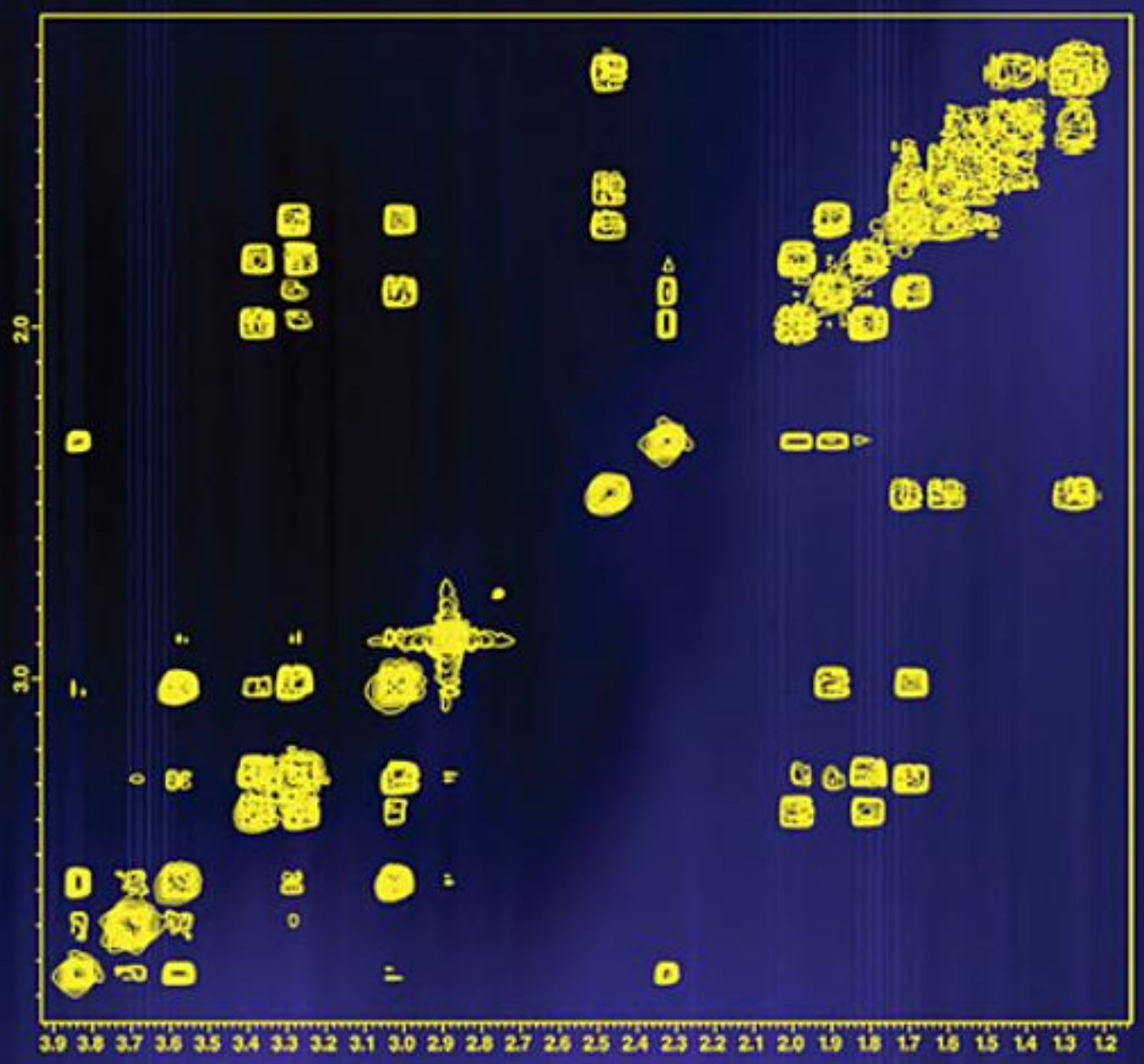

Interpretation of ORGANIC SPECTRA

Yong-Cheng Ning

With a Foreword by Nobel Prize Winner Richard R. Ernst

WILEY

Interpretation of Organic Spectra

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Interpretation of Organic Spectra

By

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Foreword

Professor Yong-cheng Ning, the well-known author of the two textbooks *Structural Identification of Organic Compounds and Organic Spectroscopy* (in Chinese), published in 2000, and *Structural Identification of Organic Compounds with Spectroscopic Techniques*, published in 2005, has written another remarkable volume *Interpretation of Organic Spectra*. The book is outstanding in its approach which follows what one might call the "Ning gold standard" in the spectroscopic textbook literature: The masterful combination of NMR, mass spectrometry, and infrared spectroscopy that has already been implemented in his first two treatises.

Indeed these three techniques are the most important "weapons" in the spectroscopic arsenal of the organic chemist. With these techniques, virtually any structural analysis problem in organic chemistry can be solved successfully. The author provides a careful exposition of each of the three tools, starting with an in-depth description of the practical aspects of NMR spectroscopy. He concentrates on those aspects that are indispensable for any organic chemist who intends to apply NMR fruitfully. He focuses the description on proton and carbon-13 NMR in their one- and two-dimensional implementations. COSY, NOESY, ROESY, and TOCSY are the well-known acronyms of the most important two-dimensional NMR techniques. Without going into all the theoretical sophistication, he is capable of providing a working knowledge for the practical organic chemist.

The second technique is mass spectroscopy that is also of great value in any structural analysis. Based on the analysis of the molecular and fragment ions, the primary structure of an organic molecule can elegantly be determined. Often, such an analysis precedes the interpretation of NMR spectra that allow one to elucidate also the secondary and tertiary structure of the molecule under consideration. The various methods of generating ions of larger molecules are discussed thoroughly, including the techniques of soft ionization, ESI, CI, FAB, MALDI, and APCI. These abbreviations illustrate the great wealth of available tools in advanced mass spectrometry.

The third tool to be discussed is infrared spectroscopy. It is complementary to the two other techniques. Its main feature is direct access to functional group identification. While NMR focuses on the individual atoms and their nuclei, infrared spectroscopy provides information on entire functional groups. However, what is missing in infrared spectroscopy is the connectivity information of the various functional groups. The connectivity can be deduced from a careful analysis of the NMR spin-coupling pattern and from the larger molecular fragments observed in mass spectrometry.

At the end of this useful book, the three techniques are applied to selected examples for demonstrating an integrated approach of analysis. This is a book that belongs in the hands of

any organic chemist who wants to determine the structure of his molecules and intermediates under actual study. I am convinced that the volume will receive a very positive reception from chemists dealing with the syntheses of molecules or with the study of natural products. It provides the information and the recipes for the successful usage of the indispensable and marvelous tools of spectroscopy.

Zürich, 8 March 2010
Richard R. Ernst
Nobel Laureate Chemistry 1991

Preface

The structural identification of organic compounds, including the confirmation of anticipated structures, is of great significance either for related disciplines or for their applications. This book deals with the structural identification with spectroscopic methods.

Another book by the same author, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, was published by Wiley-VCH in 2005. The principles of NMR, MS, IR, and Raman spectroscopy were discussed in depth. However, the book did not present enough examples about the interpretation because of the limited space.

This book contains six chapters. Chapters 1–5 present the ^{1}H spectrum, the ^{13}C spectrum, the two-dimensional NMR spectrum, the mass spectrum, and the infrared spectrum, respectively. Chapter 6, which occupies about two thirds of this book, discusses 20 examples connected with comprehensive interpretation.

This book discusses the structures of a wide range of organic compounds, containing several carbon atoms to 47 carbon atoms. Therefore, this book can be used for both beginners and researchers.

Although the interpretation for NMR spectra has become a perfect method, this book illustrates some important rules, for example, the symmetrical plane rule, which determines the complexity of the ^{1}H spectrum and the ^{13}C spectrum, by use of examples. The author has also emphasized good coordination while using the different kinds of NMR spectra for the comprehensive interpretation of several kinds of NMR spectra.

The interpretation of MS and IR spectra seems to be neglected in some existing books dealing with the structures of organic compounds. However, the application of MS and IR spectra (especially the former) can solve some structural problems, which are difficult to solve by the use of NMR spectra. Some interesting examples, which have been accumulated by the author himself in practice, show their important applications. The interpretation of the mass spectrum includes that of the mass spectra produced by the soft ionization and by the tandem mass spectrometry.

The author wishes to express his deepest gratitude to Prof. Dr. Richard R. Ernst, the single Nobel Prize winner in chemistry in 1991, who wrote the foreword to this book. The success of the Chinese version of my former book, of which more than 25 000 copies have been sold so far in China, is greatly credited to his foreword. It is certain that his current foreword to this book will continue to play an important role.

The author likes to record his appreciation to Prof. Di-hui Qin of the Department of Foreign Languages, Xidian University. He has proofread and refined the manuscript. This is the second cooperation with him, which is effective and pleasant.

Gratitude is also extended to the following professors and experts, who provide spectra for this book so that the book can cover a wide range of applications. They are Hai-jun Yang

of Tsinghua University, Wen-yi He of the Beijing Pharmaceutical Institute, Ya-fei Zhu of Zhongshan University, Hao Gao of Jinan University, Xiu-yan Sun of Yantai University, Xuan Tian of Lanzhou University, and Xiu-qing Song of the Beijing Chemical Engineering University.

Finally, the author expresses his thanks to his wife, Mrs. Chong-wei Liu, for her understanding and unswerving support in his writing books for years.

1

Interpretation of ^{1}H NMR Spectra

As described in the preface to this book, the NMR is the most important method to identify the structure of an unknown organic compound, because the information obtained from (one dimensional and two dimensional) NMR spectra is more abundant and interpretable than that obtained by other spectroscopic methods. Since ^{1}H NMR spectra have higher sensibility than other NMR spectra, ^{1}H NMR spectra can be acquired more easily in some ways, and we present ^{1}H NMR spectra in the first chapter of this book.

Because the ^{1}H NMR spectrum can be interpreted in detail, it is possible to deduce the structure of an unknown compound, whose structure is not complex, only by using its ^{1}H NMR spectrum, ^{13}C NMR spectrum and the information about its molecular weight (without two dimensional NMR spectra). When we need to select the most reasonable structure from several possible structures, the ^{1}H NMR spectrum of that compound can play a very important role.

Even when two dimensional NMR spectra were applied, the information, especially that from the analysis of coupled splittings in the ^{1}H NMR spectrum, would still be useful to deduce an unknown structure.

The main parameters of ^{1}H NMR spectra are chemical shifts, coupled constants (and splitting patterns) and peak areas. If we consider a ^{1}H NMR spectrum from the viewpoint of physics, there is a fourth parameter, that is, relaxation times. However, relaxation times are short for ^{1}H NMR spectroscopy. Therefore, the variation of relaxation times does not produce variations of peak areas of ^{1}H NMR spectra. And relaxation times do not affect the interpretation of ^{1}H NMR spectra.

The abscissa of the ^{1}H NMR spectrum is the chemical shift δ, which characterizes the position in a ^{1}H NMR spectrum of the peak of a functional group.

Because of coupling interactions between magnetic nuclei, peaks in the ^{1}H NMR spectrum will show splittings. The splitting distance between two related split peaks is characterized by the coupling constant, measured in hertz. The magnitude of coupling constants reflects the strength of coupling interaction.

The related knowledge about the chemical shift and the coupling constant will be presented later.

The ordinate of the ^{1}H NMR spectrum is the intensity of peaks. Because peaks in the ^{1}H NMR spectrum have some widths, integral values of peak areas are applied as the

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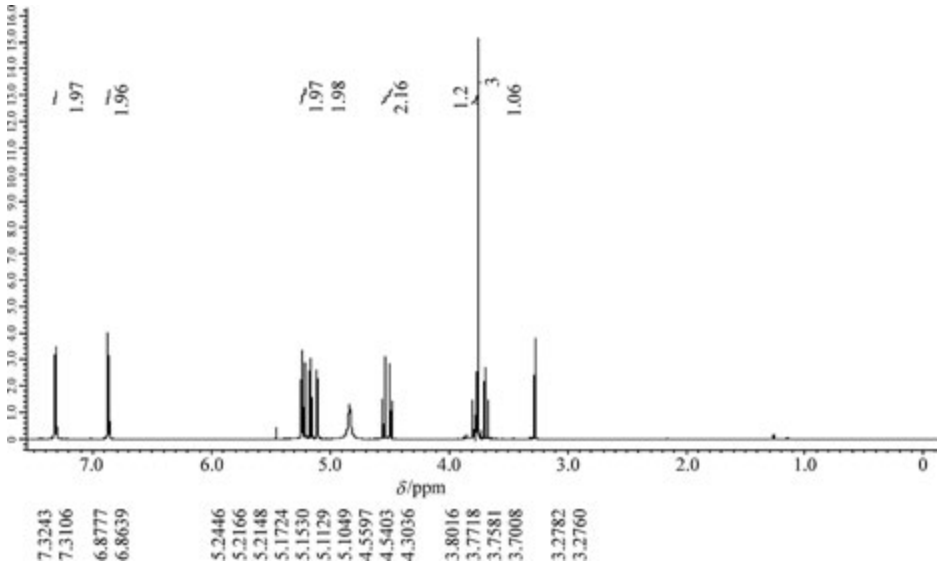


Figure 1.1 The ^{1}H spectrum of compound **C1-1**

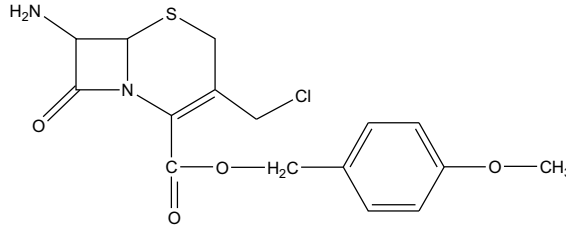
measurements of intensities of peaks. Integral values, denoted under or beside the corresponding peaks, are proportional to the numbers of hydrogen atoms of related functional groups. The quantitative relationship of the ^{1}H NMR spectrum is good with errors less than 5%.

The quantitative relationship between the integral values of peak areas and the numbers of hydrogen atoms of corresponding functional groups is important for deducing an unknown structure.

If a measured sample is a mixture, the quantitative ratio of components can be obtained from the quantitative relationship.

By using the quantitative relationship in ^{1}H NMR spectroscopy, some important results can be obtained. For example, the averaged additional number of ethylene oxides, n, in a non-ionic surfactant, $RO(CH_2)_nH$, can be measured easily by using ^{1}H NMR spectroscopy when we analyze this kind of surfactant. And the averaged value of n is more important than individual numbers which participate in average calculation for evaluating the character of this kind of surfactant. Otherwise, if we apply thin layer chromatography to analyze the surfactant, after its development by thin layer chromatography, we will get a series of spots on the thin plate. Each spot corresponds to a particular additional number and all numbers form a normal distribution shape. In this case, an average number is more important than these individual numbers which participate in average calculation.

The ^{1}H spectrum of Compound **C1-1** is shown in Figure 1.1.



C1-1

From Figure 1.1 we know that the abscissa of the ^1H spectrum is the chemical shift whose accurate values are denoted under (or above) corresponding peak sets. The ordinate of the ^1H spectrum is the peak intensity. The integral values which show the areas of peak sets are denoted above (or under) corresponding peak sets.

There are split peak sets in the ^1H spectrum. Because split distances are measured in Hz, the higher the frequency of the NMR spectrometer, the shorter the split distance in peak sets. Since Figure 1.1 was obtained through measurement by an NMR spectrometer with 600 MHz, the split distances are very short.

1.1 Chemical Shift

1.1.1 Conception of Chemical Shift

From the name of "chemical shift" one can know that in a ^1H spectrum peak positions of functional groups will be shifted compared with the peak position of a reference according to the chemical characters of different functional groups, that is, chemical shifts (values) characterize peak positions of functional groups in a ^1H spectrum. The symbol of the chemical shift is δ.

The reference of the chemical shift, which is applied most frequently, is TMS (tetramethylsilane). The position of its peak, which is a singlet, is set as the origin of the abscissa of the ^1H spectrum. Its sign is negative when a signal is positioned on the right side of the standard and positive when on the left side. Common functional groups have positive chemical shift values, that is, their peaks are situated on the left side of the signal of TMS.

The unit of chemical shift (value) is ppm (parts per million), which is dimensionless.

From the physical consideration, the chemical shift value reflects the magnitude of the extranuclear electron density of hydrogen atoms which is measured by ^1H NMR spectroscopy. Because the hydrogen atom has only s electron, the electron density is the s electron density. The greater the density of s electron around the nucleus, the smaller the chemical shift value and vice versa. If any factor makes the peak move towards the right (to decrease its chemical shift value), the function of the factors is called the shielding effect. Conversely, if any factor makes the peak move towards the left (to increase its chemical shift value), the function of the factor is called the deshielding effect.

The chemical shift values (varying ranges) of common functional groups containing hydrogen atoms are shown in Table 1.1.

From Table 1.1 we can know that δ values of functional groups are quite different. And the chemical shift value of a functional group can vary within a range.

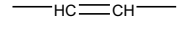
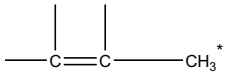
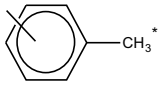
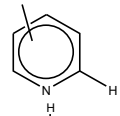
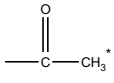
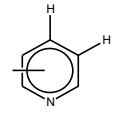
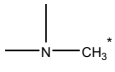
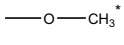
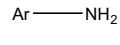
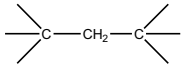
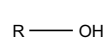
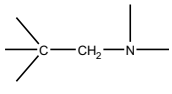
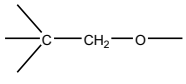
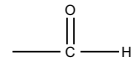
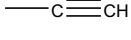
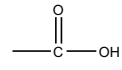
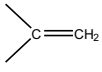
1.1.2 Factors Affecting Chemical Shifts

Factors affecting the chemical shift can be discussed from the following aspects: kind of functional groups, effects of substituents, effects of the medium, and so on.

1.1.2.1 Chemical Shift Values are Determined Mainly by the Kind of Functional Group

Functional groups have obvious differences in chemical shift value. Generally speaking, saturated groups have smaller chemical shift values than unsaturated groups.

Table 1.1 Chemical shift values (varying ranges) of common functional groups containing hydrogen atoms

Functional group	δ_H (ppm)	Functional group	δ_H (ppm)
	0.87		4.5–8.0
	1.7–2.0		6.5–8.0
	2.1–2.4		8.0–8.8
	2.1–2.6		6.5–7.3
	2.2–3.1		0.5–3.0
	0.5–3.0		
	3.5–4.0		3.0–4.8
	3.0–4.8		
	1.2–1.4		0.5–5.0
	2.3–3.5		4.0–10.0
	3.5–4.5		9.5–10.0
	2.2–3.0		9.0–12.0
	4.5–6.0		

And unsaturated groups have smaller chemical shift values than aromatic groups. The above-mentioned phenomenon can be explained by the following influence factors.

s-p Hybridization of the Connected Carbon Atom The δ value of hydrogen atoms connected with an unsaturated carbon atom is greater than that of hydrogen atoms connected with a saturated carbon atom, which can be explained by the percentages of s electrons in the

carbon atom. The increment of the percentage from 25 to 33% leads to the bond electron approaching the carbon atom, which produces a deshielding effect on the hydrogen atoms connected with the unsaturated carbon atom.

The chemical shift value of alkyne hydrogen atoms, which corresponds to sp hybridization, is between that of saturated hydrogen atoms and that of alkene hydrogen atoms, which will be otherwise explained later.

The Ring Current Effect of the Cyclic Conjugation System We discuss this effect with a benzene ring as an example. Under the effect of an applied magnet field $\boldsymbol{B_0}$, a ring current produced from the delocalized electrons of the benzene is induced. It produces an induced magnetic field, which opposes $\boldsymbol{B_0}$ in the middle of the molecule but reinforces $\boldsymbol{B_0}$ at the periphery. Although the benzene molecule tumbles in its solution, its NMR signal has the value averaged from all its directions, so the hydrogen atoms of a benzene molecule still have a larger δ value than alkene hydrogen atoms.

Anisotropic Shielding Effects of Chemical Bonds All single bonds, double bonds and triple bonds show anisotropic shielding effects, which means they have different shielding or deshielding effects in different directions.

If a six-membered ring can not reverse rapidly, two geminal hydrogen atoms (an axial hydrogen atom and an equatorial hydrogen atom) connected with the same carbon atom are not exchangeable. Their chemical shift values are different. The chemical shift value of the axial hydrogen atom is about 0.5 ppm less than that of the equatorial one, which results from the anisotropic shielding effect.

Because the π electrons of a carbon-carbon triple bond rotate around the bond axis, there is a strong shielding effect along the axial direction where the alkyne hydrogen atom lies. That is why alkyne hydrogen atoms have a smaller chemical shift value than alkene hydrogen atoms.

Stereo Effect If a hydrogen atom is close to another atom with a distance shorter than the sum of the Van der Waals radii of the two atoms, the extranuclear electron of the hydrogen atom is repelled so that the electron density will be decreased and the chemical shift value of the hydrogen atom will be increased.

1.1.2.2 Effects of Substituents

Because of effects of substituents, the chemical shift value of a functional group can be changed within a certain range. It should be noticed that a substituent can show different effects for different functional groups.

Effects of Substituents for Aliphatic Hydrogen Atoms The substitution of an electronegative functional group will increase the chemical shift value of the hydrogen atoms connected to the substituted carbon atom, that is, the δ value of the α-hydrogen atoms will be increased. The value of the β- hydrogen atoms will be increased also but by a smaller quantity. This phenomenon can be understood easily from the induction effect. The electronegative substituent attracts electrons from the substituted functional group, with the electron density of the latter to be decreased so that its δ value will be increased.

Effects of Substituents for Aromatic Hydrogen Atoms The effects of substituents for aromatic hydrogen atoms are different from those for aliphatic hydrogen atoms. In this case, the induction effect and the conjugation effect have to be considered together.

We divide substituents into three types [1].

The first type of substituents includes alkyl groups and halogen atoms. They are saturated groups and they are not, or not strongly, electronegative. Therefore, these substituents do not change obviously the electron density of the substituted benzene ring.

Groups of $-CH_3$, $-CH_2-$, –CH<, –CH=CHR, $-C\equiv CR$, –Cl, –Br, and so on, belong to this type.

The second type of substituents is the functional groups that contain saturated heteroatoms. Because of the p-π conjugation between the non-bonding electrons of the heteroatom and the delocalized electrons of the substituted phenyl ring, the electron density of the substituted phenyl ring is increased, especially at the ortho- and para- positions. From the point of view of NMR, the ortho- and para-hydrogen atoms have an upfield shift after the substitution. The ortho-hydrogen atoms have an upfield larger shift than the para-hydrogen atoms. The meta-hydrogen atoms also have an upfield shift but the shifted magnitude is less than that of the ortho- and para-hydrogen atoms.

Groups of –OH, –OR, $-NH_2$, –NHR, $-NR'R'$, and so on, belong to this type.

The third type of substituents is the groups which contain unsaturated heteroatoms. Because of the electronegativity of the heteroatom, the electron density of the substituted phenyl ring is decreased, especially at the ortho-position. From the point of view of NMR, all the remaining hydrogen atoms in the substituted phenyl ring, especially the two ortho-hydrogen atoms, have a downfield shift after the substation.

Groups of –CHO, –COR, –COOR, –COOH, –CONHR, $-NO_2$, –N=NR, and so on, belong to this type.

1.1.2.3 Effects of the Medium and Hydrogen Bond

The effects of the medium are that of the solvent which is applied in the NMR experiment.

Because the same sample molecules experience different magnetic field strengths in different solvents and because different functional groups in the same molecule are affected with different strengths by solvent molecules, NMR spectra (including the ^1H spectrum), measured in different solvents can be changed. The ^1H spectrum of a sample can change obviously with different solvents. The change relates to chemical shift values and peak shapes. Therefore, the solvent applied in the measurement of ^1H spectra should be identical in order to make a comparison between the ^1H spectrum of a sample and that of a standard substance.

Both intermolecular and intramolecular hydrogen bonds can affect chemical shift values of functional groups. The carboxyl group is an outstanding example of the effect of hydrogen bonds, whose chemical shift value can exceed 10 ppm. The chemical shift value of enol is the largest one, which can reach 16 ppm because of the effect of hydrogen bonds.

Because the chemical shift values of a functional group are related to the functional groups and with its substituents, it is possible to deduce the probable functional group and its substituent from its chemical shift value.

It is sufficient just to known factors affecting chemical shift values without related calculation equations and parameters, because chemical shift values can be estimated by the software ChemDraw (refer to section 1.5.8).

Since the range of chemical shift values of common function groups is less than 10 ppm, peak sets of a sample can overlap or partially overlap in a ^{1}H spectrum. In this case, heteronuclear shift correlation spectra, for example, the HMQC spectrum, are very important for analyzing the overlapped peak sets. We will deal with it in Section 3.2.

1.2 Coupling Constant *J*

Although the title of this section is the coupling constant *J*, our discussion in this section includes peak splittings because the coupling phenomenon and peak splittings are connected together.

1.2.1 Coupling Effect and Coupling Constant *J*

First of all, we should know which kind of nuclei has coupling effects? Simply speaking, coupling effects exist between magnetic nuclei whose magnetic quantum numbers are not zero. Non-magnetic nuclei have no coupling effect and they can not be measured by NMR.

Magnetic nuclei, which produce coupling effects, include hydrogen nuclei and other magnetic nuclei, such as ^{19}F, ^{31}P, and so on. Within some numbers of chemical bonds, hydrogen nuclei couple each other. Since 99% of carbon atoms are ^{12}C, which are non-magnetic nuclei, there are no coupling splittings between hydrogen and carbon atoms except so-called "satellite-peaks" which are situated besides the two sides of strong peaks in a ^{1}H NMR spectrum. They are produced by ^{13}C which possesses only 1% of the carbon atoms.

If the studied compound contains other magnetic nuclei, such as ^{19}F and ^{31}P, hydrogen atoms will be coupling-split by these nuclei. We will discuss it in Section 1.4.5. Because two isotopes of chlorine (^{35}Cl, ^{37}Cl) and two isotopes of bromine (^{79}Br, ^{81}Br) have a spin quantum number of 3/2, these nuclei will change their orientations rapidly, so that they have no coupling effects on hydrogen atoms. Therefore, they do not produce coupling splittings for peak sets of functional groups containing hydrogen atoms. We can interpret their ^{1}H NMR spectrum as they are non-magnetic nuclei.

Because magnetic nuclei have different orientations in an applied magnetic field, the peak set of the functional groups connected with the magnetic nuclei will be split. The peak set will be shown as multiplets.

By induction, a $2nI + 1$ rule can be introduced, where n is the number of magnetic nuclei that participate in coupling and *I* is the spin quantum number of the magnetic nuclei.

If $I = 1/2$, the $2nI + 1$ rule is simplified as the $n + 1$ rule.

In the interpretation of ^{1}H spectra, the $n + 1$ rule can be applied when the magnetic nuclei with which we deal have the spin quantum number of 1/2. This is the most frequent situation.

The $n + 1$ rule can be described as follows. The peak set of a functional group, which is connected with another functional group containing n hydrogen atoms, will be shown as a multiplet with the peak number of $n + 1$. It must be noted that n is the number of the hydrogen atoms which participate in coupling but is not the hydrogen atom number of the functional group studied.

It can be proved by the related theory or it can be known from the experience by the interpretation of ^{1}H spectra that if coupled functional groups have different chemical shift

values, their coupling splits are shown in their ^{1}H spectrum. Otherwise, if they have the same chemical shift value, their coupled splits can not be shown in their ^{1}H spectra, although their coupling effect still exists. The same chemical shift value mentioned above involves two cases. In the first case, the two functional groups have the same chemical shift value because they are symmetrical in a molecule. In the second case, the two functional groups incidentally have the same chemical shift value. The above-mentioned conclusion is very important for interpretation of ^{1}H spectra.

The magnitude of coupling effects is measured by coupling constants. The coupling effects transfer through chemical bonds. The smaller the number of the chemical bonds through which the coupling effects transfer is, the stronger the coupling effects. Therefore, an Arabic number, which means the number of the chemical bonds through which the related coupling effects transfer, is marked at the upper-left corner of the coupling constant J. For example, ^{3}J means the coupling constant across three chemical bonds.

We will discuss the coupling constant according to the number.

Coupling constants are algebra values. They have a positive or a negative sign. Since coupling constants are shown generally as an absolute value, we will not differentiate their signs in this book. Only in some special situations can the sign of a coupling constant change a ^{1}H spectrum. Readers who are interested in this topic can read the reference [1].

We use s, d, t, q and m to express the split patterns of singlet, doublet, triplet, quartet and multiplet, respectively, for the related simplification.

1.2.2 Discussion of Coupling Constants According to their Kinds

1.2.2.1 ^{1}J

^{1}J is the coupling constant across one chemical bond.

From what we have described above, we can know that $^{1}J_{C-H}$ is not shown in the ^{1}H spectrum in general. However, $^{1}J_{C-H}$ will be shown in ^{13}C NMR spectra if decoupling to hydrogen atoms is not applied.

The coupled splits, from magnetic nuclei with the chemical valence as 1, such as ^{19}F, are not shown in the ^{1}H spectrum, since its structural formula can not be continued.

The coupled splits from magnetic nuclei with multiple chemical valences, such as ^{31}P, will be shown in the ^{1}H spectrum. The ^{1}J value of ^{31}P is about 700 Hz.

1.2.2.2 ^{2}J

^{2}J is the coupling constant across two chemical bonds.

We mainly discuss ^{2}J between H–H, and the coupling is named geminal coupling.

It is important to differentiate two kinds of ^{2}J. The value of ^{2}J in a saturated structural unit is different greatly from that in an unsaturated structural unit.

The typical value of ^{2}J in a terminal alkene group is about 2.3 Hz.

The typical value of ^{2}J in a saturated chain is about 12 Hz, which is much larger than that of ^{3}J, which is encountered most frequently for interpreting the ^{1}H spectrum.

^{2}J in a saturated group is always shown in the following cases.

The two hydrogen atoms of a CH_2 group in a saturated ring have different chemical shift values because they experience different anisotropic shielding effects from their adjacent functional groups. Therefore, the coupling splits will be shown in their ^{1}H spectrum.

If the two hydrogen atoms of a CH_2 group in a saturated chain have different chemical shift values, their coupling splits will be shown in their ^1H spectrum. Since the value of ^2J in a saturated group is rather large, the coupled splits from it are prominent. We will discuss it in Section 1.4 in detail. The coupled splits by ^2J can be shown frequently for a compound whose structure is not simple.

The factors affecting the value of ^2J are as follows:

1. The absolute value of ^2J will decrease with the increase of the electronegativity of the substituent. For example,

Compounds	CH_4	CH_3Cl	CH_2Cl_2
^2J(Hz)	−12.4	−10.8	−7.5

   A vicinal π bond makes the saturated ^2J shift in the negative direction, which means the absolute value of ^2J will increase.
2. The value of ^2J is affected by the tension of a saturated ring, which is determined by the size of the ring. The special feature of the three-membered ring makes the absolute value of ^2J smaller than that of other sizes of saturated rings.

1.2.2.3 ^3J

We focus our attention on the ^3J coupling between two hydrogen atoms. Their coupling is named vicinal coupling.

Since the splits by ^2J are always absent due to two geminal hydrogen atoms frequently having approximately the same chemical shift value and the splits by long-range couplings being not obvious, the coupled splits from ^3J dominate the split shapes in general.

If a compound has several conformations, its ^3J value is the average value of those conformations.

The factors affecting the value of ^3J are as follows:

Dihedral Angle Φ Two vicinal hydrogen atoms and the two carbon atoms between these two hydrogen atoms form a dihedral angle Φ. The value of ^3J depends on the dihedral angle that is formed by the related H–C–C–H, as shown in Figure 1.2.

We can know that the value of ^3J has a minimum value when $\Phi = 90°$, and that the value of ^3J has a maximum value when $\Phi = 0°$ or 180°, while the value of ^3J when $\Phi = 180°$ is greater than that of ^3J when $\Phi = 0°$.

The following two cases will be encountered frequently.

Because the dihedral angle formed by two trans-hydrogen atoms is 180° and that by two cis- hydrogen atoms is 0°, the coupling constant value from two trans-hydrogen atoms is greater than that from two cis-hydrogen atoms. Their typical values are 15–17 Hz and 10–11 Hz, respectively.

In a saturated six-membered ring, if two vicinal hydrogen atoms are situated at axial bonds (in this case their coupling constant is denoted as J_{aa}), the coupling constant from these two hydrogen atoms is greater than that from two vicinal hydrogen atoms, which are situated at equatorial bonds (in this case their coupling constant is denoted as J_{ee}), or that from these two hydrogen atoms, with one hydrogen atoms situated at an axial bond and its vicinal hydrogen atom situated at an equatorial bond (in this case their coupling constant is

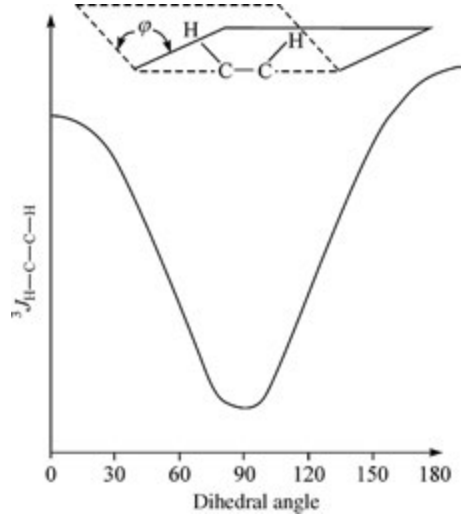


Figure 1.2 $^3J_{H\text{-}C\text{-}C\text{-}H}$ is the function of the related dihedral angle

denoted as J_{ae}). These phenomena can be explained by the following facts: Φaa ≅ 180, and Φae ≅ Φee = 60. Therefore, $J_{aa} > J_{ae} \geq J_{ee}$.

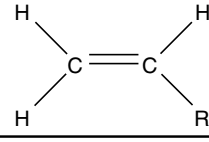
In addition, erythro- and threo- forms can be differentiated by using the relationship between coupling constants and dihedral angles. Readers who are interesting in this topic can read the reference [1].

Electronegativity of Substituents The substitution of an electronegative group will decrease the value of ^3J. This phenomenon can be encountered frequently.

The value of ^3J in a saturated alkyl group is about 7 Hz. If a saturated alkyl group is substituted by a hydroxyl group, the ^3J value will decrease by less than 5 Hz. Therefore, the decrease of ^3J value will be shown obviously in its ^{1}H spectrum.

We will discuss the split patterns in the ^{1}H spectrum later. The changes of the values of coupling constants will obviously affect split patterns.

The substitution of an alkene group by an electronegative group will decrease the values of coupling constants of trans-two hydrogen atoms and that of cis-two hydrogen atoms. For example,

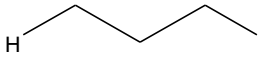
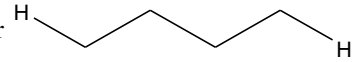


R	$^3J_{cis}$	$^3J_{trans}$
-Li	19.3	23.9
-SiR_3	14.6	20.4
-CH_3	10.0	16.8
-Cl	7.3	14.6
-F	4.7	12.8

There are other factors affecting ^3J values. However, their effects are smaller than the above-mentioned two factors. Therefore, we omit them [1].

1.2.2.4 Coupling Constants of Long-Range Couplings

In the ^1H spectrum, the couplings across four or more bonds are called long-range couplings.

The coupling constants in a saturated system decrease rapidly with an increase in the number of chemical bonds between two coupled nuclei. Only particular structural units, such as  or  have a small long-range coupling constant (generally less than 2 Hz).

In an unsaturated system, through the action of π electrons, long-range couplings can be transferred to other chemical bonds. Therefore, ^4J and even ^5J between two hydrogen atoms in an unsaturated chain can exist. There are long-range couplings in the following structural units:

① Allylic system: H–C=C–C–H.
② Homoallylic system: H–C–C=C–C–H.
③ Conjugated systems.
④ Systems containing accumulated unsaturated bonds.
⑤ Coupling between the hydrogen atom in the ortho-position of the substituted phenyl ring and those in the side chain.

An example of long-range couplings will be presented in Example 11 of Section 6.2.

1.2.2.5 Couplings in a Phenyl Ring or in a Heteroaromatic Ring

The ^3J value in a phenyl ring is larger than that in a saturated chain, because coupling effects are transferred better there than in a saturated chain. The typical ^3J value in a phenyl ring is about 8 Hz.

Because of the existence of a nitrogen atom in a pyridine ring, the electronegativity of the nitrogen atom affects ^3J values just as was mentioned above: the substitution of an electronegative group will decrease the value of ^3J. When the two vicinal hydrogen atoms are close to the nitrogen atom (for example, when they are situated in 2- and 3- positions), their ^3J typical value is about 5 Hz. When the two vicinal hydrogen atoms are far from the nitrogen atom (for example, when they are situated in 3- and 4- positions), their ^3J typical value is about 8 Hz.

The ^3J value in a five-membered heteroaromatic ring (furan, etc.) is similar to that in the pyridine ring. However, the ^3J value in a five-membered heteroaromatic ring is smaller than that in the pyridine ring, respectively.

The typical coupling constants of common functional groups are listed in Table 1.2.

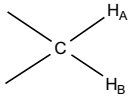
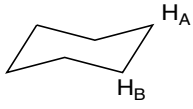
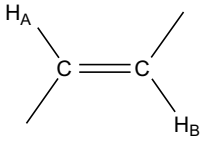
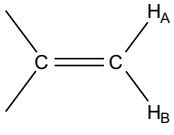
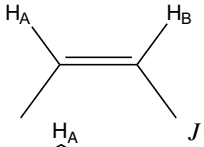
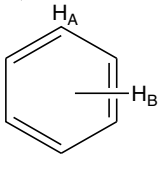
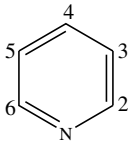
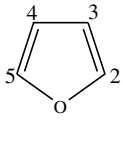
1.3 Chemical Equivalence and Magnetic Equivalence

Because of the importance of the ^1H spectrum, it is certain to measure first the ^1H spectrum of a sample for determining its structure or confirming its structure.

When we interpret a ^1H spectrum, the following question constantly arise. Why is a ^1H spectrum complicated when the structure of the sample seems uncomplicated? (Or one can even wonder if it is **really** its ^1H spectrum.)

This question just concerns the subject which we will discuss in this section: chemical equivalence and magnetic equivalence.

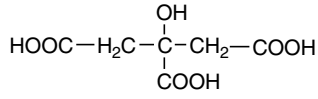
Table 1.2 Typical coupling constants of common functional groups

Structural unit	Typical coupling constant J_{AB} (Hz)
	–10– –15
CH_ACH_B	7
 *ax-ax* *ax-eq* *eq-eq*	8–11 2–3 2–3
	15–17
	0–2
	10–11
 J (ortho) J (meta) J (para)	8 2 0.3
 *J*(2–3) *J*(3–4) *J*(2–4) *J*(3–5) *J*(2–5) *J*(2–6)	5 8 1.5 1 0.8 0
 *J*(2-3) *J*(3-4) *J*(2-4) *J*(2-5)	1.8 3.6 0.8 1.5

1.3.1 Chemical Equivalence

Chemical equivalence is an important concept in stereochemistry. If two atoms (or two identical functional groups) have the same chemical environment, they are chemically equivalent. If the two functional groups are measured by NMR, they have the same chemical shift value. If two identical groups are not chemically equivalent, they may have different rates of reaction and they may have different results in their spectroscopic measurements.

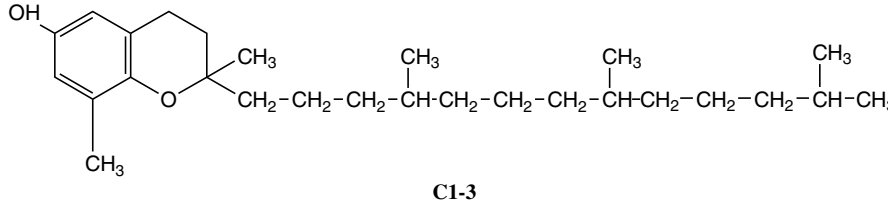
The citrate acid, **C1-2**, has the following structure:



C1-2

From the structural formula it looks as if the two carboxyl groups were chemically equivalent. However, in zymolytic reactions they have different rates, which means that the two carboxyl groups are chemically non-equivalent.

The δ-vitamin E, **C1-3**, is another example:



C1-3

The two methyl groups connected to the same carbon atom show obviously two peaks in their ^{13}C spectrum even if measured by an NMR instrument with 400 MHz, that is, its ^{13}C spectrum illustrates that the two methyl groups are chemically non-equivalent.

There are many other examples similar to those mentioned above. The problem which we encounter frequently is that since two identical functional groups in a chain are connected to the same carbon atom, so it seems reasonable that the two functional groups are chemically equivalent because their positions are exchangeable through the rotation of the carbon chain and that they should have the same chemical shift value.

The above-mentioned discussion also involves two hydrogen atoms connected to the same carbon atom, because they are a special case of two identical functional groups. It seems that these two hydrogen atoms have the same chemical shift value.

A very important rule for interpreting ^1H spectra is that there are no coupled splits of two hydrogen atoms in their ^1H spectrum if they have the same chemical shift value. Otherwise, if two hydrogen atoms have different chemical shift values, their coupled splits will be shown in their ^1H spectrum. If two geminal hydrogen atoms have different chemical shift values, their coupled splits will be prominent since the value of ^2J is much greater than that of ^3J. As a consequence, their ^1H spectrum will be complicated.

To sum up, whether for two hydrogen atoms attached to a carbon atom or for two identical functional groups attached to a carbon atom, it is not possible to determine in a simple way whether they are chemically equivalent or not.

It is necessary for us to apply the symmetrical plane rule to determine whether two identical functional groups (including two hydrogen atoms) attached to a common carbon

atom are chemically equivalent or not. If two identical functional groups are determined to be chemically equivalent, they have the same chemical shift value and as a consequence, their coupled splits will not be shown in their ^1H spectrum. Otherwise, if two identical functional groups do not satisfy the requirement of the symmetric plane rule, they will have different chemical shift values and they will produce a complex ^1H spectrum.

The symmetrical plane rule can be presented as follows.

If the molecule to be discussed has a symmetrical plane and the symmetrical plane bisects the angle of XCX, where two X groups are the two identical functional groups attached to a common carbon atom, these two X groups are enantiotopic (if rapid intramolecular motions exist, this symmetrical plane should bisect the angle for every conformer). If the solvent applied for NMR measurement is an achiral solvent, these two X groups are chemically equivalent and they have the same chemical shift value. If the solvent applied is chiral, these two X groups may have different chemical shift values.

If this condition is not totally satisfied, these two X groups are not chemically equivalent.

The symmetrical plane rule can be presented further as follows.

If the molecule to be discussed has no symmetrical plane, two identical functional groups (including two hydrogen atoms) attached to a common carbon atom are not chemically equivalent. This means that they should have different chemical shift values from the theoretical consideration. However, it is not certain that this difference in chemical shift values can be measured. The measurement of this difference can also be determined depending on experimental conditions, such as the frequency of the NMR spectrometer applied, solvent applied, temperature in measurement, and so on.

Because the compound **C1-3** has no symmetrical plane, the two methyl groups attached to a common CH are not chemically equivalent. They have two signals in their ^{13}C spectrum.

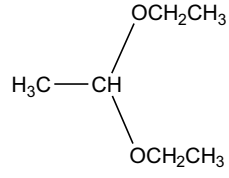
If the molecule has a symmetrical plane but rapid intramolecular motions exist, the condition that two identical functional groups be chemically equivalent is that the symmetrical plane bisects the XCX angle for every conformer and the measurement is carried out in an achiral solvent.

It is a pity that this important rule is seldom dealt with in detail in the existing books on nuclear magnetic resonance. Some books only describe a few examples concerning this rule without a theoretical discussion of the rule itself; some books describe the rule briefly without a discussion of related examples.

According to many years of research and teaching of the author himself, the above question about the rule may be the one most frequently encountered in interpreting ^1H spectra for students.

We now explain the rule further with a simple example.

The compound **C1-4** has a simple structure:



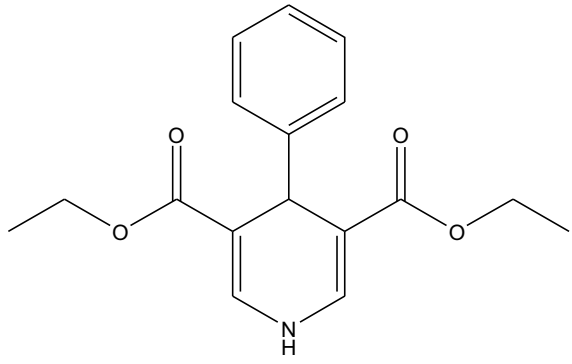
C1-4

Since the compound has a symmetrical plane, two ethoxyl groups are chemically equivalent. Therefore, the two CH_3 groups have the same chemical shift value and so do

the two CH_2 groups. However, the symmetrical plane does not bisect the H–C–H angle, and the two hydrogen atoms in a CH_2 group are not chemically equivalent. Therefore, the two hydrogen atoms produce geminal coupling, that is, the two hydrogen atoms form two sets of doublets. By splitting from its adjacent methyl groups, the CH_2 group produces 16 peaks. However, because the two CH_2 groups are symmetrical in the molecule, their lines are strictly overlapped in the same positions.

Next we shall discuss a more complicated example.

The compound **C1-5** has the following structure:



C1-5

The ^1H spectrum of the compound **C1-5** is shown in Figure 1.3.

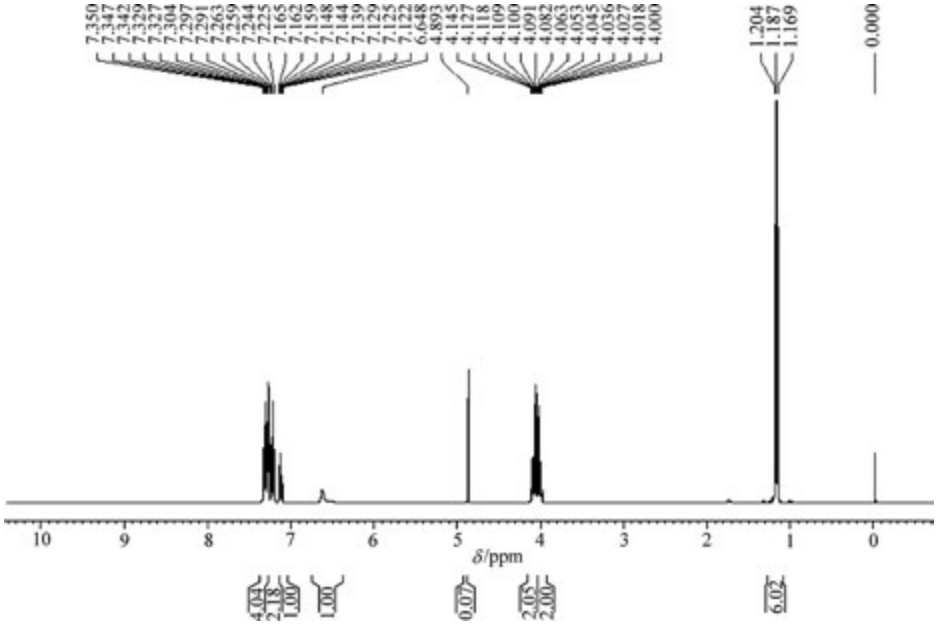


Figure 1.3 The ^1H spectrum of the compound **C1-5**

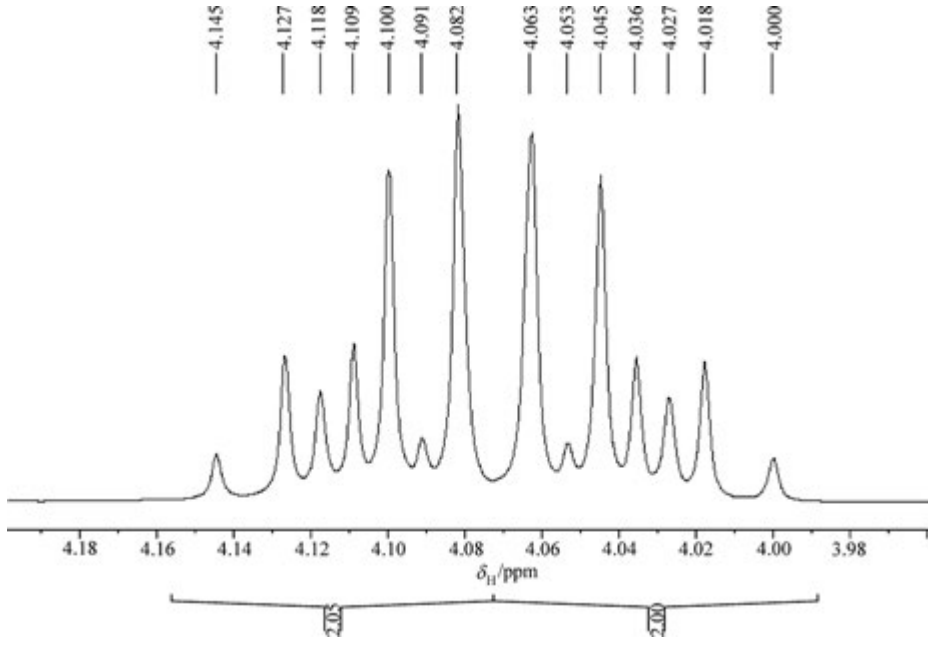


Figure 1.4 The locally enlarged ^{1}H spectrum in the region near 4.1 ppm in Figure 1.3

The locally enlarged ^{1}H spectrum in the region near 4.1 ppm in Figure 1.3 is shown in Figure 1.4.

We will present a detailed interpretation of the ^{1}H spectrum later. We just assign Figures 1.3 and 1.4 now. The integral value of the peak sets at about 7.3 ppm is 7 which corresponds to aromatic hydrogen atoms. The triplet at 1.19 ppm, whose peak area is 6, corresponds to two methyl groups. The peak sets, whose area at about 4.07 ppm is 4, correspond to two CH_2 groups. Readers will ask the question: Why do two CH_2 groups produce so many peaks?

First of all, let us analyze the split pattern of the peak sets.

Fourteen peaks are shown in Figure 1.4. In fact, the 14 peaks result from the partially overlapped 16 peaks. Since the 14 peaks show a symmetrical distribution, we can mark them with 1 to 14, respectively, either from the left side or from the right side. After a careful interpretation, we can find four quartets. They are:

1,	2,	4,	6;
3,	5,	7,	8;
7,	8,	10,	12;
9,	11,	13,	14

The distance between two adjacent peaks in a quartet corresponds to the ^3J coupling constant between CH_3 and CH_2. Since Figure 1.4 was measured by an NMR spectrometer with 400 MHz, the coupling constant can be calculated as 7.2 Hz by using related data.

The distance between two adjacent centre positions of quartets corresponds to the ^2J between two hydrogen atoms connected to a common carbon atom. These couplings come from the non-equivalence of the two hydrogen atoms. In calculation, it is not necessary to calculate the centre positions of quartets. For example, we can calculate the distance between No. 2 and No. 5. The calculated ^2J value is 10.8 Hz.

Why can a CH_2 group produce 14 peaks (in fact, 16 peaks)? The answer can be obtained immediately by using the symmetrical plane rule. The molecule has a symmetrical plane. Therefore, the ethoxyl group on the left side and that on the right side of the structural formula are chemically equivalent. However, this symmetrical plane does not bisect the H–C–H angle of the CH_2, so the two hydrogen atoms are not chemically equivalent. The signal of each hydrogen atom is split by its geminal hydrogen atom. Then the signal is split further by the methyl group. Therefore, each CH_2 group produces 16 peaks.

There are many more examples like that, including examples in Section 1.6. Because the molecule discussed has no symmetrical plane, it produces a complex ^1H spectrum.

The symmetrical plane rule is applied not only in the interpretation of the ^1H spectrum, but also in the interpretation of the ^{13}C spectrum, so it is a general rule.

1.3.2 Magnetic Equivalence

We have discussed the symmetrical plane rule above. If two identical functional groups are not chemically equivalent, their ^1H spectrum will be complex. Like chemical equivalence, magnetic equivalence is also an important concept, which also relates to the complexity of the ^1H spectrum.

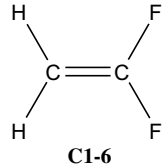
The concept of magnetic equivalence of several identical nuclei is as follows.

1. They are chemically equivalent.
2. They have the same coupling constant (both absolute value and sign) for any other magnetic nucleus.

Only when the above-mentioned two conditions are satisfied will several identical nuclei be magnetic equivalent.

Since we have understood chemical equivalence already, here we just emphasize the second condition which can be misunderstood sometimes.

A classic example is about the compound **C1-6**.

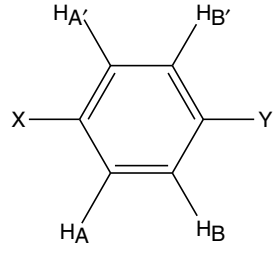


C1-6

From the structural formula, we can know that the molecule is of symmetry. The two hydrogen atoms as well as the two fluorine atoms are chemically equivalent. However, if we select a fluorine atom, a hydrogen atom couples with the selected fluorine atom through cis-coupling, and the other hydrogen atom through trans-coupling. Therefore, they do not satisfy the second condition above, neither do the two fluorine atoms.

Because the two hydrogen atoms in the compound **C1-6** are chemically equivalent, their peaks have the same position in their ^{1}H spectrum. However, since the two hydrogen atoms are not magnetically equivalent, they have more than 10 peaks.

Now we discuss the compound **C1-7**.



C1-7

Because the compound **C1-7** has a symmetrical axis, H_A and $H_{A'}$ as well as H_B and $H_{B'}$ are chemically equivalent. However, the coupling between H_A and H_B is an ortho-coupling with the coupling constant ^3J while the coupling between $H_{A'}$ and H_B is a para-coupling with the coupling constant ^5J. Therefore, H_A and $H_{A'}$ are magnetically non-equivalent.

If a compound has magnetically non-equivalent atoms, its ^{1}H spectrum will be complicated. If we do not consider magnetic equivalence of a structure, we can underestimate the complexity of its ^{1}H spectrum.

1.3.3 Classification of ^{1}H Spectra

On the basis of the concepts of chemical equivalence and magnetic equivalence, we can discuss the classification of ^{1}H spectra.

^{1}H spectra can be classified into the first-order and the second-order spectra.

The first-order spectra can be interpreted by the $n + 1$ rule (or by the $2nI + 1$ rule for a nucleus with $I \neq 1/2$). The second-order spectra are those which cannot be interpreted by the $n + 1$ rule (or by the $2nI + 1$ rule for a nucleus with $I \neq 1/2$).

The conditions for producing the first-order spectra are as follows:

1. The ratio of $\Delta\nu/J$ is large enough (when the relevant parameters are calculated with the same unit, for example, in Hz).
2. The nuclei in a group are magnetically equivalent.

The characteristics of the first-order spectra are as follows:

1. The number of peaks is illustrated by the $n + 1$ rule, where n is the number of adjacent hydrogen atoms that are magnetically equivalent.
2. The intensities of peaks within a peak set can be expressed approximately as the coefficients of the developed binomial. Relative peak intensities are 1: 1 for a doublet, 1: 2: 1 for a triplet, 1: 3: 3: 1 for a quartet, and so on.
3. Both δ and J can be read directly from the spectrum. δ is the centre position of the peak set while J is the interval between two adjacent peaks (in Hz).

If the above-mentioned conditions cannot be satisfied at the same time, the second-order spectra will result, which are differentiated from the first-order spectra by the following facts:

1. In general, the number of peaks is larger than that calculated by the $n + 1$ rule.
2. The intensities in a peak set show complicated ratios.
3. Generally neither δ nor J can be read out directly from the spectrum.

Because of the characteristics of the second-order spectra, their interpretation is complicated.

When we interpret the first-order spectra, we consider only two sets of magnetic nuclei, to which we apply the $n + 1$ rule. However, when we interpret the second-order spectra, we must consider more magnetic nuclei, for example, three sets of magnetic nuclei.

From the first condition mentioned above, we know that the large enough ratio of $\Delta v/J$ is a key condition. Therefore, the frequency of the NMR spectrometer applied is of particular importance. Compared with the spectrometer with 100 MHz which was applied long ago, the NMR spectrometers with at least 400 MHz are now applied. Therefore, the ratio of $\Delta v/J$ increases at least by four times. If the ratio of $\Delta v/J$ might not be large enough before, it is large enough now. Therefore, the ^1H spectra can be interpreted as the first-order spectra in almost each case, that is, the spectra can be analyzed by using the $n + 1$ rule. Of course, we should also pay attention to the second condition mentioned above, that is, magnetic equivalence. When we apply the $n + 1$ rule, the nuclei group, which has the number of n, should have the same coupling constant. If they have different coupling constants, the $n + 1$ rule should be applied several times. Each time the $n + 1$ rule is applied just for one coupling constant. We will discuss this point in Section 1.5.5.

1.4 Characteristics of the ^1H Spectra of Some Functional Groups

Now we discuss the characteristics of the ^1H spectra of some functional groups, most of which are common functional groups.

1.4.1 Substituted Phenyl Ring

The substituted phenyl rings are common structural units. We shall discuss them according to the number of substituents.

1.4.1.1 Mono-Substituted Phenyl Ring

When five hydrogen atoms are found in the aromatic region from the integral curve, the existence of a mono-substituted phenyl ring can be estimated.

The concept of "three types of substituents" is very useful for interpreting the spectra of substituted phenyl rings.

When a phenyl ring is substituted by a substituent, which belongs to the first type, the remaining hydrogen atoms, including the ortho-, meta- and para- hydrogen atoms, have approximately δ values. The five hydrogen atoms form a peak set within a narrow δ region

with the appearance that the peaks in the middle are strong and the peaks at two edges are weak.

In the substitution of a phenyl ring by a substituent, which belongs to the second type, because of the p-π conjugation between the non-bonding electrons of the hetroatom and the delocalized electron of the phenyl ring, the electron density of the substituted phenyl ring increase, especially at the ortho- and para-positions. These hydrogen atoms have smaller chemical shift values than those at the meta-positions. The two hydrogen atoms at the meta-positions have a larger δ value than that at ortho- and para-positions, but their δ value is still smaller than that of the unsubstituted phenyl ring. Since the two hydrogen atoms at meta-positions have two adjacent hydrogen atoms, they show triplets from ^3J coupling. The triplets are split further by ^4J and ^5J.

In the substitution of a phenyl ring by a substituent, which belongs to the third type, because of electronegativity of the hetroatom, the electron density of the substituted phenyl ring decreases. All the five remaining hydrogen atoms, especially the two hydrogen atoms at the ortho-positions, have a downfield shift after the substitution. Since each ortho-hydrogen atom has one adjacent hydrogen atom, its peaks are illustrated as a doublet with respect to ^3J, which is split further by ^4J and ^5J.

Therefore, from the peak shapes, the type of a substituent can be estimated.

1.4.1.2 Di-Substituted Phenyl Ring

The para-substituted phenyl ring has the simplest ^{1}H spectra among those of all substituted phenyl rings. Because of the symmetry of the para-substituted phenyl ring and the existence of two sets of adjacent hydrogen atoms, the para-substituted phenyl ring shows two sets of doublets with respect to ^3J, which is split further by ^4J and ^5J. Since the magnitude of ^3J is much larger than those of ^4J and ^5J, the shape of the doublet is clear.

According to the concept of "three types of substituents of the phenyl ring," the more different the two functional groups of a para-substituted phenyl ring are the more difference between the δ values of the two sets of doublets exists. Of course, if one substituent belongs to the second type and another substituent belongs to the third type, the difference between the δ values of the two sets of doublets will be obvious.

If a substituent in the para-substituted phenyl ring is an alkyl group, the height of the doublet of the two hydrogen atoms at the ortho-position with respect to the alkyl will be smaller than that of another doublet because of the ^4J coupling between the α-hydrogen atom in the alkyl and the hydrogen atoms at its ortho-position.

The peak shape of the ortho-substituted phenyl ring is complicated.

If the two functional groups of an ortho-substituted phenyl ring are different, all the four remaining aromatic hydrogen atoms have different chemical shift values. As a result, they show the most complicated spectra among all the substituted phenyl rings. If the two substituents are greatly different, for example, one belongs to the second type and another belongs to the third type, it is possible that the ^{1}H spectrum be analyzed by using the $n + 1$ rule.

If the two functional groups of an ortho-substituted phenyl ring are identical, the ^{1}H spectrum of the remaining four aromatic hydrogen atoms is symmetrical with respect to their middle point because of the symmetry of its structure. However, its ^{1}H spectrum is still complicated.

In general, the spectrum of the meta-substituted phenyl ring is complex. However, an isolated hydrogen atom, which is situated between the two substituents, always shows a singlet without the split by ^{3}J. However, the spectrum of the remaining three hydrogen atoms is complicated.

1.4.1.3 Multi-Substituted Phenyl Ring

The spectrum of a multi-substituted phenyl ring can be interpreted using the method mentioned above.

After the tri-substitution, the phenyl ring's remaining three hydrogen atoms can be either adjacent or separated. The isolated hydrogen atom shows a singlet without the split by ^{3}J. The adjacent hydrogen atoms show the coupling split by ^{3}J, ^{4}J and ^{5}J.

After the substitution of four functional groups, the remaining two aromatic hydrogen atoms show two sets of doublets.

After the substitution of five functional groups, the remaining one aromatic hydrogen atom shows a singlet.

1.4.2 Substituted Heteroaromatic Ring

Because of the existence of the heteroaromatic atom, the hydrogen atoms in a heteroaromatic ring have different δ values. The substitution further increases these differences in δ. Therefore, the spectra of a substituted heteroaromatic ring can be analyzed by the $n + 1$ rule even if one uses an NMR spectrometer with a low frequency.

Because of the existence of the heteroatom, ^{3}J changes positions with respect to the heteroatom. This phenomenon can be understood as follows. The substitution of an electronegative functional group will decrease the value of ^{3}J. As a result, the coupling constant ^{3}J concerning the α-hydrogen atom with respect to the heteroatom is smaller than the other ^{3}J. For example, in a pyridine ring, the typical value of ^{3}J between the hydrogen atoms at 2- and 3-positions is 5 Hz, but that between the hydrogen atoms at 3- and 4-positions is 8 Hz. We can consider that the former is affected by the nitrogen atom since it is near to its two adjacent hydrogen atoms.

1.4.3 Normal Long-chain Alkyl Group

The peak of the terminal methyl group in a normal long-chain alkyl group can be easily determined because it shows a triplet at about 0.87 ppm. The peaks of the methylene group, which is connected to the methyl group, and the peaks of its adjacent methylene groups are situated around 1.25 ppm within a narrow range. The last methylene group, which is connected with a substituent, has a δ value larger than 1.25 ppm because all substituents are more electronegative than an alkyl group. The peak shape of the last methylene group is a triplet and its chemical shift value is determined by the electronegativity of the substituent.

1.4.4 Carbonyl Compounds

Carbonyl groups can not be measured directly by the ^{1}H spectrum. However, aldehydes and carboxyl groups can be measured by the ^{1}H spectrum. Aldehydes have chemical shift values within a narrow range of 9.5–10.0 ppm, while carboxyl acids have chemical shift values greater than 10 ppm in general.

1.4.5 Reactive Hydrogen Atom

The hydrogen atom, which is connected with a heteroatom, is called the reactive hydrogen atom. Because the chemical bond between the reactive hydrogen atom and the heteroatom is weak, the hydrogen atom is changeable. This situation is different from that of the hydrogen atoms which are connected with carbon atoms. Therefore, the peaks of reactive hydrogen atoms are different from those of hydrogen atoms connected to carbon atoms.

First of all, the peak area (integral value) of the reactive hydrogen atom is less than a unit value, because of the interaction between the reactive hydrogen atom and deuterated solvent. Therefore, its peak should not be assigned as an impurity peak. Because the existence of a reactive hydrogen atom will affect the chemical shift value of its adjacent functional group, one can use this point to prove the existence of a reactive hydrogen atom.

Secondly, the peak shape of a reactive hydrogen atom is different from that of a non-reactive hydrogen atom. The peak shape of the latter is sharp and the peak shape of a reactive hydrogen atom is blunt. The bluntness varies with the types of reactive hydrogen atom and its structural environments.

The formation of blunt shapes of reactive hydrogen atoms comes from two factors.

The first factor producing a blunt shape is the rate of interchange reactions.

When an interchange reaction of a reactive hydrogen atom takes place rapidly, the reactive hydrogen atom will show a rather sharp peak although the peak is blunter than that of non-reactive hydrogen atoms. When an interchange reaction of a reactive hydrogen atom takes place slowly, the reactive hydrogen atom will show a blunt peak. Generally speaking, the rates of interchange reactions of reactive hydrogen atoms decrease according to the order: OH > NH > SH. Their peak shapes become blunter and blunter also in this order.

If a compound contains several reactive hydrogen atoms and the rates of interchange reactions of these reactive hydrogen atoms are high, these several reactive hydrogen atoms show only one peak of an average value

$$\delta_{observed} = \sum N_i \times \delta_i \tag{1.1}$$

where N_i is the molar fraction of the i-th reactive hydrogen atom and δ_i is the chemical shift value of the i-th reactive hydrogen atom.

If a compound contains several reactive hydrogen atoms and the rates of interchange reactions of these reactive hydrogen atoms are low, these several reactive hydrogen atoms show several peaks with their own δ values.

The second factor producing a blunt shape is the rate of quadrupole moment relaxation.

Among the three types of reactive hydrogen atoms, we just discuss amino group, because only the nitrogen atom has a quadrupole moment.

A primary amine, $-NH_2$, will be taken as an example.

If the quadrupole moment relaxation of the nitrogen atom is rapid, its spin orientation varies rapidly. As a result, its adjacent magnetic nuclei will not be split by the nitrogen atom. If the quadrupole moment relaxation of the nitrogen atom is slow, the peak of the hydrogen atoms of the amine group will be split by the nitrogen atom. Since the spin quantum number of the nitrogen atom is 1, the split peak shows a triplet according to the

$2nI+1$ rule. However, it should be noted that the intensity ratio of the triplet is 1: 1: 1 (not 1: 2: 1).

If the rate of the interchange reaction of the amine group is high and the quadrupole moment relaxation is rapid, the amine group will produce a rather narrow singlet. If the reaction occurs slowly, or the quadrupole moment relaxation is slow, the amine group will show a triplet. If both the reaction rate and the quadrupole moment relaxation are of the middle value between the above two limits, the peak shape of the NH_2 should show a blunt singlet.

In common situations, an amine group shows a blunt peak. Sometimes, the peak of an amine group appears slightly blunt.

1.4.6 Compounds Containing Fluorine or Phosphor Atoms

Since the spin quantum numbers of either fluorine or phosphor is 1/2, both of them are magnetic nuclei. They will split the peaks of hydrogen nuclei (and ^{13}C nuclei) which are near them. The isotope abundance of either fluorine or phosphor is 100%.

Since the NMR frequency of fluorine or phosphor is greatly different from that of hydrogen nuclei, the peak split pattern in the ^1H spectrum coincides with that estimated by the $n+1$ rule.

Since a fluorine atom can not connect directly with a hydrogen atom (otherwise it is an HF molecule), the shortest distance between a fluorine atom and a hydrogen atom is that across two chemical bonds, which corresponds to a coupling constant ^2J. The largest distance, over which the coupling between a fluorine atom and a hydrogen atom exists, is that across five chemical bonds. It corresponds to ^5J.

The value of $^2J_{F\text{-}H}$ in a saturated chain is 45–80 Hz, that of $^3J_{F\text{-}H}$ 0–30 Hz, and that of $^4J_{F\text{-}H}$ 0–4 Hz.

The value of $^2J_{F\text{-}H}$ in an alkylene group is 70–90 Hz, that of $^3J_{F\text{-}H}$ (trans-) 10–50 Hz, that of $^3J_{F\text{-}H}$ (cis-) 3–20 Hz. We can see the influence of the dihedral angle again.

In an aromatic unit, the coupling constants between a fluorine atom and hydrogen atoms are ^3J-^5J. The value of $^3J_{F\text{-}H}$ is 6–9 Hz, and 4–8 Hz for $^4J_{F\text{-}H}$, 0–3 Hz for $^5J_{F\text{-}H}$.

Because the value of $^3J_{F\text{-}H}$ approximates that of $^3J_{H\text{-}H}$ in an aromatic unit, sometimes it happens that a triplet appears instead of a doublet, which results, in fact, from the split from the $^3J_{F\text{-}H}$.

Generally speaking, the coupling from the phosphor atom is less strong than that from the fluorine atom. In crossing the same number of chemical bonds, the coupling constants from phosphor are less than those from fluorine.

Because the chemical valence of phosphor is 5, there may exist $^1J_{P\text{-}H}$, whose value can be 700 Hz.

The value of $^2J_{P\text{-}H}$ in a saturated chain is 10–20 Hz, and that of $^3J_{P\text{-}H}$ 1–10 Hz.

The value of $^2J_{P\text{-}H}$ in an alkylene group is 10–40 Hz, that of $^3J_{P\text{-}H}$ (trans-) 30–60 Hz, and that of $^3J_{P\text{-}H}$ (cis-) 10–30 Hz.

1.5 Interpretation of ^1H NMR Spectra

In what follows, we will present the method for interpreting ^1H NMR spectra.

Table 1.3 The chemical shift values of common solvent peaks

Deuterated Solvent	$CDCl_3$	D_2O	CD_3SOCD_3 (DMSO)	CD_3OD	CD_3COCD_3
δ (ppm)	7.26	4.79	2.49	3.31	2.05

The interpretation of the second-order spectra (such as, ABX, AB_2 systems) will not be dealt with here, because ^1H spectra can be simplified as the first-order spectra in general by using an NMR spectrometer with a high frequency. If the ^1H spectrum shows the characteristic of the second-order spectra, that is, there are no equally spaced peaks, readers can consult the reference [1], which is the companion volume of this book.

The following steps are proposed to readers as the reference.

1.5.1 Find Impurity Peaks, Pay Attention to the Solvent Applied

The ^1H NMR spectrum has a good quantitative relationship. Because the amount of impurities should be much less than that of the sample to be measured, the peaks of impurities can be discerned from those of the sample.

Deuterated solvents are applied in the measurement of the ^1H spectrum.

It should be noticed that the ^1H spectrum is related with the solvent applied. It is possible that a ^1H spectrum changes with a different solvent. Therefore, if you compare a ^1H spectrum obtained from your measurement with that from a reference, you should use the same solvent as that in the reference.

Deuterated solvents can not be 100% deuterated isotopically. The remaining trace hydrogen atoms will produce corresponding peaks, that is, the solvent peaks. The solvent applied can be known from the δ value of the solvent peak.

The δ values of common deuterated solvents are shown in Table 1.3.

Because the sample to be measured may contain a small quantity of water, the peak from the water will be shown in the ^1H spectrum. Its δ value is related to the solvent applied. The δ values of the peaks from the water in common solvents are listed in Table 1.4.

1.5.2 Calculation of the Unsaturation Number of the Unknown Compound

If the molecular formula of an unknown compound is known, its unsaturation number, which is helpful for deducing an unknown structure, can be calculated. When the nitrogen atoms are trivalent, the unsaturation number can be calculated according to the following formula

Table 1.4 The δ values of the peaks from the water in common solvents

Deuterated Solvent	$CDCl_3$	D_2O	CD_3SOCD_3 (DMSO)	CD_3OD	CD_3COCD_3
δ (ppm)	~1.6	4.79[a]	~3.3	4.79	2.80

[a] If deuterated water is applied as the solvent, the water in the sample and the water remaining in the deuterated water show a common peak.

$$\Omega = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2} \tag{1.2}$$

where Ω is the unsaturation number;

C, H, X, and N are the numbers of carbon, hydrogen, halogen and nitrogen atoms in the molecule, respectively.

If the nitrogen atoms are pentavalent, we have

$$\Omega = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{3N}{2} \tag{1.3}$$

where N is the number of pentavalent nitrogen atoms.

1.5.3 Determination of the Number of Hydrogen Atoms Corresponding to Every Peak Set in the ^{1}H Spectrum

Because the ^{1}H NMR spectrum has good quantitative relationship, the integral value of each peak set is proportional to the number of hydrogen atoms.

If the molecular formula of the unknown compound is known, the numbers of hydrogen atoms corresponding to peak sets can be determined by the ratios of the total integral value to individual integral values.

If the structure of the unknown compound is simple, it is possible that the structure be deduced only by its ^{1}H spectrum and its molecular formula.

The following functional groups can be used as the references to determine hydrogen atoms corresponding to peak sets:

1. The terminal methyl group in a normal long-chain unit
   The signal of the terminal methyl group is situated at about 0.87 ppm as a triplet.
2. Methoxy groups
   The chemical shift values of methoxy groups are situated in 3.5–3.9 ppm in general. Because the signals of the methoxy group are singlets, these signals can be easily determined.
3. The para-substituted phenyl ring
   The para-substituted phenyl ring shows two sets of doublets, which can be discerned from their peak shape. Their chemical shift values are 6.8–8.0 ppm in general.

According to the characteristics of the structure measured, it is possible to choose some functional groups of the compound as the reference to determine the numbers of other functional groups.

If the structure of the unknown compound is complicated and the number of hydrogen atoms of the compound is large, the above-mentioned method will not work well. The trouble comes from two aspects. First, there are overlapped peak sets. Secondly, the integral values are not near to integers. In this case, the application of two-dimensional NMR spectra (mainly the category of HSQC) is quite necessary.

1.5.4 Determination of Functional Groups of the Unknown Compound

According to the chemical shift value and the number of hydrogen atoms of a peak set, the functional group, which corresponds to the peak set, can be determined. Even its adjacent functional group can be estimated from the chemical shift value. For example, the chemical shift values of methoxy groups are 3.5–3.9 ppm. Its adjacent functional group is an aromatic group which can be estimated if the δ value of the methoxy group is near to 3.9 ppm.

If the structural formula of the sample to be measured is of symmetry or local symmetry, some identical functional groups will produce peaks at the same position. For example, the three methyl groups in a tert-butyl have the same δ value.

1.5.5 Analysis of Coupling Splittings of Peak Sets

Because of the interactions between magnetic nuclei, split peak sets are shown in the ^{1}H spectra. As we have described before, the probability of encountering the second-order spectra is small because of the application of high frequency NMR spectrometers. The spectra can be treated as the first-order spectra which means we can interpret ^{1}H spectra by using the $n + 1$ rule.

The precondition for the $n + 1$ rule is the same coupling constant of the nuclei which participates in the coupling, that is, they are magnetically equivalent. If the nuclei have different coupling constants, the $n + 1$ rule should be applied once just for one particular coupling constant.

Suppose that there is a structural unit –CH–CH_2– in an aliphatic chain. Because of the rotation around the carbon-carbon bond, there is only one coupling constant between the CH and the CH_2. Therefore, the CH is split as a triplet by the CH_2.

Now we discuss again the structural unit –CH–CH_2–, but it is situated in a six-membered ring. Suppose that the hydrogen atom of the CH is along an axial direction. And the two hydrogen atoms of the CH_2 should be along an axial and an equatorial direction, respectively. These two hydrogen atoms have different coupling constants for the CH: one coupling constant is J_{aa} and other is J_{ae}. Since the two coupling constants are different, the coupling between the CH and the CH_2 must be considered twice, that is, the $n + 1$ rule should be applied twice. As a result, the CH shows the peak pattern of 2×2.

To sum up, the $n + 1$ rule will be applied once for a particular coupling constant. Then a split pattern is obtained. If a group of hydrogen atoms is coupled by two groups of hydrogen atoms with two coupling constants, respectively, what is the result?

If these two coupling constants are closed, the two groups of hydrogen atoms can be considered together. Therefore, the $n + 1$ rule will be applied just once. For example, a CH group is connected to two CH_2 groups on both its sides. If these two CH_2 groups are not connected to electronegative groups, their ^3J values will not decrease. Therefore, the CH group shows a quintet.

If these two coupling constants are obviously different, for example, one CH_2 group is connected with an electronegative group and the other CH_2 group is not, the coupling effect will be considered twice. The coupled split of the CH group shows a split pattern of $(n_1 + 1)(n_2 + 1)$, where n_1 and n_2 are the numbers of these two groups of hydrogen atoms, respectively. This consideration is similar to that of –CH–CH_2– in a six-membered ring.

If two coupling constants are neither closed, nor obviously different, the split pattern is complicated. The peak number is less than that calculated from $(n_1 + 1)(n_2 + 1)$ and greater than that of $n_1 + n_2 + 1$. In fact, the spectrum belongs to the second-order spectrum.

As a coupling exists in two coupled peak sets, the distance that corresponds to this coupling constant can be found in both related peak sets. Therefore, the connection of these two functional groups corresponding to the two peak sets can be determined. Since ^3J is the coupling constant encountered most frequently, the direct connection of the two related functional groups can be determined.

From the experience of the author, the information from the analysis of coupled splittings is more important and more reliable than that from the analysis of chemical shift values. If there are some discrepancies between the two conclusions obtained, the result obtained from the coupled splitting analysis deserves to be considered first. This is because a δ value can not be estimated precisely from any calculation or found from the same structural unit in a similar chemical environment. What is more, there are exceptions to δ values, but there are few exceptions to coupled splittings. Therefore, the analysis of coupled splittings is the most important thing for interpreting the ^{1}H spectrum. The analysis is to find the pairs of equally spaced peaks in a coupled split peak set and in related peak sets.

First, we will describe how to find the pairs of equally spaced peaks in a peak set. If the peak set is split by one coupling constant, it is easy to find the pairs, because any adjacent two peaks have the same distance, which corresponds to the coupling constant. If the peak set is split by several coupling constants, the split pattern will be complicated. In this case, one must take great skill to find the pairs of equally spaced peaks. It is recommended that one find first the smallest coupling constant from the complicated split peak set, and then other coupling constants according to the order from small to large. Some examples will be discussed in Section 1.6.

If a group of magnetically equivalent nuclei is split by several long-range coupling constants, the coupled splittings of the peak set of the group will not be clear. As a result, it is difficult to find the coupled splittings from related long-range coupling constants.

To sum up, in a peak set, we find out first one or several equal distances, with every distance obtained from equally spaced peaks. Therefore, we can know by how many coupling constants the peak set is split.

Then, we will find equal distances from related coupled peak sets. Like the coexistence of an action force and its reaction force, if a peak set A couples to a peak set B with a coupling constant, which corresponds to a particular distance between two peaks in the peak set A, the peak set B must also couple to the peak set A with the same coupling constant. Therefore, the particular distance can be found in both peak set A and peak set B. If the coupling constant is ^3J, the hydrogen atoms of peak set A are the vicinal hydrogen atoms of peak set B.

The above-mentioned method is very important for interpreting the ^{1}H spectrum. Sometimes the analysis of split patterns provides important structural information although the COSY (spectrum) is available.

1.5.6 Combination of Possible Structural Units

From the equal distances in related peak sets, adjacent functional groups can be found. The related chemical shift values of the related peak sets is also proof of determining the related

connections. To repeat this operation, we can, step by step, deduce the unknown structure from a small structural unit to a larger structural unit gradually, and finally we get one or several possible structures of the unknown compound.

Of course, it is often difficult, or even impossible, that an unknown structure is deduced only on the basis of its ^{1}H spectrum. When an unknown compound contains heteroatoms or quaternary carbon atoms, the connections found from the equal distances will be stopped at these points. In this case, two-dimensional NMR spectra are quite necessary.

In some cases, the coupling constants read out from ^{1}H spectra may have errors. As a result, the determination of connections by using the above-mentioned method can be difficult. In this case, the application of 2D NMR spectra is also necessary.

1.5.7 Assignment of the ^{1}H spectrum According to the Deduced Structure

The assignment of a ^{1}H spectrum means analyzing whether every peak set or multiplet which should have an appropriate δ value and reasonable coupled splittings corresponds to the deduced structure. Then the δ value of every functional group is marked beside the related positions of the deduced structural formula.

As mentioned above, to analyze to see whether coupled splittings are reasonable is very important.

If we can deduce several possible structures, the most reasonable structure can be selected from the assignment.

It must be pointed out that the assignment is a necessary step for postulating a structure or confirming a structure from its ^{1}H spectrum.

As mentioned above, we pay more attention to the analysis of coupled splittings than to that from δ values in the assignment. If there are some discrepancies between the two conclusions obtained, what results from the coupled splitting analysis deserves to be considered first.

1.5.8 Checking of the Deduced Structure

If the ^{1}H spectrum, the ^{13}C spectrum and necessary spectra (DEPT, COSY, HMQC or equivalent spectra, HMBC or equivalent spectra) are available, a correct structure can be deduced in general. Even in this case, it is advisable that the deduced structure will be verified by other methods. If the structure is deduced only by the ^{1}H spectrum and related data, the verification of the deduced structure by other methods is strongly recommended.

Some methods are proposed as follows:

ChemDraw is the software to draw structural formulae by a computer. This software is accepted by publishing houses. Because many structural units are stored in the software and these structural units can be assembled easily by the software, it is easy to draw a structure.

After a structure is drawn, a tool is used to frame the drawn structural formula. Then click "structure" at the upper part of the screen once and a dialog frame will appear. Then click once "Predict 1H-NMR shift" and calculated δ values will be shown beside the drawn structural formula and a simulated ^{1}H spectrum is shown in the lower part of the screen.

The software simulates the ^{1}H spectrum according to the drawn structure and calculates chemical shift values by using related data. It should be known that the calculation through ChemDraw is rough. Therefore, the result calculated from ChemDraw acts only as a reference.

Because the simulated ^{1}H spectrum by ChemDraw consists of a series of bars, and the software does not consider the frequency of NMR spectrometers, the simulated spectrum is not a good reference.

A reliable method is the comparison of the measured ^{1}H spectrum with that of the standard sample.

Sadtler standard spectra, which contain ^{1}H spectra, ^{13}C spectra, IR spectra, and so on, are useful references. A standard spectrum can be found from the molecular formula, the name of the compound, and so on. However, the information that Sadtler spectra provide is limited and the Sadtler ^{1}H spectra were measured with rather low frequency NMR spectrometers. And because the aspect of the ^{1}H spectrum is tightly related to the NMR spectrometer frequency, Sadtler ^{1}H spectra are not ideal for the comparison between the measured spectra and standard spectra.

There are several ways to find standard spectra from the Web.

① Find standard ^{1}H spectra (and ^{13}C spectra, IR spectra and EI mass spectra) free of charge.

The National Institute of Advanced Industrial Science and Technology (Japan) set up the following Web site:

http://www.aist.go.jp/RIODB/SDBS/cgi-bin/direct_frame_top.cgi?lang = eng,

One could find standard spectra there free of charge. This Web site has now been changed into

http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang = eng,

After one signs an agreement for listed terms, one can use this data base. The compounds, which have the same elemental composition, are found from the data base by inputting a particular elemental composition. After one chooses an item (a compound), its structural formula, ^{1}H spectra, ^{13}C spectra, IR spectra and EI mass spectra are shown. The solvent applied in NMR measurement and the frequency of the NMR spectrometer applied are listed, too.

② If the institute or the university with which the reader is associated has an agreement with the following two Web sites, abundant spectra and spectral data can be found. However, individuals can not access it.

http://166.111.120.35/database/crossfire.htm

https://scifinder.cas.org

③ After registration, standard ^{1}H spectra, ^{13}C spectra and IR spectra can be obtained from the BIO-RAD Company by payment. Its Web site is as follows:

http://www.bio-rad.com/B2B/BioRad/product/br_category.jsp?BV_SessionID = @@@@0825957604.1237457197@@@@&BV_EngineID = ccciadegkhhjflhcfngcfkmdhkkdfll.0&categoryPath = Catalogs%2fInformatics + %7c + Sadtler2fSpectral + Databases&divName = Informatics + %7c + Sadtler&catLevel= 3&lang = English&country = HQ&loggedIn = false&catOID = -40660&isPA = false&serviceLevel = Lit + Request

1.6 Examples of ^{1}H Spectrum Interpretation

Because several kinds of spectra are needed to deduce an unknown structure, the ^{1}H spectrum interpretation is only one part of this task. However, the importance of the ^{1}H

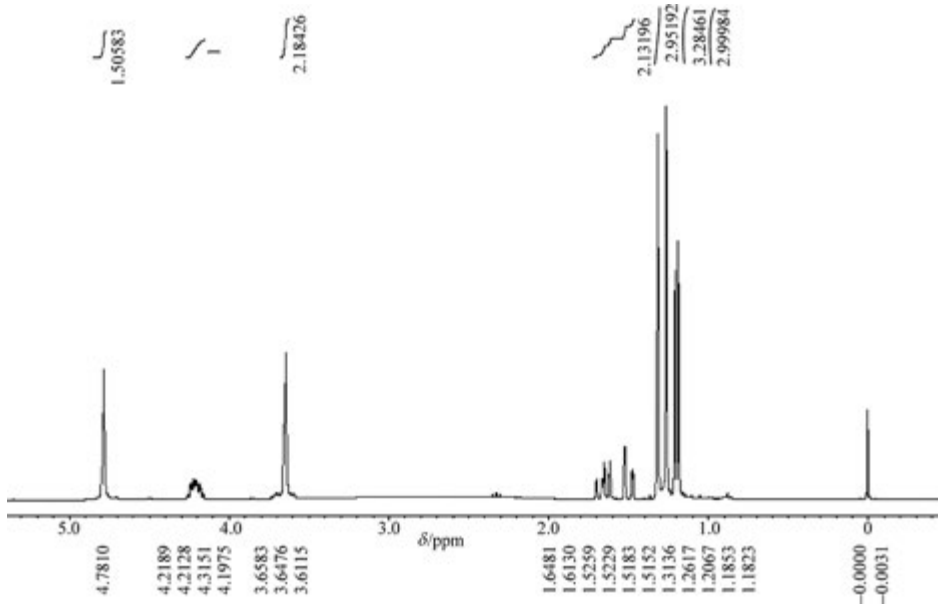


Figure 1.5 ^{1}H spectrum of an unknown compound

spectrum interpretation can be understood from these examples. The method for the interpretation is described here.

Example 1.1 The molecular formula of an unknown compound is determined as $C_6H_{12}O_2$ by mass spectrometry. Its ^{1}H spectrum is shown in Figure 1.5. Try to postulate what its structure is. The frequency of the spectrometer applied is 300 MHz.

Solution

The unsaturation number, 0, is calculated from the molecular formula. This value means the unknown compound is a saturated compound without rings.

The peak at 4.78 ppm is the peak from water in the sample.

The singlet at 3.65 ppm, which corresponds to two hydrogen atoms, was removed after several drops of D_2O were added to the sample solution and the solution was shaken and measured again. Therefore, this peak is that of two reactive hydrogen atoms. Since the elemental formula of the unknown compound contains only oxygen atoms as heteroatoms, the reactive hydrogen atom must correspond to two hydroxyl groups. Because the two hydroxyl groups have a rapid exchange rate, the two hydroxyl groups show only one peak.

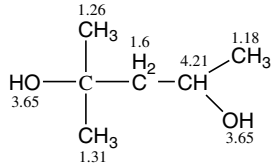
The doublet at 1.18 ppm, which has the smallest δ value in the ^{1}H spectrum, should correspond to a methyl group connected with a CH group.

Two singlets at 1.26 and 1.31 ppm, each of which has the integral value of 3, should be two isolated methyl groups connected to quaternary carbon atoms.

The multiplet at 4.21 ppm corresponds to a hydrogen atom. The multiplet is formed from the couplings by its adjacent functional groups.

The peak set at 1.6 ppm corresponds to two hydrogen atoms. Therefore, it is a methylene group. The left side of the peak set shows clearly a quartet. The right side of the peak set seems to be a doublet. In fact, the latter has a peak shaped as d × d, because the value of the second coupling constant is small. The distance from the left first line to the left third line, which is equal to that from the left second line to the left fourth line, corresponds to the larger coupling constant, the ^2J between the two geminal hydrogen atoms. And the smaller coupling constant is that between the CH_2 group and its adjacent CH group.

By the above-mentioned analysis, we can postulate the structure of the unknown compound and complete its assignment:



C1-8

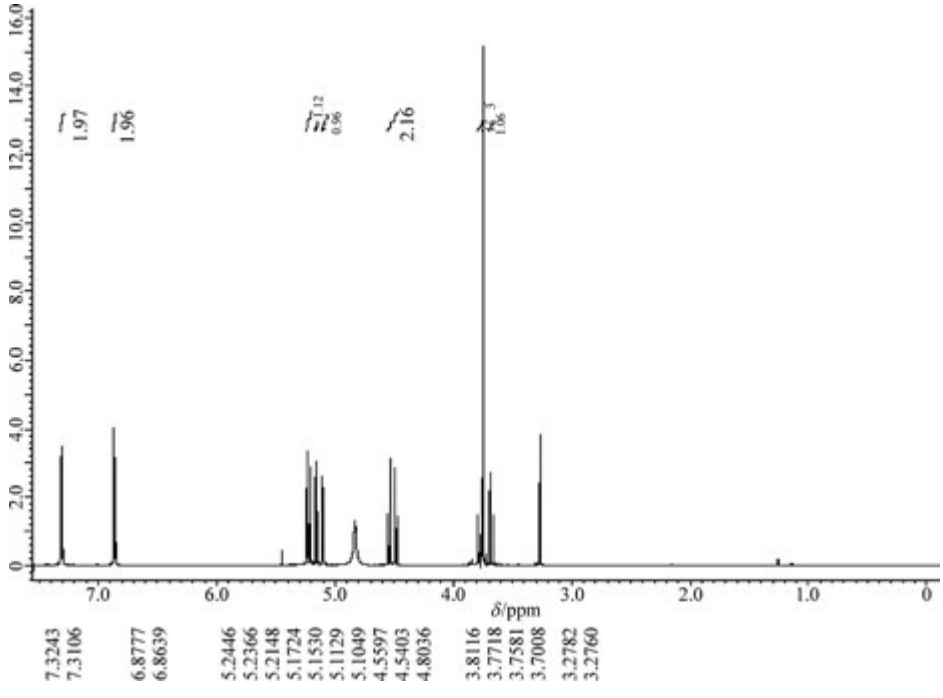


Figure 1.6 ^1H spectrum of a compound

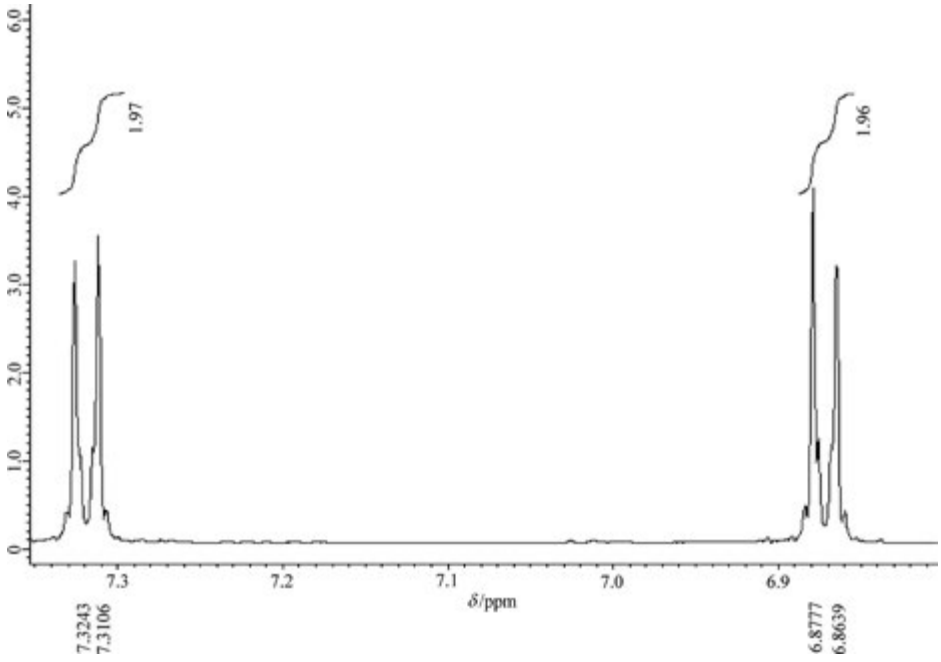
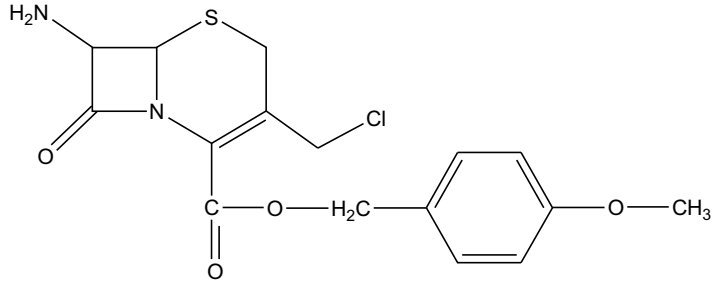


Figure 1.7 Locally enlarged spectrum (1) of the ^{1}H spectrum

Example 1.2 A compound has the following structure:



C1-9

Its ^{1}H spectrum is shown in Figure 1.6. Its locally enlarged spectra of the ^{1}H spectrum are shown in Figures 1.7–1.9, respectively. Try to analyze these spectra if they coincide with the structure above. All NMR spectra were measured with a 600 MHz spectrometer. The solvent applied for the measurement is deuterated methanol.

Solution

This example concerns only the analysis of the coincidence between the ^{1}H spectra and the structure. If an unknown structure needs to be deduced, the ^{1}H spectrum, ^{13}C spectrum, DEPT spectrum, COSY spectrum, HSQC spectrum, HMBC spectrum and mass spectrometry data are necessary.

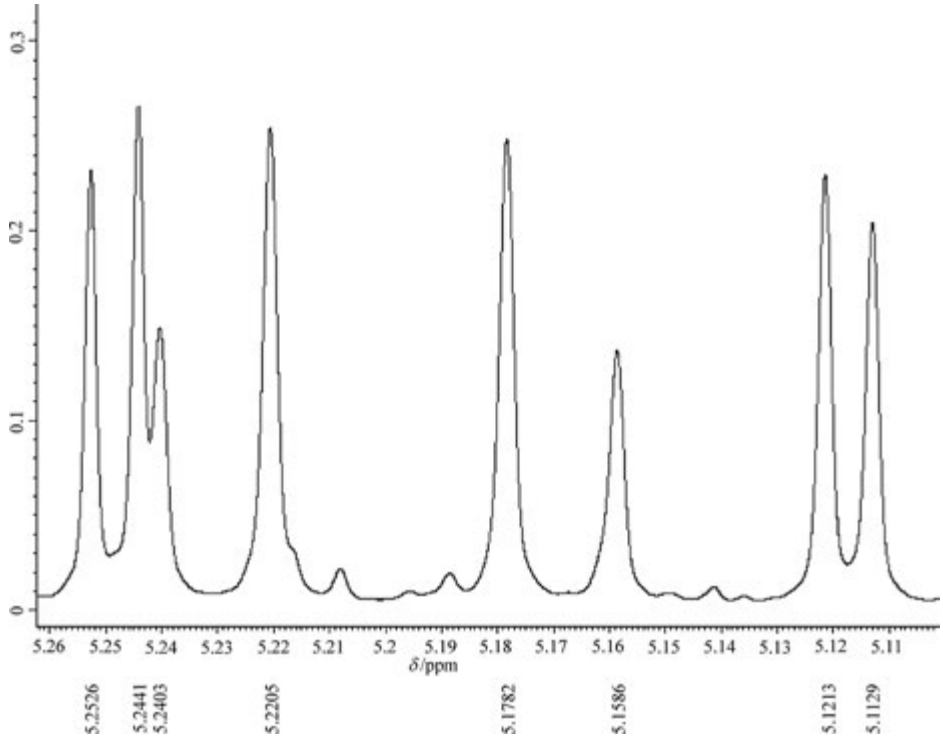


Figure 1.8 Locally enlarged spectrum (2) of the ^{1}H spectrum

The peak at 3.38 ppm is the solvent peak and the peak at about 4.75 ppm is the water peak. The peak of the amino group merges into the water peak.

The data of the ^{1}H spectrum can be summarized in Table 1.5.

It should be noticed that coupling constants read from coupled splittings may have errors because of the measured errors.

We now interpret the spectra from the left side to the right side.

The two sets of doublets at the left edge of the ^{1}H spectrum correspond to two hydrogen atoms, respectively. We are familiar with the peak shape, which corresponds to a para-substituted phenyl ring. Since the ^3J plays a leading role in the split shape, the peak shape shows roughly a doublet. The two peaks at 7.324 and 7.311 ppm, respectively, which correspond to altogether two hydrogen atoms, should be the peaks of two hydrogen atoms, which are situated at the ortho-position respective to the CH_2 group. Since the CH_2 group belongs to the first type of substituents, its ortho-hydrogen atoms have a δ value close to that of the unsubstituted phenyl ring. The two peaks at 6.878 and 6.864 ppm, respectively, which correspond to two hydrogen atoms, should be the peaks of two hydrogen atoms, which are situated at the ortho- position respective to the methoxy group. Since the methoxy group belongs to the second type of substituents, its ortho-hydrogen atoms have a δ value less than that of the unsubstituted phenyl ring.

