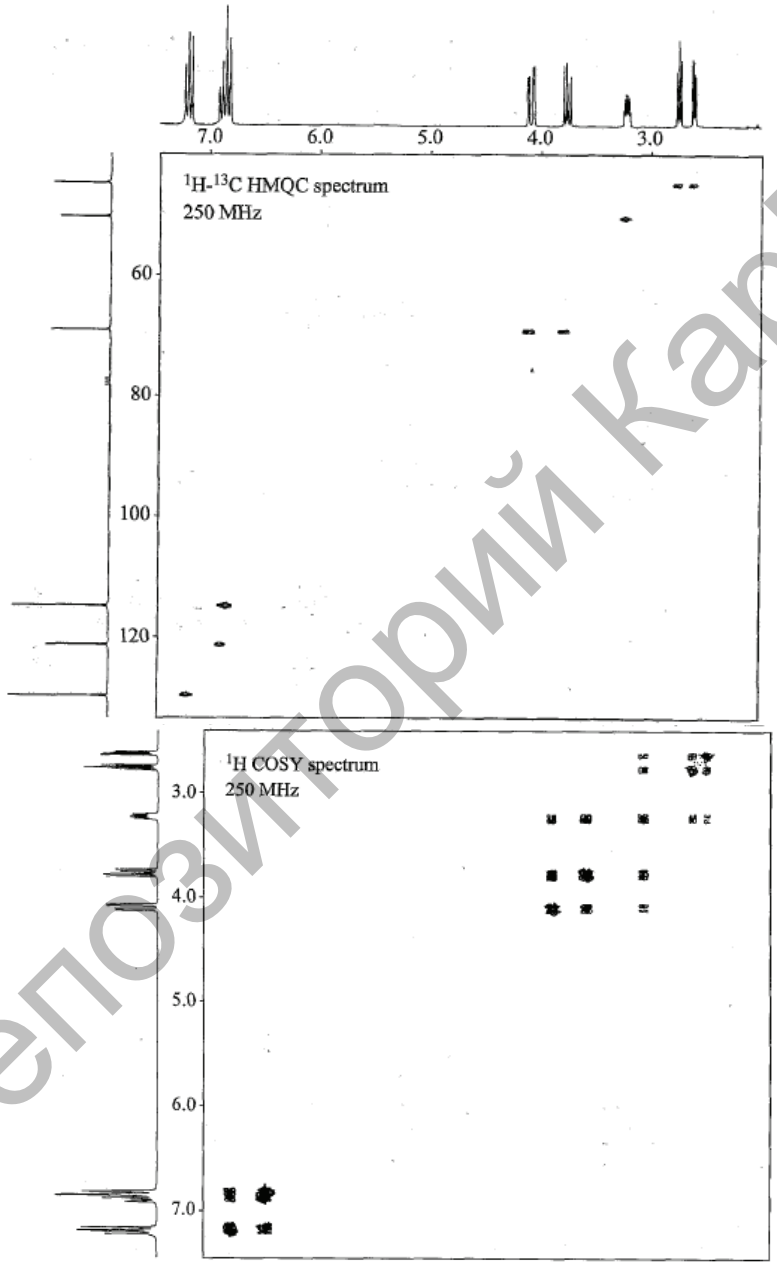
9. Analyze the spectra and solve the structure of the molecule for which data are provided.





10. Analyze the spectra and solve the structure of the molecule for which data are provided below.





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