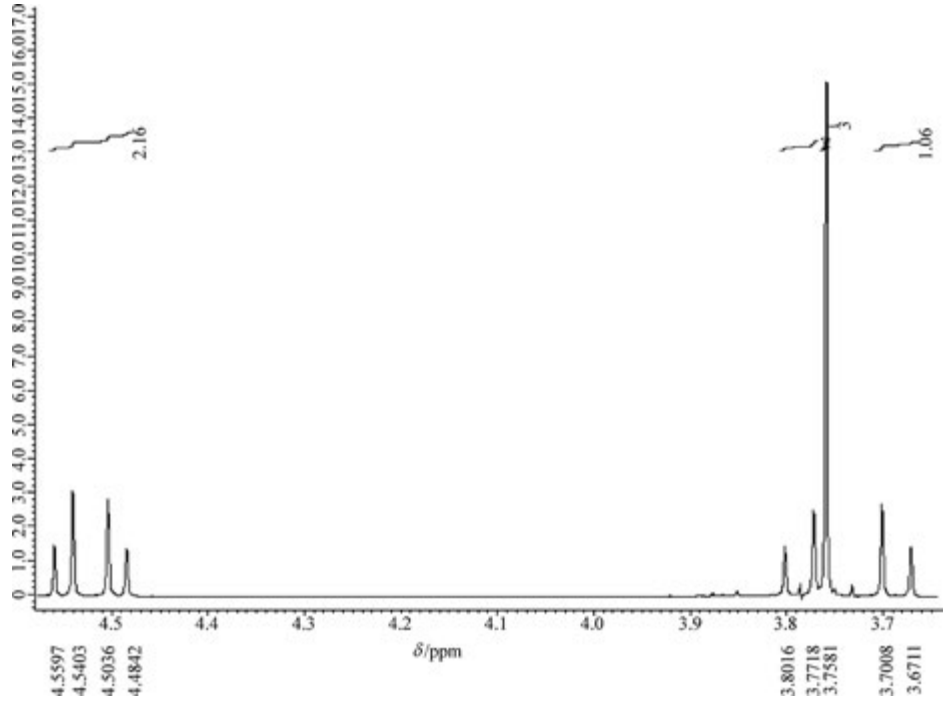


Figure 1.9 Locally enlarged spectrum (3) of the ^{1}H spectrum

Now we are to interpret the peak sets in the 5.253–5.113 ppm region. There are four doublets in this region and every doublet corresponds to one hydrogen atom. Coupling constants can be calculated from the distances in doublets. They are listed in Table 1.5. From the distances between 5.253 and 5.244 ppm and between 5.121 and 5.113 ppm, coupling constants 5.1 and 5.0 Hz are calculated, respectively. We can know these two doublets are coupled to each other. An error of between 5.0 and 5.1 is tolerable. The value of

Table 1.5 Summarized data from the ^{1}H spectra

δ_H (ppm)	Peak area	Peak shape	Coupling constant (Hz)
7.324, 7.311	2	d	8.2
6.878, 6.864	2	d	8.3
5.253, 5.244	1	d	5.1
5.240, 5.221	1	d	11.9
5.178, 5.159	1	d	11.8
5.121, 5.113	1	d	5.0
4.559, 4.540	1	d	11.6
4.504, 4.484	1	d	11.6
3.802, 3.772	1	d	17.9
3.758	3	s	
3.701, 3.671	1	d	17.8

5.0 Hz is smaller than that of the ordinary ^3J because of the effect from the substitution of an electronegative group and the effect of the four-membered ring. Therefore, these two doublets can be assigned as the two hydrogen atoms which are situated in the four-membered ring. And the further assignment in the four-membered ring is accomplished by using other NMR data.

The other two doublets: 5.240 and 5.221; 5.178 and 5.159 ppm have almost the same distance, from which two coupling constants 11.8 and 11.9 Hz are calculated. Obviously, this value corresponds to ^2J. Therefore, these two doublets are assigned as the CH_2 group situated between the phenyl ring and the carboxyl group.

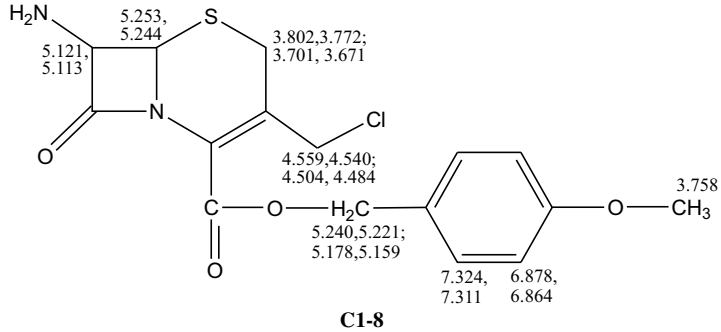
There are two doublets in the region of 4.559–4.484 ppm. Because the two doublets correspond to two hydrogen atoms and the two doublets have the same coupling constant 11.6 Hz, which is the value of ^2J, these two doublets should be a CH_2 group.

There are two doublets and a singlet in the region of 3.802–3.671 ppm. The isolated singlet can be assigned as the methoxy group on the basis of its δ value, number of hydrogen atoms and peak shape. The two doublets correspond to two hydrogen atoms. Their coupling constants can be calculated as 11.9 and 11.8 Hz. Therefore, the two doublets should be a CH_2 group also.

Because two hydrogen atoms of a CH_2 group in a ring have larger chemically non-equivalents than those in a chain, we assign the two doublets in 3.802–3.671 ppm as the CH_2 group in the six-membered ring and the two doublets in 4.559–4.484 as the CH_2 group between the chlorine atom and the double bonds.

On the basis of the interpretation above, we come to the conclusion that the structure coincides with the ^1H spectra and that the related assignments are reasonable.

The assignments are shown as follows:

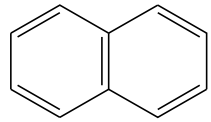


C1-8

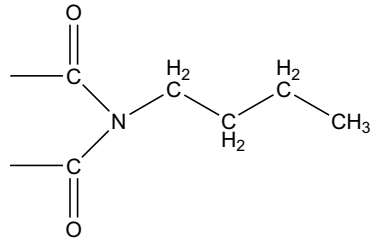
The assignment of the ^1H spectrum is proved by the assignments of other NMR spectra of the compound.

From this example, the symmetrical plan rule can be understood further. Because the structure has no symmetrical plan, the two hydrogen atoms of the three CH_2 groups are not chemically equivalent. And their differences in chemical non-equivalence are obvious.

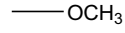
Example 1.3 The following structural units of an unknown compound have been determined from related spectra:



,



and



Try to combine these structural units to get their structure by using their ^{1}H spectra, which were measured by two NMR spectrometers with the frequencies of 300 MHz and 600 MHz, respectively.

Solution

From the comparison between Figures 1.10 and 1.11, we can understand the strongpoint of the NMR spectrometer with a higher frequency. The peak set at 7.70 ppm in Figure 1.10 seems to show a triplet. However, the peak intensity ratio is not 1: 2: 1. That is why the author called for the use of another ^{1}H spectrum with a higher resolution. The peak set at 7.70 ppm in Figure 1.12 shows clearly two doublets, which is very important for the combination of the structural units.

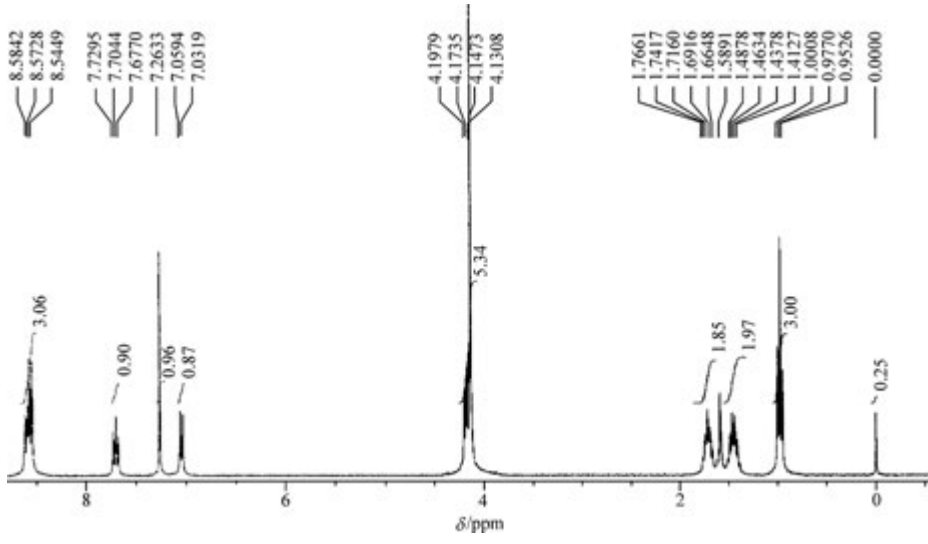


Figure 1.10 The ^{1}H spectrum of the unknown compound (measured by a 300 MHz spectrometer)

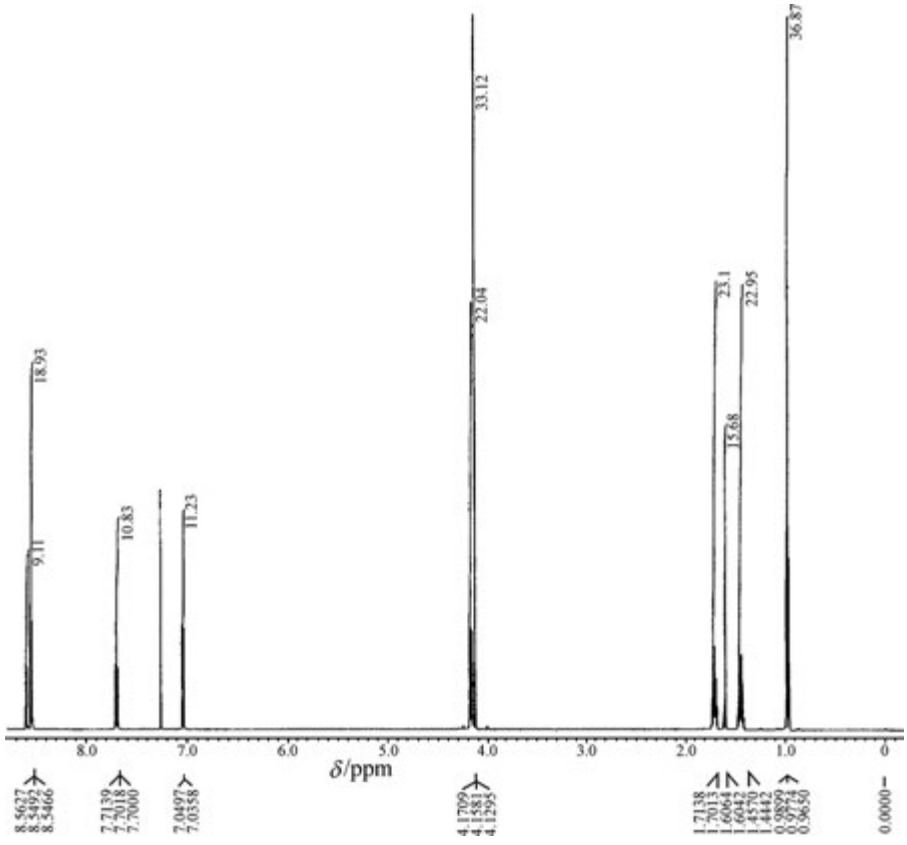


Figure 1.11 The ^{1}H spectrum of the unknown compound (measured by a 600 MHz spectrometer) (Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA.)

Our task is to connect the two latter structural units to the naphthalene ring. There will be many possibilities for the connection if the ^{1}H spectra are not available. Now, on the basis of the information on chemical shift values and the peak shape analysis, especially the latter, the correct and most reasonable structure can be found.

The key to finding the structure is the analysis of the peak shapes of the aromatic hydrogen atoms. The ^{1}H spectrum data (with a 600 MHz spectrometer) are summarized in Table 1.6. The five peak sets in the aromatic region are denoted as Roman numbers I, II, III, IV, and V from the left side to the right side.

Two coupled systems: I–IV–III and II–V can be known from Table 1.6. Since both II and V are doublets, they are two coupled functional groups. The connection of I–IV–III can be determined from the ^3J coupling between I–IV and between IV–III and the ^4J coupling between I–III.

There are four hydrogen atoms on each side of the naphthalene ring (without substitution). II and V are vicinal hydrogen atoms. Since II has a smaller δ value, its adjacent substituent should belong to the second type of substituents. From the structural unit above, the methoxy group can be selected. Since V has a larger δ value, its adjacent substituent should belong to the third type of substituents. From the structural unit above, the carboxyl group can be selected.

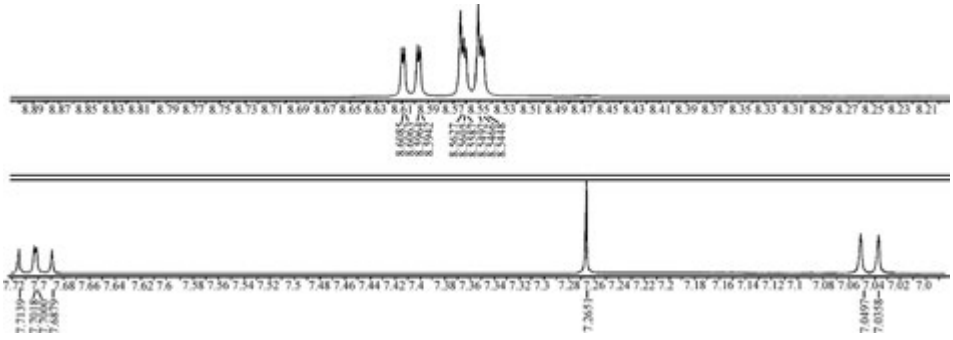


Figure 1.12 The locally enlarged spectrum in the down field region from Figure 1.11 (Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA.)

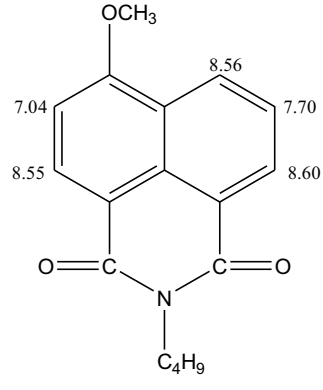
Table 1.6 Summarized ^{1}H spectrum data of the unknown compound

No.	I	II	III	IV	V
δ (ppm)	8.6014	8.5560	8.5227	7.7009	7.0428
Splittings	$d(^3J) \times d(^4J)$	$d(^3J)$	$d(^3J) \times d(^4J)$	$d(^3J) \times d(^3J)$	$d(^3J)$

On the other side of the substituted naphthalene ring, there are three adjacent hydrogen atoms. The only substituent can be determined as the carboxyl group, which is the last substituent mentioned above. Because of the effect of the carboxyl group, it is reasonable that the substituted position of the carboxyl group is the ortho-position of I.

On the basis of the discussion above, the structure of the unknown compound has been postulated, and its assignment is accomplished.

The assignment is shown as follows:



C1-9

Reference

[1] Ning, Yong-Cheng (2005) *Structural Identification of Organic Compounds with Spectroscopic Techniques*, Wiley-VCH, Weinheim, Ch.2.

2

Interpretation of ^{13}C NMR Spectra

The interpretation of the ^{13}C spectrum starting with the characteristics and the advantages will be discussed in this chapter.

2.1 Characteristics and Advantages of the ^{13}C NMR Spectra

We know that the main parameters for the ^{1}H spectrum are chemical shift, coupled split and coupling constant J, and peak area. These parameters are shown in the ^{1}H spectrum.

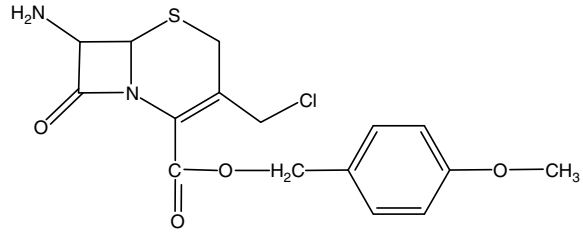
The appearance of the ^{13}C spectrum is greatly different from that of the ^{1}H spectrum.

What we measure for the ^{13}C spectrum are the ^{13}C nuclei. Since the isotopic abundance of ^{13}C is just 1%, the ^{13}C-^{13}C coupling can be omitted. And because the hydrogen atoms are decoupled during the ^{13}C spectrum measurement, in the ^{13}C spectrum there are no split peaks of ^{13}C nuclei caused by the hydrogen atoms connected to carbon atoms, in the ^{13}C spectrum. As a result, only lines are shown in the ^{13}C spectrum. In some special cases, it is possible that there be some blunt peaks in a ^{13}C spectrum. The aim of the decoupling for hydrogen atoms is to focus the split peaks into a singlet. Therefore, the possibility of the overlapping of split peaks of ^{13}C nuclei is reduced to a minimum. And through the decoupling, the intensities of signals of ^{13}C nuclei are enhanced by the Overhauser effect. To sum up, in the ^{13}C spectrum there are only narrow peaks, which appear as lines in general. The heights of the lines reflect approximately the numbers of the related carbon atoms. Only in some special cases, for example, when some peaks are blunt, are the integral values of peak areas necessary.

The abscissa of the ^{13}C spectrum is the chemical shift, and the ordinate the intensities of peaks, which are approximately proportional to the numbers of related carbon atoms. When accurate quantitative data are necessary, or the result gained from ordinary decoupling is not clear, a special pulse sequence is used to obtain an accurate quantitative ^{13}C spectrum.

Interpretation of Organic Spectra, First Edition. Yong-Cheng Ning.
 Published 2011 by John Wiley & Sons (Asia) Pte Ltd. ISBN: 978-0-470-82516-7

The ^{13}C spectrum of compound **C2-1** is shown in Figure 2.1.



C2-1

Because of the characteristics of the ^{13}C spectrum, which will be described below, it is important for the identification of the structure of an unknown compound or for the confirmation of an anticipated structure.

The characteristics and the importance of the ^{13}C spectrum can be known from the following facts.

1. Carbon atoms form the skeleton of the organic compound. Some functional groups, such as carbonyl group and cyanide group, contain no hydrogen atoms but carbon atoms.
2. The varying region of the chemical shift value of the ^{13}C spectrum is much larger than that of the ^{1}H spectrum. The latter is less than 10 ppm but the former can exceed 200 ppm.
3. Besides the narrow varying region, the ^{1}H spectrum has a certain peak width and coupled splits which often lead to the overlapped peak sets. As a result, peak sets in

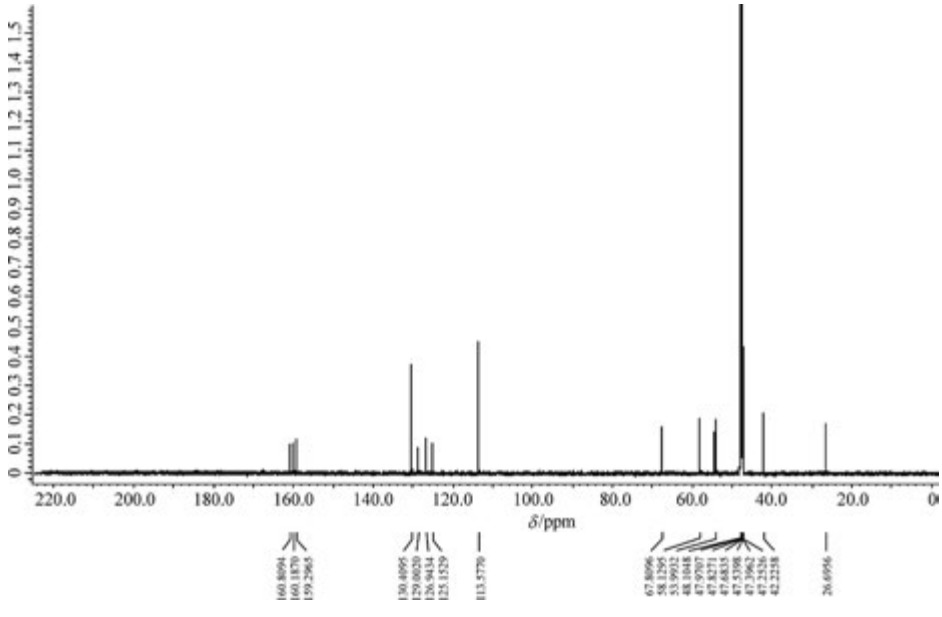


Figure 2.1 The ^{13}C spectrum of compound **C2-1**

the ^{1}H spectrum may be difficult to resolve. So the ^{1}H spectra of natural products can be differentiated with difficulty as fingerprints. However, there are a small number of overlapped peaks in the ^{13}C spectrum. Because the varying region of the chemical shift value of the ^{13}C spectrum is roughly 20 times that of the ^{1}H spectrum and peaks in the ^{13}C spectrum appear as lines, peaks in the ^{13}C spectrum are separate in general. Thus, a slight variation in the structure of a compound can be distinguished clearly in its ^{13}C spectrum. For a compound, which is of no symmetry and has a molecular weight less than about 400, every carbon atom can correspond to a peak in its ^{13}C spectrum. Therefore, natural compounds can be differentiated easily by their ^{13}C spectra.

4. The determination of the order of carbon atoms has been a routine method now, which is useful for the identification of an unknown compound. Therefore, the information, which includes the number of the carbon atoms of the compound, the numbers of primary, secondary, tertiary, and quaternary carbon atoms in the compound, and possible functional group in the compound, can be obtained from its ^{13}C spectrum and the determination of the order of carbon atoms.
5. The ^{13}C spectrum is sensitive to a stereo structure. As a result, it is suitable for the determination of the stereo structure of an unknown compound.

Here it is necessary to describe the low sensitivity of the ^{13}C spectrum.

The ratio of signal to noise, S/N, of an NMR signal is described by Equation 2.1.

$$S/N \propto N\gamma_{\text{exe}}\,\gamma_{\text{det}}^{3/2}\,B_0^{3/2}(NS)^{1/2}\,T_2/T \tag{2.1}$$

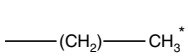
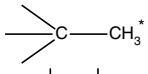
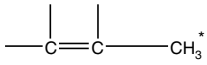
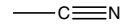
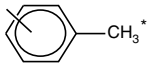
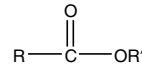
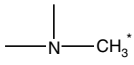
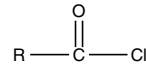
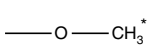
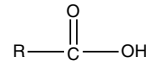
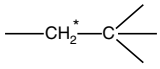
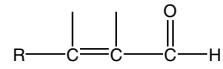
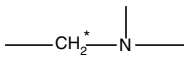
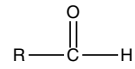
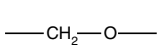
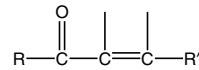
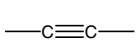
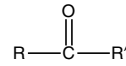
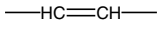
where N is the number of the nuclei to be measured in the effective volume, which is proportional to the isotopic abundance, γ_{exe} is the magnetogyric ratio of the nuclei to be excited, γ_{det} is the magnetogyric ratio of the nuclei to be detected, B_0 is the magnetic induction strength, (NS) is the number of scans for the measurement, T_2 is the transverse relaxation time, and T is the absolute temperature. Only from the consideration based on the isotopic abundance, whose value is 1% for ^{13}C and 100% for ^{1}H, is the ratio of ^{13}C 1% that of ^{1}H. If we consider the value of γ, which for ^{13}C is 1/4 that for ^{1}H, the ratio of ^{13}C will be decreased further. Therefore, the acquisition of the measurement of the ^{13}C spectrum must be accumulated. In 2D NMR experiments, their measurement is not so difficult as that of the ^{13}C spectrum, because there are some mechanisms by which the sensitivity of the detection of ^{13}C nuclei (in 2D NMR experiments) is increased. Therefore, the measurement of the ^{13}C spectrum is the most time-consuming for all NMR experiments.

2.2 The Main Parameter of the ^{13}C Spectrum is the Chemical Shift

We have known that there are lines (peaks) without coupled split in the ^{13}C spectrum and that there are blunt peaks in some extremely special cases. The heights of the peaks are roughly proportional to related carbon atoms. Therefore, the main parameter of the ^{13}C spectrum is the chemical shift (value).

That is why in this chapter we will just discuss the subject of the chemical shift.

Table 2.1 The varying region of the chemical shifts of functional groups in the ^{13}C spectrum

Functional group	δ_C (ppm)	Functional group	δ_C (ppm)
	10–15		110–150
	25–30		125–155
	15–28		110–130
	15–25		165–175
	25–45		165–180
	45–60		172–185
	23–37		165–175
	41–60		200–205
	45–75		195–205
	70–100		205–220
	110–150		

The varying region of the chemical shifts of functional groups in the ^{13}C spectrum is shown in Table 2.1.

We will discuss the chemical shifts of several common functional groups later.

2.3 Chemical Shift Values of Common Functional Groups and Main Factors Affecting Chemical Shift Values

Functional groups have varying regions of their chemical shifts in the ^{13}C spectrum. The factors affecting the chemical shifts are different for different functional groups. We will discuss them according to common functional groups.

From the viewpoint of mechanisms about the chemical shift, there is a great difference between the ^{13}C spectrum and the ^{1}H spectrum. Although the factor dominating the chemical shift for the ^{13}C spectrum is the paramagnetic shielding and that for the ^{1}H

spectrum is the diamagnetic shielding, the effects of these factors have something in common for both the ^{1}H spectrum and the ^{13}C spectrum.

2.3.1 Alkanes and their Derivatives

2.3.1.1 Electronegativity of Substituents

For chain aliphatic alkyl groups, the electronegativity of its substituents is the main factor for its δ value. In the case of the substitution by an electronegative functional group the α-carbon atom will have a considerable downfield shift. The β-carbon atom will have a slight downfield shift. These effects come from the induction effect of the electronegativity of the substituent.

The shifted increments of common functional groups for chain aliphatic alkyl groups are listed in Table 2.2, where the subscripts of α and β denote the relative positions of the discussed carbon atom with respect to the substituent, the subscript of n means the substituent is situated at the terminal edge of the chain aliphatic alkyl group, and the subscript of iso means the substituent is situated as a branch chain.

From Table 2.2, it can be known that some increments are very large.

Table 2.2 The shifted increments of common functional groups for chain aliphatic alkyl groups

Functional group	$Z_{\alpha n}$	$Z_{\alpha iso}$	$Z_{\beta n}$	$Z_{\beta iso}$
–F	70	63	8	6
–Cl	31	32	10	10
–Br	20	26	10	10
–I	–7	4	11	12
$-OCOCH_3$	52	45	6.5	5
–OH	49	41	10	8
$-NH_2$	28.5	24	11.5	10
–NHR	36.5	30	8	7
$-NO_2$	61.5	57	3	4
–CHO	30	—		–2.5
$-COCH_3$	29	23	3	1
–COOH	20	16	2	2
$-CONH_2$	22	—	2.5	—
$-C_5H_5$	23	17	9	7

2.3.1.2 Steric Effect

The steric effect is one of the characteristics of the ^{13}C spectrum.

1. The relationship between the number of substituents and the chemical shift value of the substituted carbon atom

If the hydrogen atoms of an alkyl chain are substituted by alkyl groups, the chemical shift value of the substituted carbon atom will increase. The more branches the substituted carbon atom has, the greater the chemical shift of the substituted carbon atom. This phenomenon can be illustrated by the following data.

$R = CH_3$:	CH_3R	CH_2R_2	CHR_3	CR_4
δ(ppm)	5.7	15.4	24.3	31.4

Table 2.3 Empirical increments used in Equation 2.2 for the calculation of the chemical shift values of a substituted benzene. J.T. Clerc, Structure Analysis of Organic Compounds, Budapest Akademiai Kiado, 1981

Substituent	Z_1	Z_2	Z_3	Z_4
——H	0.0	0.0	0.0	0.0
——CH_3	9.3	0.6	0.0	–3.1
——CH_2CH_3	15.7	–0.6	–0.1	–2.8
——$CH(CH_3)_2$	20.1	–2.0	0.0	–2.5
——$CH_2CH_2CH_2CH_3$	14.2	–0.2	–0.2	–2.8
——$C(CH_3)_3$	22.1	–3.4	–0.4	–3.1
——CH_2Cl	9.1	0.0	0.2	–0.2
——CH_2Br	9.2	0.1	0.4	–0.3
——CF_3	2.6	–3.1	0.4	3.4
——CH_2OH	13.0	–1.4	0.0	–1.2
——C≡CH	–6.1	3.8	0.4	–0.2
——Ph	13.0	–1.1	0.5	–1.0
——F	35.1	–14.3	0.9	–4.4
——Cl	6.4	0.2	1.0	–2.0
——Br	–5.4	3.3	2.2	–1.0
——I	–32.3	9.9	2.6	–0.4
——OH	26.9	–12.7	1.4	–7.3
——OCH_3	30.2	–14.7	0.9	–8.1
——NH_2	19.2	–12.4	1.3	–9.5
——$NHCH_3$	21.7	–16.2	0.7	–11.8
——$N(CH_3)_2$	22.4	–15.7	0.8	–11.8
——NO_2	19.6	–5.3	0.8	6.0
——CHO	9.0	1.2	1.2	6.0
——$COCH_3$	9.3	0.2	0.2	4.2
——COOH	2.4	1.6	–0.1	4.8
——$COOCH_3$	2.1	1.2	0.0	4.4

2. The relationship between the size of the substituent and the chemical shift value of the substituted carbon atom

The larger the size of the substituent, the greater the increment in the chemical shift of the substituted carbon atom.

In the following series, the chemical shift of the carbon atom marked at its upper-left with "*" increases from the left side to the right side.

$$R—*CH_3, R—*CH_2CH_3, R—*CH_2CH_2CH_3, R—*CH_2CH(CH_3)_2, R—*CH_2C(CH_3)_3.$$

This phenomenon can be explained as follows. From a hydrogen atom to a methyl group, an ethyl group, a propyl group, an isobutyl group, and a tert-butyl group, the size of the substituent increases gradually. As a result, the carbon atom marked with * is affected more and more by the steric effect. Therefore, the chemical shift value of the substituted carbon atom increases gradually.

3. γ-Gauche effect of the substituent

Any substitution decreases the chemical shift value of the carbon atom, which is at the γ-position with respect to the substituent. It is known that the substitution of an electronegative functional group increases the chemical shift values of the α- and β-carbon atoms; on the contrary, the chemical shift value of the γ-carbon atom decreases, a phenomenon called the γ-gauche effect, which can be explained by the steric effect. The alkyl chain can rotate. γ-gauche conformation takes about one third of the time. Under this conformation, extranuclear electrons of the γ-carbon atom are "pressed" by the substituent so that they move towards the carbon atom, the latter having a fairly small δ value. After averaging all three conformations, the γ-gauche effect still exists.

2.3.1.3 Heavy Atom Effect

After the substitution by an iodine atom, the substituted carbon atom will have a smaller δ value. This phenomenon can be explained as follows. The numerous electrons of the iodine atom increase the shielding effect of the substituted carbon atom, so that the peak of the latter shifts is upfield.

2.3.2 Cycloalkanes and their Derivatives

The factors affecting the chemical shift value of the alkyl group are still applicable to cycloalkanes and their derivatives.

The factor, which is characteristic of cycloalkanes and their derivatives, is their cyclic tension. The chemical shift values of cycloalkanes vary only slightly from a five-membered to a 17-membered ring. Their δ values are about 26 ppm. Because of the strong tension of the three-membered ring, its δ value decreases greatly to about –2.8 ppm. Also because of the strong tension of the three-membered ring, its absorption band position in the IR spectrum is abnormal compared with those of other saturated rings. We will discuss it in Chapter 5.

2.3.3 Alkylenes and their Derivatives

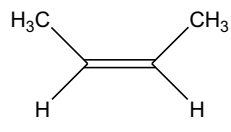
The chemical shift of ethylene is 123.3 ppm and that of its derivatives 100–150 ppm in general. There is roughly the following order

$$\delta_{C=} \rangle \delta_{CH=} \rangle \delta_{CH_2=}.$$

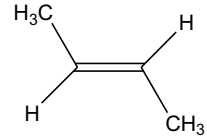
The discussion for alkanes and their derivatives are still applicable to alkylenes and their derivatives.

Two items need to be added here.

γ-Gauche Effect Because of the γ-gauche effect, trans-isomer and cis-isomer can be differentiated from each other by their chemical shift values. Examples are as follows.



C2-2



C2-3

The chemical shifts of the two methyl groups of cis-butene-2, **C2-2**, are smaller than those of trans-butene-2, **C2-3,** by 5 ppm. By using this method, the satisfactory differentiation of cis-isomer from trans-isomer was achieved by the author on many occasions.

Conjugation Effect When two double bonds are conjugated, the chemical shift values of the two middle carbon atoms will decrease because their bond orders decrease.

2.3.4 Benzene and its Derivatives

The chemical shift of benzene is 128.5 ppm.

The discussion about the chemical shift values of substituted benzene concerns two aspects: the chemical shift value of the substituted carbon atom and those of remaining carbon atoms in the substituted benzene.

2.3.4.1 Chemical Shift Value of the Substituted Carbon Atom

The factors (the electronegativity of the substituents, the steric effect, the heavy atom effect) discussed in Section 2.3.1 are still applicable to the substituted carbon atom.

The chemical shift value of the substituted carbon atom by a hydroxyl group will increase by an amount of 26.9 ppm and that of an amino group by an amount of 19.2 ppm.

The following examples refer to the steric effect.

When a benzene is substituted by $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, and $-C(CH_3)_3$, respectively, the chemical shift of the substituted carbon atom will increase correspondingly, 9.3, 15.7, 20.1, and 22.1 ppm, respectively.

2.3.4.2 Chemical Shift Values of Ortho-, Meta-, and Para-Carbon Atoms

The concept of the three types of substituents is still suitable for the consideration of δ values of these remaining carbon atoms.

The first type of substituents contains alkyl, alkylene, and halogen atoms. After the substitution, the remaining carbon atoms, including the ortho-, meta- and para-carbon atoms, roughly have their original δ values.

The second type of substituents contains saturated heteroatoms. After the substitution, the signals of the carbon atoms of the ortho-, meta-, and para-positions shift toward the upfield, with this shift occurring especially the ortho-carbon atoms, and then the para-carbon atoms.

The third type of substituents contains unsaturated heteroatoms. After the substitution, the signals of the carbon atoms of the ortho-, meta-, and para-positions shift toward downfield. However, the downfield shifts are slight, not like in the ^1H spectrum.

The chemical shifts of a substituted benzene molecule can be calculated approximately by Equation 2.2.

$$\delta = 128.5 + \sum_i Z_j(R_i) \tag{2.2}$$

where j = 1, 2, 3, and 4, which illustrate the relative positions of considered carbon atoms with respect to the substituent; Z_1, Z_2, Z_3 and Z_4 are the chemical shift increments for the substituted ortho-, meta-, and para-carbon atoms, respectively.

If a benzene molecule is substituted by several functional groups, the chemical shift of a considered carbon atom can be calculated approximately from the addition of the calculation obtained by using Equation 2.2.

2.3.5 Carbonyl Groups

The peaks of carbonyl groups are situated at the leftmost side in the ^{13}C spectrum.

There are two main factors affecting the chemical shift values of carbonyl groups.

2.3.5.1 Substitution by Heteroatoms

After the substitution of a carbonyl group by a heteroatom, the chemical shift of the substituted carbonyl group will shift towards the upfield. The chemical shifts of ketone groups are slightly greater than 200 ppm. After the substitution by a heteroatom, their chemical shifts will be less than 180 ppm. This effect is stronger than the conjugation effect, which will be discussed below.

2.3.5.2 Conjugation Effect

When a carbonyl group is substituted by a double bond, they form a larger conjugation system. As a result, the chemical shift value of the substituted carbonyl group will decrease. However, this effect is weaker than that of the substitution by a heteroatom. The shifted amount is about 10 ppm.

2.4 Determination of the Carbon Atom Orders

The determination of the carbon atom order means the ascription of every carbon atom to primary, secondary, tertiary, or quaternary carbon atom, respectively, that is, the ascription of every carbon atom to CH_3, CH_2, CH, or C, respectively. Obviously, it is very important to identify the structure of an unknown structure.

The ordinary method to determine the carbon atom order is DEPT experiment. This method uses a pulse sequence, whose last tipped angle is one of the following angles: 45°, 90°, or 135°.

When the last tipped angle is 45°, in which case the experiment is named DEPT-45, the spectrum shows the peaks of all CH, CH_2, and CH_3 groups with all peaks upward.

When the last tipped angle is 90°, in which case the experiment is named DEPT-90, the spectrum shows only the peaks of all CH groups, with all peaks upward.

When the last tipped angle is 135°, in which case the experiment is named DEPT-135, the spectrum shows the peaks of all CH and CH_3 groups with the peaks upward and the peaks of all CH_2 groups with the peaks downward.

There are no peaks of the quaternary atoms in all of the three types of DEPT spectrum.

It should be known that the heights of peaks in DEPY spectra may not be the maximum signals. For example, the heights of the peaks of CH_2 are the maximum signals (absolute values) but those of CH and CH_3 are not maximum signals in the DEPT-135 spectrum.

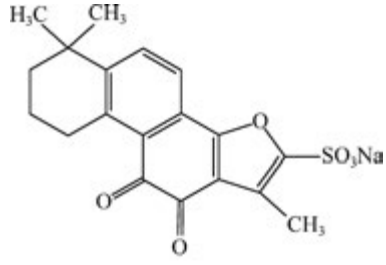
Because the information obtained from the DEPT-135 almost contains that obtained from DEPT-45 and DEPT-90, the routine method to determine the carbon atom orders is just DEPT-135, which can be simplified as DEPT.

It must be pointed out that the results mentioned above are ideal. Generally speaking, the precondition for the determination of carbon atom orders is that the values of the $^1J_{C-H}$'s be kept the same. However, the $^1J_{C-H}$ values vary with the structural environments of carbon atoms. The advantages of the DEPT experiment are that it does not require strictly the same value of the $^1J_{C-H}$ and that it can give a good result in general. However, it can be true that the result obtained from the DEPT experiment is not clear (for example, the ambiguity between a positive peak and a negative peak in a narrow region) if the $^1J_{C-H}$ values vary by a large amount.

The $^1J_{C-H}$ value in a saturated structural unit is about 125 Hz and that in an unsaturated structural unit is about 157 Hz. To compromise these two different values, 140 Hz is used for the ordinary DEPT experiment. It is possible that the result obtained from the DEPT experiment is not clear in some cases.

If the result obtained by a DEPT experiment is not clear, the heteronuclear shift correlation spectroscopy, such as HMQC or HSQC (spectra), can clearly determine the carbon atom orders through the correlation between the signals in the ^1H spectrum and those in the ^{13}C spectrum.

The DEPT spectrum of compound **C2-4** is shown in Figure 2.2.



C2-4

2.5 Steps for ^{13}C NMR Spectrum Interpretation

From the description above, we have known that the main parameter of the ^{13}C spectrum is the chemical shift. Although the ^{13}C spectrum can characterize the structure of an unknown compound, several kinds of NMR spectra are needed for the identification of an unknown structure. Therefore, we will not describe how to deduce an unknown structure but emphasize how to get related structural information from its ^{13}C spectrum.

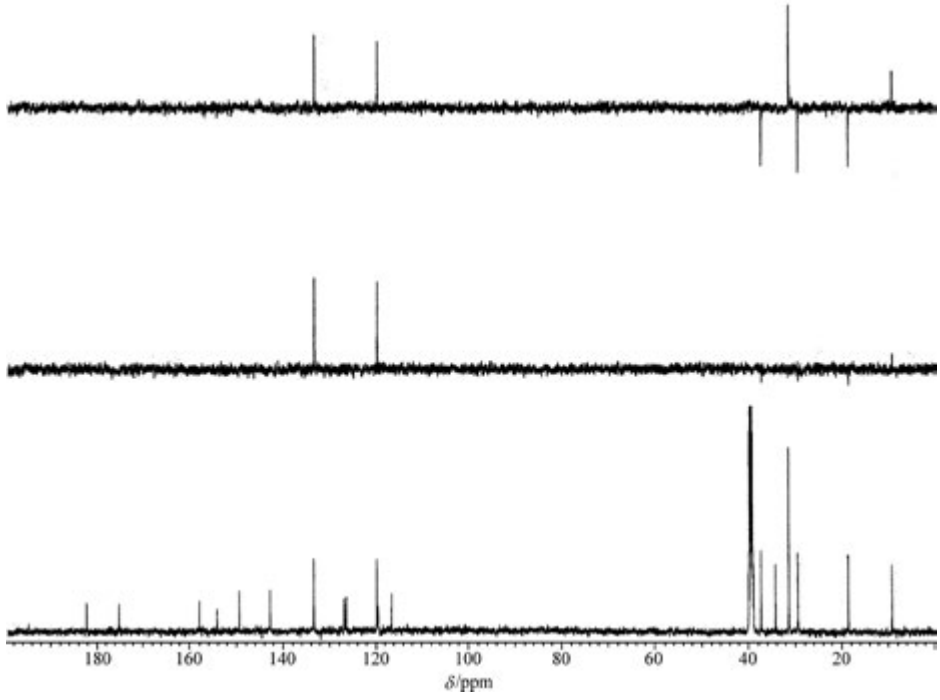


Figure 2.2 The DEPT spectrum of the compound **C2-4**

The method to analyze the ^{1}H spectrum for the identification of an unknown structure, especially the skill, that is, the peak shape analysis, is systematically presented in Chapter 1. However, the peak shape analysis is not needed in the interpretation of the ^{13}C spectrum.

To sum up, we will present only how to obtain related structural information from the ^{13}C spectrum. It does not include how to deduce an unknown structure in an all-round way.

The following steps are recommended.

2.5.1 Recognizing Impurity Peaks and Identifying Solvent Peaks

Because the amount of impurities should be much fewer than that of the sample to be measured, the peaks of impurities can be discerned from those of the sample.

Since before and after the measurement of the ^{13}C spectrum the measurements of the ^{1}H spectrum and related 2D NMR spectra are necessary, and one of deuterated solvents must be used as the solvent for the measurement of the ^{13}C spectrum.

There is a great difference between the measurement of the ^{13}C spectrum and that of the ^{1}H spectrum. The solvent peak in the ^{1}H spectrum comes from the fact that deuterated solvents can not be 100% deuterated isotopically. However, the solvent peak in the ^{13}C spectrum comes from the signals of the carbon atoms of the solvent applied, which does not concern the degree of deuteration. It must be known that the amount of the carbon atoms of the derterated solvent in the measured volume is much larger than that of the sample measured. If we estimate the intensity of the solvent peak according

Table 2.4 The chemical shifts of common solvents

Deuterated solvent	$CDCl_3$	CD_3SOCD_3 (DMSO)	CD_3OD	CD_3COCD_3	
δ (ppm)	77.0	39.7	49.3	30.2	206.8
Peak shape (number of peaks)	3	7	7	7	1

to ordinary consideration, the intensity of the solvent peak would be much higher than that of the sample peaks. Fortunately the intensities of solvent peaks are much lower than those of the samples in general because some relaxation mechanisms of solvents decrease its signal intensity.

The signals for common solvents in ^{13}C spectra are shown in Table 2.4. If one does not know what solvent was used in the measurement, the solvent can be known from the chemical shift value of the solvent peak.

By "peak shape" in Table 2.4 is meant the number of peaks, which is expressed with an Arabic number. Because the spin number of the derterium element is 1, the peak number split by derterium atoms should be calculated by the $2nI + 1$ rule. And the peak intensity ratio for a peak set is not the coefficient of a developed binomial. For example, the peak intensity ratio of the solvent CDCl is 1 : 1 : 1 (not 1 : 2 : 1). However, the intensity of the middle peak of the three peaks may be slightly higher than those of the other two peaks.

When deuterated water is used as the solvent, of course there is no solvent peak.

2.5.2 Calculation of the Unsaturation Number of the Unknown Compound

Please refer back to Section 1.5.2.

2.5.3 Consideration of Chemical Shift Values of Peaks

On the basis of the chemical shift value, the corresponding functional group can be roughly determined.

δ_C can be divided into three regions.

1. The region of carbonyl groups and accumulated alkenes where $\delta_C > 150$ ppm, and in general $\delta_C > 165$ ppm

   Because it is situated in the lowest downfield region, it is easy to recognize.

   In general, the signals of carbonyl groups are weak, because of their long relaxation times.

   If $\delta_C > 200$ ppm, it means the existence of an aliphatic aldehyde or ketone group. The connection of a carbonyl group with a heteroatom shifts δ_C within the range of 160–180 ppm. Carboxylic acids, esters, acid halides and so on belong to this case.

   The conjunction of a carbonyl group with a double or triple bonds shifts δ_C in the upfield direction by less than 10 ppm.

   If a compound contains an accumulated alkene, C = C = C, the central carbon atom has a peak in this region. However, the two other carbon atoms have two peaks in the region of double bonds.

2. The region of double bonds and phenyl rings where $\delta_C = 90–160$ ppm, and in general $\delta_C = 100–150$ ppm

Alkenes, phenyl rings, nitrile groups (their carbon atoms), and so forth have their signals in this region.

From the carbon atoms whose signals are situated in these two regions, their corresponding unsaturation number can be calculated. The difference between this number and that calculated from the molecular formula equals the number of the formed cycles of the molecule.

3. The region of saturated carbon atoms

In general $\delta_C < 100$ ppm. If a carbon atom is not connected with a heteroatom, its chemical shift is less than 55 ppm in general.

Alkyne groups have signals within the range of 70–100 ppm, which is an exception of unsaturated carbon atoms.

2.5.4 Determination of Carbon Atom Orders

The carbon atom orders are determined by the DEPT experiment in general. If the result obtained is not clear, the combination of the heteronuclear shift correlation spectrum and the ^1H spectrum can lead to a good result.

After the determination of the orders, the number of the hydrogen atoms, which are directly connected with carbon atoms, can be determined. If this number is less than that in the molecular formula, the other hydrogen atoms should be reactive hydrogen atoms, that is, the hydrogen atoms connected with heteroatoms.

2.5.5 Postulation of Possible Functional Groups

Functional groups can be deduced from the chemical shifts and the carbon atom orders of its related peaks.

How is it possible to assemble these deduced functional groups? It needs to combine related spectra.

Please refer back to Section 1.5.8 to find standard spectra from the web. The data base of the ^{13}C spectrum and that of the ^1H spectrum are closely linked.

3

Interpretation of 2D NMR Spectra

The development and perfection of 2D NMR spectra is a milestone in NMR spectroscopy. Professor R. R. Ernst, who has made outstanding contributions to 2D NMR theories, won solely the Nobel Prize in chemistry in 1991, a brilliant illustration.

The structure of an unknown compound can be deduced objectively and reliably by using its ^{1}H spectrum, ^{13}C spectrum and related 2D NMR spectra without related chemical knowledge, such as the relationship between the chemical shift value and the chemical properties of functional groups. Of course, the mass data of the unknown compound are necessary in general. If an unknown compound has a very complicated structure, even all related NMR spectra, including its ^{1}H spectrum, ^{13}C spectrum and 2D NMR spectra, are not sufficient to deduce its structure. In this case, the application of other methods, for example, the X-ray diffraction of the single crystal, is necessary.

In Chapter 6, we will deal with the limited kinds of 2D NMR spectra which are in common use. Considering the fact that readers may use other kinds of 2D NMR spectra in practice, the kinds of 2D NMR spectra presented here are more than those introduced in Chapter 6.

Since each kind of 2D NMR spectrum has its own features, we will present them according to their kinds. However, our aim is to determine an unknown structure or to confirm the structure of an anticipated structure, so only 2D NMR spectra, which are tightly related to the aim, will be presented.

3.1 General Knowledge about 2D NMR Spectra

Before the discussion of 2D NMR spectra, it is necessary to introduce some general knowledge about 2D NMR spectra.

Each 2D NMR spectra is obtained by the application of a particular pulse sequence. The principle of 2D NMR spectroscopy is the explanation of the function of pulse sequences. Readers who are interested in this topic can read the reference [1].

Interpretation of Organic Spectra, First Edition. Yong-Cheng Ning.
 Published 2011 by John Wiley & Sons (Asia) Pte Ltd. ISBN: 978-0-470-82516-7

The appearance of 2D NMR spectra is the contour plot, which is similar to the contour map. The number of circles denotes the intensity of the peak. The form of 2D NMR spectra is a rectangle. Its abscissa is denoted as ω_2 or F_2, and the ordinate, ω_1 or F_1. Both ω and F mean the frequency. Either the abscissa or the ordinate corresponds to the chemical shifts in the ^1H spectrum or in the ^{13}C spectrum. To sum up, a 2D NMR spectrum correlates two kinds of NMR spectra, for example, the heteronuclear shift correlation spectrum, or reveals the correlation between two peak sets for one kind of NMR spectra, such as the COSY spectrum or the NOESY spectrum. Therefore, a "cluster" obtained by changing experimental parameters, such as temperature, concentration, pH values, and so forth, is not a 2D NMR spectrum, although the cluster has a three dimensional appearance. Based on the same reason, the spectrum cluster obtained from the T_1 measurement is not a 2D NMR spectrum either.

The nuclei, whose chemical shift values are shown in the abscissa, are the nuclei measured in the 2DNMR experiment. For example, an H, C-COSY spectrum correlates the ^{13}C spectrum and the ^1H spectrum of a compound. The abscissa of the spectrum is the chemical shift of the ^{13}C spectrum. The nuclei measured in this experiment are ^{13}C nuclei. The ordinate (ω_1 or F_1) of the spectrum is the chemical shift of the ^1H spectrum. In this experiment, ^1H nuclei are not measured. The δ_H values and the correlation between the δ_H values and those of the δ_C are obtained by using a particular pulse sequence.

Some instrument companies exchange the abscissa and the ordinate of some 2D NMR spectra in an inverse mode. We will present them later.

The useful information on 2D NMR comes from cross peaks, also named correlated peaks. A cross peak corresponds to two chemical shift values, the abscissa and the ordinate, respectively. The cross peak illustrates the correlation between these two chemical shift values.

For some experimental factors, artifacts may exist in a 2D NMR spectrum. A simple method to judge if a (cross) peak is an artifact is to examine its abscissa and ordinate. If the abscissa and the ordinate of a "correlated" peak do not correspond to any chemical shift values in the ^1H spectrum or in the ^{13}C spectrum, either of which corresponds to the 2D NMR spectrum, this "correlated" peak is certainly an artifact.

There are some exceptions, which will be discussed in the HMBC spectrum.

3.2 Homonuclear Shift Correlation Spectroscopy, COSY (H, H-COSY)

Homonuclear Shift Correlation Spectroscopy, COSY (H, H-COSY) is the commonest 2D NMR spectrum, because it is very useful and the measurement of the COSY spectrum is more rapid than that of all other 2D NMR spectra.

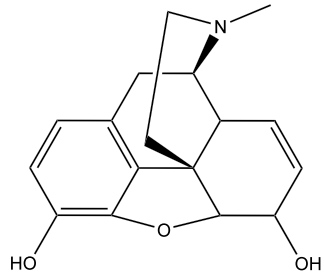
The projection of a COSY spectrum on the ω_2 (F_2, horizontal) axis or on the ω_1 (F_1, vertical) axis is the ^1H spectrum of the sample, which is set at the top and the right (or left) of the COSY spectrum. The lateral ^1H spectrum can be omitted. A COSY spectrum has the form of a square or a rectangle (when F_2 and F_1 are not in the same scale). The form of a rectangle is common. There is a diagonal, which is the symmetrical axis of the COSY spectrum. Frequently the diagonal rises from left to right. The peaks on the diagonal are called diagonal peaks or auto-correlated peaks while the peaks, which are situated off the diagonal, are called cross peaks or correlated peaks. The cross peaks are symmetrically

distributed about the diagonal. Therefore, from the cross peaks under (or above) the diagonal all information on the COSY spectrum is obtained. The COSY spectrum mainly illustrates coupling relations through ^3J. If we draw a vertical line through the center of a cross peak to be discussed, the vertical line will pass through the center of a peak set in the ^{1}H spectrum at the top of the COSY spectrum. This peak set is one of the two coupled peak sets that lead to the cross peak. Then we draw a horizontal line through the center of the cross peak, which will pass through a diagonal peak. A vertical line passing through the diagonal peak will cross the center of another peak set in the ^{1}H spectrum. This peak set is another one contributing to the cross peak. Therefore, a pair of the coupled peak sets can be found from any cross peak of a COSY spectrum. This information replaces the peak shape analysis in the ^{1}H spectrum interpretation.

Although the COSY reveals the coupling relationships through ^3J, some couplings through ^4J (even ^5J in some aromatic systems) with a rather large coupling constant can be found in a COSY spectrum. On the other hand, some couplings through ^3J with a rather small value can not be found in COSY spectra, for example, when the related dihedral angle is near 90°.

The interpretation method and the function of the COSY spectrum can be illustrated through the following example.

Compound **C3-1** has the following structure.



C3-1

Its ^{1}H spectrum, its locally enlarged spectra of the ^{1}H spectrum in the high field region, its COSY spectrum, and its locally enlarged spectra of the COSY spectrum (in the high field) are shown in Figures 3.1–3.4, respectively. All NMR spectra were measured by using an NMR spectrometer with a frequency of 600 MHz. The applied solvent is deuterated DMSO.

Try to assign the ^{1}H spectrum.

Solution

We interpret the ^{1}H spectrum first. The strong peak at 2.50 ppm is the solvent peak. The peak at 3.33 ppm is the water peak. Their integral values are not denoted in the ^{1}H spectrum.

The peak set at about 2.20 has its integral value of 2.3. An important clue about this peak set is its peak shape, which is not symmetrical. Splits are shown in the right part of the peak set. And a blunt shape is shown in the left part of the peak set. These facts reveal that the peak set is composed of two partially overlapped peak sets, which correspond to two hydrogen atoms.

After every peak set in the ^{1}H spectrum has been analyzed, all data can be summarized in Table 3.1.

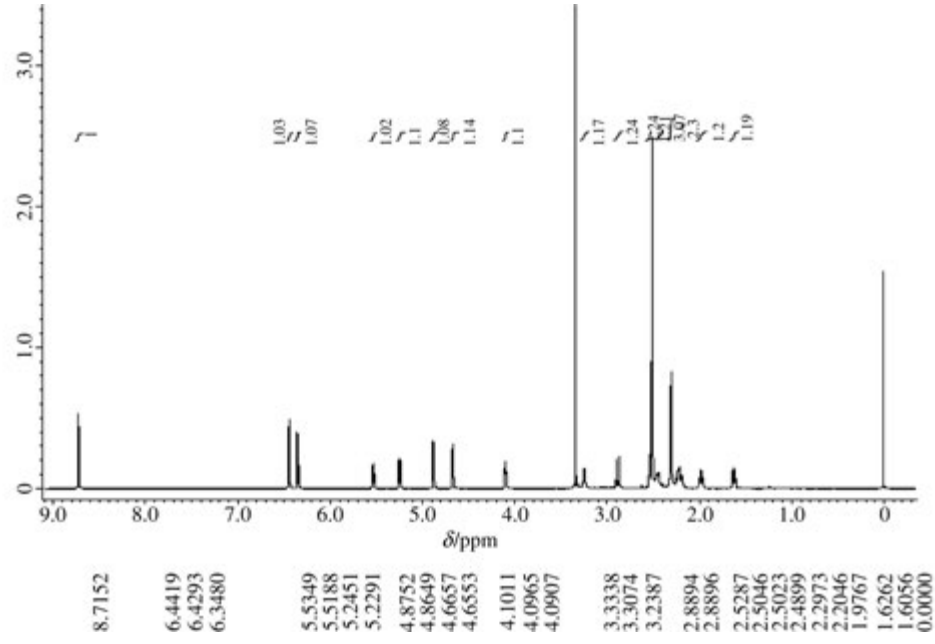


Figure 3.1 The ^{1}H spectrum of compound **C3-1**

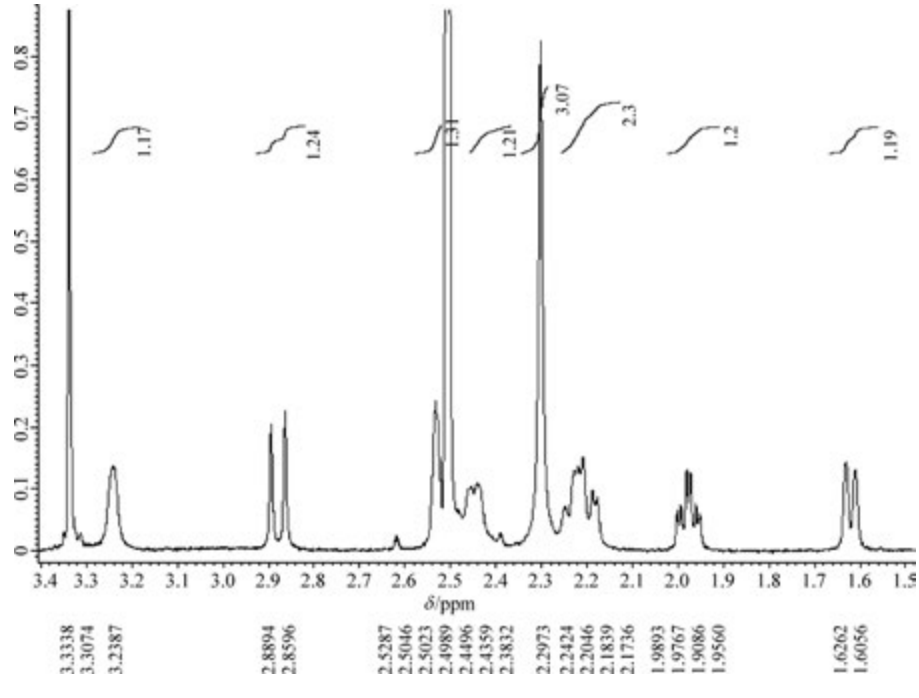


Figure 3.2 The locally enlarged ^{1}H spectra in the high field of Figure 3.1

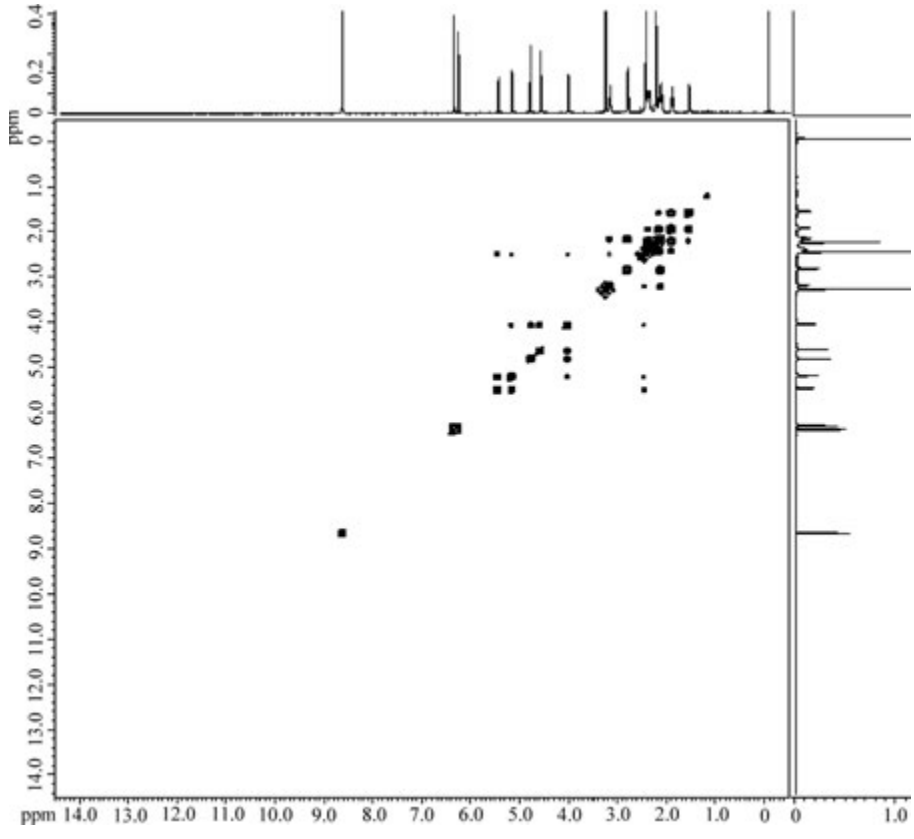


Figure 3.3 The COSY spectrum of compound **C3-1**

Although all information on every peak set are shown clearly in Table 3.1, the assignment of the ^{1}H spectrum can not be accomplished. Therefore, we must analyze the correlated peaks in the COSY spectrum.

Draw a vertical line through $F_2 = 1.62$ ppm. This vertical line passes through two correlated peaks, whose ordinates are $F_1 = 1.97$ppm and $F_1 = 2.21$ ppm, respectively. These two correlated peaks illustrate the two couplings between the peaks at 1.62–1.97 ppm and at 1.62–2.21 ppm. We write these results into the first row of Table 3.2. From the correlated peak at 2.21 ppm, we can know that the coupled peak is situated at 2.21 ppm, which is differentiated from the peak at 2.19 ppm. It means the result of the COSY spectrum has a higher resolution and a clearer result than that of the ^{1}H spectrum.

Below the peak at 1.62 ppm, the next peak set is situated at 1.97 ppm. Draw a vertical line through $F_2 = 1.97$ ppm. This vertical line passes through three correlated peaks, whose ordinates are $F_1 = 1.62$ ppm, $F_1 = 2.21$ ppm and $F_1 = 2.44$ ppm, respectively. The correlated peak with $F_2 = 1.97$ ppm and $F_1 = 1.62$ ppm and the correlated peak with $F_2 = 1.62$ ppm and $F_1 = 1.97$ ppm are equivalent. That is why we analyze only the correlated peaks, which are

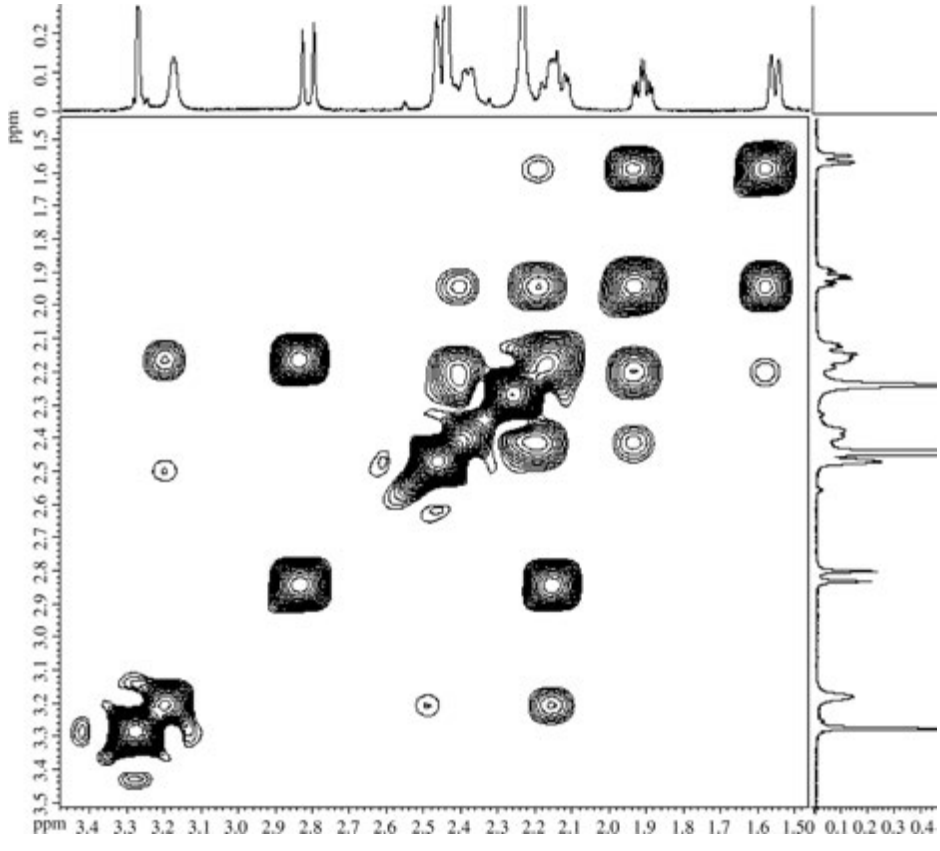


Figure 3.4 The locally enlarged COSY spectrum of Figure 3.3 (in the high field)

situated on one side of the diagonal. The three correlated peaks mean that the peak at 1.97 ppm has coupled correlations with the three peaks, whose chemical shift values are 1.62, 2.21, and 2.44 ppm, respectively. We write this result into the second row of the Table 3.2.

Below the peak at 1.97 ppm, the next peak set is situated at about 2.20 ppm. Draw a vertical line through $F_2 = 2.20$ ppm. There are two columns of correlated peaks (they can be seen clearly further if the electronic spectrum is used). The right column contains two correlated peaks, whose ordinates are $F_1 = 1.62$ and 3.24 ppm, respectively. The left column contain three correlated peak, whose ordinates are $F_1 = 1.62$, 1.97, and 2.44 ppm, respectively. From the two columns, we can know that the resolution of the COSY spectrum is better than that of the ^{1}H spectrum.

Similarly, Table 3.2 can be obtained.

Now we assign the ^{1}H spectrum of compound **C3-1**.

The peaks of the nitro-methyl group, the hydroxyl group and the phenol group can be assigned easily, because they are isolated hydrogen atoms. They show three singlets in their

Table 3.1 Summarized data from the ^{1}H spectrum of compound **C3-1**

δ_H (ppm)	Integral value of peak set	Peak shape, *J* (Hz)
1.62	1.19	d (12.4)
1.97	1.2	d, t (12.4, 7.56)
2.19	one of 2.3	m
2.21	one of 2.3	m
2.29	3.07	s
2.44	1.21	d, broad,
2.52	1.24	s
2.88	1.24	d (17.9)
3.24	1.17	s, broad
4.09	1.1	m
4.66	1.14	d (6.2)
4.87	1.04	d (6.2)
5.24	1.1	d, t (9.6, 2.7)
5.53	1.02	d (9.7)
6.34	1.07	d (7.6)
6.43	1.03	d (7.6)
8.72	1	s

Note: s, d, t, q and m denote singlet, doublet, triplet, quartet, and multiplet respectively.

^{1}H spectrum and no correlated peaks in the COSY spectrum. Therefore, the three singlets at 2.29, 4.87, and 8.72 ppm with the hydrogen atom numbers of 3, 1, and 1, respectively, can be assigned as the nitro-methyl group, the hydroxyl group, and the phenol group, respectively.

Table 3.2 Summarized data from the COSY spectrum of compound **C3-1**

No.	δ_H (ppm)	Coupled hydrogen atoms, δ_H (ppm)	Weakly coupled hydrogen atoms, δ_H (ppm)
1	1.62	1.97	(2.21)
2	1.97	1.62, 2.21, 2.44	
3	2.19	2.88, 3.24	
4	2.21	1.97, 2.44	(1.62)
5	2.29	isolated	
6	2.44	1.97, 2.21	
7	2.52	5.24, 5.53	(3.24)
8	2.88	2.19	
9	3.24	2.19	(2.52)
10	4.09	4.66, 4.87, 5.24	
11	4.66	4.09	
12	4.87	4.09	
13	5.24	4.09, 5.53	(2.52)
14	5.53	2.52, 5.24	
15	6.34	6.43	
16	6.43	6.34	
17	8.72	isolated	

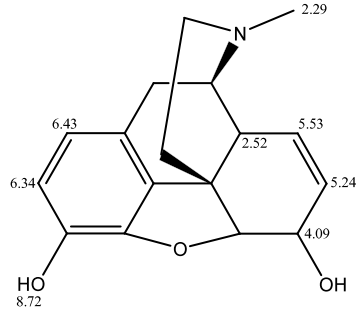
Parenthesis () stands for weak couplings.

Since the hydrogen atoms in the substituted benzene ring and in double bonds have rather large chemical shift values, their peaks can be differentiated from other hydrogen atoms.

The two peak sets, which have the largest chemical shift values, should belong to aromatic hydrogen atoms. Because the phenol group belongs to the second type of substituents of the benzene, which will decrease the chemical shift value of the remaining hydrogen atoms, especially the ortho-hydrogen atoms, the peak set at 6.34 ppm should be assigned as the ortho-hydrogen atoms with respect to the phenol group, and the peak set at 6.43 ppm as the meta-hydrogen atoms.

The peak sets at 5.24 and 5.53 ppm can be assigned as those of two alkene hydrogen atoms. According to row No. 14 of Table 3.2, in the alkane groups region only one peak set at 2.52 ppm couples with the peak set at 5.53 ppm. Again according to row No. 7 of Table 3.2, the peak set at 2.52 ppm couples with the peak sets at 5.24 and 5.53 ppm, respectively. According to row No. 13 of Table 3.2, the peak set at 5.24 ppm couples with three peak sets at 2.52, 4.09, and 5.53 ppm, respectively, with the coupling between the peak set at 2.52 ppm being weak. And according to row No. 10 of Table 3.2, the peak set at 4.09 ppm couples with three peak sets at 4.66, 5.24, and 4.87 ppm, respectively. Notice that the peak set at 4.87 ppm is assigned as the hydroxyl group. To sum up, it is reasonable that the peak set at 5.24 ppm is closer to the hydroxyl group than that at 5.53 ppm. The result of the COSY spectrum about the peak set at 4.09 ppm coincides with the peak shape analysis at 4.09 ppm in the ^{1}H spectrum, with multiple splits shown.

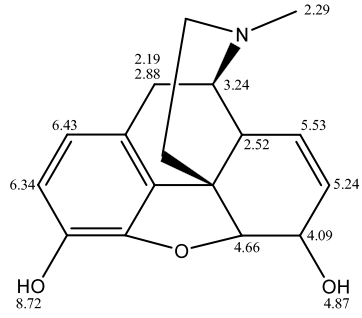
On the basis of the discussion above, we can assign the structure at the following positions.



According to row No. 7 of Table 3.2, the peak set at 2.52 ppm couples with three peak sets at 3.24, 5.24, and 5.53 ppm, respectively, with only the peak set at 3.24 ppm belonging to an alkane group. And according to row No. 9 of Table 3.2, the peak set at 3.24 ppm couples with two peak sets at 2.52 and 2.19 ppm, respectively. The peak set at 2.19 ppm strongly couples with the peak set at 2.88 ppm, which means the coupling of these two hydrogen atoms is geminal coupling (^2J coupling).

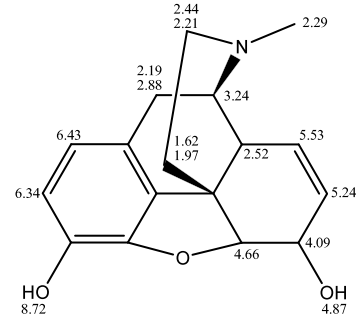
According to row No. 10 of Table 3.2, the peak set at 4.09 ppm couples with peak sets at 4.66 and 4.87 ppm besides its coupling with the peak set at 5.24 ppm. Because in this measurement, DMSO is used as the solvent, the peak set of the hydroxyl group shows splits. After the exchange of the measured solution with D_2O, the area of the peak set at 4.87 ppm decreases. It is also proof that the peak set at 4.87 ppm can be assigned as the hydroxyl group.

The discussion above can further assign the ^{1}H spectrum as follows.



Now only two methylene groups are not yet assigned. From their coupled relationships (row No. 1, 2, 4 and 6 of Table 3.2), and the consideration that the methylene group connected with the nitrogen atom should have a larger δ value than the other, these two methylene groups can be assigned.

The final assignment is as follows.



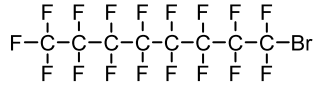
C3-1

From this example, we can know the function of the COSY spectrum, by which the ^{1}H spectrum of the compound **C3-1** was assigned. In addition, we can know that the resolution of the COSY spectrum is better than that of the ^{1}H spectrum.

In the above-mentioned assignment, we applied some knowledge about the chemical shift. This is because we use only the COSY spectrum for the assignment. If we have HMBC and HSQC spectra, the assignment can be accomplished without the knowledge and the assignment can be accomplished more easily.

Because of the importance of the COSY spectrum, we will present another example. Although this example does not concern an H, H-COSY but an F, F-COSY spectrum, the method of the interpretation for the F, F-COSY is similar to that for the H, H-COSY. In addition, this example is interesting.

The compound **C3-2** has the following structure.



C3-2

In this example, the fluorine atoms replace the hydrogen atoms. Therefore, we list the fluorine spectrum and the F, F-COSY spectrum (Figures 3.5–3.9).

Try to assign the fluorine spectrum.

Solution

Although this example concerns an F, F-COSY spectrum, the analysis method is similar to that for the last example. Figure 3.5 shows a quantitative fluorine spectrum, which is similar to a quantitative ^{13}C spectrum. From Figure 3.5, the number of the fluorine atoms related to peaks of the fluorine spectrum is quantitatively illustrated. There are eight peaks in the fluorine spectrum, which indicates the existence of eight types of fluorine atoms. Except the peak at –81.8 ppm, which corresponds to three fluorine atoms, all other peaks correspond to two fluorine atoms, which implies that the compound is a straight chain compound, a coincidence with the structural formula.

Our task is to assign the fluorine spectrum.

The highest peak at –81.8 ppm corresponds to three fluorine atoms. And it shows a triplet in its highly enlarged spectrum, which means it is split by two fluorine atoms. These two

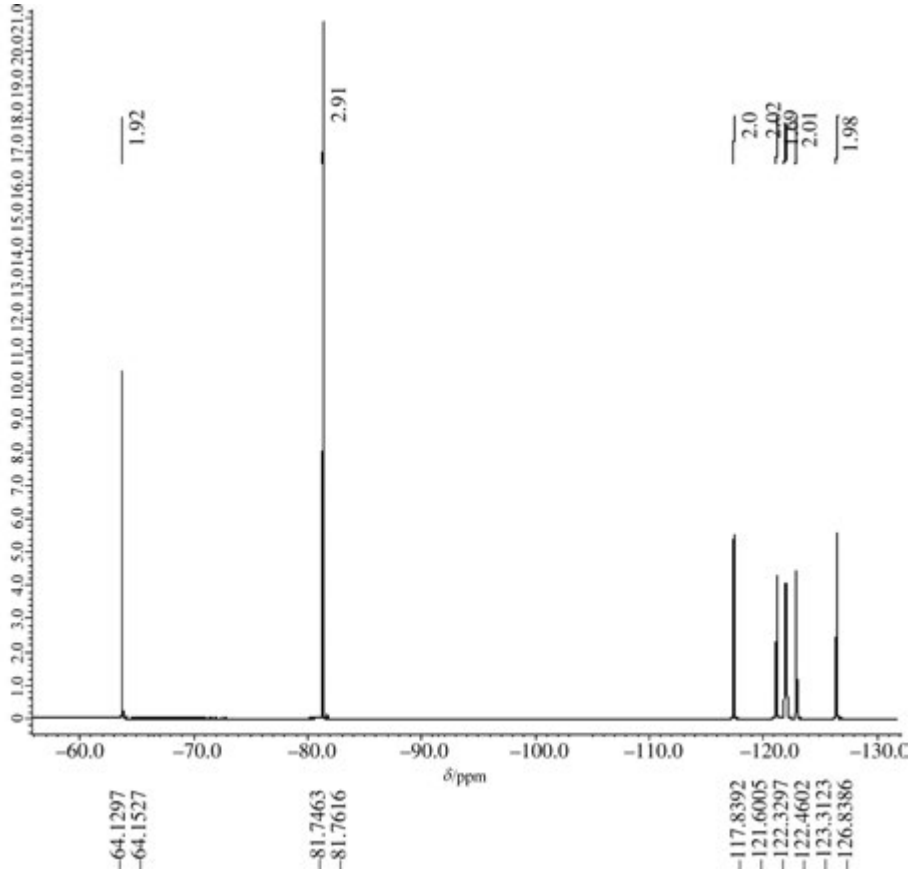


Figure 3.5 The fluorine spectrum of the compound **C3-2**

considerations lead to the result that this peak can be assigned as the terminal three fluorine atoms.

The assignment of the other peaks in the fluorine spectrum needs the F, F-COSY spectrum.

As in the last example, draw a vertical line through –81.8 ppm, which passes through the correlated peak at $F_2 = -81.8$ ppm and $F_1 = -123.3$ ppm. Of course, this correlated peak is equivalent to the correlated peak at $F_2 = -123.3$ ppm and $F_1 = -81.8$ ppm. This correlated peak illustrates that the two fluorine atoms, whose chemical shift value is –123.3 ppm, have ^3J coupling with the three fluorine atoms.

Then draw a vertical line through –123.3 ppm, which passes through three correlated peaks at $F_1 = -81.8, -122.4$, and –126.9 ppm, respectively. It seems impossible that a peak has three correlated peaks, because one peak has at most two correlated peaks in its COSY spectrum for a straight chain compound, which correspond to its left group and its right group. We will explain the cause of the formation of the three correlated peaks later.

Repeat these operations and we can read all data from the F, F-COSY spectrum. The data are listed in Table 3.3.

From Table 3.3, it can be known that the peak at –121.7 ppm and the peak at –123.3 each have three correlated peaks.

Because compound **C3-2** is a straight chain one, the existence of three correlated peaks can only be explained by the consideration that the intensities of some ^4Js are comparable with that of ^3Js. There is not any other explanation for this fact. This consideration coincides with reference [2].

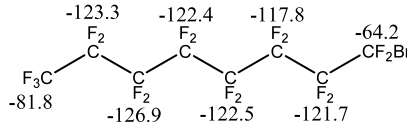
The F, F-COSY spectrum of compound **C3-2** can be interpreted as follows.

As the terminal group, the three fluorine atoms have only the correlated peak produced by a ^3J coupling. For the next group, because the intensity of the correlated peak produced by a ^4J coupling is comparable with that of the correlated peak produced by a ^3J coupling, three correlated peaks are observed (one correlated peak from a ^4J coupling and two correlated peaks from ^3J couplings). After that, two correlated peaks are observed (one correlated peak from a ^4J coupling and the other correlated peak from a ^3J coupling). And for the still next group, because the intensity of the correlated peak produced by a ^4J coupling is stronger than that of the correlated peak produced by a ^3J coupling, only two correlated peaks produced by ^4J couplings are observed. Passing the middle position of the structure, we have the reverse result, that is, two correlated peaks produced by ^4J couplings for the group in the

Table 3.3 Coupled data read from the F, F-COSY spectrum

δ_F (ppm)	Coupled fluorine atoms, δ_F (ppm)
–64.2	–121.7
–81.8	–123.3
–117.8	–121.7, –122.4
–121.7	–64.2, –117.8, –122.5
–122.4	–117.8, –123.3
–122.5	–121.7, –126.9
–123.3	–81.8, –122.4, –126.9
–126.9	–122.5, –123.3

middle position of the structure, then two correlated peaks (one correlated peak from a ^4J coupling and the other correlated peak from a ^3J coupling), then three correlated peaks produced by a ^4J coupling and two ^3J couplings, and finally one correlated peak produced by a ^3J coupling for the terminal group. On the basis of the consideration above, we can assign the fluorine spectrum of compound **C3-2**. The result is shown as follows.



The coupling relations of compound **C3-2** are listed in Table 3.4.

From the two examples above, we can know the important function of the COSY spectrum. The couplings between vicinal hydrogen atoms can be determined by the COSY spectrum. In addition to the heteronuclear shift correlation spectrum, the connections between the carbon atoms of an unknown compound can be determined. The determination of the connections between the carbon atoms is very important either for the identification of an unknown compound or for the confirmation of an anticipated structure.

It should be emphasized that the result obtained from the COSY spectrum is more reliable than that obtained from the HMBC spectrum. Of course, the precondition is that the correlated peaks in the COSY spectrum be clear.

The homonuclear shift correlation spectra have many variations, which can be selected on different requirements. The COSY spectrum as shown in Figure 3.3 is the conventional type. We will present two other types of COSY which are also applied frequently.

Phase-Sensitive COSY

For a compound with a complicated structure, it is possible that correlated peaks be close, and that they can even be overlapped. In this case, the interpretation of the COSY spectrum may be difficult. This situation comes from the fact that fine structure of correlated peaks is not clear and that the sections of correlated peaks are too large.

Because the conventional COSY only needs the simplest pulse sequence and it can lead to a good result in general, the conventional COSY spectrum will be the first selected. If the result obtained is not satisfactory, the phase-sensitive COSY, whose pulse sequence is more complicated than that of the conventional COSY, can be considered.

Table 3.4 The result of the COSY spectrum of compound **C3-2**

δ_F (ppm)	Coupled fluorine atoms, δ_F (ppm)	Related J
–64.2	–121.7	^3J
–121.7	–64.2, –117.8, –122.5	^3J, ^3J, ^4J
–117.8	–121.7, –122.4	^4J, ^3J
–122.5	–121.7, –126.9	^4J, ^4J
–122.4	–117.8, –123.3	^4J, ^4J
–126.9	–122.5, –123.3	^4J, ^3J
–123.3	–81.8, –122.4, –126.9	^3J, ^3J, ^4J
–81.8	–123.3	^3J

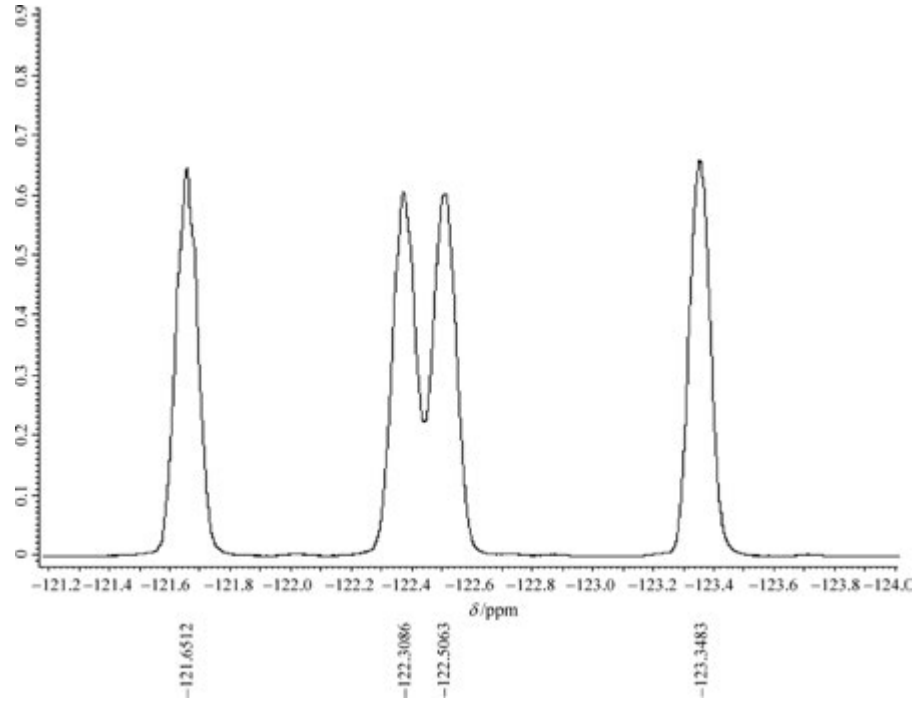
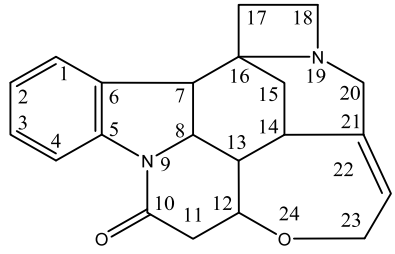


Figure 3.6 The locally enlarged spectrum of Figure 3.5

The appearance of the phase-sensitive COSY spectrum is similar to that of the conventional COSY spectrum except the fine structures of the correlated peaks. From the viewpoint of the NMR spectroscopy, the correlated peaks of the conventional COSY spectrum are not pure absorption peak shapes, but those of the phase-sensitive COSY spectrum are. The fine structures of the phase-sensitive COSY spectrum are clear, from which coupling constants can be read out. The trouble about the partially overlapped correlated peaks is greatly reduced. As for the interpretation method for the phase-sensitive COSY spectrum is the same as that for the conventional COSY spectrum.

The phase-sensitive COSY spectrum of compound **C3-3** is shown in Figure 3.10.



C3-3

From Figure 3.10, we can see that all correlated peaks show rectangles with clear fine structures.

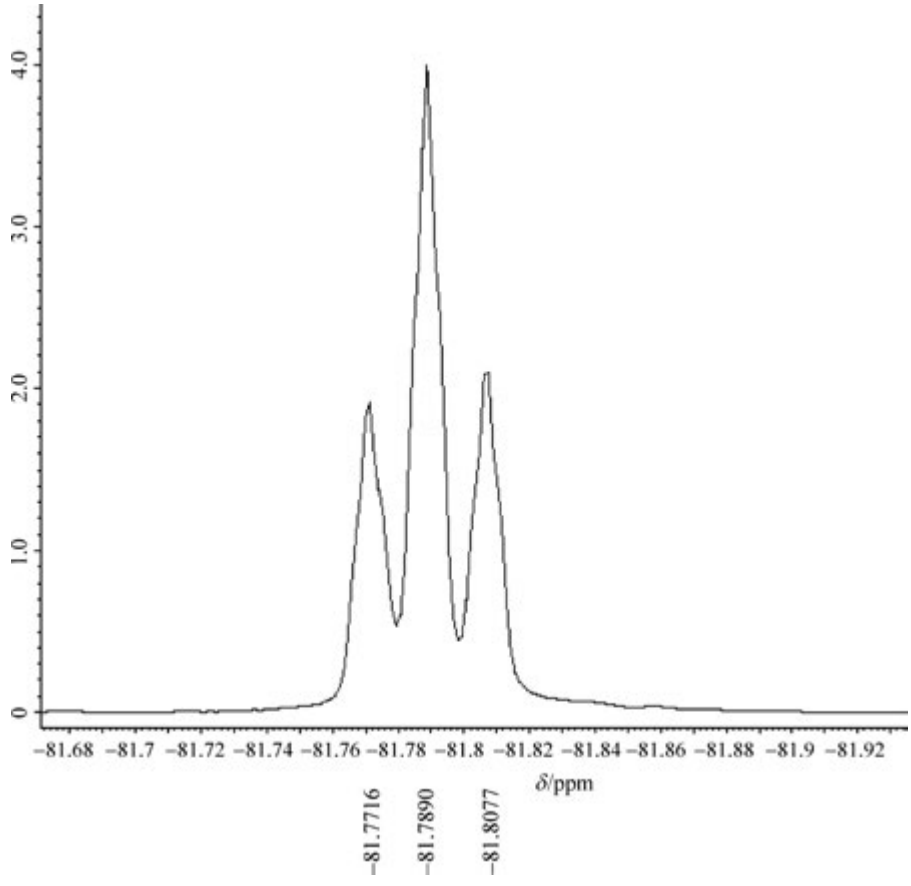


Figure 3.7 The highly enlarged spectrum at –81.8 ppm of Figure 3.5

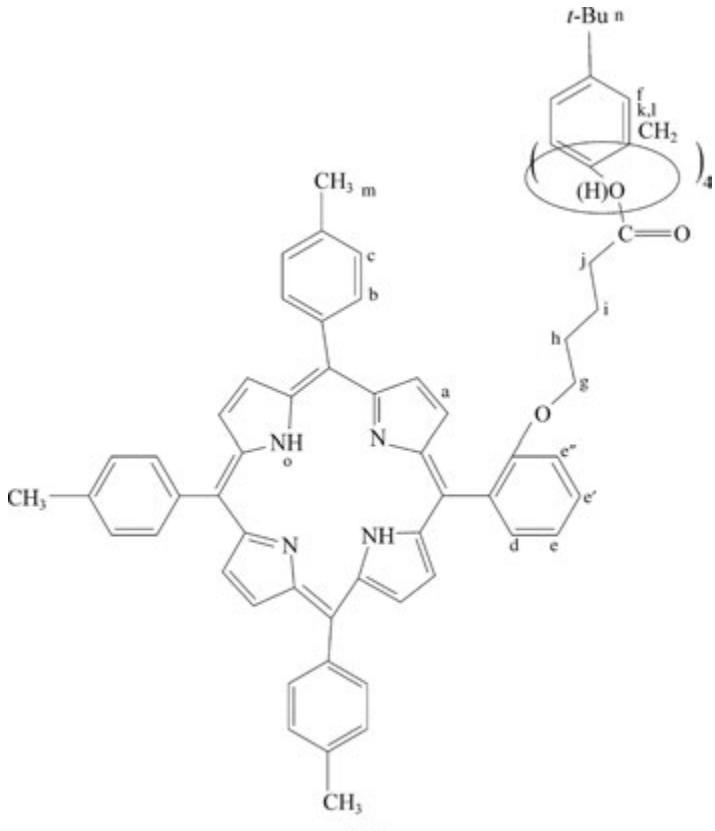
DQF-COSY

DQF-COSY is the abbreviation of double-quantum filtered COSY.

Although their mechanisms are different, the appearance of the DQF-COSY spectrum is similar to that of the conventional COSY spectrum and their interpretation method is the same.

If an organic compound contains a tert-butyl group, or a methoxy group, and so on, which produces a strong singlet in its ^{1}H spectrum, then in its conventional COSY spectrum the correlated peaks produced from weak peaks of the ^{1}H spectrum have weak intensities, or even these correlated peaks may be absent. In such cases, the DQF-COSY spectrum can give a better result than the conventional COSY spectrum. The correlated peaks of the strong peaks in their ^{1}H spectrum (including the solvent peak) are suppressed and the peak shapes of all the correlated peaks in the DQF-COSY spectrum are improved.

The DQF-COSY spectrum of compound **C3-4** is shown in Figure 3.11.



C3-4

In Figure 3.11, the correlated peaks of weak peaks in the ^{1}H spectrum are shown.

COSYLR

COSYLR is an abbreviation of the COSY optimized for long-range couplings, and it can also be named LRCOSY.

In the ^{1}H spectrum, long-range couplings are illustrated as the slight increments in width at the half-height of the peaks. Sometimes, they can be illustrated as tiny splits at the top of related peaks. Either the slight increments or tiny splits are not reliable enough.

The COSYLR spectrum deals with long-range couplings. Its appearance is similar to that of the COSY spectrum. And the interpretation method for it is the same as that for the COSY spectrum. The correlations of long-range couplings can be shown in the COSYLR spectrum. Long-range couplings can be determined through their related correlated peaks. It should be known that there are also the correlated peaks produced by ^{3}J couplings. Therefore, each correlated peak in the COSYLR spectrum should be analyzed carefully.

The high field portion of the COSYLR spectrum of compound **C3-3** is shown in Figure 3.12.

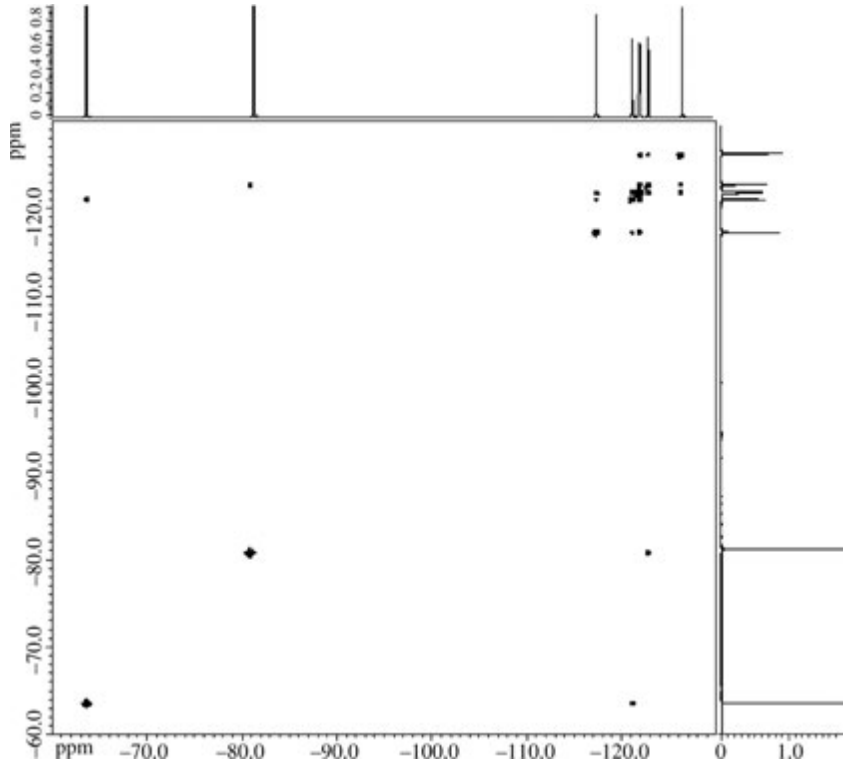


Figure 3.8 The F, F-COSY spectrum of the compound **C3-2**

Many correlations from long-range couplings, such as H-13, H-15′; H17, H-15′; H-13, H20′; H20′, H-23 and so on, are shown in Figure 3.12.

3.3 Heteronuclear Shift Correlation Spectroscopy

The heteronuclear shift correlation spectrum correlates the peak (set) of different kinds of nuclei, with the commonest correlation being the peak (set) between the ^{1}H spectrum and the ^{13}C spectrum.

The earliest heteronuclear shift correlation spectrum is H, C-COSY, which can also be written as C, H-COSY. The form of the spectrum is a rectangle with the abscissa (F_2) of the δ_C and the ordinate (F_1) of the δ_H. The ^{13}C spectrum is set on the top of the H, C-COSY spectrum and the ^{1}H spectrum is set beside the left side. There are correlated peaks in the H, C-COSY spectrum. Every correlated peak has its abscissa, which corresponds to a peak in the ^{13}C spectrum, and its ordinate, which corresponds to a peak set in the ^{1}H spectrum. The correlated peak reveals the correlation between the peak in the ^{13}C spectrum and the peak set in the ^{1}H spectrum.

In the H, C-COSY experiment the ^{13}C nuclei are measured. Although the application of the pulse sequence of the H, C-COSY experiment can improve the sensitivity of the ^{13}C

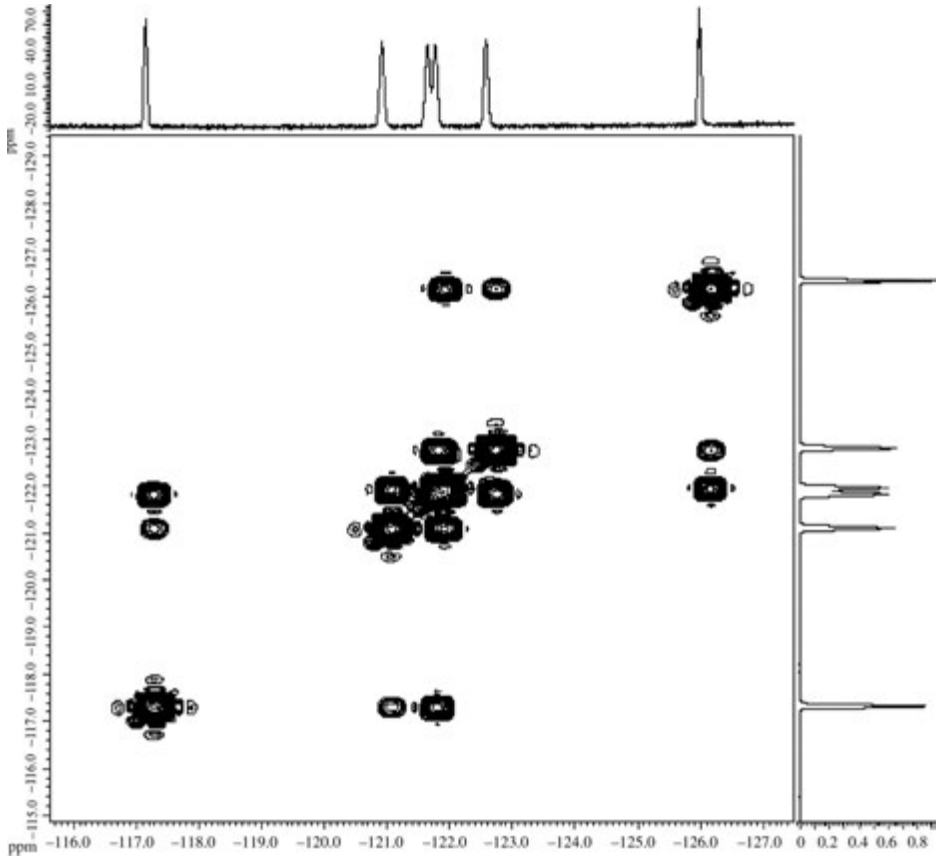


Figure 3.9 The locally enlarged spectrum of Figure 3.8

nuclei measurement, the H, C-COSY experiment is still time-consuming. As a result, the so-called inverse mode, in which the ^{1}H nuclei are measured, developed. Since the measurement is carried out from the ^{13}C nuclei to the ^{1}H nuclei, the sensitivity of the heteronuclear shift correlation experiment is improved greatly.

Because the inverse mode is applied, the abscissa of the spectrum obtained by the inverse mode is the δ_H and the ordinate, the δ_C. The ^{1}H spectrum is set on the top of the 2D NMR spectrum and the ^{13}C spectrum beside the left side.

It should be noticed that some instrument companies still put the δ_C on the abscissa and the δ_H on the ordinate as the normal mode. Doing so is only a simple treatment, and it does not mean that sampling should concern carbon nuclei.

Two types of heteronulcear shift correlation spectra in the inverse mode are HMQC and HSQC, both of which are applied frequently. The interpretation methods for them are the same.

Now we take the HMQC spectrum of compound **C3-5** as the example to illustrate its interpretation method and its function.

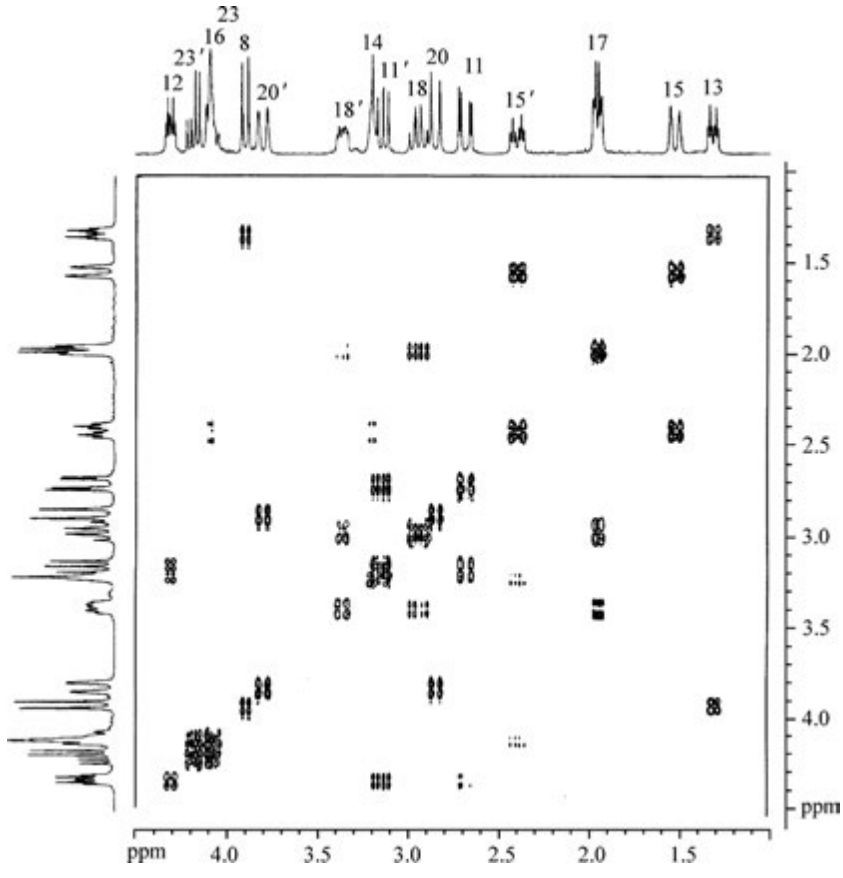
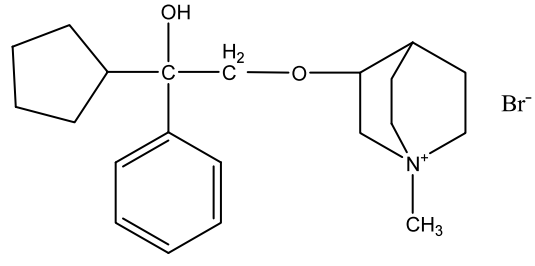


Figure 3.10 The high field portion of the phase-sensitive COSY spectrum of compound **C3-3**. (Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA.)

Compound **C3-5** has the structure as follows.



C3-5

Since correlated peaks are close in the high field region, the locally enlarged HMQC spectrum in the high field region is shown in Figure 3.14.

From Figures 3.13 and 3.14 we know that the spectrum on the right side of the HMQC spectrum is the ^{13}C spectrum. Each correlated peak in the HMQC spectrum corresponds to a peak in the ^{13}C spectrum and a peak (set) in the ^{1}H spectrum. The correlated peak

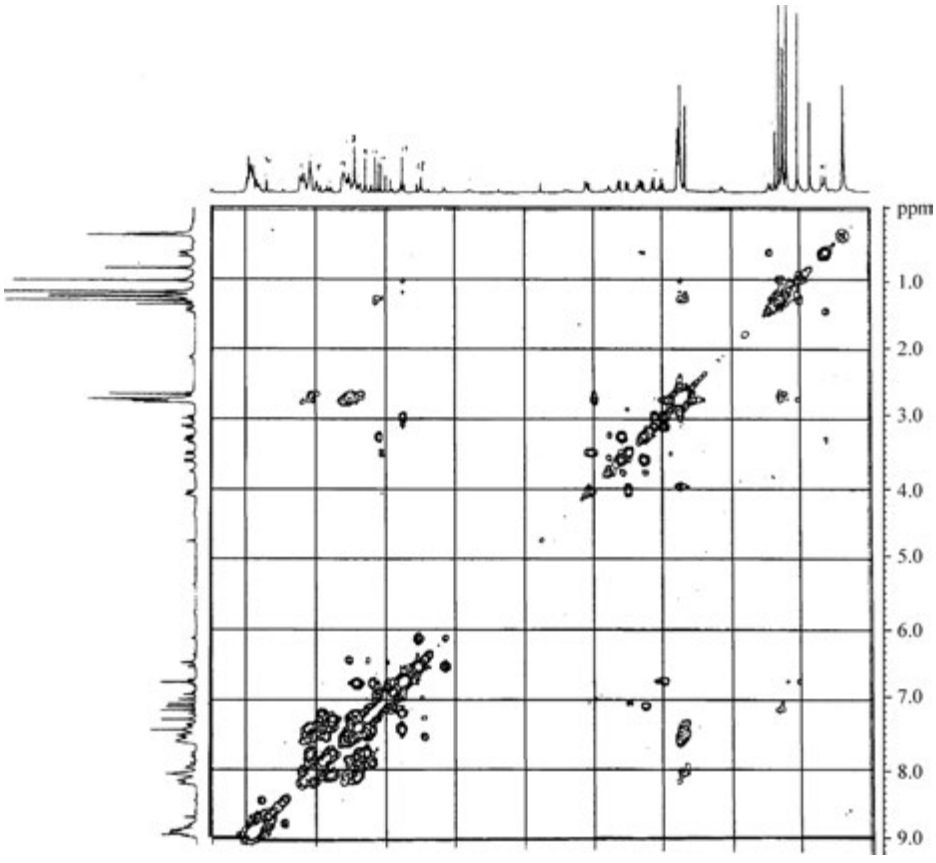


Figure 3.11 The partial DQF-COSY spectrum of compound **C3-4**. [Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA and *Structural Identification of Organic Compounds and Organic Spectroscopy* 2nd ed., © 2000 Science Press (Chinese Edition).]

illustrates the correlation between these two peaks. If a functional group, which contains no hydrogen atoms, such as a quaternary carbon atom, its ^{13}C spectrum peak does not correspond to a correlated peak.

The function of the heteronuclear shift correlation spectrum is as follows, with the HMQC spectrum of compound **C3-5** as an example.

1. The correlation between the two peaks of the ^{13}C spectrum and the ^{1}H spectrum.

By drawing a vertical line and a horizontal line through a correlated peak, they will pass through a peak set in the ^{1}H spectrum and a peak in the ^{13}C spectrum. These two peaks are correlated by the correlated peak.

The correlation between the ^{13}C spectrum and the ^{1}H spectrum is very important for the determination of the related functional group and for the postulation of the environment of the functional group. For example, a CH_2 group has its δ_H value of 5.2 ppm. It seems an alkene group. However, according to its δ_C value of 74 ppm, it should be an alkane group. For another example, a CH group has a rather small δ_H value but a rather large δ_C value. It can be known that the CH group does not connect with an electronegative group

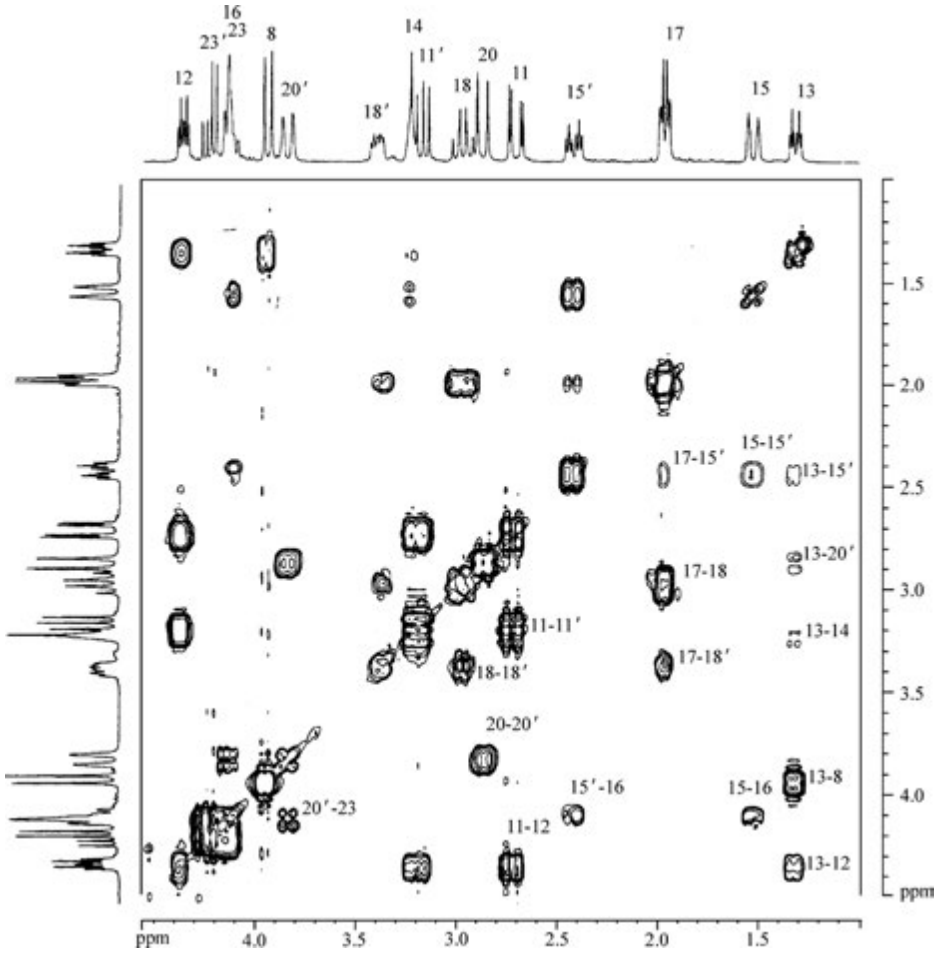


Figure 3.12 The high field portion of the COSYLR spectrum of compound **C3-3**. [Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA and *Structural Identification of Organic Compounds and Organic Spectroscopy* 2nd ed., © 2000 Science Press (Chinese Edition).]

but connects with a functional group of a large size, which has a stereo effect on the δ_C value.

After all correlations between the ^{13}C spectrum and the ^{1}H spectrum are completed, the ascription of all hydrogen atoms of the compound has been accomplished. If some hydrogen atoms have no connection with carbon atoms, these hydrogen atoms should be reactive hydrogen atoms.

2. The combination of the COSY spectrum and the heteronuclear shift correlation spectrum can determine the connections of carbon atoms.

The COSY spectrum can find all vicinal couplings (^{3}J couplings). And the heteronuclear shift correlation spectrum correlates the peak sets of the ^{1}H spectrum with the peak of the

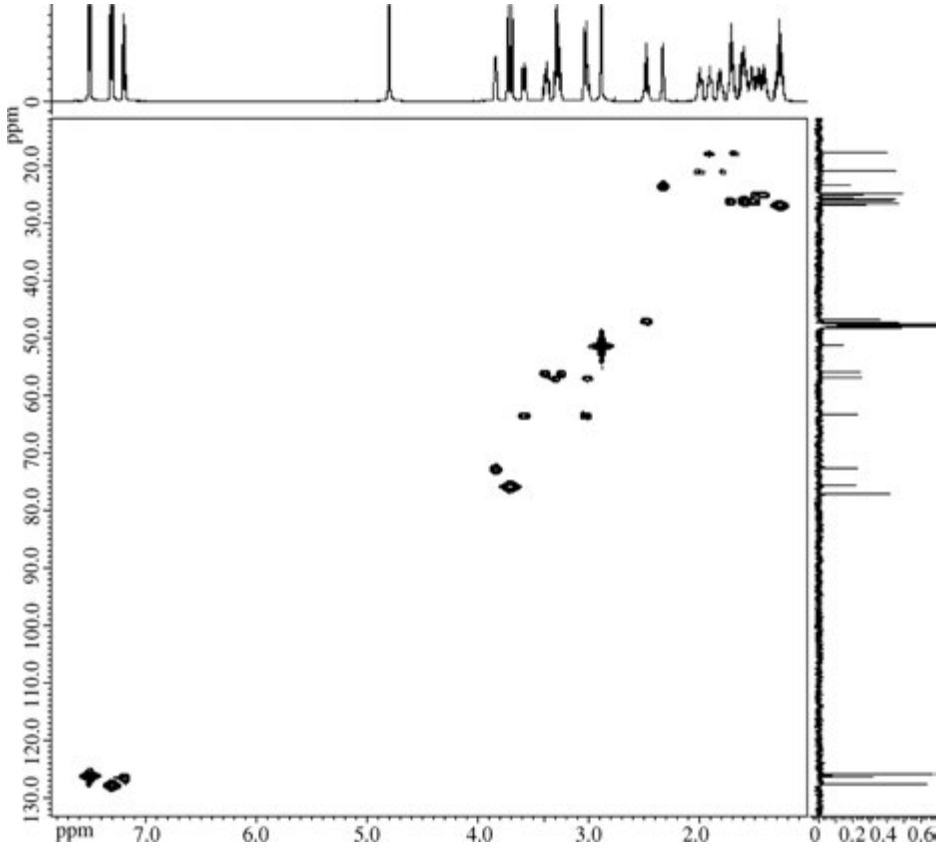


Figure 3.13 The HMQC spectrum of compound **C3-5**

^{13}C spectrum. Therefore, the combination of these two 2D NMR spectra can determine the connections of carbon atoms.

The two points mentioned above are very important for the identification of an unknown compound or for the assignments of the ^{13}C spectrum and the ^{1}H spectrum.

3. The heteronuclear shift correlation spectrum makes one know the ^{1}H spectrum in detail.

It is frequently encountered that peak sets are partially overlapped (even overlapped seriously) in the ^{1}H spectrum for a compound with a complex structure. On the basis of the heteronuclear shift correlation spectrum, the overlapped peak sets can be analyzed clearly. If the integral value of overlapped peak sets is not close to an integer, for example, if it is 2.5, the number of related hydrogen atoms can be determined definitively by analyzing the heteronuclear shift correlation spectrum. For example, from Figure 3.14 we can see clearly that the peak sets in the region of about 1.7 ppm is composed of two peak sets. One peak set comes from a hydrogen atom of the CH_2 group, whose δ_C value is about 18 ppm. Another peak set comes from a hydrogen atom of the CH_2 group, whose δ_C value is about 27 ppm. The former has a slightly smaller δ_H value and the latter has

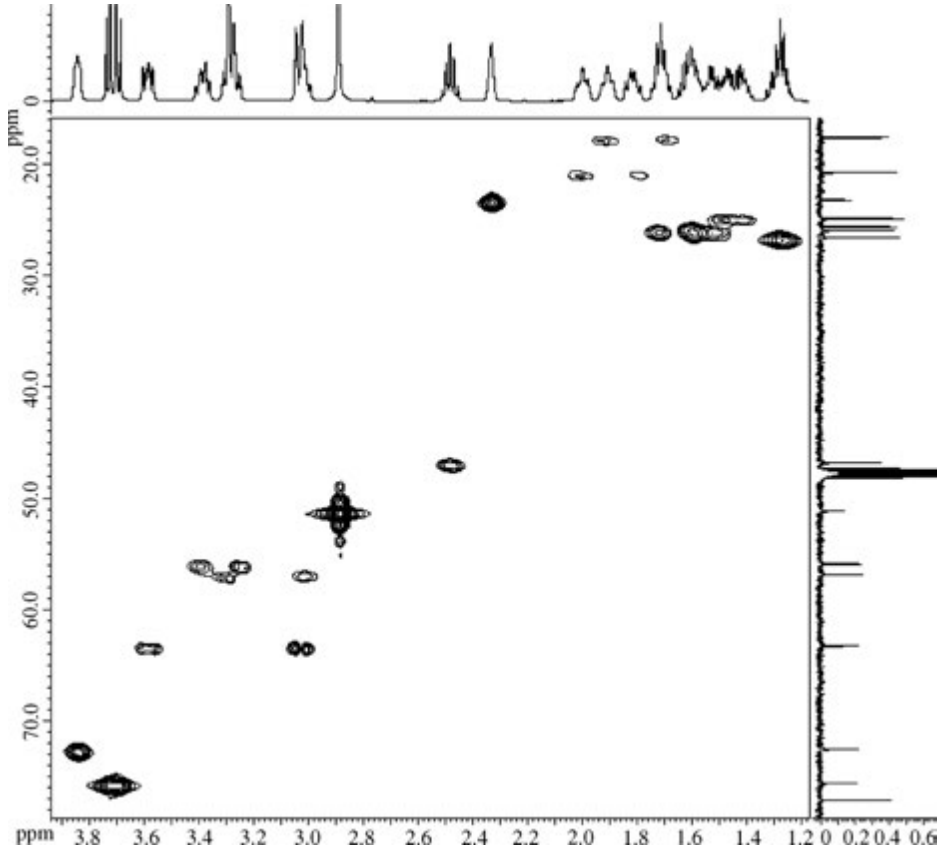


Figure 3.14 The locally enlarged spectrum in the high field region of Figure 3.13

a slightly larger δ_H value. Similarly, we can analyze the peak sets of two hydrogen atoms at about 3.02 ppm in Figure 3.14.

4. The heteronuclear shift correlation spectrum is very effective for the recognition of two chemically non-equivalent hydrogen atoms of a CH_2.

In fact, the item above has dealt with this subject already. If a CH_2 group has two chemically non-equivalent hydrogen atoms, that is, the two hydrogen atoms have different chemical shift values, a horizontal line, which passes through the peak of the ^{13}C spectrum of the CH_2 group, will pass through two correlated peaks. The two CH_2 groups discussed above, whose δ_C are about 18 and 27 ppm, respectively, are the examples. The result obtained from the heteronuclear shift correlation spectrum is more reliable than that obtained from the DEPT spectrum. Therefore, all CH_2 groups, which have two chemically non-equivalent hydrogen atoms, can be recognized immediately from the heteronuclear shift correlation spectrum, as shown in Figures 3.13 and 3.14 for compound **C3-5**. The COSY spectrum can not differentiate the correlation of ^2J couplings from that of ^3J couplings. Therefore,

two chemically non-equivalent hydrogen atoms can not be recognized by the COSY spectrum. However, the heteronuclear shift correlation spectrum can do it well.

5. Because the ordinate of the heteronuclear shift correlation spectrum is the chemical shift of the ^{13}C spectrum, whose resolution is much better than that of the ^{1}H spectrum, the heteronuclear shift correlation spectrum is effective to resolving overlapped peak sets in the ^{1}H spectrum and those in the COSY spectrum.

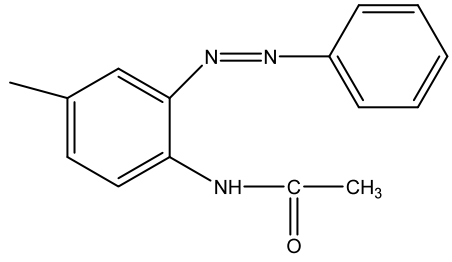
3.4 Long-Range Heteronuclear Shift Correlation Spectroscopy

The long-range heteronuclear shift correlation spectrum correlates the long-range couplings between carbon atoms and hydrogen atoms. The correlated peaks in the spectrum correlate the heteronuclear long-range couplings usually across three chemical bonds. However, it is possible that the passed chemical bonds be two chemical bonds or four chemical bonds. In the aromatic system, the correlated peaks, which correspond to the long-range couplings across five chemical bonds, can be shown.

Like the HMQC or HSQC spectrum, long-range heteronuclear shift correlation spectroscopy usually applies the inverse mode, by which the HMBC spectrum is obtained.

Like the HMQC or HSQC spectrum, the abscissa of the HMBC spectrum is the δ_H and the ordinate is the δ_C. Some instrument companies still put the ^{13}C chemical shift on the abscissa and the ^{1}H chemical shift on the ordinate as the normal mode. Doing so is only a simple treatment, and it does not mean that sampling concerns the carbon nuclei.

Compound **C3-6** has the following structure.



C3-6

The HMBC spectrum of compound **C3-6** is shown in Figure 3.15.

The locally enlarged spectrum in the low field region in Figure 3.15 is shown in Figure 3.16.

From Figures 3.15 and 3.16 we can know that to read the data from the HMBC spectrum is more complicated than from the HMQC (or HSQC) spectrum. It is possible that three types of correlated peaks be shown in the HMBC spectrum.

1. The long-range correlated peaks. This type of correlated peaks can be easily cognized. Draw a horizontal line and a vertical line through the correlated peak. These two lines will pass through a peak in the ^{13}C spectrum and a peak set in the ^{1}H spectrum, respectively. The correlated peak of the HMBC spectrum correlates these two peaks. The HMBC spectrum differs from the HMQC (or HSQC) spectrum in the fact that the latter shows the heteronuclear ^{1}J couplings but the former shows the long-range couplings.

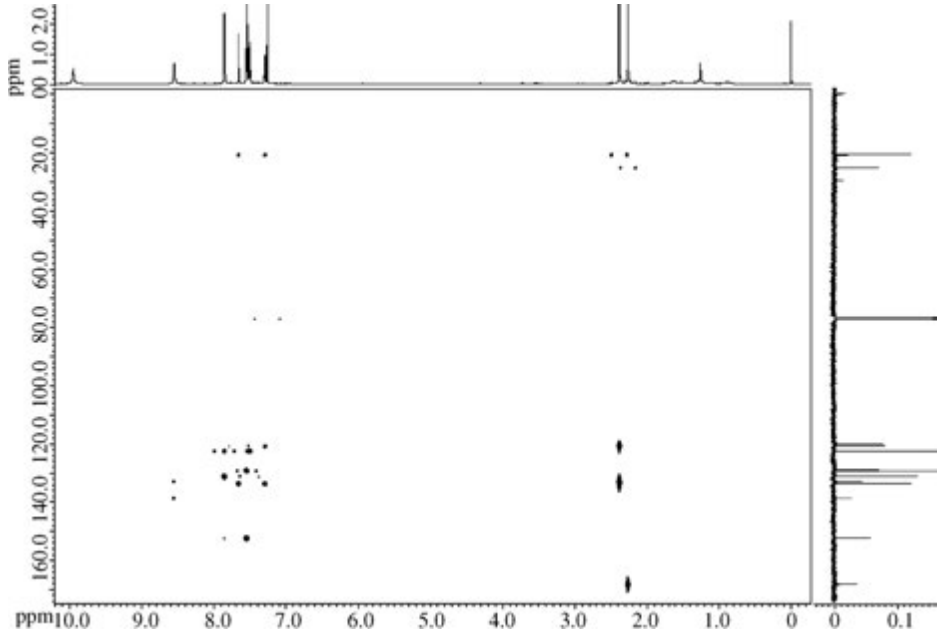


Figure 3.15 The HMBC spectrum of compound **C3-6**

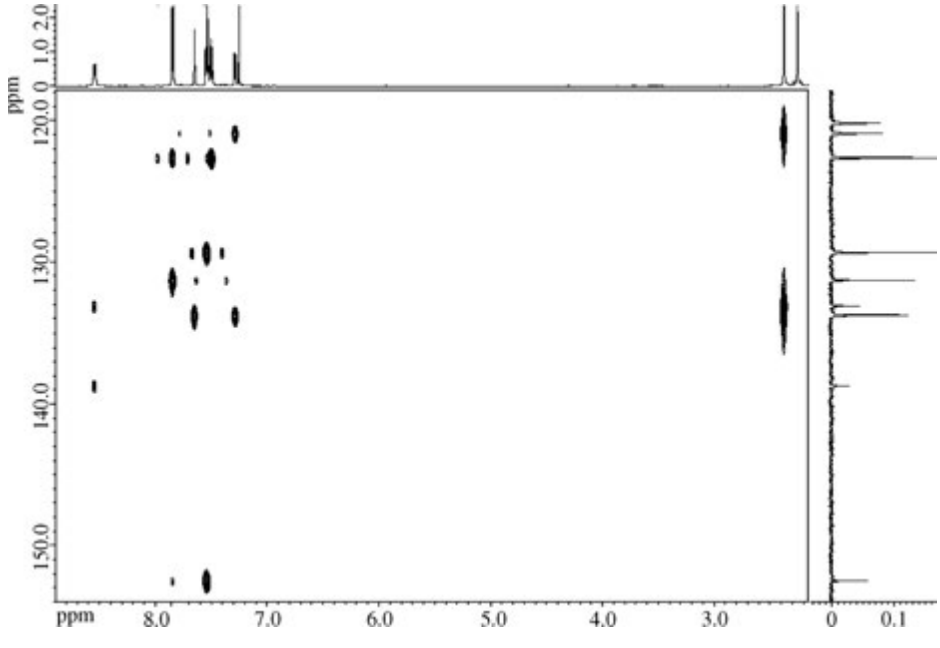


Figure 3.16 The locally enlarged spectrum in the low field region of Figure 3.15

This type of the correlated peaks corresponds to the δ_C and the δ_H.

2. A pair of correlated peaks is situated on a horizontal line. Their center aims at the center of a peak set in the ^{1}H spectrum. The pair of the two peaks at about $F_1 = 22$ ppm and that at about $F_1 = 26$ ppm in Figure 3.15 belong to the type mentioned above. Their middle points aim at $F_2 = 2.35$ ppm (singlet) and at $F_2 = 2.25$ ppm (singlet), respectively. The correlated peaks of this type illustrate the couplings from ^1J couplings. The correlated peak with $F_1 = 22$ ppm and $F_2 = 2.35$ ppm illustrates the correlation between the peak at 22 ppm in the ^{13}C spectrum and the peak set at 2.35 ppm in the ^{1}H spectrum, that is, the related carbon atom and the related hydrogen atom are connected directly. The correlated peak with $F_1 = 26$ ppm and $F_2 = 2.25$ ppm illustrates that the carbon atom with $\delta_C = 26$ ppm is connected directly with the hydrogen atom with $\delta_H = 2.25$ ppm. Of course, this information has been obtained from the HMQC (or HSQC) spectrum already. However, we need to know the meaning of this type of correlated peaks in the HMBC spectrum. And they can serve as an additional proof of the result from the HMQC spectrum.

3. A pair of two peaks with another peak at their center is situated on a horizontal line. The central peak aims at a peak set in the ^{1}H spectrum. The meaning of the three peaks is the same as that mentioned above. That is, the three peaks illustrate the coupling from ^1J. Item 2 and 3 are the two manifestations about the ^1J couplings. The three peaks at $F_1 = 129$ ppm with their center position at 7.55 ppm belong to such an example. For the three peaks, the intensity of the central peak is stronger than that of two other peaks.

In the HMBC spectrum, it is certain that there exists the first kind of correlated peaks. Whether two other kinds exist or not depends on the conditions for the measurement.

It should be noticed that artifacts may be shown in the HMBC spectrum. The artifacts can be recognized from the following two facts.

1. The ordinate or/and abscissa of an artifact do not coincide with any δ_C or δ_H except what we described in items 2 and 3 above.

2. Artifacts have low intensities.

The function of the HMBC spectrum can be summarized as follows.

1. The connection across several chemical bonds between a carbon atom and a hydrogen atom can be found from the HMBC spectrum.

This function is very important for the identification of an unknown compound. We have known that the connection between carbon atoms and hydrogen atoms of a structural unit can be determined by the comprehensive interpretation of the COSY spectrum and the heteronuclear shift correlation spectrum, for example, the HMQC spectrum. This operation can be extended to the structural unit and even to the whole structure. However, it will stop at any quaternary carbon atom or heteroatom. Therefore, we can only obtain some structural units in this way. How can we connect the found structural units? The unique method is the application of the HMBC spectrum.

Because the correlated peaks in the HMBC spectrum illustrate the correlation across two to five chemical bonds, the interpretation of the HMBC spectrum is rather difficult. Therefore, we must consider many possibilities about one correlated peak. For the

deduction of an unknown structure, it is essential to deal with many correlated peaks in the HMBC spectrum. Thus, the number of the total possibilities of an unknown structure will be a huge number, because the total possibilities are the product of individual possibilities.

From the beginning of the 1980s, the computer-assisted structure elucidation developed rapidly. The related commercial software appeared in the 1990s. The great superiority of the computer-assisted structure elucidation over the human interpretation lies in the interpretation of the HMBC spectrum, because the computer can deal with many possibilities resulting from the HMBC spectrum.

Anyhow, in spite of the difficulties in the interpretation of the HMBC spectrum, the interpretation of the HMBC spectrum is the unique method to find an unknown structure containing quaternary atoms and heteroatoms, which exist in a compound in general.

2. Because the ordinate (F_1) of the HMBC spectrum is δ_C, which has a high resolution, the fact that the HMBC spectrum has a high resolution is very important. From related comparisons it can be known that the resolution of the HMBC spectrum is often higher than that of the HMQC (or HSQC) spectrum. And it is certain that the resolution of the HMBC spectrum is much better than that of the COSY spectrum. Of course, the resolution of the ^{1}H spectrum is not comparable to that of the HMBC spectrum. Therefore, when peak sets are overlapped in a ^{1}H spectrum, we will examine the HMQC (or HSQC) spectrum first. If the result is still not clear, we will see the HMBC spectrum. When correlated peaks are overlapped in a COSY spectrum so that to read data is difficult, the result from its HMBC spectrum can help to obtain related correct information.

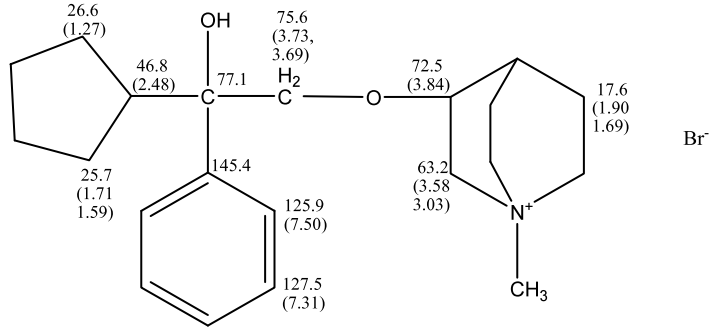
3. The correlated peaks from the ^1J couplings in the HMBC spectrum can be an additional check of the result from the HMQC (or HSQC) spectrum.

The HMBC spectrum of compound **C3-5** is shown to illustrate its function.

Because of the complexities of the structure and its corresponding HMBC spectrum, we list only partial data read out from the spectrum and partial assignments of the structure.

The partial result of Figures 3.17 and 3.18 is shown in Table 3.5

The partial assignment is shown as follows.



The function of the HMBC spectrum can be known from the assignment above. The connection around the quaternary carbon atom with $\delta_C = 77.1$ in particular can not be found without the HMBC spectrum.

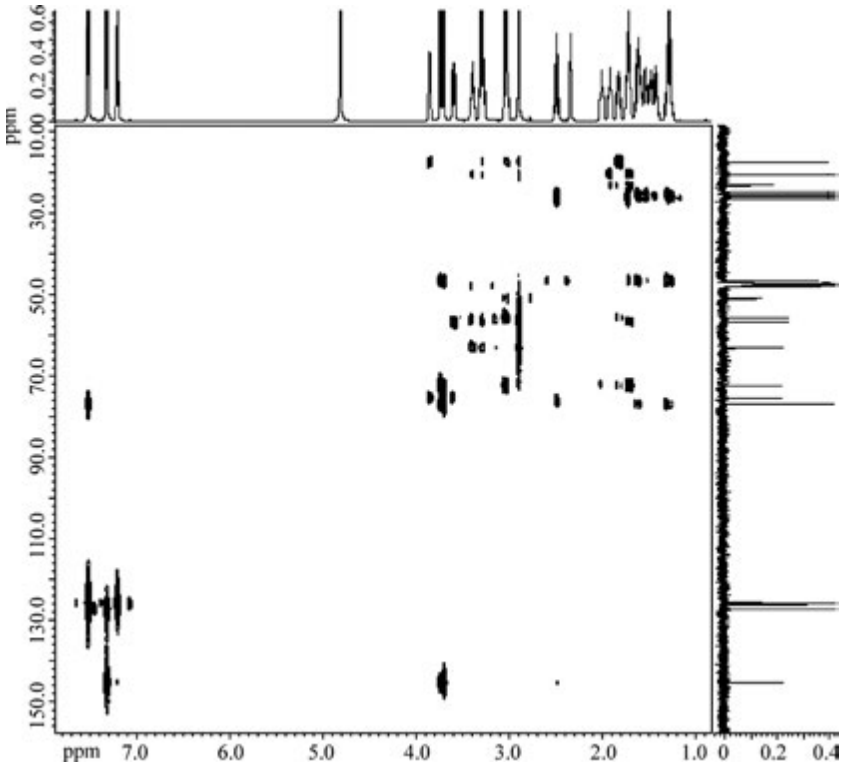


Figure 3.17 The HMBC spectrum of compound **C3-5**

3.5 NOESY and ROESY

NOESY and ROESY belong to the two-dimensional NMR spectra about the NOE effect. They are somewhat different in principle and function. However, their appearances and the interpretation methods for them are the same. Readers who want to know more about the topic can read the reference [1].

After the measurement of a conventional ^{1}H spectrum, if two hydrogen atoms less than 5 angstroms apart exist in a molecule, when the ^{1}H spectrum is again measured under the irradiation of the peak set of one of the two hydrogen atoms, the area of the peak set of the other hydrogen atom will change. This phenomenon is called the NOE effect. The difference in NOE spectrum comes from the subtraction of the first (conventional) ^{1}H spectrum from the second ^{1}H spectrum, and a peak will appear where there exists a change in area, which indicates the existence of the NOE effect.

The above-mentioned method is to measure the NOE effect by one dimensional spectrum. This kind of difference NOE spectrum shows only the NOE effect from one pair of hydrogen atoms. If a structure contains many pairs of hydrogen atoms with the NOE effect, the measurement of all pairs having NOE effects will be tedious and time-consuming. The two dimensional NOE spectrum can show all pairs of hydrogen atoms having the NOE

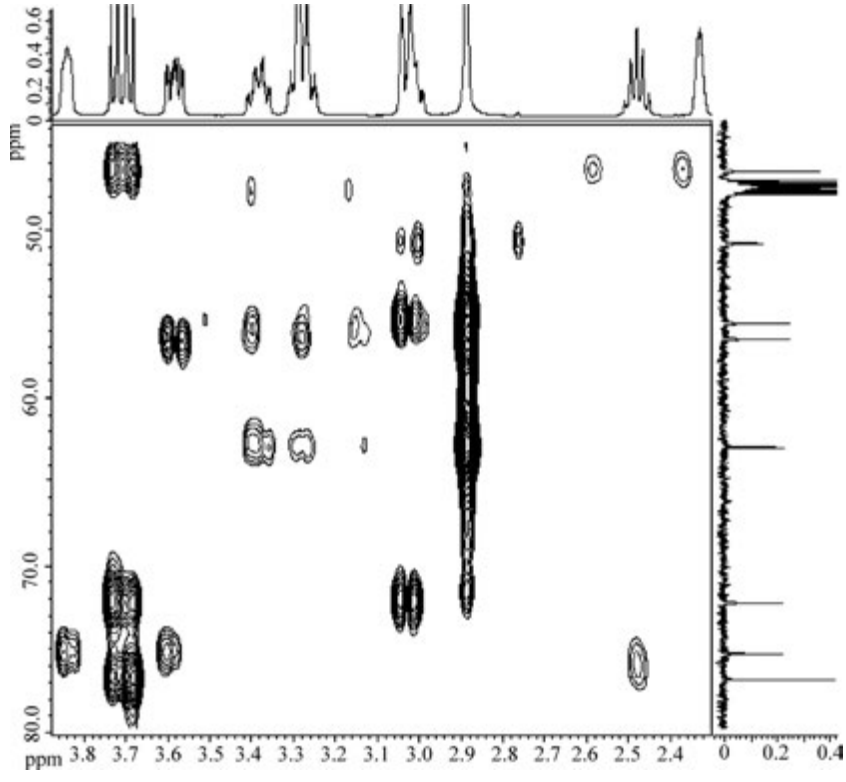


Figure 3.18 The locally enlarged spectrum of Figure 3.17

effect in a spectrum. Therefore, all pairs of hydrogen atoms less than 5 angstroms apart can be found.

The appearance of NOESY (or ROESY) is the same as that of the COSY spectrum. However, the correlated peaks in the NOEY (or ROESY) spectrum illustrate the NOE effect between two hydrogen atoms but the COSY spectrum illustrates the correlation between two hydrogen atoms from ^{3}J couplings. Because two vicinal hydrogen atoms are also less than 5 angstroms apart, the correlated peaks in the COSY spectrum can be shown in the NOESY spectrum even when some operations are applied in the NOESY (or ROESY) measurement. Therefore, one needs to differentiate the correlated peaks in the NOESY

Table 3.5 The partial result of Figures 3.17 and 3.18

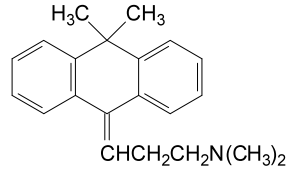
δ_C (ppm)	Coupled hydrogen atom with a long-range J coupling, δ_H (ppm)	Coupled hydrogen atom with ^{1}J couplings, δ_H (ppm)
145.4	7.31, 3.73, 3.69	
77.1	7.50, 3.73, 3.69, 1.59, 1.27	
75.6	2.48,	3.73, 3.69
72.5	3.73, 3.69, 3.03, 1.69	

spectrum from those in the COSY spectrum. The more chemical bonds two hydrogen atoms cross, the more important the significance of the NOE effect is.

The two dimensional spectra about the NOE effect are a powerful tool to resolve stereochemistry problems.

Now we will give two examples here.

The compound **C3-7** has the structure as follows.



• HCl

C3-7

The NOESY spectrum and its locally enlarged spectrum of compound **C3-7** are shown in Figures 3.19 and 3.20, respectively.

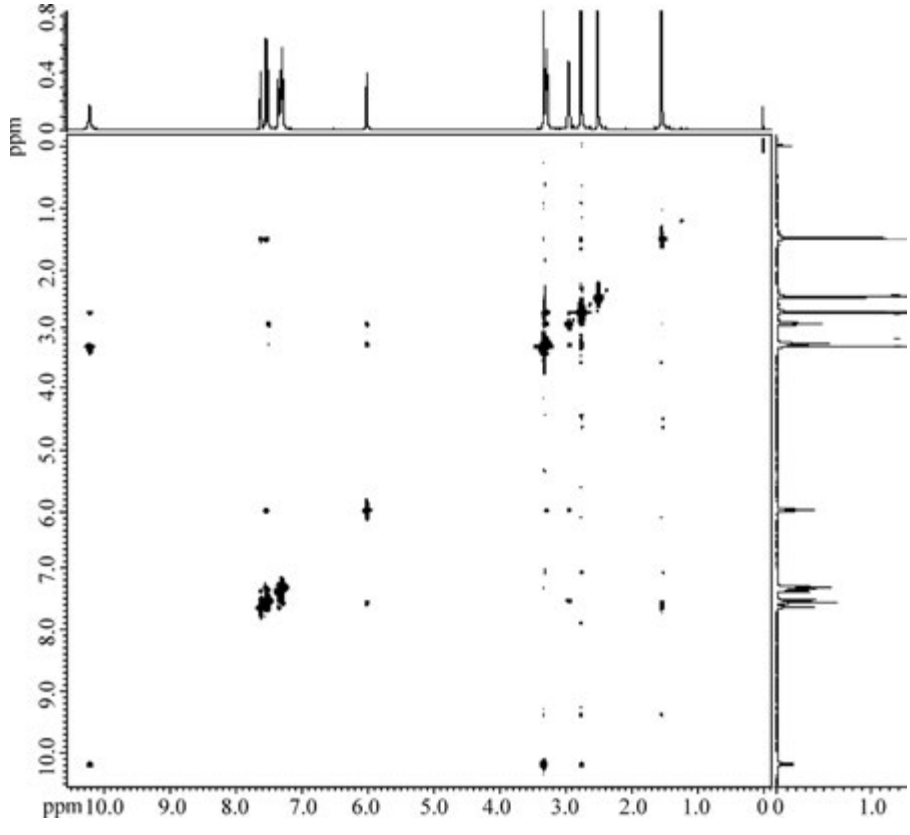


Figure 3.19 The NOESY spectrum of compound **C3-7**

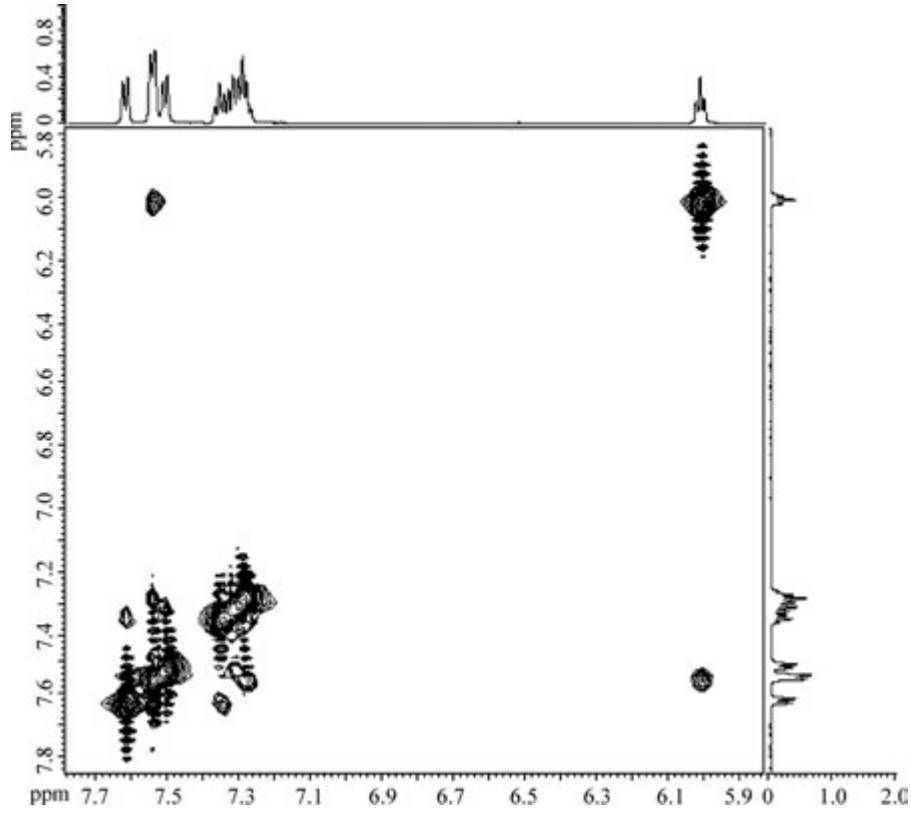
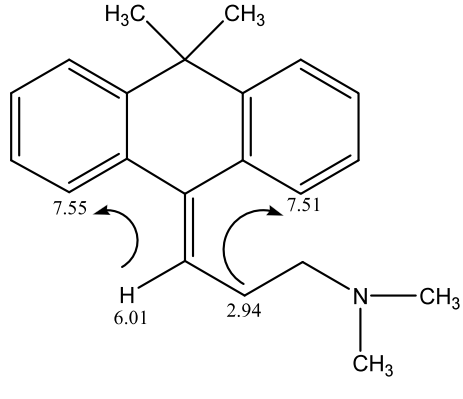


Figure 3.20 The locally enlarged spectrum of Figure 3.19

On the basis of the data obtained from the NOESY spectrum, the NOE effect in the structure is determined, so that the direction of the side-chain of the structure can be determined.



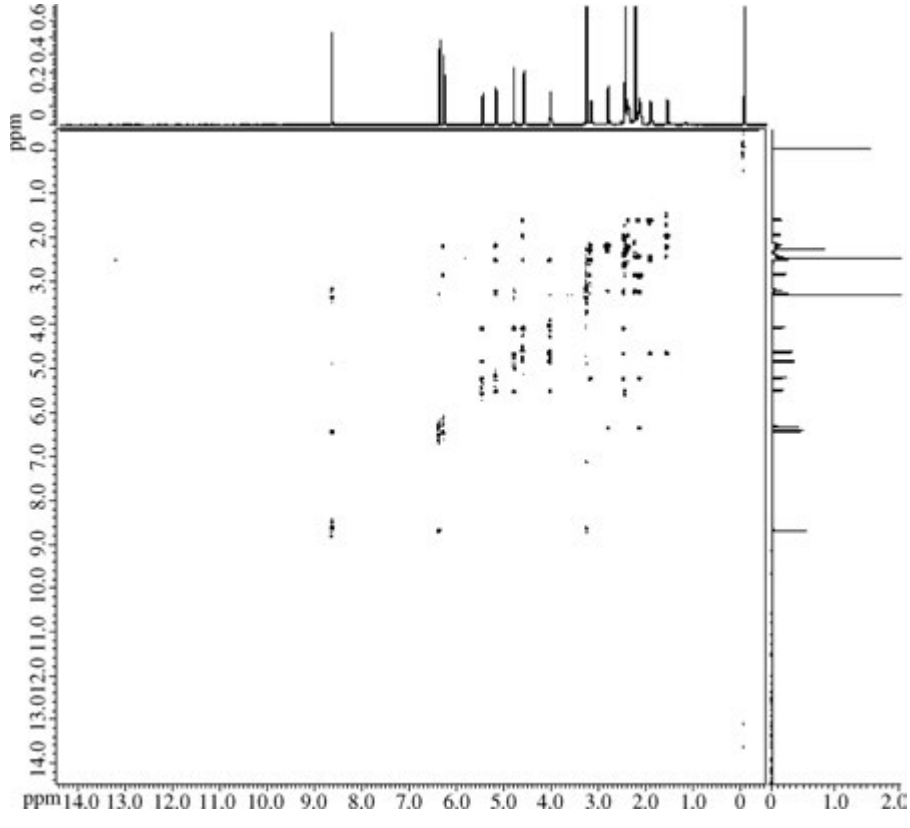
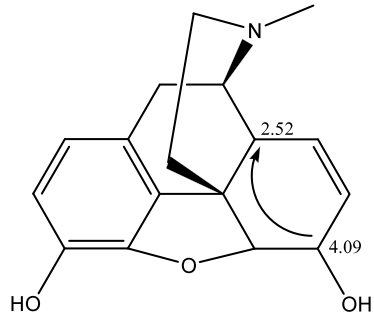


Figure 3.21 The NOESY spectrum of compound **C3-1**

We now give another example of the NOESY spectrum with compound **C3-1** discussed above.

The NOESY spectrum and its locally enlarged spectrum of compound **C3-1** are shown in Figures 3.21 and 3.22, respectively.

From the NOESY spectra, the NOE effect can be illustrated as follows.



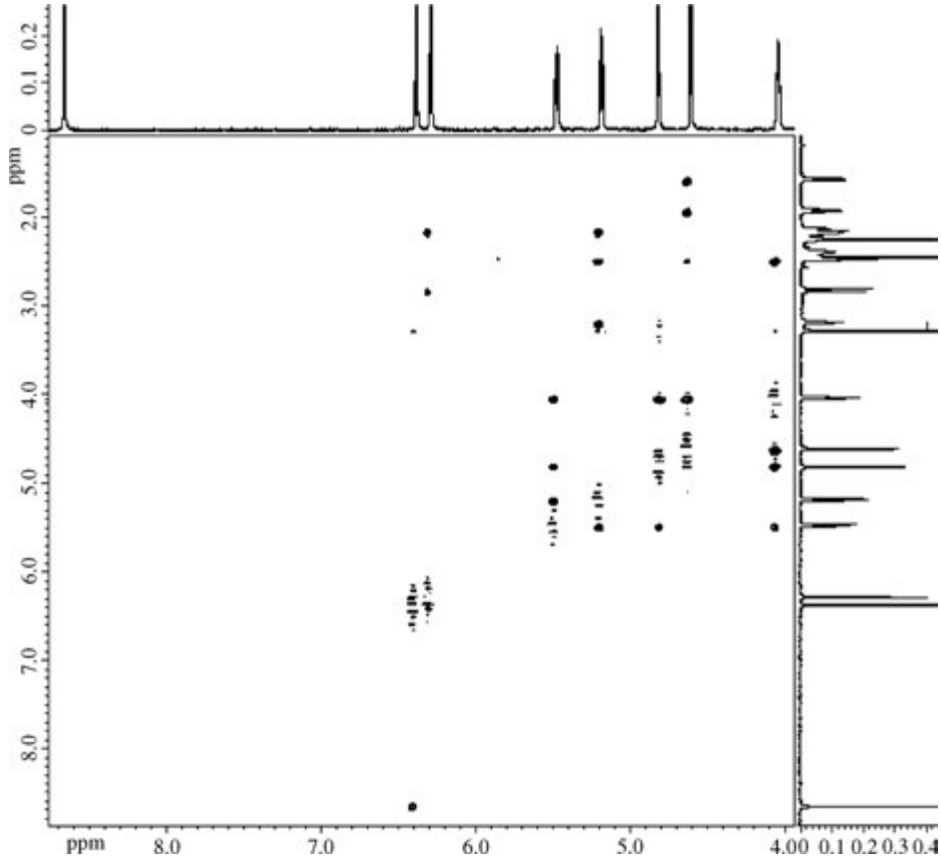


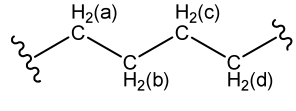
Figure 3.22 The locally enlarged spectrum of Figure 3.21

Because the two hydrogen atoms are across five chemical bonds, they can not have any couplings and the NOE effect illustrates that the six-membered ring possesses a boat configuration.

3.6 Total Correlation Spectroscopy, TOCSY

We discussed already the COSY spectrum in Section 3.2. From the spectrum, the vicinal couplings can be found (we do not discuss the ^4J couplings for the moment). By drawing a horizontal line or a vertical line through a correlated peak, they will pass through several other correlated peaks, which correlate vicinal hydrogen atoms. That means the related carbon atoms are connected directly.

If there is the following structural unit:



By using the COSY spectrum, just vicinal couplings can be determined. Supposing we can find the coupling from CH_2(a) to CH_2(d), it will be very useful for the deduction of the unknown structure.

The appearance of the TOCSY spectrum is similar to that of the COSY spectrum. The projections on the abscissa (ω_2, F_2) or on the ordinate (ω_1, F_1) are the ^1H spectrum. Therefore, its abscissa (ω_2, F_2) and ordinate (ω_1, F_1) are denoted by δ_H. The ^1H spectrum is put on the top and on the side. The TOCSY spectrum has also a diagonal line. The useful information comes from the correlated peaks, which are situated off the diagonal line. The difference between the COSY spectrum and the TOCSY spectrum is that the number of the correlated peaks of the latter is much greater than that of the former. For the above structural unit, by drawing the vertical line through the peak set of CH_2(a), the line will pass through the correlated peaks of CH_2(b), CH_2(c) and CH_2(d).

Supposing an unknown structure contains several saccharine rings, by using its TOCSY spectrum the couplings in every saccharine ring can be found and the signals of different saccharine rings can be differentiated.

The example about the TOCSY spectrum will be discussed in the last example in Section 6.2.

References

[1] Ning, Yong-Cheng (2005) Chapter 4, in *Structural Identification of Organic Compounds with Spectroscopic Techniques*, Wiley-VCH.
[2] Berger, S. (1995) *Journal of Fluorine Chemistry*, **72**, 117–119.

4

Interpretation of Mass Spectra

The mass spectrum is the unique method to obtain the molecular weight of an unknown compound. The mass spectrum with a high resolution can give the molecular formula of an unknown compound. The mass spectrum is necessary in principle for the identification of the structure of an unknown compound.

EI mass spectra, which are produced by using electron impact ionization, will be discussed mainly in this chapter. Mass spectra produced by soft ionization will be presented later in this chapter.

4.1 Basic Knowledge of Organic Mass Spectrometry

4.1.1 Mass Spectra

The abscissa of the mass spectrum is the mass-to-charge ratio, which is the ratio of the mass of an ion to its carried charge. In general, the direction from left to right denotes an increase in the mass-to-charge ratio. Since recorded ions are frequently singly charged ions, the abscissa illustrates the masses of the ions.

The ordinate of the mass spectrum represents the intensities of the peaks of ions. The highest peak in the mass spectrum is called the base peak.

4.1.2 Ionization in Organic Mass Spectrometry

Because different types of ionization have different principles, the mass spectra obtained by using different types of ionization are different, and their interpretation methods are different.

We will discuss mass spectra respectively according to the type of ionization.

Interpretation of Organic Spectra, First Edition. Yong-Cheng Ning.
 Published 2011 by John Wiley & Sons (Asia) Pte Ltd. ISBN: 978-0-470-82516-7

4.1.2.1 Electron Impact Ionization

The electron impact ionization is the earliest developed ionization.

Many fragment ions (in a broad sense) can be obtained by using the ionization. Therefore, the electron impact ionization is useful for the postulation of an unknown structure.

The disadvantage of the electron impact ionization may be a low intensity of the molecular ion peak. In some cases the molecular ion peak is absent by using the electron impact ionization.

4.1.2.2 Soft Ionization

All ionization methods belong to soft ionization except the electron impact ionization.

Soft ionization mainly produces quasi-molecular ions, from which the molecular weight of the sample can be deduced.

Soft ionization includes chemical ionization (CI), fast atom bombardment (FAB), matrix-assisted laser desorption-ionization (MALDI), electrospay ionization (ESI), atmospheric pressure chemical ionization (APCI), and so on.

The mass spectra by using soft ionization will be presented in Section 4.4.

4.1.3 Ion Types in Organic Mass Spectrometry

4.1.3.1 Molecular Ions

A molecular ion is produced from the ionization of an organic molecule. It is denoted as "$M^{+\bullet}$." The symbol "+" indicates that the ionized molecule has lost an electron and "." indicates that the ionized molecule has lost one of its paired electrons, leaving an unpaired electron, which means that the ionized molecule is a radical.

The mass-to-charge ratio of the molecular ion is numerically equal to its molecular weight when it is a singly-charged ion.

4.1.3.2 Quasi-Molecular Ions

Quasi-molecular ions are produced from soft ionization. A quasi-molecular ion contains an organic molecule (M) and another composition, such as $M + H]^+$, $M-H]^+$, $M + Met]^+$, where H means the hydrogen atom, and Met means a metal atom.

The quasi-molecular ions do not contain an unpaired electron, and they are stable in structure.

4.1.3.3 Fragment Ions

Fragment ions in a broad sense are ions that result from fragmentation of molecular ions. Fragment ions (in a narrow sense) are the ions that are produced from the molecular ions through simple fragmentation reaction only. In this text, the expression "fragment ions" is used in the narrow sense unless otherwise stated, that is, they do not contain rearrangement ions.

4.1.3.4 Rearrangement Ions

Rearrangement ions result from rearrangement reactions of the molecular ion or other ions. The structure of rearrangement ions is not that in their original structure.

4.1.3.5 Parent Ions and Daughter Ions

If an ion produces another ion, the former is called the parent ion and the latter the daughter ion. Of course, the molecular ion is the earliest parent ion.

4.1.3.6 Metastable Ions

"Metastable" implies "lying between stable and unstable." Metastable ions are those produced from the ion source exit to the detector. Through metastable ions the pair of a parent ion and the daughter ion can be found.

4.1.3.7 Odd-Electron Ions and Even-Electron Ions

Ions with unpaired electrons are called odd-electron ions. They are highly reactive because of the presence of the unpaired electrons.

Ions without any unpaired electron are called even-electron ions, which are more stable than odd-electron ions.

4.1.3.8 Multiply-Charged Ions

Ions with more than one charge are called multiply-charged ions. As a result, their mass-to-charge ratios decrease correspondingly, which is useful for the determination of the molecular weight of the molecule with a large molecular weight.

The peak for the mass-to-charge ratio of a half-integer should be the peak of double-charged ions.

4.1.3.9 Isotopic Ions (Cluster)

Molecules that contain an element with a non-unique isotopic composition will produce isotopic ions. Isotopic ions form isotopic ion clusters.

4.2 Isotopic Ion Clusters in Mass Spectra

Before we discuss the interpretation of the mass spectrum, we need to present the concept of isotopic ion cluster. When an organic molecule contains an element with a non-unique isotopic composition, the isotopic ion clusters will appear in its mass spectrum. Isotopic ion clusters can exist in the molecular ion or quasi-molecular ion region, or in the fragment ion region, that is, the region with a smaller mass-to-charge ratio than that of the molecular ion. When the compound contains bromine or/and chlorine elements, the isotopic ion clusters are prominent.

Since it is a cluster, there is a ratio of the intensities of the peaks in the cluster. The ratio of the intensities of the peaks in an isotopic ion cluster will be discussed in this section.

The relative isotopic abundances of common elements in organic chemistry are shown in Table 4.1.

Table 4.1 is differentiated from the common isotopic abundance table, in which all isotopic contents are set as 100% for an element. For the convenience of the calculation of the intensity ratio of isotopic ion clusters, the abundance of the lightest isotope, which is also the most abundant isotope, A, is set as 100% in Table 4.1. A + 1 and A + 2 are the isotopes whose atomic weights are heavier than A by 1 and by 2 (u), respectively.

First of all, we discuss the simplest case. Assume that an element possesses two isotopes and that m atoms of the element exist in a compound. The relative intensities of the cluster peaks can be calculated by the binomial $(a + b)^m$.

Table 4.1 The relative isotopic abundances of common elements in organic chemistry

	A	A + 1	A + 2
C	100	1.11	
H	100	0.015	
N	100	0.37	
O	100	0.04	0.20
F	100		
Si	100	5.06	3.36
P	100		
S	100	0.79	4.43
Cl	100		31.99
Br	100		97.28
I	100		

$$(a+b)^m = a^m + ma^{m-1}b + \frac{m(m-1)}{2!}a^{m-2}b^2 + \cdots + \frac{m(m-1)\ldots(m-k+1)}{k!}a^{m-k}b^k + \cdots + b^m \tag{4.1}$$

Equation 4.1 can be easily understood, because the developed binomial is an equation for probabilities.

In Equation 4.1 a is the isotopic abundance of the lighter isotope, and b is the isotopic abundance of the heavier isotope.

The values of the terms in Equation 4.1 describe the intensities of the cluster peaks. The plus sign in the developed equation implies "coexistence" of the cluster peaks or relatively intensity ratio of the cluster peaks.

Assume a compound contains two chlorine atoms. Since 100 : 31.99 can be approximately 3 : 1, we can substitute $m = 2$, $a = 3$, and $b = 1$ into Equation 4.1 and then obtain the following result:

$$(a+b)^m = (a+b)^2 = a^2 + 2ab + b^2 = 9:6:1$$

The expression above means that three peaks at M, M + 2, and M + 4 can be shown in its mass spectrum. And the intensity ratio of the three peaks is 9 : 6 : 1.

If a compound contains i elements and every element has two isotopes, Equation 4.1 becomes the following equation:

$$(a_1+b_1)^{m_1}(a_2+b_2)^{m_2}(a_3+b_3)^{m_3}\ldots(a_i+b_i)^{m_i} \tag{4.2}$$

where a_1, b_1, and m_1 correspond to the element No. 1, and so on.

In the application of Equation 4.2, the equation should be developed. After the development, the terms that correspond to the same mass should be added. The remaining plus sign has the same meaning as before.

The function of the application of Equations 4.1 and 4.2 will be shown in examples in Section 4.3.6.

Because the isotopic cluster of the molecular ion is situated at the right edge of the mass spectrum, its appearance is very clear. Therefore, its information is more reliable than that in the lower mass region.

In the lower mass region, the isotopic cluster peaks can overlap the peaks of fragment ions and rearrangement ions.

Besides isotopic clusters, there are clusters from fragment ions (in a narrow sense) in the EI mass spectrum. Of course, the appearances of fragment ions and those of isotopic clusters are different.

4.3 Interpretation of EI MS

For the postulation of an unknown structure, at least the molecular weight of the unknown compound should be known. Of course, it is better if one is aware of its molecular formula. Therefore, first of all, we hope to get the molecular weight of the unknown compound from the mass spectrometry, including those from various types of ionization. In addition, the information on fragment ions (in a broad sense) can help to postulating an unknown structure.

Since there are many fragment ion peaks (in broad sense) in the EI mass spectrum, the EI mass spectrum has more structural information than that from soft ionization if the molecular ion peak is shown in the EI mass spectrum.

4.3.1 Determination of Molecular Ion Peak

If a molecular ion peak can be determined in an EI mass spectrum, the molecular weight of the sample can be determined.

Since the molecular ion has the largest mass-to-charge ratio, the molecular ion peak should appear at the right edge of the mass spectrum.

When the sample to be measured cannot be vaporized or it can be vaporized but with decomposition, or when there are no intact ionized molecules on the ionization, it is possible that there be no molecular ions in the EI ionization. On the other side, the impurities, especially the volatile impurities, may interfere with the recognition of the molecular ion peak, because their peaks may situate in the high mass-to-charge region. Therefore, it is not easy to judge if the peak at the right edge of the mass spectrum is the molecular ion peak.

The molecular ion peak can be recognized with the help of the following facts.

4.3.1.1 The Peak with the Largest m/z could be the Molecular Ion Peak

The peak with the largest *m/z* value could be the molecular ion peak. When it is one peak of an isotopic cluster, it can be recognized according to specific rules (refer to Section 4.2).

4.3.1.2 The Logical Loss of Neutral Fragments is the Key to the Recognition

The molecular ion will produce a fragment ion (in a broad sense) with a lower mass by losing a neutral fragment. Because the neutral fragment has some limitations in its mass, the verification of the mass difference between the molecular ion peak and the next peak towards the direction of the decrease of *m/z* is very important. The mass difference should be reasonable. This is the most important criterion for the recognition of the molecular ion peak.

An organic molecule can lose one or two hydrogen atoms during its ionization. However, it is not possible that a molecule lose three even four hydrogen atoms during its ionization

without its fragmentation. On the other hand, the smallest fragment, which an organic molecule can lose, is a methyl group. On the basis of the consideration above, there should be no fragment ion peaks (in a broad sense) in the region from M−3 to M−15. If this case exists, the peak with the largest mass will not be the molecular ion peak.

For example, the last two peaks at the right edge of a mass spectrum have the mass difference of 3. These two peaks may be the peak produced from the loss of a molecular ion by a methyl group and from the loss of the molecular ion by a water molecule. Therefore, the peak with the largest mass is not the molecular ion peak.

4.3.1.3 A Molecular Ion Peak Should Have a Complete Elemental Composition

A molecular ion peak should have a complete elemental composition. If an elemental composition deduced from any cluster in the mass spectrum is more complete than that of the supposed molecular ion peak, the latter cannot be the molecular ion peak.

The mass of the multiply-charged ions after the number of charges is modified should be less than (or equal to) that of the supposed molecular ion.

4.3.1.4 The Nitrogen Rule can Help to Judge the Molecular Ion Peak

The nitrogen rule can be stated as follows.

If a compound contains an even number or no nitrogen atoms, its molecular weight will be an even number. If a compound contains an odd number of nitrogen atoms, its molecular weight will be an odd number.

This rule can be understood easily as follows. The main isotopes of common elements existing in organic compounds have an odd number of atomic weight and an odd number of chemical equivalence, such as ^{1}H, ^{19}F, ^{31}P, ^{35}Cl, and ^{79}Br; or an even number of atomic weight and an even number of chemical equivalence, such as ^{12}C, ^{16}O, and ^{32}S. However, ^{14}N, which is the main isotope of the element nitrogen, has an even number of atomic weight but an odd number of chemical equivalence. Therefore, if we know that the compound to be analyzed contains no nitrogen atom, its molecular weight should be an even number. If its mass spectrum shows an odd number as a molecular ion peak, it is certain that this consideration is wrong.

The nitrogen rule can be used to help to judge a molecular ion peak. It can also be used for the analysis of the peak of a fragmental ion (in a broad sense).

4.3.1.5 The Intensity of the Molecular Ion Peak is Related with the Types of Compounds

If the type of the compound to be analyzed has been determined by using any other kind of spectra, the information can help to judge a molecular ion peak.

In EI mass spectra, the intensities of molecular ion peaks can be divided into three classes:

1. The following compounds give prominent molecular ion peaks whose intensities are arranged in the following order: aromatic compounds > conjugated polyenes > alcyl compounds > short chain alkanes > some sulphur-containing compounds.
2. Straight chain compounds, such as ketones, esters, aldehydes, amides, ethers, and halogenides in general show molecular ion peaks.
3. The following compounds with large molecular weights: aliphatic alcohols, amines, nitrates and nitrites have no molecular ion peaks, especially for those with highly branched chains.

Since a compound contains several functional groups and a practical situation may be complicated, the above-mentioned three classes are a rough classification.

4.3.1.6 Differentiation of a Molecular Ion Peak from the Peaks of $M+1]^+$ or $M-1]^+$

Ethers, esters, amines, amides, cyanides, and so forth may show a remarkable $M+1]^+$ peak while aromatic aldehydes, some nitrogen-containing compounds, and so forth may have a strong $M-1]^+$ peak. The determination of these functional groups by other spectroscopic techniques can help to differentiate the peak of $M^{+\bullet}$ from that of $M-1]^+$ or $M+1]^+$.

Sometimes the analysis of the relationship among fragment ion peaks in the large mass region is also helpful to determining the molecular ion peak.

A molecular ion produces fragment ions (in a broad sense), which can be divided as fragment ions (in a narrow sense) and rearrangement ions according to their reactive mechanism. The aim of the interpretation of the peaks of these ions is to deduce the structural units of the sample molecules.

4.3.2 Interpretation of Fragment Ion Peaks

The so-called simple cleavage is the process in which only one chemical bond is cleaved so that the products are the structural units of the original molecule. The ions produced by simple cleavage reactions are called fragment ions (in a narrow sense). What will be discussed in this section is limited to the recognition of the peaks of fragment ions (in a narrow sense).

4.3.2.1 Three Types of Simple Cleavages

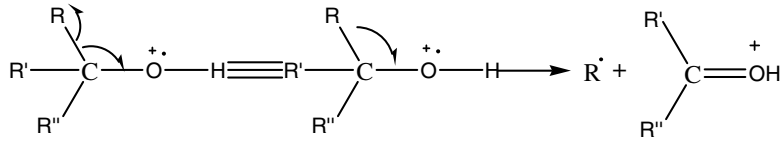
Simple cleavages start from the molecular ion.

When the charge can be localized on one particular atom, the sign "+" is shown on that atom. If the ion possesses an unpaired electron, it is noted as "+.," for example, $CH_3\text{-}CH_2\text{-}CH_2\text{-}O^{+\bullet}H$.

When the charge can not be localized on one particular atom or the labeling of its position is not necessary, square brackets "[ ]" or "] " should be marked at the end of the structural formula of the ion, for example, $[CH_3\text{-}CH_2\text{-}CH_2\text{-}OH]^{+\bullet}$ or $CH_3\text{-}CH_2\text{-}CH_2\text{-}OH]^{+\bullet}$.

The simple cleavage, in which a chemical bond in the original structure is broken, is classified into the following three types:

Homolytic Cleavage The two electrons of a chemical bond transfer to two sides, so that the process produces a radical and an even-electron ion, for example:



The driving force of this type of cleavage is the strong tendency of a radical to form electron pairing.

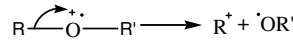
The tendency to produce this type of cleavage generally parallels that of the radical site to donate electrons:

$$N > S, O, \pi, R > Cl, Br > H,$$

where π signifies an unsaturated site and $R^{\bullet}$, an alkyl radical.

The possibility of producing this type of cleavage is high, especially when the compound contains some heteroatoms, such as nitrogen or oxygen atoms.

Heterolytic Cleavage The two electrons of a chemical bond transfer to one side, so that the process produces an even-electron ion and a radical, for example:

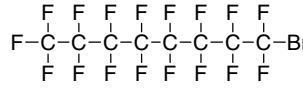


Notice that the charge-site has been changed after the cleavage.

The cause to produce this type of cleavage comes from the induction effect of the original charge. The production of a stable alkyl ion is favorable. The order for producing this type of cleavage is:

$$\text{halogens} > O, S \gg N, C$$

We discussed a compound in Section 3.2, which has the following structure:



A bromine atom situates at the terminal edge of the structural formula. Because the bromine atom can strongly induce this type of cleavage, there is no molecular ion peak in its EI mass spectrum, which is replaced by the fragment ion peak of $C_8F_{17}^{+}$ with a high abundance.

Hemiheterolytic Cleavage In the case where a chemical bond is ionized, the leaving electron transfers toward one side to produce a radical and an even-electron ion, for example:

$$R^{\bullet +} R' \rightarrow R^{\bullet} + R'^{+}$$

This type of cleavage occurs when the compound contains neither heteroatoms (such as nitrogen or oxygen atoms) nor π bond.

4.3.2.2 Rules for the Simple Cleavage

The simple cleavages possess high-degree regularities. With the addition of the generalization by the author, the rules for the simple cleavages are concise.

If the structure of a compound is known, its possible ways of simple cleavages can be easily predicted and so can the ions produced by simple cleavages. Conversely, the information of the ions produced by simple cleavages can help to deduce the structure of the unknown compound.

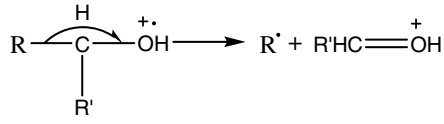
The rules for the simple cleavages can be generalized as follows.

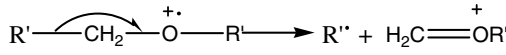
1. There are three types of cleavages for compounds containing heteroatoms:

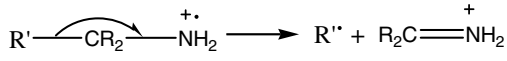
   ① The C–C bond next to the heteroatom is cleaved; or more generally, the bond to be broken is the bond between the α- carbon atom and another atom, including a hydrogen atom, a (β-) carbon atom, and another heteroatom.

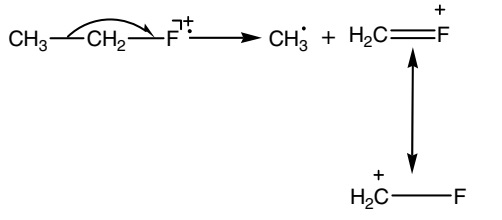
   Fragment ions produced from this cleavage are frequently given in EI mass spectra. In most cases the ions contain the heteroatom that initiates the fragmentation. The charge may be kept at the other side in some cases.

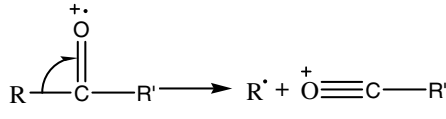
   Some examples are presented below.

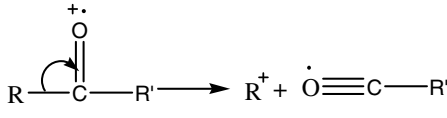




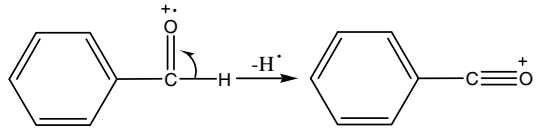




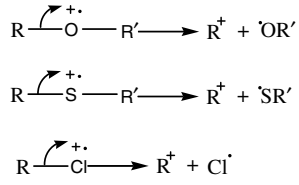




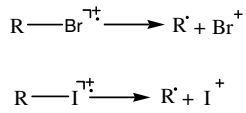
A special example of the fragmentation is the formation of $M-1]^+$.



② The bond between the heteroatom and its α- carbon atom is cleaved and the charge is kept at the site of alkyl. For example:



③ The bond between the heteroatom and its α- carbon atom is cleaved and the charge is kept at the site of the heteroatom.

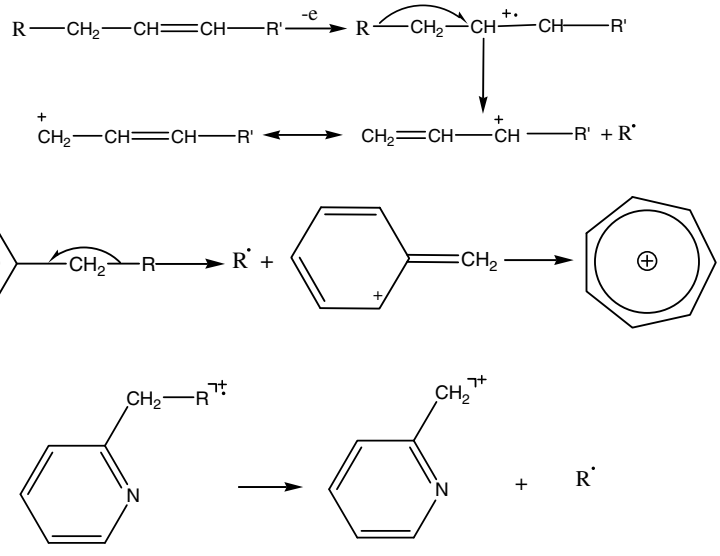


The cleavage in ③ happens less frequently than those in ① and ②.

Obviously, unsaturated heteroatoms which connect the α- carbon atom with σ and π bonds can carry out only the cleavage in ①. Saturated heteroatoms can carry out reactions from ① to ③ but only one reaction is apt to occur.

If the heteroatom is situated in the upper part of the periodic table, especially in upper left part, the cleavage in ① is apt to occur. On the other hand, if the heteroatom is situated in the lower-right part of the periodic table, the cleavage in ③ tends to occur.

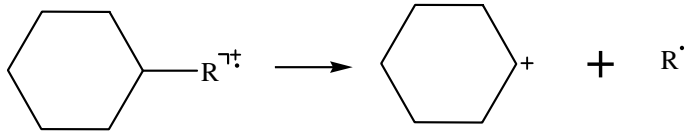
2. The C–C bond next to an unsaturated C=C bond, or a phenyl ring, or a heteroaromatic ring can be easily cleaved. For example:



3. A cleavage is possible at an alkyl-substituted carbon atom. The more of the carbon atom is substituted, the more easily a cleavage takes place, which is a consequence of the following cation stability order:

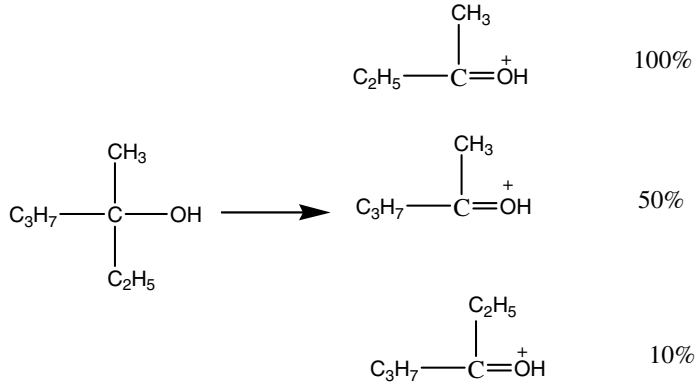
$$^{+}CR_3 > {}^{+}CHR_2 > {}^{+}CH_2R > {}^{+}CH_3$$

Saturated rings tend to lose alkyl side chains at the branch, which is a special case of (3)



m/z 83

4. If several alkyls are connected with a carbon atom, the cleavage, which results in the loss of the largest subsistent, will be the most likely to occur.



Having known these rules mentioned above, one can predict the existence of ions resulting from simple cleavages for a known structural formula, which is very useful for the interpretation of mass spectra.

4.3.2.3 Recognition of Fragment Ions

Fragment ions can be recognized by their mass-to-charge ratios. If a compound contains no nitrogen atoms, its fragment ions will have odd numbers of their mass-to-charge ratios. If a compound contains nitrogen atoms, its fragment ions which contain no nitrogen atom will have odd numbers of their mass-to-charge ratios while its fragment ions which contain a nitrogen atom will have even numbers of their mass-to-charge ratios.

Alkyl fragment ions appear in the EI mass spectrum in general. They can be used as the starting point of the interpretation. Their mass-to-charge ratios are 15, 29, 43, 57, 71....

If an alkyl fragment ion is replaced by an oxygen atom, the series of its mass-to-charge ratios is 31, 45, 73, 87....

4.3.3 Interpretation of Rearrangement Ion Peaks

A rearrangement concerns the cleavage of at least two bonds and the formation of new bonds. A rearrangement produces ions that are not structural units of the precursor ion.

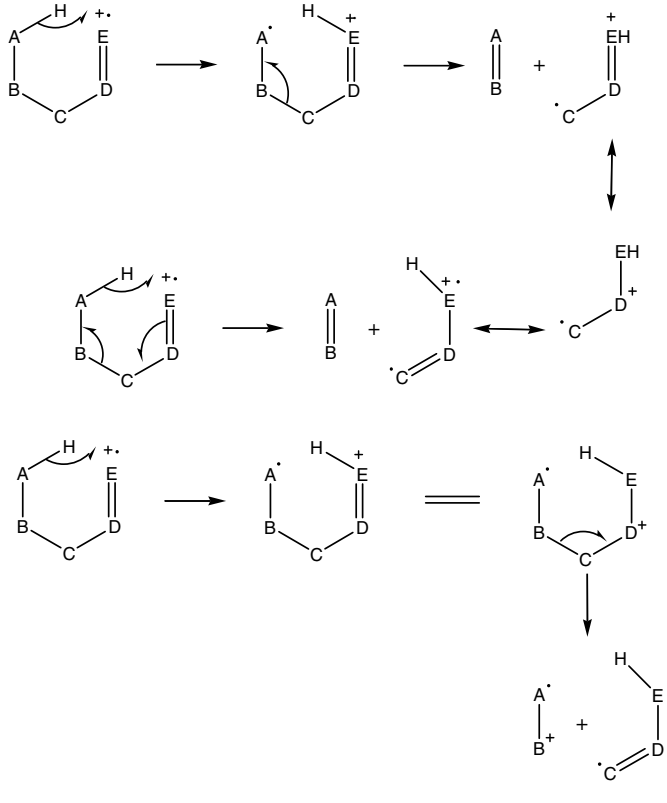
The most common rearrangement leads to the loss of a smaller molecule. Since a molecular ion is an odd-electron ion, an ion rearranged from a molecular ion is an odd-electron ion, too. If the molecule contains no nitrogen atom, the mass of the rearrangement ion will be an even number, while that of the ions resulting from a simple cleavage will be an odd number. Therefore, the rearranged ion can be differentiated from the ions resulting from simple cleavages by the odd or even numbers of the ion mass. For example, the ion *m/z* 91 is produced from an alkyl benzene through a simple cleavage while the ion *m/z* 92 through a rearrangement. The mass difference of 1 u concerns different reactive mechanisms. Ions produced from compounds containing nitrogen atoms by rearrangements and simple cleavages can be analyzed in a way similar to the above.

The small molecules, which are lost during rearrangement reactions, are H_2O, CO, HX (where X is a halogen atom), and so on.

Because of rearrangement reactions, new structural units are produced. Therefore, it is necessary to know common rearrangement reactions.

4.3.3.1 Common Rearrangement Reactions

McLafferty Rearrangement The rearrangement can be shown as follows.



where D = E is a double or triple bond;

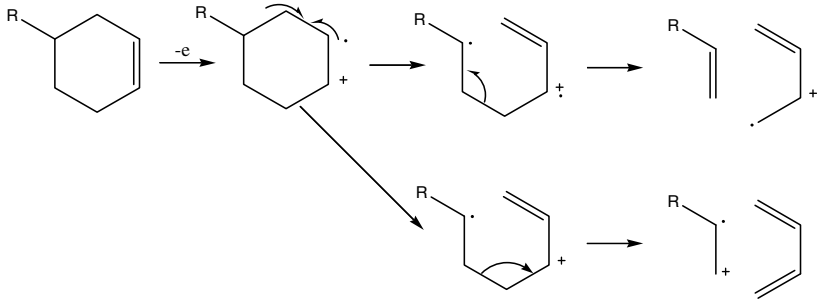
C is a carbon atom or a heteroatom;
H is a hydrogen atom at the γ- position with respect to the unsaturated bond.

Because the hydrogen atom is close to the unsaturated bond, its transfer through a six-membered ring is favorable. If the specific condition (that is, the existence of an unsaturated bond and its γ- H) is satisfied, this rearrangement may well take place.

Although it is possible to produce two kinds of ions by this rearrangement, the formation of the ion containing the unsaturated bond is slightly more probable.

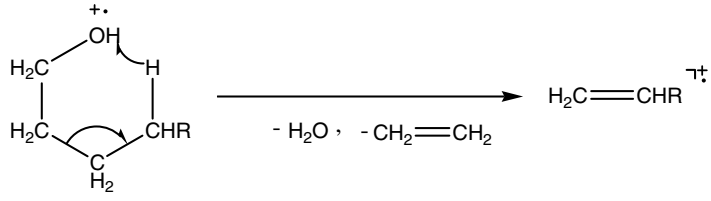
Retro-Diels–Alder Reaction (RDA) Compounds containing a double bond in a six-membered ring can undergo a retro-Diels–Alder reaction, which is the reverse reaction of Diels–Alder reaction, hence the name.

The rearrangement can be shown as follows.



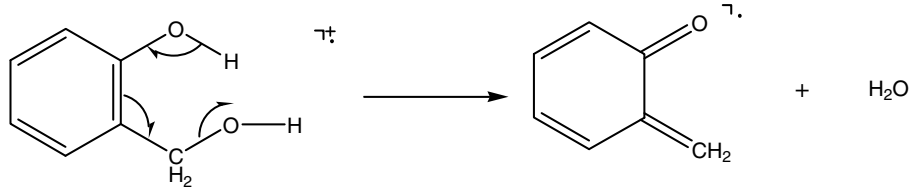
The formation of the ion containing the double bond is more probable.

Rearrangements with the Loss of a Small Molecule This type of rearrangement can take place in many types of compounds by losing a small molecule, for example, an alcohol molecule loses a water molecule or loses a water molecule and an ethylene molecule.

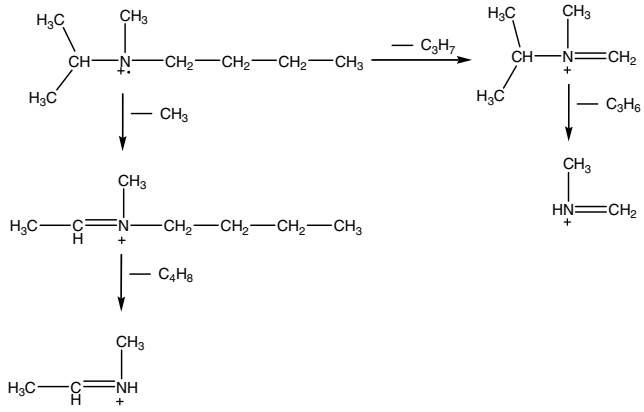


Other examples are the loss of a hydrogen halide from a halide, the loss of HCN from cyanides and the loss of H_2S from thiols. Other small molecules that may be lost include CH_3COOH, CH_3OH, $CH_2{=}C{=}O$, and so forth.

Two substituents at adjacent positions of a benzene ring may lose a small molecule. This is called an ortho- effect, for example:



Rearrangements through the Transfer of a Four-Membered Ring This rearrangement mainly takes place in a fragment ion containing a saturated heteroatom. The rearrangement reaction is shown as follows.



There are other rearrangements, which are omitted here, because the probabilities that these arrangements take place, are small.

4.3.3.2 Recognition of Rearrangement Ions

If a compound contains no nitrogen atom, its rearrangement ions have an even mass number. Therefore, the rearrangement ions can be easily differentiated from ions produced by simple cleavages.

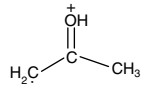
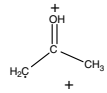
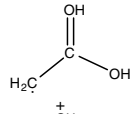
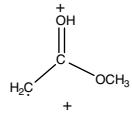
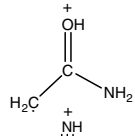
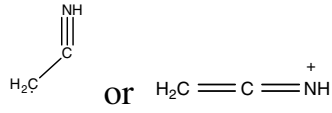
The rearrangement ions produced by McLafferty can be found from Table 4.2.

4.3.4 Complex Cleavages of Alicyclic Compounds

The complex cleavages of alicyclic compounds are different from the RDA rearrangement. An alicyclic molecule must cleave two bonds in order to lose a fragment. And this process includes the migration of a hydrogen atom.

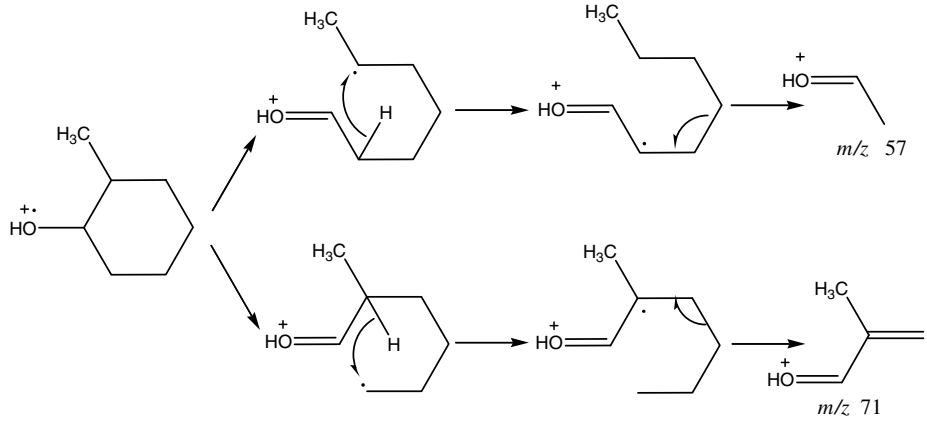
When the complex cleavage takes place in an alicyclic compound, the ionization will take place where the ionization occurs easily, such as in the heteroatom in the compound, and its lonely electron pair will be ionized first. Since an electron is ejected, the other unpaired electron stays. The staying electron needs to be paired, which will be accomplished by a migration of a hydrogen atom carrying an electron. The migration takes place through a six-membered ring (if this condition can not be satisfied, the migration will be accomplished through a five-membered ring). The migrated hydrogen atom takes an electron away. The remaining electron also needs to be paired. Finally, the chemical bond, which is adjacent to

Table 4.2 The smallest mass number of McLafferty rearrangement ions

Compound	Smallest rearrangement ion	Smallest mass number
Alkenes		42
Alkyl benzenes		92
Aldehydes		44
Ketones		58
Carboxylic acids		60
Carboxylates		74
Amides		59
Nitriles		41

the leaving electron, will be broken to give an electron to pair the leaving electron. Therefore, a fragment is lost.

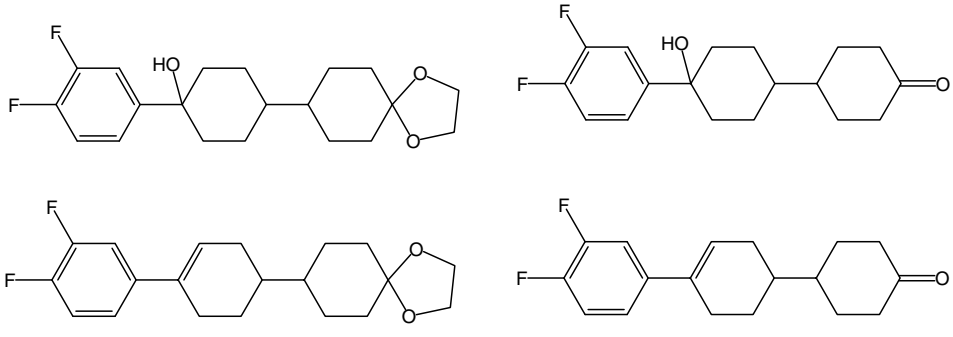
The fragmentation of 2-methyl cyclohexanol is given here as an example:



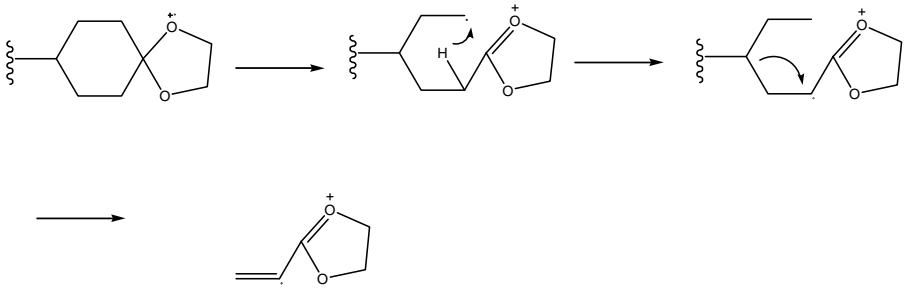
This fragmentation produces ions with *m/z* 57 and 71.

One more example, which was interpreted by the author, is presented here.

The mixture composed of the following four components was analyzed by EI mass spectrometry.



The author discovered the fragment ion with *m/z* 99, which can be interpreted by the following mechanism:



m/z 99

Therefore, from the ion with *m/z* 99, the related structural unit can be determined.

4.3.5 Mass Spectrum Patterns of Common Functional Groups

Although the ions produced by simple cleavages, rearrangements, and complex cleavages have been presented, to get the structural information as much as possible is still difficult, because what we see in the mass spectrum is only the mass-to-charge ratios, some numbers.

Therefore, it is necessary to discuss mass spectrum patterns according to common functional groups. If we analyze a mass spectrum not from one or two peaks but from an integral appearance, that is, patterns, it will be rather easy to find corresponding functional groups.

The compound to be analyzed may contain several functional groups. However, we can find functional groups one by one if we apply the method presented here. On the basis of awareness of functional groups, it is possible to deduce the structure.

How can one illustrate the patterns of common functional groups? A good way is to discuss corresponding simple compounds, and then the characteristics, that is, the patterns, can be known.

4.3.5.1 Alkanes

Alkanes are very common organic compounds, and with the help of their mass spectrum patterns the compounds containing alkyl groups can be interpreted.

Straight Chain Alkane The characteristics of normal chain alkanes are as follows.

① The straight chain alkane produces a weak molecular ion peak.
② Its mass spectrum consists of a series of clusters of peaks spaced by 14 u. The elemental composition of the strongest peak in a cluster is C_nH_{2n+1}, and the other peaks have elemental compositions of C_nH_{2n}, C_nH_{2n-1}, and so forth. C_nH_{2n-1} is produced by removing H_2 from C_nH_{2n+1}.
③ The tops of the clusters form a smooth curve with the highest point at C_3 or C_4. Because every C–C bond has a certain cleavage probability and the produced ions can undergo consecutive cleavages, the ion C_3 or C_4 has the greatest abundance.
④ The top of the cluster next to the molecular ion peak is M−29, which is a very important criterion to distinguish a straight chain alkane from a branched one.

The above-mentioned ③ and ④ are very important to determining a normal chain alkyl group.

Branched Alkanes The characteristics of branched chain alkanes are as follows.

① Branched alkanes produce a weaker molecular ion peak than straight chain alkanes. When a compound has multiple branched chains, its molecular ion peak may disappear.
② Although the mass spectrum of the branched chain alkane still consists of a series of clusters of peaks spaced by 14 u, the top of the clusters does not form a smooth curve. Because cleavages are likely to occur at a branched chain, the abundance of related ions will overpass the smooth curve.
③ There arise noticeable ions C_nH_{2n} at a branched chain, whose intensities may sometimes be larger than those of C_nH_{2n+1}. They are produced from C_nH_{2n+1} with the loss of an H.
④ The branched methyl produces the ion M−15.

Cycloalkanes The mass spectra of cycloalkanes have the following characteristics:

① Because of the existence of the ring, their molecular ion peak has a higher intensity than that of straight chain alkanes.
② The cleavage at a side chain is common, which leads to the production of the ion C_nH_{2n-1}. The loss of a hydrogen atom from C_nH_{2n-1} results in the production of the ion C_nH_{2n-2}.
③ The consecutive loss of C_2H_4 (or sometimes C_2H_5) is a common characteristic of the fragmentation of cycloalkanes.

Because of ② and ③, cycloalkanes produce abundant ions with even mass numbers.

4.3.5.2 Unsaturated Hydrocarbons

Unsaturated Straight Chain Hydrocarbon The mass spectra of unsaturated straight chain hydrocarbons have the following characteristics:

① Compared with saturated hydrocarbons, the unsaturated hydrocarbons have the higher intensities of the molecular ions owing to the existence of the double bond.
② Like saturated hydrocarbons, there exists a series of peak clusters spaced by 14 u, but the largest peaks in the clusters have the elemental composition of C_nH_{2n-1}. The tops of the clusters form a smooth curve, which illustrates that the double bond can migrate along the chain during its ionization.
③ If a γ- H with respect to an unsaturated group exists, McLaferty rearrangement will occur.
④ *Cis*- and *trans*-isomers have similar mass spectra.

Unsaturated and Branched Hydrocarbons The mass spectra of unsaturated and branched hydrocarbons are similar to those of unsaturated straight chain hydrocarbons with two exceptions which follow:

① Some tops of the clusters overpass the smooth curve because of the possible cleavages at branched chains.
② If conjugated double bonds exist, they can not migrate along the chain.

Unsaturated Alicyclic Hydrocarbons

① RDA will occur if its condition is satisfied. RDA plays an important role in its mass spectrum.
② For the ions produced by cleavages at its side chains the reader can refer to those of alkanes.

4.3.5.3 Aliphatic Compounds Containing Heteroatoms

Aliphatic Compounds Containing Saturated Heteroatoms

① Simple cleavages

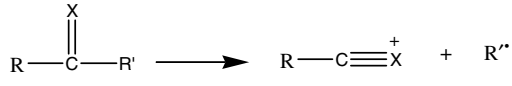
Refer to the item (1) in Section 4.3.2.

② Rearrangement

The main rearrangement reactions of aliphatic compounds containing heteroatoms are those of the losses of small molecules, such as the loss of a water molecule (or a water molecule and an ethylene molecule) from an alcohol molecule, the loss of a hydrogen halide from a halide, the loss of HCN from cyanides and the loss of H_2S from thiols. However, amines do not lose NH_3 in general, because the nitrogen atom ionized can easily initiate cleavage reactions.

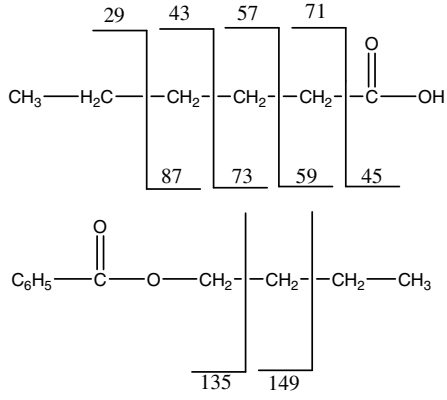
Aliphatic Compounds Containing Unsaturated Heteroatoms The following reactions take place for this kind of compounds.

① The simple cleavage is initiated by the ionized heteroatom, such as



In some cases, alkyl ions are produced.

② One C–C bond, which is situated far from the heteroatom, is broken apart. In fact, this type of reaction does not belong to the simple cleavage. It is just a simplified treatment. Some examples are as follows:



③ McLafferty rearrangement

The reader is referred to Section 4.3.2 and Table 4.2.

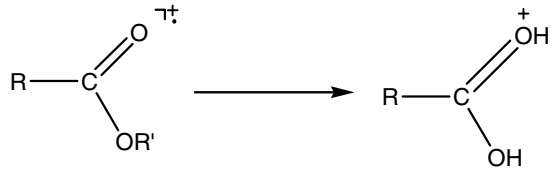
④ The rearrangement for losing a small molecule

The examples are the loss of H_2O, CO from an aldehyde molecule, the loss of an acetic acid molecule from an acetate molecule, and so on.

The four types of reactions mentioned above can easily take place.

⑤ The rearrangement with two hydrogen atoms

This kind of rearrangement mainly takes place for carboxylates, for example,

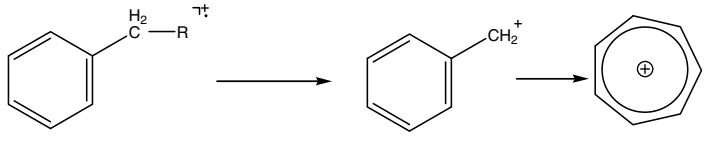


4.3.5.4 Alkyl Benzenes

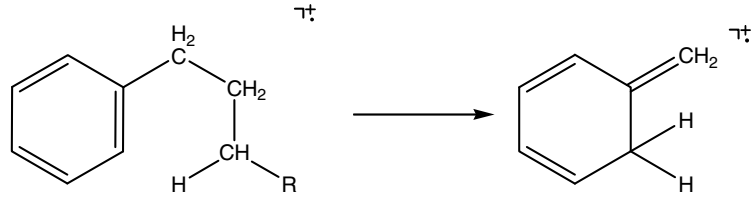
The mass spectra of alkyl benzenes are characterized as follows.

① A remarkable molecular ion peak.
② A simple cleavage resulting in the production of a tropylium ion.

If the benzene ring is connected with a CH_2 group, the peak of *m/z* 91 will be strong; and if the benzene ring is connected with a CH group, the peak will still appear.



③ McLafferty rearrangement



If a γ-H with respect to the benzene ring exists, the peak of *m/z* 92 will be remarkable.

④ The fragment ions containing the benzene ring can successively lose C_2H_2.

m/z 91 → *m/z* 65 → *m/z* 39
m/z 77 → *m/z* 51 (metastable ion m^*:33.8).

Thus, the mass spectra of phenyl derivatives usually show the peaks of *m/z* 39, 51 65, 77, and so on.

4.3.5.5 Aromatic Compounds with Heteroatom Substitutions

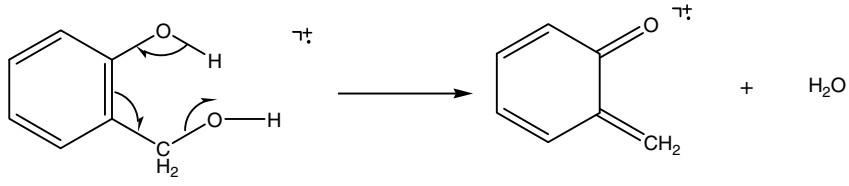
Their mass spectra can be interpreted according to the following three cases.

The Heteroatom Substitutes the Aromatic Ring at Its Side Chain In this case, the mass spectrum is formed by superimposing that of an alkyl benzene on that of the substituted side chain.

The Heteroatom is Connected to the Benzene Ring Directly In this case, the most common reaction is the loss of neutral fragments that are in general produced by rearrangements. Table 4.3 is useful for finding the substituent.

The fragments produced by simple cleavages are indicated in parentheses.

Multiple Substitutions The *ortho*- effect resulting from two substituents on an aromatic ring can be used to differentiate *ortho*- isomer from the *para* – and meta- isomers. One example is as follows:



The mass spectra of the *para*- and meta- isomers can be roughly predicted from Table 4.3.

4.3.5.6 Heteroaromatic Compounds and their Derivatives

The mass spectra of these compounds show their remarkable molecular ion peaks.

Pyridine, pyrrole, furan and thiophene may lose the fragment containing the heteroatom so as to produce the ion *m/z* 39, which is identical to *m/z* 39 produced from benzene. Five-membered heteroaromatics also produce the fragment:  , where X represents NH, O, or S.

Table 4.3 Lost neutral fragments from phenyl derivatives by rearrangements [2]

Substituent	Lost neutral fragments
$-NO_2$	NO, CO, (NO_2)
$-NH_2$	HCN
$-NHCOCH_3$	C_2H_2O, HCN
–CN	HCN
–F	C_2H_2
$-OCH_3$	CH_2O, CHO, (CH_3)
–OH	CO, CHO
–SH	CS, CHS, (SH)
$-SCH_3$	CS, CH_2S, SH, (CH_3)

The mass spectra of the substituted heteroaromatics can be interpreted in the same way as those of the substituted benzene.

4.3.6 Interpretation of the EI Mass Spectrum and Examples

4.3.6.1 Interpretation of the EI Mass Spectrum

For the deduction of an unknown structure, it is necessary to comprehensively analyze several kinds of spectra. The mass spectrum is just one kind of spectra. We will present the interpretation of the EI mass spectrum here.

If we deduce an unknown structure only by its mass spectrum, this structure must be a simple one and the conclusion is not reliable. If the structure is complex and we use only the mass data, what we obtain is partial structural information.

The steps of the interpretation of the EI mass spectrum are as follows.

Determination of the Molecular Ion Peak The reader is referred to Section 4.3.1. We emphasize again that the logical loss of a neutral fragment is the most important criterion for the determination of the molecular ion peak.

Determination of the Molecular Formula If the high resolution mass spectrum is available, the molecular formula can be obtained from it. (See Section 4.5.)

The discussion here is limited to the determination of the molecular formula on the basis of the low resolution mass spectrum.

If the ^1H spectrum and the ^{13}C spectrum are available, the most reliable method to determine the molecular formula is as follows: the number of the hydrogen atoms is determined by the ^1H spectrum; the number of the carbon atoms is determined by the ^{13}C spectrum; and the number of the heteroatoms is determined by the mass spectrum (with a low resolution).

If the ^1H spectrum and the ^{13}C spectrum are not available and only the low resolution mass spectrum is available, the steps for the determination of the molecular formula are as follows.

① Determination of the number of the carbon atoms

From Table 4.1 we can know that the isotopic abundance of ^{12}C is determined as 100, and that of ^{13}C as 1.11. Therefore, we have

$$n_C \cong \frac{I(M+1)}{I(M)} \div 1.1\% \tag{4.3}$$

where n_C is the carbon atom number in a molecular formula.

I (M + 1) and I (M) are the relative intensities of the M + 1 peak and the M peak, respectively.

Equation 4.3 should be applied flexibly. For example, when the intensity of M−15 is much stronger than that of M, for the calculation of Equation 4.3 one should use I(M−14)/I (M−15) and the final result is the calculated result plus 1. And for the compound (4-1), one should use I(M–Br–1)/I(M–Br).

Of course, the result obtained from Equation 4.3 is not accurate. If the unknown structure is simple, it is possible that the number of carbon atoms be determined on the basis of the molecular weight and of the series of the fragment ions (in a broad sense) in the high mass region.

② Determination of the numbers of the chlorine, bromine, and sulfur atoms

From Table 4.1 we can know that the isotopic abundances of A + 2 of these three elements are high. Therefore, the numbers of the chlorine, bromine, and sulfur atoms, especially for chlorine and bromine, can be determined by the calculation of (M + 2)/M.

③ Determination of the existence of oxygen atoms

Compared with the series of alkyl ions with *m/z* 29, 43, 57, 71. . ., the existence of oxygen atoms can be found from the series of ions with *m/z* 31, 45, 59, 73. . . .

The number of the oxygen atoms can be deduced from the difference between the molecular weight and the masses obtained from the addition of the mass of deduced units.

④ Determination of the existence of other heteroatoms

The existence of fluorine can be verified by the M−20 (M−HF) peak and/or the M−50 (M−CF_2) peak.

The existence of iodine can be verified by the M−127 peak. Furthermore, the existence of iodine leads to a smaller value of I (M + 1)/I (M).

⑤ Determination of the number of the hydrogen atoms

The H atom number can be calculated from the difference between the molecular weight and the mass of all the other elemental compositions.

After the awareness of the molecular formula, the unsaturation number of the unknown compound can be calculated.

Deduction of the Existing Functional Groups According to the patterns of common functional groups, the existing functional groups can be deduced by comparing the mass spectrum with the patterns.

Interpretation of Important Ions The interpretation of the mass spectrum and that of NMR spectra are totally different. In the NMR spectrum, almost all peaks can be interpreted in general. However, only few peaks in the mass spectrum can be interpreted. Therefore, we should focus our attention on some important peaks in the mass spectrum. We should be aware that the peaks in a mass spectrum are greatly different in importance.

① Ions close to the high mass edge

The importance of these ions is much greater than that of ions with smaller masses because the former have a closer relationship with the molecular ion than the latter. Whether these ions are produced by simple cleavage or rearrangement, they possess some structural information about the unknown compound.

② Rearrangement ions

Because rearrangement reactions can take place only under some conditions, the probabilities of rearrangement reactions taking place are lower than those of fragment reactions taking place. Therefore, all discernible rearrangement ions show more structural characteristics than fragment ions. Thus, interpreting rearrangement ions is an important way to deduce an unknown structure.

Most rearrangement ions are odd-electron ions which are distinguishable from ions produced by simple cleavages. If the compound contains no nitrogen atom, rearrangement ions have even mass numbers.

③ Metastable ions

If the information about metastable ions is shown in an EI mass spectrum, it should be utilized sufficiently. The parent ion and its daughter ion can be found from the related metastable ion, which is very important for deducing an unknown structure. The metastable ion of the molecular ion is even more important.

Without the information about metastable ions, the pairs of parent ion and its daughter ion can not be determined.

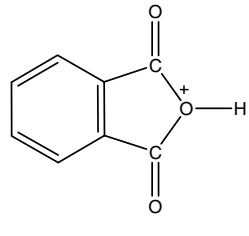
From reference [1], readers can obtain related knowledge about metastable ions.

④ Characteristic ions

These ions show the characteristics of unknown compounds.

Many kinds of natural products have their own characteristic ions.

All phthalates produce a strong peak at *m/z* 149, with the ion structure which follows:



Trying one's Utmost to Deduce the Structure of the Unknown Compound It is necessary to try one's utmost to deduce structural units. The structural extension from a small structural unit depends on the mass spectrum measured.

Library Retrieval of Unknown Mass Spectra If the mass spectrum is obtained from your own measurement, the computer attached to the commercial mass spectrometer will give several searched results from its mass spectrum data bank, which are possible structures corresponding to the measured mass spectrum. After the comparisons between the mass spectrum measured and the mass spectra searched from the data bank, the Purity, FIT, and RFIT (which are possibly named otherwise according to different companies) will be listed for every searched compound. These data reflect the possibilities that the searched compounds would be of the unknown structure. Readers who are interesting in this topic can read the Reference [1].

Finding Standard Mass Spectra from the Web If the mass spectrum is not obtained from your own measurement, you can not use the library retrieval by the computer. Therefore, you need to find standard mass spectra from the Web.

The standard mass spectra can be found from the following Web sites.

① Finding standard mass spectra (also ^{13}C spectra, ^{1}H spectra, and IR spectra) free of charge.

The National Institute of Advanced Industrial Science and Technology (Japan) sets up the following Web site:

http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng,

Please refer to Section 1.5.8.

② If the institute or the university with which the reader is associated has an agreement with the following two Web sites, abundant spectra and spectral data can be found. However, individuals can not access it.

http://166.111.120.35/database/crossfire.htm
https://scifinder.cas.org

③ Standard mass spectra can be found by payment from the following Web site: http://webbook.nist.gov/chemistry/

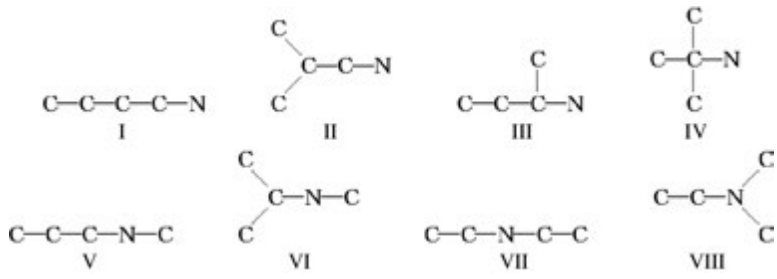
4.3.6.2 Examples for the Interpretation of EI Mass Spectra

The following examples illustrate the interpretation method and function of the EI mass spectrum from different aspects.

Example 4.1 The molecular formula for an unknown compound is $C_4H_{11}N$. Try to postulate seven isomer structures from the relative abundances of the isomers shown in the following Table 4.4.

Solution

It is possible to draw all the eight isomer structures according to the formula. For simplicity, only the skeletons are drawn.



Each set of data can be correlated with an isomer.

This example is a logical training for interpreting the mass spectra of the compounds containing saturated heteroatoms. For these isomers, the nitrogen atom will initiate fragmentation. Therefore one bond of the α- carbon atom except the bond of C–N will be broken apart, thus forming a stable even-electron ion by losing any of CH_3, C_2H_5 and C_3H_7, or even an H atom. Because an even-electron ion can not produce an odd-electron ion by losing a radical group, the successive fragmentations must be the four-membered rearrangements.

Since the molecular weight is 73, the mass discrimination effect is not great.

The assignment starts from the base peak, followed by other important peaks.

From the data, it follows that four isomers (A, B, C and G) have the base peak at *m/z* 58, and that five structures (III, IV, VI, VII and VIII) may lose a methyl so as to produce the ion *m/z* 58. However, III will also produce the abundant ion *m/z* 44, which does not agree with any set of the data. Thus III can be eliminated.

It is easy to assign IV as G since the carbon atom adjacent to the nitrogen atom is connected with three methyls, and IV loses a methyl easily so as to produce a strong peak at *m/z* 58 with its molecular ion peak missing.

In the three remaining sets of data (A, B and C), the abundances of *m/z* 30 which show the probability of undergoing four-membered ring rearrangements can be used for the assignment.

VII can undergo the rearrangement at both sides of the nitrogen atom. Each of the two rearrangements leads to the production of the ion *m/z* 30. Thus VII can be assigned as B, which has the highest abundance of the ion *m/z* 30.

VI can produce the ion *m/z* 30 by two successive rearrangements. First it rearranges at the right side to produce the ion M−1, which rearranges at its left side so as to produce the ion *m/z* 30. From the medium intensity of the ion *m/z* 30 of isomer C, VI can be assigned as C.

Table 4.4 Relative abundance of the peaks of the EI mass spectra of seven isomers

m/z	A	B	C	D	E	F	G
30	13.7	73.6	29.2	10.9	100	100	11.6
43	7.1	3.6	7.3	4.1	<2	<3	4.3
44	24.9	29.4	9.4	100	<2	<3	<1
58	100	100	100	10.7	<2	0	100
72	19.3	19.1	10.5	2.8	<2	<2	0
73	31.5	31.5	11.4	1.0	4.9	10.1	0

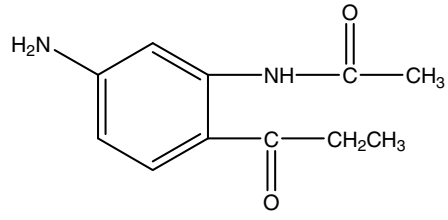
No rearrangements of VIII can lead to the production of the ion *m/z* 30. Instead, VIII can undergo two successive rearrangements to form the ion *m/z* 44, which suggests the assignment of VIII as A.

Only isomer D has the base peak at *m/z* 44, which agrees with III and V. Since V can not produce the ion *m/z* 58, only III satisfies D (the base peak *m/z* 44 and a peak of *m/z* 58).

Finally only isomers E and F are left, both of which are characterized by the base peak at *m/z* 30. The two remaining structures I and II agree with the base peak. Because II has a methyl branch, it must have a lower molecular ion intensity. Therefore, II can be assigned as E and I as F.

Through this example, you can understand the fragmentation initiated by a saturated heteroatom, which is the training for the logic thinking about the mass spectrum interpretation.

Example 4.2 The EI mass spectrum of compound **C4-1** is shown in Figure 4.1. Try to interpret this spectrum.



C4-1

Solution

There are many tiny peaks beside which only numbers are marked in the mass spectrum, and they can be omitted.

The peak at *m/z* 222 has the largest mass-to-charge ratio. The next peak is situated at *m/z* 180. Their mass difference is 42 u. The loss of a neutral fragment is reasonable. Therefore, the peak at *m/z* 222 can be considered as the molecular ion peak. The value of 222 coincides with the elemental formula $C_{11}H_{14}O_3N_2$.

Although there is no related metastable ion, the ion with *m/z* 180 should be produced from the molecular ion, because it is the ion nearest to the molecular ion. Since the mass difference between the molecular ion and the ion is 42 u, which is an even number, the related mass reaction should be a rearrangement reaction with the loss of a small molecule.

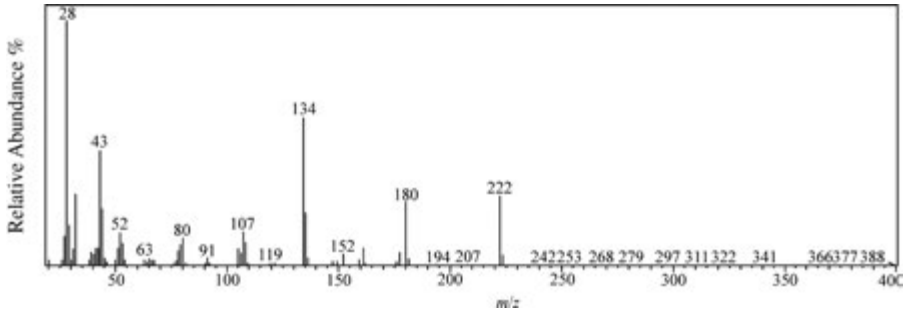
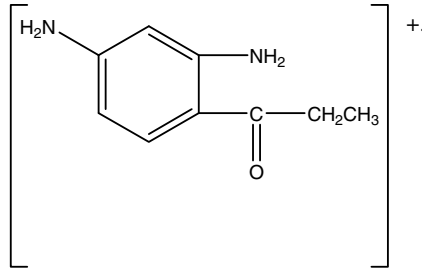
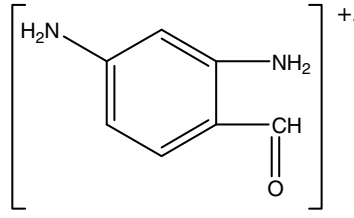


Figure 4.1 The EI mass spectrum of compound **C4-1**

Compared with the structure of the sample, it can be known that the reaction corresponds to McLafferty rearrangement through $NHCOCH_3$. The structure of the ion produced should be as follows:



Similarly, it can be concluded that the ion with *m/z* 152 should be produced by McLafferty rearrangement (for the second time) through $COCH_2CH_3$. The ion should have the following structure:

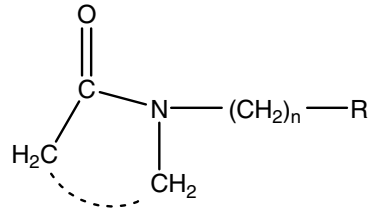


m/z 152

It is possible that the ion with *m/z* 124 be produced from the ion with *m/z* 180 by the loss of CH_3CH_2OH.

Example 4.3 The mass spectrum of an unknown compound is shown in Figure 4.2.

The molecular formula $C_{18}H_{35}ON$ of the compound and a structural unit have been determined by the combination of several kinds of spectra. The structural unit has the following structure:



Try to determine the structure.

Solution

The unsaturation number of the compound, 2, can be calculated from its molecular formula $C_{18}H_{35}ON$. Since the structural unit has the unsaturation number of 2 already, the remaining structural units contain neither a double bond nor the formation of rings.

Since the structural unit contains two heteroatoms, the remaining structural units contain only carbon atoms and hydrogen atoms.

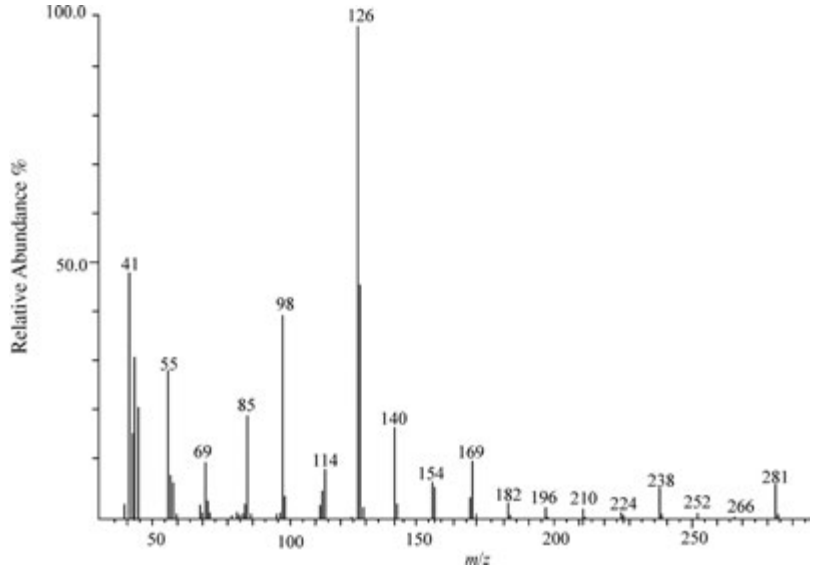
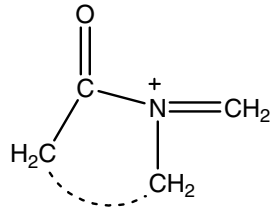


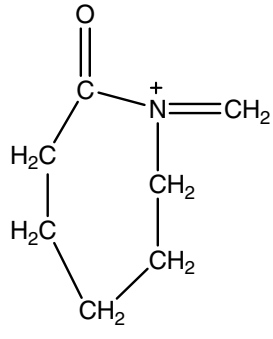
Figure 4.2 The mass spectrum of an unknown compound. [Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA and *Structural Identification of Organic Compounds and Organic Spectroscopy* 2nd ed., © 2000 Science Press (Chinese Edition).]

To sum up the conclusions above, our task is the determination of the size of the ring and the number of methylene groups in the side chain.

Because the nitrogen atom plays the most important role in the fragmentation and the nitrogen atom is situated at the branched point, the ion with the following structure should exist in the mass spectrum.

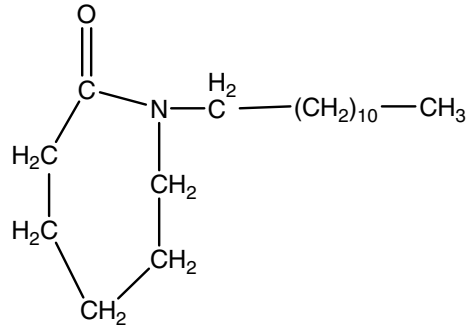


The base peak of the mass spectrum is situated at *m/z* 126. According to this mass-to-charge ratio, we can obtain the structure of the ion with *m/z* 126:



m/z 126

There are peak clusters spaced by 14 u in Figure 4.2 from *m/z* 238 to *m/z* 98, which is the characteristic of a normal alkyl chain. The ^{13}C spectrum and the ^{1}H spectrum confirm again the existence of a normal alkyl chain. Therefore, we obtain the structure of the unknown compound which follows.



C4-2

This example belongs to the most impressive ones of innumerous examples about the deduction of unknown compounds. This example illustrates thoroughly the advantages of the mass spectrum. If the data of a high resolution mass spectrum without the information about fragment ions and NMR spectra are available, the structure of the unknown compound can be deduced. However, this result is not so reliable as the result obtained mainly from the base peak in the mass spectrum, because the differentiation of the methylene groups in the ring from those in the side chain is not easy by using NMR spectra. Although we can deduce unknown structures mainly on the basis of NMR spectra, it is better to apply the information about the mass spectrum as far as possible.

4.4 Interpretation of the Mass Spectra from Soft Ionization

When the molecular ion information can not be provided by the EI mass spectrum, the mass spectrum from soft ionization is necessary.

The information provided by the mass spectrum from soft ionization is mainly that about molecular weight.

4.4.1 Mass Spectra from ESI (Electrospray Ionization)

ESI is the abbreviation of electrospray ionization, which is the interface between the HPLC and the mass spectrometer. Therefore, the mass spectrum from ESI concerns only the combination of HPLC-MS. The solution containing a sample to be analyzed flows out of a capillary tube, which is encircled by a larger tube with flowing nebulized vapor, and the sample molecules are ionized when they are sprayed. The ionization is called electrospray ionization.

The electrospray ionization is very soft. There are few fragment ions in the ESI mass spectrum. One of the advantages of the ESI mass spectrum is a clear background.

Another attractive advantage of ESI mass spectra is the formation of multiply-charged ions, which leads to the realization of the detection of macromolecules by mass

spectrometry with reduced values of *m/z*. Therefore, a compound with a high molecular weight can be analyzed with mass spectrometry by using inexpensive mass analyzers, for example, a quadrupole which has a mass-to-charge range of less than 4000.

The ESI can be operated in a positive ion mode or in a negative ion mode.

In the positive ion mode, $[M + nH]^{n+}$ or $[M + nNa]^{n+}$ are detected. In the negative mode, $[M-nH]^{n-}$ are detected.

The molecular weight is calculated from the cluster of multiply-charged ions.

A typical cluster of multiply-charged ion peaks in an ESI spectrum is shown in Figure 4.3.

The abscissa of the Figure 4.3 is the mass-to-charge ratio and the ordinate is the intensity of the ion current. The numbers marked in Figure 4.3 are carried charges by ions, which ranges from 10 to 19.

The molecular weight can be calculated from any two successive peaks.

The following two equations can be deduced easily [1]:

$$n_1 = \frac{m_2 - X}{m_1 - m_2} \tag{4.3}$$

and

$$M = n_1(m_2 - X) \tag{4.4}$$

where M is the molecular weight of the sample;

n is the number of the charges corresponding to the right peak of the two successive peaks;
m_1 and m_2 are the "apparent" mass-to-charge ratios of the right peak and the left peak of the two successive peaks, respectively;
X is the mass of the charged species. When the *p*H value of the sample solution is low, the species is H^+ and $X = 1$. The other charged species may be Na^+.

The computer with the combination of HPLC-MS can calculate any two successive peaks to give a more accurate result.

The ESI technique can also be used for the mass analysis of small molecules.

In positive ion ESI mass spectra, there are the peaks produced from intact analyte molecules which may include $[M + H]^+$, $[M + Met]^+$, $[M + NH_4]^+$, and so forth, where Met denotes a metal atom.

In negative ion ESI mass spectra, the main quasi-molecular ion is $[M-H]^-$.

The tendency to form $[2M + H]^+$ or $[2M + Na]^+$ increases with the analyte concentration, which is common to all soft ionization techniques.

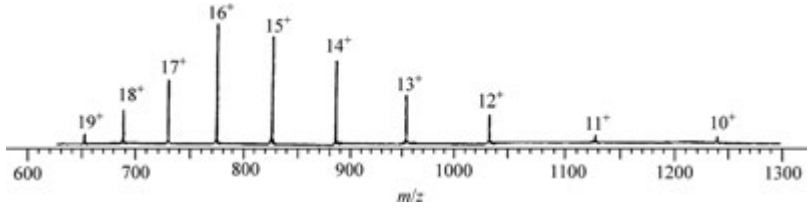


Figure 4.3 A typical cluster of multiply-charged ion peaks in an ESI spectrum. [Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA and *Structural Identification of Organic Compounds and Organic Spectroscopy* 2nd ed., © 2000 Science Press (Chinese Edition).]

The ESI produces ions with low internal energy. Peaks of fragment ions are rare in ESI mass spectra. The structural information from fragment ions is obtained mainly by using CID [1]. It should be noted that the ions obtained by using CID are even-electron ions, whose fragmentation pathways are different from those from the odd-electron ion. They have different fragmentation pathways.

4.4.2 Mass Spectra from CI

A reagent gas is used in the chemical ionization. Since molecules of the reagent gas are the overwhelming majority compared with sample molecules, they are ionized by energized electrons, and then a series of complicated chemical reactions takes place. Finally the quasi-molecular ion containing the sample molecule is obtained.

Since EI and CI can be switched rapidly in an ionization source in a commercial mass spectrometer, both the CI mass spectrum and the EI mass spectrum of an unknown compound can be obtained in one single experiment.

The CI can be operated in a positive mode, that is, positive ions are detected, or in a negative mode, that is, negative ions are detected. This subsection is mainly focused on the positive ion CI because it is more frequently applied than the negative ion CI.

The information about the molecular weight and the information about stereochemistry can be obtained from the CI mass spectrum. This information is tightly related with the reagent gas in the CI.

Methane, isobutene, and ammonia are used frequently as the reagent gases in the CI.

When the molecular ion of a sample is decomposed easily by EI, CI can give the information about its molecular weight. Quasi-molecular ions, precisely the adduct ions which contain the intact molecules of the sample measured, can be found in a CI spectrum. The peak of the quasi-molecular ion is quite clear in the CI mass spectrum.

The formation of quasi-molecule ions is related with the reagent gas to be used. For example, if NH_3 is used as the reagent gas, $[M+H]^+$ is likely to be produced when the sample molecules are more basic than NH_3, while $[M-H]^+$ is likely to be produced when they are more acid.

The adduct ions which appear most frequently in CI mass spectra are $[M+H]^+$s, protonated molecules. Since they are even-electron ions which are more stable than odd-electron ions and the ions produced in CI possess much lower internal energy than parent ions produced in EI, the peak of $[M+H]^+$ is distinguished in a CI mass spectrum in general. Therefore, the molecular weight of the unknown compound can be postulated from the *m/z* value minus 1. However, the existence of other quasi-molecular ions can complicate this situation. Three possibilities should be considered.

First, in addition to $[M+H]^+$, there are other adduct ions which consist of the analyte molecule and an ion produced from the reagent gas. For example, $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ coexist in a CI mass spectrum when methane is used as the reagent gas. If isobutane is used as the reagent gas, the peak of $[M+C_4H_9]^+$ can appear.

Secondly, the ionization process in CI is tightly related with the reagent gas to be used. In general, the proton affinity of an analyte is greater than that of reagent gases, in which case the formation of $[M+H]^+$ is the main result during the CI process. If the proton affinities of the analyte and the reagent gas are nearly equal, adduct ions formed by the association of the reagent ion with the analyte molecule may be observed instead of the

protonated molecule. For example, the ions of $[M+NH_4]^+$ can be formed when ammonia is used as the reagent gas.

Thirdly, when a hydrocarbon sample is analyzed, the ions of $[M-H]^+$ are formed because the proton affinities of hydrocarbons are less than those of reagent gases. This phenomenon can be observed for other kinds of sample, such as some alcohols, esters, and so forth.

In any case the molecular weight of an unknown compound can be determined from its CI mass spectrum. If methane is used as the reagent gas, the molecular weight can be determined from the three peaks which have the *m/z* difference of 28 (between the peaks of $[M+H]^+$ and $[M+C_2H_5]^+$), and 40 (between the peaks of $[M+H]^+$ and $[M+C_3H_5]^+$). Since the reagent gas is known, the molecular weight can be deduced from the ions of $[M+NH_4]^+$ when ammonia is used as the reagent gas.

Besides the information about the molecular weight of an unknown compound, the information about the structure and about the stereochemistry of the compound can be known from CI mass spectra. Isomers can show distinguishable CI mass spectra.

The fragmentation in CI is related tightly with the reagent gas to be used. In general the proton affinity of an analyte is greater than that of reagent gases. The greater the difference in proton affinity between an analyte and the reagent gas to be used in CI, the more energy will be transferred to the protonated molecule of the analyte, the more degree of fragmentation of the analyte will occur, and the more fragment ions exist in the CI spectrum. Since the order of proton affinities of reagent gases is ammonia > isobutane > methane, the order of the number of fragment ion peaks of an analyte with reagent gases is methane > isobutane > ammonia in CI mass spectra.

The fragmentation reactions which occur frequently in CI are the elimination of HX from the protonated molecule of the analyte where X is a heteroatom or a functional group existing in the molecule. These reactions are different from those in EI because these reactions start from an even-electron ion while the reactions in EI start from an odd-electron ion.

In the negative ion mode in CI, the quasi-molecular ion is most frequently $[M-H]^-$.

4.4.3 Mass Spectra from FAB

FAB is the abbreviation of fast atom bombardment.

Inert gas atoms are ionized first, and then accelerated by an electric field in an atomic gun to get high kinetic energy. Then they become atoms with high kinetic energy through a charge-exchanging reaction. Finally, the atoms with high kinetic energy bambard a target, which consists of the sample to be analyzed and a matrix, to produce the ionization of the sample molecule.

The FAB can be operated in a positive mode or a negative mode.

Since a matrix is used in the FAB, many intense peaks produced from the matrix exist in the mass spectrum from FAB. In the positive ion mode of FAB, these peaks represent X_nH^+ where X is the matrix molecule, and dehydration products of X_nH^+. The ions produced from the matrix also exist in the mass spectrum from FAB. Therefore, these ions dominate the mass spectrum from FAB. If glycerol is used as the matrix, there are peaks at *m/z* 45, 57, 75, 93, 185, 277, 369, 461, 553, and so forth. Similarly, there are peaks at *m/z* 91, 183, 275, 367, 459, 551, and so forth in the mass spectrum in the negative ion mode.

The adduct ions of the matrix molecule and Na^+ and/or K^+ which are added into the sample or exist as contaminants can also appear in the mass spectrum from FAB. For example, the peaks of *m/z* 115, $[glycerol + Na]^+$, 131, $[glycerol + K]^+$, 207, [2 glycerol $+ Na]^+$, 223, [2glycerol $+K]^+$, and so forth can exist in the mass spectrum in the positive ion mode.

In addition to these peaks, randomly fragmented pieces mainly from the matrix appear as background peaks. Sometimes this background looks like "grass growing everywhere."

The information about the molecular weight of an unknown compound can be postulated from adduct ions which appear in the region of high mass-to-charge ratios in a mass spectrum from FAB.

When the positive ion mode is operated, the protonated sample molecule, $[M + H]^+$, and the cationized molecules, $[M + Na]^+$ and $[M + K]^+$, frequently exist in the spectrum. From the differences in the region of high mass-to-charge ratios of 22 (between $[M + Na]^+$ and $[M + H]^+$) and 16 (between $[M + K]^+$ and $[M + Na]^+$), the molecular weight of the compound can be deduced. The intensities of the peaks of $[M + K]^+$ and $[M + Na]^+$ can be enhanced by adding sodium and potassium salts into the mixture of the matrix and analyte intentionally.

For an intense $[M + H]^+$ ion signal, the matrix should be more acidic than the sample molecule.

Other adduct ions can appear in the region of high mass-to-charge ratios. If the analyte has basic sites, species such as $[M-H + 2Na]^+$, $[M-H + 2K]^+$, and so forth will be observed. Sometimes cluster ions of sample molecules, such as $[2M + H]^+$, can appear in the mass spectrum.

If the negative ion mode is operated, the quasi-molecular ion is most frequently $[M-H]^-$. For its good signal, the matrix should be more basic than the analyte molecule.

There are fragment ions in the mass spectra from FAB. They can be used to postulate the structure of the unknown compound. The degree of the fragmentation in FAB is related to the matrix to be used.

If an ionic compound, A^+B^-, is studied by FAB, A^+ will be detected in the positive ion mode and B^- in the negative ion mode.

4.4.4 Mass Spectra from MALDI

MALDI is the abbreviation of Matrix-assisted laser desorption-ionization.

The solution of a sample to be analyzed is mixed with a matrix. After the vaporization of the solvent used, the mixture is radiated by laser pulses with a certain wavelength. The matrix transfers the absorbed energy into the sample molecules so that they are vaporized and ionized.

MALDI is only applied for TOF. This ionization is appropriate for the mass analysis of the molecules with a high molecular weight. The low-mass region (<500 *m/z*) in MALDI mass spectra is obscured by the noise produced from the matrices used in MALDI. However, this disadvantage does not affect its application for high-mass region in MALDI mass spectra.

MALDI gives only the information about the molecular weight of the sample because few fragment ions appear in MALDI mass spectra. Therefore, MALDI is appropriate for the analysis of a mixture.

In the MALDI mass spectrum, the peak of $[M+H]^+$, the protonated molecule, is significant. There can exist other peaks produced from the analyte molecule, such as $[M+2H]^{2+}$, $[M+3H]^{3+}$, $[2M+H]^+$, and so forth. The peak intensity of $[2M+H]^+$ increases with the enhancement of its concentration in the sample. The peak of $[M+Met]^+$, where Met denotes a metal atom, can be found in a MALDI mass spectrum.

4.4.5 Mass Spectra from APCI

Because APCI (atmospheric pressure chemical ionization) is the interface between an HPLC and a mass spectrometer, the mass spectrum from APCI appears only in the LC-MS experiment.

The principle of APCI is similar to that of CI. However, they are different in two aspects. First, the pressure in the ionization chamber of CI is approximately 100 Pa but that of APCI 100 k Pa. Therefore, the ionization efficiency in APCI is much higher than that in CI. Secondly, the ionization in CI starts from the electrons emitted from a filament while the ionization in an APCI starts from a corona discharge.

The mass spectrum from APCI is similar to that from CI except that there are peaks of $[(H_2O)_nH]^+$ in APCI mass spectrum, because H_2O has the highest proton affinity of those gases normally present in the ionization.

4.4.6 Examples of the Interpretation of Mass Spectra from Soft Ionization

Example 4.4 Compound **C4-3** has the following structure:

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2OCH_2COOH$$

C4-3

The synthesized compound **C4-3** is a liquid, which produced some crystal grains after the placement. One dimensional and two dimensional NMR spectra were measured but both the NMR spectra are the same as those of compound **C4-3**, respectively.

The IR spectrum of the crystal grains was measured. The peak of the carboxyl group was shifted from 1735 to 1753 cm^{-1}. In this case, the mass spectrum of the crystal grains is very important.

The FAB mass spectrum of the crystal grains produced from compound **C4-3** is shown in Figure 4.4.

Try to find the structure of the crystal grains.

Solution

Figure 4.4 is the mass spectrum by using FAB. It is a nice spectrum, because the interference from the matrix is not acute.

We have known that there are adduct ions in the FAB mass spectrum.

The molecular weight of compound **C4-3** is 188, which is calculated from its molecular formula.

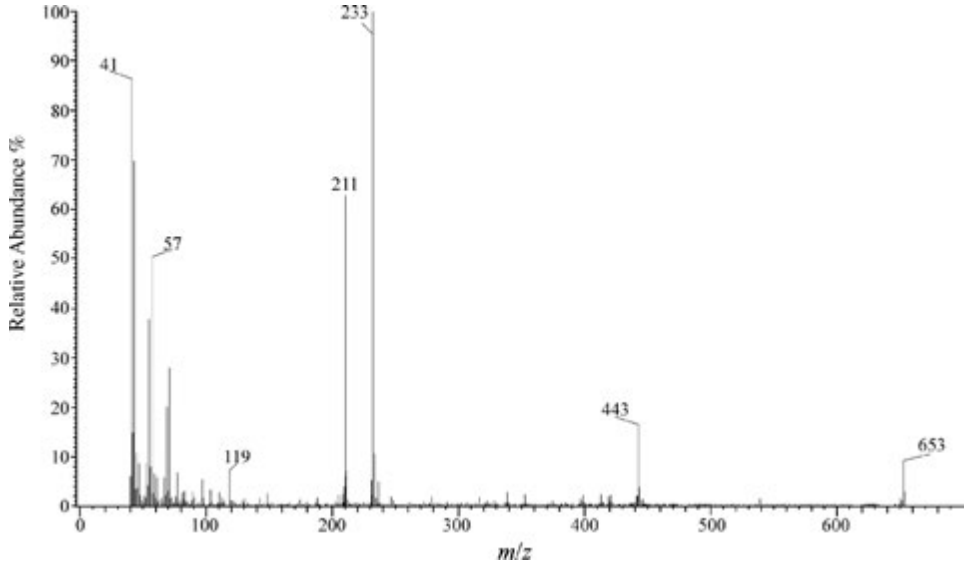


Figure 4.4 The FAB mass spectrum of the crystal grains produced from compound **C4-3**

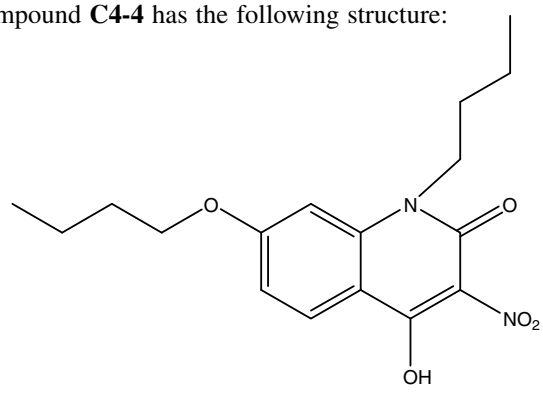
According to the molecular weight of 188 and the possibilities of adduct ions in the FAB mass spectrum, the ions situated in the high mass range can be analyzed as follows:

$$211 = [M + Na]^+$$
$$233 = [M + 2Na - H]^+$$
$$399 = [2M + Na]^+$$
$$443 = [2M + 3Na - 2H]^+$$

From the data above, the conclusion that the crystal grains are the dimer of compound **C4-3** can be drawn.

This example is interesting and important. Because all NMR spectra do not change with respect to the original sample, the mass spectrum data show an outstanding function. It is one of the most impressive examples of the author.

Example 4.5 Compound **C4-4** has the following structure:



C4-4

The ESI mass spectra in a positive mode and in a negative mode of compound **C4-4** are shown in Figures 4.5 and 4.6, respectively. Try to confirm the structure by these two mass spectra.

Solution

We will interpret Figure 4.5 first.

Calculate the molecular weight of compound **C4-4**, which is 334.

Because Figure 4.5 shows the ESI mass spectrum in a positive mode, the ions in the mass spectrum are the addition of the organic molecule and species with positive charge.

It can be seen that the mass spectrum coincides with the structure of compound **C4-4**.

$$373 : M + K;$$
$$357 : M + Na;$$
$$335 : M + H;$$
$$317 : M + H - H_2O;$$
$$261 : M + H - C_4H_9OH;$$

Almost all peaks can be interpreted.

The peaks and the intensities of ions in Figure 4.5 decrease with the decrease of the cone voltage from 40 volts to 20 volts.

Now we are going to interpret Figure 4.6.

m/z 333: quasi-molecular ion, $M–H]^-$;

m/z 276: $M–H–C_4H_9$.

Therefore, the structure of compound **C4-4** has been confirmed by its mass spectra.

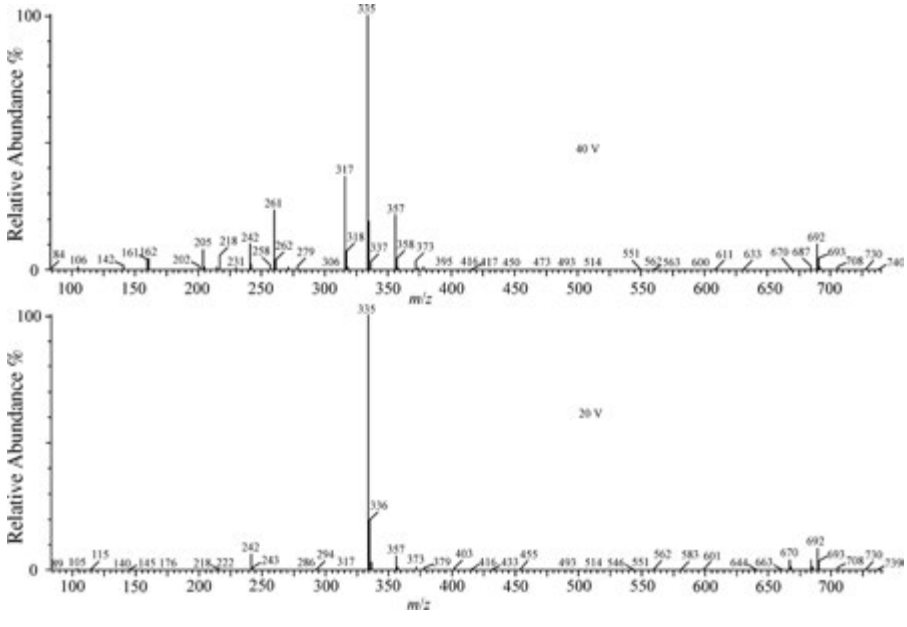


Figure 4.5 The ESI mass spectrum of compound **C4-4** in a positive mode

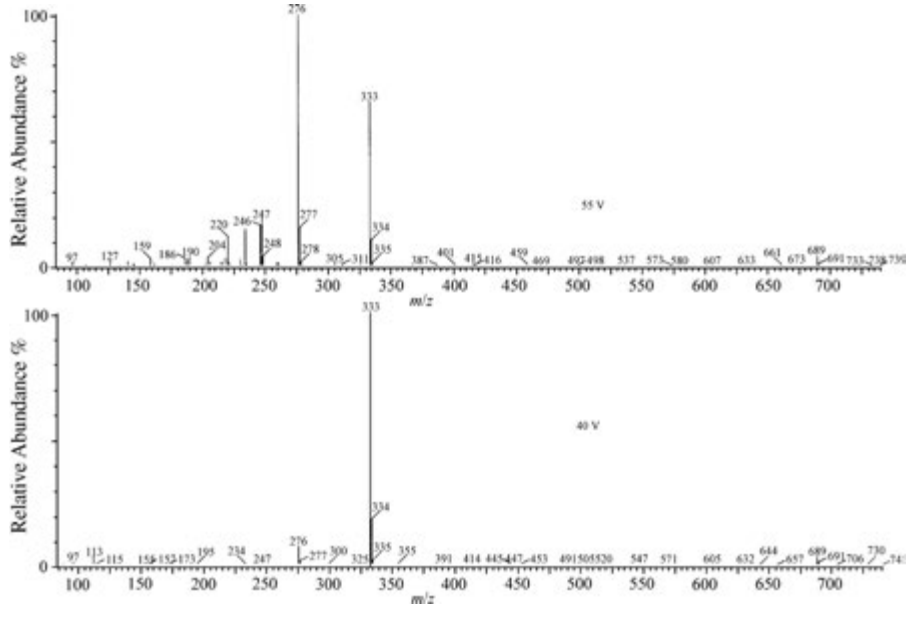


Figure 4.6 The ESI mass spectrum of compound **C4-4** in a negative mode

4.5 Interpretation of High Resolution Mass Spectra

Here the high resolution mass spectra include those from EI and from soft ionization.

The precise masses of the isotopes that are important in organic chemistry are listed in Table 4.5.

The basis of Table 4.5 is that the precise mass of ^{12}C is determined as 12, so that the calculation of organic mass spectrometry can be simplified.

Thanks to the fact that the precision of the data obtained from high resolution mass spectrometry can reach the order of magnitude of ppm, or even better, and because there

Table 4.5 The precise masses of the isotopes which are important in organic chemistry

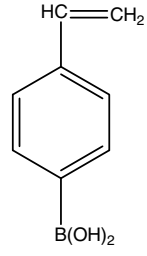
Isotope	Atomic weight	Isotope	Atomic weight
^{1}H	1.00782504	^{31}P	30.9737634
^{2}H	2.01410179	^{32}S	31.9720718
^{13}C	13.0033548	^{34}S	33.9678677
^{14}N	14.0030740	^{35}Cl	34.9688527
^{15}N	15.0001090	^{37}Cl	36.9659026
^{16}O	15.9949146	^{79}Br	78.9183360
^{18}O	17.9991594	^{81}Br	80.9162900
^{19}F	18.9984033	^{127}I	126.904477
^{28}Si	27.9769284		

exists some limitation on the numbers of heteroatoms of the compound to be analyzed, the elemental composition of the molecular ion (or quasi-molecular ion) and some fragment ions (in a broad sense) can be determined. Of course, the awareness of the elemental composition is very important for the deduction of an unknown structure. However, the mass spectrum with a low resolution can not accomplish this task.

In the previous part, we introduced the determination of a molecular weight by the cluster of multi-charged ions of ESI mass spectrum. If the sample is a mixture, their clusters will be superposed, which leads to the difficulty in determining their molecular weights. However, this problem can be resolved by using high resolution mass spectrometry.

For the determination of a molecular weight, only "one peak" in the ESI mass spectrum is necessary to the calculation. In fact the "one peak" is an isotope cluster in a high resolution spectrum. A compound contains a certain number of carbon atoms. The replacement of ^{12}C by ^{13}C results in an isotope cluster. The charge of an associated ion can be calculated from the isotope cluster. For example, if $\Delta m/z = 0.1$, we have $z = 10$ because $\Delta m = 1$ (a ^{12}C is replaced by a ^{13}C). The molecular weight can be directly calculated from the m/z and z.

Example 4.6 The structure of compound **C4-5** is determined by the combination of its ^{1}H spectrum, ^{13}C spectrum, and mass spectrum as follows:



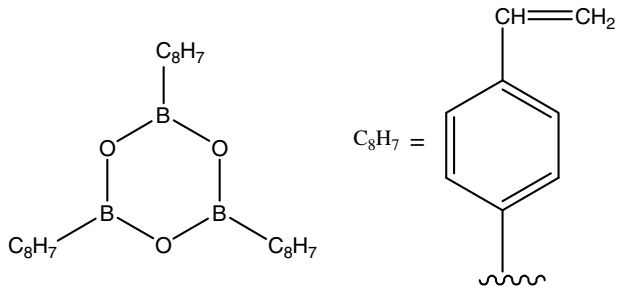
C4-5

A new compound is isolated from the products of compound **C4-5**. That the new compound contains a para-substituted benzine ring and an ethylene group is deduced from its ^{1}H spectrum. The remaining problem is the determination of another substituent of the substituted benzine ring. Try to find it from its high resolution mass spectrum, which is shown in Figure 4.7.

Solution

We focus our attention on the base peak (m/z 390) of Figure 4.7 and its vicinity. Because the peaks at m/z 389 and 388 are successive loss of 1 u from the peak at m/z 390, these two peaks can be considered as M−1 and M−2, respectively. Therefore, the peak at m/z 390 can be considered temporarily as the molecular ion peak, and the peak at m/z 391 the isotopic peak.

On the basis of the structure of compound **C4-5** and the possible molecular weight of the new compound, the possible structure of the new compound can be considered as follows:



C4-6

The element boron has two isotopes: ^{11}B and ^{10}B, whose precise atomic weights are 11.00931 and 10.01294, respectively.

By the application of the data above and those of Table 4.5, the calculation of the precise molecular weight of compound **C4-6** is 390.1770. Compared with the measured precise mass of 390.1793, the error is 5.8 ppm, which is acceptable. Therefore, the deduction of the structure of compound **C4-6** can be initially affirmed.

Now we are going to analyze the isotopic cluster produced by the isotopes of boron.

We will analyze the abscissa of Figure 4.7, that is, the precise mass, first.

Because the isotopic abundance of ^{11}B is 81.17%, and that of ^{10}B 18.83%, the peak containing three ^{11}B atoms is the most intensive peak in the isotopic cluster. If a ^{11}B atom is replaced by a ^{10}B atom, the molecular weight will decrease by the following amount:

$$11.00931 - 10.01294 = 0.99637(\text{u})$$

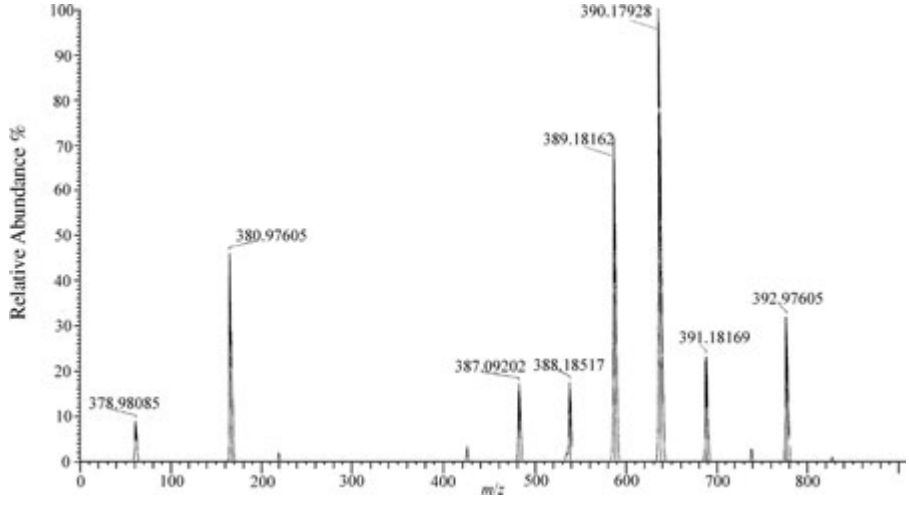


Figure 4.7 The mass spectrum with a high resolution of an unknown compound

According to the data above, from the peak position at 390.1793, we can calculate the precise masses of 389 and 388u. They are respectively at:

$$390.1793 - 0.99637 = 389.1829$$
$$390.1793 - 2 \times 0.99637 = 388.1866$$

And their precise masses shown in Figure 4.7 are 389.1816 and 388.1852, respectively, with errors of 3.3 and 3.6 ppm, respectively. Their coincidence is very good.

Then we will analyze the ratios of the peak intensities of the isotopic cluster.

According to the isotopic abundances of ^{11}B and ^{10}B, the ratios of the peaks at *m/z* 387 (containing no ^{11}B), 388 (containing one atom of ^{11}B), 389 (containing two atoms of ^{11}B), and 390 (containing three atoms of ^{11}B) can be calculated by Equation 4.1. They are:

$$0.01 : 0.15 : 0.67 : 1.00$$

The data above coincide with the measured data from Figure 4.7.

By the combination of the two conclusions above, the possible structure of compound **C4-6** has been confirmed.

4.6 Interpretation of Mass Spectra from Tandem Mass Spectrometry

As its name implies, the tandem mass spectrometry means mass spectrometers in a series.

MS/MS or MS^2 denotes the operation by two times of mass spectrometry, MS^3 the operation by three times of mass spectrometry.

Tandem mass spectrometry is divided into two types: tandem-in-space and tandem-in-time.

For the tandem-in-space type, each mass analysis needs to use a mass analyzer. For conducting the subsequent mass analysis, some ions need to be broken up, which occurs in a mass analyzer in general. Therefore, three mass analyzers are needed to conduct MS^2. Obviously, the tandem mass spectrometry in space needs a high cost.

The tandem mass spectrometry is tightly related with the measurement of metastable ions. The advantage of the tandem-in-space type is that it can realize different kinds of measurements of metastable ions, so as to find daughter ions from a parent ion, to find a selected daughter ion from different parent ions, and so on.

The concept of tandem-in-time can be presented as follows. By analyzing a sample by the mass spectrometry, its mass spectrum is obtained. Then the ions with a certain mass-to-charge ratio are selected and then broken up so that one can obtain the mass spectrum of the daughter ions of the selected ion. This is an MS^2. Repeat this operation, MS^3 and so forth can be operated.

In this process, daughter ions are measured successively. This is the experiment most frequently conducted in tandem mass spectrometry.

Only one mass analyzer is needed in the tandem-in-time type. An FT-MS or an ion trap, especially the latter, can be used for the mass analyzer. Therefore, the instrument fee for the tandem-in-time type is much lower than that for the tandem-in-space type. Although only the successful measurement of daughter ions can be realized in the tandem-in-time type, this measurement is the most important one for the measurement of metastable ions.

From above-mentioned presentation, we can understand the important function of tandem mass spectrometry. Its prominent advantage is that we can focus our attention to some specific part of the unknown structure. A simple and clear conclusion may be obtained from tandem mass spectrometry.

The examples for the tandem mass spectrometry will be presented in the last example (No. 20) in Section 6.2.

References

[1] Ning, Yong-Cheng (2005) *Structural Identification of Organic Compounds with Spectroscopic Techniques*, Wiley-VCH.
[2] Rose, M.E. and Johnstone, R.A.W. (1982) *Mass Spectrometry for Chemists and Biochemists*, Cambridge University Press, Cambridge.

5

Interpretation of Infrared Spectra

Although NMR spectra are applied as a main tool for the identification of an unknown structure or the confirmation of an anticipated structure, the function of IR spectra still should not be neglected because of the following facts.

1. The IR spectrum emphasizes the information on some functional groups, especially strong polar functional groups. This is important to determining the existence of such functional groups.
2. The measurement of IR spectra is the easiest one of all another spectroscopic methods. Any sample in a solid, liquid or gaseous phase can be measured by infrared spectroscopy.
3. Commercial products, which are mixtures in general, are mostly suitable for being measured by IR spectroscopy. IR spectra can be used for the comparison between an unknown sample with a standard sample, and between a mixture with a commercial product.
4. An organic compound can be differentiated easily from an inorganic compound by the IR spectrum, which is impossible or inconvenient if we use other spectroscopic methods.

5.1 Elementary Knowledge of Infrared Spectroscopy

5.1.1 Infrared Spectrum

The abscissa of the infrared spectrum is the wavenumber, cm^{-1}. The commercial IR spectrometers usually cover 400–4000 cm^{-1} (medium IR region). The ordinate of the infrared spectrum is the intensity of the IR absorption, which uses transmittance in general.

The peaks in the IR spectrum usually are not sharp peaks, some of which even have a rather broad width. Therefore, IR absorption signals can be named absorption peaks or absorption bands.

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Because in the IR measurement, the intensity measurement relates to the slit width of the spectrometer used and the slit usually can not be set to have an ideal narrow width, exact molar absorptivity can not be obtained in IR spectrometry.

5.1.2 Two Regions of the Infrared Spectrum

The IR spectrum is divided into two regions: a fingerprint region and a functional group region, with the boundary of $1300\,cm^{-1}$. The region below $1300\,cm^{-1}$ is the fingerprint region and that beyond $1300\,cm^{-1}$ the functional group region. In fact, the boundary at $1300\,cm^{-1}$ is generalized by experiments.

The absorption peaks (bands) in the functional groups illustrate the existence of the functional groups, which exist in the sample to be measured. Generally speaking, every peak (band) in the functional group region corresponds to a certain functional group. In other words, every peak (band) can be interpreted.

The fingerprint region is quite different from the functional group region. Although there are some bands in this region which also reveal the existence of some functional groups also, many bands show only an overall characteristic of a compound, just like the human fingerprint. Because every organic compound has its own fingerprint absorptions, the absorption bands in the fingerprint region are very useful to comparing an unknown compound with a known compound so that the conclusion about whether the unknown compound is identical to the known compound or not can be drawn.

5.2 Characteristic Absorption Frequencies of Functional Groups

5.2.1 Elemental Equation of IR Spectroscopy

The elemental equation, which is shown in Equation 5.1, expresses the relation between IR absorption wavenumbers and related parameters.

$$\bar{v} = \frac{1}{2\pi C}\sqrt{\frac{k}{\mu}} \tag{5.1}$$

where $\bar{v}$ is the IR absorption wavenumber;

C is the propagation velocity of electromagnetic wave;
k is the force constant of the chemical bond;
μ is the reduced mass, with $\mu = \frac{m_1 m_2}{m_1 + m_2}$ or $\frac{1}{\mu} = \frac{1}{m1} + \frac{1}{m_2}$.

Equation 5.1 is deduced from the model of a diatomic molecule. However, its application can be extended to polyatomic functional groups.

5.2.2 Factors Affecting Absorption Frequencies

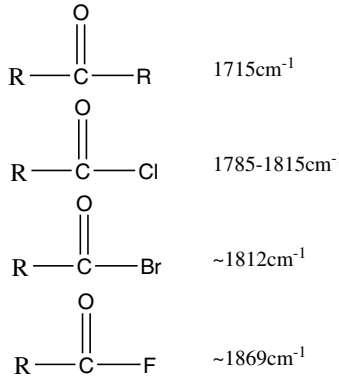
The discussion here is mainly focused on structural factors. The absorption bands of carbonyls are sensitive to structural changes. Therefore they will be discussed as an example.

5.2.2.1 Electron Effect

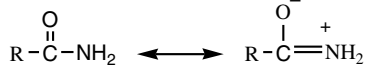
The carbonyl has a double bond to produce an absorption band with a great intensity at a large wavenumber, whose shift produced by a structural change is evident. Therefore, the IR bands of carbonyl groups can be used as the examples. If a structural change induces it from ($\delta+$)C=O ($\delta-$) towards (+) C–O (–), this can displace the absorption band towards a smaller wavenumber.

Induction Effect The normal absorption band of the aliphatic ketone is situated at about 1715 cm^{-1}. The substitution of a carbonyl by a halogen atom increases the double bond tendency of the carbonyl (it has more difficulty changing towards C–O) so that the IR absorption band of the carbonyl group will shift to a larger wavenumber.

The following data illustrate the effect of the substitution of the carbonyl group by a halogen atom, which is mainly the induction effect.



Mesomeric Effect The mesomeric effect is also called the resonance effect, which can be considered as a way of thinking. Some spectroscopic phenomena can be explained by the mesomeric effect. The most typical example is the IR absorption of amides. According to the mesomeric effect, the connection of an amine group with a carbonyl group will produce the following reaction.



The resonance reduces the double bond tendency of the amide, so its absorption band is displaced towards a rather smaller wavenumber. All amides have an IR absorption frequency lower than 1690 cm^{-1}.

Conjugation Effect If a carbonyl is conjugated with another double bond, the delocalization of electrons of the carbonyl decreases the bond order of the carbonyl double bond. Therefore, its absorption band is displaced towards a rather smaller wavenumber. The absorption bands of unsaturated ketones and those of aromatic ketones are situated at about 1675 cm^{-1} and 1690 cm^{-1} respectively. Both values are smaller than those of aliphatic ketones.

A substitution may take effect on several aspects, so it has an overall influence. The above discussion is focused only on the effect which plays a dominant role in the structural change.

5.2.2.2 Steric Effect

Cyclic Tension Generally speaking, the stronger tension a cycle has, the larger wavenumber its absorption band shifts towards. A typical example is the IR absorption band of a CH_2 in a ring, for example:

cyclohexane	cylcopropane
2925 cm^{-1}	3050 cm^{-1}

Steric hindrance

A conjugated system has to keep coplanarity. If the coplanarity is deviated or distorted by a structural change, for example, a substitution, the absorption band of the system will shift into a larger wavenumber.

Influence of the Hydrogen Bond Both inter- and intra-hydrogen bonds weaken the bond which participates in the formation of the hydrogen bond. As a result, the absorption band of the bond shifts into a smaller wavenumber. However, the dipolar moment change of the bond affected by the hydrogen bond increases, so that its absorption intensity increases as well. The absorption bands of alcohol can be listed as an example:

Isolated	Dimer	Polymer
3610–3640 cm^{-1}	3500–3600 cm^{-1}	3200–3400 cm^{-1}

The strong hydrogen bonds between carboxylic acid molecules can shift the hydroxyl absorption band to about 3000 cm^{-1} with its tail at about 2500 cm^{-1}.

5.2.2.3 Mass Effect (Deuteration Effect)

If a group containing hydrogen atoms is deuterated, its absorption frequency will decrease because of the augmentation of its reduced mass.

Except of the structural changes above, the conditions of the measurement, the size of crystal grains of the sample, the method of the crystallization, and so on, may change the IR absorption frequencies of the sample.

5.2.3 Characteristic Frequencies of Common Functional Groups

The characteristic frequencies of common functional groups can be found from references.

5.3 Discussion on the IR Spectrum According to Regions

From Equation 5.1, it can be easily understood that the IR absorption frequencies are determined by the reduced mass of the functional group and the force constant of the chemical bond. When the reduced mass is small, or the force constant of the chemical bond is large, the IR absorption frequencies are high, and vice versa.

The functional groups containing hydrogen atoms have small reduced masses (close to 1). This effect is stronger than that of the force constants of chemical bonds. Therefore, these functional groups have the highest IR absorption frequencies.

The force constants of chemical bonds decrease in the order of triple bonds, double bonds, and single bonds, and the IR absorption frequencies decrease in the same order.

The vibrations can be classified into two types: stretching vibrations and bending vibrations. The motions along chemical bonds are stretching vibrations while all the other vibrations are bending vibrations. Therefore, the frequencies of stretching vibrations are higher than those of bending vibrations. Because the force constants decrease in the order of triple bonds, double bonds, and single bonds, the corresponding IR absorption frequencies decrease in the same order, which happens under the precondition that the comparison is made on the same vibration mode (both in stretching vibrations or both in bending vibrations). For example, the stretching vibration frequency of a single bond may be higher than the bending vibration frequency of a double bond.

We will discuss IR absorption frequencies in six regions, of which the first four belong to the functional group region, and the remaining two the fingerprint region.

5.3.1 Functional Group Region

5.3.1.1 4000–2500 cm^{-1}

This is the frequency region of stretching vibrations of the functional groups containing hydrogen atoms, that is, the group of X–H (X: C, H, O, S, etc.).

Hydroxyl Group The absorption bands of hydroxyl groups are situated in the 3200–3650 cm^{-1} region. Inter- or intra- hydrogen bonds between hydroxyl groups have a great influence on the position, shape and intensity of hydroxyl absorption bands. Isolated hydroxyl groups, which exist in the solution of a low polar solvent with a low concentration or in gaseous phase, have absorption bands at the large wavenumber edge (3640–3610 cm^{-1}) with a sharp shape. An associated hydroxyl group has an absorption band at about 3300 cm^{-1} with a broad and blunt shape. If isolated and associated hydroxyl groups coexist, two related absorption bands, a sharp one with a rather large wavenumber and a blunt one with a rather small wavenumber, will appear in the IR spectrum. Because of the influence of strong hydrogen bonds, carboxylic acids form a strong absorption band at about 3000 cm^{-1}. The weak absorption bands of C–H stretching vibrations, which will be discussed later, overlap that of carboxylic acids. Trace moisture in KBr crystal powder, which is used for sampling, may produce a small band at about 3300 cm^{-1}. If a sample contains isolated water, it shows an absorption band at 1630 cm^{-1} and an overtone at about 3300 cm^{-1}, which may interfere with the detection of hydroxyls. Therefore, it is necessary to determine the existence of a hydroxyl group by checking the hydroxyl absorption bands in the fingerprint region.

Amino Groups The absorption bands of isolated primary amino groups are situated in the 3300–3500 cm^{-1} region. Their association decreases the frequency about 100 cm^{-1}.

A primary amino has two absorption bands because of the symmetric and asymmetric stretching vibrations of NH_2. Primary amino groups are differentiated from hydroxyl groups distinctly by the absorption band shape, although their absorption frequencies are close.

A secondary amino has only one absorption band because it has only one stretching vibration mode. The absorption band of the associated secondary amino is situated at about 3300 cm^{-1} with a band shape less blunt than that of the associated hydroxyl. The aromatic

secondary amino has an absorption band at a larger wavenumber with a stronger intensity than that of the aliphatic secondary amino.

The tertiary amino has no absorption band in this region because of the absence of hydrogen atoms.

Alkyls The boundary between saturated and unsaturated C–H stretching absorption is $3000\,cm^{-1}$ except the cyclopropyl group. All saturated C–H stretching absorption bands are situated below $3000\,cm^{-1}$, which is very useful for differentiating the saturated C–H from the unsaturated C–H. Because of the low intensity, the absorption bands of unsaturated C–H bonds usually appear to be "shoulder peaks," which are small peaks overlapping upon a strong band.

The absorption bands of C≡C–H groups are situated at about $3300\,cm^{-1}$ with a sharp shape distinguished from other bands of other functional groups at about $3300\,cm^{-1}$.

Four absorption bands at ~2960, ~2870, ~2925, and ~$2850\,cm^{-1}$, respectively, are assigned as saturated C–H stretching vibrations. The first two result from CH_3 groups which are assigned as v_{as} and v_s of the CH_3 group, respectively, while the last two result from CH_2 groups which are assigned as v_{as} and v_s of the CH_2 group, respectively. Therefore, the quantitative ratio between CH_3 and CH_2 can be estimated roughly from the intensities of the four bands.

Both CH_3 and CH_2, when connecting with an oxygen atom or other electronegative atom, have lower absorption frequencies than those listed above.

The aldehyde group has two absorption bands at about $2820\,cm^{-1}$ and at $2720\,cm^{-1}$ respectively, which result from the Fermi resonance of ν_{C-H} and the overtone of δ_{C-H}.

On the basis of the discussion above, it can be known that the bands at about $3000\,cm^{-1}$ can be used for the differentiation of an organic compound from an inorganic compound.

5.3.1.2 2500–2000 cm^{-1}

This is the region for triple bonds (–C≡C, –C≡N) and accumulated double bonds (–C=C=C–, –N=C=O, etc.). Except the two bands resulting from CO_2 background (~2365, $2335\,cm^{-1}$), all the other bands in this region should not be ignored even when their intensities are small.

5.3.1.3 2000–1500 cm^{-1}

This is the region for double bonds, which is important for the interpretation of IR spectra.

A carbonyl usually shows a strong band in 1650–$1900\,cm^{-1}$. Except carboxylic acid salts, the carbonyl group shows a strong band with a sharp or slightly broad shape. The strong band is usually the strongest or the second strongest band in the IR spectrum.

The absorption bands of stretching vibrations of carbon-carbon double bonds appear in this region with low intensities.

The skeleton vibrations of the phenyl group have absorption bands at about 1450, 1500, 1580, and $1600\,cm^{-1}$. The band near $1450\,cm^{-1}$ may not be distinct from the absorption bands of CH_2 and CH_3, while the other three banks can indicate the existence of a phenyl ring.

Heteroaromatic rings have absorption bands similar to those of the phenyl ring. For example, pyran has absorption bands at about 1600, 1500, and $1400\,cm^{-1}$ and pyridine at about 1600, 1570, 1500, and $1435\,cm^{-1}$.

In addition to the above-mentioned absorption bands, there are the absorption bands of C=N, N=O, and so forth in this region. The nitro group has two absorption bands because two oxygen atoms connect with the same nitrogen atom. Its asymmetric stretching absorption band is situated in this region while the symmetric stretching absorption band appears at about 1350 cm^{-1}.

5.3.1.4 1500–1300 cm^{-1}

In addition to the above-mentioned bands in the region, the absorption bands of CH_3 and CH_2 should be paid attention to.

The methyl group has two absorption bands at about 1380 and 1460 cm^{-1}, respectively. A branching of the band at about 1380 cm^{-1} shows that two or three methyl groups connect to the same carbon atom (e.g., t-butyl and isopropyl groups).

The CH_2 group has an absorption band at about 1470 cm^{-1}.

5.3.2 Fingerprint Region

The region below 1300 cm^{-1} is the fingerprint region of the IR spectrum. The boundary of the fingerprint region at 1300 cm^{-1} is obtained by experiments. And it coincides with the theoretical consideration.

Many absorption bands in the fingerprint region form the characteristics of the sample to be measured, like the human fingerprint. Most absorption bands in the fingerprint region are difficult to interpret.

The fingerprint region can be classified as the following two regions.

5.3.2.1 1300–910 cm^{-1}

All stretching vibrations and bending vibrations of the single bonds of the functional groups containing no hydrogen atoms, bending vibrations of the part of the function groups containing hydrogen atoms, stretching vibrations of the double bonds containing heavy atoms, skeleton vibrations of compounds, and so on, appear in this region. This region is rich in the IR absorption information.

5.3.2.2 Below 910 cm^{-1}

The bands resulting from substitutions of a phenyl ring appear in this region. Therefore, these bands offer related structural information. However, if the substituents are strong polar groups, the substitutions can not be determined by the absorption bands in this region.

The absorption bands of carbon-hydrogen bending vibrations of alkenes appear in this region and its preceding region (1500–1300 cm^{-1}).

5.4 Interpretation of IR Spectra According to Regions

This section is very important for the readers who are not familiar with the interpretation of the IR spectrum.

The discussion in this section is different from that in Section 5.3 from the following two aspects.

1. What the discussion in Section 5.3 emphasizes is the analysis of the functional groups existing in each region, while the discussion in this section emphasizes the

interpretation of IR absorption bands in the order of the decrease of the absorption wavenumber.

2. The discussion in this section emphasizes the combination of related absorption bands of a certain functional group to identify whether the functional group exists or not.

We still follow the order of the six regions.

5.4.1.1 4000–2500 cm^{-1}

We have said that this is the region of stretching vibration frequencies of the functional groups containing hydrogen atoms, that is, the group of X–H (X: C, H, O, S, etc.).

Because hydroxyl groups and amino groups are associated under ordinary sampling, there are no absorption bands in the region of 4000–3600 cm^{-1}. If there are IR absorption bands in the region of 4000–3600 cm^{-1}, the existence of isolated hydroxyl groups can be determined.

If associated hydroxyl groups of alcohols and phenols exist, a broad and blunt band will appear at about 3300 cm^{-1}.

When the sample to be measured contains a small quantity of water or when the crystal powder of KBr, which is used for sampling, contains a small quantity of water, a band will appear at about 3300 cm^{-1}. If the quantity of water is considerable, an IR absorption band will also appear at about 1630 cm^{-1}, which can be assigned as the absorption band of the isolated water. Hydroxyl groups have not this band (at about 1630 cm^{-1}). A hydroxyl group can be differentiated from a small quantity of water by the absorption of C–O bond in the region of 1250–1050 cm^{-1}.

The IR absorption of the carboxyl group was presented in Section 5.3.

The isolated amino groups have an IR absorption band below 3600 cm^{-1}.

If two blunt bands formed in a saddle shape appear at about 3300 cm^{-1}, the conclusion that a primary amino group exist can be drawn immediately. The two bands come from the symmetrical and asymmetrical stretching vibrations of the primary amino, whose two N–H bonds produce these two stretching vibrations. If only a blunt band appears at about 3300 cm^{-1}, it reveals the existence of a hydroxyl group or a secondary amino group. It is not difficult to differentiate a hydroxyl group from a secondary amino group, because the former has strong absorption bands among C–O bands in the region of 1050–1250 cm^{-1}.

If a sharp band (like a peak) appears at about 3300 cm^{-1}, it is possibly the absorption of an alkyne group. It is necessary to confirm the alkyne group by the inspection of the absorption of the C≡C triple bonds in the region of 2100–2260 cm^{-1}. However, this absorption is weak, and even disappears when the two parts at the two sides of the triple bonds are symmetrical.

After the bands at about 3300 cm^{-1}, the bands near 3000 cm^{-1} need to be analyzed carefully. 3000 cm^{-1} is the boundary between the absorption bands of saturated C–H and those of unsaturated C–H. Except the absorption bands of the saturated three-membered ring, which have unordinary spectroscopic parameters in IR and in NMR, all saturated C–H produce IR absorption bands below 3000 cm^{-1}. Unsaturated C–H vibrations give IR absorption bands beyond 3000 cm^{-1}. However, the intensities of their bands are low, so that these bands may appear as "shoulder peaks," which are small bands overlapping upon a strong band. If an IR absorption band extends from 3000 cm^{-1} towards a higher wavenumber with a slippery curve shape, the fact reveals that the sample to be measured

contains no unsaturated C–H groups. Contrarily, if some small bands overlap on the curve, the conclusion that the sample contains unsaturated C–H groups can be drawn.

Below 3000 cm^{-1}, there are IR absorption bands at about 2960, 2920, 2870, and 2850 cm^{-1}, of which 2960 and 2870 cm^{-1} belong to the CH_3 groups and 2920 and 2850 cm^{-1} belong to the CH_2 groups. We insert some knowledge about Raman spectroscopy here. Although the principle of IR spectroscopy and that of Raman spectroscopy are different, the positions of IR absorption bands and those of Raman scattering peaks are close for the same sample. However, the positions of IR absorption bands of CH_3 and CH_2 are considerably different from those of Raman scattering peaks of CH_3 and CH_2. Now we return to the IR spectrum. If the intensities of the peaks at 2920 and 2850 cm^{-1} are considerably stronger than those at 2960 and 2870 cm^{-1}, it reveals that the sample contains many CH_2 groups but few CH_3 groups, just like the situation with a normal alkyl group. For the confirmation of the existence of a normal alkyl group, it is necessary to check if there is a weak band at about 720 cm^{-1}, which comes from the connection of four (or more) CH_2 groups.

If there are IR absorption bands at about 2820 and 2720 cm^{-1}, which corresponds to the vibration of C–H in an aldehyde group, it should be considered that the sample contains the group. This postulation needs to be proved by the absorption band of the carbonyl group of the aldehyde group.

Because any organic compound contains C–H bond, it is certain that there are IR absorption bands in the region of 2800–3100 cm^{-1}, while there are not with inorganic compounds. Therefore, the bands at about 3000 cm^{-1} can be used for the differentiation of an organic compound from an inorganic compound.

5.4.1.2 2500–2000 cm^{-1}

This region belongs to that of triple bonds and accumulated double bonds.

Because two light beams are not in balance in the IR spectrometer, CO_2 in air always produces IR absorption bands at about 2365 and 2335 cm^{-1}. Except these two IR absorption bands, all other bands including weak bands should attract one's attention, because even a weak band provides related structural information.

The absorption bands of alkyne groups are weak. If the two structural units at both sides of the alkyne group are symmetrical, the band of the alkyne group may disappear.

5.4.1.3 2000–1500 cm^{-1}

This region is very important for the IR spectrum.

The most important IR bands in this region belong to carbonyl groups, which produce the strongest or the second strongest band in their IR spectra. These situations do not exist only in a compound which contains tens of carbon atoms.

Weak IR absorption bands in this region reveal the existence of carbonyl groups as impurities.

If a compound contains a carboxyl group, besides the absorption band of the carbonyl group in this region, there is a strong and broad absorption band at about 3000 cm^{-1}.

If a compound contains an aldehyde group, there are also weak bands at about 2820 and 2720 cm^{-1}.

If a compound is a carboxylate, there are also absorption bands in the region of 1050–1300 cm^{-1} from C–O–C vibrations.

C=C double bonds produce IR absorption bands in the region of 1600–1670 cm^{-1} with medium or low intensities. For the confirmation of C=C double bonds, the bands at about 3050 cm^{-1} from unsaturated C–H stretching vibrations and the bands in the region of 970–800 cm^{-1} from unsaturated C–H bending vibrations should be checked.

The skeleton vibrations of the benzene ring produce IR absorption bands at about 1450, 1500, 1580, and 1600 cm^{-1}. Since the band at about 1450 cm^{-1} is close to the bands of CH_3 groups and CH_2 groups, the existence of a benzene ring can be postulated from the last three bands, that is, at about 1500, 1580, and 1600 cm^{-1}. These three bands may not appear in the same IR spectrum. However, if there is a band at about 1500 or 1600 cm^{-1}, the existence of a benzene ring can be known in principle. Of course, there should be IR absorption bands at about 3050 cm^{-1}, which are from unsaturated C–H of the benzene ring. Remember that these bands are weak, so that they always appear as "shoulder peaks."

The positions of IR absorption bands of heteroaromatic rings are similar to those of the benzene ring. For example, the furan ring produces bands at about 1400, 1500, and 1600 cm^{-1}, and the pyridine ring produces bands at about 1435, 1500, 1570, and 1600 cm^{-1}.

The nitro groups produce two sharp and strong bands in the region of 1550–1530 cm^{-1} and in the region of 1370–1340 cm^{-1}. The latter has a lower intensity than the former. Because the nitro group has a symmetrical stretching vibration and an asymmetrical vibration, it produces two IR absorption bands. The asymmetrical stretching vibration has a rather high frequency.

The imine group (C=N) produces IR absorption band in the region of 1690–1630cm^{-1} with a medium intensity.

5.4.1.4 1500–1300 cm^{-1}

Except the IR absorption bands of the benzene ring, the heteroaromatic ring, nitro group, and so on, the bands in this region provide the information on the bending vibrations of C–H bonds.

The methyl groups produce absorption bands at about 1380 and 1460 cm^{-1}. Branching at the band at about 1380 cm^{-1} shows that two or three methyl groups connect to the same carbon atom (for example, t-butyl and isopropyl groups).

The methylene group, CH_2, produces only one IR absorption band in this region (at about 1470 cm^{-1}). If four (or more) CH_2 groups connect together, they produce an absorption band at about 720 cm^{-1} with low intensity.

CH_3 groups and CH_2 groups produce absorption bands also in the region of 2970–2850 cm^{-1}.

5.4.1.5 1300–910 cm^{-1}

The absorption bands from the C–O bond should be paid attention to first. The bands from C–O–C bonds of carboxylates appear in the region of 1300–1050 cm^{-1}, in which aromatic carboxylates produce bands in the higher wavemumber region and aliphatic carboxylates in the lower region.

Associated hydroxyl or phenol groups produce IR absorption bands in the region of 1250–1050 cm^{-1} from the C–O bond, in which the aliphatic C–O bond produces a band at a lower wavemumber while the aromatic C–O bond produces one at a higher wavenumber.

Another IR absorption band in this region which should be considered is the absorption band of the added products from ethylene oxides or propylene oxides, which are commonly

applied as nonionic surfactants. Their absorption bands are situated at about 1110 and at about 940 cm^{-1}. The latter has a lower intensity than the former.

5.4.1.6 Below 910 cm^{-1}

Substituted benzene rings produce IR absorption bands in the region of 900–650 cm^{-1}, which are useful for the interpretation of this region. Before the application of the ^1H spectrum, these bands are used as a main way to determine substituted positions of the substituted benzene rings. The related data can be remembered if we keep to the following thinking: the less the hydrogen atoms remaining in the benzene ring, the higher the absorption frequencies. Therefore, of the five substituted benzene rings, only an isolated hydrogen atom remains, and it has an IR absorption band in the region of 900–850 cm^{-1}. Contrarily, when the benzene ring remains rather more hydrogen atoms, it has a lower frequency absorption. When the benzene leaves four or five hydrogen atoms, its absorption band is situated in the region of 770–730 cm^{-1}. In addition, another band should be added for some substitutions.

When a benzene ring is substituted by strongly polar functional groups, the thinking about the substitutions will not be judged by the absorption bands.

5.5 Interpretation of IR Spectra

5.5.1 Key Points for the Interpretation of IR Spectra

5.5.1.1 Three Characteristics of the IR Spectrum (Frequency, Intensity, and Band Shape)

The IR absorption band has three characteristics: frequency, that is, the position of the absorption band, the intensity, and the band shape, which should be analyzed together for the interpretation of an IR spectrum.

Of course, the frequency of the absorption band is the most important data. However, a correct conclusion can be obtained only on the basis of the combined consideration of the three characteristics mentioned above.

For example, an alkyne group, a hydroxyl group, or a primary amine group produces an absorption band at about 3300 cm^{-1}. However, they can be recognized from their band shapes. The alkyne group shows a sharp band, while the hydroxyl group a blunt band, and the primary amine group a blunt and branched band.

The intensity of an absorption band should also be considered. For example, there is a weak band at about 1700 cm^{-1}, which does not reveal the existence of a carbonyl group, because the carbonyl group has a strong absorption band. Therefore, it should be considered that a carbonyl group exists as an impurity.

To sum up, an unknown absorption band can be assigned only if all of the three characteristics of the absorption band coincide with those of a known absorption band.

5.5.1.2 Co-Existence of all Related Absorption Bands of a Functional Group

A functional group has several vibration modes (stretching vibration modes and bending vibration modes), so it shows several absorption bands in an IR spectrum. These bands are called related absorption bands of the functional group.

For example, a hydroxyl group shows an absorption band from its stretching vibration (the associated hydroxyl groups produce a band at about 3300 cm^{-1}) and a band from the C–O stretching vibration in the region of 1050–1150 cm^{-1}.

The methylene groups of a normal alkyl group show the bands at about 2920 and 2850 cm^{-1}, whose intensities are stronger than those at about 2960 and 2870 cm^{-1} from the terminal methyl group, and a weak band at about 720 cm^{-1} when the number of the connected methylene groups is greater than 4.

A functional group can be determined only if all related absorption bands appear and each of them coincides with the corresponding known band in the three characteristics: frequency, intensity and band shape.

5.5.2 Steps for the Interpretation of an IR Spectrum

Generally speaking, the structure of an unknown compound can not be obtained only by the interpretation of its IR spectrum. What we can get is the structural information related with the IR spectrum.

It is recommended that the interpretation of the IR spectrum start from the high wavenumber edge, 4000 cm^{-1} to the low wavenumber edge in the order described in Section 5.4. Every band in the functional group region can find a corresponding functional group in principle.

After the interpretation of the bands in the functional group, we analyze the bands in the fingerprint region. It should be noticed that the information obtained from the fingerprint region coincides with that from the functional group. As described above, many bands in the fingerprint can not be assigned.

If the structure of an unknown compound can be guessed or postulated, to find a standard IR spectrum from IR spectrum collections or from Websites is very important.

5.5.3 Searching Standard IR Spectra from IR Spectrum Collections or Websites

Sadtler IR spectra have been most widely used for the comparison of the measured spectrum with a standard spectrum. Saddler standard spectra include the ^{1}H spectrum, the ^{13}C spectrum, the IR spectrum, and the UV spectrum. Several kinds of indexes can be used in different ways. The search is convenient. In addition, Sadtler spectra include those of commercial products. Therefore, it is convenient for the study of commercial products.

There are the following ways to search standard spectra from Websites.

① Find standard IR spectra (^{1}H spectra, ^{13}C spectra, or EI mass spectra) free of charge.

http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng.

Please refer to Section 1.5.8.

② If the institute or the university with which the reader is associated has an agreement with the following two Websites, abundant spectra and spectral data can be found. However, individuals can not access it.

http://166.111.120.35/database/crossfire.htm

https://scifinder.cas.org

③ After the registration, standard IR spectra, ^{1}H spectra, and ^{13}C spectra can be obtained from the BIO-RAD Company by payment. Please refer to Section 1.5.8.

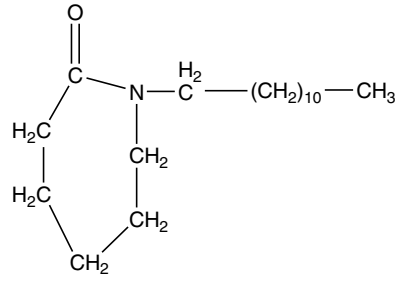
5.5.4 Examples of Interpreting IR Spectra

We have known the function of the IR spectrum. The interpretation of the IR spectrum can leads to related structural information to help the deduction of an unknown structure.

The following four examples are the author's practice to postulate unknown structures.

To use only the IR spectrum to postulate a simple structure is not very useful for readers.

Example 5.1 An unknown compound has been tentatively identified as follows.



C5-1

Its IR spectrum is shown in Figure 5.1.

Try to analyze if the IR spectrum coincides with the structural formula.

Solution

We start the interpretation from the high wavenumber side.

There are no IR absorption bands in the region of 4000–3000 cm^{-1}, which reveals that the compound contains neither hydroxyl groups nor amine groups.

The two strong absorption bands at 2924 and 2853 cm^{-1} reveal that the compound contains many methylene groups. Because these two bands are too strong, the bands from methyl groups can not be seen. This postulation is proved further by the band at 721 cm^{-1}. It should be known that the intensity of the band at about 720 cm^{-1} from the connection of four (or more) methylene groups is low.

The strong absorption at 1649 cm^{-1} illustrates the existence of a carbonyl group. The frequency, which is less than 1700 cm^{-1} by a considerable amount, reveals that the carbonyl group is an amide group.

A special feature in Figure 5.1 is that there are four absorption bands in the region of 1482–1422 cm^{-1}, which is rarely encountered. The four bands in the region of the methylene group manifest that methylene groups of the compound have different structural environments, that is, the groups connected with these methylene groups have different electronegative properties. This postulation coincides with the structural formula above, in which at least four types of methylene groups (that is, those connected with the nitrogen atom and with the carbonyl group, and those situated in the normal chain and in the ring) exist.

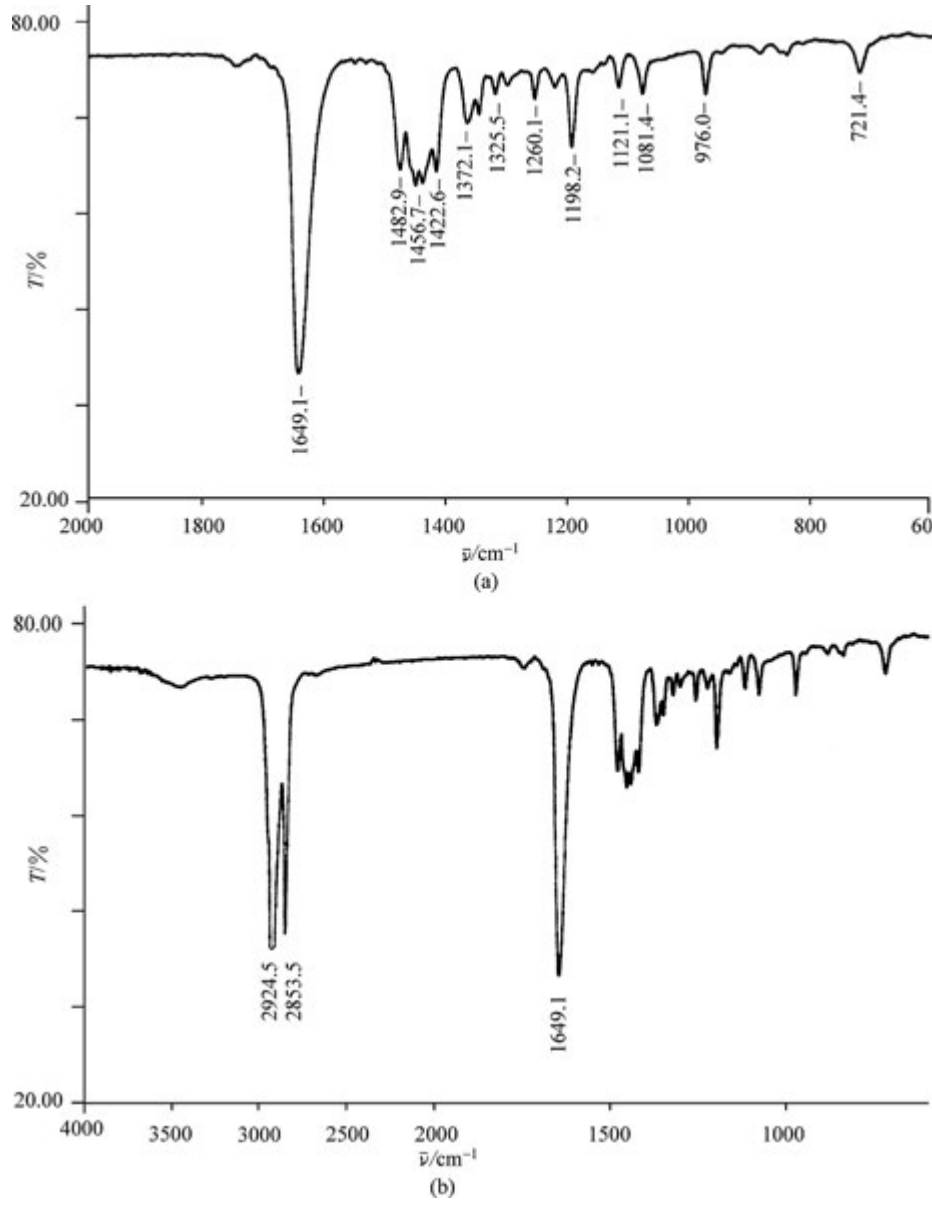
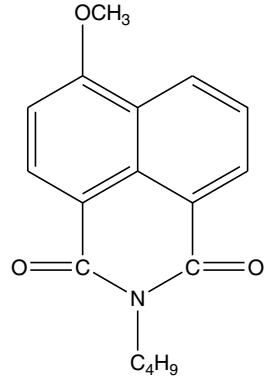


Figure 5.1 The IR spectrum of compound **C5-1**. (Lower part: the IR spectrum of the compound; Upper part: the locally enlarged IR spectrum of the lower wavenumber region of the IR spectrum). (Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA.)

From the weak absorption band at 1372 cm^{-1} it can be known that the compound contains few methyl groups.

To sum up, the conclusion that Figure 5.1 coincides with the structural formula can be drawn. Therefore, the structure deduced should be correct.

Example 5.2 An unknown compound has been tentatively identified as the following structure.



C5-2

Its IR spectrum is shown in Figure 5.2.

Try to analyze if the IR spectrum coincides with the structural formula.

Solution

We still start the interpretation from the high wavenumber side.

There are no IR absorption bands in the region of 4000–3000 cm^{-1}, which reveals that the compound contains neither hydroxyl groups nor amine groups.

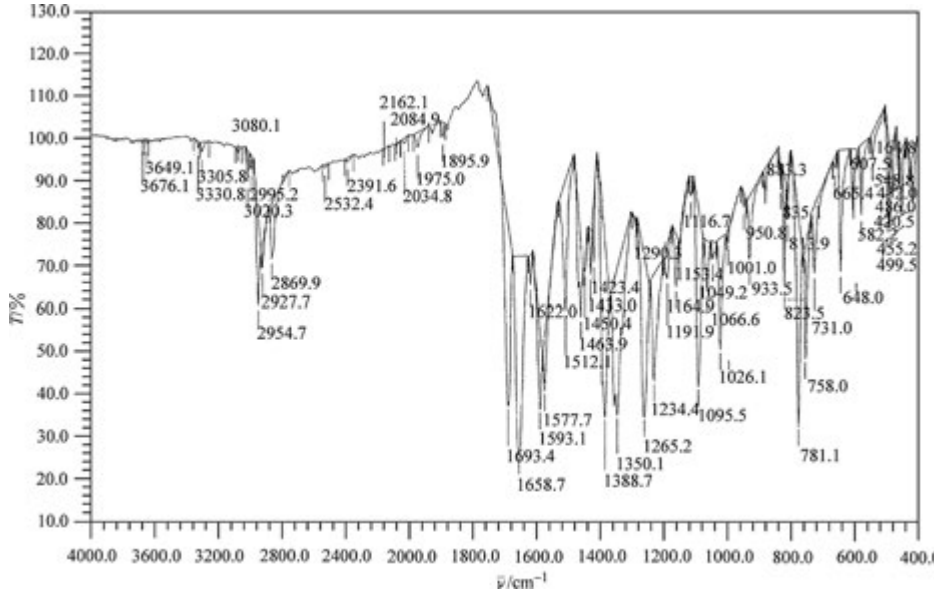


Figure 5.2 The IR spectrum of compound **C5-2** (Reprinted with permission from Yong-Cheng Ning, *Structural Identification of Organic Compounds with Spectroscopic Techniques*, © 2005 Wiley-VCH Verlag GmbH & Co. KGaA.)

The band at 3020 cm^{-1} has a certain intensity. It should be known that the bands of unsaturated C–H vibrations have low intensities. Therefore, the band at 3020 cm^{-1} should be paid attention to. Obviously, this band corresponds to the aromatic ring in compound **C5-2**. The bands at 1593 and 1512 cm^{-1} provide a further proof.

The bands at 2954, 2927, and 2869 cm^{-1} can be assigned to the absorptions of methyl groups and methylene groups.

The bands at 1693 and 1658 cm^{-1} reveal that the compound contains two carbonyl groups. The band at 1658 cm^{-1} can be determined as an amide group according to its absorption frequency. The band at 1693 cm^{-1} can not be determined in detail at this time.

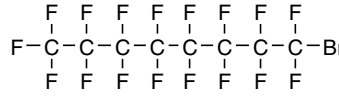
The assignment of these two absorption bands will be clarified when we combine the structural formula. Because two carbonyl groups connect with the same nitrogen atom, they are both amide groups with absorption frequencies less than 1700 cm^{-1}. Since these two carbonyl groups are connected through a common nitrogen atom, they form a symmetrical stretching vibration and an asymmetrical stretching vibration. Because the asymmetrical vibration has a higher frequency, its absorption band possesses a higher frequency than that of a common amide.

The bands at 1463, 1450, and 1388 cm^{-1} can be assigned to the absorption bands of methyl groups and methylene groups.

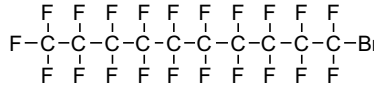
The bands at 1265 and 1095 cm^{-1} are assigned to C–O–C vibrations.

To sum up, the IR spectrum coincides with the structural formula. Therefore, the tentatively postulated structure is finally confirmed by the spectrum.

Example 5.3 Compounds **C5-3** and **C5-4** have the following structures.



C5-3



C5-4

Their IR spectra are shown in Figures 5.3 and 5.4, respectively. What conclusion can be drawn by interpreting these two IR spectra?

Solution

The following conclusions can be drawn from the comparison between these two IR spectra.

1. Normally speaking, the two IR spectra should be similar because these two structures are similar. However, there are obvious differences between these two IR spectra.
2. This fact can be explained only by the difference of the phases of these two samples. Compound **C5-3** is a liquid while compound **C5-4** is a solid.

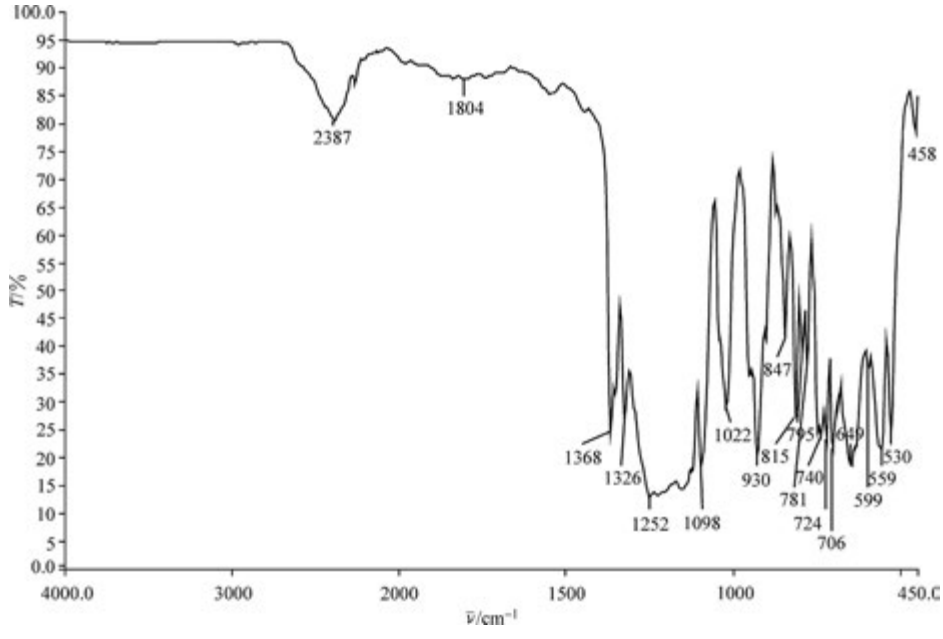


Figure 5.3 The IR spectrum of compound **C5-3**

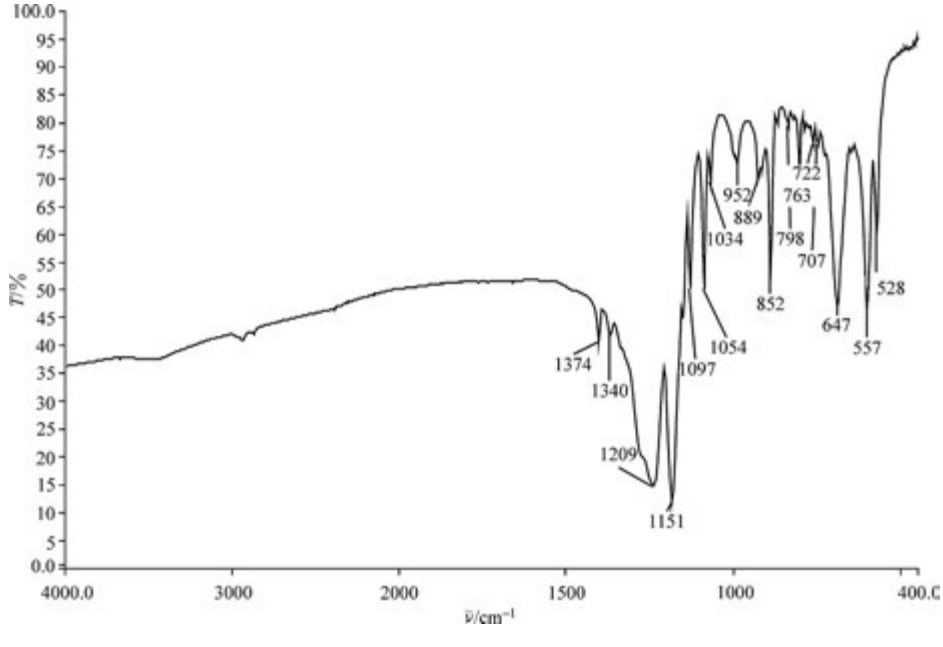


Figure 5.4 The IR spectrum of compound **C5-4**

3. These two IR spectra still have the common feature that there are strong absorption bands in the region of 1000–1400 cm^{-1}. These bands come from the C–F vibrations. Because the fluorine atom is strongly polar, the related absorption bands are strong.

Example 5.4 In Example 5.1 for the interpretation of mass spectra from soft ionization in Section 4.4.6, the new compound was identified as the dimer by its FAB mass spectrum. Before and after the formation of the dimer, the IR absorption band of the carbonyl group shifts its frequency from 1735 to 1753 cm^{-1} by an amount of 18 cm^{-1}, which is much greater than the measurement error (8 cm^{-1}). All NMR spectra of the dimer are the same as those of the monomer, so that NMR spectra can not provide the information on the new compound. However, the IR spectrum of the dimer at least reveals the formation of the new compound.

6

Identification of Unknown Compounds or Confirmation of Structures through Comprehensive Interpretation of Spectra

We have discussed the interpretations of the ^{1}H spectrum, the ^{13}C spectrum, the 2D NMR spectra, the mass spectrum, and the IR spectrum in the preceding five chapters, respectively. Now we shall present the interpretation combining the spectra mentioned above.

First, it should be known that we focus our attention on the commonest method, that is, we deduce an unknown structure or confirm a structure by the application of the ^{1}H spectrum, the ^{13}C spectrum, the DEPT spectrum, the COSY spectrum, the HMQC (or HSQC) spectrum, the HMBC spectrum of the unknown compound, and other necessary data (for example, the molecular weight obtained from the mass spectrum, the data obtained from tandem mass spectrometry). The method mentioned above is used most frequently and it is very reliable in general. Other methods are not used frequently because of a low sensitivity, for example, 2D INADEQUATE, or because of other reasons. Therefore, they are not presented here.

Then, it should be known that the examples for assignments of spectra to confirm anticipated structures occupy a rather large proportion of this chapter, because it is difficult to deduce a very complicated structure by using the method mentioned above. In such a case, the complicated structure needs to be identified by the application of X-ray diffraction of the single crystal of the unknown compound. On the basis of the identification of an unknown structure by the application of X-ray diffraction of the single crystal, the structures of its derivatives can be determined by using the spectroscopic method. In some literature it is also possible that some structures be determined by the comparison of the spectroscopic data of the studied structure with published spectroscopic data of the known compound in other literature.

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When an unknown compound contains a lot of quaternary carbon atoms or heteroatoms, the functions of its COSY, HSQC and HMBC spectra are limited. Therefore, it may be difficult to identify its structure. This situation can take place even if a structure is not complicated. In this case, the method mentioned above may not work well.

In any case, readers can understand the method mentioned above well after the study of this chapter and know how to obtain as much structural information as possible from related spectra.

6.1 Commonly Used Method and Steps

Generally speaking, the molecular weight or the elemental composition of an unknown compound, which is obtained from its mass spectrum, is necessary for its structural identification. The ^{13}C spectrum and ^{1}H spectrum can give direct information in detail about the carbon atoms and the hydrogen atoms of the studied compound and the indirect information about the heteroatoms of the compound. For example, the existence of heteroatoms can be known from the peaks of NH and OH in the ^{1}H spectrum, and the splits by fluorine atoms in the ^{1}H spectrum and the ^{13}C spectrum. However, some information about some heteroatoms, for example, the existence of SH, can not be obtained from NMR spectra. Therefore, the deduction of the structure of the compounds containing heteroatoms should be accomplished by use of mass spectrum data.

For the deduction of an unknown structure, we should analyze the spectra one by one, and then we get structural units. Finally, we assemble structural units obtained to form the whole structure or to confirm an anticipated structure.

The steps for the spectrum interpretation and related information, which should be obtained for each step, are as follows.

6.1.1 ^{1}H Spectrum

The interpretations of the spectra of a compound start from the interpretation of the ^{1}H spectrum in general.

The ^{1}H spectrum gives the following abundant structural information on the compound.

1. A global understanding of the compound to be studied can obtained from its ^{1}H spectrum, including the information which follows:
   Is this compound an aliphatic or an aromatic compound?
   Does it have a normal chain or a branched chain if this compound is an aliphatic compound?
   Does it have many branches if this compound is a branched one?
2. Even if mass spectrum data are not available, how many hydrogen atoms exist in the compound can be known from its ^{1}H spectrum. Because some peak sets in the ^{1}H spectrum can be used as a standard, for example, the singlet of a terminal methyl group or a methoxyl group corresponds to three hydrogen atoms, the hydrogen atom number of the compound can be determined by its ^{1}H spectrum from the addition of integral values of all peak sets.

3. From coupled splits of peak sets in the ^{1}H spectrum, some connections of the compound can be found. As the described in Chapter 1, the analysis of coupled splittings is very important for the deduction or the confirmation of a structure even when 2D NMR spectra are available.
4. From chemical shift values and coupled splittings in the ^{1}H spectrum, some functional groups can be determined directly, such as methoxyl, aldehyde, carboxyl, enol, normal chain alkyl, para-substituted benzene ring, and so on. The heteroatoms related with the functional groups mentioned above can also be determined.

6.1.2 ^{13}C Spectrum

The following information can be obtained from the ^{13}C spectrum.

1. Because of the high resolution of the ^{13}C spectrum, it shows clearly the number of the groups of the carbon atoms, which have the same chemical shift value. If the molecule has no symmetrical plane, the number of the peaks in the ^{13}C spectrum is equal to the number of the carbon atoms of the molecule. If the molecule has a local symmetry, the carbon atom numbers of the related peaks, which are the overlapped peaks with the same δvalue, still can be estimated even without the quantitative ^{13}C spectrum. Therefore, the total carbon atom number of the molecule can be calculated from the number of all peaks.
2. The structural information obtained from the ^{13}C spectrum is clearer than that from the ^{1}H spectrum. The numbers of alkyl, alkene, aromatic, and carbonyl carbon atoms can be determined from the ^{13}C spectrum.
3. Compared with the DEPT spectrum, the peaks of quaternary carbon atoms can be known, because their peaks are absent in the DEPT spectrum.

6.1.3 DEPT Spectrum

The following information can be obtained from the DEPT spectrum.

1. From the DEPT spectrum, the peaks of CH, CH_2, and CH_3 in the ^{13}C spectrum can be resolved.
2. Because quaternary carbon atoms have no peaks in the DEPT spectrum, their peaks in the ^{13}C spectrum can be discerned.
3. By the combination of the DEPT spectrum and the ^{13}C spectrum, the number of the hydrogen atoms, which are connected directly with carbon atoms, can be calculated. The subtraction of the number from the number of the hydrogen atoms in the molecular formula gives the number of the reactive hydrogen atoms of the molecule.

6.1.4 COSY Spectrum

The COSY spectrum has the following functions:

1. The COSY spectrum shows the coupling relationships between groups containing hydrogen atoms, which concern mainly the couplings with ^{3}J.

2. For some systems, such as aromatic systems, the systems containing double bonds, and some special configurational systems, the couplings with ^4J may be shown in the COSY spectrum. This information is also useful for the postulation of the structure of an unknown compound.

It should be emphasized that the reliability of the information obtained from the COSY spectrum is higher than that obtained from the HMBC spectrum. If the overlapping of correlated peaks in the COSY spectrum is not serious, the information obtained from the COSY spectrum should be considered first.

6.1.5 HMQC (or HSQC) Spectrum

The functions of the HMQC (or HSQC) spectrum are as follows.

1. The peaks in the ^{13}C spectrum and the peak sets in the ^1H spectrum are correlated through the HMQC (or HSQC) spectrum. The determination of the correlation is very important for the deduction of an unknown structure.
2. The overlapped peak sets in the ^1H spectrum can be resolved and they can be interpreted well. Many such examples will be shown in Section 6.2.
3. Because the resolution of the HMQC (or HSQC) spectrum is higher than that of the COSY spectrum, the interpretation of the HMQC (or HSQC) spectrum can help to interpret the overlapped correlated peaks in the COSY spectrum.

6.1.6 HMBC Spectrum

The HMBC spectrum is necessary for the deduction of an unknown structure.

1. Only the HMBC spectrum can be used to find the connections around quaternary carbon atoms.
2. The correlation in the HMBC spectrum can cross a heteroatom. Therefore, the HMBC spectrum is very important for the determination of the position where the heteroatom situates and for the connection of two structural units which are separated by a heteroatom or a quaternary carbon atom.
3. Because the resolution of the HMBC spectrum is higher than that of the COSY spectrum, the interpretation of the HMBC spectrum can help to analyze the overlapped correlated peaks in the COSY spectrum.

6.2 Examples for the Deduction of the Structure of an Unknown Compound or for the Confirmation of an Anticipated Structure

On the basis presented above, we can deduce the structure of an unknown compound or confirm an anticipated structure.

For every example, several tables are listed to summarize the data obtained from related spectra. These tables, from which the reader can understand how to read out spectroscopic data correctly, are necessary to solve structural problems.

The examples are arranged roughly in order from the simple one to the complicated one.

Example 6.1 The mass spectrum, the ^{1}H spectrum, the quantitative ^{13}C spectrum, the DEPT-135 spectrum, the COSY spectrum, the H, C-COSY spectrum, and the HMBC spectrum of an unknown compound are shown in Figures 6.1–6.7, respectively. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used in the NMR measurement is deuterated methanol. Try to deduce the structure of the unknown compound.

Solution

The step for the interpretation follows this order: first the determination of its molecular formula, then the determination of its structural units, and finally the assembling of structural units.

We now interpret the mass spectrum first. The mass spectrum of an unknown compound always plays an important role in the determination of its molecular formula. The peak at *m/e* 140 in Figure 6.1 meets the conditions of the molecular ion peak, because the mass difference between the *m/e* 140 and the next peak is 28 (u), which is one of the reasonable mass differences in the EI mass spectrum.

Figure 6.2 is the ^{1}H spectrum of the unknown compound. The peaks at 3.21 and 4.75 ppm are the solvent peak and the water peak, respectively. The peaks at 1.18, 1.81 ppm, and so on belong to impurities, because their peak areas are very small.

The ^{1}H spectrum shows four peak sets. Every one of the integral values of the four peak sets is close to 1. It can not be determined for the moment whether the integral value of 1 corresponds to one or to two hydrogen atoms.

Now we analyze the quantitative ^{13}C spectrum, in which the peak at about 48 ppm is the solvent peak. Since the ^{13}C spectrum shows 7 peaks, the unknown compound should contain 7 groups of carbon atoms.

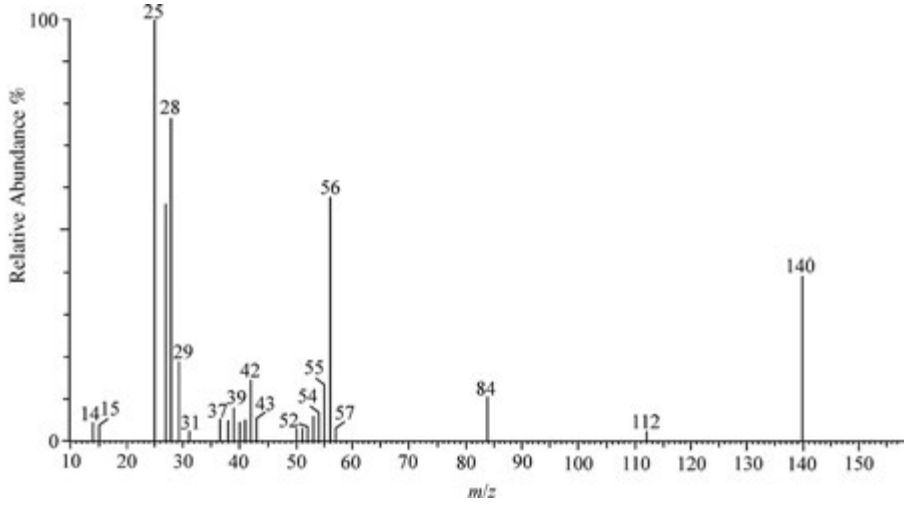


Figure 6.1 The mass spectrum of the unknown compound

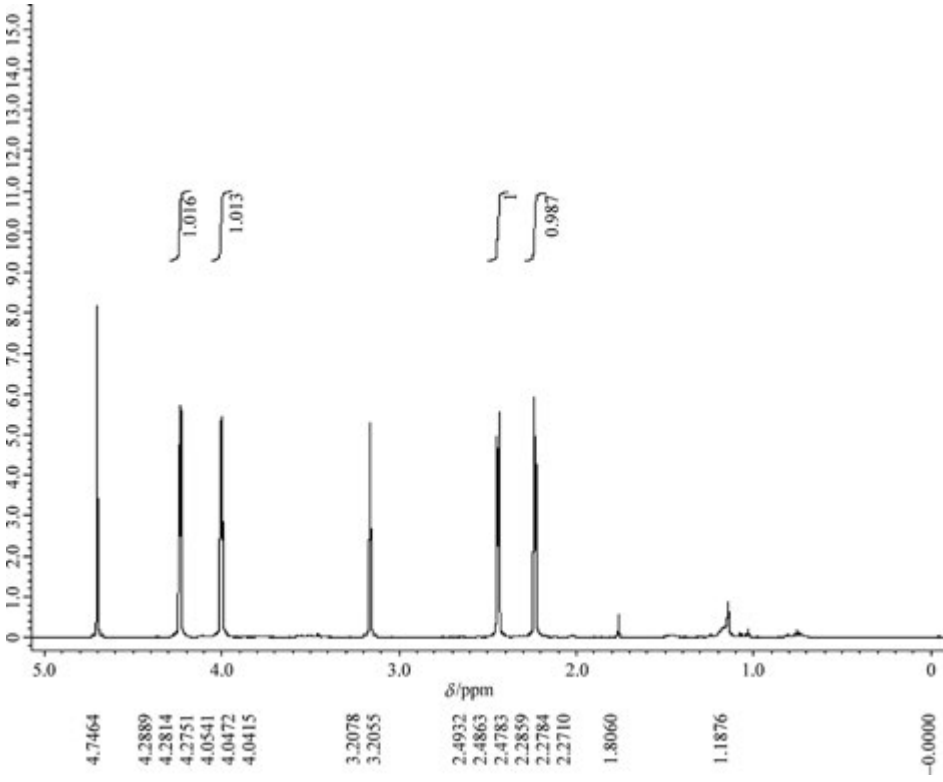


Figure 6.2 The ^{1}H spectrum of the unknown compound

By the combination of the quantitative ^{13}C spectrum and the DEPT-135 spectrum, we get Table 6.1.

From the special chemical shift value of 196.6 ppm in the ^{13}C spectrum, this peak can be assigned as a carbonyl group, and furthermore a ketone or an aldehyde group, because if a carbonyl group connects with a heteroatom, its chemical shift value will be less than 180 ppm.

Since the unknown compound contains a carbonyl group, it is possible that the compound lose a carbonyl group to produce an ion with the m/e ratio, which is the molecular weight minus 28, especially when the carbonyl group is situated in a ring. To sum up, the data from the ^{13}C spectrum coincide with those from the mass spectrum.

By the combination of the ^{13}C spectrum and the DEPT spectrum, the above-mentioned question can be known, that is, every peak set in the ^{1}H spectrum corresponds to two hydrogen atoms, because the four peaks in the DEPT spectrum are downwards (CH_2).

On the basis of the interpretation of the ^{1}H spectrum, the ^{13}C spectrum, and the DEPT-135 spectrum, it can be known that the unknown compound contains at least C_7H_8O, whose mass is 108. Compared with its molecular weight of 140, the remaining mass is 32 (u). Because there are peak sets at 4.05 and 4.28 ppm in the ^{1}H spectrum, and there are peaks at 63.7 and 67.1 ppm in the ^{13}C spectrum, it is reasonable that the unknown compound contains two

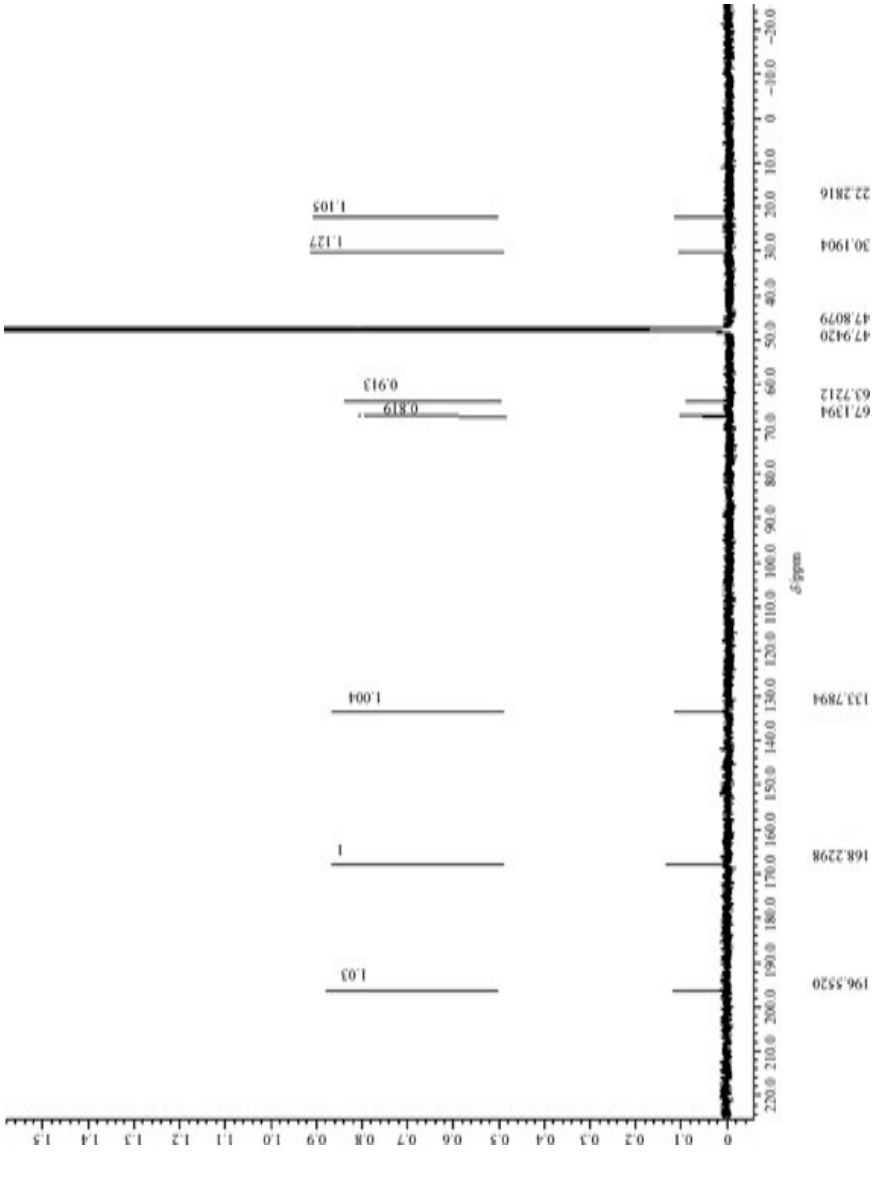


Figure 6.3 The quantitative ^{13}C spectrum of the unknown compound

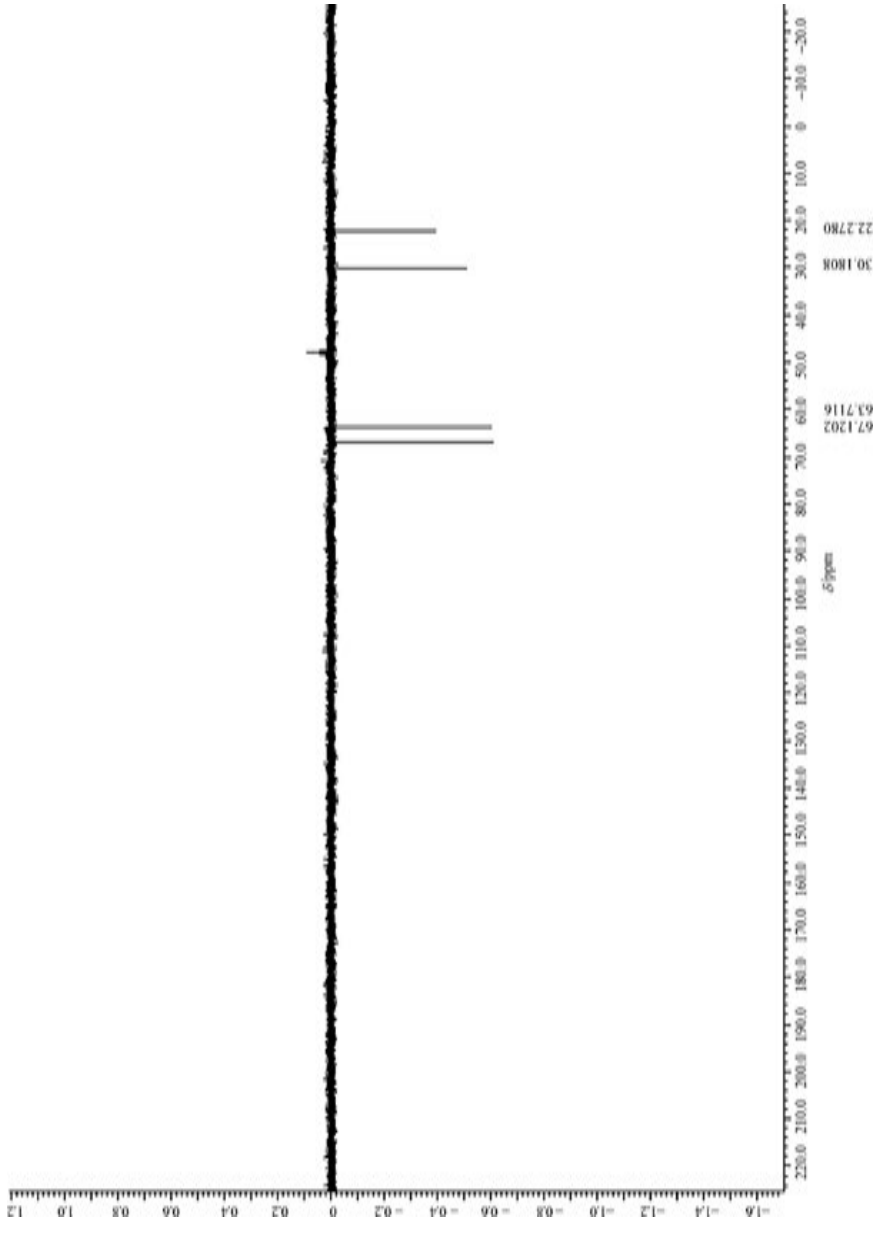


Figure 6.4 The DEPT-135 spectrum of the unknown compound

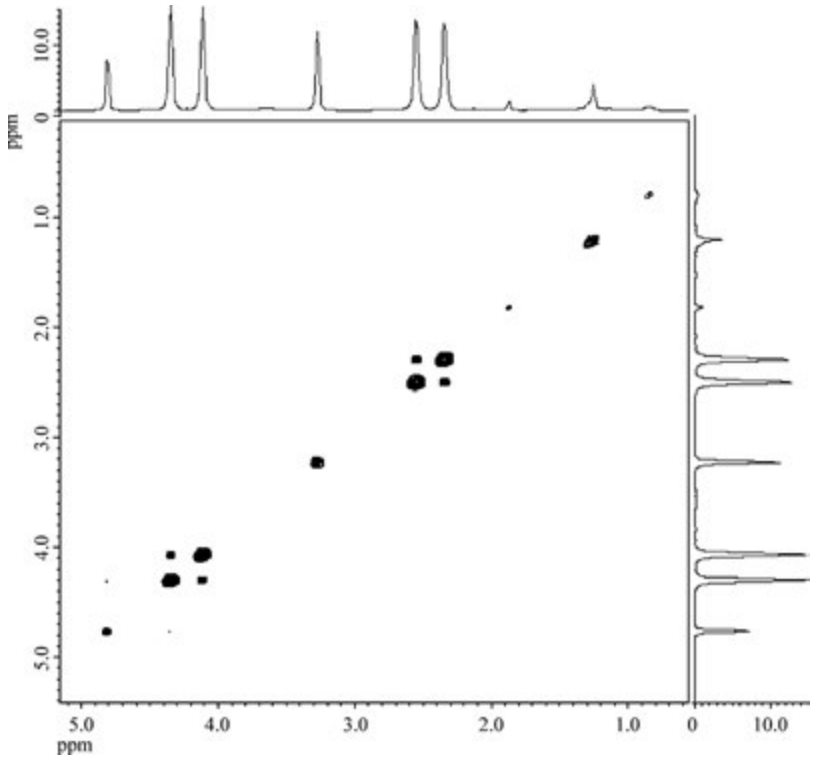


Figure 6.5 The COSY spectrum of the unknown compound

other oxygen atoms, whose mass is 32 (u). There is not any other solution for the remaining mass. Therefore, we can finally determine that the molecular formula of the unknown compound is $C_7H_8O_3$.

The unsaturation number of 4 can be calculated from the above-mentioned molecular formula. Since the unknown compound contains a carbonyl group and a double bond, whose peaks are situated at 133.8 and 168.2 ppm in the ^{13}C spectrum, respectively, the unknown compound should contain two rings to match the unsaturation number of 4.

The COSY spectrum gives the following structural units:

CH_2 (2.28) –CH_2 (2.49); CH_2 (4.05) –CH_2 (4.28).

The H, C-COSY spectrum correlates the peaks in the ^{13}C spectrum and above-mentioned peak sets in the ^{1}H spectrum. These two structural units can be rewritten as follows.

CH_2 (30.19, 2.28) –CH_2 (22.28, 2.49); CH_2 (63.72, 4.05) –CH_2 (67.14, 4.28).

The first number in the parenthesis is δ_C and the second one is δ_H.

From the chemical shift values of 63.72 and 67.14 ppm, it can be known that these two carbon atoms should connect with one oxygen atom, respectively, that is, we have

–O– CH_2 (63.72, 4.05) –CH_2 (67.14, 4.28) –O–

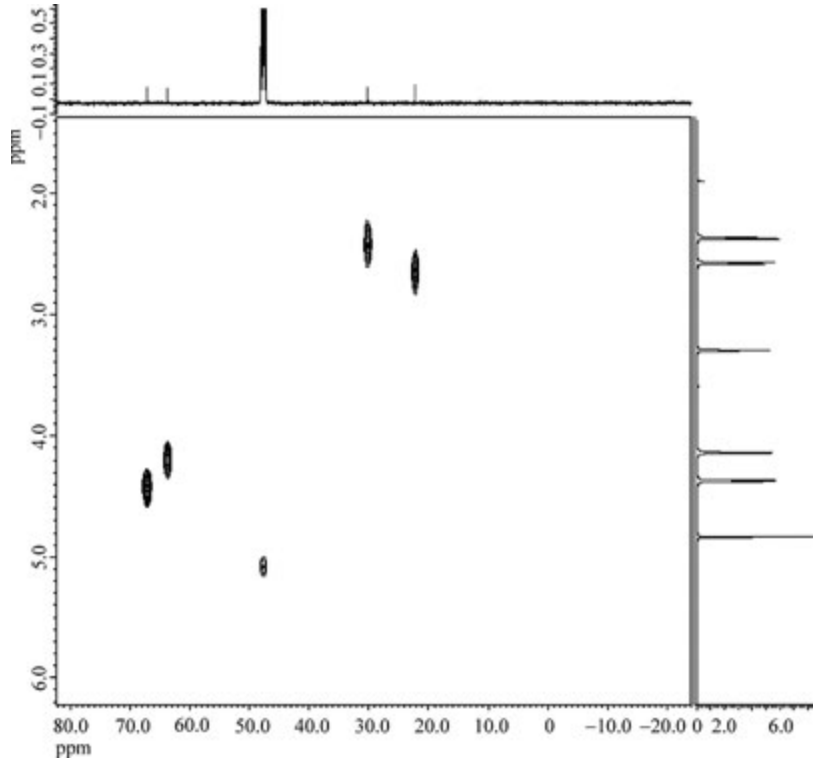


Figure 6.6 The H, C-COSY spectrum of the unknown compound

From the chemical shift values of 30.19 and 22.28 ppm, it can be known that the other pair of methylene groups should connect with a quaternary carbon atom, respectively.

Because the unknown compound contains quaternary carbon atoms and oxygen atoms, the assembling of structural units needs the help of the HMBC spectrum.

There are two pairs of correlated peaks, which are symmetrical about two δ_H values (2.28 and 2.49 ppm). Their ordinates are 22.28 and 30.19 ppm, respectively. These two pairs of correlated peaks are those about ^1J couplings (see Section 3.4). The other correlated peaks are those about C–H long-range couplings.

The data summarized from the HMBC spectrum are listed in Table 6.2.

Because of the correlations of δ_C (196.56)/δ_H (2.28, 2.49) and δ_C (168.23)/δ_H (2.28, 2.49) in the HMBC spectrum, these two methylene groups should connect with the carbonyl group (196.56 ppm) and the alkylen carbon atom (168.2 ppm), respectively. In addition to the correlation of δ_C (133.79)/δ_H (2.49), it is reasonable that these two methylene groups are connected with the carbonyl group and a double bond, which consists of the two alkylene carbon atoms mentioned above.

Because these two alkylene carbon atoms have rather larger chemical shift values, it is reasonable that both of them should connect with oxygen atoms.

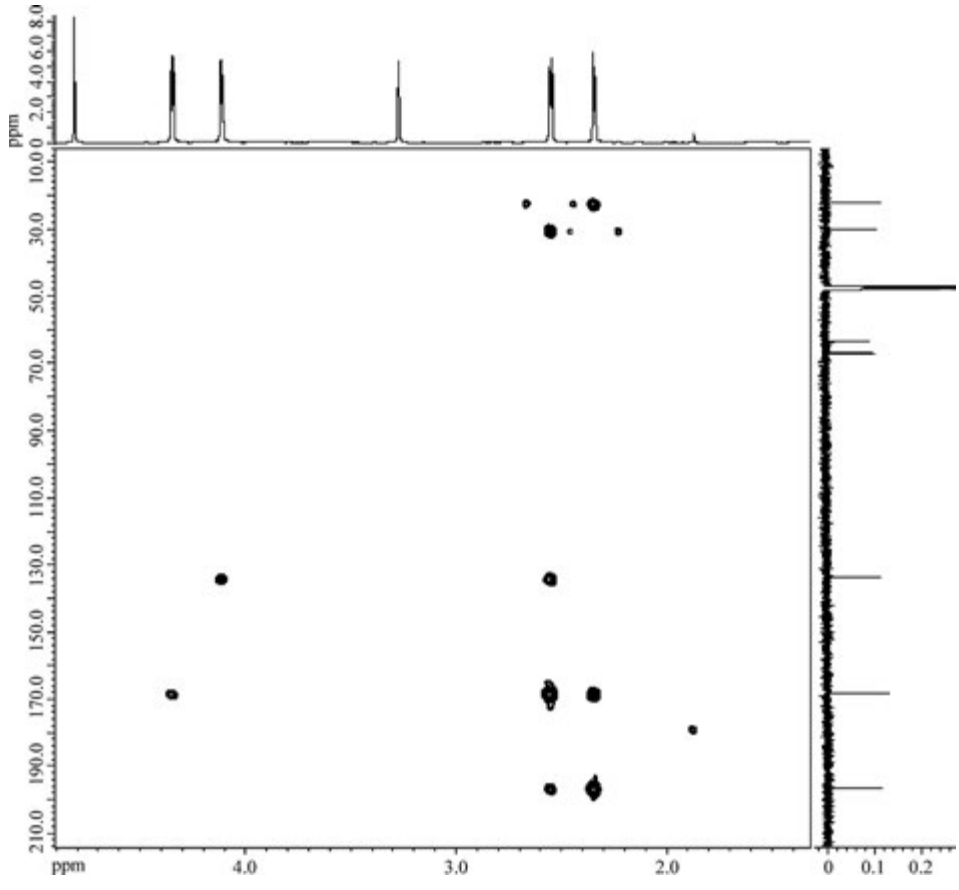


Figure 6.7 The HMBC spectrum of the unknown compound

The above postulation coincides with the unsaturation number of 4.

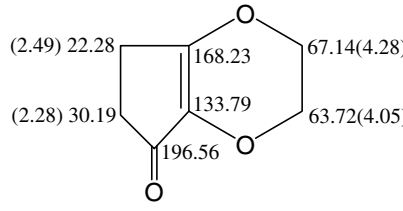
To sum up, the unique possible structure of the unknown compound is as follows. The assignments of the ^{13}C spectrum and the ^{1}H spectrum (in parenthesis) are marked besides the structural formula.

Table 6.1 The data summarized from the quantitative ^{13}C spectrum and the DEPT-135 spectrum

δ_C (ppm)	Number of C atoms	Order of C atoms
22.28	1	CH_2
30.19	1	CH_2
63.72	1	CH_2
67.14	1	CH_2
133.79	1	C
168.23	1	C
196.56	1	C

Table 6.2 The data summarized from the HMBC spectrum

δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
22.28	2.28	2.49
30.19	2.49	2.28
63.72		
67.14		
133.79	2.49, 4.05	
168.23	2.28, 2.49, 4.28	
196.56	2.28, 2.49	

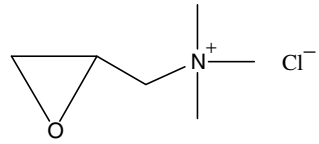


C6-1

We supplement the assignment of the ^{13}C spectrum.

Because of the formation of a conjugated system from the double bond and the carbonyl group, the alkylene carbon atom, which is situated in the middle of the conjugated system, has its δ_C of 133.79 ppm. Another alkylene carbon atom, which is situated at the terminal position of the conjugated system, has its δ_C of 168.23 ppm. These data show the function of a conjugated system for chemical shift values.

Example 6.2 A purchased industrial chemical has the following structure:



C6-2

Its ^1H spectrum, the locally enlarged spectrum of the ^1H spectrum in the high field region, its DEPT-135 spectrum, its COSY spectrum, and its HSQC spectrum are shown in Figures 6.8–6.13, respectively. All NMR spectra were measured by an NMR spectrometer with a frequency of 400 MHz. The solvent used is D_2O. Try to confirm the structure and try to determine the structure of the major impurity in the chemical.

Solution

We start from the interpretation of the ^1H spectrum of compound **C6-2**.

This ^1H spectrum is much more complicated than expected. The complexity of the ^1H spectrum comes from the following factors: the structural characteristic of compound **C6-2** and impurities in the chemical.

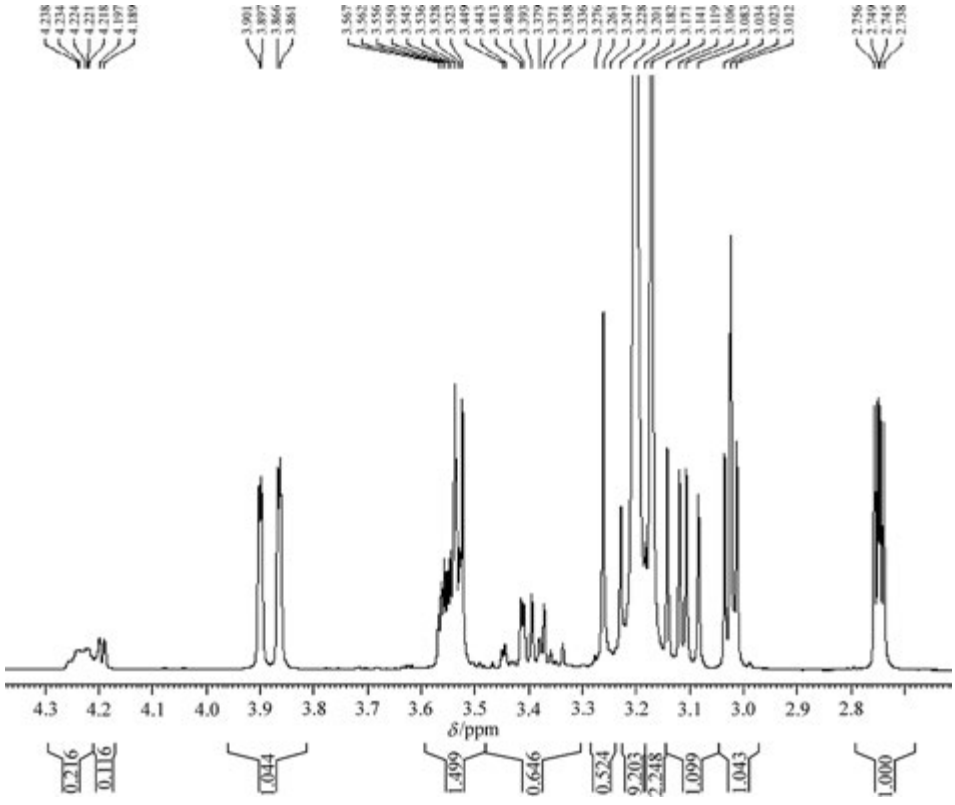


Figure 6.8 The ^{1}H spectrum of compound **C6-2**

The peak sets of the impurities in the ^{1}H spectrum can be found from their peak areas, whose integral values are much smaller than those of the sample. Therefore, the peak sets at 3.40 and 4.22 ppm can be determined as impurity peak sets. The peak set at 3.26 ppm can be determined as that of impurities by using the HSQC spectrum.

Now we set to interpreting the ^{13}C spectrum.

There are several triplets, whose intensities are prominent, in the ^{13}C spectrum. These triplets come from the couplings by the nitrogen atom of the quaternary salt. Because the main isotope of the nitrogen element is ^{14}N, whose isotopic abundance is 99.6%, according to the $2nI + 1$ rule, the coupled nuclei will be split in triplets, in which the peaks have approximate intensities. These triplets, which are rare in the ^{13}C spectrum of a compound containing the nitrogen atom, concern the nitrogen atom of the quaternary salt.

The first task before us is the differentiation of the impurities from the main composition. And it should be determined if the impurity peaks belong to a composition. For the differentiation, we need to interpret several kinds of NMR spectra.

We shall start from the interpretation of the ^{13}C spectrum. The three strong peaks at 44.82 ppm, 45.68 ppm, and 53.69 ppm (a triplet) in the high field region of the ^{13}C spectrum can be determined as the peaks of the main composition from their prominent intensities.

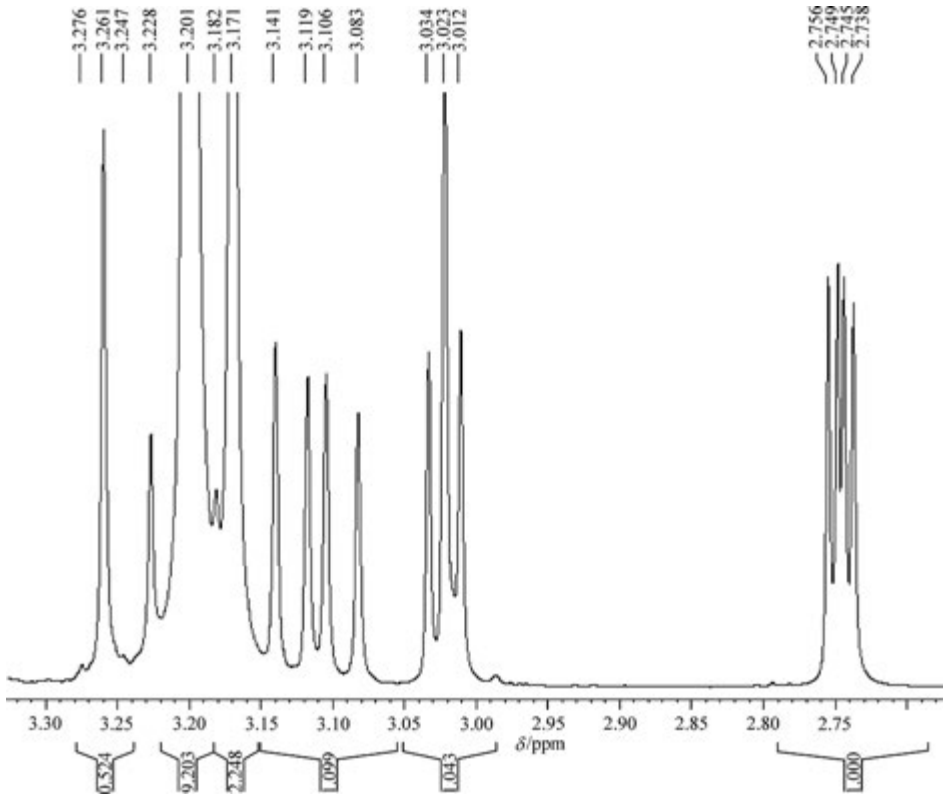


Figure 6.9 The locally enlarged spectrum of Figure 6.8 in the high field region

Because this ^{13}C spectrum is not a quantitative one, the height of the peak corresponding to each carbon atom is not the same. However, the numbers of the carbon atoms for the peaks (or peak sets, such as the triplets) can be determined if we interpret the ^{13}C spectrum with the help of the HSQC spectrum. For example, from the correlation of δ_C (53.69)/δ_H (3.20) in the HSQC spectrum, it can be known that the triplet at 53.69 ppm corresponds to three carbon atoms (three methyl groups), because the singlet at 3.20 ppm has an integral value of 9.2.

It is difficult to determine the peaks in the low field region of the ^{13}C spectrum as those of the major composition or those of the impurities at this time. However, this differentiation can be done by a comprehensive interpretation of the DEPT spectrum, the HSQC spectrum, and the ^{1}H spectrum.

We now take the triplet at 68.67 ppm (at the left edge) in the ^{13}C spectrum as an example. From the heights of the triplet, which has the fourth intensity in the ^{13}C spectrum, we can guess that the triplet belongs to the major composition. However, it is not reliable.

From the DEPT spectrum it can be known that this triplet belongs to a CH_2 group. And two correlated peaks at 68.67 ppm on the F_1 axis in the HSQC spectrum further manifest that the triplet belongs to a CH_2 group, and that these two hydrogen atoms are not chemically equivalent. Since the areas of the two peak sets are equal to 2, it is certain that they belong to

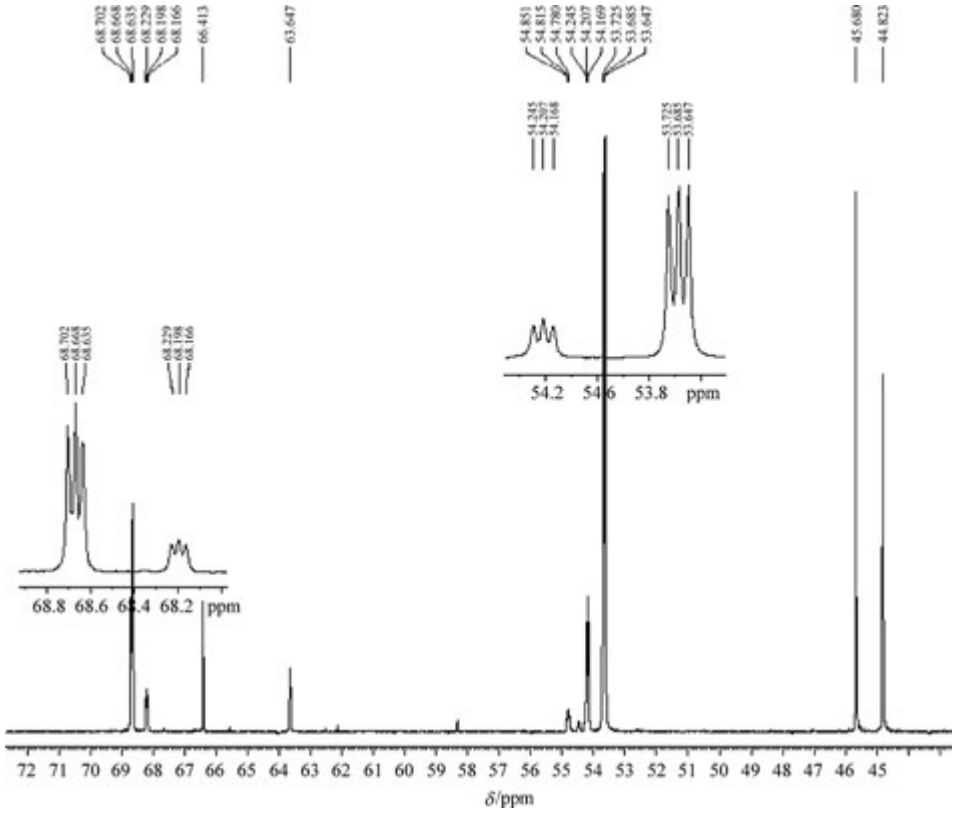


Figure 6.10 The ^{13}C spectrum of compound **C6-2**

the major composition. The correlated peaks between these two peak sets and other peak sets of the major composition in the ^{1}H spectrum further illustrate that they belong to the major composition.

By using the method mentioned above, related data can be summarized in Table 6.3 by the combination of the ^{13}C spectrum, the DEPT spectrum, the HSQC spectrum, and the ^{1}H spectrum. The related data include the carbon atom number of the peaks of the major composition and the integral values of the peak sets in the ^{1}H spectrum. The integral value which corresponds to one hydrogen atom of the major composition is set as 1.

There are some tiny peaks in the ^{13}C spectrum. They are not listed in Table 6.3, because they belong to the impurities with very small quantities.

We supplement some details for Table 6.3.

There are a prominent doublet and a series of multiple peaks in the region of 3.51–3.58 ppm, whose integral value is marked in Table 6.3 as 1.49. From their peak areas, it is difficult to know which belongs to the major composition? The solution can be obtained by using the HSQC spectrum. In the HSQC spectrum, the doublet on the F_2 axis correlates with the peak at 63.65 ppm in the F_1 axis. From the height of the peak at 63.6 ppm,

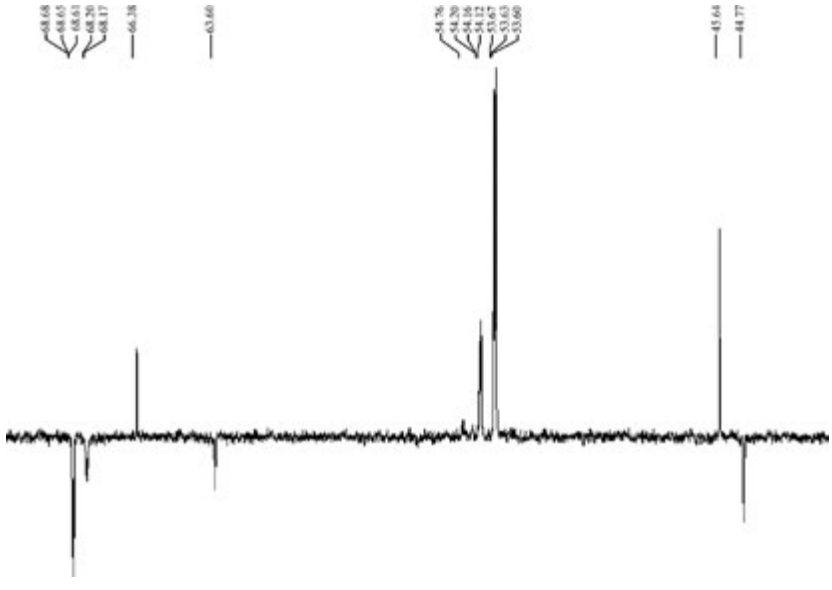


Figure 6.11 The DEPT-135 spectrum of compound **C6-2**

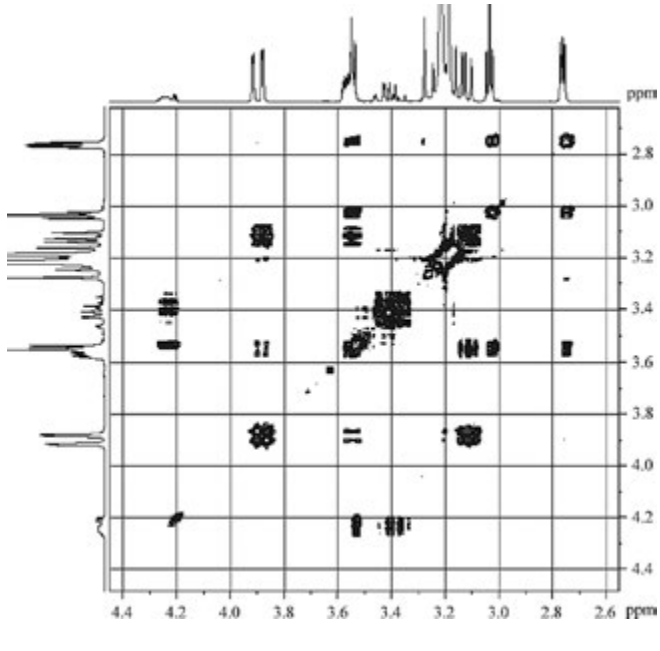


Figure 6.12 The COSY spectrum of compound **C6-2**

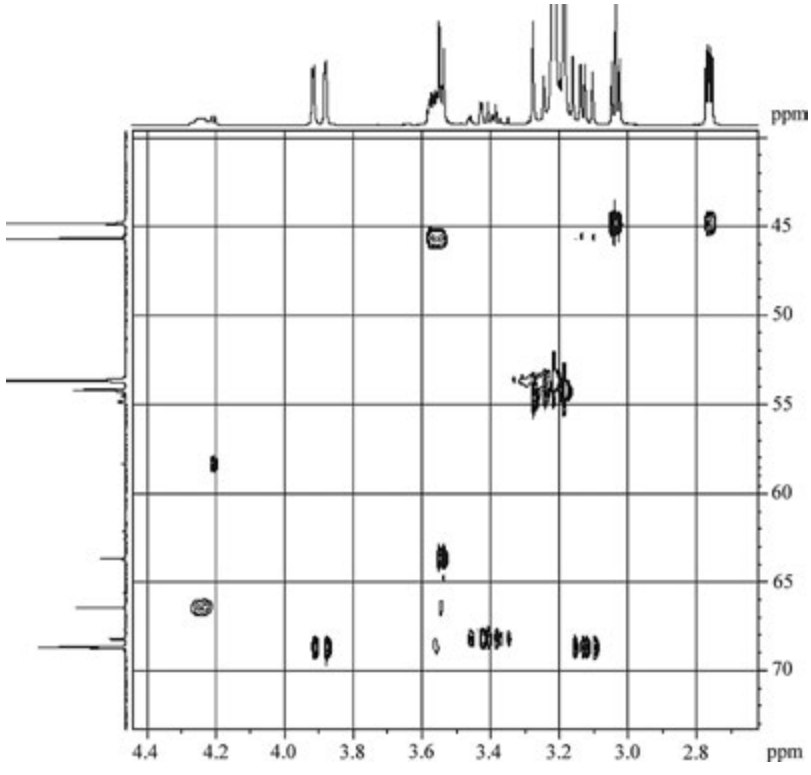


Figure 6.13 The HSQC spectrum of compound **C6-2**

Table 6.3 The related data summarized from the ^{13}C spectrum, the DEPT spectrum, the HSQC spectrum, and the ^{1}H spectrum

δ_C (ppm)	Order of C atoms	δ_H (ppm)	Integral value of peak sets in the ^{1}H spectrum	Number of C atoms	Remarks
44.82	CH_2	2.75, 3.02	1, 1	1	
45.68	CH	3.55	One part of 1.49	1	
53.69	CH_3	3.20	9.20	3	A triplet in the ^{13}C spectrum
54.21	CH_3	3.17	2.25		A triplet in the ^{13}C spectrum
63.65	CH_2	3.53	One part of 1.49		
66.41	CH	4.23	0.22		
68.19	CH_2	3.40	0.65		A triplet in the ^{13}C spectrum
68.67	CH_2	3.11, 3.88	1, 1	1	A triplet in the ^{13}C spectrum

Table 6.4 The data of the major composition summarized from the ^{13}C spectrum and the ^{1}H spectrum

δ_C (ppm)	Number of C atoms	Order of C atoms	δ_H (ppm)	Peak shape in the ^{1}H spectrum	Remarks
44.82	1	CH_2	2.75, 3.02	d,d	
45.68	1	CH	3.55	t	
53.69	3	CH_3	3.20	s	A triplet in the ^{13}C spectrum
68.67	1	CH_2	3.11, 3.88	d,d; d,d	A triplet in the ^{13}C spectrum

we know that the peak belongs to the impurities. On the other hand, the multiple peaks in the F_2 axis correlate with the peak at 45.68 ppm in the F_1 axis, from whose intensity it can be ascribed to the major composition. The correlation peaks of the multiple peaks in the COSY spectrum further prove the conclusion.

On the basis of the discussion above, the peaks in the ^{13}C spectrum and the peak sets in the ^{1}H spectrum of the major composition can be selected. We will further interpret them.

The data of the major composition are summarized in Table 6.4.

Now we analyze the COSY spectrum. Our attention is focused on the major composition. Related data are summarized in Table 6.5.

The "^{2}J coupling" marked in the remarks in Table 6.5 is determined by using the HSQC spectrum.

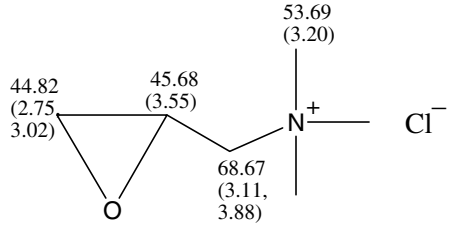
From the peak shape of the two triplets at 53.69 and 68.67 ppm in the ^{13}C spectrum, it can be determined that the two triplets correspond to the two carbon atoms, which connect directly with the nitrogen atom. In the HSQC spectrum, the triplet at 53.69 ppm on F_1 axis correlates with the singlet at 3.20 ppm on F_2 axis, which means the three methyl groups have no coupling with other functional groups containing hydrogen atoms. Therefore, these three methyl groups should be those connected directly with the nitrogen atom. The triplet at 68.67 ppm corresponds to a methylene group, whose two hydrogen atoms are greatly chemically non-equivalent, which coincides with the connection of the CH_2 group with the nitrogen atom.

The coupling relationships: δ_H (ppm) (2.75, 3.02)/3.55/(3.11, 3.88) illustrate the connection of related groups.

Table 6.5 The coupling relationships of the major composition

δ_H (ppm)	Coupled H, δ_H (ppm)	Remarks
2.75	3.02, 3.55	
3.02	2.75, 3.55	The former is ^{2}J coupling
3.11	3.55, 3.88	
3.20	isolated	
3.55	2.75, 3.02, 3.11, 3.88	
3.88	3.11, 3.55	The former is ^{2}J coupling

To sum up, it can be concluded that the anticipated structure coincides with all NMR spectra. Therefore, the anticipated structure is correct. And reasonable assignments can be shown as follows:



Now we give some explanation of the ^{1}H spectrum about the major composition. Because the molecule has no symmetrical plane, the two hydrogen atoms of the two CH_2 groups in the molecule are not chemically equivalent. As a result, they will show the coupling from their ^2J s. Because the value of ^2J is greater than that of ^3J, the coupling splits from the ^2J are evident. We take the peak sets at 3.11 and 3.88 ppm as examples. There is the same spaced distance in these two peak sets: 3.901–3.866 and 3.141–3.106. Both of them correspond to 14 Hz, which is the value of ^2J. The spaced distance of 3.901–3.897, which corresponds to 1.6 Hz, is the value of the related ^3J. The spaced distance of 3.141–3.119, which corresponds to 8.8 Hz, is the value of another ^3J. It should be known that the two hydrogen atoms have different ^3Js even if they have the same vicinal hydrogen atom.

Other peak sets can be interpreted similarly.

Now we interpret the spectra of the impurities by the similar method.

The data of the impurities are taken from Table 6.3 and then they form Table 6.6.

We have following considerations about the tabulation of Table 6.6.

1. According to the DEPT spectrum, the order of carbon atoms of the impurities can be determined.
2. Because of the co-existence of the major composition and the impurities, and because of the overlapping of peak sets in the ^{1}H spectrum, the integral values of peak sets of the impurities in the ^{1}H spectrum may not be accurate. However, the numbers of carbon atoms are correct.
3. There are a doublet and a series of multiple peaks in the region of 4.19–4.26 ppm. The doublet can be ascribed to the main impurity by using the HSQC spectrum. The series of multiple peaks should be ascribed to another impurity, whose quantity is smaller than

Table 6.6 The data of the impurities summarized from Table 6.3

δ_C (ppm)	Order of C atoms	δ_H (ppm)	Integral value of peak sets in the ^{1}H spectrum	Number of C	Peak shape in the ^{1}H spectrum
54.21	CH_3	3.17	2.25	3	s
63.65	CH_2	3.53	One part of 1.49	1	d
66.41	CH	4.23	0.22	1	m
68.19	CH_2	3.40[a]	0.65	1	m

[a] There are many peaks in the region near 3.40 ppm.

Table 6.7 The coupling relationships of the main impurity

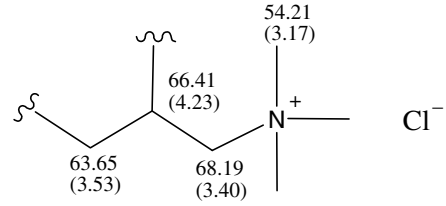
δ_H (ppm)	Coupled H, δ_H (ppm)
3.17	isolated
3.40	4.23
3.53	4.23
4.23	3.40, 3.53

that of the maim impurity. The correlated peaks in the COSY spectrum provide an additional proof.

We will interpret the correlations of the main impurity in the COSY spectrum. The related data are listed in Table 6.7.

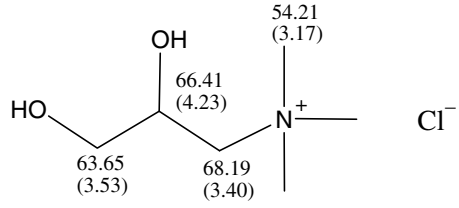
The two triplets at 54.21 and 68.19 ppm in the ^{13}C spectrum manifest that they correspond to the two groups of carbon atoms of the main impurity, which connect directly with the nitrogen atom. From the DEPT spectrum, they can be determined as the peaks of CH_3 and CH_2 group, respectively. From the COSY spectrum, related connections can be found.

To sum up, we get the (partial) structure of the main impurity which follows:



From the chemical shift values mentioned above ($\delta_C > 63.6$ ppm, $\delta_H > 3.53$ ppm), the existence of hydroxyl groups can be postulated. Because the solvent to be used in the measurement is D_2O, the peaks of the hydroxyl groups merge in that of D_2O. Therefore, there are no signals of the hydroxyl groups in the ^1H spectrum.

Finally, we obtain the structure and the assignments of the main impurity which follow:



C6-3

Because these two compounds have simple structures, the HMBC spectrum is not used for the structural deduction and for the assignments.

Example 6.3 The molecular weight of an unknown compound is 187. Its ^1H spectrum, its ^1H spectrum measured after the exchange with D_2O, its ^{13}C spectrum, its DEPT-135

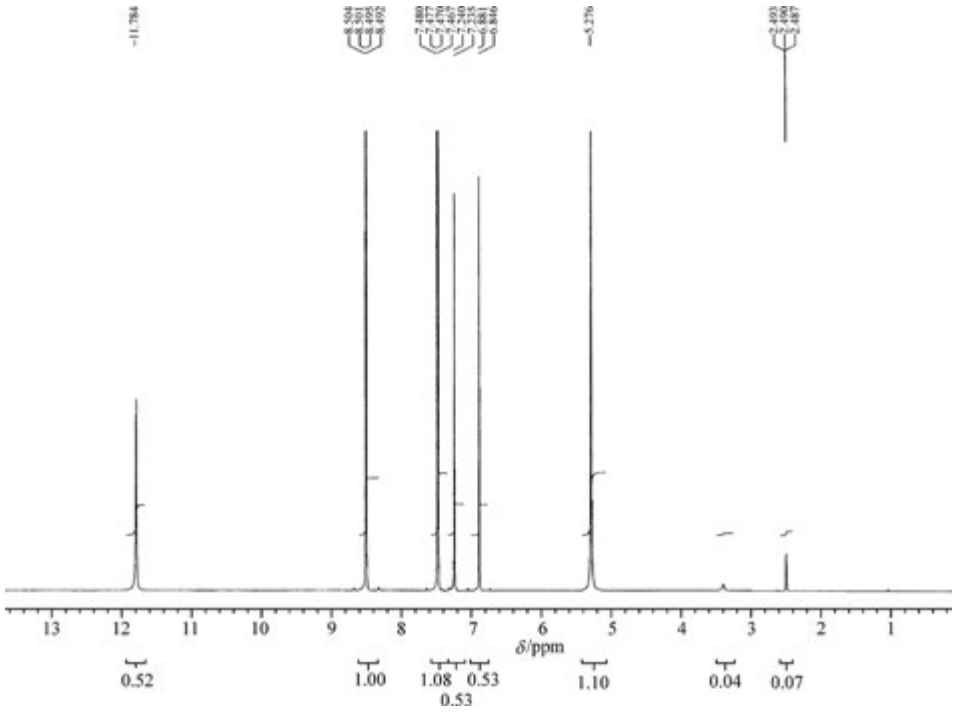


Figure 6.14 The ^{1}H spectrum of the unknown compound

spectrum, its COSY spectrum, its HSQC spectrum, and its HMBC spectrum are shown in Figures 6.14–6.20, respectively. Try to deduce its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated DMSO.

Solution

First we will determine its molecular formula, then its structural units, and finally we assemble its structural units to obtain its structure.

As usual, we start from the interpretation of its ^{1}H spectrum. The peak set at 2.49 ppm is the solvent peak. The peak at 3.2 ppm is the water peak, when deuterated DMSO is used as the solvent. Three peaks at 6.88, 7.24, and 11.78 ppm all have their integral value of 0.5. The hydrogen atom number of every peak of these three peaks should be 1. Similarly, other three peaks all have their integral value of 1. The hydrogen atom number of every peak of the other three peaks should be 2. Therefore, the total hydrogen atom number of the unknown compound is 9.

Figure 6.15 is the ^{1}H spectrum of the unknown compound measured after the exanchge with D_2O. The singlet at 11.78 ppm and the singlet at 5.28 ppm in the Figure 6.14 almost disappear in Figure 6.15. Therefore, these two singlets should be those of reactive hydrogen atoms.

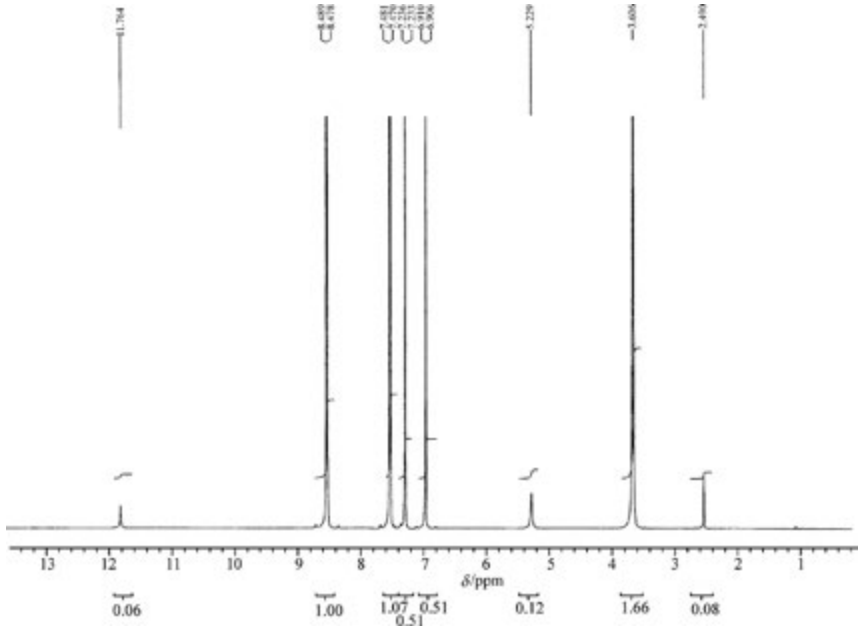


Figure 6.15 The ^{1}H spectrum of the unknown compound measured after the exchange with D_2O

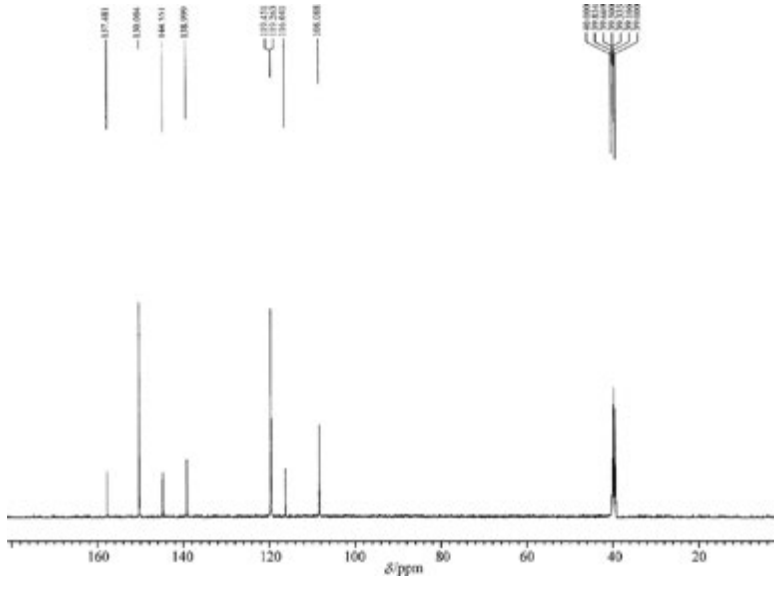


Figure 6.16 The ^{13}C spectrum of the unknown compound

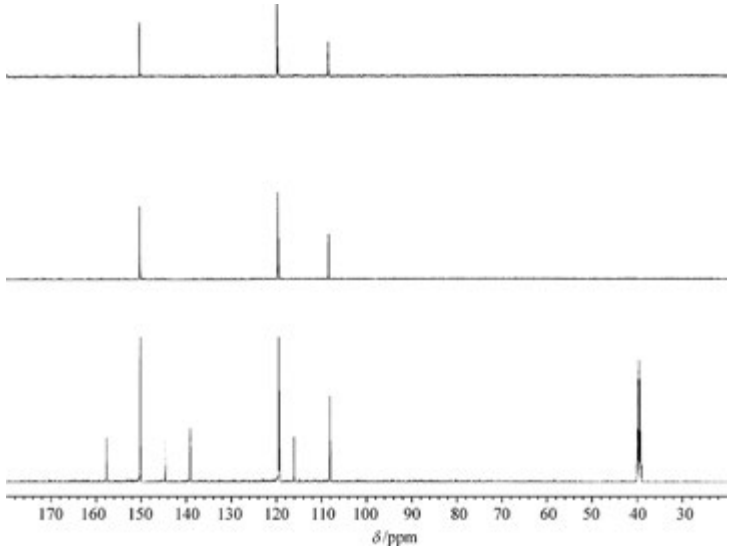


Figure 6.17 The DEPT-135 spectrum of the unknown compound

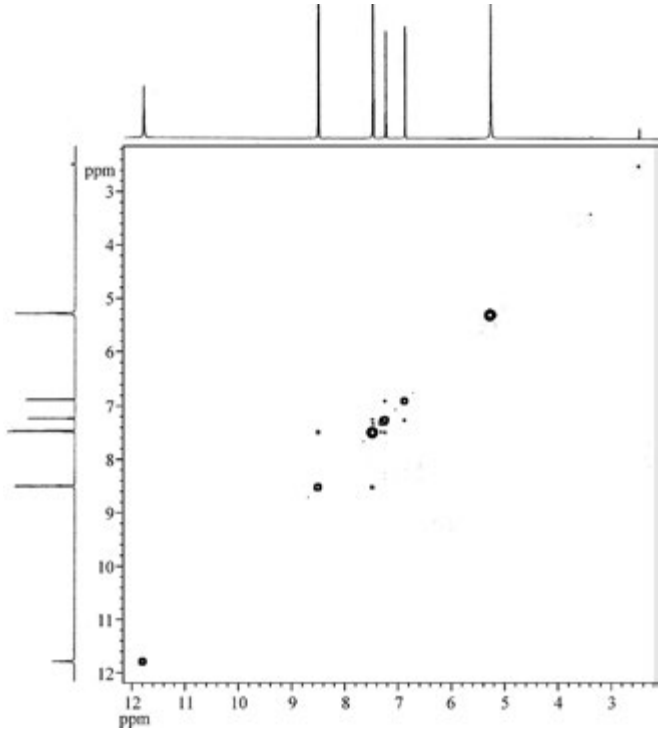


Figure 6.18 The COSY spectrum of the unknown compound

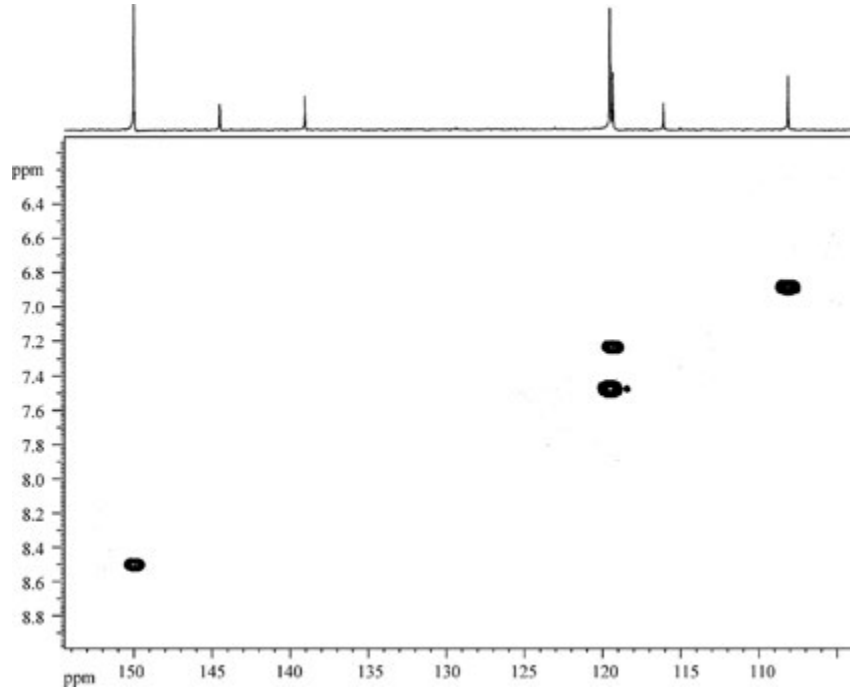


Figure 6.19 The HSQC spectrum of the unknown compound

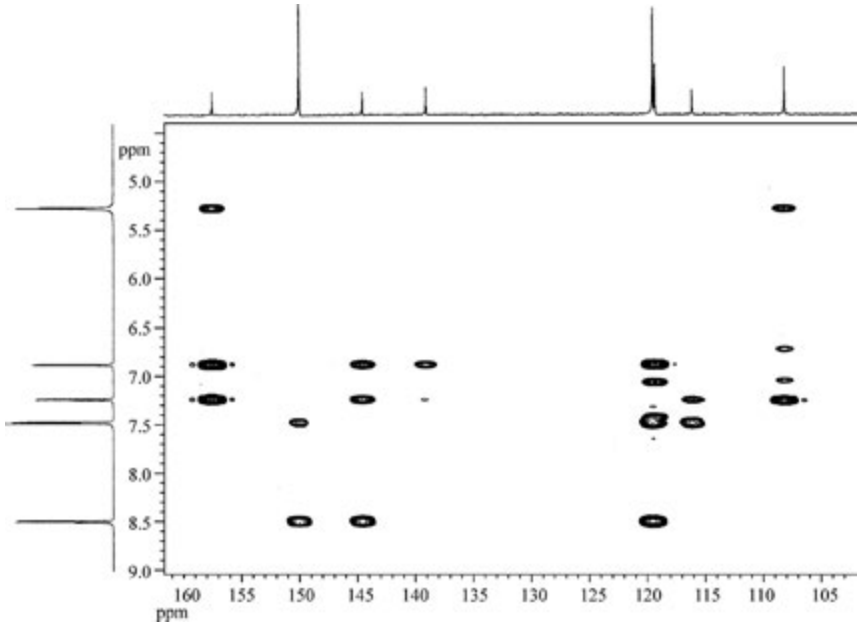


Figure 6.20 The HMBC spectrum of the unknown compound

Table 6.8 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Hydrogen atom number	Peak shape	Coupling constant (Hz)
5.28	2	s	
6.88	1	d	2.5
7.24	1	d	2.5
7.47	2	d, d	5.0, 1.5
8.50	2	d, d	4.5, 1.5
11.78	1	s	

The data summarized from the ^{1}H spectrum are listed in Table 6.8.

The value of the coupling constant, 2.5 Hz, corresponds to a long-range coupling constant. The value of the coupling constant, 5.5 Hz, corresponds to a ^{3}J value affected by a substitution of an electronegative group.

Now we interpret the ^{13}C spectrum. The peaks at 39.5 ppm are the solvent peak. There are eight peaks in the ^{13}C spectrum, of which two peaks have a prominent height. These two peaks should correspond to two carbon atoms, respectively. Therefore, the unknown compound contains 10 carbon atoms. This conclusion can be approved by the HSQC spectrum, because these two peaks correlate two hydrogen atoms in the aromatic region, respectively.

The HSQC spectrum correlates the peaks in the ^{13}C spectrum with the peak sets in the ^{1}H spectrum. The DEPT spectrum determines the order of the carbon atoms.

The data summarized from the ^{13}C spectrum, the DEPT spectrum, the ^{1}H spectrum, and the HSQC spectrum are listed in Table 6.9.

From Table 6.9, we know that the unknown compound contains $C_{10}H_9$, which has the mass of 129 (u). Compared with its molecular weight of 187, the difference of 58 should come from the contribution of heteroatoms. Because the molecular weight is an odd number, the unknown compound should contain an odd number of nitrogen atoms. Therefore, the unique solution for the mass difference of 58 is the addition of the mass of three nitrogen atoms and that of one oxygen atom.

Table 6.9 The data summarized from the ^{13}C spectrum, the DEPT spectrum, the ^{1}H spectrum, and the HSQC spectrum

δ_C (ppm)	C atom number	Order of carbon atom	Directly connected H, δ_H (ppm)	Remarks
108.09	1	CH	6.88	
116.04	1	C		
119.26	1	CH	7.24	
119.45	2	CH	7.47	
138.99	1	C		
144.51	1	C		
150.00	2	CH	8.50	
157.48	1			
			5.28	2 reactive hydrogen atoms
			11.78	1 reactive hydrogen atoms

To sum up, the molecular formula of the unknown compound is $C_{10}H_9ON_3$.

Now we inspect the ^1H spectrum. The singlet at 5.28 ppm has no correlation peak in the HSQC spectrum. Because the singlet corresponds to two hydrogen atoms, it should be an NH_2 group. From the peak shape and δ_H values of the peak sets at 8.50 and 7.47 ppm in the ^1H spectrum, these two peak sets should be those of the remaining four hydrogen atoms of the pyridine ring substituted at 4- position. Because the molecular weight is an odd number, the unknown compound should contain another nitrogen atom.

Because the unknown compound contains only one oxygen atom, the existence of a NO_2 group in the unknown compound is impossible. Therefore, the three nitrogen atoms should have the chemical valence of 3. The unsaturation number of the unknown compound can be calculated as 8.

Either from the ^1H spectrum or from the ^{13}C spectrum, it can be known that the unknown compound contains no aliphatic functional groups. Therefore, it contains only aromatic functional groups.

The information obtained from the COSY spectrum is just the correlation: δ_H (ppm) 8.50/7.47 and the weak correlation: δ_H (ppm) 7.24/6.88, which coincides with their coupling constant of 2.5 Hz.

Because the COSY spectrum provides little structural information, the assemblage of structural units depends mainly on the HMBC spectrum.

It should be noticed that Figures 6.19 and 6.20 are not the normal illustration in the inverse mode, in which the abscissa is the chemical shift value of the acquired nuclei (hydrogen nuclei). The abscissa of Figures 6.19 or 6.20 is δ_C, like that in the normal mode. In fact, the abscissa of Figures 6.19 or 6.20 is the F_1 axis. Therefore, these illustrations are obtained after a treatment by the computer. They are not concerned with which mode to use. Similar illustrations will be seen later.

Because the abscissa in Figure 6.20 is the F_1 axis, that is, the δ_C, the correlated peaks, which reflex the ^1J couplings, are the two symmetrical peaks about a peak (or a peak set) on the F_2 axis.

The data summarized from the HMBC spectrum are listed in Table 6.10.

Table 6.10 The data summarized from the HMBC spectrum

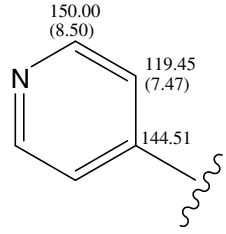
δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)	Remarks
108.09	5.28, 7.24	6.88	
116.04	7.24, 7.47		
119.26	6.88[a]	7.24	
119.45	7.47[a], 8.50		
138.99	6.88		
144.51	6.88, 7.24, 8.50		
150.00	7.47	8.50	Determined from the HSQC spectrum
157.48	5.28, 6.88, 7.24		

[a] They are determined from the enlarged HMBC spectrum, which is not given in this example.

A 4-substituted pyridine ring can be determined from the following facts.

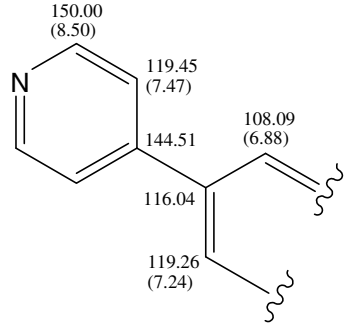
1. The peak sets at 7.47 and 8.50 ppm in the ^{1}H spectrum coincide with those of the remaining four hydrogen atoms of a 4-substituted pyridine ring from chemical shift values or from their peak shapes or from their coupling constants.
2. The correlated relationship, δ_H (ppm) 8.50/7.47, in the COSY spectrum.
3. The long-range couplings of δ_C (144.51)/δ_H (8.50) and δ_C (150.00)/δ_H (7.47) ppm in the HMBC spectrum.

From the three facts mentioned above, we can obtain the following structural units:



Now we analyze the two peak sets at 6.88 and 7.24 ppm in the ^{1}H spectrum. They have the same split distance of 2.5n Hz. Because these two hydrogen atoms belong to two alkene carbon atoms, their coupling constant is a long-range coupling constant, which should be across four chemical bonds.

According to the following long-range couplings in the HMBC spectrum: δ_C (116.04)/δ_H (7.47), δ_C (116.04)/δ_H (7.24), δ_C (119.26)/δ_H (6.88), and δ_C (108.09)/δ_H (7.24) ppm, the structural unit can be extended as follows:

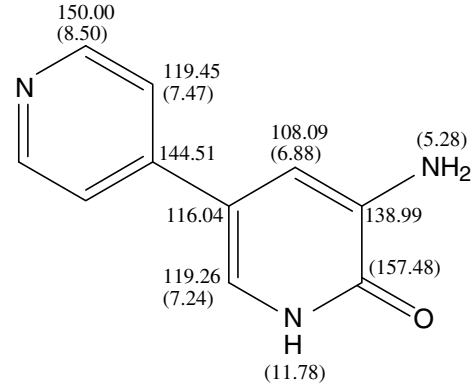


Because the unknown compound contains only one oxygen atom, the singlet at 11.78 ppm in the ^{1}H spectrum should not correspond to one carboxyl group. Therefore, the singlet may belong to an amide group. The peak at 157.5 ppm in the ^{13}C spectrum coincides with an amide group. To sum up, the unknown compound contains an amide group.

Since the unknown compound has an unsaturation number of 8, after subtracting the unsaturation of 4 (a pyrindine ring), 1 (an amide group), and 2 (two sets of double bond) from it, there remains an unsaturation number of 1, so that the unknown compound should contain one ring.

According to the long-range couplings in the HMBC spectrum of $\delta_C(157.48)/\delta_H(6.88)$ ppm and $\delta_C(157.48)/\delta_H(7.24)$ ppm, the position of the amide group can be determined. According to the long-range couplings in the HMBC spectrum of $\delta_C(157.48)/\delta_H(5.28)$ and $\delta_C(108.09)/\delta_H(5.28)$ ppm, the position of the amino group can be determined. According to the long-range coupling in the HMBC spectrum of $\delta_C(138.99)/\delta_H(6.88)$ ppm, the chemical shift value of the last alkene carbon atom can be determined.

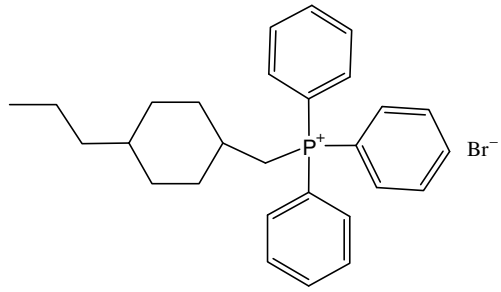
To sum up, we have accomplished the assemblage of the structure of the unknown compound and the assignment of the ^1H spectrum and the ^{13}C spectrum.



C6-4

We would like to supplement the following fact. Because the carbonyl group in the amide group is situated in a conjugated system, its chemical shift value is smaller than that of an isolated amide group.

Example 6.4 A synthesized compound has the following anticipated structure:



C6-5

Its ^1H spectrum, ^{13}C spectrum, DET-135 spectrum, COSY spectrum (the locally enlarged one in the high field region), HSQC spectrum, HMBC spectrum, and two locally enlarged HMBC spectra are shown in Figures 6.21–6.29. Try to confirm the structure and to assign the ^1H spectrum and the ^{13}C spectrum. All NMR spectra were measured by an NMR spectrometer with a frequency of 400 MHz. The solvent used is $CDCl_3$.

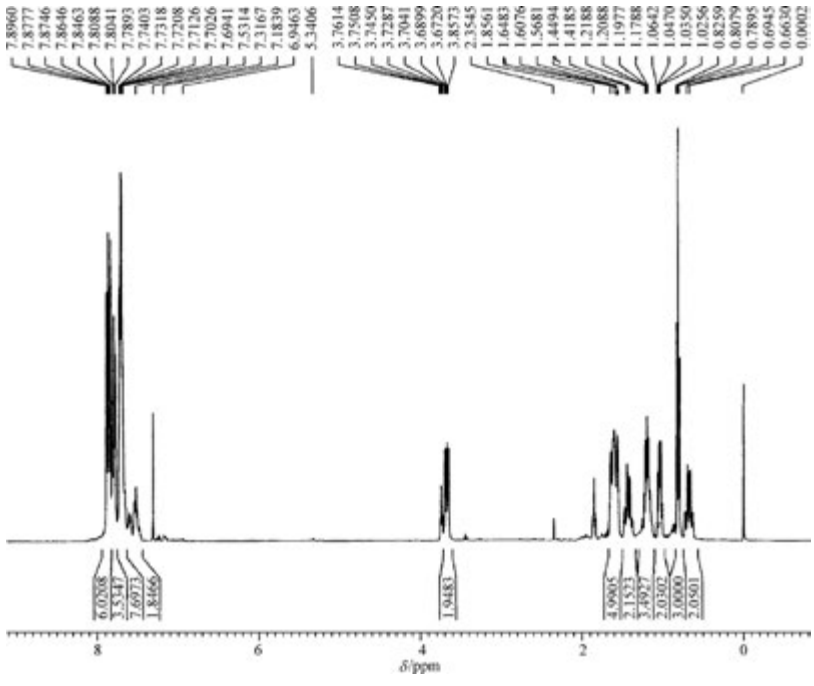


Figure 6.21 The ^{1}H spectrum of compound **C6-5**

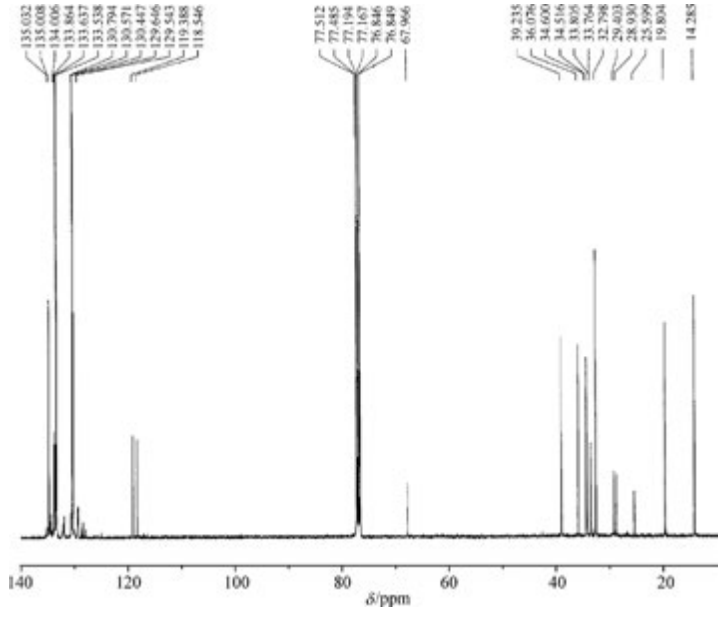


Figure 6.22 The ^{13}C spectrum of compound **C6-5**

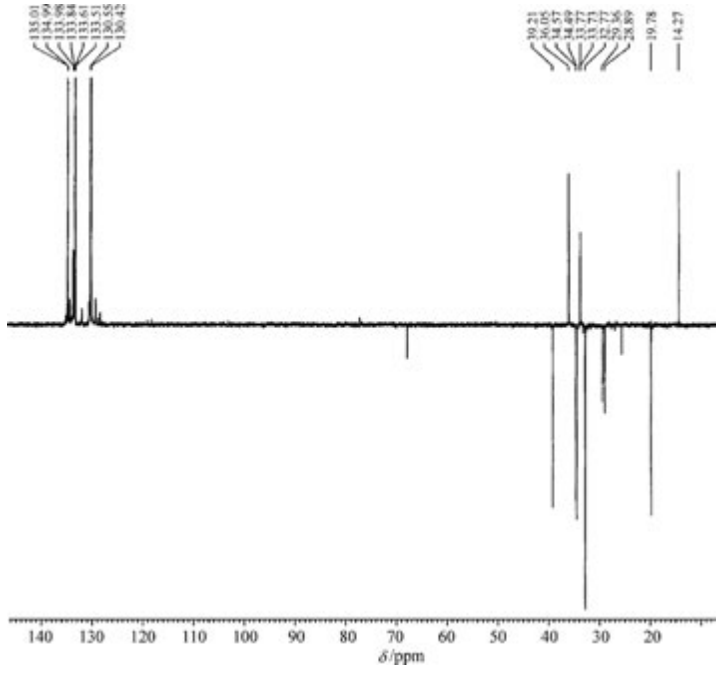


Figure 6.23 The DEPT-135 spectrum of compound **C6-5**

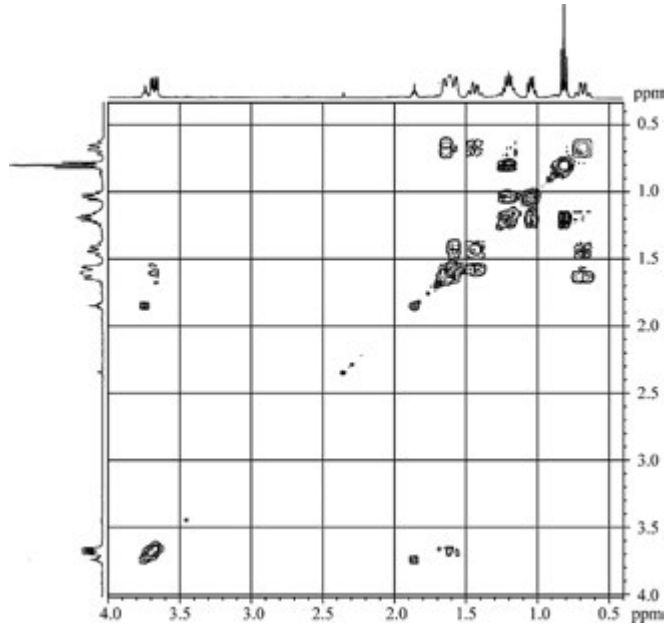


Figure 6.24 The COSY spectrum of compound **C6-5** (locally enlarged in the high field region)

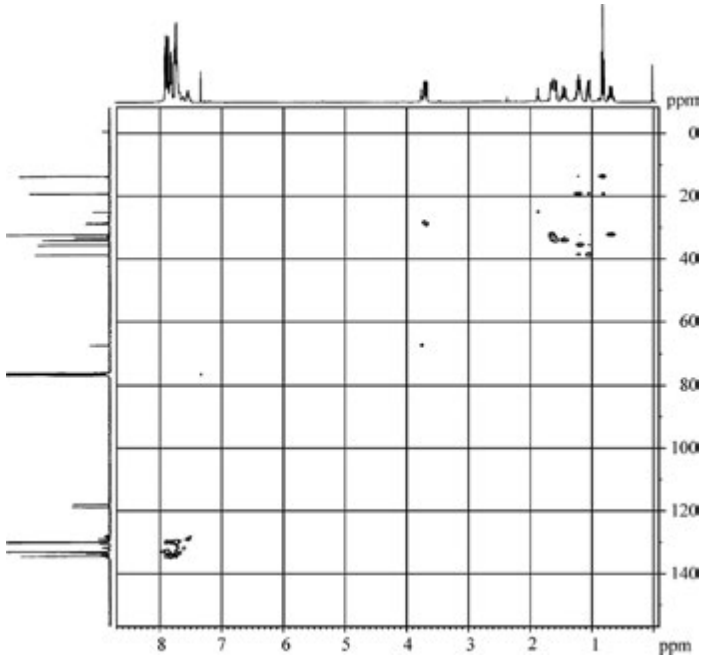


Figure 6.25 The HSQC spectrum of compound **C6-5**

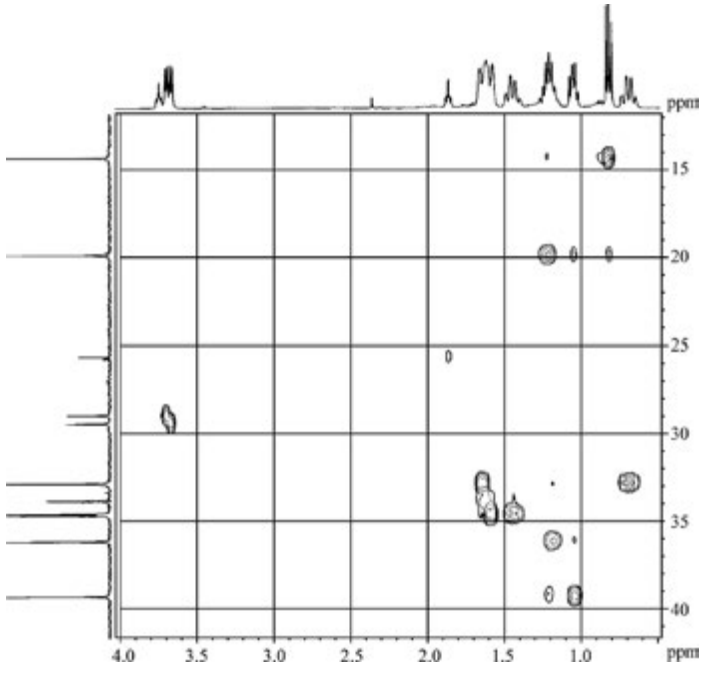


Figure 6.26 The locally enlarged HSQC spectrum of compound **C6-5**

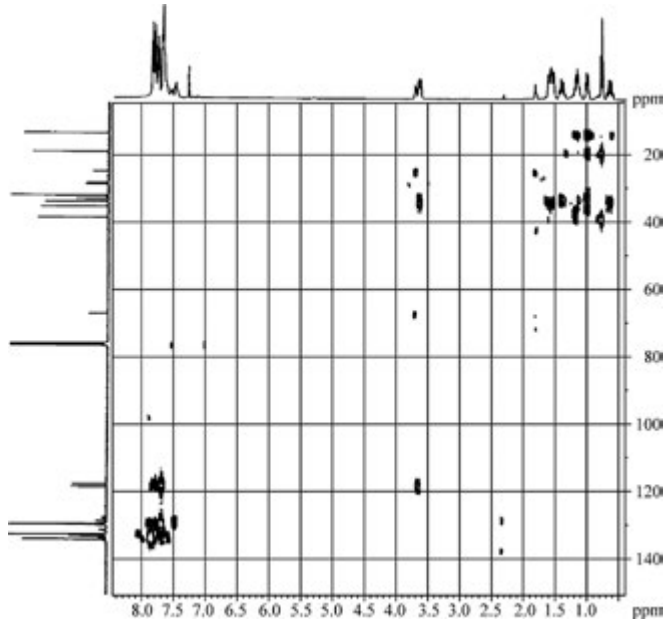


Figure 6.27 The HMBC spectrum of compound **C6-5**

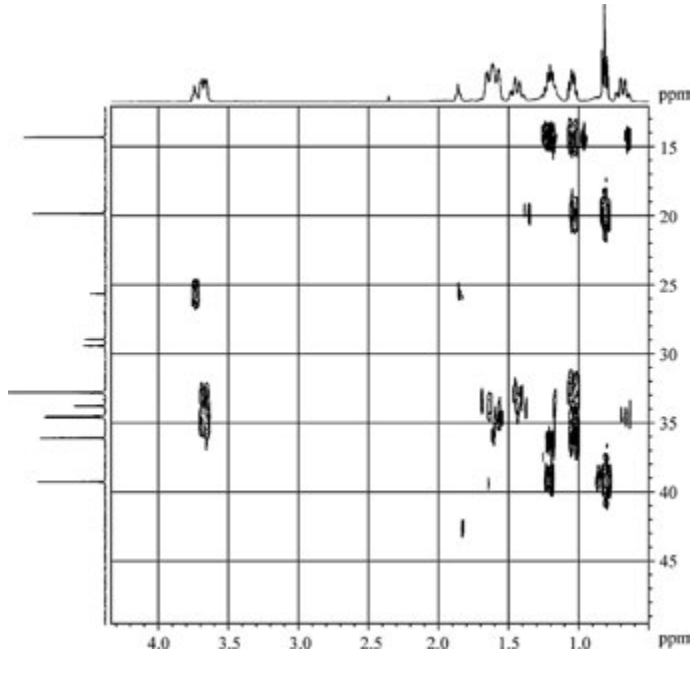


Figure 6.28 The locally enlarged HMBC spectrum (1) of compound **C6-5**

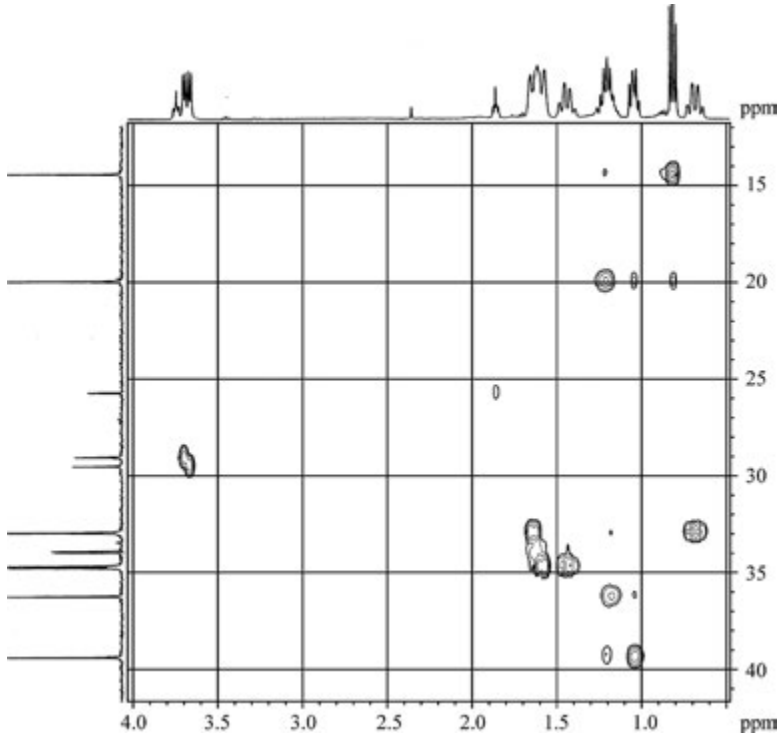


Figure 6.29 The locally enlarged HMBC spectrum (2) of compound **C6-5**

Solution

We now start from the interpretation of the ^{1}H spectrum.

There are small peaks at 1.86, 2.35, 3.76, 6.95, 7.18, 7.53, and 7.58 ppm. From their small peak areas, which are not comparable to those of the sample, they can be determined as impurity peaks.

The peak at 7.31 ppm is the solvent peak.

The integral values of some peaks (or peak sets) are not close to integers. In this case, the hydrogen atom numbers can be determined with the help of the HSQC spectrum.

The data summarized from the ^{1}H spectrum and the HSQC spectrum are listed in Table 6.11.

When we interpret the ^{13}C spectrum, it should be noted that related peaks in the ^{13}C spectrum will be split by the phosphorus atom, which consists of the unique isotope ^{31}P with the spin quantum number of 1/2. The coupling constants between a phosphorus atom and a carbon atom are about 40–150 Hz for ^{1}J, 5–10 Hz for ^{2}J, and smaller values for long range couplings. Because the decouplings are carried out only for hydrogen atoms, the coupling splits by phosphorus atoms can not be removed by the decoupling. Phosphorus atoms also produce the splits for related peak sets in the ^{1}H spectrum. However, because peak sets in the ^{1}H spectrum have complicated shapes in general, the splits produced by phosphorus atoms may not be obvious. But the splits produced by phosphorus atoms in the ^{13}C spectrum will be obvious, because peaks in the ^{13}C spectrum are not splits.

Table 6.11 The data summarized from the ^{1}H spectrum and the HSQC spectrum

δ_H (ppm)	Integral value	Hydrogen atom number	Peak shape	Remarks
0.68	2.05	2	q	
0.81	3.00	3	t	
1.04	2.02	2	m	
~1.21	3.49	4	m	From the HSQC spectrum we know that this region corresponds to two CH_2 groups.
1.43	2.15	2	m	
~1.61	4.99	5	m	From the HSQC spectrum we know that this region corresponds to 2 CH_2 and 1 CH.
3.69	1.95	2	m	
7.72	7.69	6	m	Peak shape is not clear.
7.80	3.53	3	m	Peak shape is not clear.
7.87	6.02	6	m	Peak shape is not clear.

The reader may have the following question: how can we know whether two adjacent peaks in the ^{13}C spectrum belong to two carbon atoms or just to one carbon atom whose peak is split by a heteroatom? If that carbon atom connects with hydrogen atoms, the answer can be obtained by using the ^{1}H spectrum, the DEPT spectrum, and the HSQC spectrum. If that carbon atom does not connect with hydrogen atoms, it should be considered from several aspects.

Although the peak at 67.96 ppm has a certain height, it is a peak of an impurity, because its correlated peak set in the ^{1}H spectrum has a small area. Similarly, the peak at 25.59 ppm can be determined as a peak of an impurity.

The data summarized from the ^{1}H spectrum, the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum are listed in Table 6.12.

Table 6.12 The data summarized from the ^{1}H spectrum, the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum

No.	δ_C (ppm)	Number of carbon atoms	Order of carbon atom	Directly connected hydrogen atoms, δ_H (ppm)
1	135.03, 135.01	1	CH	7.80
2	133.64, 133.54	2	CH	7.87
3	130.57, 130.45	2	CH	7.72
4	119.39, 118.55	1	C	
5	39.24	1	CH_2	1.04
6	36.08	1	CH	1.20
7	34.60, 34.52	2	CH_2	1.59, 1.43
8	33.81, 33.76	1	CH	1.61
9	32.79	2	CH_2	1.63, 0.68
10	29.40, 28.93	1	CH_2	3.69
11	19.80	1	CH_2	1.22
12	14.29	1	CH_3	0.81

Table 6.13 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)
1	3.69	1.61*
2	1.63	0.68
3	1.61*	3.69
4	1.59	1.43
5	1.43	1.59, 0.68
6	1.21*	1.04, 0.81, (0.68)
7	1.04	1.21*
8	0.81	1.21*
9	0.68	1.63, 1.43, (1.21*)

Notes:
1. The correlated peaks in the aromatic region are not listed in the table.
2. The chemical shift values marked "*" mean that the values are situated in a narrow region, for example, 1.21* ppm covers 1.20 and 1.22 ppm.
3. The parenthesis () represents weak couplings.

From correlated peaks in the HSQC spectrum, the overlapped peak sets in the ^{1}H spectrum can be resolved. For example, the correlated peak corresponding to the peak at $F_1 = 36.08$ ppm has a slightly smaller δ_H than that of the correlated peak corresponding to the peak at $F_1 = 19.80$ ppm. The information is manifested in Table 6.12. Similarly, from the HSQC spectrum, we can know that three peak sets in the ^{1}H spectrum overlap in the region of $F_2 = 1.57$–1.65 ppm (1.59, 1.61, and 1.63 ppm). Their correlated peaks are situated at $F_1 = 34.56$, 33.78 and 32.79 ppm, respectively.

Now we interpret the COSY spectrum. The overlapping of peak sets in the ^{1}H spectrum leads to the overlapping of correlated peaks in the COSY spectrum. Therefore, it is necessary to interpret correlated peaks in the COSY spectrum with the help of the HMBC spectrum.

The data obtained from the COSY spectrum are summarized in Table 6.13.

Now we interpret the HMBC spectrum. Figure 6.29, that is, the locally enlarged HMBC spectrum of compound **C6-5** (2), has less correlated peaks than Figure 6.28, that is, the locally enlarged HMBC spectrum of compound **C6-5** (1), because the latter is drawn with lower sections. However, Figure 6.29 shows correlated peaks clearly. Therefore, it is better to summarize related long-range heteronuclear correlations by using the three figures (Figures 6.27–6.29).

The data summarized from Figures 6.27–6.29 are listed in Table 6.14.

From Nos. 4, 2, and 1 of Table 6.14, it can be known that the unknown compound contains a mono-substituted benzene ring. Combining the HSQC spectrum, the related δ_C can be denoted.

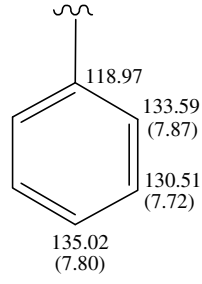


Table 6.14 The data summarized from the HMBC spectra

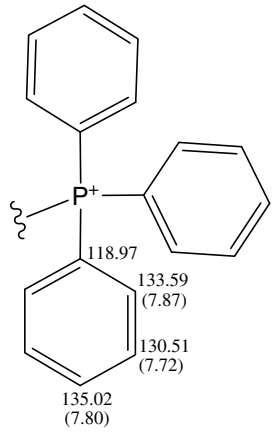
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H ato H atoms with the ^1J coupling, δ_H (ppm)
1	135.03, 135.01	7.87	
2	133.64, 133.54	7.80	
3	130.57, 130.45		7.72
4	119.39, 118.55	7.72, 3.69	
5	39.24	1.21*, 0.81	
6	36.08	1.21*, 1.04	
7	34.60, 34.52	3.69, (0.68)	1.59
8	33.81, 33.76	3.69, 1.61*	
9	32.79	1.04	
10	29.40, 28.93		3.69
11	19.80	1.04, 0.81	
12	14.29	1.22, 1.04	0.81

Notes:
1. The chemical shift values marked "*" mean that the values are situated in a narrow region, for example, 1.21* ppm covers 1.20 and 1.22 ppm.
2. The parenthesis () represents weak couplings.

It should be noticed that δ_C values mentioned above are averaged values from the peaks split by the couplings of the phosphorus atom. For example, 118.97 ppm is the averaged values of 119.39 and 118.55 ppm. This value corresponds to the quaternary carbon atom, which directly connects with the phosphorus atom.

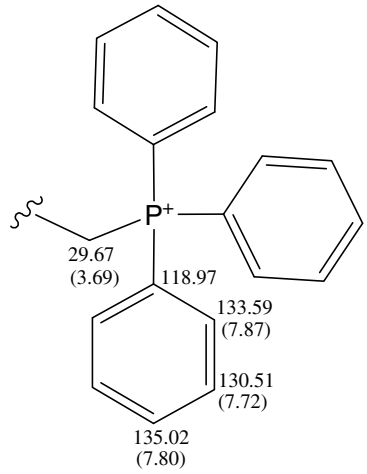
Because the peak sets at 7.87, 7.80, and 7.72 ppm in the ^{1}H spectrum correspond to 6, 3, and 6 hydrogen atoms, respectively, the phosphorus atom should connect with three mono-substituted benzene rings.

To sum up, we get the following structural unit:

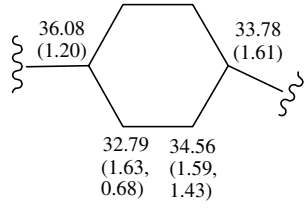


According to the split peaks in the ^{13}C spectrum, the carbon atom, whose chemical shift value is 29.67 ppm (averaged from 29.40 and 28.93 ppm), is the aliphatic carbon atom connected directly with the phosphorus atom. The chemical shift value of 3.69 ppm of the

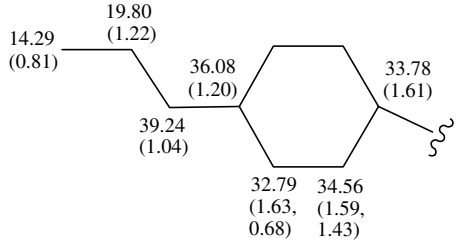
hydrogen atom connected directly with the above-mentioned carbon atom can be determined from the HSQC spectrum. Therefore, the structural unit can be extended as follows:



Either from the ^{13}C spectrum, or from the ^{1}H spectrum, the existence of a six-membered ring can be determined, because two sets of two CH_2 groups are chemically equivalent. From Nos. 2, 4, 5, 6, and 9 of Table 6.13, and from Nos. 6, 7, and 8 of Table 6.14, we can get the following structural unit. Combining the HSQC spectrum, the related δ_C can be denoted.



According to Nos. 8, 7, and 6 of Table 6.13, and according to Nos. 12 and 11 of Table 6.14, the structural unit can be extended as follows:

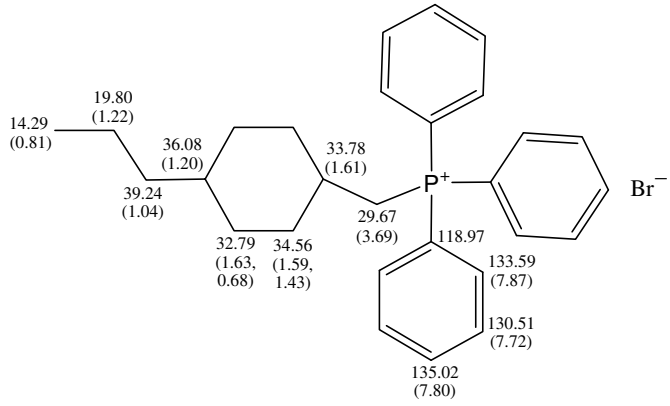


The marked chemical shift values in the above-mentioned structural unit are obtained from the HSQC spectrum.

According to No. 1 of Table 6.13, and according to Nos. 7 and 8 of Table 6.14, the two structural units mentioned above can be connected toghther, which leads to the whole

structure of the compound. Because the phosphorus atom connects with four functional groups, the phosphorus atom forms an ion. Its counter ion is a bromine ion.

To sum up, we have confirmed the structure of the compound with complete assignments. The result is as follows:



C6-5

Example 6.5 The molecular weight of an unknown compound was determined as 331 by its mass spectrum. From the isotopic cluster in its mass spectrum, the existence of one chlorine atom in the unknown compound can be determined. Its ^{1}H spectrum (with $CDCl_3$ as the solvent its), ^{1}H spectrum (after the exchange with D_2O), its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum, its DEPT-135 spectrum, its COSY spectrum, its HSQC spectrum, and its HMBC spectrum are shown in Figures 6.30–6.36, respectively. Try to deduce its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is $CDCl_3$.

Solution

We now start from the interpretation of the ^{1}H spectrum. The peak at 7.26 ppm is the solvent peak.

From the ^{1}H spectrum it can be known that the unknown compound contains 26 hydrogen atoms. Compared with Figure 6.30, three peaks at 9.54, 8.51, and 5.16 ppm, respectively, disappear in Figure 6.31, which is measured after the exchange with D_2O. Therefore, these three peaks should correspond to three reactive hydrogen atoms. In fact, because these three peaks are singlets with a blunt shape, they can be tentatively considered as reactive hydrogen atoms.

Figure 6.30 shows a better resolution so that peak shapes can be clearly seen.

We mainly use the data obtained from Figure 6.30 and consult the peak shapes shown in Figure 6.31 to form Table 6.15.

From Table 6.15, it can be known that the unknown compound contains 26 hydrogen atoms, of which 23 hydrogen atoms connect directly with carbon atoms. This result coincides with that obtained from the DEPT-135 spectrum.

Now we are going to interpret the ^{13}C spectrum.

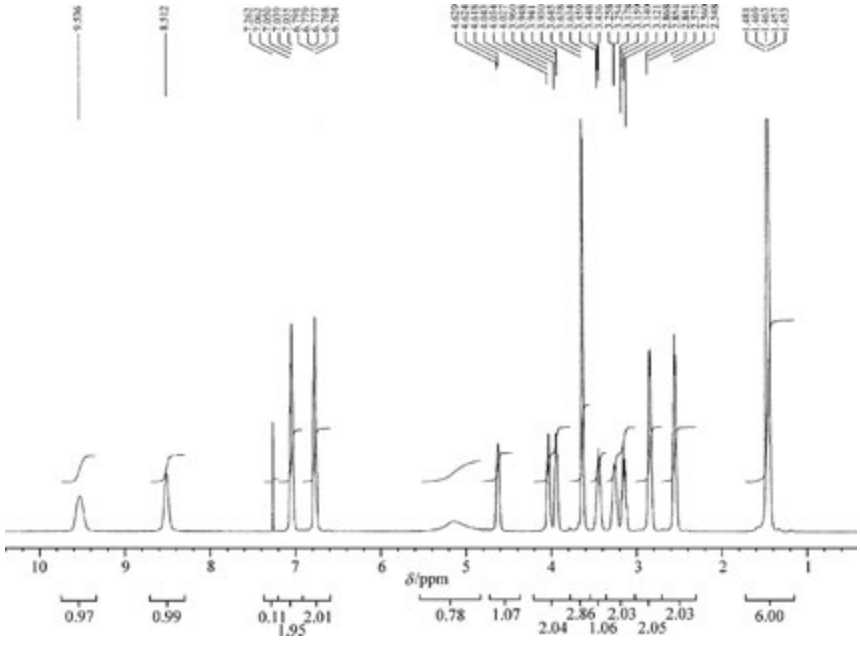


Figure 6.30 The ^{1}H spectrum of the unknown compound (with $CDCl_3$ as the solvent)

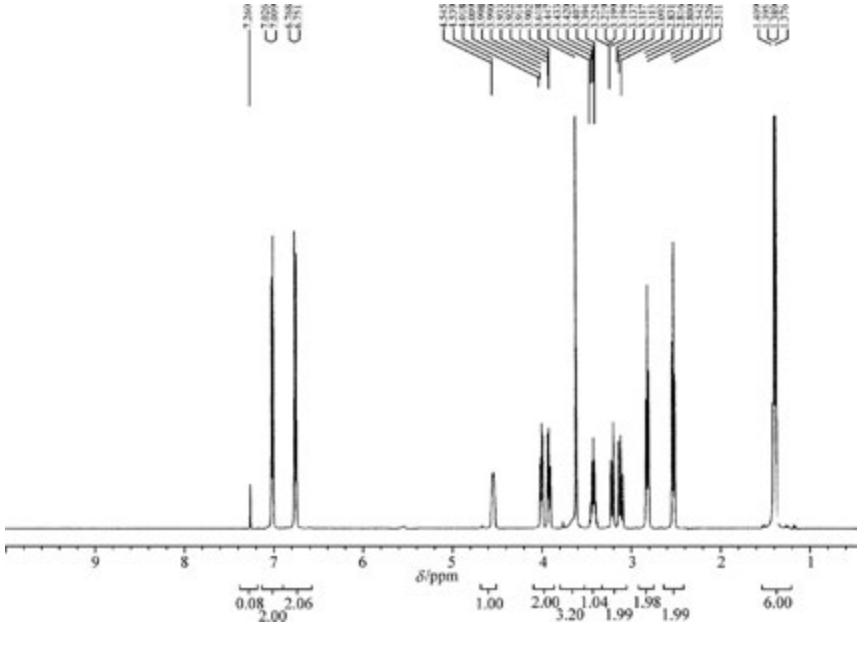


Figure 6.31 The ^{1}H spectrum of the unknown compound (after the exchange with D_2O)

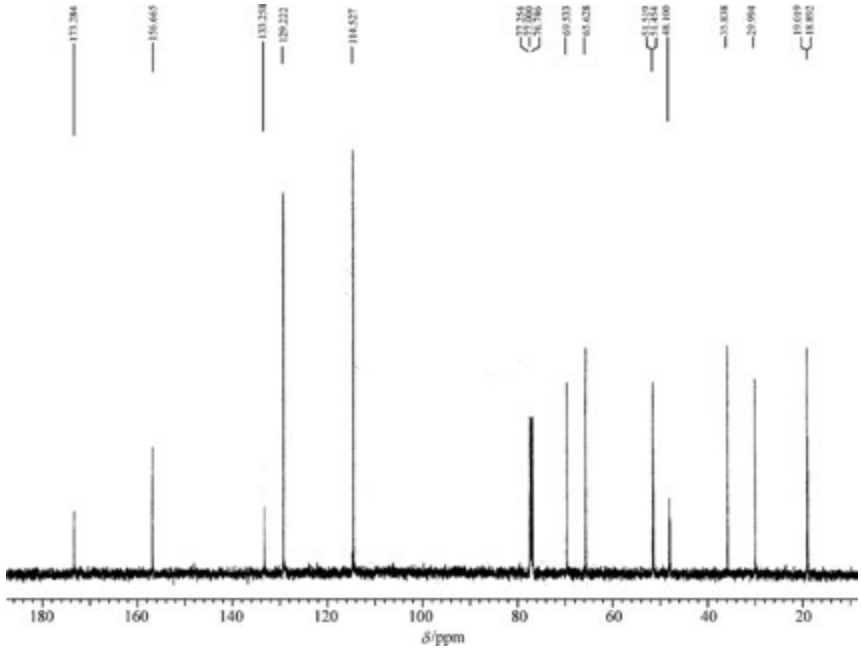


Figure 6.32 The ^{13}C spectrum of the unknown compound

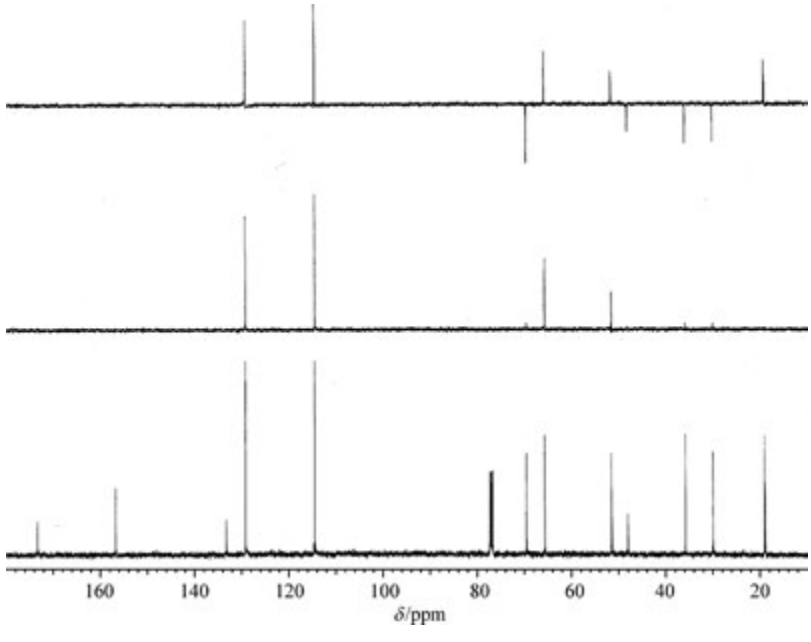


Figure 6.33 The DEPT-135 spectrum of the unknown compound

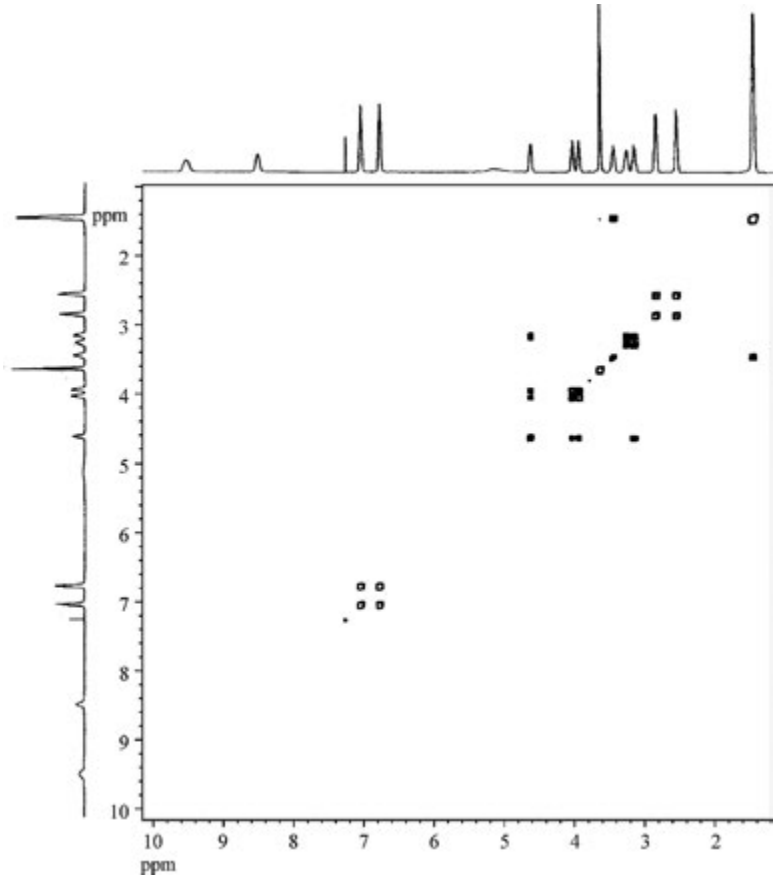


Figure 6.34 The COSY spectrum of the unknown compound

The three peaks at about 77.0 ppm are the solvent peaks ($CDCl_3$). There are 14 peaks in the ^{13}C spectrum, of which two close peaks are situated at about 19.0 ppm, and other two close peaks at about 51.5 ppm. Because the two peaks at 114.53 and 129.22 ppm have prominent heights, they should correspond to two carbon atoms, respectively. To sum up, the unknown compound contains 16 carbon atoms.

Because two sets of peaks in the ^{13}C spectrum are close, the interpretation of the spectrum needs the help of the HSQC spectrum and the DEPT spectrum, in which from the bottom to top the spectra are arranged according to the ^{13}C spectrum, DEPT-90 spectrum, and DEPT-135 spectrum, respectively. The related data are listed in Table 6.16.

According to the δ_C value of 173.24 ppm, this peak can be ascribed as a carbonyl group. Therefore, the unknown compound should contain an oxygen atom.

From the HSQC spectrum it can be known that the two peaks at about 51.5 ppm correspond to a CH_3 and a CH, respectively. Similarly, it can be known that the two peaks at 114.5 and 129.2 ppm correspond to two CH, respectively. To sum up, the unknown compound contains 3 CH_3, 4 CH_2, and 6 CH.

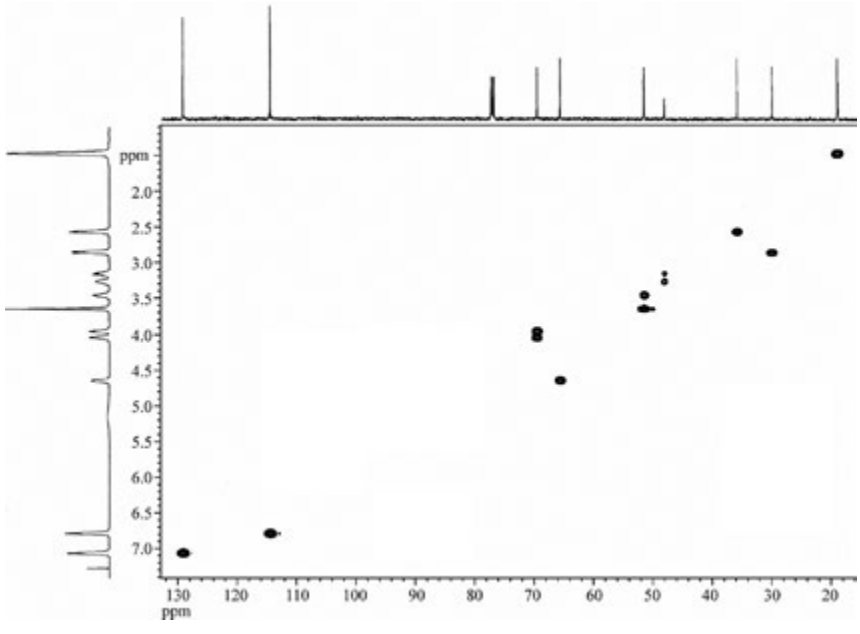


Figure 6.35 The HSQC spectrum of the unknown compound

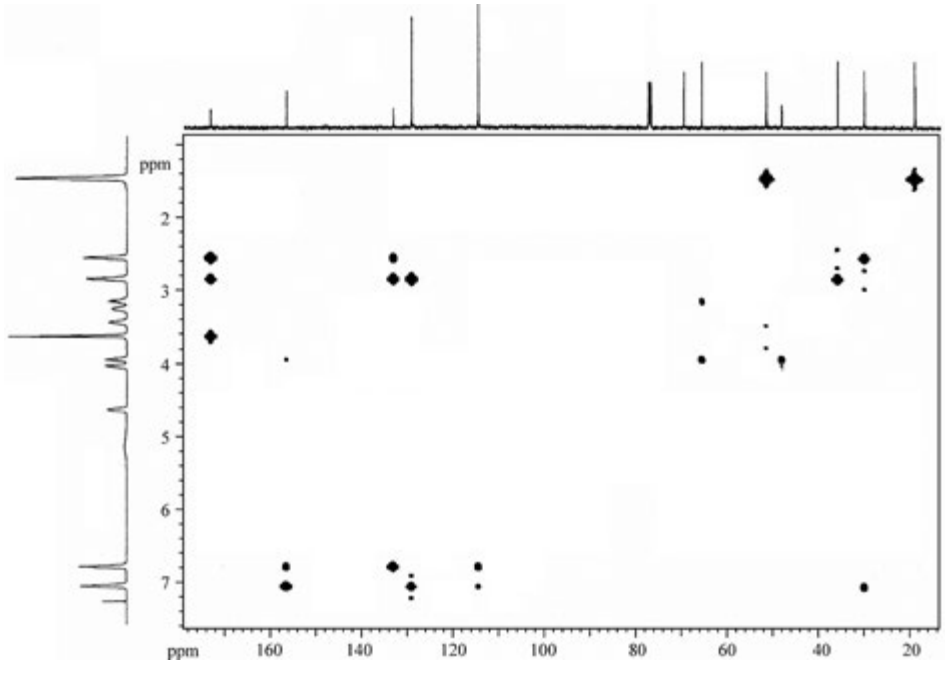


Figure 6.36 The HMBC spectrum of the unknown compound

Table 6.15 The data summarized from the ^{1}H spectra (Figures 6.30 and 6.31)

δ_H (ppm)	Number of H atoms	Peak shapes	Remarks, coupling constant (Hz)
1.46	6	d (two doublets)	Overlapped two doublets, 7.0, 6.5
2.56	2	t	7.5
2.85	2	t	7.5
3.14	1	d, d	
3.25	1	d, d	
3.45	1	m	6.5
3.64	3	s	
3.92	1	d, d	
4.00	1	d, d	
4.62	1	blunt	
5.16	1	s, blunt	Reactive hydrogen atom
6.78	2	d	
7.05	2	d	
8.51	1	s, blunt	Reactive hydrogen atom
9.54	1	s, blunt	Reactive hydrogen atom

Because the molecular weight of the unknown compound is an odd number, it should contain an odd number of nitrogen atoms, at least one nitrogen atom. Now we have found its elemental composition of $C_{16}H_{26}ONCl$, which has a mass of 283 (u). Compared with its molecular weight, the remaining mass is 48 (u), which corresponds to three oxygen atoms. Any other combination of isotopic mass can not coincide with the remaining mass difference of 48 (u). The postulation of the existence of three oxygen atoms coincides with the peaks at 69.5 and 65.6 ppm in the ^{13}C spectrum and with the peak sets at 4.62, 4.00, and 3.92 ppm in the ^{1}H spectrum. To sum up, the unknown compound has its molecular formula of $C_{16}H_{26}O_4NCl$.

Table 6.16 The data summarized from the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum

δ_C (ppm)	Number of C atoms	Order of C atoms	Directly connected H atoms, δ_H (ppm)
18.89	1	CH_3	1.46
19.02	1	CH_3	1.46
29.99	1	CH_2	2.85
35.84	1	CH_2	2.56
48.10	1	CH_2	3.14, 3.25
51.45	1	CH	3.45
51.52	1	CH_3	3.64
65.63	1	CH	
69.53	1	CH_2	3.92, 4.00
114.53	2	CH	6.78
129.22	2	CH	7.05
133.36	1	C	
156.67	1	C	
173.24	1	C	

Table 6.17 The data summarized from the COSY spectrum

δ_H (ppm)	Coupled H, δ_H (ppm)
1.46	3.45
2.56	2.85
2.85	2.56
3.14	3.25, 4.62
3.25	3.14
3.45	1.46
3.64	isolated
3.92	4.00, 4.62
4.00	3.92, 4.62
4.62	3.14, 3.92, 4.00
6.78	7.05
7.05	6.78

We will find its structural units and then combine them to form a structure. Therefore, the COSY spectrum and the HMBC spectrum are needed.

The data summarized from the COSY spectrum are listed in Table 6.17.

Now we interpret the HMBC spectrum. It should be noticed that the abscissa is the δ_C value.

The data summarized from the HMBC spectrum are listed in Table 6.18.

Now we will find its structural units and then assemble them.

If the information obtained from the COSY spectrum is certain, the information should be used first, because it is more reliable than that obtained from the HMBC spectrum. The COSY spectrum shows the correlations with ^3J couplings, that is, the vicinal couplings. By the combination of the information obtained from the COSY spectrum and that from the HSQC spectrum, the connection between two vicinal carbon atoms can be determined.

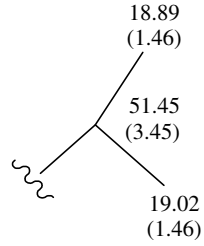
Table 6.18 The data summarized from the HMBC spectrum

δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
173.24	2.56, 2.85, 3.64	
156.67	(3.92), 6.78, 7.05	
133.26	2.56, 2.85, 6.78	
129.22	2.85	7.05
114.53	7.05	6.78[a]
69.53		
65.63	3.14, 3.92	
51.45, 51.52[b]	1.46	3.64
48.10	3.92	
35.84	2.85	2.56
29.99	2.56, 7.05	2.85
18.89, 19.02		1.46[a]

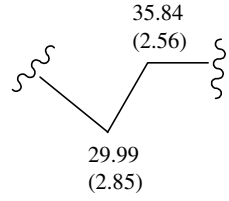
Parenthesis (·) stands for weak couplings.
[a] Indicates that the value is determined by the HSQC spectrum.
[b] There are two close peaks, whose correlated peaks are difficult to be differentiated.

However, the connections will be stopped at quaternary carbon atoms or heteroatoms. In these cases, the connection can only be extended by using the HMBC spectrum.

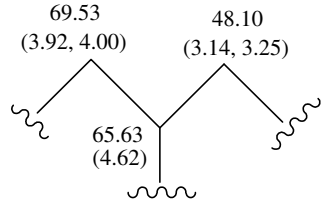
From the correlation between the peak set of six hydrogen atoms at 1.46 ppm and the peak set of one hydrogen atom at 3.45 ppm, the existence of an isopropyl group can be known. By using the HSQC spectrum, the related δ_C can be determined.



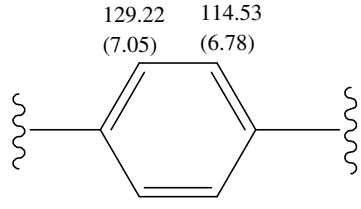
From the correlation between the peak set of two hydrogen atoms at 2.56 ppm and the peak set of two hydrogen atoms at 2.85 ppm, the connection of two CH_2 groups can be determined. By using the HSQC spectrum, the related δ_C can be determined.



From the correlations of δ_H (ppm) 3.14/3.25, 4.62; 3.92/4.00, 4.62; 4.62/3.14, 3.92, 4.00; we can find the following structural unit. By using the HSQC spectrum, the related δ_C can be determined.



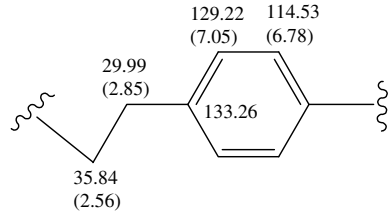
From the correlation of δ_H (ppm) 6.78 (two hydrogen atoms)/7.05 (two hydrogen atoms), the existence of a para-substituted benzene ring can be known. By using the HSQC spectrum, the related δ_C can be determined.



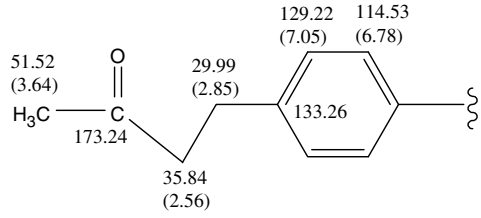
The remaining singlet at 3.62 ppm is the peak of an isolated methyl group.

We will connect the structural units mentioned above by using the HMBC spectrum.

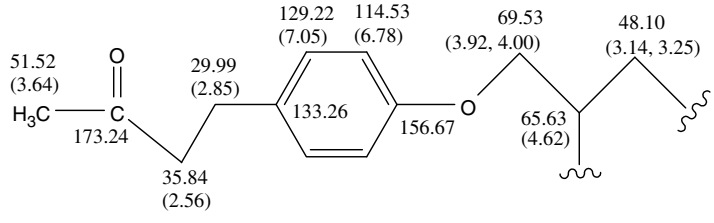
We now start from the para-substituted benzene ring. According to the correlation ofδ_C(133.26)/δ_H(6.78) in the HMBC spectrum, the peak at 133.26 ppm can be determined as one quaternary carbon atom of the substituted benzene ring. Because this is the correlation with a ^3J coupling, the intensity of the correlated peak is strong. According to the correlations of δ_C(133.26)/δ_H(2.56, 2.85),δ_C(29.99)/δ_H(7.05), andδ_C(129.22)/δ_H(2.56, 2.85) in the HMBC spectrum, the connection between the substituted benzene ring and the two connected CH_2 groups can be determined. And the chemical shift value of the CH_2 group, which connects directly with the benzene ring can be determined as 29.99 ppm from the correlation of δ_C(29.99)/δ_H(7.05) in the HMBC spectrum. To sum up, we get the following structural unit:



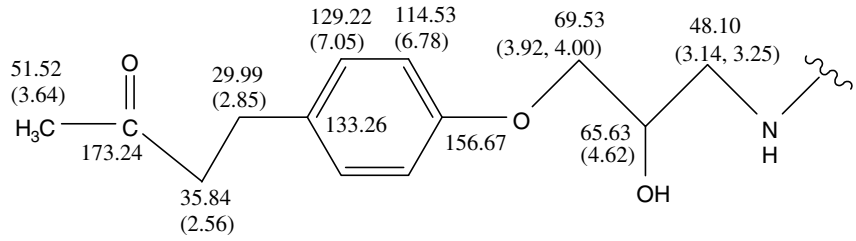
According to the correlation of δ_C(173.24)/δ_H(2.56, 2.85, 3.64) from the HMBC spectrum and the fact that the singlet at 3.64 ppm in the ^{1}H spectrum is an isolated singlet in the H spectrum, the structural unit can be extended as follows:



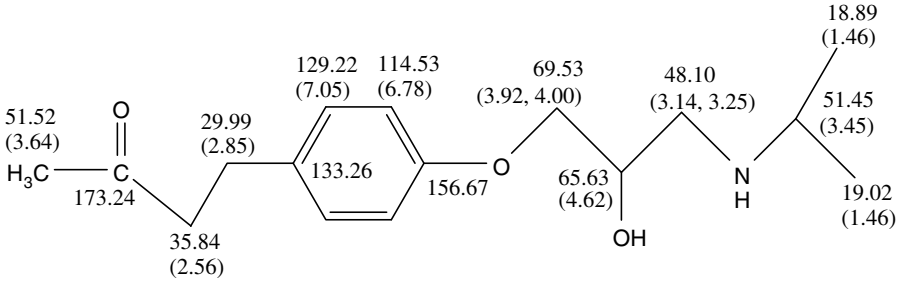
Now we will extend the structure of the other side of the para-substituted benzene ring. From the correlation of δ_C(156.67)/δ_H(6.78, 7.05) in the HMBC spectrum, the chemical shift value of the other quaternary carbon atom of the substituted benzene ring can be determined as 156.67 ppm. This value means that this quaternary carbon atom should connect with a strongly electronegative group. Compared with the molecular formula, the connection of the quaternary carbon atom with one oxygen atom is reasonable. Another important clue is the correlation of δ_C(156.67)/δ_H(3.92) in the HMBC spectrum. Therefore, the structural unit mentioned above can be extended further as follows:



Because the unknown compound contains three reactive hydrogen atoms, and because the remaining elemental composition of the unknown compound leaves ON, the CH and the CH_2 of the structural unit mentioned above should connect with an OH and an NH, respectively. Therefore, the structure mentioned above can be extended further as follows:



Only an isopropyl group is left in the structural units. Its CH should connect with the NH. Therefore, the structure of the unknown compound can be completed as follows:



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Remember that the unknown compound contains a chlorine atom and a reactive hydrogen atom. Therefore, the unknown compound is a chloride.

It should be noted that the compound has no symmetrical plane. Therefore, the two methyl groups of the isopropyl group are not chemically equivalent. Because they have different chemical shift values, they show two doublets, which can be seen in the enlarged ^{1}H spectrum.

Example 6.6 The molecular weight of an unknown compound has been determined as 205 by its mass spectrum. Its ^{1}H spectrum, the locally enlarged spectrum of the ^{1}H spectrum, its ^{1}H spectrum (measured after the exchange with D_2O), its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum, its DEPT-135 spectrum, its COSY spectrum, the locally enlarged spectrum of the COSY spectrum, its HSQC spectrum, its HMBC spectrum, and the locally enlarged spectrum of the HMBC spectrum are shown in Figures 6.37–6.49, respectively. Try to deduce its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated DMSO.

Solution

We now start to determine the elemental composition of the unknown compound.

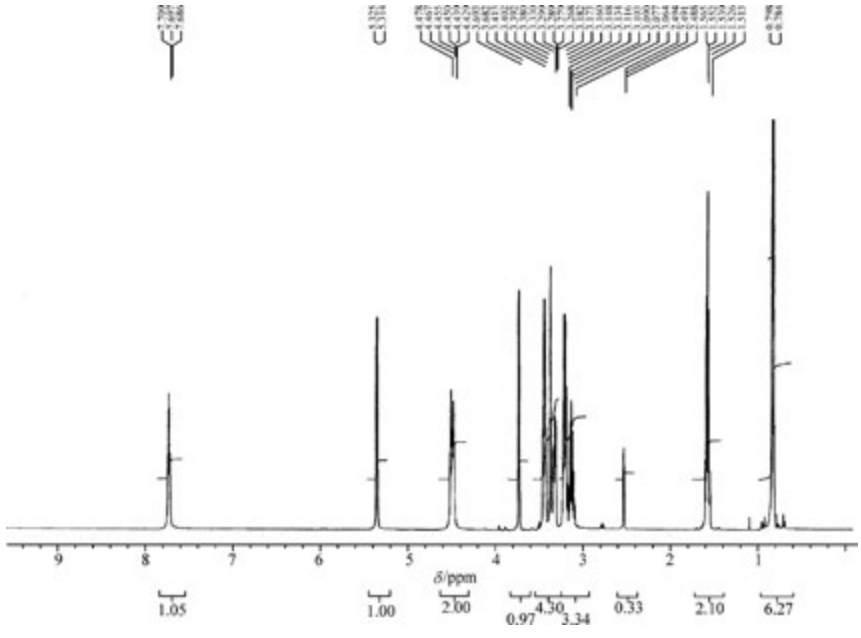


Figure 6.37 The ^{1}H spectrum of the unknown compound

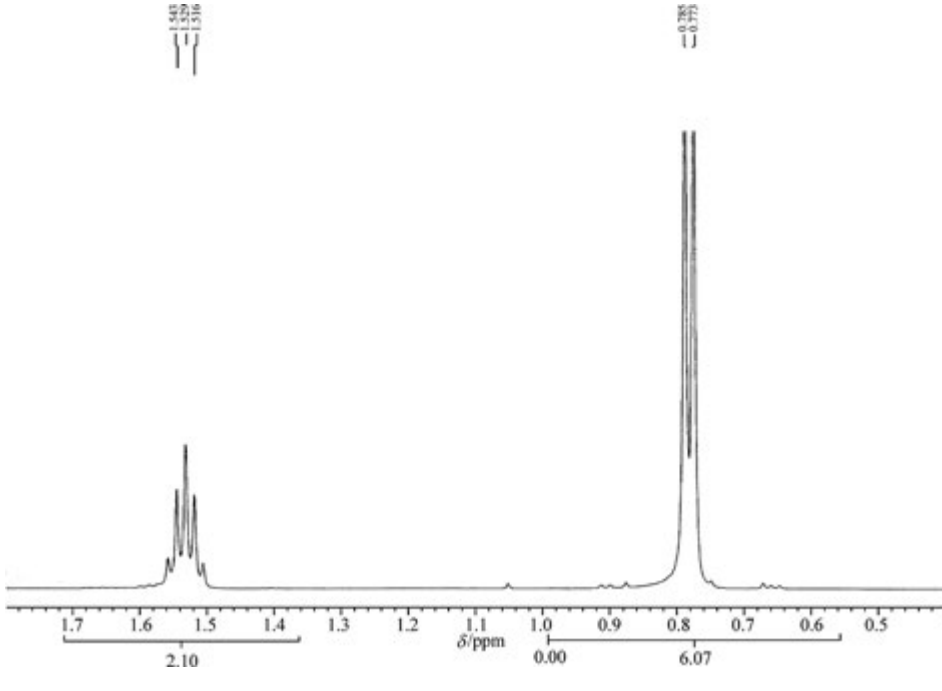


Figure 6.38 The locally enlarged spectrum (1) of Figure 6.28

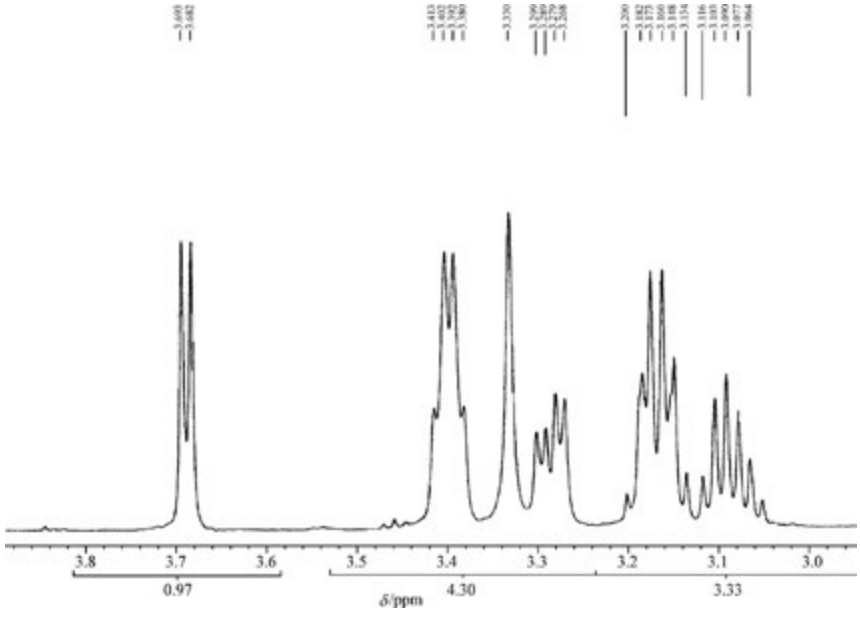


Figure 6.39 The locally enlarged spectrum (2) of Figure 6.28

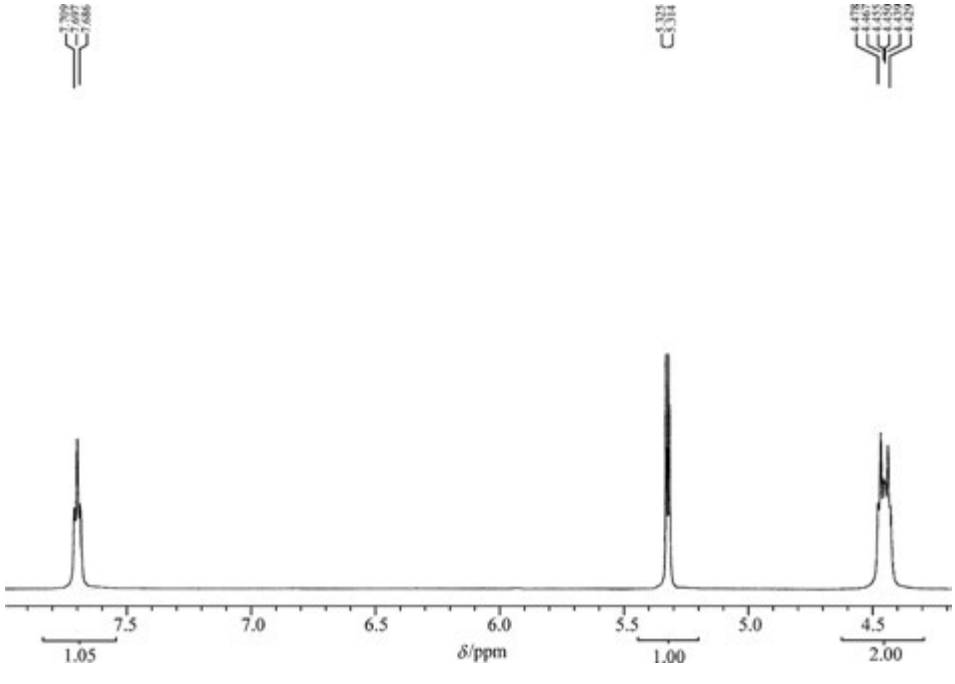


Figure 6.40 The locally enlarged spectrum (3) of Figure 6.28

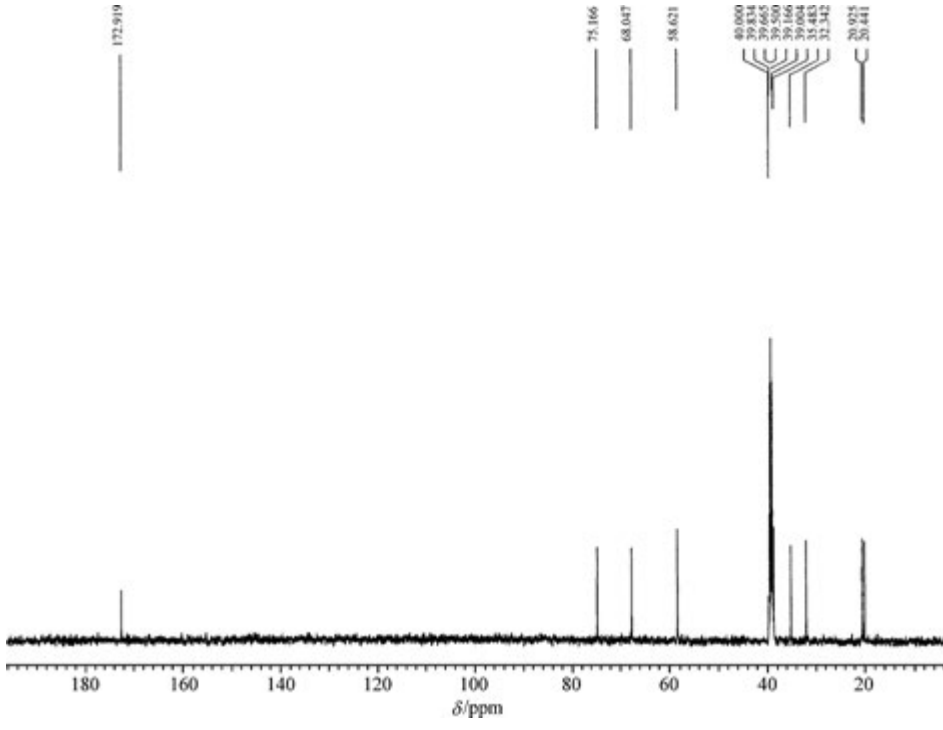


Figure 6.41 The ^{13}C spectrum of the unknown compound

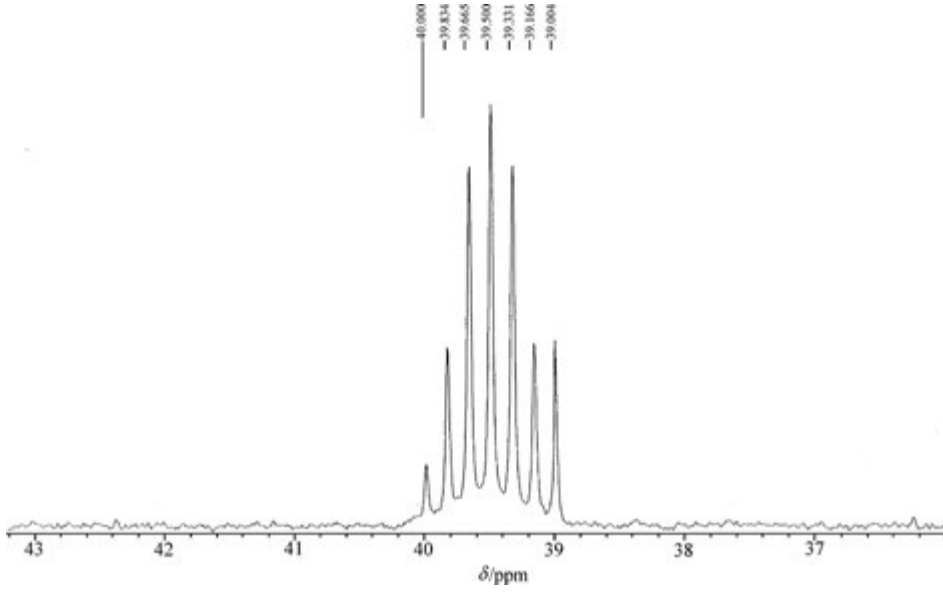


Figure 6.42 The locally enlarged spectrum of Figure 6.41

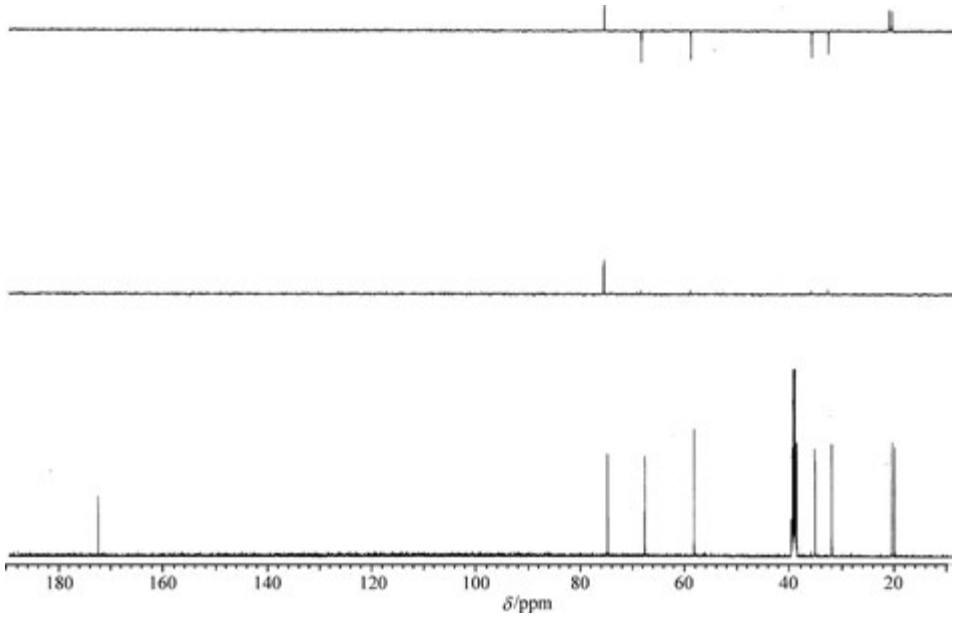


Figure 6.43 The DEPT-135 spectrum of the unknown compound

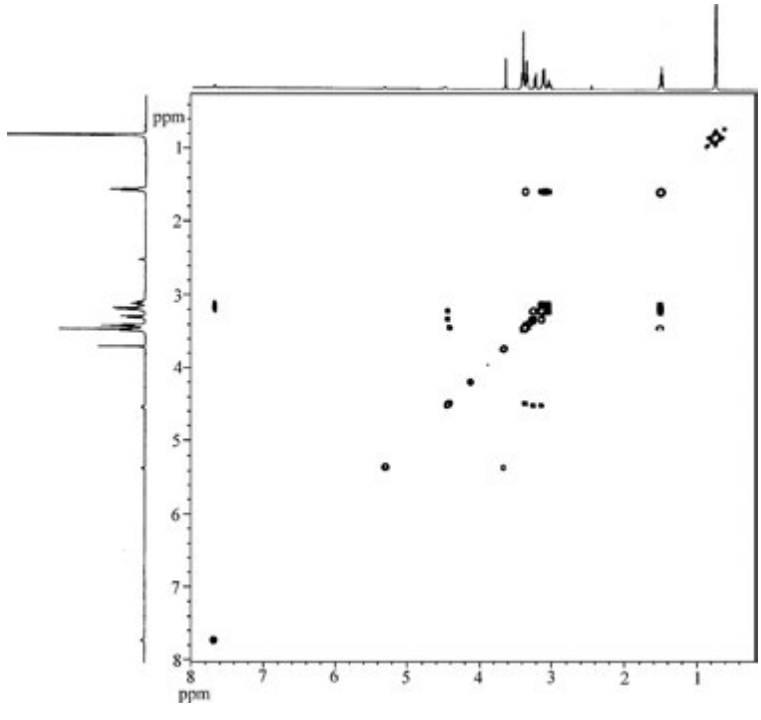


Figure 6.44 The COSY spectrum of the unknown compound

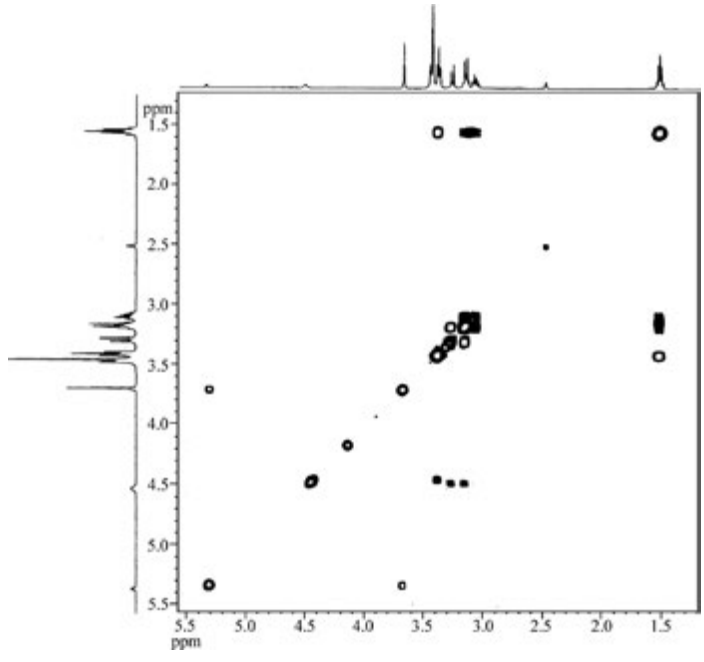


Figure 6.45 The locally enlarged spectrum of Figure 6.44

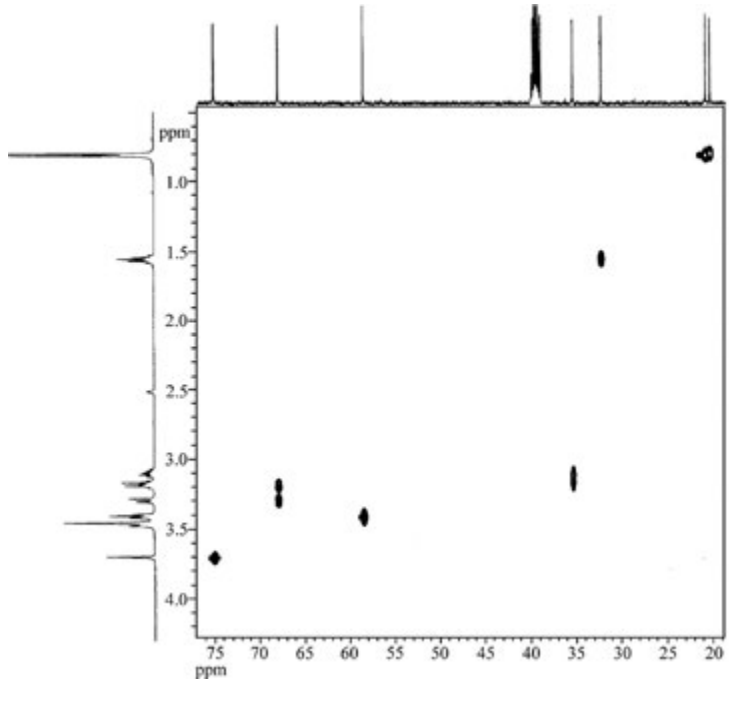


Figure 6.46 The HSQC spectrum of the unknown compound

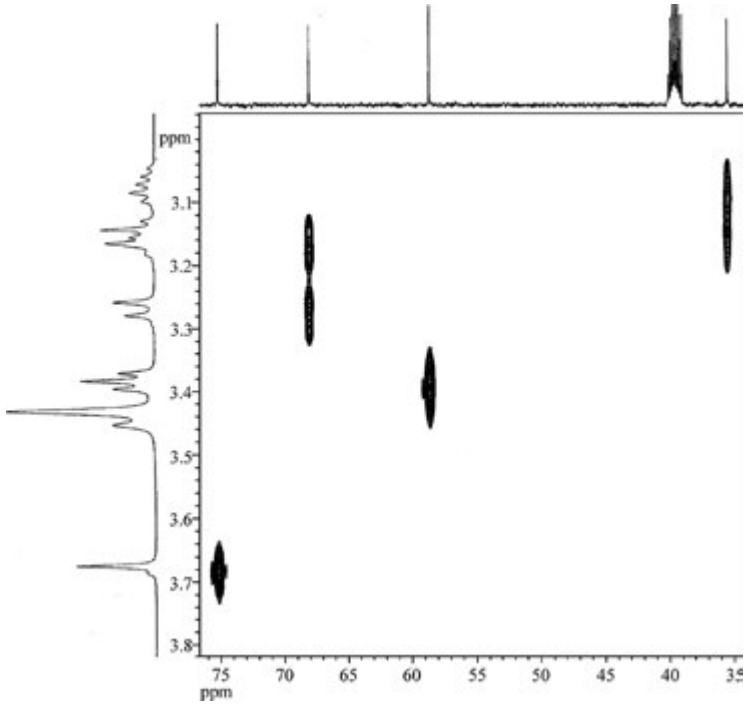


Figure 6.47 The locally enlarged spectrum of Figure 6.46

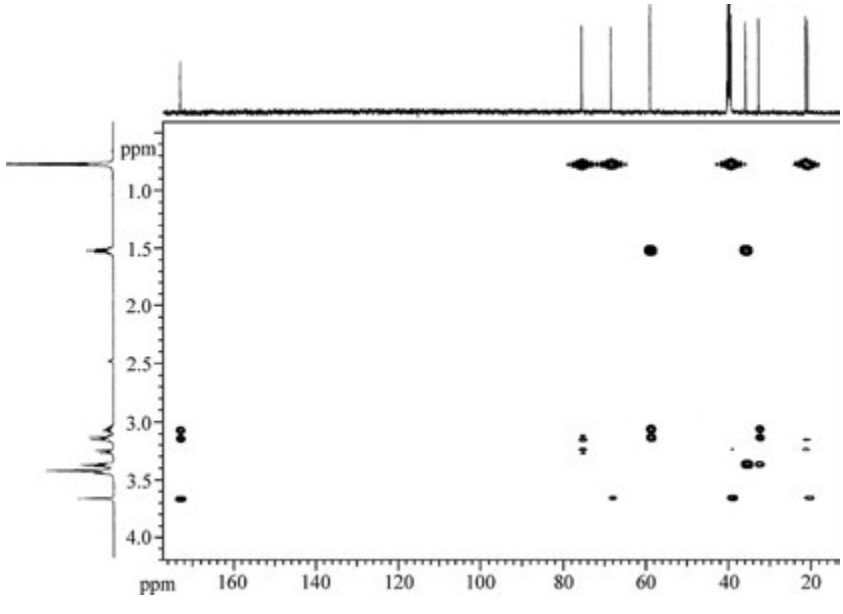


Figure 6.48 The HMBC spectrum of the unknown compound

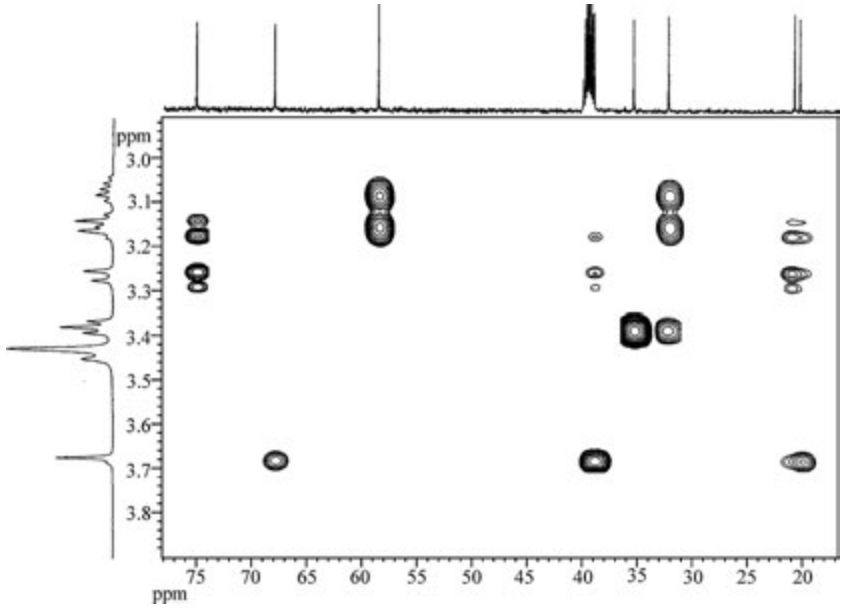


Figure 6.49 The locally enlarged spectrum of Figure 6.48

The peak set at 2.49 ppm in the ^{1}H spectrum is the solvent peak. The singlet at 3.30 ppm is the water peak when the solvent is deuterated DMSO.

After the exchange with D_2O (the spectrum is not shown here), the areas of the four peak sets at 4.44, 4.47, 5.32, and 7.69, respectively, are reduced to less than 28%. This fact means that these four peak sets correspond to four reactive hydrogen atoms, whose exchange rate is not rapid enough.

All reactive hydrogen atoms can be recognized by using the HSQC spectrum, in which the reactive hydrogen atoms have no correlated peaks. The number of the total reactive hydrogen atoms can also be known from the DEPT spectrum. The subtraction of the hydrogen atoms counted from the DEPT spectrum from the number of the hydrogen atoms of its molecular formula gives the total number of the reactive hydrogen atoms.

Because the exchange reaction takes place slowly, the peaks of the reactive hydrogen atoms show split shapes by the couplings of their vicinal hydrogen atoms. Therefore, they have correlated peaks in the COSY spectrum.

According to the ^{1}H spectra, its locally enlarged spectrum, and other related 2D spectra, we obtain Table 6.19.

Now we supplement some explanations about Table 6.19.

The two peaks at 0.78 and 0.79 ppm may be mistaken for a doublet, because their distance corresponds to 6 Hz. However, since they have no correlated peaks in the COSY spectrum, they should be two singlets.

The peak set at 1.53 ppm is a typical quintet, with the peak intensity having the ratio close to 1 : 4 : 6 : 4 : 1.

Table 6.19 The data summarized from the ^{1}H spectrum and related 2D spectra

δ_H (ppm)	Number of H	Peak shape	Remarks, ^{3}J (Hz)
0.78	3	s	
0.79	3	s	
1.53	2	quintet	6.5
3.09	1	m	
3.15	1	m	
3.18	1	m	
3.28	1	d, d	10, 5
3.40	2	d, d	11, 5.5
3.69	1	d	5.5
4.44	1	t	5.5
4.47	1	t	5.5
5.32	1	d	5.5
7.69	1	t	6.0

The peak sets at about 3.17 ppm correspond to two hydrogen atoms. Since their peak shape is approximately symmetrical, they may be considered as the peak set of a CH_2 group. However, by using the HSQC spectrum, the HMBC spectrum, and the COSY spectrum, it can be known that the peak set consists of two partially overlapped peak sets, which belong to two hydrogen atoms of two functional groups, respectively. And these two hydrogen atoms have no relationship. These two hydrogen atoms are listed in two rows (3.15 and 3.18 ppm).

The peak set at about 4.45 ppm consists of two triplets, which are close to each other. Notice that there are two small peaks between two high peaks. This analysis can be proved further by the COSY spectrum, in which correlated peaks at about 4.45 ppm are arranged in two rows (and in two columns).

From Table 6.19 it can be known that the unknown compound contains 19 hydrogen atoms.

Now we will interpret the ^{13}C spectrum.

The ^{13}C spectrum shows 8 peaks. However, from the locally enlarged spectrum of Figure 6.41 it can be seen that a peak of the sample is just overlapped on the furthest right peak of the peak set of deuterated DMSO. Because of this overlapping, the peak set is not symmetrical. Therefore, this unknown compound contains 9 carbon atoms. This postulation coincides with the HMBC spectrum, in which the correlated peak at about 39.0 ppm exists.

The data summarized from the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum are listed in Table 6.20.

We would like to give the following explanation for Table 6.20. The correlation between $\delta_C = 35.5$ ppm and $\delta_H = 3.09$, 3.15 ppm and the correlation between $\delta_C = 68.1$ ppm and $\delta_H = 3.18$, 3.28 ppm are determined with the help of the HMBC spectrum.

From Table 6.20 it can be known that the unknown compound contains $2CH_3$, $4CH_2$, 1CH, and 2 quaternary carbon atoms.

Because its molecular weight is an odd number, the unknown compound should contain an odd number of nitrogen atoms. First we suppose the unknown compound contains one nitrogen atom. The subtraction of the mass of $C_9H_{19}N$ from the molecular weight gives

Table 6.20 The data summarized from the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum

δ_C (ppm)	Number of C atoms	Order of C atoms	Directly connected H atoms, δ_H (ppm)
20.4	1	CH_3	0.78
20.9	1	CH_3	0.79
32.3	1	CH_2	1.53
35.5	1	CH_2	3.09, 3.15
39.0	1	C	
58.6	1	CH_2	3.40
68.1	1	CH_2	3.18, 3.28
75.2	1	CH	3.69
172.9	1	C	

64 (u), which corresponds to the mass of four oxygen atoms. This postulation coincides with the fact that the unknown compound has four reactive hydrogen atoms. Therefore, its molecular formula is $C_9H_{19}O_4N$.

The unsaturation number of the unknown compound can be calculated as 1 from its molecular formula. Since the unknown compound contains a carbonyl group, it has neither any other unsaturated group nor rings.

The data summarized from the COSY spectrum are listed in Table 6.21.

Because the peak sets at 3.15 and 3.18 ppm are close, their correlated peaks are resolved with the help of the HMBC spectrum.

The data summarized from the HMBC spectrum are listed in Table 6.22.

Now we will find its structural units, and then extend them.

We now start from the interpretation of the ^1H spectrum. The two singlets at 0.77 and 0.78 ppm correspond to two isolated methyl groups. Therefore, they have no correlated peaks in the COSY spectrum.

Table 6.21 The data summarized from the COSY spectrum

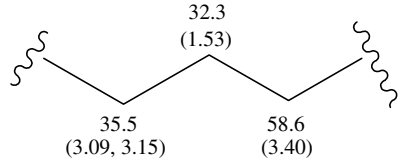
δ_H (ppm)	Coupled H, δ_H (ppm)	Remarks
0.78	isolated	
0.79	isolated	
1.53	3.09, 3.15, 3.40	Refer to No. 7 of Table 6.22
3.09	1.53, 3.15, 7.69	Refer to No. 7 of Table 6.22
3.15	1.54, 3.09	
3.18	3.28, 4.47	
3.28	3.18, 4.47	Refer to No. 2 of Table 6.22
3.40	1.53, 4.44	
3.69	5.32	
4.44	3.40	
4.47	3.18, 3.28	Refer to No. 2 of Table 6.22
5.32	3.69	
7.69	3.09	

Table 6.22 The data summarized from the HMBC spectrum

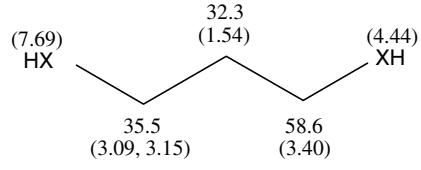
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	172.9	3.09, 3.15, 3.69	
2	75.2	0.78, 0.79, 3.18, 3.28	
3	68.1	0.78, 0.79, 3.69	
4	58.6	1.53, 3.09, 3.15	
5	39.0	(3.28), 3.69	
6	35.5	3.38	
7	32.3	3.09, 3.15, 3.38	
8	20.4, 20.9	3.18, 3.28, 3.69	0.78[a], 0.79[a]

Parenthesis () stands for weak couplings.
[a] The correlations are proved by the HSQC spectrum.

We start again from the quintet at 1.53 ppm in the ^{1}H spectrum, which should be a CH_2 group between two CH_2 groups. According to the correlations of δ_H (ppm)1.53/3.09, 3.15, 3.40, and the related data in Table 6.20, we obtain the following structural unit:



The unit above can be extended by using the correlations of δ_H (ppm) 4.44/3.40 and 7.69/3.09 obtained from Table 6.21. Because the two peaks at 4.44 and 7.69 ppm have no correlated peaks in the HSQC spectrum, they correspond to reactive hydrogen atoms. However, we can not determine whether they are OH or NH at this time. Therefore, we use XH to express a reactive hydrogen atom. As a result, the structural unit is extended as follows:

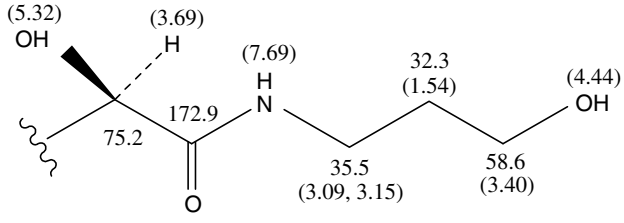


If XH=OH, it will be a terminal group. If XH=NH, it will connect with another group.

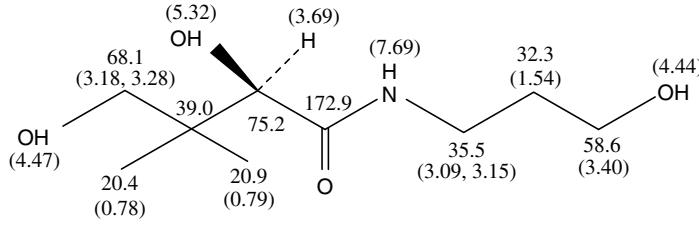
We have known that the unknown compound contains four reactive hydrogen atoms, one nitrogen atom, and four oxygen atoms. From the peak at 172.9 ppm, it can be determined that this peak is that of a carbonyl group. Therefore, these four reactive hydrogen atoms should be 3 OH and 1 NH. The δ_C value of 172.9 ppm manifest that the carbonyl group should connect with a heteroatom. If the carbonyl group connects with an OH, the carboxyl

group will form a terminal group of the unknown compound. If the carbonyl group connects with an NH, the amide group will connect with another functional group. Because the HMBC spectrum shows the correlation of $\delta_C(172.9)/\delta_H(3.09, 3.15, 3.69)$ ppm, this carbonyl group should connect with an NH group. At the same time, the peak at 7.69 ppm can be determined as that of an NH. Since the unknown compound contains just one nitrogen atom, the peak at 4.44 ppm can be ascribed as an OH group. Therefore, the structural unit can be extended as follows:

The correlation of $\delta_C(172.9)/\delta_H(3.69)$ ppm has been mentioned above. From Table 6.20 it can be known that the peak at 3.69 ppm belongs to a CH, which has a correlation with the peak at 5.32 ppm in the COSY spectrum. This CH has $\delta_C = 75.2$ ppm, which coincides with the connection with an OH group. Therefore, the above-mentioned structural unit can be extended as follows:



The correlations in the HMBC spectrum: $\delta_C(75.2)/\delta_H(0.78, 0.79, 3.18, 3.28)$, $\delta_C(68.1)/\delta_H(0.78, 0.79, 3.69)$, $\delta_C(39.0)/\delta_H(3.28, 3.69)$, and $\delta_C(20.4, 20.9)/\delta_H(3.18, 3.28, 3.69)$ ppm, and the correlation in the COSY spectrum ofδ_H(ppm) 4.47/3.18, 3.28 leads to the whole structure of the unknown compound and to the complete assignment.



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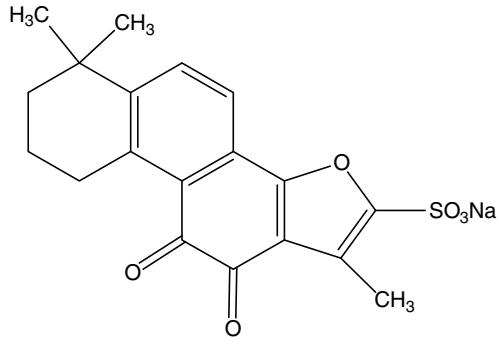
The coincidence of all spectra and the reasonable assignments manifest that the structure of the unknown compound is correct.

Example 6.7 A compound has the anticipated structure which follows:



C6-8

Its ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum, its DEPT spectrum, its COSY spectrum, its HSQC spectrum, and its HMBC spectrum, the locally enlarged spectrum of the HMBC spectrum (two spectra) are shown in Figures 6.50–6.57, respectively. Try to confirm its structure by using its NMR spectra. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated DMSO.

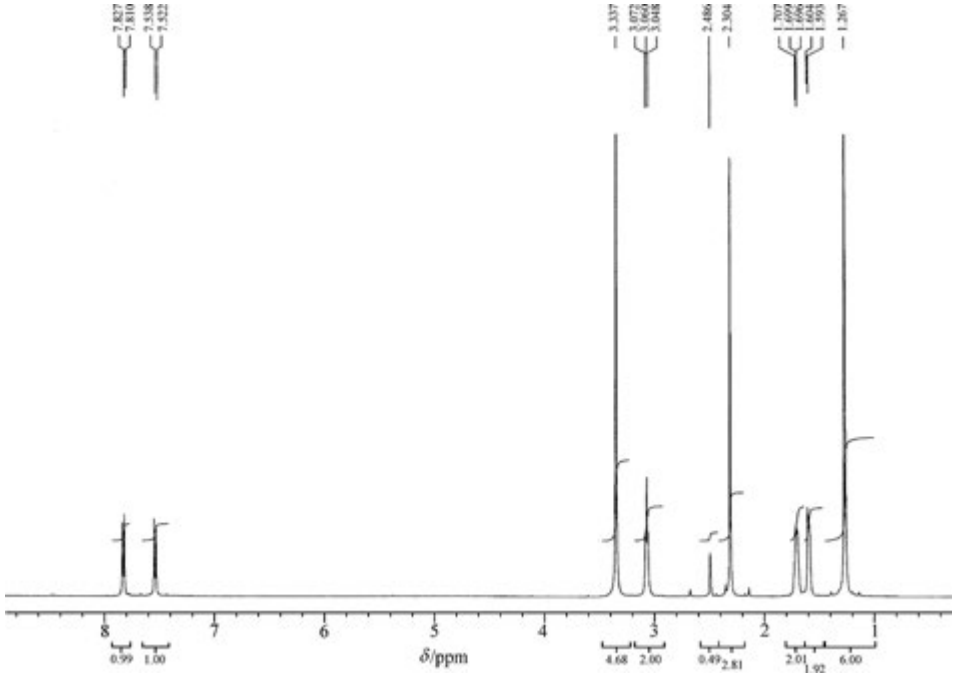


Figure 6.50 The ^{1}H spectrum of the compound

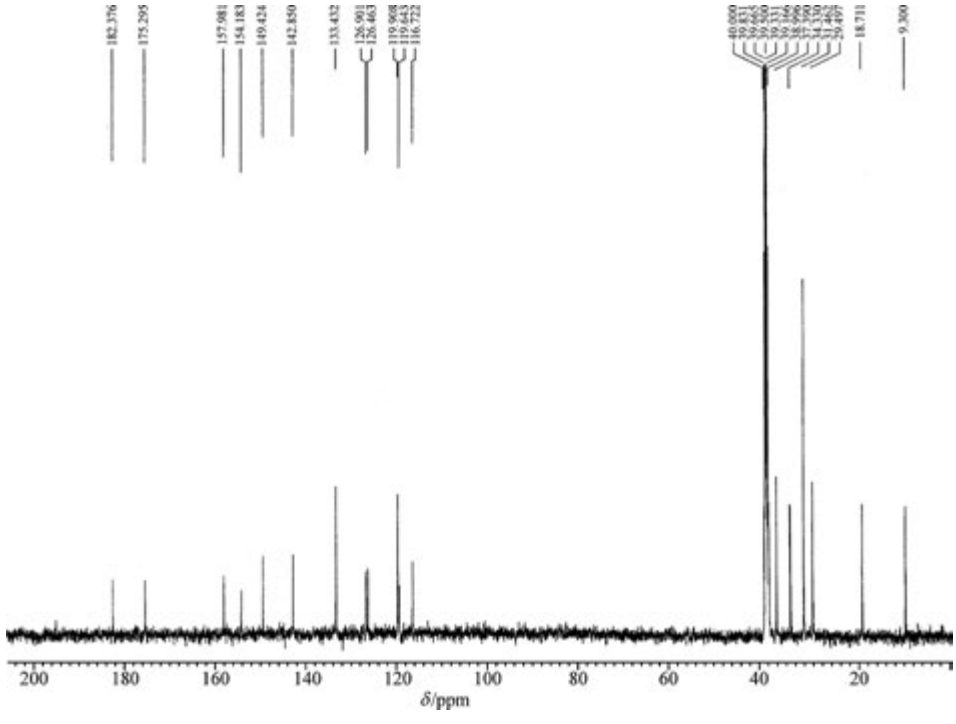


Figure 6.51 The ^{13}C spectrum of the compound

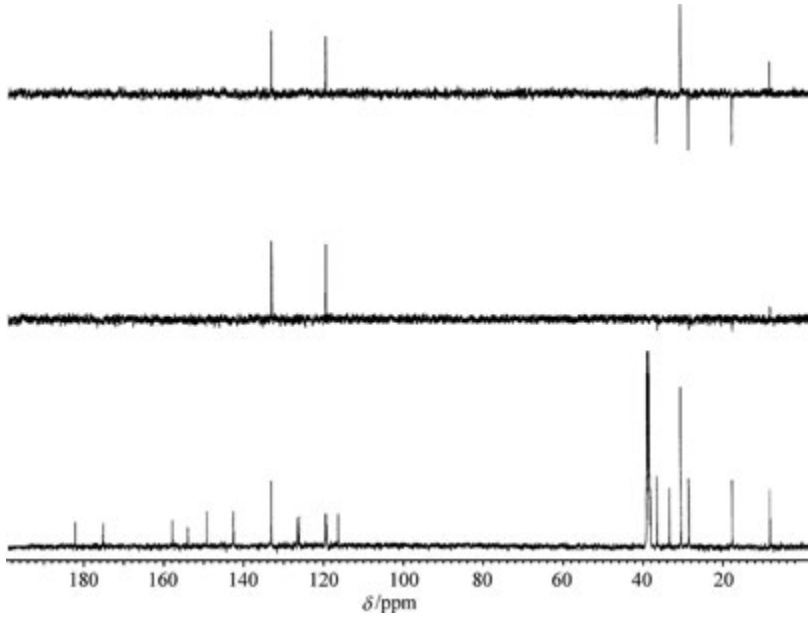


Figure 6.52 The DEPT spectrum of the compound

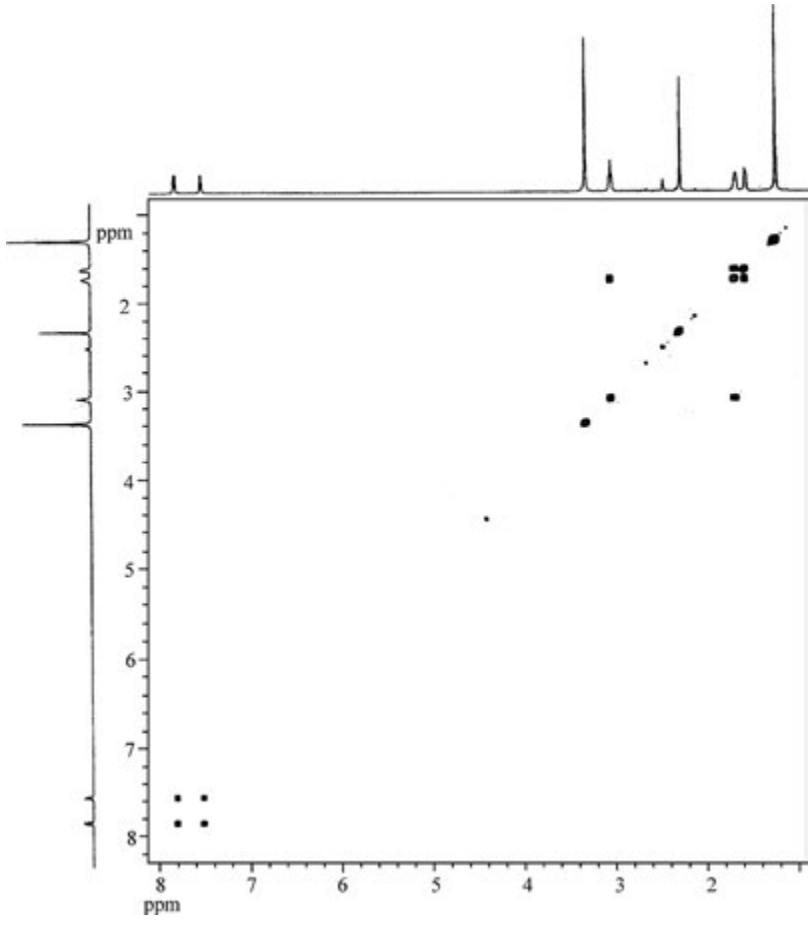


Figure 6.53 The COSY spectrum of the compound

Solution

The peak at about 2.50 ppm in the ^{1}H spectrum is the solvent peak (deuterated DMSO), and the peak at 3.30 ppm is the peak from water, which exists in the sample.

Because the ^{1}H spectrum is so simple, it is very easy to analyze their peak shapes. The related data are listed in Table 6.23.

From Table 6.23 it can be known that the unknown compound contains 3 CH_3, 3 CH_2, and 2 CH. The number of its total hydrogen atoms is 17, which coincides with its structural formula.

Now we will interpret its ^{13}C spectrum.

The peaks at 39.5 ppm are the solvent peaks. Since the spectrum has 16 peaks, of which three peaks (at 31.5, 119.9, and 133.4 ppm, respectively) have a prominent height, the ^{13}C spectrum shows the existence of 19 carbon atoms, which coincides with its structural formula.

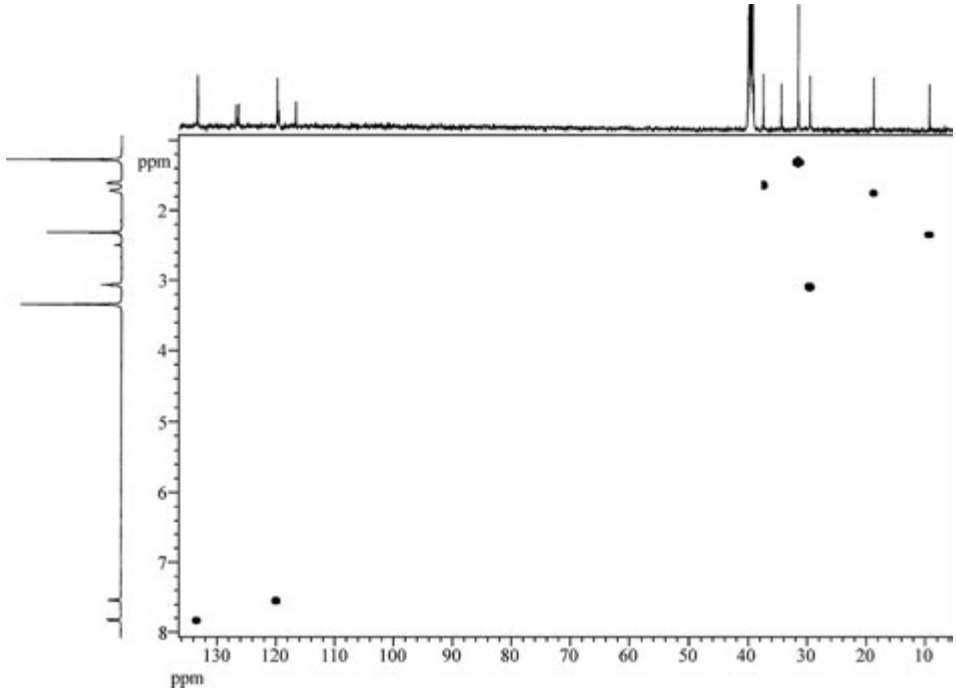


Figure 6.54 The HSQC spectrum of the compound

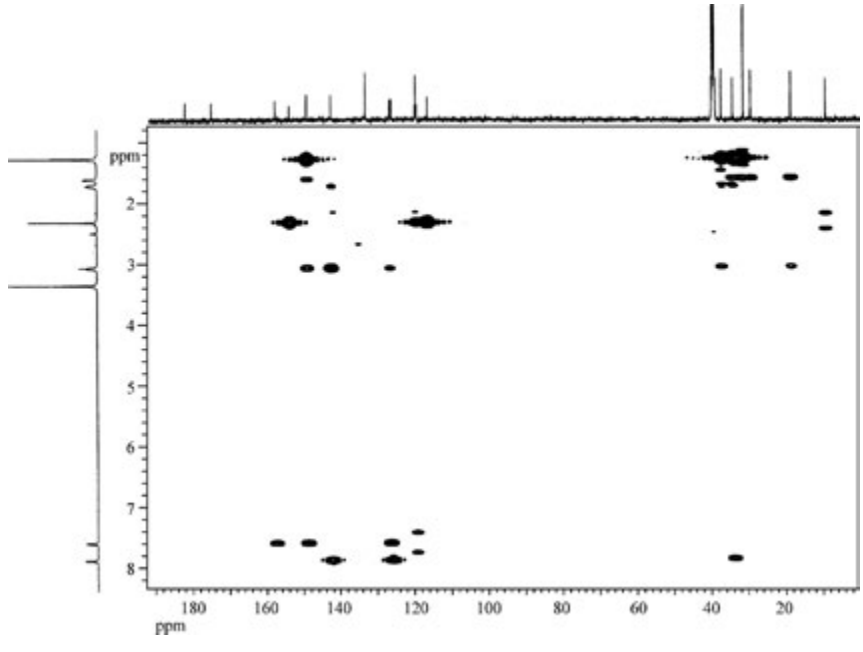


Figure 6.55 The HMBC spectrum of the compound

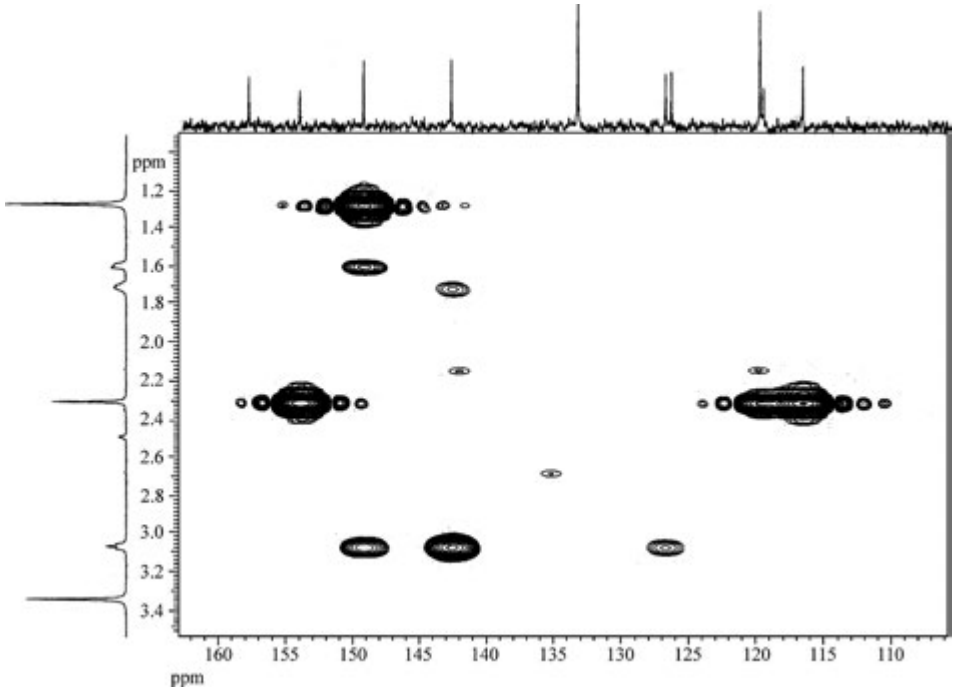


Figure 6.56 The locally enlarged spectrum (1) of Figure 6.55

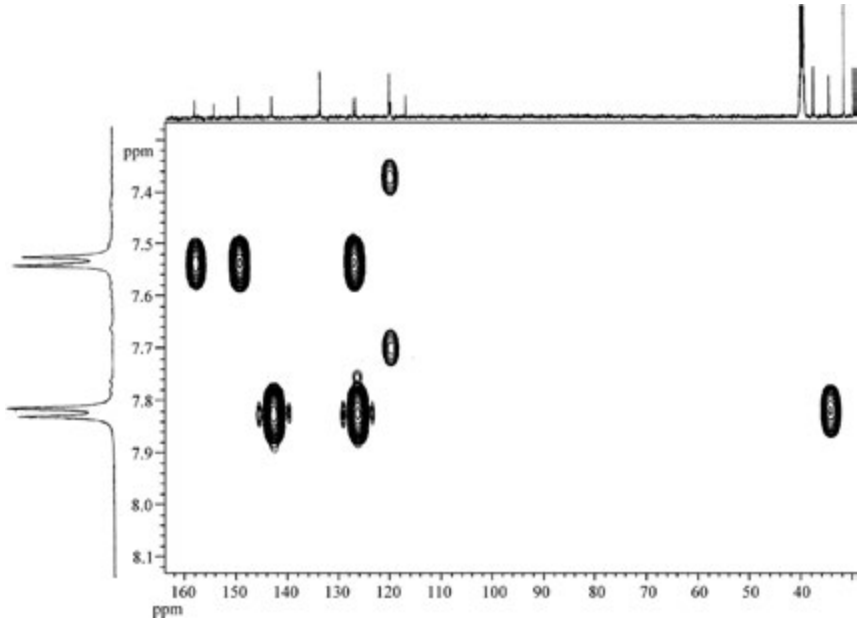


Figure 6.57 The locally enlarged spectrum (2) of Figure 6.55

Table 6.23 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape
1.27	6	s
1.60	2	m
1.70	2	m
2.30	3	s
3.06	2	t
7.53	1	d
7.81	1	d

There are three spectra in the DEPT spectrum, in which the ^{13}C spectrum, the DEPT-90 spectrum, and the DEPT-135 spectraum are arranged from bottom to top.

The data summarized from the ^{13}C spectrum, the DEPT spectrum, the HSQC spectrum, and the ^{1}H spectrum are listed in Table 6.24.

The two peaks at 119.6 and 119.9 ppm are close. From Figure 6.52 it can be known that the peak at 119.6 ppm corresponds to a quaternary carbon atom and the peak at 119.9 ppm to a CH group. This information coincides with that obtained from the HSQC spectrum.

The data summarized from the COSY spectrum are listed in Table 6.25.

The data summarized from the HMBC spectrum are listed in Table 6.26.

Table 6.24 The data sammurized from the ^{13}C spectrum, the DEPT spectrum, the HSQC spectrum, and the ^{1}H spectrum

δ_C (ppm)	Number of C atoms	Order of C atom	Directly connected H atoms, δ_H (ppm)
9.3	1	CH_3	2.30
18.7	1	CH_2	1.70
29.5	1	CH_2	3.06
31.5	2	CH_3	1.27
34.3	1	C	
37.4	1	CH_2	1.60
116.7	1	C	
119.6	1	C	
119.9	1	CH	7.53
126.5	1	C	
126.9	1	C	
133.4	1	CH	7.81
142.9	1	C	
149.4	1	C	
154.2	1	C	
157.9	1	C	
175.3	1	C	
182.3	1	C	

Table 6.25 The data summarized from the COSY spectrum

δ_H (ppm)	Coupled H, δ_H (ppm)
1.27	isolated
1.60	1.70
1.70	1.60, 3.06
2.30	isolated
3.06	1.70
7.53	7.81
7.81	7.53

According to the correlations of δ_H (ppm)1.60/1.70/3.06 in the COSY spectrum, with the help of the HSQC spectrum, we obtain the following structural unit:

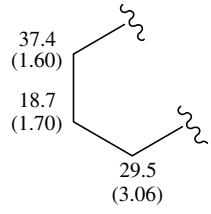
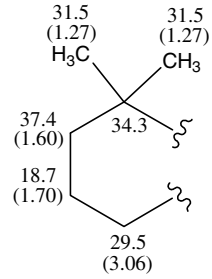


Table 6.26 The data summarized from the HMBC spectrum

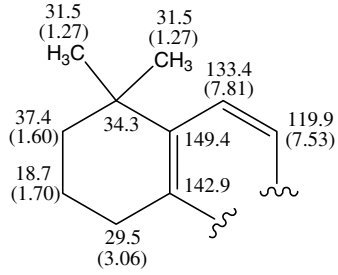
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	9.3		2.30
2	18.7	1.60, 3.06	
3	29.5	1.60	
4	31.5	1.60	1.27[a]
5	34.3	1.27, 1.60, 1.70, 7.81	
6	37.4	1.27, 1.70, 3.06	1.60
7	116.7	2.30	
8	119.6, 119.9	2.30	7.53
9	126.5	7.81	
10	126.9	3.06, 7.53	
11	133.4		
12	142.9	1.70, 3.06, 7.81	
13	149.4	1.27, 1.60, 3.06, 7.53	
14	154.2	2.30	
15	157.9	7.53	
16	175.3		
17	182.3		

[a] Only one correlated peak exists in the HMBC spectrum but without the pair of the correlated peaks. However, the correlation with the ^1J coupling can be determined from the HSQC spectrum.

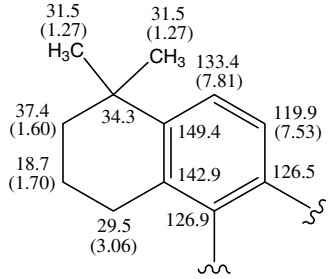
According to the correlations of $\delta_C(37.4)/\delta_H(1.27, 1.70, 3.06)$, $\delta_C(34.3)/\delta_H(1.27, 1.60, 1.70)$, and $\delta_C(31.5)/\delta_H(1.60)$ ppm in the HMBC spectrum, with the help of the HSQC spectrum, the structural unit mentioned above can be extended as follows:



According to the correlations of $\delta_C(34.3)/\delta_H(7.81)$, $\delta_C(142.9)/\delta_H(1.70, 3.06, 7.81)$, $\delta_C(149.4)/\delta_H(1.27, 1.60, 3.06, 7.53)$ ppm in the HMBC spectrum and the correlation of δ_H(ppm) 7.81/7.53 in the COSY spectrum, with the help of the HSQC spectrum, the structural unit mentioned above can be extended as follows:



According to the correlations of $\delta_C(126.9)/\delta_H(3.06, 7.53)$, $\delta_C(126.5)/\delta_H(7.81)$ ppm in the HMBC spectrum, with the help of the HSQC spectrum, the structural unit mentioned above can be extended as follows:

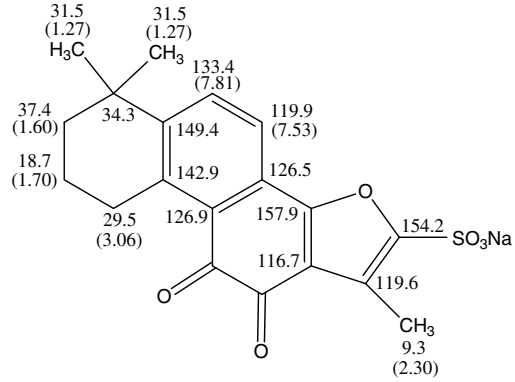


When we extend a structural unit by using the HMBC spectrum, it should be noticed that the correlated peak between the carbon atom and the hydrogen atom across three chemical bonds has a stronger intensity than that across two chemical bonds.

The above-mentioned postulation is made strictly on the basis of the interpretation of 2D NMR spectra step by step. Even if we have no anticipated structural formula, the result mentioned above can be obtained.

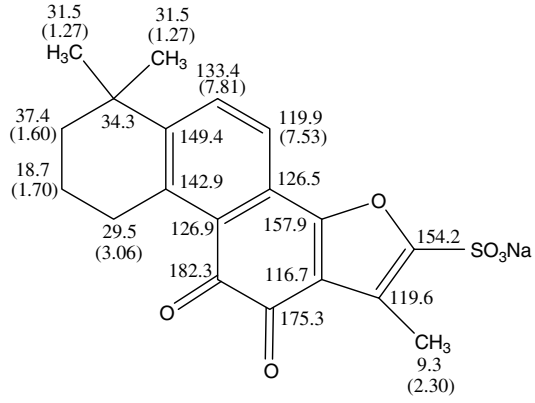
However, because the compound contains ten quaternary carbon atoms and they are connected together, the structural unit can not be extended by using the HMBC spectrum. This situation shows the limitation of the method which we commonly use. If we need to postulate an unknown structure, the only method is based on the comparison of the measured spectra with those of a standard sample.

To complete the assignment, we start from the remaining methyl group ($\delta_C = 9.3$ ppm, $\delta_H = 2.30$ ppm) of the anticipated structural formula. If we consider the correlations of $\delta_C(116.7)/\delta_H(2.30)$, $\delta_C(119.6)/\delta_H(2.30)$, $\delta_C(154.2)/\delta_H(2.30)$, and$\delta_C(157.9)/\delta_H(7.53)$ ppm in the HMBC spectrum, we can complete the assignments except the two carbonyl groups.



When we assign the structure mentioned above, we consider the correlated peak between the carbon atom and the hydrogen atom across three chemical bonds has a stronger intensity than that across two chemical bonds. Because the intensity of the correlated peak of $\delta_C(116.7)/\delta_H(2.30)$ is stronger than that of$\delta_C(119.6)/\delta_H(2.30)$, the former should be that across three chemical bonds, and the latter that across two chemical bonds.

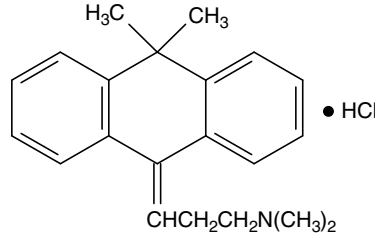
These two carbonyl groups can not be assigned by the HMBC spectrum, because they have no correlated peaks in the HMBC spectrum. We estimate their chemical shift values from chemical knowledge.



C6-8

We would like to state the following conclusion again: if a compound contains many quaternary carbon atoms or heteroatoms and there are no hydrogen atoms between them, the conventional method does not work well.

Example 6.8 A compound has the anticipated structure which follows:



C6-9

Its ^{1}H spectrum, the locally enlarged spectrum of the ^{1}H spectrum (in the low field region and in the high field region), its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum (in the low field region and in the high field region), its DEPT spectrum, the locally enlarged DEPT spectrum (in the low field region), its COSY spectrum, its HMQC spectrum, the locally enlarged HMQC spectrum (in the low field region), its HMBC spectrum, the locally enlarged HMBC spectrum (in the low field region), its NOESY spectrum, and locally enlarged NOESY spectrum (in the low field region) are shown in Figures 6.58–6.72, respectively. Try to confirm its structure by using its NMR spectra. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is deuterated DMSO.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The peak set at 2.50 ppm in the ^{1}H spectrum is the solvent peaks, and the peak at 3.32 ppm the water peak.

The peak shapes in the high field region are clear. The peak shapes in the low field region (aromatic hydrogen region) are complicated. However, it can be found by careful inspection that the peak shapes in the low field region are those of the typically substituted benzene ring, in which the splits by ^{3}J couplings play the principal role and the splits by ^{4}J couplings play the secondary role. The peak shapes of all peak sets are given in Table 6.27.

The peak which belongs to a reactive hydrogen atom is shown in the low field region of the ^{1}H spectrum.

The peak sets in the aromatic region correspond to 9 hydrogen atoms. The peak sets in the aliphatic region correspond to 16 hydrogen atoms. These results coincide with the structural formula.

Now we will interpret the ^{13}C spectrum.

There are 14 peaks in the aromatic region, and 4 peaks in the aliphatic region.

The use of the HMQC spectrum and the HMBC spectrum is very helpful for the interpretation of the ^{13}C spectrum of this unknown compound.

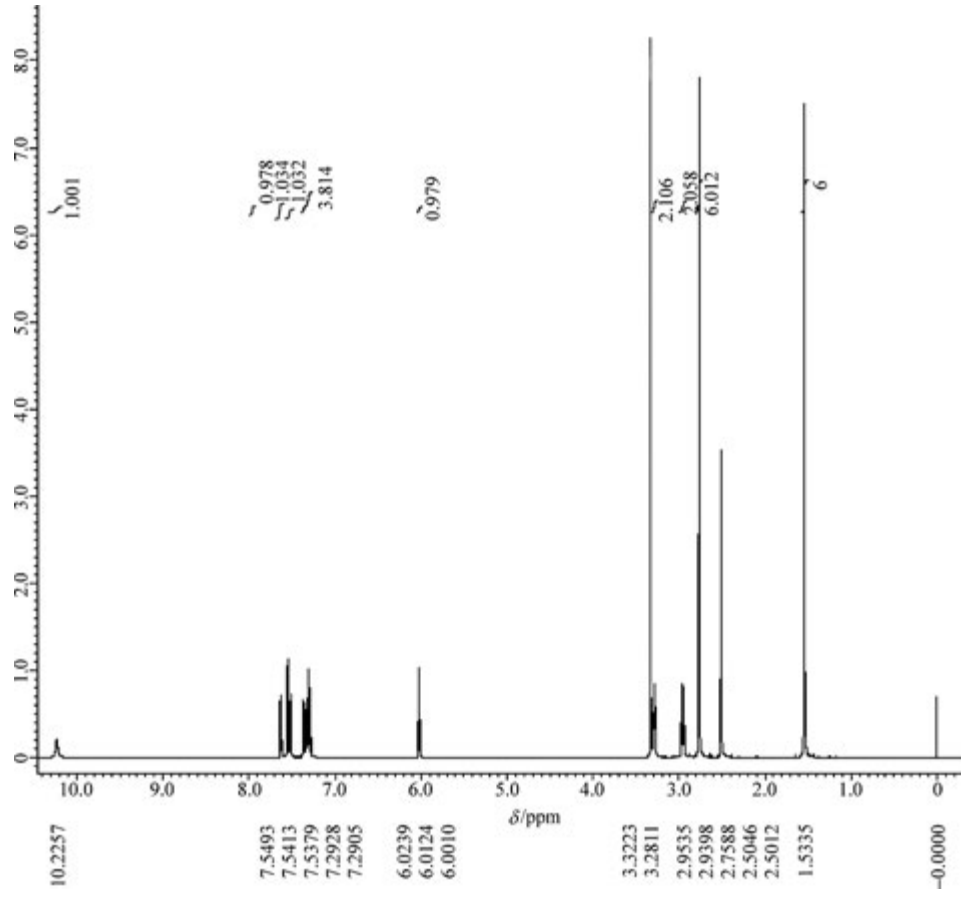


Figure 6.58 The ^{1}H spectrum of the compound

From the HMQC spectrum, it can be known that the two peaks at 42.6 and 28.9 ppm correspond to six hydrogen atoms, respectively, that is, these two peaks correspond to two methyl groups, respectively. Therefore, the compound should have 20 carbon atoms.

From the locally enlarged spectrum of the HMBC spectrum, a small peak besides the solvent peaks, which has a correlated peak, can be seen. Therefore, we know that a peak of the compound hides in the solvent peaks. Because this peak has no correlated peak in the HSQC spectrum, it belongs to a quaternary carbon atom.

To sum up, this compound contains 21 carbon atoms, which coincides with the structural formula.

By using the ^{13}C spectrum, the ^{1}H spectrum, the DEPT spectrum, the HMQC spectrum, and the HMBC spectrum, the data of the ^{13}C spectrum and the ^{1}H spectrum can be listed in Table 6.27.

Because the peak sets in the aromatic region of the ^{1}H spectrum are closed even if overlapped, some rows in Table 6.27 are accomplished with the help of the locally enlarged spectrum of the ^{1}H spectrum, which gives precise chemical shift values.

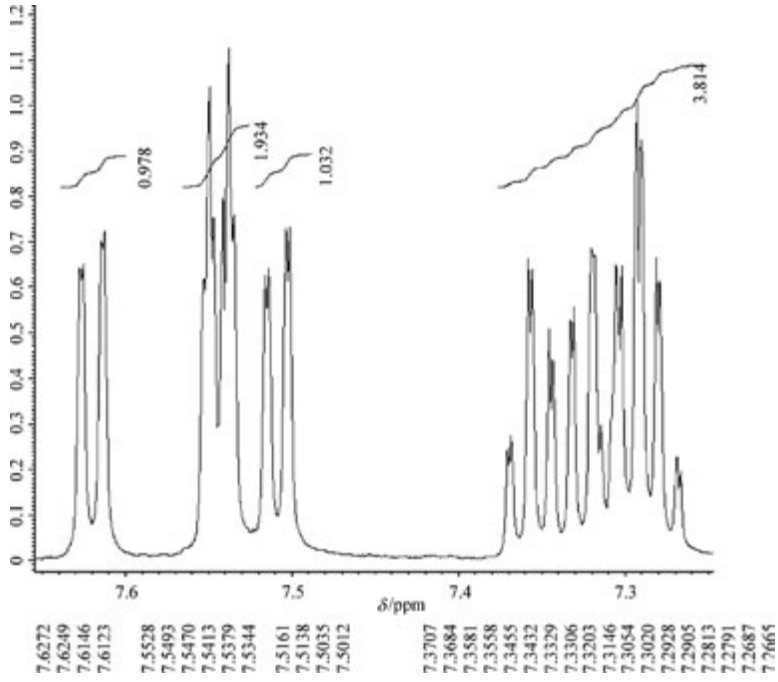


Figure 6.59 The locally enlarged spectrum of Figure 6.58 (in the low field region)

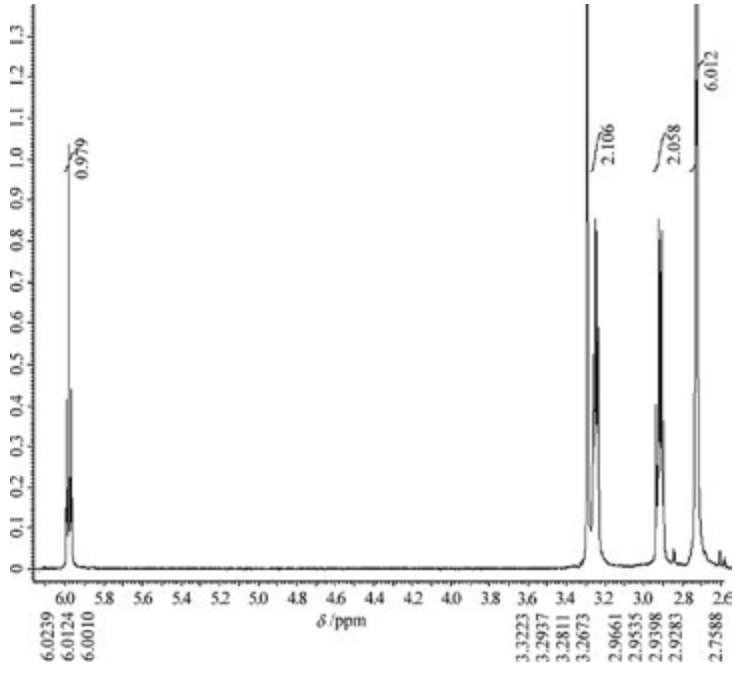


Figure 6.60 The locally enlarged spectrum of Figure 6.58 (in the high field region)

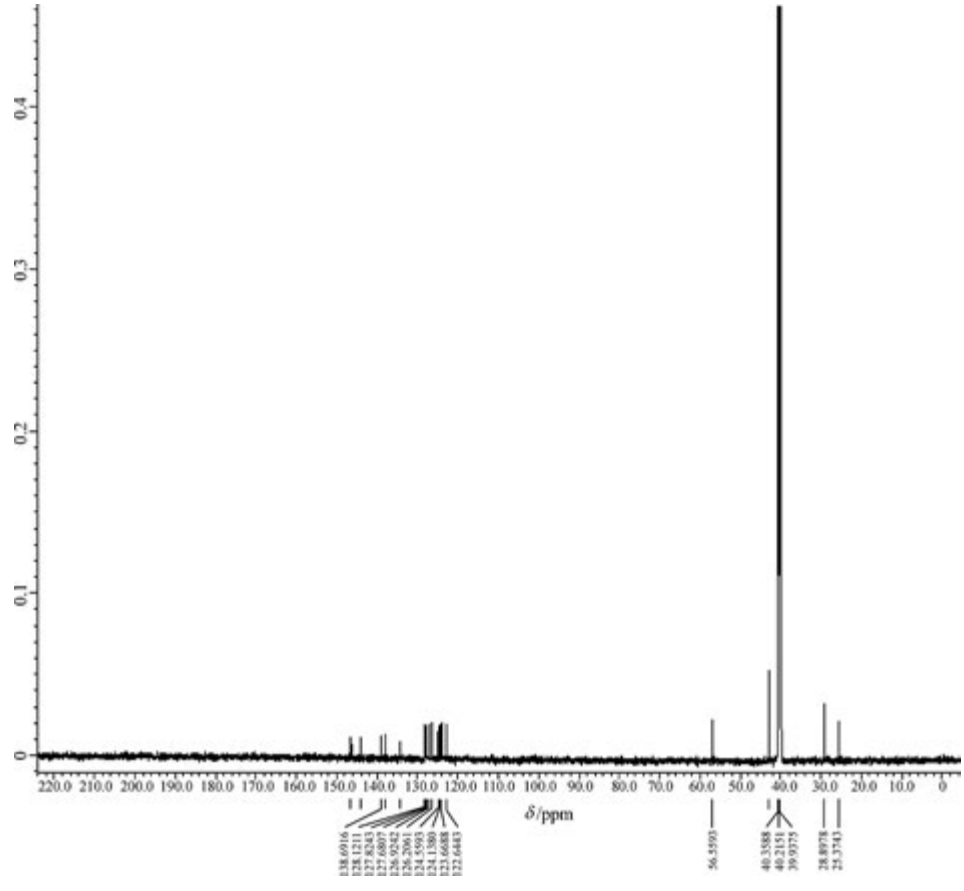


Figure 6.61 The ^{13}C spectrum of the compound

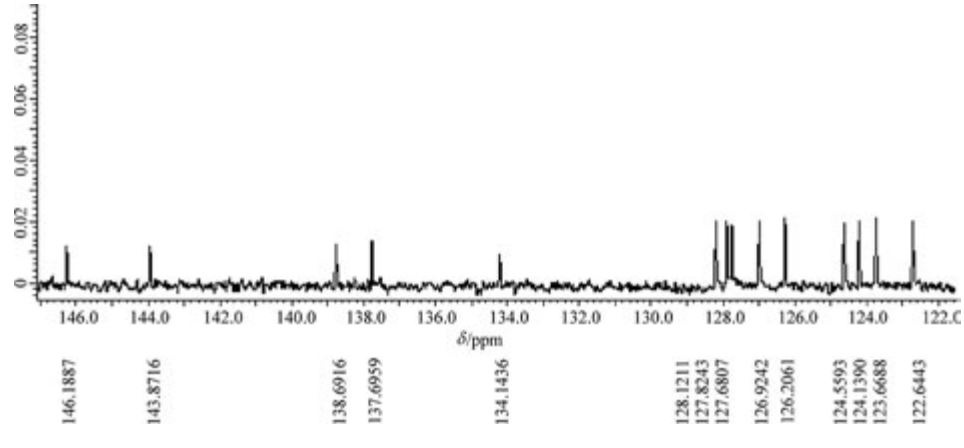


Figure 6.62 The locally enlarged spectrum of Figure 6.61 (in the low field region)

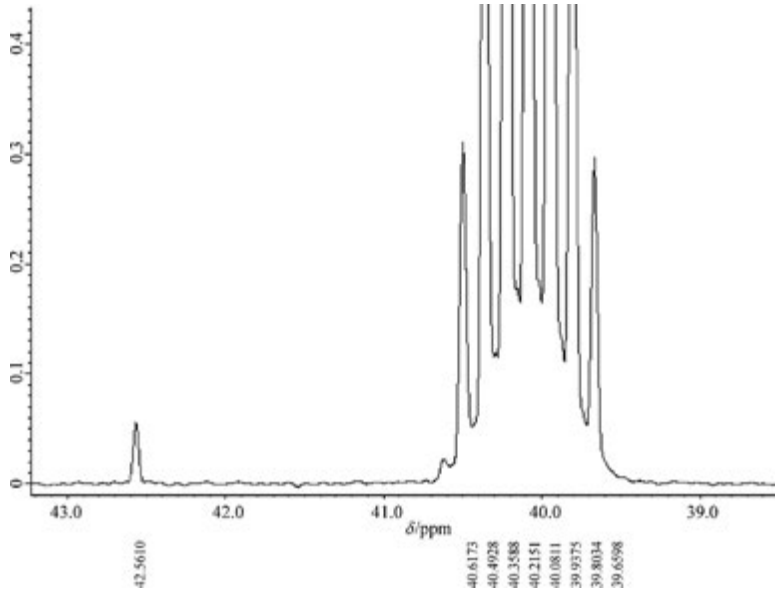


Figure 6.63 The locally enlarged spectrum of Figure 6.61 (in the high field region)

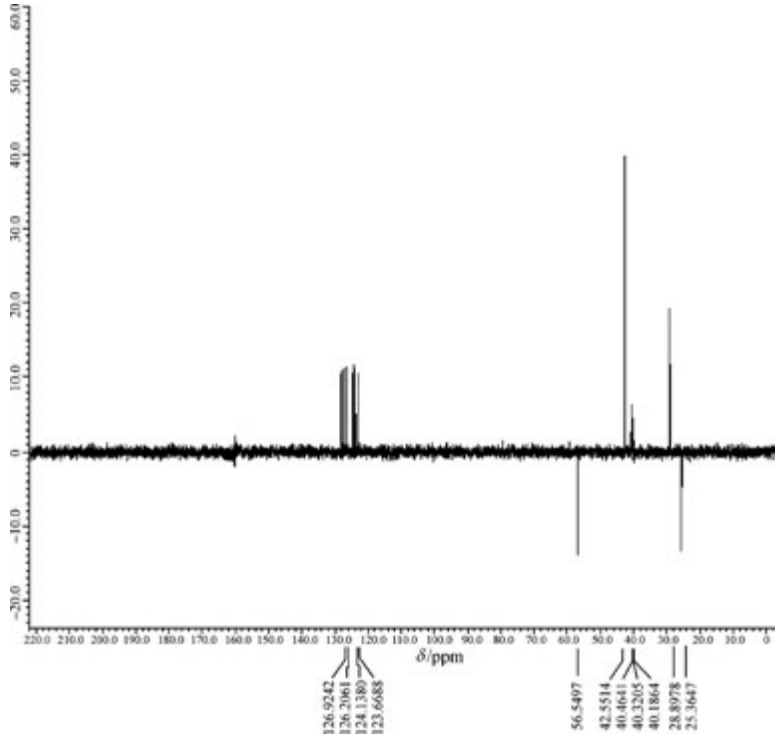


Figure 6.64 The DEPT spectrum of the compound

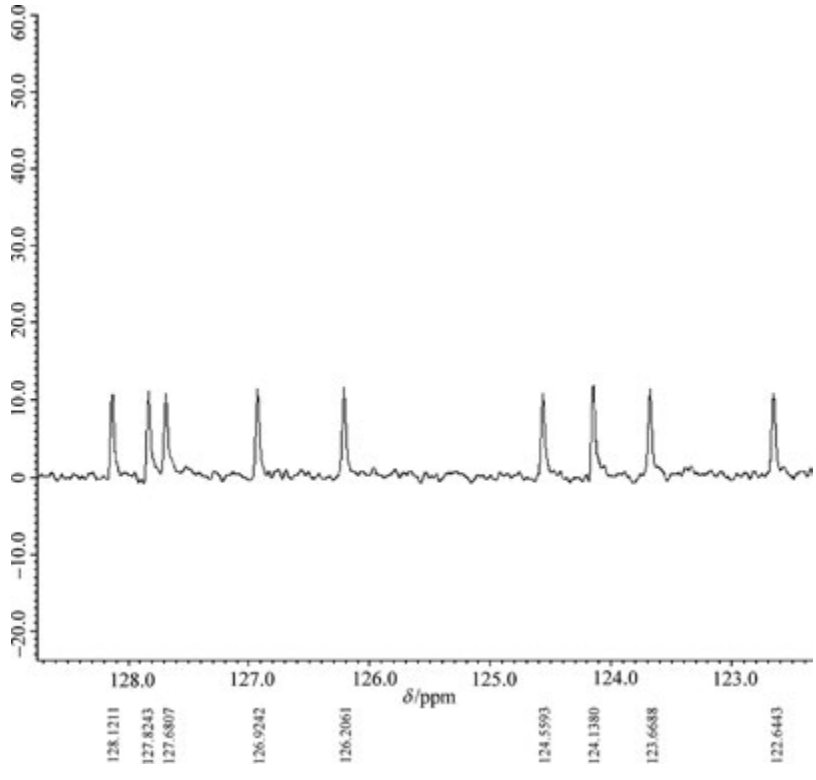


Figure 6.65 The locally enlarged spectrum of Figure 6.63 (in the low field region)

Now we will interpret the COSY spectrum.

Although all NMR spectra are measured by the NMR spectrometer with a frequency of 600 MHz, some correlated peaks in the COSY spectrum are still not clear. They need be read out carefully. In addition, the HMBC spectrum, in which the centers of correlated peaks are shown precisely, can be used to resolve close correlated peaks in the COSY spectrum.

The data summarized from the COSY spectrum are listed in Table 6.28.

From Table 6.28 it can be known that the compound contains two aromatic systems. (The aromatic systems can be differentiated from double bonds by their chemical shift values.) Combining the HMQC spectrum, we have:

$$124.1(7.62)-128.1(7.36)-126.2(7.32)-127.8(7.51),$$
$$124.6(7.55)-126.9(7.28)-127.7(7.29)-123.7(7.54),$$

and a chain of $122.6(6.01)-25.4(2.94)-56.6(3.28)$, where $\delta_H = 6.01$ should belong to an alkene hydrogen atom.

The data summarized from the HMBC spectrum are listed in Table 6.29.

When we read the data of 2D NMR spectra, to read the chemical shift values of correlated peaks according to their relative positions in a 2D NMR spectrum is better than to read the data directly from the abscissa. For example, to read the data in the region of

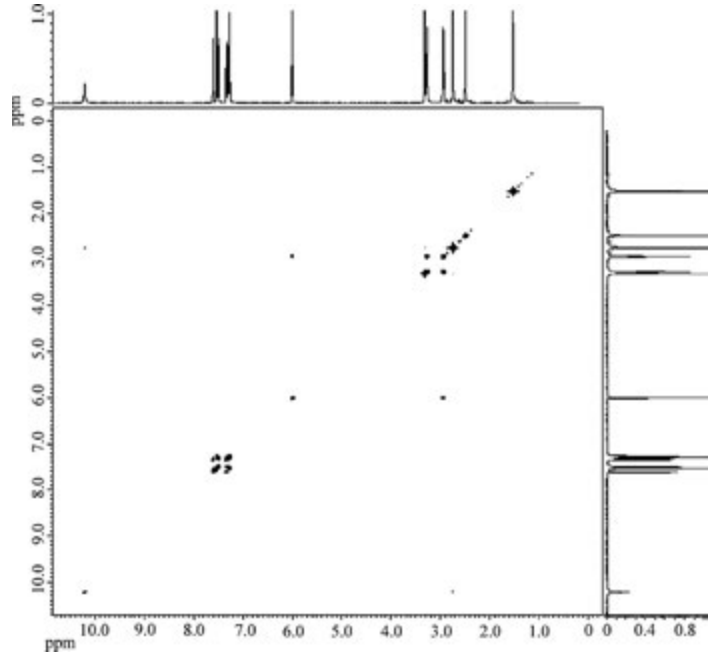


Figure 6.66 The COSY spectrum of the compound

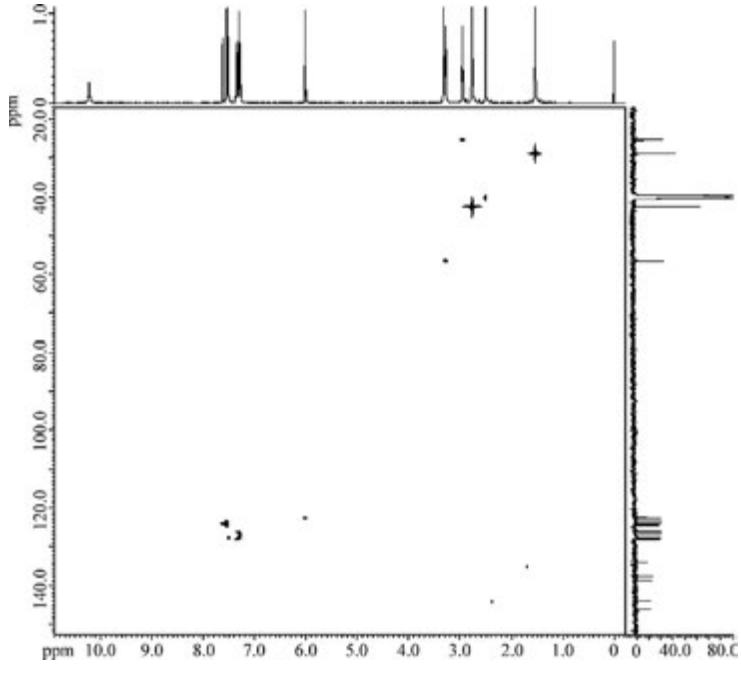


Figure 6.67 The HMQC spectrum of the compound

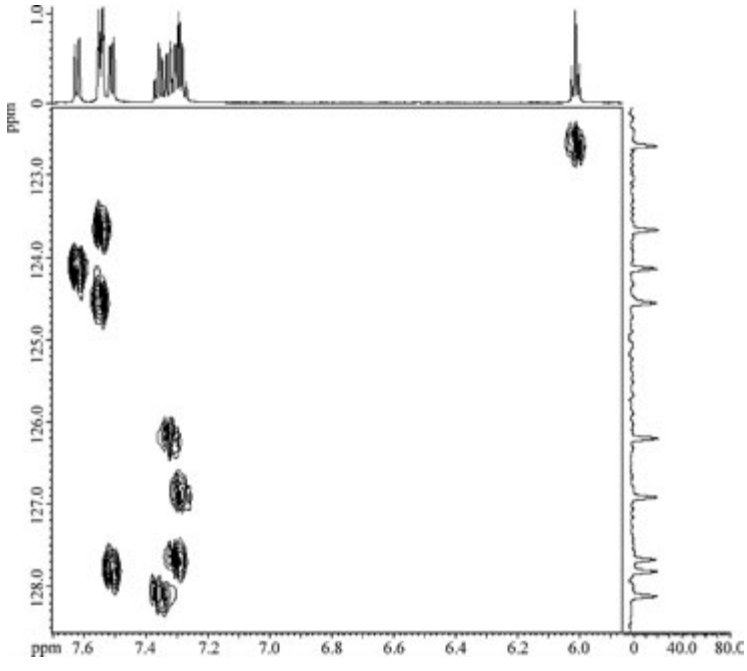


Figure 6.68 The locally enlarged spectrum of Figure 6.67 (in the low field region)

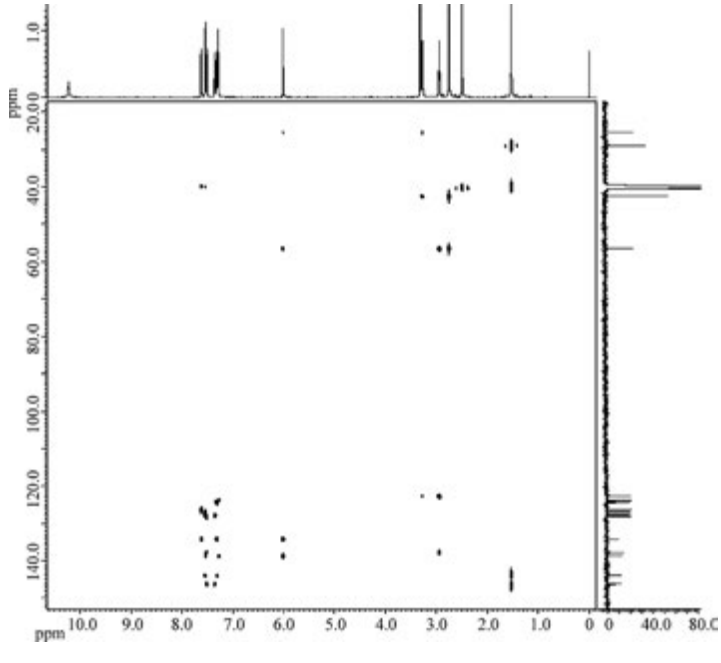


Figure 6.69 The HMBC spectrum of the compound

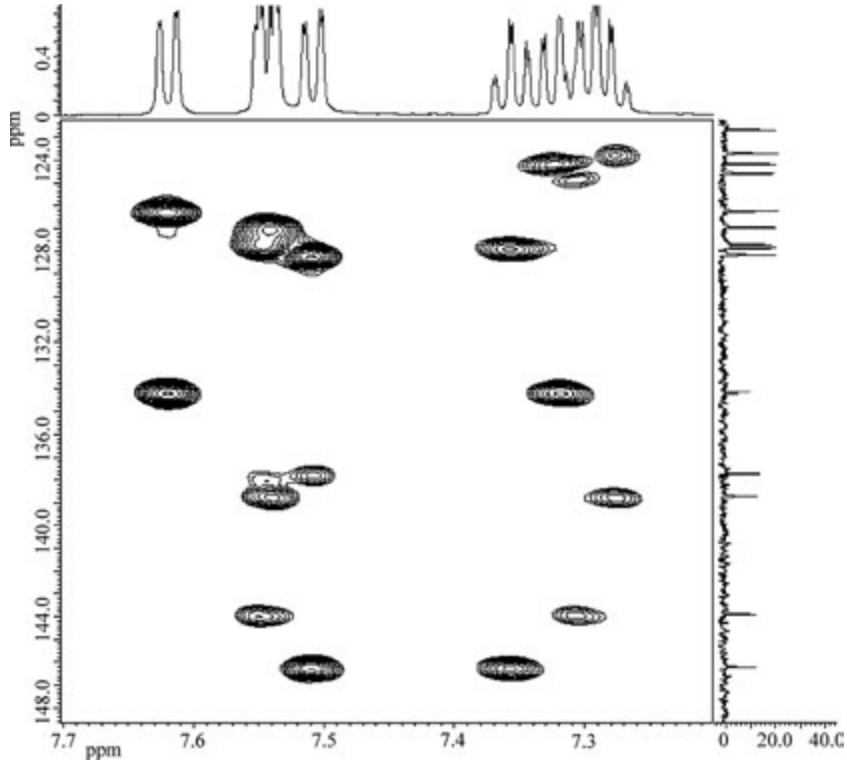
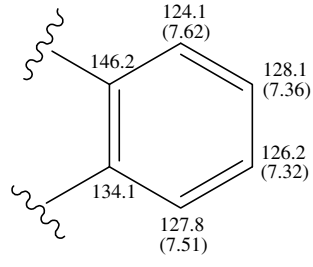


Figure 6.70 The locally enlarged spectrum of Figure 6.69 (in the low field region)

$F_2 = 7.26$–7.37 ppm of the HMBC spectrum can introduce errors. However, if we divide this region into four columns, which correspond to the four peak sets in the region of the ^1H spectrum, and we read the chemical shift values of the correlated peaks according to these columns, the results should be precise.

Now we will deduce structural units and assemble them.

We start from the first aromatic system. From Nos. 1 and 5 of Table 6.29, and from the fact that the correlated peaks across three chemical bonds have strong intensities, we obtain the following structural unit:



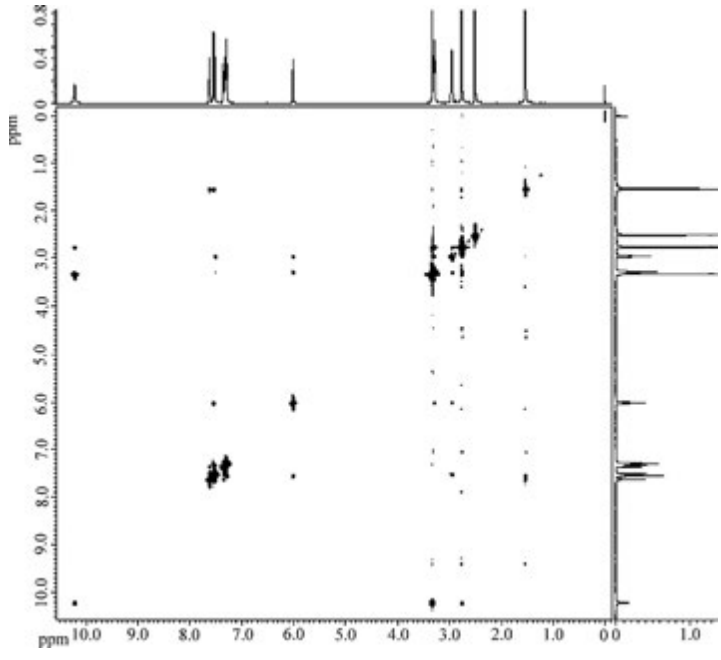


Figure 6.71 The NOESY spectrum of the compound

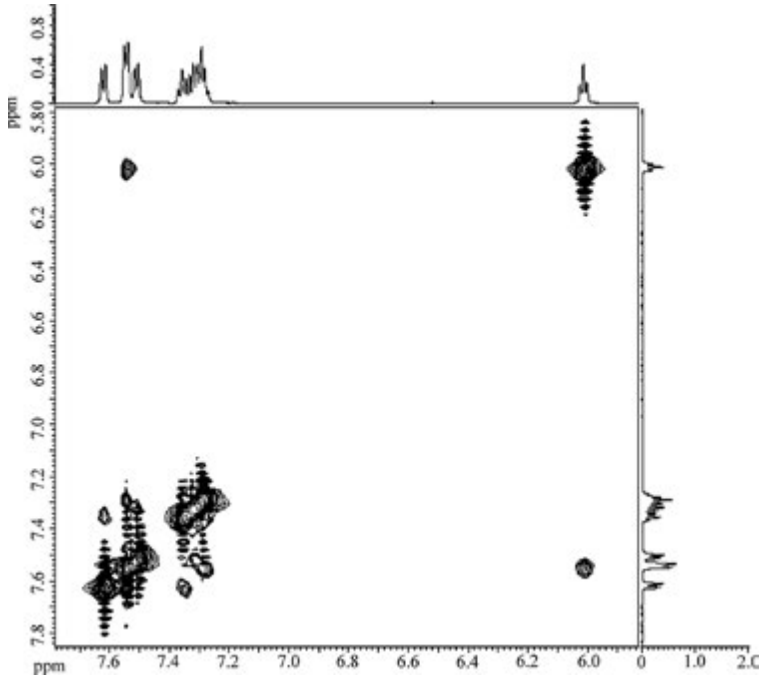


Figure 6.72 The locally enlarged spectrum of Figure 6.71 (in the low field region)

Table 6.27 The data of the ^{13}C spectrum and the ^{1}H spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atoms	δ_H (ppm)	Number of H atoms	Peak shape
1	146.2	1	C			
2	143.9	1	C			
3	138.7	1	C			
4	137.7	1	C			
5	134.1	1	C			
6	128.1	1	CH	7.36	1	t, d
7	127.8	1	CH	7.51	1	d, d
8	127.7	1	CH	7.29	1	t, d
9	126.9	1	CH	7.28	1	t, d
10	126.2	1	CH	7.32	1	t, d
11	124.6	1	CH	7.55	1	m
12	124.1	1	CH	7.62	1	d, d
13	123.7	1	CH	7.54	1	m
14	122.6	1	CH	6.01	1	t
15	56.6	1	CH_2	3.28	2	m
16	42.6	1	CH_3	2.76	6	s
17	40.6	1	C			
18	28.9	2	CH_3	1.53	6	s
19	25.4	1	CH_2	2.94	2	m

Table 6.28 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)
1	1.53	isolated
2	2.76	isolated
3	2.94	6.01, 3.28
4	3.28	2.94
5	6.01	2.94
6	7.28	7.55, 7.29
7	7.29	7.54, 7.28
8	7.32	7.51, 7.36
9	7.36	7.62, 7.32
10	7.51	7.33
11	7.54	7.29
12	7.55	7.28
13	7.62	7.36

Similarly, from the second aromatic system mentioned above, according to Nos. 2 and 3 of Table 6.29, and from the fact that the correlated peaks across three chemical bonds have strong intensities, we obtain the following structural unit:

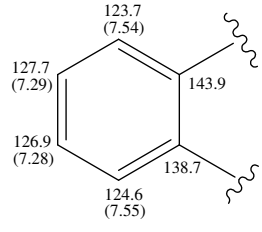
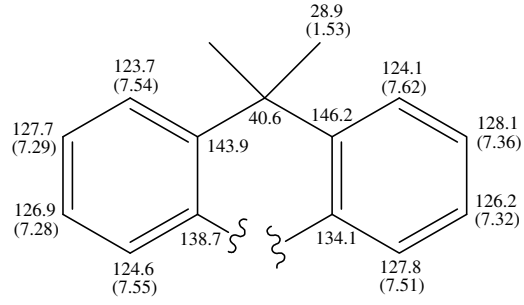


Table 6.29 The data summarized from the HMBC spectrum

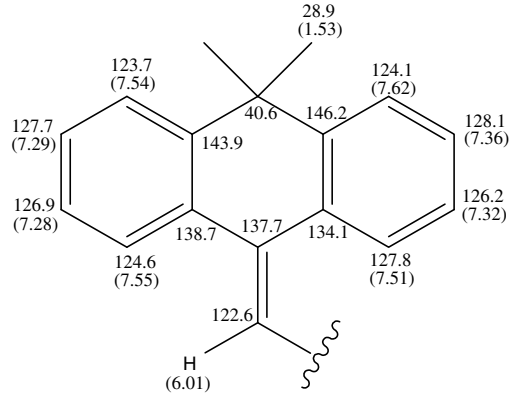
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	146.2	7.51, 7.36, 1.53	
2	143.9	7.55, 7.29, 1.53	
3	138.7	7.54, 7.28, 6.01	
4	137.7	7.51, 2.94	
5	134.1	7.62, 7.32, 6.01	
6	128.1	7.51	
7	127.8	7.36	
8	127.7	7.55	
9	126.9	7.54	
10	126.2	7.62	
11	124.6		
12	124.1	7.32	
13	123.7	7.28	
14	122.6	3.28, 2.94	
15	56.6	5.95, 2.94, 2.76	
16	42.6	3.28	2.76[a]
17	40.6	1.53	
18	28.9		1.53
19	25.4	5.95, 3.28	

[a] Indicate that it is not a typical peak set for the ^1J coupling. However, it can be determined from the HMQC spectrum.

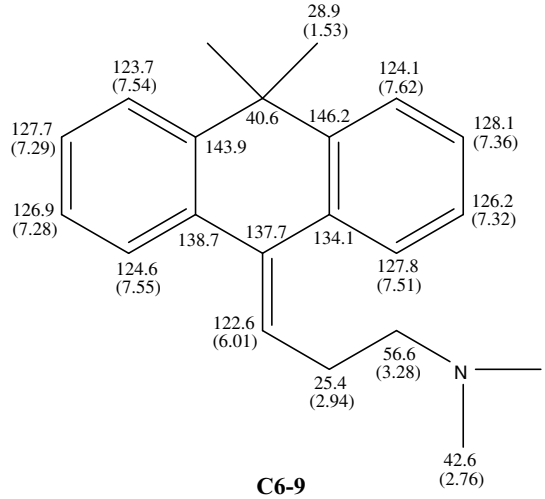
According to Nos. 1, 2, and 17 of Table 6.29, that is, the correlations of δ_C(146.2)/δ_H(1.53), δ_C(143.9)/δ_H(1.53), and δ_C(40.6)/δ_H(1.53) ppm, the two aromatic systems can be connected through a quaternary carbon atom and two methyl groups.



According to Nos. 4, 5, and 3 of Table 6.29, that is, the correlations of δ_C(137.7)/δ_H(7.51), δ_C(134.1)/δ_H(6.01), and δ_C(138.7)/δ_H(6.01) ppm, the structural unit mentioned above can be extended as follows:



From the third structural unit obtained from the COSY spectrum, according to No. 16 of Table 6.29, that is, the correlation of $\delta_C(42.6)/\delta_H(3.28)$ ppm, we obtain the whole structure of the compound.



C6-9

In fact, the structure mentioned above leaves a question, that is, the direction of the side chain. Although the two aromatic systems are symmetrical, the direction of the side chain will affect the chemical shift values of related aromatic carbon atoms and aromatic hydrogen atoms.

From two NOESY spectra, we have found the following correlations of δ_H (ppm) 2.94/7.51, and 6.01/7.55 ppm. Therefore, the structure drawn above is correct.

The peak situated in the lowest field in the ^1H spectrum is that of the reactive hydrogen atom of the hydrochloric acid.

To sum up, the anticipated structure is correct.

Example 6.9 The EI mass spectrum, the IR spectrum (in the high wavenumber region), the IR spectrum (in the low wavenumber region), the ^1H spectrum, the locally enlarged ^1H spectrum (in the low field region), the ^{13}C spectrum, the locally enlarged ^{13}C spectrum (in

the low field region), the DEPT-135 spectrum, the COSY spectrum, the locally enlarged COSY spectrum (in the low field region), the HMQC spectrum, the locally enlarged HMQC spectrum (in the low field region), the HMBC spectrum, and the locally enlarged HMBC spectrum (in the low field region) of an unknown compound are shown in Figures 6.73–6.86. Try to deduce the structure of the unknown compound. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is $CDCl_3$.

Solution

The peak with the largest mass-to-charge ratio in the mass spectrum is situated at *m/z* 253. The next peak is situated at *m/z* 238. The mass difference between these two ions is 15 (u), which manifests a reasonable loss of a neutral fragment. Therefore, the peak at *m/z* 253 can be considered tentatively as the molecular ion peak. Because the molecular weight is an odd number, the unknown compound should contain an odd number of nitrogen atoms.

The peak at 7.26 ppm in the ^{1}H spectrum is the solvent peak. Although the peak at 1.25 ppm in the ^{1}H spectrum has an integral value of 0.73, it can be determined as an impurity peak from the very low intensities of its related cross peaks in the HMQC spectrum. Other peaks with low intensities in the ^{1}H spectrum can be determined as impurity peaks. From integral values of all peak sets in the ^{1}H spectrum it can be known that the unknown compound contains 15 hydrogen atoms.

The peaks in the low field region of the ^{13}C spectrum are not shown clearly. Therefore, the inspection of the locally enlarged ^{13}C spectrum is necessary. There are small peaks besides every normal peak with the same distance between the normal peak and related small peak. These small peaks are not signals of the sample, and they may be produced by the drift of the magnetic field of the spectrometer.

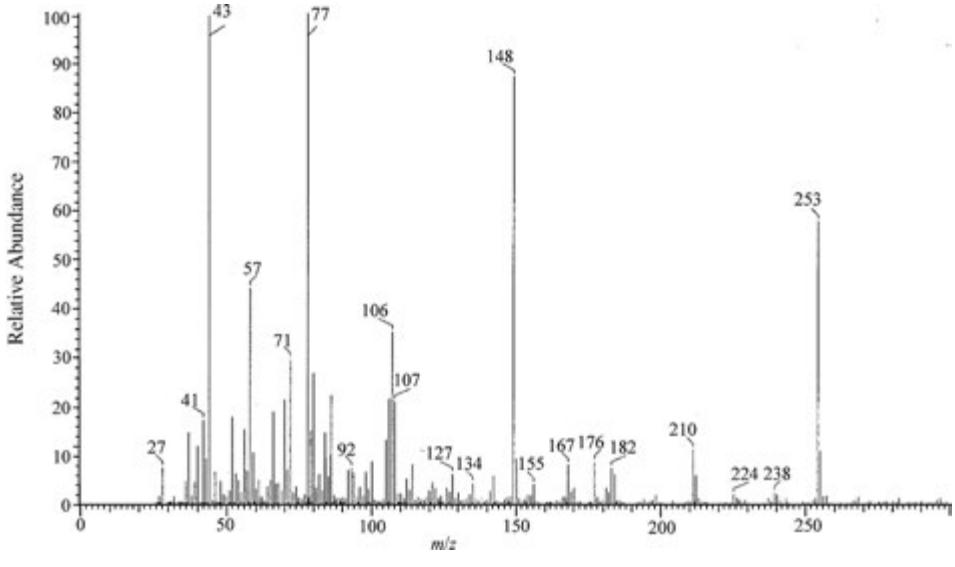


Figure 6.73 The EI mass spectrum of the unknown compound

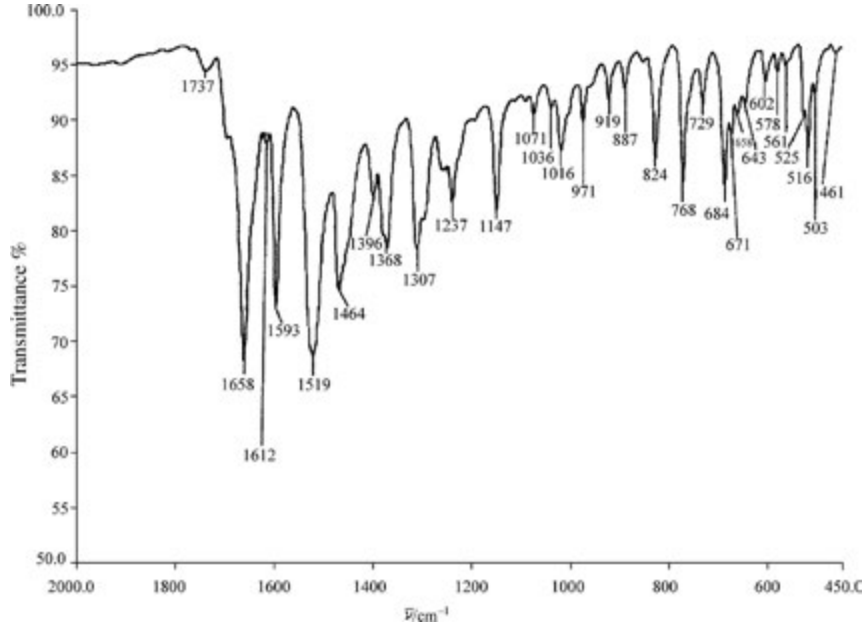


Figure 6.74 The IR spectrum (in the high wavenumber region)

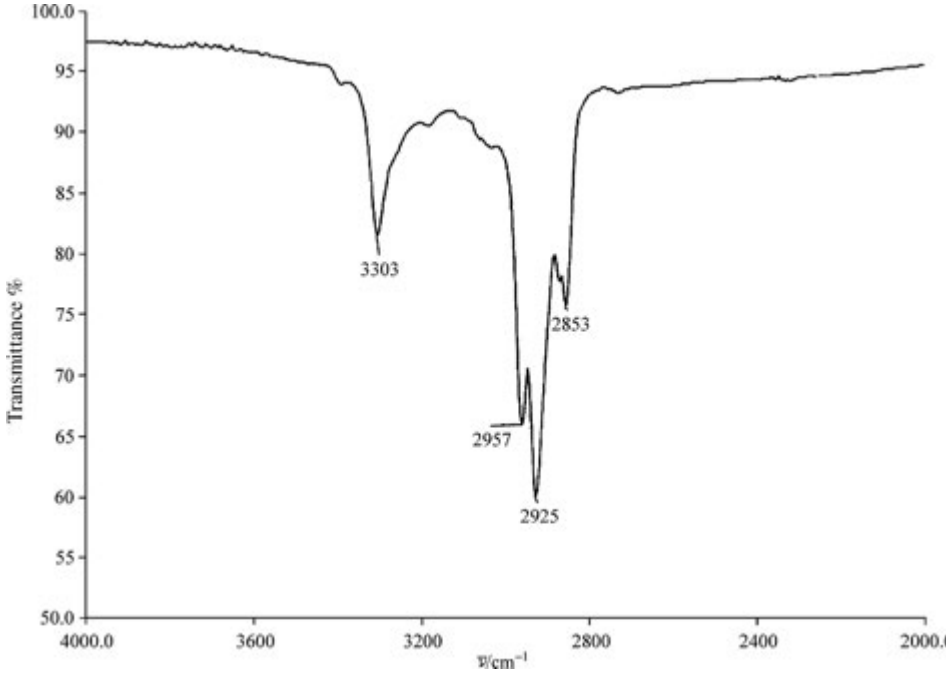


Figure 6.75 The IR spectrum (in the low wavenumber region)

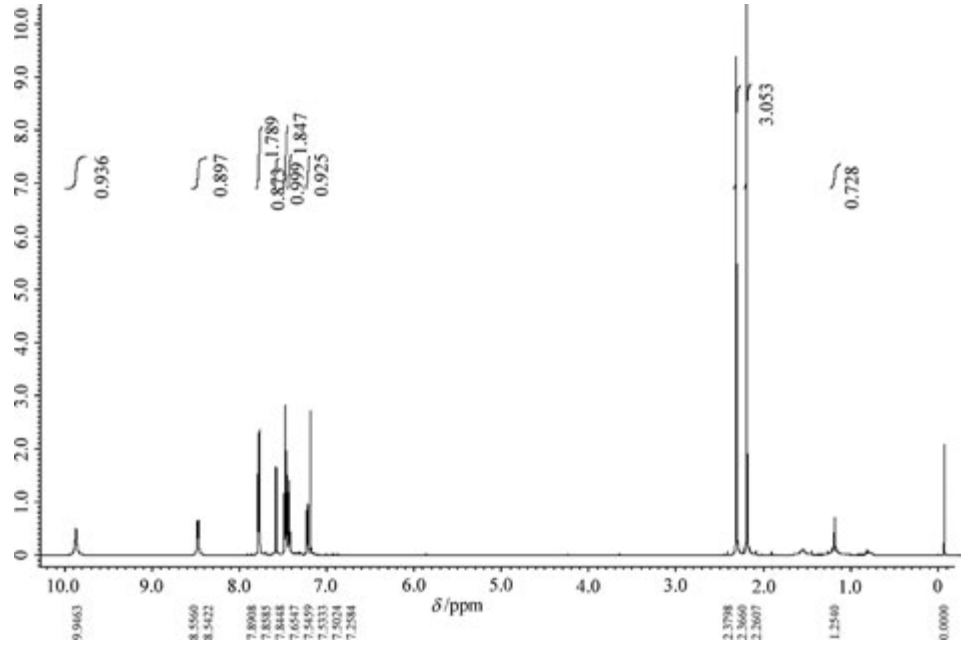


Figure 6.76 The ^{1}H spectrum of the unknown compound

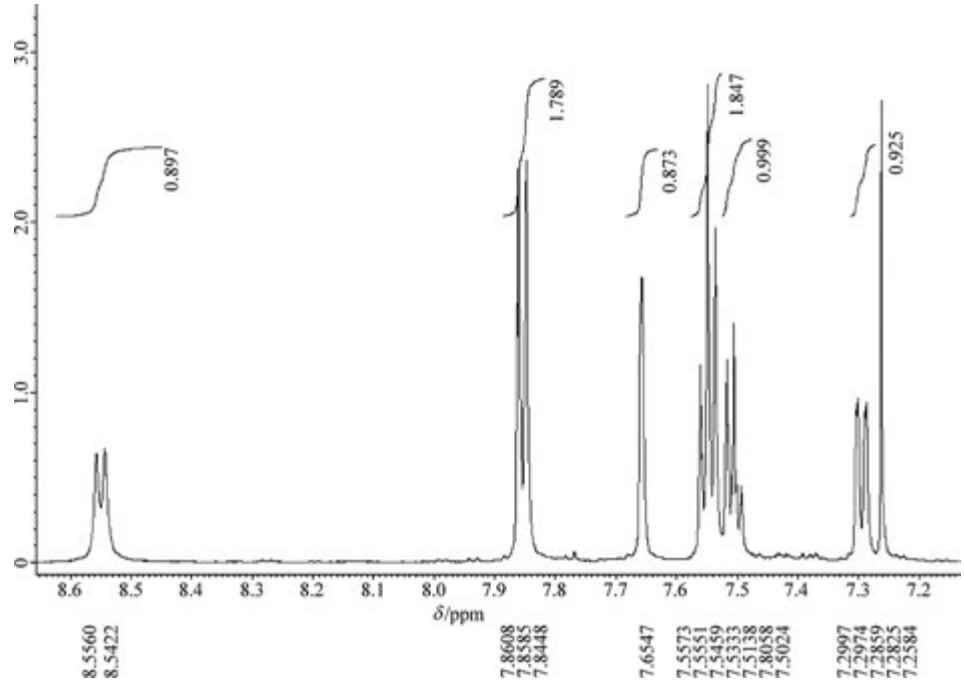


Figure 6.77 The locally enlarged ^{1}H spectrum (in the low field region)

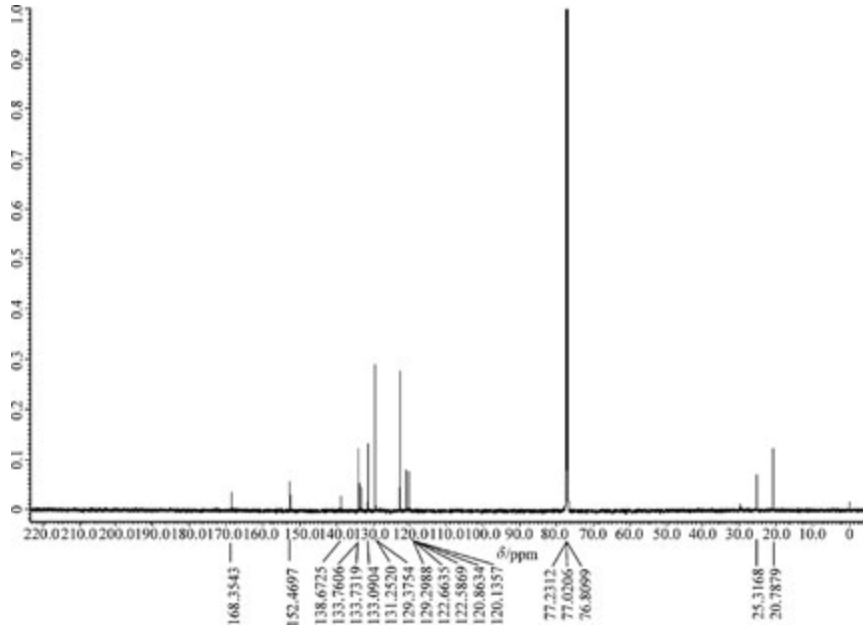


Figure 6.78 The ^{13}C spectrum of the unknown compound

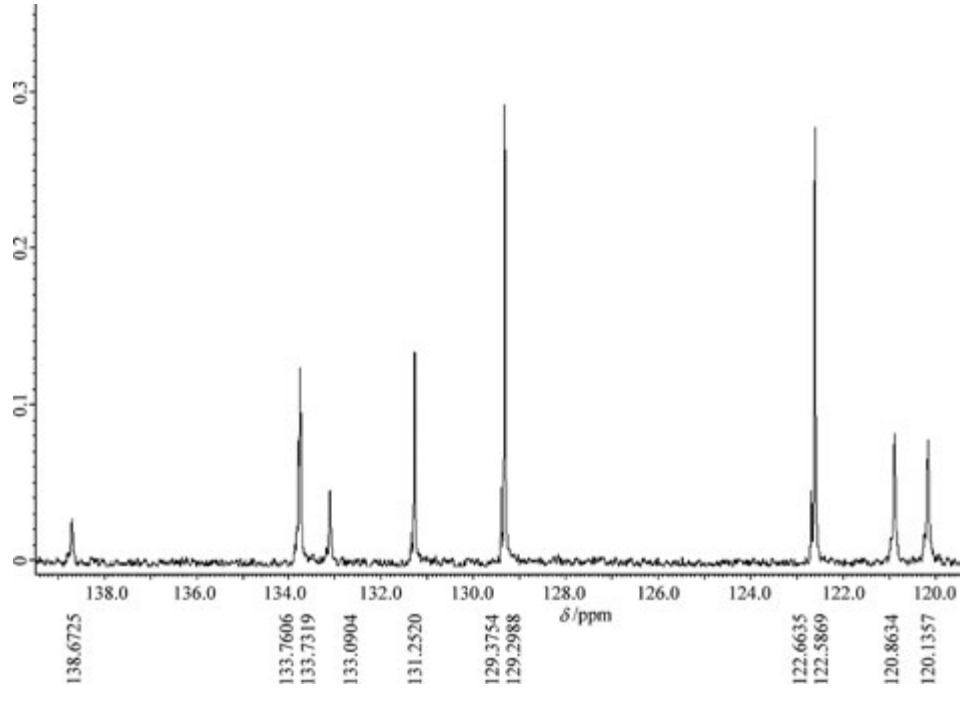


Figure 6.79 The locally enlarged ^{13}C spectrum (in the low field region)

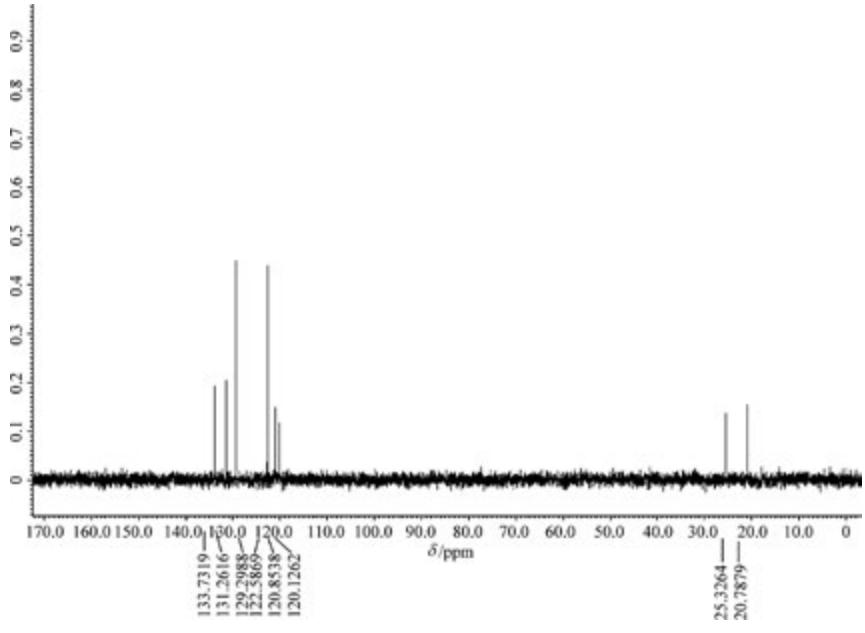


Figure 6.80 The DEPT-135 spectrum of the unknown compound

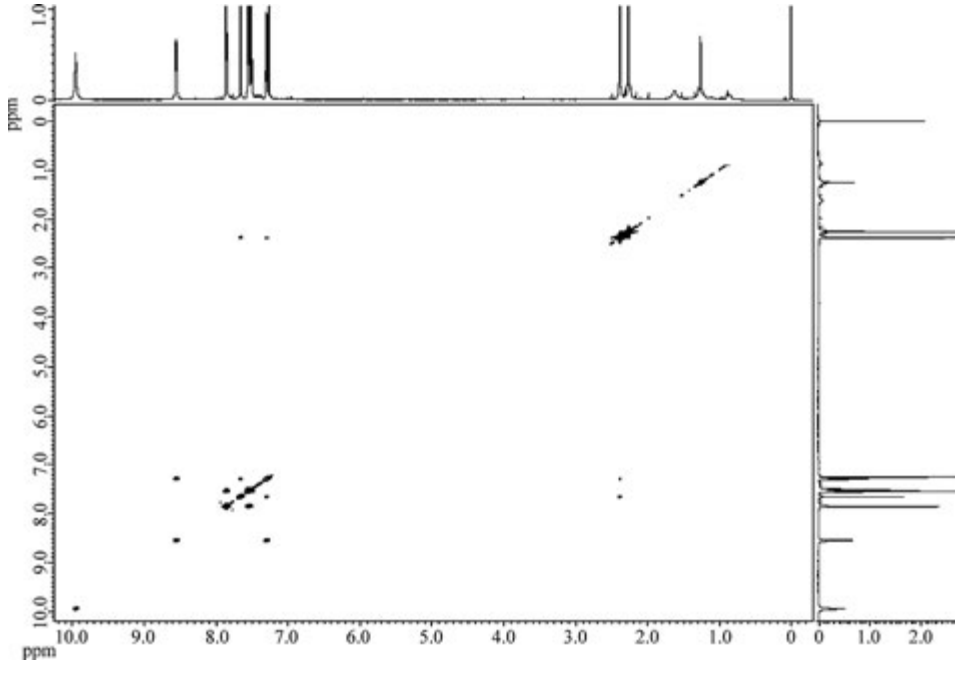


Figure 6.81 The COSY spectrum of the unknown compound

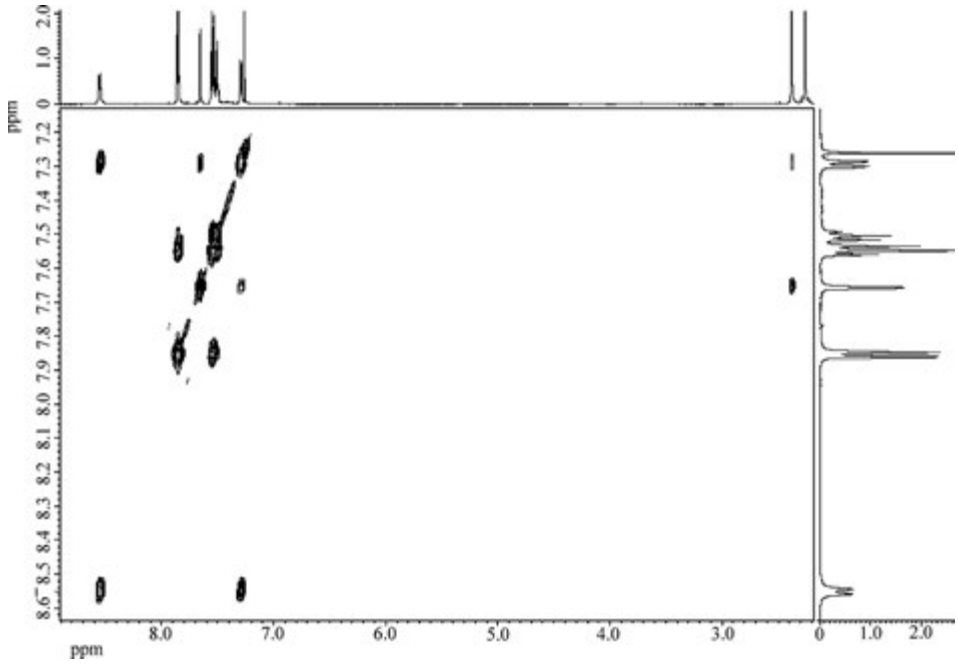


Figure 6.82 The locally enlarged COSY spectrum (in the low field region)

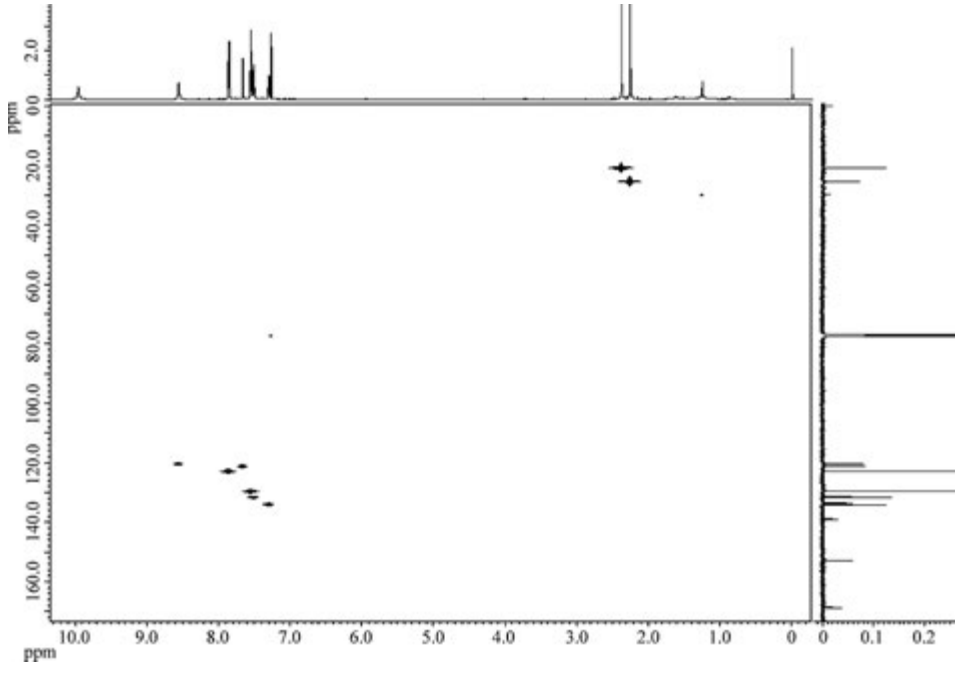


Figure 6.83 The HMQC spectrum of the unknown compound

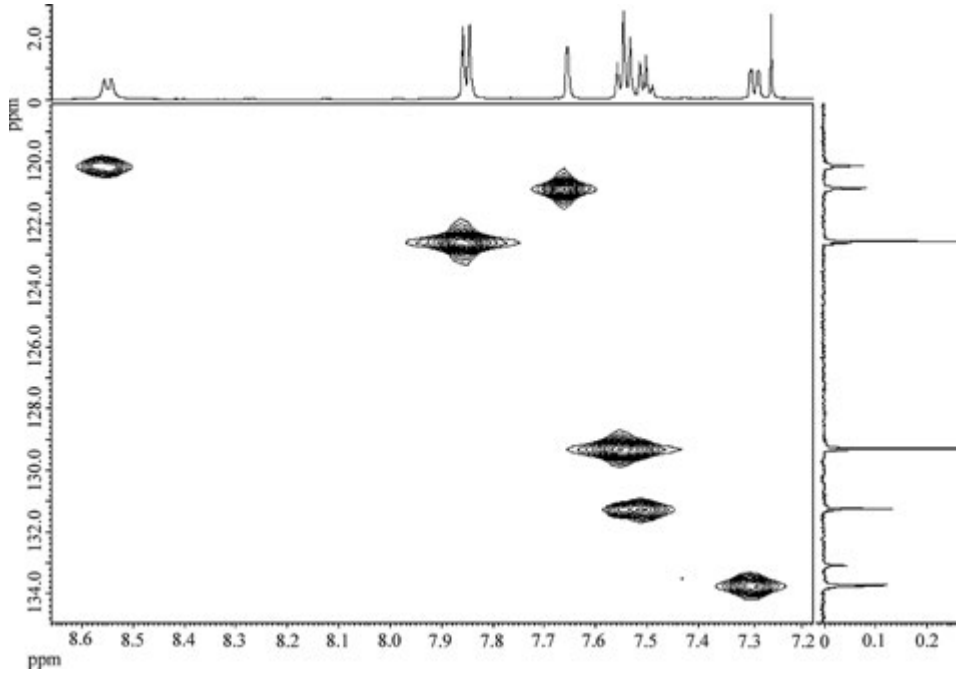


Figure 6.84 The locally enlarged HMQC spectrum (in the low field region)

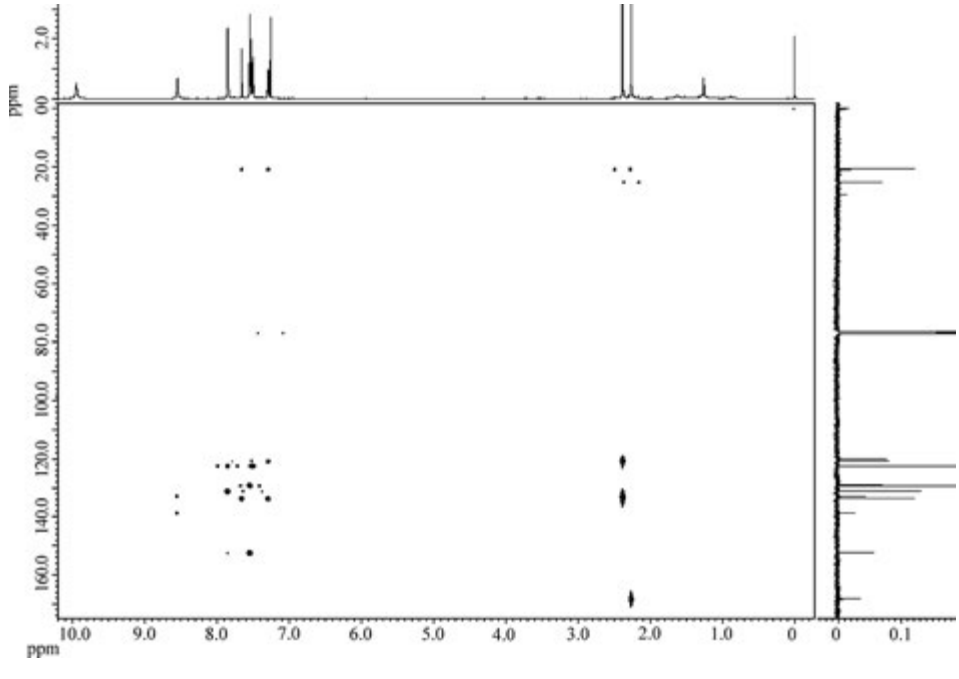


Figure 6.85 The HMBC spectrum of the unknown compound

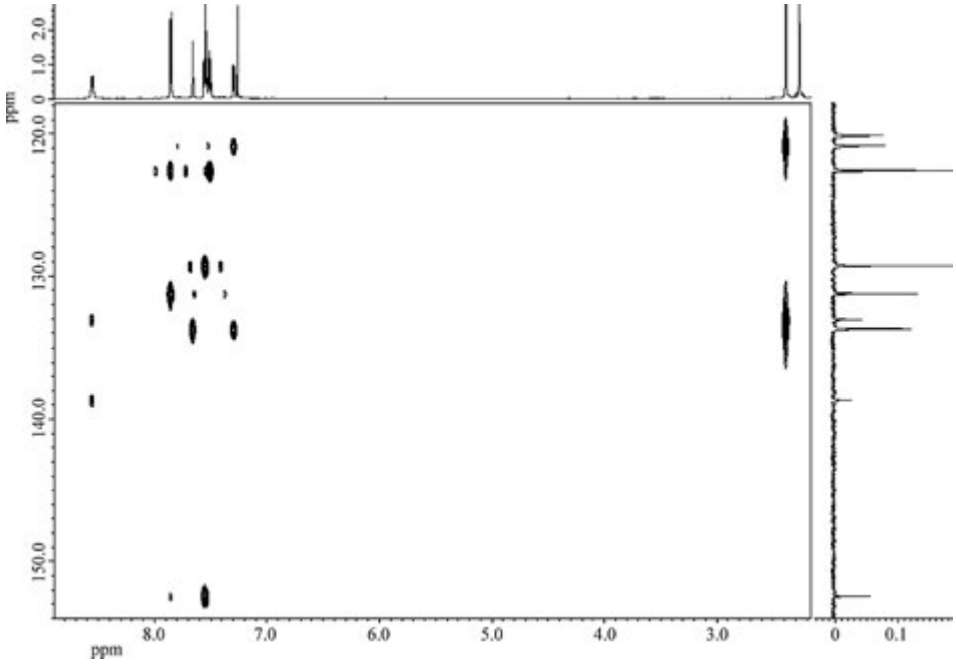


Figure 6.86 The locally enlarged HMBC spectrum (in the low field region)

The peak at 133.76 ppm is a strong peak, which can not be considered as a drift peak. Therefore, it should be one of signals. The two peaks at 122.6 and 129.3 ppm have prominent intensities, which correspond two carbon atoms, respectively. Therefore, the 13 peaks in the ^{13}C spectrum correspond to 15 carbon atoms.

The peak at 168.35 ppm, which is situated at the lowest field position, should be the signal of a carbonyl group from its chemical shift value.

To sum up, this unknown compound contains 15 hydrogen atoms, 15 carbon atoms, and 1 oxygen atom. The addition of the masses of the elemental composition mentioned above is 211 (u), the difference between which and the molecular weight of the unknown compound is 42 (u).

Because the unknown compound should contain an odd number of nitrogen atoms, the mass difference of 42 just corresponds to three nitrogen atoms. Therefore, the molecular formula of the unknown compound is $C_{15}H_{15}ON_3$, from which its unsaturation number of 10 can be calculated. The value of 10 is a rather large number.

Now we will interpret the ^1H spectrum.

The peak shapes in the ^1H spectrum are simple so that they can be easily analyzed.

The data summarized from the ^1H spectrum are listed in Table 6.30.

According to Table 6.30, the related data of Table 6.31 can be summarized with the help of the HSQC spectrum and the DEPT spectrum.

The data summarized from the COSY spectrum are listed in Table 6.32.

The data summarized from the HMBC spectrum are listed in Table 6.33.

Tables 6.30–6.33 contain all NMR information.

Table 6.30 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape	Remarks[a] (^{3}J, Hz)
2.26	3	s	
2.37	3	s	
7.29	1	d	8.28
7.51	1	t	6.84
7.55	2	t	7.56
7.65	1	s	
7.85	2	d	8.22
8.55	1	d	8.28
9.95	1	s	

[a] Indicates that because ^{3}J values read from the ^{1}H spectrum are not precise, the couplings between peak sets are determined mainly by the COSY spectrum.

Table 6.31 The attribution of the signals of the ^{1}H spectrum and the ^{13}C spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atoms	Directly connected H atoms, δ_H(ppm)
1	20.78	1	CH_3	2.37
2	25.32	1	CH_3	2.26
3	120.14	1	CH	8.55
4	120.86	1	CH	7.65
5	122.59	2	CH	7.85
6	129.29	2	CH	7.55
7	131.25	1	CH	7.51
8	133.09	1	C	
9	133.73	1	CH	7.29
10	133.76	1	C	
11	138.67	1	C	
12	153.47	1	C	
13	168.35	1	C	

Table 6.32 The data summarized from the COSY spectrum

No.	δ_H(ppm)	Coupled H atoms, ppm
1	2.26	
2	2.37	(7.29), (7.65)
3	7.29	(2.37), (7.65), 8.55
4	7.51	7.55
5	7.55	7.51, 7.85
6	7.65	(2.37), (7.29)
7	7.85	7.55
8	8.55	7.29
9	9.95	isolated

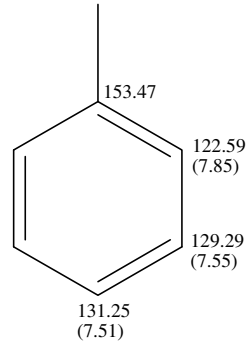
Parenthesis () stands for weak couplings.

Table 6.33 The data summarized from the HMBC spectrum

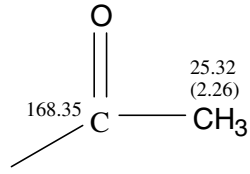
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	20.78	7.29, 7.65	2.37
2	25.32		2.26
3	120.14		
4	120.86	2.37, 7.29	7.65
5	122.59	7.51, 7.55	7.85
6	129.29		7.55
7	131.25	7.85	7.51
8	133.09	2.37, (8.55)	
9, 10	133.73, 133.76	7.65	7.29
11	138.67	(8.55)	
12	153.47	7.55, (7.85)	
13	168.35	2.26	

Now we will postulate the structure of the unknown compound. We start from finding structural units.

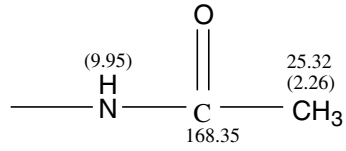
According to Nos. 4, 5, and 7 of Table 6.32, and Nos. 5, 7, and 12 of Table 6.33, we can find a mono-substituted benzene ring which follows:



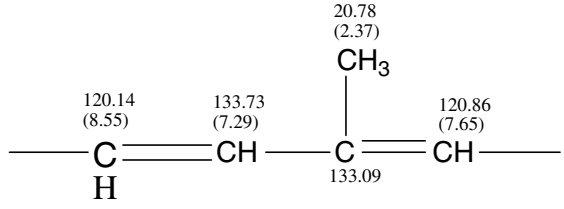
From No. 13 of Table 6.33, we can find another structural unit:



Because the carbonyl group has a rather small chemical shift value, this carbonyl group should connect with a heteroatom. If we consider the peak at 9.95 ppm, which has no correlated peak in the HMQC spectrum, is an NH, we can get the following extension of the structural unit mentioned above:



According to Nos. 2, 3, 6, and 8 of Table 6.32, we can find a conjugated system with four unsaturated carbon atoms. If we consider the two hydrogen atoms whose peaks are situated at 7.29 and 7.65 ppm have a small coupling constant, their coupling should stride over a quaternary carbon atom,, which is additionally proved by the weak couplings of δ_H (ppm) 2.37/7.29 and 2.37/7.65 in the COSY spectrum. Therefore, we have the following structural unit:

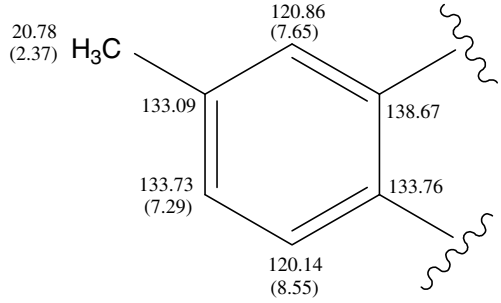


The elemental composition of the structural units mentioned above is $C_{13}H_{15}ON$ whose unsaturation number is 7, and the remaining elemental composition of the unknown compound is C_2N_2 whose unsaturation number is 3. Compared with Table 6.31, we know that the remaining two carbon atoms are quaternary carbon atoms.

We may first consider that the remaining two carbon atoms with the above-mentioned four unsaturated carbon atoms form a benzene ring, which is based on the following facts:

1. From Nos. 10 and 11 of Table 6.33, it can be known that these two peaks at 133.76 and 138.67 ppm have long-range couplings with the two peaks at 7.65 and 8.55 ppm, respectively. These two unsaturated hydrogen atoms belong to the third structural unit.
2. Because we miss the unsaturation number of 3 so far, it should be considered that the unknown compound contains another benzene ring. The formation of the second benzene ring will increase the unsaturation number by 2.

Therefore, the third structural unit can be extended as follows:



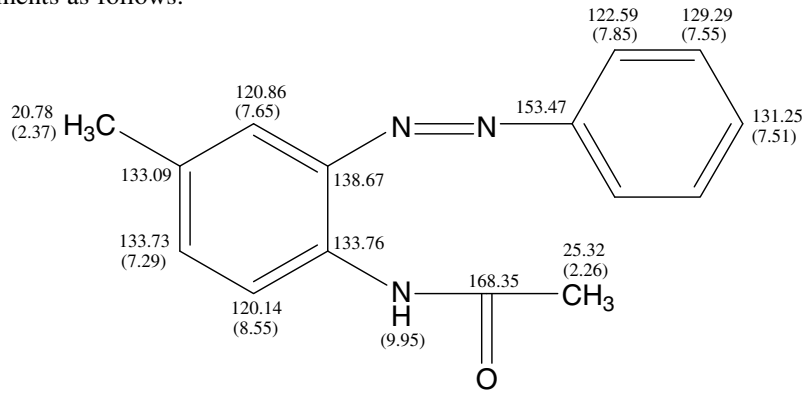
When we draw the structural unit mentioned above, we consider that the correlated peaks across three chemical bonds have strong intensities.

These two quaternary carbon atoms should connect with the other functional group, respectively.

The remaining elemental composition leaves only two nitrogen atoms, which can be considered as a diazo bond, because there is no other possible functional group. In addition, the chemical shift value of the quaternary carbon atom of the mono-substituted benzene ring coincides with that of the substitution of a benzene ring by a diazo bond.

According to the chemical shift values of the two carbon atoms of the third structural unit, the related substitutions can be determined.

To sum up, we obtain the structure of the unknown compound, and we complete its assignments as follows:



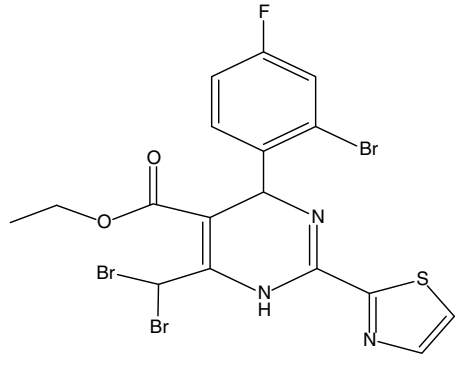
Now we will supplement the assignments about the IR spectrum:

3303 cm^{-1} represents the absorption of the NH group;
1658 cm^{-1} represents the absorption of the amide group;
1593 cm^{-1} represents the absorption of the benzene ring;
1519 cm^{-1} represents the absorption of the diazo group.

Now we will supplement the assignments about the EI mass spectrum:

m/z 210 stands for M^+–43($COCH_3$);
m/z 148 stands for M^+–107;
m/z 107 stands for $C_6H_5N_2H_2$.

Example 6.10 A synthesized compound has an anticipated structure which follows:



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Its ^{1}H spectrum, the locally enlarged spectrum of peak sets of the ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum (in the low field region), its DEPT-135 spectrum, its COSY spectrum, its HSQC spectrum, its HMBC spectrum, and the locally enlarged spectrum of the HMBC spectrum are shown in Figures 6.87–6.95, respectively. Try to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 400 MHz. The solvent used is $CDCl_3$.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The sample is of high purity, so that the peaks of impurities are very weak. The peak at 7.26 ppm is the solvent peak ($CDCl_3$).

The locally enlarged spectrum of peak sets of the ^{1}H spectrum shows clearly the shapes of the peak sets, from which coupling constants can be read out. The related data are listed in Table 6.34.

The following two facts should be noticed when we interpretate the ^{13}C spectrum.

First, because this ^{13}C spectrum is not a quantitative ^{13}C spectrum, and furthermore the widths of the peaks of the ^{13}C spectrum are different, the heights of the peaks are obviously different.

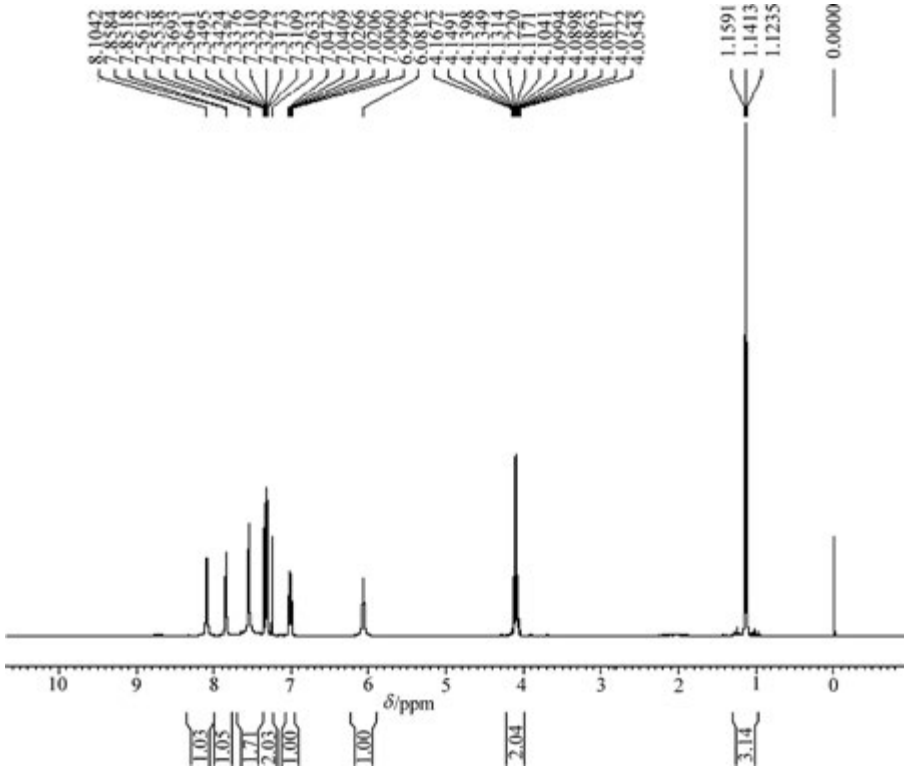


Figure 6.87 The ^{1}H spectrum of the compound

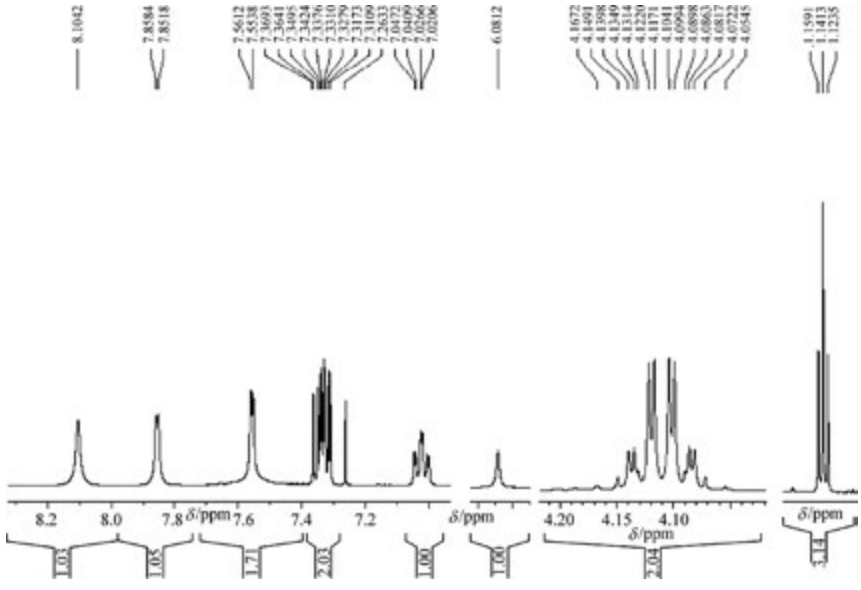


Figure 6.88 The locally enlarged spectrum of peak sets of Figure 6.87

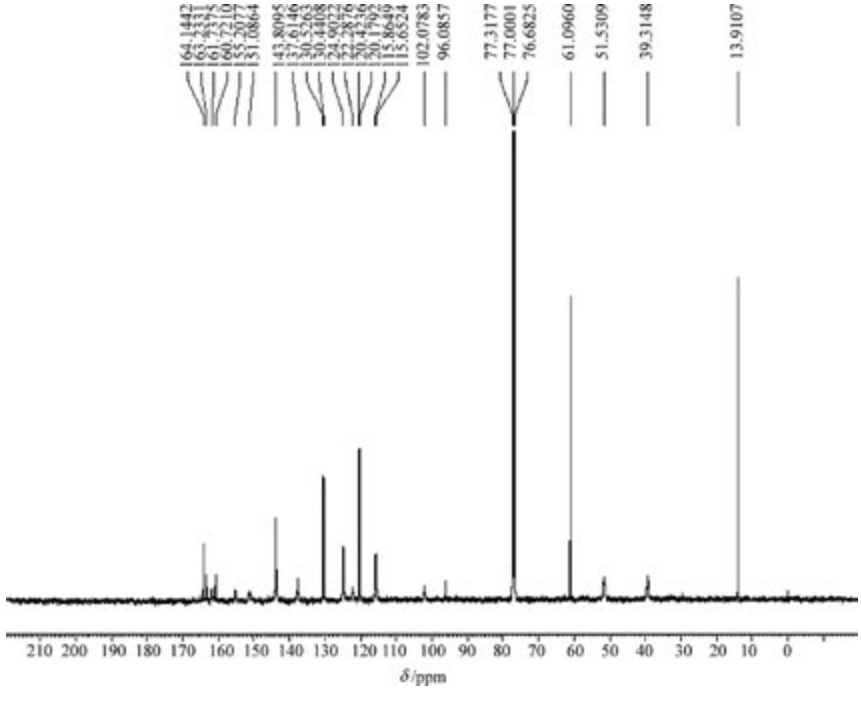


Figure 6.89 The ^{13}C spectrum of the compound

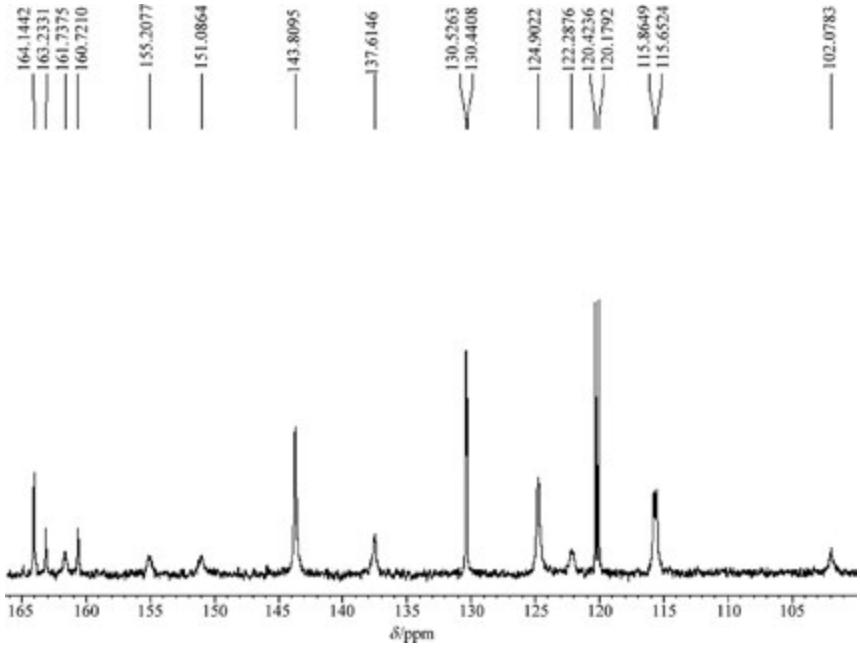


Figure 6.90 The locally enlarged spectrum of Figure 6.89 (in the low field region)

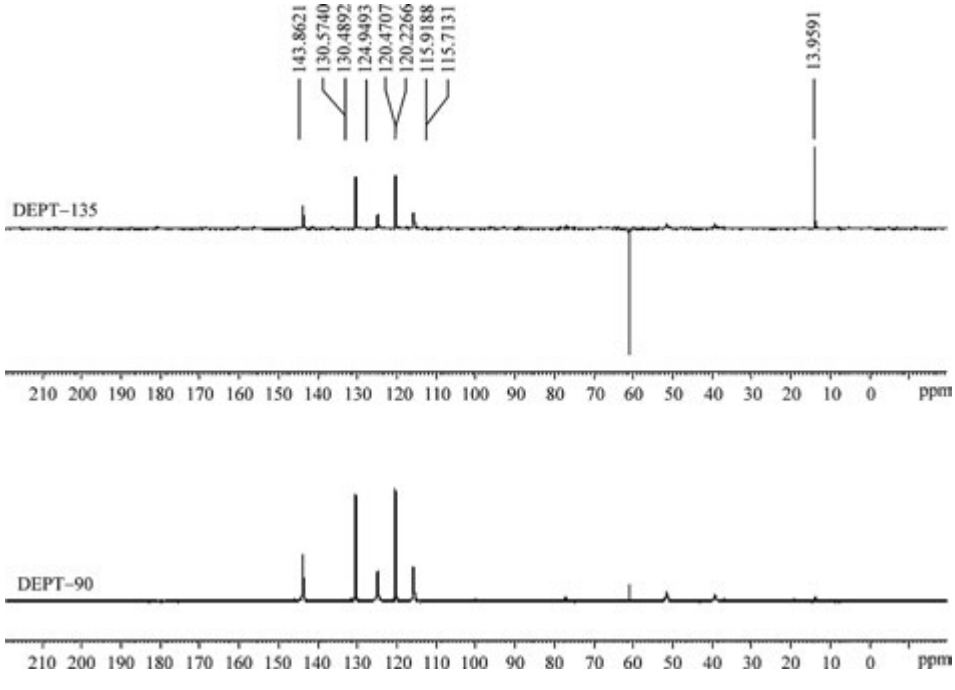


Figure 6.91 The DEPT-135 spectrum of the compound

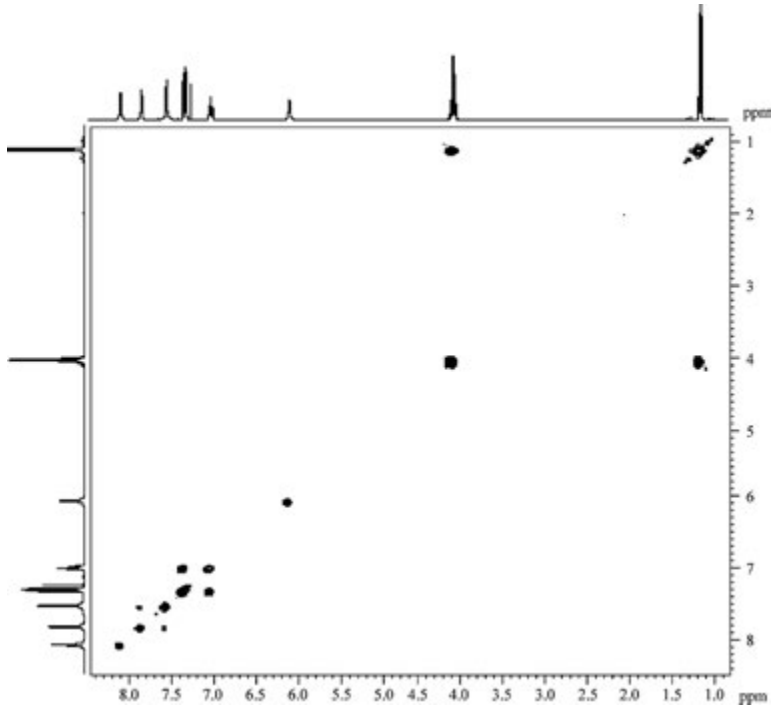


Figure 6.92 The COSY spectrum of the compound

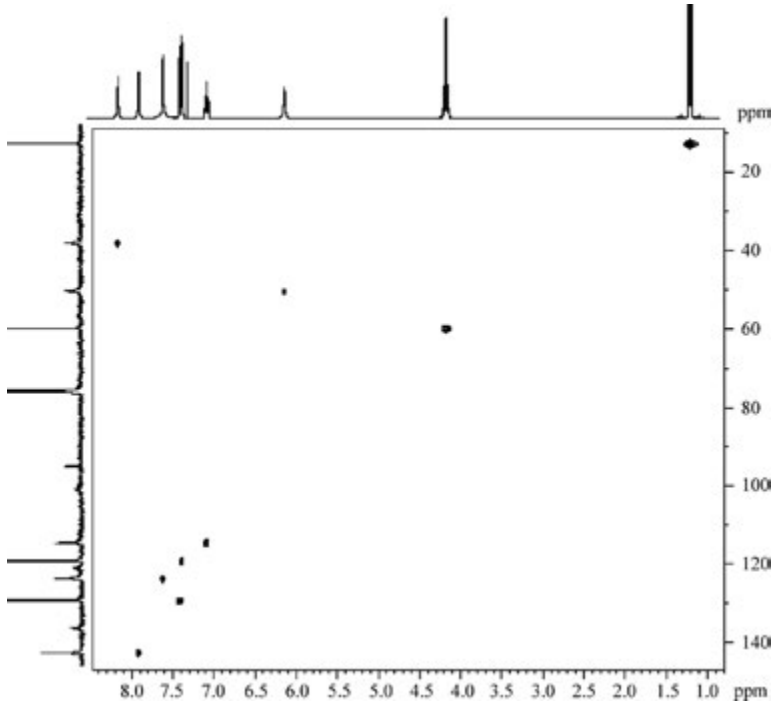


Figure 6.93 The HSQC spectrum of the compound

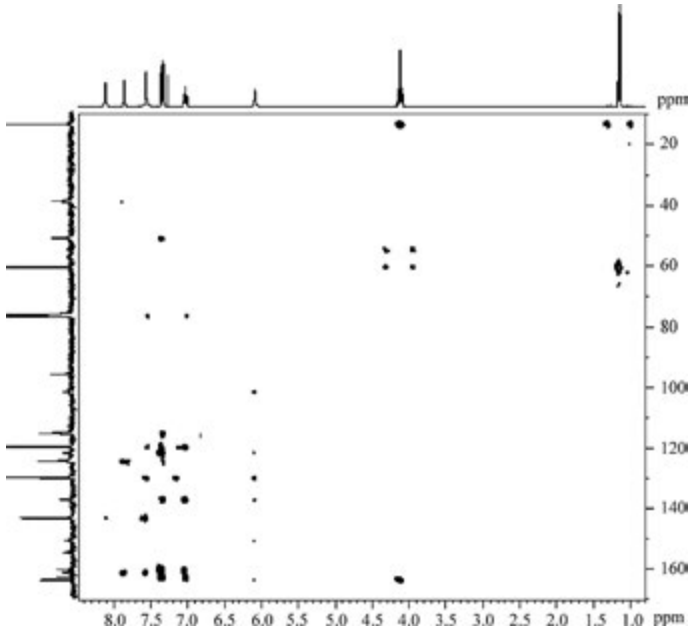


Figure 6.94 The HMBC spectrum of the compound

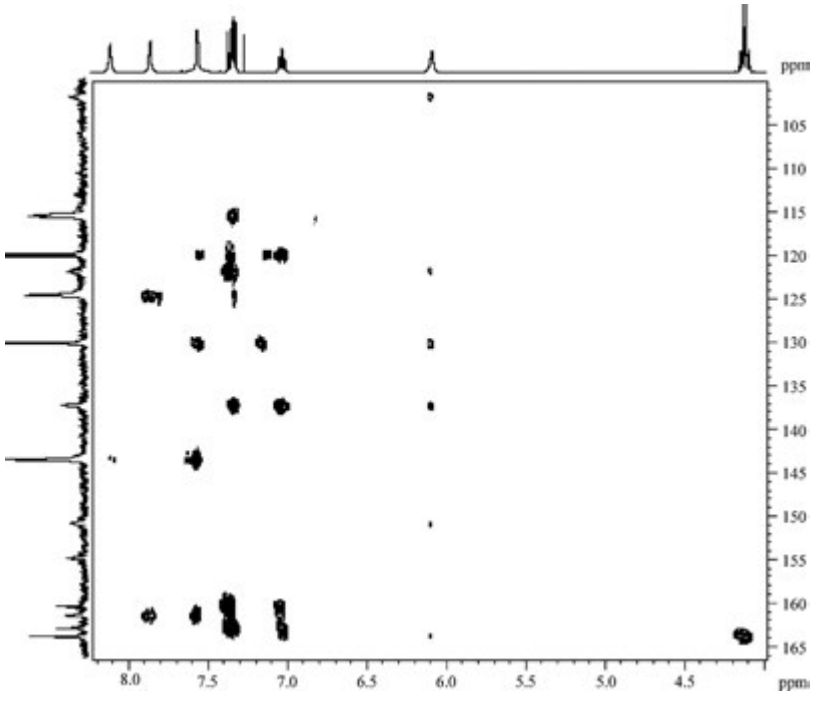


Figure 6.95 The locally enlarged spectrum of Figure 6.94

Table 6.34 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape	Coupling constant (Hz)	Remarks
1.14	3	t	7.12	
4.11	2	q, d	7.12, 1.92	Averaged values
6.08	1	s		
7.03	1	t, d	8.24, 2.52	
7.33	1	m		
7.35	1	m		
7.56	1	d	2.96	
7.86	1	d	2.72	
8.10	1	s		

Second, we should pay attention to the coupled splits by the fluorine atom. Because only the hydrogen atoms are decoupled during the measurement of the ^{13}C spectrum, the coupled splits by fluorine atoms can not be removed by the ordinary decoupling. $^{1}J_{CF}$ is about 240–270 Hz. $^{2}J_{CF}$ is about 20-50 Hz. There are couplings across more chemical bonds between fluorine atoms and carbon atoms. When we interpret this ^{13}C spectrum, attention should be paid to the peak pairs with a similar intensity and with a certain distance and it is necessary to inspect if they are produced by the couplings from the fluorine atom. The coupling constants between a fluorine atom and related carbon atoms are helpful when making judgments.

By the comprehensive interpretation of the ^{13}C spectrum, the DEPT spectrum, and the HSQC spectrum, the data of the ^{13}C spectrum and the ^{1}H spectrum can be summarized in Table 6.35.

Table 6.35 The summarized data of the ^{13}C spectrum and the ^{1}H spectrum

δ_C (ppm)	Number of C atoms	Order of C atom	Directly connected H atoms, δ_H (ppm)	Remarks J_{CF} (Hz)
13.91	1	CH_3	1.14	
39.31	1	CH	8.10	
51.53	1	CH	6.08	
61.09	1	CH_2	4.11	
102.08	1	C		
115.65, 115.86	1	CH	7.03	21
120.18, 120.42	1	CH	7.33	24
122.29	1	C		Splits are observed
124.90	1	CH	7.56	
130.44, 130.53	1	CH	7.35	9
137.61	1	C		
143.81	1	CH	7.86	
151.09	1	C		
155.21	1	C		
161.73	1	C		
160.72, 163.23	1	C		251
164.14	1	C		

Table 6.36 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H atoms, δ_H (ppm)	Remarks
1	1.14	4.11	
2	4.11	1.14	
3	6.08	isolated	
4	7.03	7.35	
5	7.33		
6	7.35	7.03	
7	7.56	7.86	
8	7.86	7.56	
9	8.10	isolated	

Now we will interpret the COSY spectrum.

The data summarized from the COSY spectrum are listed in Table 6.36.

Now we will interpret the HMBC spectrum.

The data summarized from the HMBC spectrum are listed in Table 6.37.

Tables 6.34–6.37 contain all NMR information.

We will confirm the structure through the assignments of the NMR spectra.

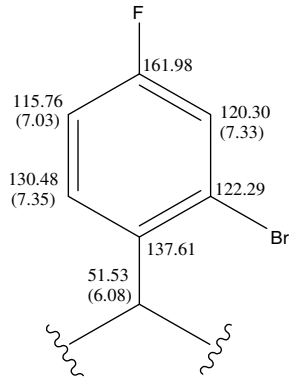
Because of the coupled splits by the fluorine atom, we use averaged data to simplify related data.

Table 6.37 The data summarized from the HMBC spectrum

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	13.91	4.11	1.14
2	39.31		
3	51.53	7.35	
4	61.09	1.14	4.11
5	102.08	6.08	
6	115.65, 115.86	7.33	
7	120.18, 120.42	7.03, 7.35	7.33
8	122.29	6.08, 7.33, 7.35	
9	124.90	7.86	
10	130.44, 130.53	6.08	7.35
11	137.61	6.08, 7.03, 7.33	
12	143.81	7.56	
13	151.09	(6.08)	
14	155.21		
15	161.73	7.56, 7.86	
16	160.72, 163.23	7.03, 7.33, 7.35	
17	164.14	4.11, (6.08)	

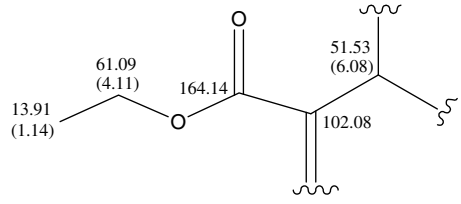
Parethesis () indicates weak correlations.

The assignment of the structural unit below can be completed by the following analyses.



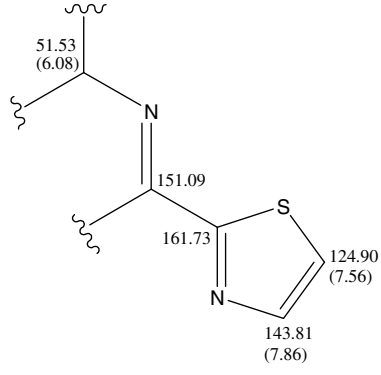
1. The fluorine atom produces splits of peaks in the ^{13}C spectrum. The less chemical bonds the coupling spans, the larger the coupling constant. For example, from a split with 251 Hz the ^{1}J coupling can be determined.
2. According to No. 4 (6) of Table 6.36, the coupling of δ_H (ppm) 7.03/7.35 exists.
3. According to Nos. 6, 8, 10, 11, and 16 of Table 6.37, the related heteronuclear long-range couplings exist.

The assignment of the structural unit below can be completed by the following analyses.



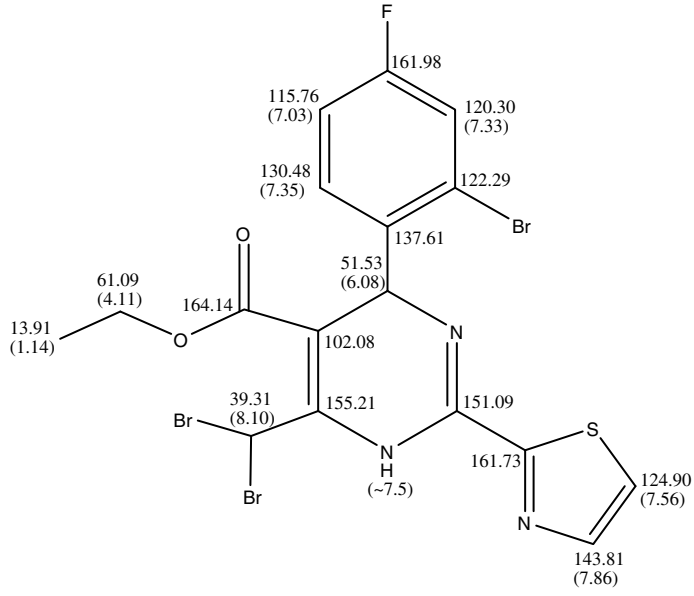
1. According to No. 1 (2) of Table 6.36, the coupling of δ_H (ppm) 1.14/4.11 exists.
2. According to Nos. 1, 4, 5 and 17 of Table 6.37, the related heteronuclear long-range couplings exist.

The assignment of the structural unit below can be completed by the following analyses.



1. According to No. 7 (8) of Table 6.36, the coupling of δ_H (ppm) 7.56/7.86 exists.
2. According to Nos. 9, 12, 13 and 15 of Table 6.37, the related heteronuclear long-range couplings exist.

In addition to the remaining CH($\delta_C = 39.31$ and $\delta_H = 6.08$ ppm), two bromine atoms, and an NH, the assignment can be completed.



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The peak of NH is situated at about 7.5 ppm.
To sum up, the anticipated structure is correct.

Example 6.11 The molecular weight of an unknown compound has been determined as 294 by its mass spectrum. Its ^{1}H spectrum, the locally enlarged spectrum of the ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged spectrum of the ^{13}C spectrum, its DEPT-135 spectrum, its COSY spectrum, the locally enlarged spectrum of the COSY spectrum, its HSQC spectrum, the locally enlarged spectrum of the HSQC spectrum, its HMBC spectrum, and the locally enlarged spectrum of the HMBC spectrum are shown in Figures 6.96–6.107, respectively. Try to deduce its structure. All NMR spectra were measured by an NMR spectrometer with 400 MHz. The solvent used is deuterated DMSO.

Solution
The peak at about 2.50 ppm in the ^{1}H spectrum is the solvent peak (deuterated DMSO), and the peak at 3.30 ppm is the peak from water, which exists in the sample. The small peaks at 1.14–1.24, 1.34, 1.38, 1.56, 1.67, 3.10, 3.16, 3.27, 4.13, and so on are impurity peaks, whose low intensities manifest that they are due to impurities.

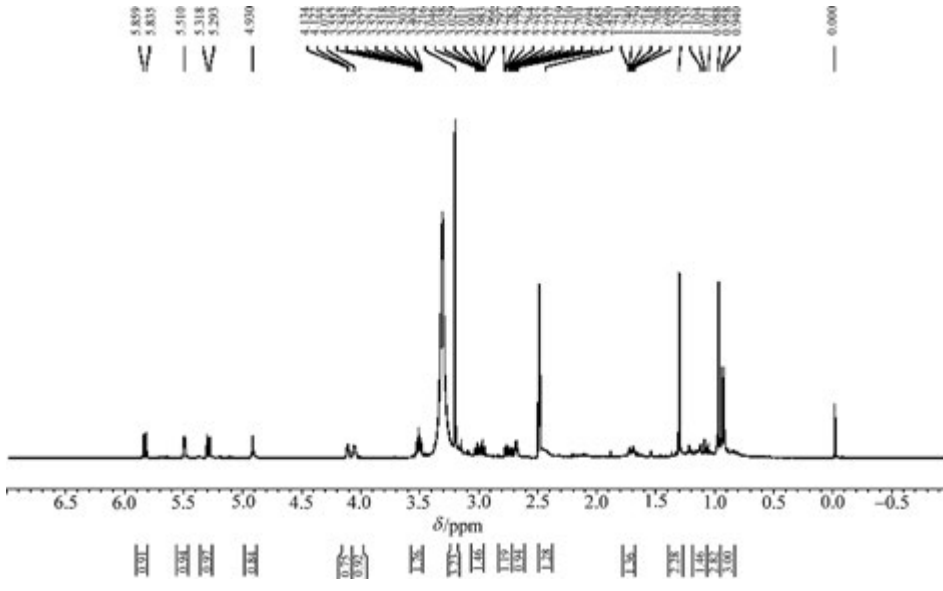


Figure 6.96 The ^{1}H spectrum of the unknown compound

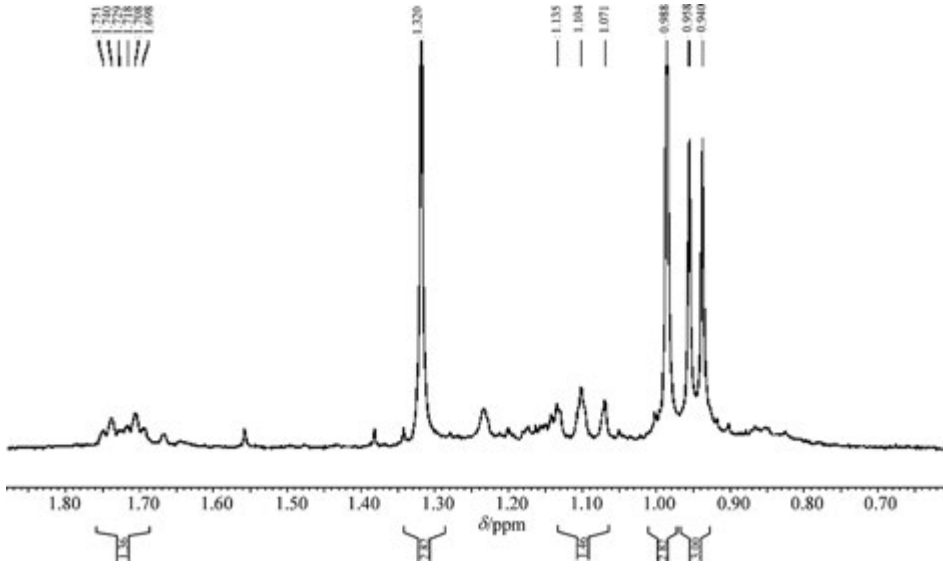


Figure 6.97 The locally enlarged spectrum of Figure 6.96 (1)

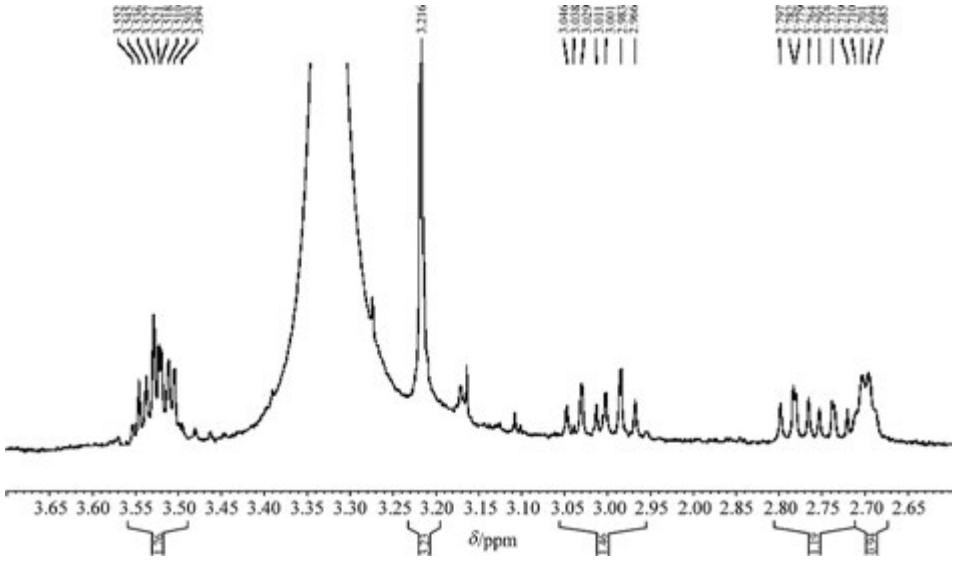


Figure 6.98 The locally enlarged spectrum of Figure 6.96 (2)

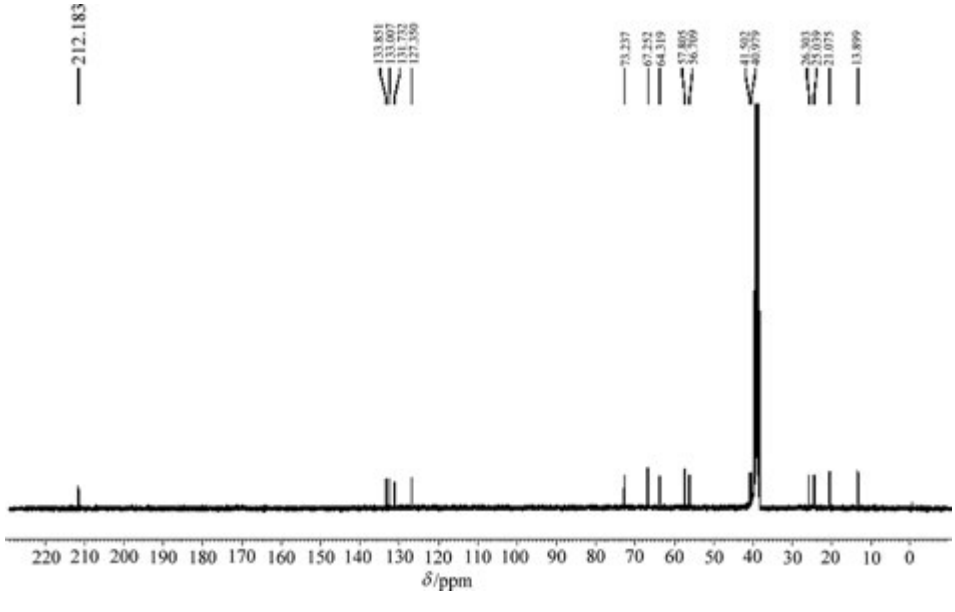


Figure 6.99 The ^{13}C spectrum of the unknown compound

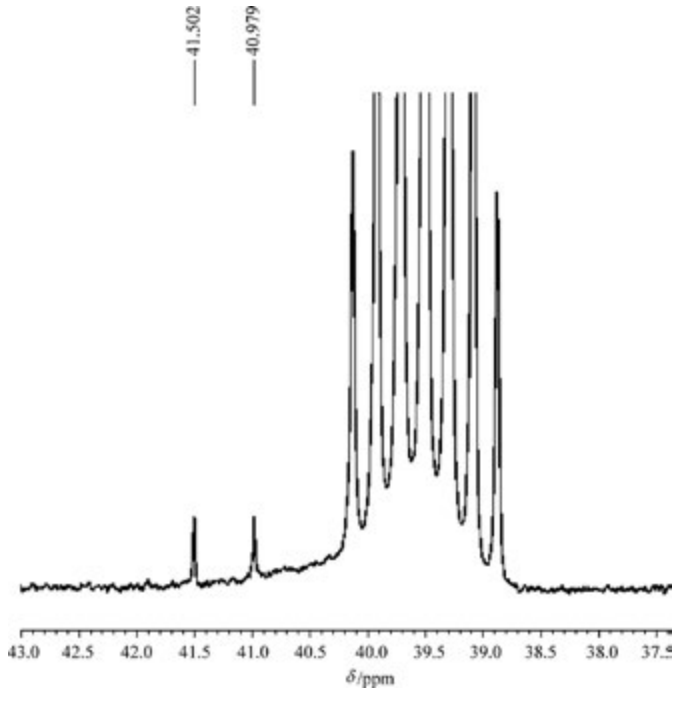


Figure 6.100 The locally enlarged spectrum of Figure 6.99

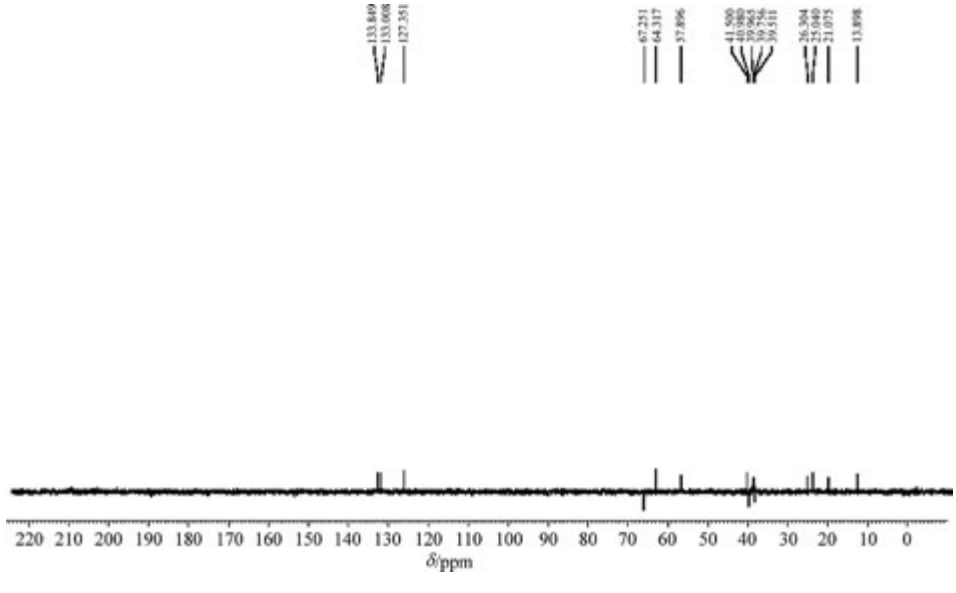


Figure 6.101 The DEPT-135 spectrum of the unknown compound

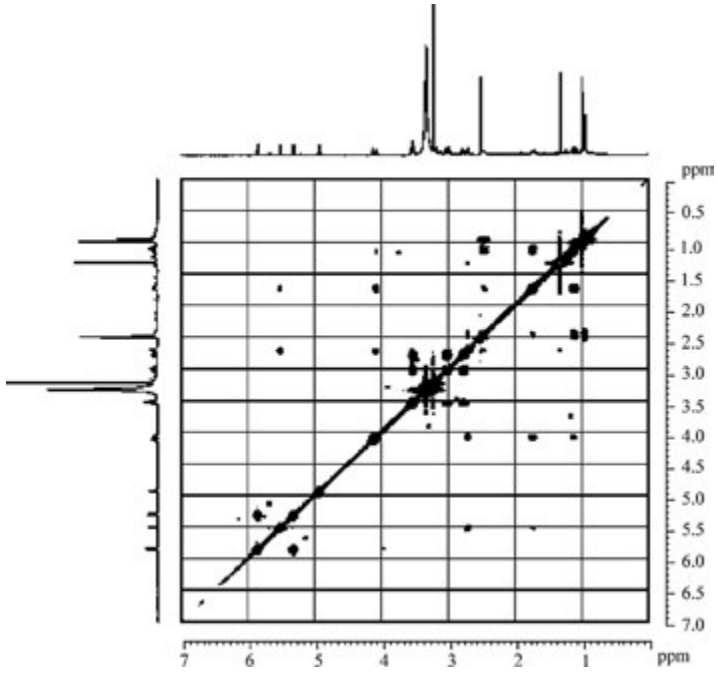


Figure 6.102 The COSY spectrum of the unknown compound

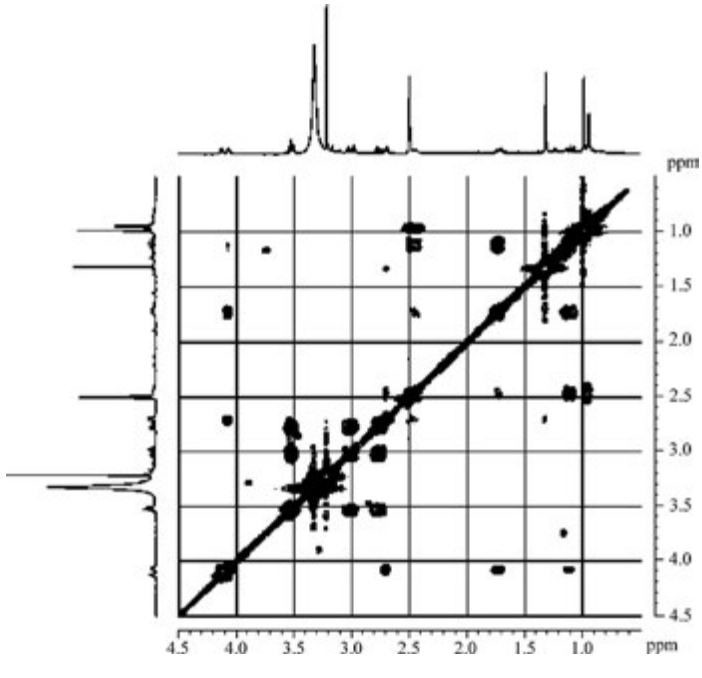


Figure 6.103 The locally enlarged spectrum of Figure 6.102

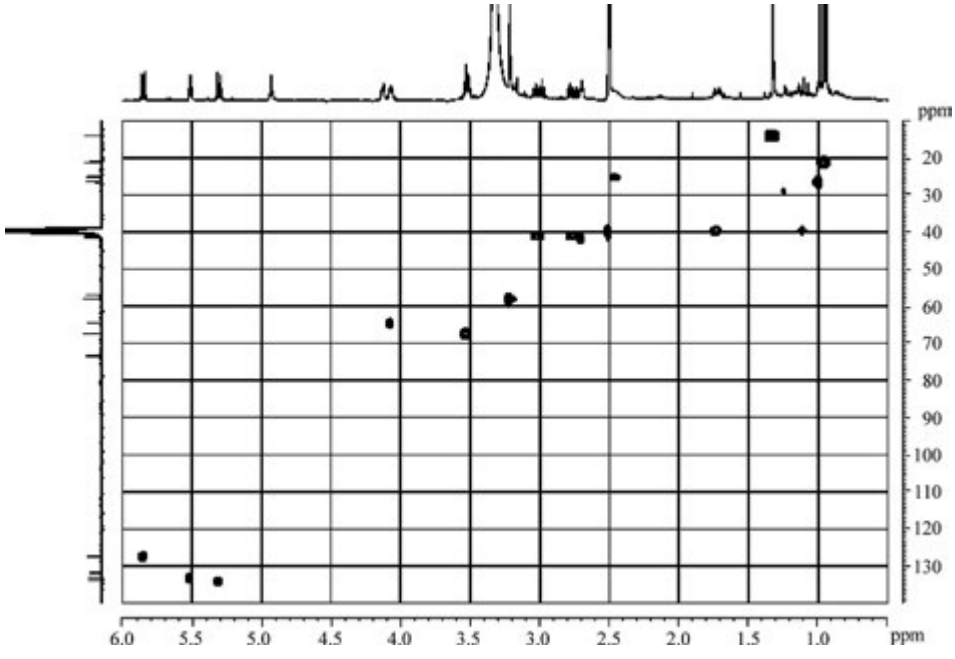


Figure 6.104 The HSQC spectrum of the unknown compound

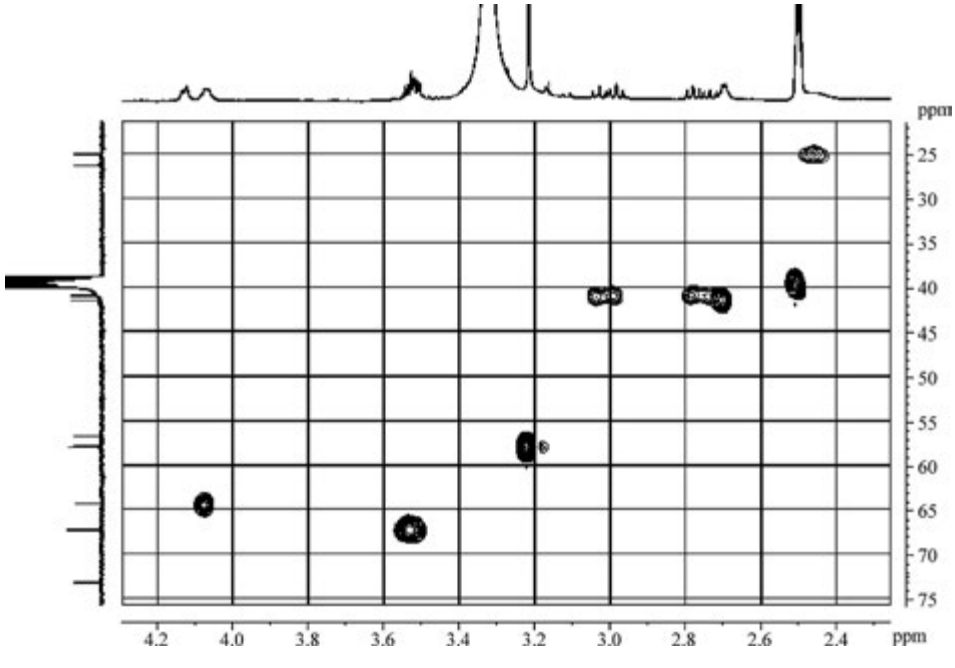


Figure 6.105 The locally enlarged spectrum of Figure 6.104

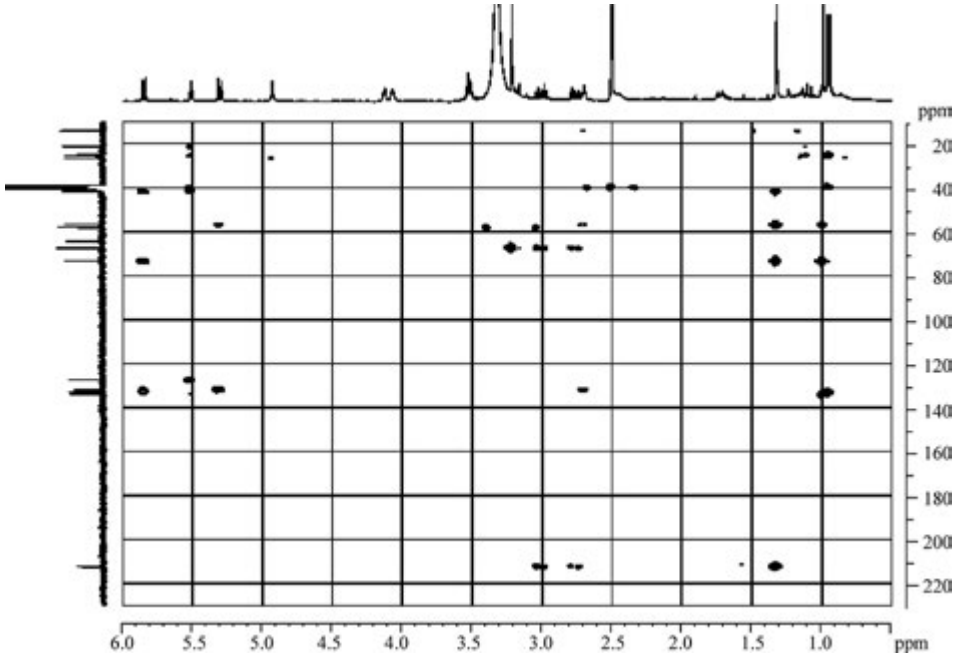


Figure 6.106 The HMBC spectrum of the unknown compound

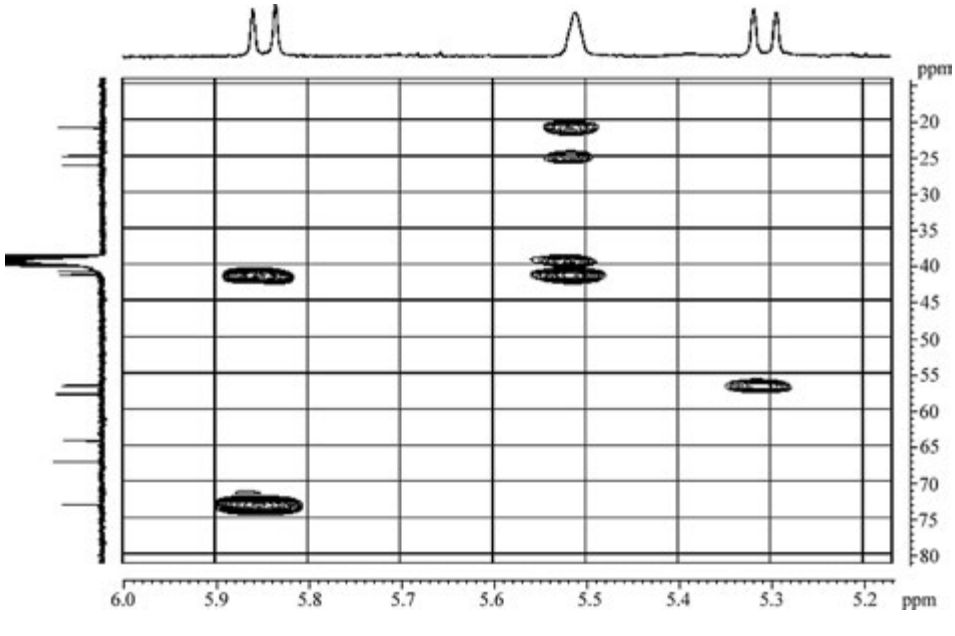


Figure 6.107 The locally enlarged spectrum of Figure 6.106

The related data summarized from the ^{1}H spectrum are listed in Table 6.38.

The peak at 2.45 ppm shows a very blunt shape. It seems that this peak does not belong to the sample. However, it really belongs to the sample from its correlated peak in the HSQC spectrum.

Some numbers of hydrogen atoms corresponding to peak sets can be determined from the ^{1}H spectrum, for example, those peak sets with integral values of 3.00, 2.82, and 2.82 obviously correspond to three hydrogen atoms, respectively. It is difficult to determine the integral values of some peak sets in the H spectrum, such as those with the integral values of 1.46 and 1.36, which can not be treated by the rounding-off method. In order to find the correct hydrogen atom numbers, it is necessary to interpret the ^{1}H spectrum with the help of the HSQC spectrum. For example, the peak set at 3.01 ppm has an integral value of 1.46. From the HSQC spectrum, its correlation with the peak at 40.98 ppm in the ^{13}C spectrum can be found. The peak at 40.98 ppm correlates with two peak sets at 3.01 and 2.76 ppm, respectively. Therefore, the peak set at 3.01 ppm corresponds only to one hydrogen atom, which belongs to a CH_2 group. Other peak sets can be analyzed similarly. Finally, we get Table 6.38.

If the hydrogen atom number of a peak set in the ^{1}H spectrum is still difficult to determine with the help of the HSQC spectrum, the DEPT spectrum and the COSY spectrum can be used to help the determination.

From the addition of numbers of the third column of Table 6.38, it can be known that the unknown compound contains 26 hydrogen atoms.

It is necessary to analyze the ^{13}C spectrum very carefully. The peak at 40.98 ppm is clearly different from the peak at 41.50 ppm in the solvent peak region in the locally enlarged ^{13}C spectrum. However, another peak of the sample is overlapped totally with a peak of the solvent peak set. The peak can not be found even from the locally enlarged ^{13}C spectrum (Figure 6.100). In this case, the interpretation of the HSQC spectrum can give the correct result. In the HSQC spectrum, three correlated peaks at $F_2 = 1.10$, 1.72, and 2.50 ppm, respectively, can be found at the position of the solvent peak set on the F_1 axis.

Table 6.38 Related data summarized from the ^{1}H spectrum

δ_H (ppm)	Integral value	Number of H atoms	Peak shape
0.95	3.00	3	d
0.99	2.82	3	s
1.10	1.46	1	t
1.32	2.82	3	s
1.72	1.36	1	d, t
2.45	1.28	1	broad
2.70	0.94	1	q
2.76	1.19	1	m
3.01	1.46	1	m
3.22	3.23	3	s
3.52	1.76	2	m
4.07	0.92	1	broad
4.12	0.75	1	s
4.93	0.84	1	s
5.31	0.97	1	d
5.51	0.94	1	s
5.85	0.91	1	d

The correlated peak at $F_2 = 2.50$ ppm is ascribed to the correlated peak of the solvent, while the two other correlated peaks at $F_2 = 1.10$ and 1.72 ppm belong to a CH_2 group, whose peak in the ^{13}C spectrum overlaps with the peak set of the solvent.

If it is difficult to use the ^{13}C spectrum to resolve peaks even with the help of the HSQC spectrum, both the DEPT spectrum and the HMBC spectrum should also be used. In the example above, the downward peak at 39.51 ppm in the DEPT spectrum implies the CH_2 group, which is an additional proof of the preceding conclusion.

If we interpret several kinds of NMR spectra together, the error which may arise from the interpretation of only one kind of NMR spectrum can be avoided.

The related data summarized from the ^{13}C spectrum, DEPT spectrum, HSQC spectrum, and the ^{1}H spectrum are listed in Table 6.39.

From Table 6.39 it can be known that the unknown compound contains 4 CH_3, 3 CH_2, 6 CH, and 4 C (quaternary carbon atoms). From δ values it can be known that one methyl group is a methoxyl group, and that one quaternary carbon atom belongs to a carbonyl group. To sum up, the unknown compound contains 17 carbon atoms.

It can be known that the subtraction of the hydrogen atoms counted from Table 6.39 from those counted from the ^{1}H spectrum leaves two hydrogen atoms, whose δ values are 4.12 and 4.93 ppm, respectively. Obviously, these two hydrogen atoms should be reactive hydrogen atoms.

To sum up, the unknown compound contains $C_{17}H_{26}O$ (a carbonyl group). And the two reactive hydrogen atoms correspond to two heteroatoms. The subtraction of the mass of $C_{17}H_{26}O$ from the molecular weight leaves 48 (u), which corresponds to three oxygen atoms. Since the molecular weight is an even number, the unknown compound contains no nitrogen atom or an even number of nitrogen atoms. The latter situations do not coincide with the mass difference of 48 (u). Therefore, the mass difference of 48 leads to the unique

Table 6.39 The NMR data summarized from the ^{13}C spectrum, DEPT spectrum, HSQC spectrum, and the ^{1}H spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atom	Directly connected H atoms, δ_H (ppm)
1	13.89	1	CH_3	1.32
2	21.08	1	CH_3	0.95
3	25.04	1	CH	2.45
4	26.30	1	CH_3	0.99
5	39.51	1	CH_2	1.10, 1.72
6	40.98	1	CH_2	2.76, 3.01
7	41.50	1	CH	2.70
8	56.71	1	C	
9	57.89	1	CH_3	3.22
10	64.32	1	CH	4.07
11	67.25	1	CH_2	3.52
12	73.24	1	C	
13	127.35	1	CH	5.85
14	131.73	1	C	
15	133.01	1	CH	5.51
16	133.85	1	CH	5.31
17	212.18	1	C	

Table 6.40 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H atoms, δ_H (ppm)
1	0.95	2.45
2	0.99	
3	1.10	1.72, 2.45, 4.07
4	1.32	(2.70)
5	1.72	1.10, 2.45, 4.07
6	2.45	0.95, 1.10, 1.72, (5.51)[a]
7	2.70	(1.32), 4.07
8	2.76	3.01, 3.52
9	3.01	2.76, 3.52
10	3.22	
11	3.52	2.76, 3.01
12	4.07	1.10, 1.72, 2.70, 4.12
13	4.12	4.07
14	4.93	
15	5.31	5.85
16	5.51	(1.72), (2.45)[a], 2.70
17	5.85	5.31

[a] The datum is btained from the COSY spectrum with a low section, which is not shown in this example.

solution of three oxygen atoms. Chemical shift values read from the ^{13}C spectrum and the ^{1}H spectrum accord with this postulation, because their values imply the connection with an oxygen atom.

Therefore, the molecular formula of the unknown compound can be determined as $C_{17}H_{26}O_4$, from which the unsaturation number of 5 can be calculated.

Because the unknown compound contains one carbonyl group and four unsaturated carbon atoms ($\delta_C = 127–134$ ppm), which correspond to two double bonds, the unknown compound must contain two rings.

The data summarized from the COSY spectrum are listed in Table 6.40.

The data summarized from the HMBC spectrum are listed in Table 6.41.

Now we assemble the structural units step by step through the use of the COSY spectrum and the HMBC spectrum.

We now start from No. 9 of Table 6.39, the methoxyl group with $\delta_C = 57.89$ ppm and δ_H 3.22 ppm. It is certainly a terminal group of the structure. Since the oxygen atom cuts off the ^{3}J coupling of the methyl group, the structural unit connected to the methoxyl group can be known only by using related correlated peaks in the HMBC spectrum. On the basis of the correlation of δ_C (67.25)/δ_H (3.22) known from No. 11 of Table 6.41, the correlation of δ_C (67.25)/δ_H (3.52) known from No. 11 of Table 6.39, the correlation of δ_H (ppm) 3.52/2.76, 3.01 known from No. 11 of Table 6.40, and the correlation ofδ_C (40.98)/δ_H (2.76, 3.01) known from No. 6 of Table 6.39, we get the following structural unit:

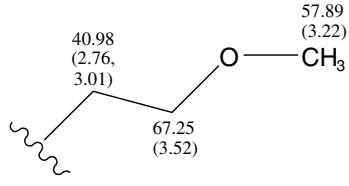
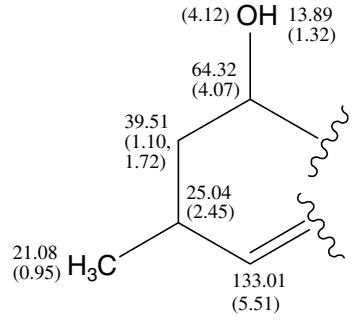


Table 6.41 The data summarized from the HMBC spectrum

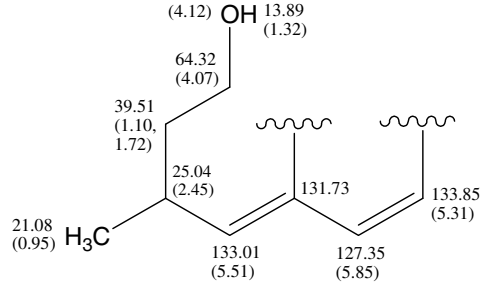
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	13.89	2.70	1.32
2	21.08	(1.10), 5.51	
3	25.04	0.95, 1.10, 5.51	
4	26.30		0.99
5	39.51	0.95, 5.51	
6	40.98		
7	41.50	1.32, 5.51, 5.85	
8	56.71	0.99, 1.32, 2.70, 5.31	
9	57.89		3.22
10	64.32		
11	67.25	2.76, 3.01, 3.22	
12	73.24	0.99, 1.32, 5.85	
13	127.35	5.51	
14	131.73	2.70, 5.31	
15	133.01	0.95, 5.85	
16	133.85	0.99	
17	212.18	1.32, 2.76, 3.01	

According to No. 17 of Table 6.41, the carbon atom with $\delta_C = 212.18$ ppm couples with the hydrogen atom with $\delta_H = 2.76$, 3.01 ppm. Because the correlation with $^3J_{CH}$ is not the unique solution in the HMBC spectrum, the connection between the carbonyl group and the CH_2 can not be determined so far.

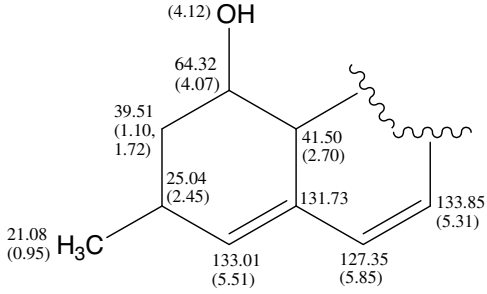
Then we look at No. 10 of Table 6.39, the CH group with $\delta_C = 64.32$ and $\delta_H = 4.07$ ppm. According to No. 12 of Table 6.40, the CH group connects the hydroxyl group with $\delta_H = 4.12$ ppm and the CH_2 group with $\delta_C = 39.51$ and $\delta_H = 1.10$, 1.72 ppm. Also from the result of the COSY spectrum and the HSQC spectrum (No. 3 and No. 5 of Table 6.40 and No. 3 of Table 6.39), we know that the CH_2 group connects another CH group with $\delta_C = 25.04$ and $\delta_H = 2.45$ ppm. According to No. 6 of Table 6.40, the CH group connects the methyl group with $\delta_C = 21.08$ and $\delta_H = 0.95$ ppm and the alkene CH group with $\delta_C = 133.01$ and $\delta_H = 5.51$ ppm. To sum up, we get the following structural unit:



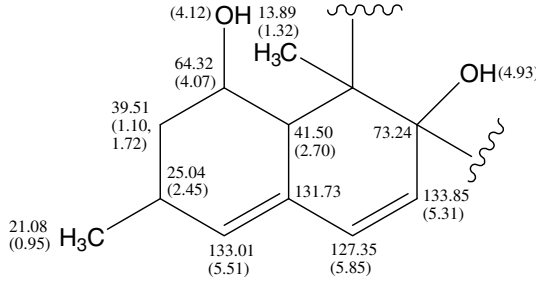
From Nos. 13, 14, and 15 of Table 6.41, the following correlations can be known: $\delta_C(127.35)/\delta_H(5.51)$, $\delta_C(131.73)/\delta_H(5.31)$, and $\delta_C(133.01)/\delta_H(5.85, 0.95)$ ppm. The double bonds mentioned above can extend to a conjugated double bond which follows:



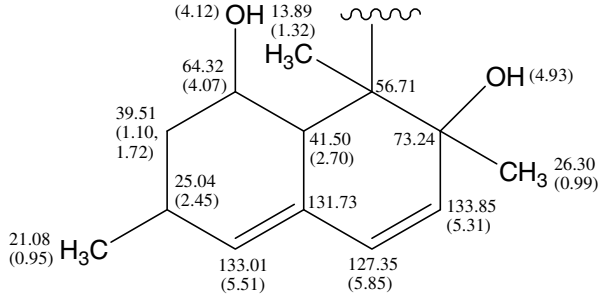
According to No. 7 of Table 6.41, that is, the correlation of $\delta_C(41.50)/\delta_H(5.51, 5.85)$, the CH_2 group connected with the alkene carbon atom with $\delta_C = 131.73$ ppm can be found. The correlation, which exists between the quaternary carbon atom ($\delta_C = 131.73$ ppm) and the peak sets at $\delta_H = 5.51$ and 5.85 ppm, should be paid attention to. Therefore, the position of the quaternary carbon atom can be determined correctly. Therefore, the structural unit extents as follows:



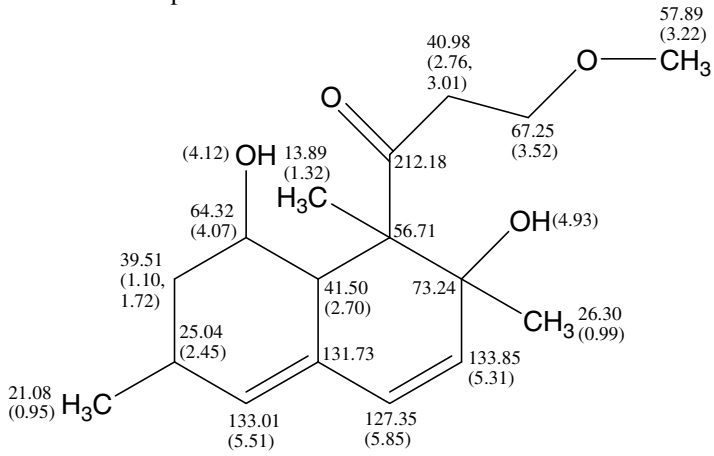
According to No. 12 of Table 6.41, that is, the correlation of $\delta_C(73.24)/\delta_H(5.85)$, the quaternary carbon atom with $\delta_C = 73$ ppm which is connected with the alkene carbon atom with $\delta_C = 133.85$ ppm can be found. From its chemical shift value, it can be known that the quaternary carbon atom should connect with a hydroxyl group. In addition, from the long-range coupling correlation of the quaternary carbon atom with the hydrogen atom with $\delta_H = 1.32$ ppm (No. 12 of Table 6.41), the above structural unit can be extended as follows:



According to No. 8 of Table 6.41, that is, the correlation of $\delta_C(56.71)/\delta_H(0.99, 2.70, 5.31)$, the position of the quaternary carbon atom with $\delta_C = 56.71$ ppm, which connects with another quaternary carbon atom with $\delta_C = 73.24$ ppm, can be determined. The position of the methyl group connected with the quaternary carbon atom can also be determined:



Finally, according to No. 17 of Table 6.41, that is, the correlation of $\delta_C(212.18)/\delta_H(1.32, 2.76, 3.01)$, the above-mentioned two structural units can be connected through the carbonyl group. Therefore, we assemble all functional groups and accomplish the assignments of the ^{13}C spectrum and the ^{1}H spectrum:



(C6-12)

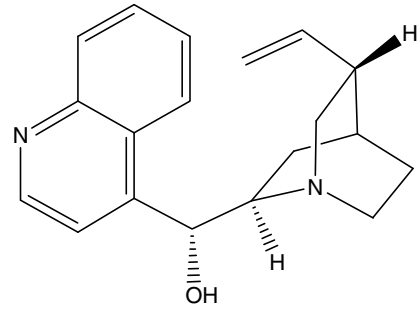
According to the above structural formula and the assignment, it seems that we should see the correlation between $\delta_C = 64.32$ and $\delta_H = 2.45$. However, there does not exist the related correlated peak in the HMBC spectrum. The cause of this phenomenon is that the peak at $\delta_H = 2.45$ has a blunt shape. The intensity of the peak is low, so that the intensity of the related correlated peak in the HMBC spectrum is also low.

We would like to supplement the assignment of the ^{1}H spectrum as follows.

1. Because the structure has no symmetrical plane, the two hydrogen atoms of all the three CH_2 groups are not chemically equivalent. Therefore, their ^{2}J couplings are shown in the ^{1}H spectrum. Even the ^{2}J coupling of the peak set at 3.52 ppm is shown clearly, although we mark only one chemical shift value for it. The other two CH_2 groups show greater chemical non-equivalence than the first one. Therefore, we mark two chemical shift values.

2. The CH group with $\delta_C = 41.50$ and $\delta_H = 2.70$ ppm shows a quartet shape in the ^1H spectrum. The formation of the quartet relates with long-range couplings. Long-range couplings will be shown clearly when a double bond, an allylic system, a homoallylic system, or W-type structural unit exists.

Example 6.12 A synthesized compound has the following anticipated structure:



C6-13

Its ^1H spectrum, the locally enlarged ^1H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its DEPT-135 spectrum, the locally enlarged DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HMQC spectrum, the locally enlarged HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.108–6.128, respectively. All NMR spectra were

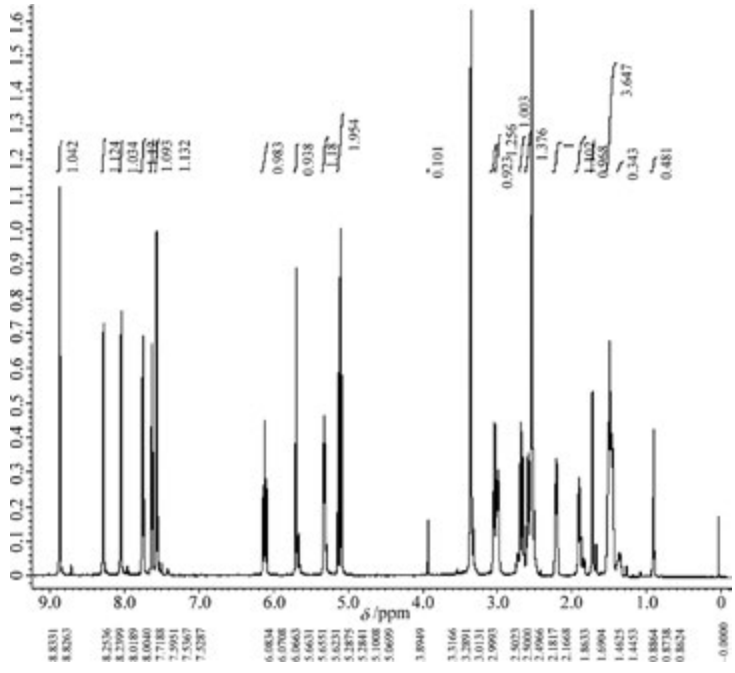


Figure 6.108 The ^1H spectrum of the compound

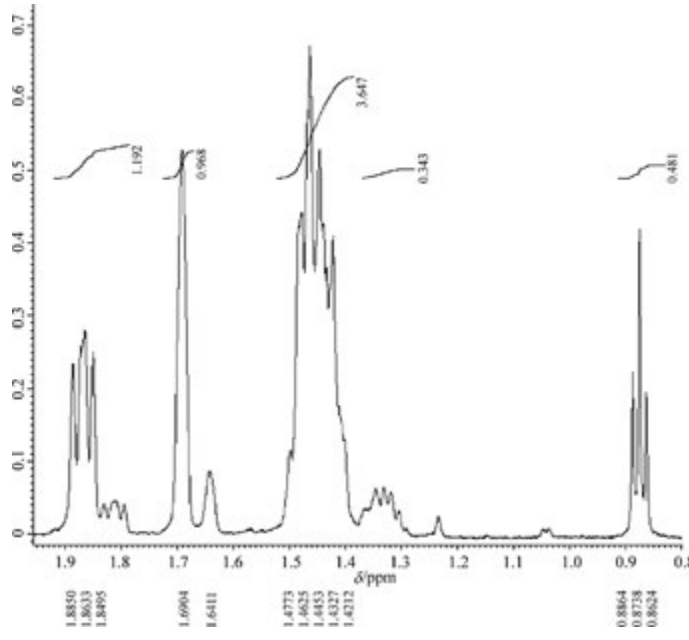


Figure 6.109 The locally enlarged ^{1}H spectrum (1)

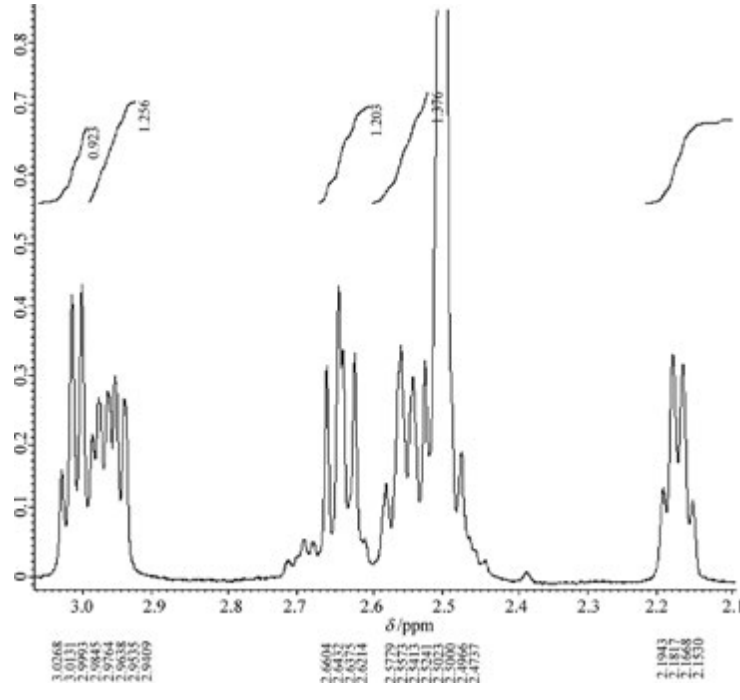


Figure 6.110 The locally enlarged ^{1}H spectrum (2)

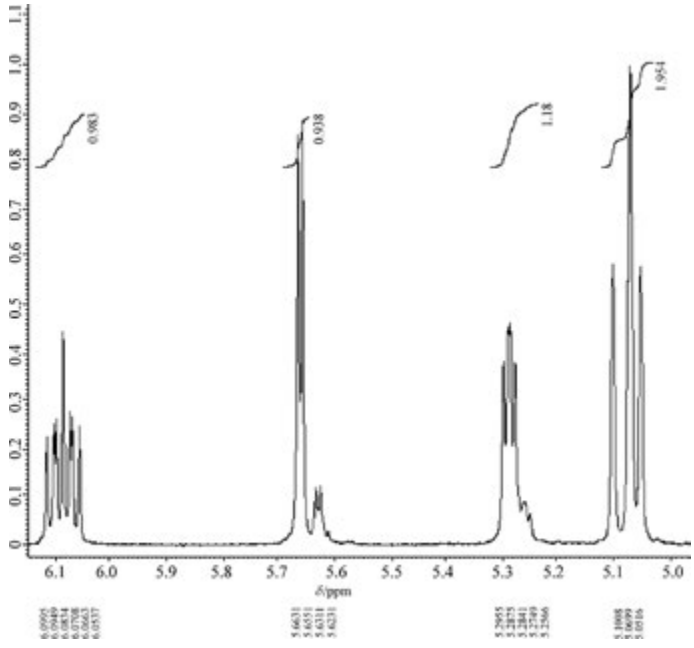


Figure 6.111 The locally enlarged ^{3}H spectrum (3)

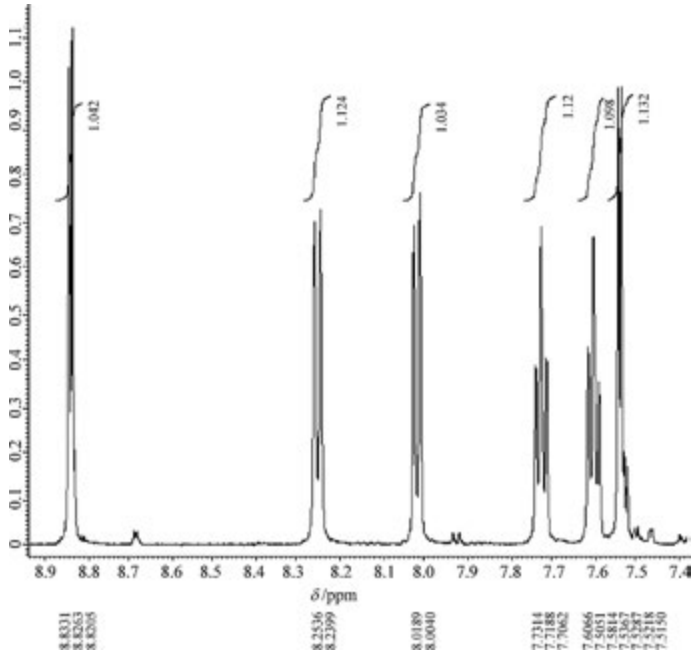


Figure 6.112 The locally enlarged ^{1}H spectrum (4)

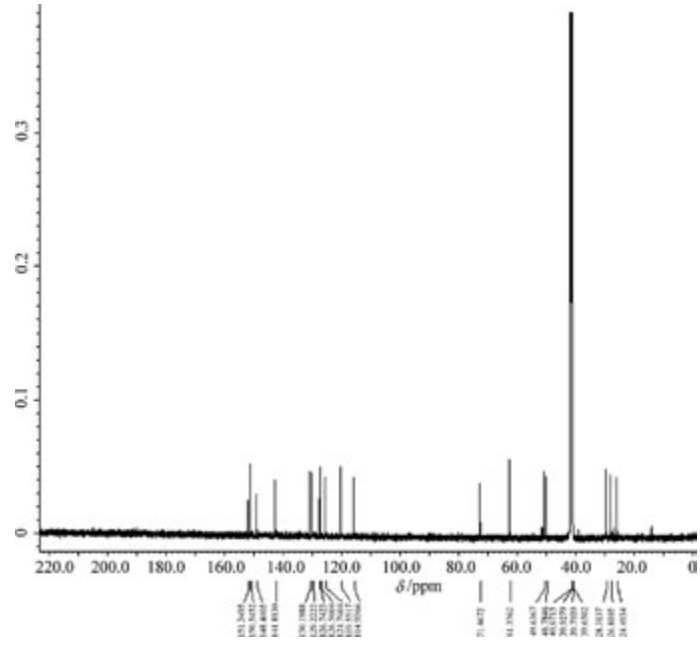


Figure 6.113 The ^{13}C spectrum of the compound

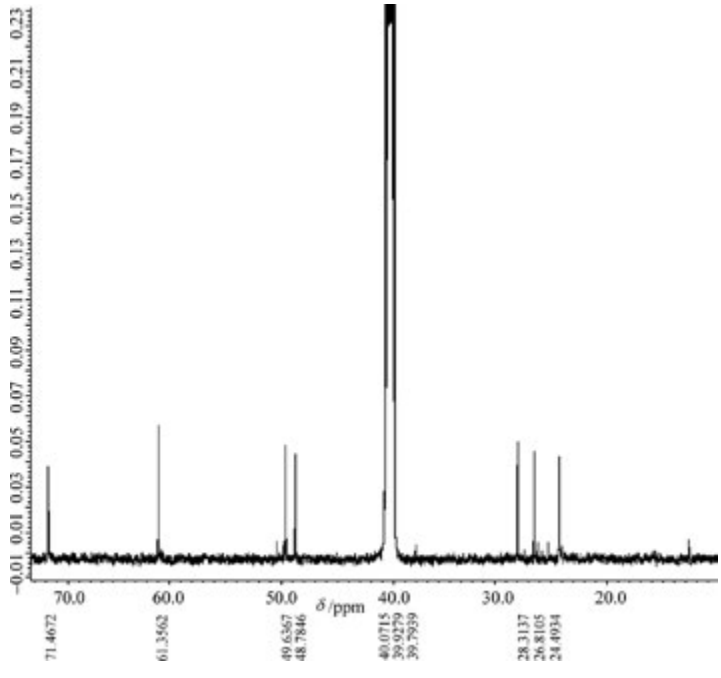


Figure 6.114 The locally enlarged ^{13}C spectrum (1)

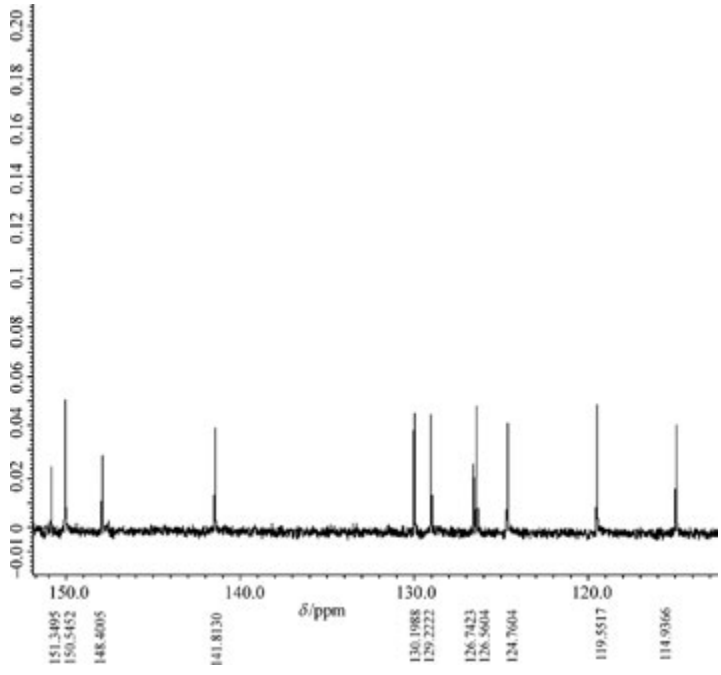


Figure 6.115 The locally enlarged ^{13}C spectrum (2)

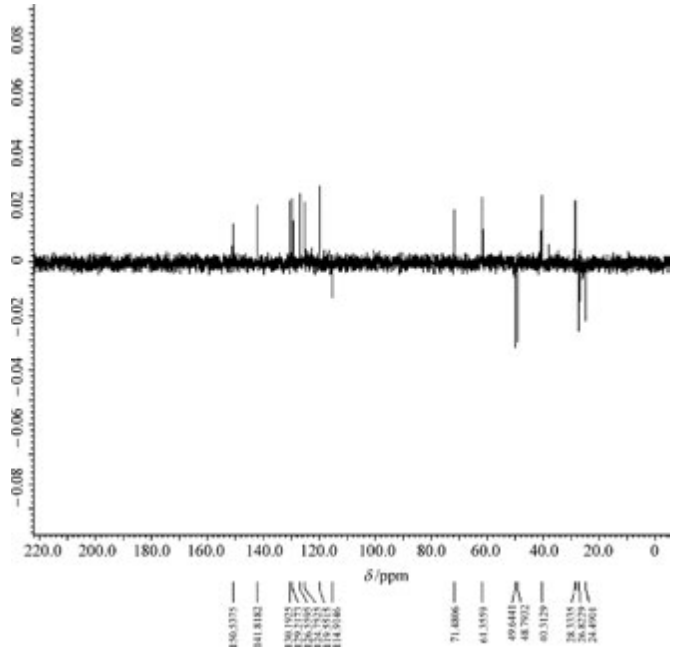


Figure 6.116 The DEPT-135 spectrum of the compound

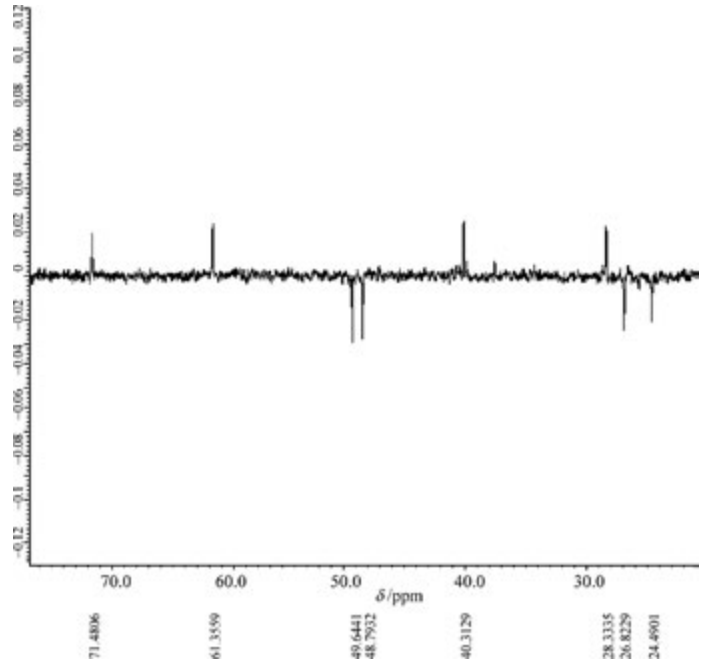


Figure 6.117 The locally enlarged DEPT-135 spectrum (1)

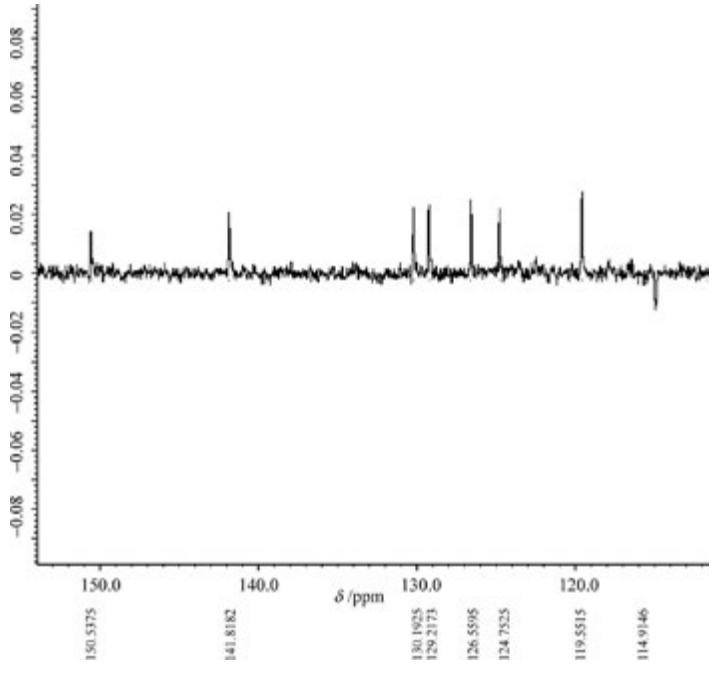


Figure 6.118 The locally enlarged DEPT-135 spectrum (2)

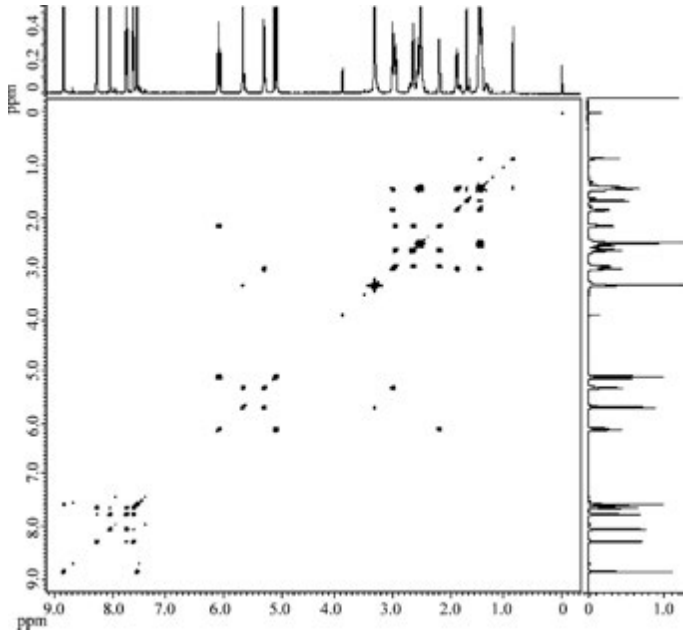


Figure 6.119 The COSY spectrum of the compound

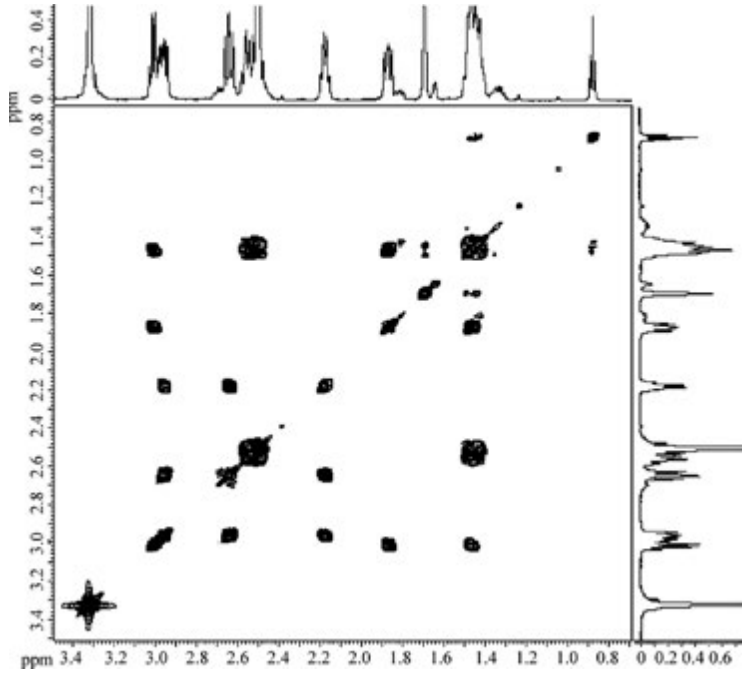


Figure 6.120 The locally enlarged COSY spectrum (1)

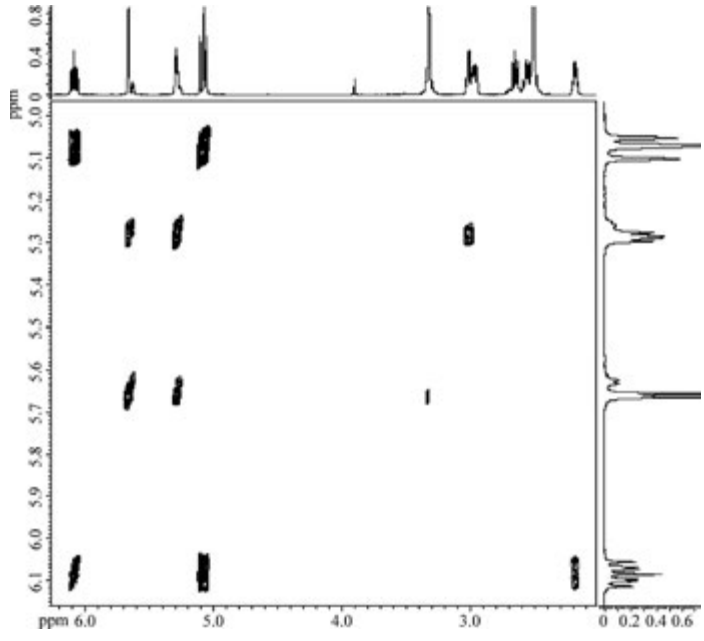


Figure 6.121 The locally enlarged COSY spectrum (2)

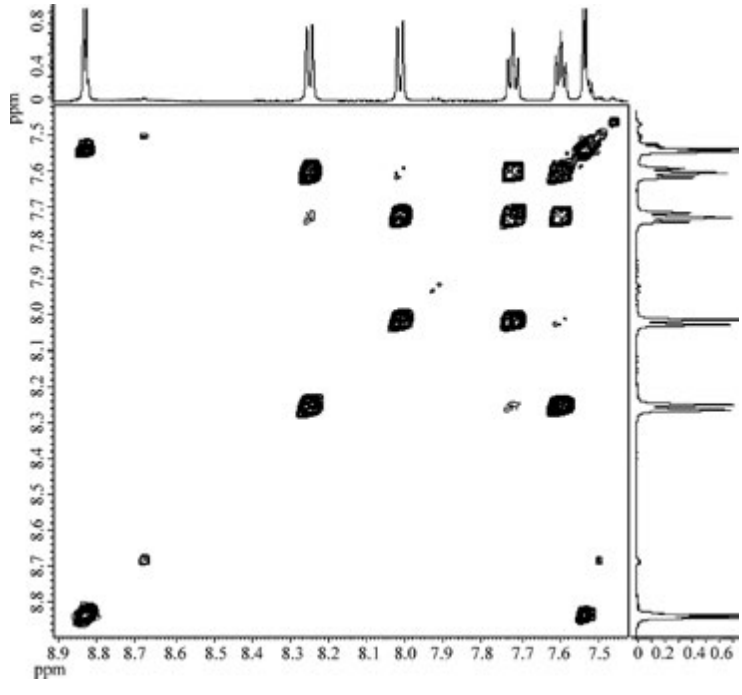


Figure 6.122 The locally enlarged COSY spectrum (3)

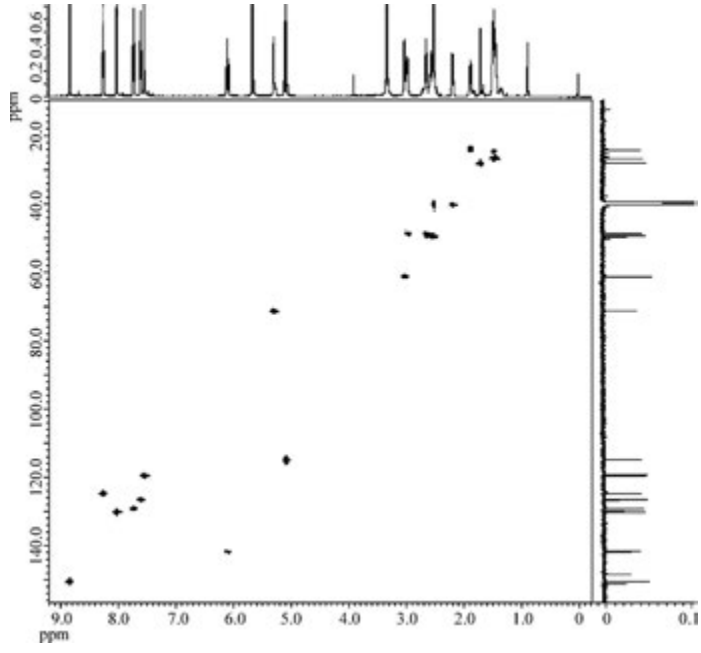


Figure 6.123 The HMQC spectrum of the compound

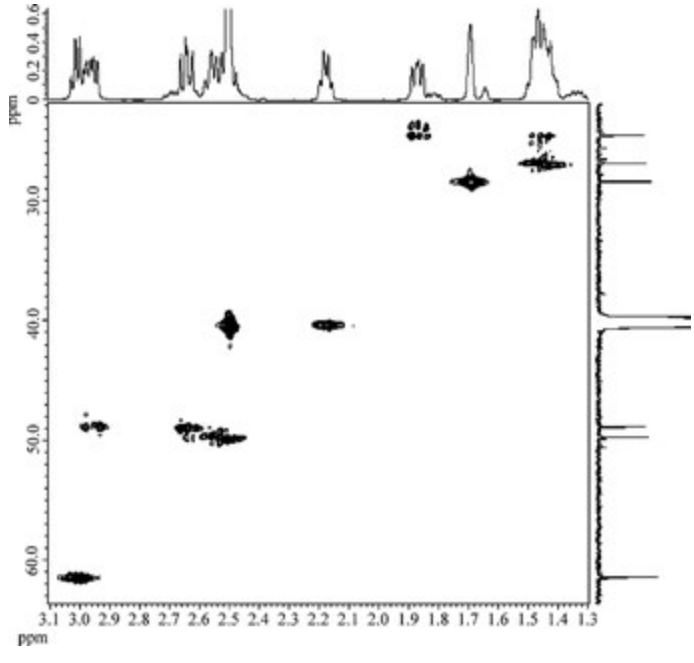


Figure 6.124 The locally enlarged HMQC spectrum (1)

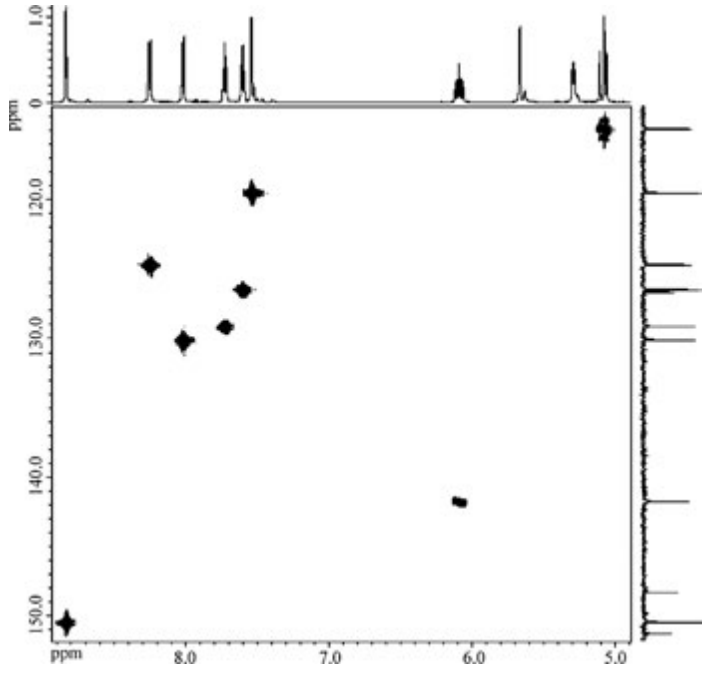


Figure 6.125 The locally enlarged HMQC spectrum (2)

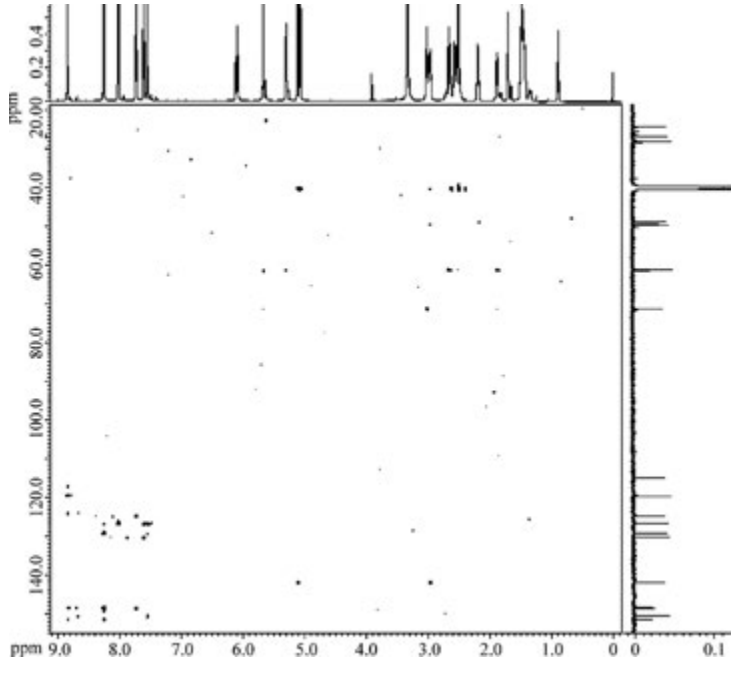


Figure 6.126 The HMBC spectrum of the compound

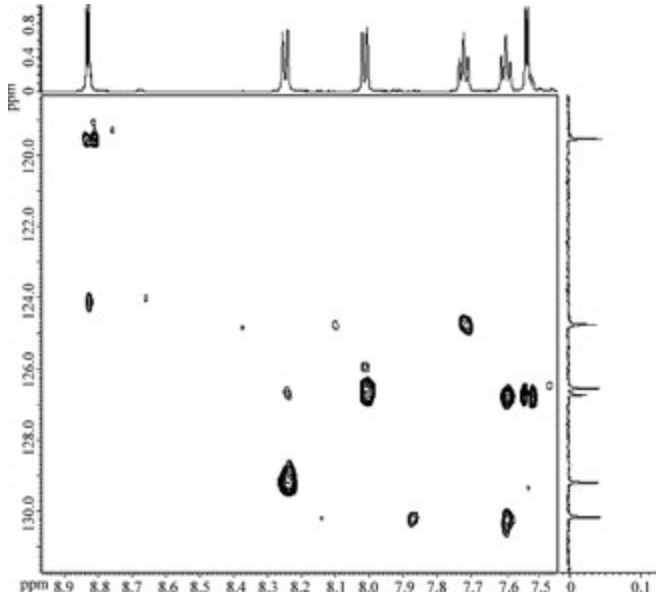


Figure 6.127 The locally enlarged HMBC spectrum (1)

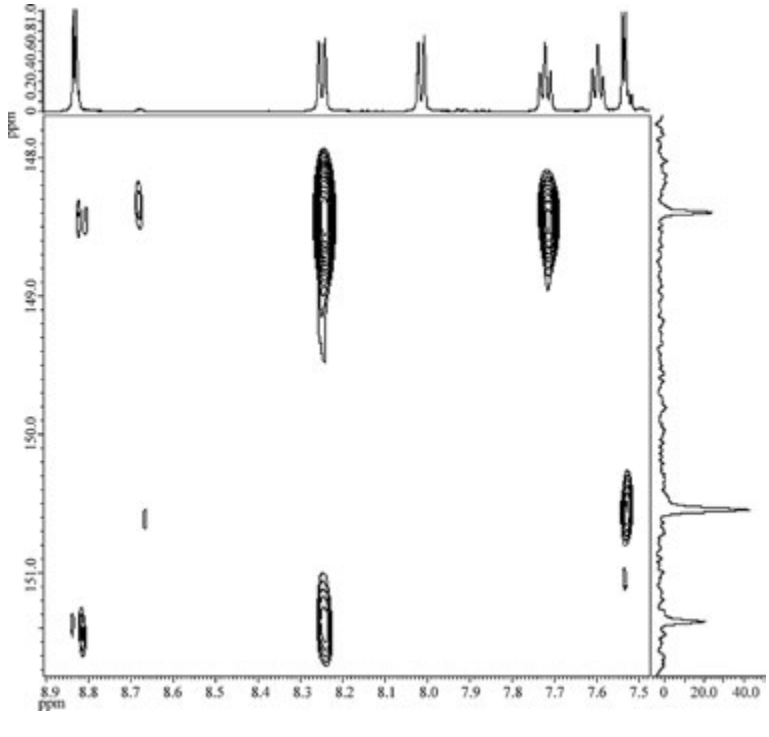


Figure 6.128 The locally enlarged HMBC spectrum (2)

measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is deuterated DMSO.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The peak at about 2.50 ppm is the solvent peak. The peak at about 3.30 ppm is the water peak when deuterated DMSO is used as the solvent.

From peak areas, many tiny peaks in the ^{1}H spectrum can be determined as impurity peaks. Another method to find impurity peaks is the inspection of the HMQC (or HSQC) spectrum. The impurity peaks have small correlated peaks in the HMQC spectrum. For example, the peak at 0.87 ppm in the ^{1}H spectrum has an integral value of 0.87, from which the peak seems to be a sample peak. However, it has a correlated peak with a low intensity in the HMQC spectrum, so that it can be determined as an impurity peak.

If the integral value of a peak is close to an integer, the hydrogen atom number corresponding to the peak can be determined. If the integral value deviates from an integer, the hydrogen atom number corresponding to the peak should be determined with the help of the HMQC spectrum.

With the help of the HMQC spectrum, the data summarized from the ^{1}H spectrum are listed in Table 6.42.

Because coupling constants to be read out may suffer from errors, the couplings are determined mainly by the COSY spectrum.

Now we will interpret the ^{13}C spectrum.

Table 6.42 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Integral value	Number of H atoms	Peak shape	J (Hz)	Remarks
1.46	3.65	3	m		The number of H atoms is determined with the help of the HSQC spectrum.
1.69	0.97	1	s		
1.86	1.19	1	m		
2.17	1.00	1	q	8.9	
2.53	1.72	2	m		
2.64	1.21	1	m		
2.95	1.26	1	m		
3.01	0.92	1	q	8.2	
5.07	1.95	2	m		
5.28	1.18	1	m		
5.66	0.94	1	d	4.8	
6.08	0.98	1	m		
7.52	1.13	1	d	4.1	
7.59	1.09	1	t	8.2	
7.72	1.12	1	t	7.6	
8.01	1.03	1	d	8.9	
8.24	1.12	1	d	8.2	
8.83	1.04	1	d	4.1	

Table 6.43 The summarized data from the ^{13}C spectrum and the ^{1}H spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atom	Directly connected H atoms, δ_H (ppm)	Remarks
1	24.49	1	CH_2	1.46, 1.86	
2	26.81	1	CH_2	1.43, 1.48	
3	28.31	1	CH	1.69	
4	40.03	1	CH	2.17	
5	48.78	1	CH_2	2.64, 2.95	
6	49.64	1	CH_2	2.49, 2.56	The order is determined by the DEPT spectrum.
7	61.36	1	CH	3.01	
8	71.47	1	CH	5.28	
9	114.94	1	CH_2	5.07	
10	119.55	1	CH	7.52	
11	124.76	1	CH	8.24	
12	126.56	1	CH	7.59	Determined by the enlarged spectrum
13	126.74	1	C		
14	129.22	1	C	7.72	
15	130.19	1	CH	8.01	
16	141.81	1	CH	6.08	
17	148.40	1	C		
18	150.55	1	CH	8.83	
19	151.35	1	C		

Because the intensities of peaks of impurities and those of the peaks of the sample are greatly different, the peaks of impurities can be easily recognized. It should be noticed that there is a sample peak overlapped on the solvent peaks. There are two correlated peaks at about 40 ppm on the F_1 axis and at about 2.5 and 2.17 ppm on the F_2 axis, respectively, in the HMQC spectrum. The former is the correlated peak of the solvent, and the latter belongs to the sample. Therefore, we know that a sample peak is overlapped on the peaks of the solvent.

The comprehensive interpretation of the ^{13}C spectrum, the DEPT spectrum, the HMQC spectrum, and the ^{1}H spectrum gives Table 6.43.

The correlated peaks in the COSY spectrum are close, even in the enlarged COSY spectrum.

First we read the data of the COSY spectrum and its enlarged spectrum to give Table 6.44, and then analyze the data.

The correlated peak at $F_2 = 3.30$ ppm and $F_1 = 5.66$ ppm in the COSY spectrum is not listed in Table 6.44, because the peak at 3.30 ppm in the ^{1}H spectrum is the water peak. This correlated peak in the COSY spectrum implies that the peak at 5.66 ppm in the ^{1}H spectrum may be the peak of a reactive hydrogen atom. This postulation is proved by the HMQC spectrum, in which the peak has no correlated peak.

It is difficult to interpret the correlated peaks at about 1.46 ppm, because the peak sets in this region come from three hydrogen atoms. Every peak set of the three hydrogen atoms has its correlated peaks. Therefore, their correlated peaks are close in the COSY spectrum.

Table 6.44 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H atoms, δ_H (ppm)
1	1.46	(1.69), 1.86, 2.53, 3.01
2	1.69	1.46
3	1.86	1.46, 3.01
4	2.17	2.64, 2.95, 6.08
5	2.53	1.46
6	2.64	2.17, 2.95
7	2.95	2.17, 2.64
8	3.01	1.46, 1.86, 5.28
9	5.07	6.08
10	5.28	3.01, 5.66
11	5.66	5.28
12	6.08	2.17, 5.07
13	7.52	8.83
14	7.59	7.72, (8.01), 8.24
15	7.72	7.59, 8.01, (8.24)
16	8.01	(7.59), 7.72
17	8.24	7.59, (7.72)
18	8.83	7.52

Parenthesis () reresents weak couplings.

When we have trouble interpreting the close correlated peaks in a COSY spectrum, the use of the HMBC spectrum is helpful. Because the F_1 axis of the HMBC spectrum is δ_C, the resolution of the HMBC spectrum is in general higher than that of the COSY spectrum. However, the HMBC spectrum of the compound has no correlated peaks in this region. Therefore, we should use as many data of the HMQC spectrum as possible.

From the HMQC spectrum (Table 6.43), it can be known that three peak sets are situated at 1.43, 1.46, and 1.48 ppm in the ^{1}H spectrum. The peak sets at 1.43 and 1.48 ppm belong to a CH_2 group and the peak sets at 1.46 and 1.86 ppm belong to another CH_2 group. The aim in interpreting the COSY spectrum is to find vicinal couplings. Since ^2J couplings of chemically non-equivalent two hydrogen atoms of a CH_2 group can be found from the HMQC spectrum, we can focus our attention on their ^3J couplings.

The data summarized from the HMBC spectrum are listed in Table 6.45.

The correlated peak at about 2.50 ppm on the F_2 axis and that at about 40 ppm on the F_1 axis are not listed in Table 6.45, because they are from the solvent.

The above four tables contain the information on all NMR spectra, with which we will assign the ^{1}H spectrum and the ^{13}C spectrum.

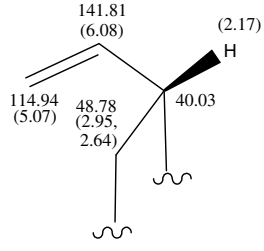
We now start from the substituted ethylene. According to No. 9 (12) of Table 6.44, the correlation of δ_H (ppm) 5.07/6.08 exists. Combining the HSQC spectrum, the related δ_C can be assigned. Therefore, we obtain the following structural unit, which is a terminal group of the compound.

Table 6.45 The data summarized from the HMBC spectrum

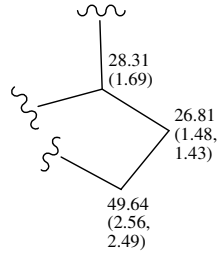
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	24.49		
2	26.81		
3	28.31		
4	40.03	2.95, 5.07	
5	48.78	2.17	
6	49.64	2.95	
7	61.36	1.86, 2.64, 5.28, 5.66	
8	71.47	(1.86), 3.01	
9	114.94		
10	119.55	8.83	
11	124.76	7.72	
12	126.56	8.01	
13	126.74	7.52, 7.59	
14	129.22	8.24	
15	130.19	7.59	
16	141.81		
17	148.40	7.72, 8.24	
18	150.55	7.52	
19	151.35	8.24, 8.83	

Parenthesis () represents weak couplings.

According to Nos. 12 and 4 of Table 6.44, the correlations of δ_H (ppm) 6.08/2.17 and 2.17/2.64, 2.95 exist. Combining the HSQC spectrum, the related δ_C can be assigned. Therefore, the above structural unit can be extended as follows:

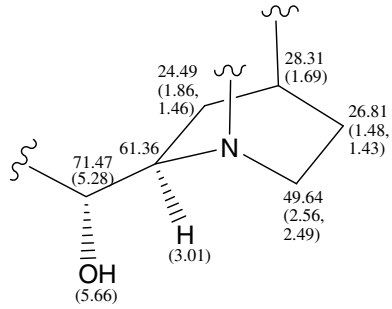


Then we start from No. 6 of Table 6.43, that is, the CH_2 group with $\delta_C = 49.64$ and $\delta_H = 2.49, 2.56$ ppm. According to Nos. 5 and 2 of Table 6.44, that is, the correlations of δ_H (ppm) 2.53/1.46 and 1.46/1.69 exist. Combining the HSQC spectrum, the related δ_C can be assigned. Therefore, we obtain the following structural unit:

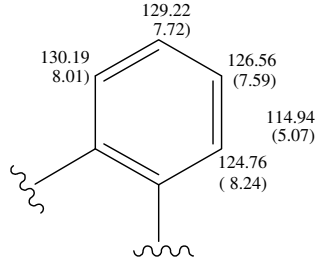


For the above postulation, the 2.53 ppm is the averaged value of 2.49 and 2.56 ppm, and the 1.46 ppm is the averaged value of 1.43 and 1.48 ppm.

According to Nos. 1, 8, and 10 of Table 6.44, the correlations of δ_H (ppm) 1.46/1.69, 1.86, 3.01/1.46, 1.86, 5.28; 5.28/3.01, 5.66 exist. Combining the HSQC spectrum, the related δ_C can be assigned. Therefore, the above structural unit can be extended further as follows:

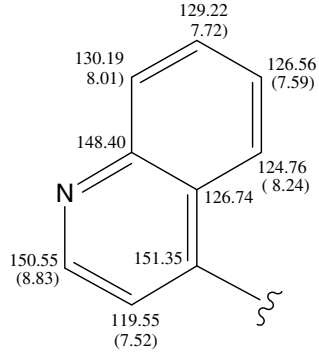


In addition, from Nos. 16, 15, 14, and 17 of Table 6.44, the couplings of δ_H (ppm) 8.01/7.72/7.59/8.24, and the weak couplings of δ_H (ppm) 8.01/7.59 and 7.72/8.24 exist. These couplings represent the existence of an ortho-substituted benzene ring. Therefore, we have

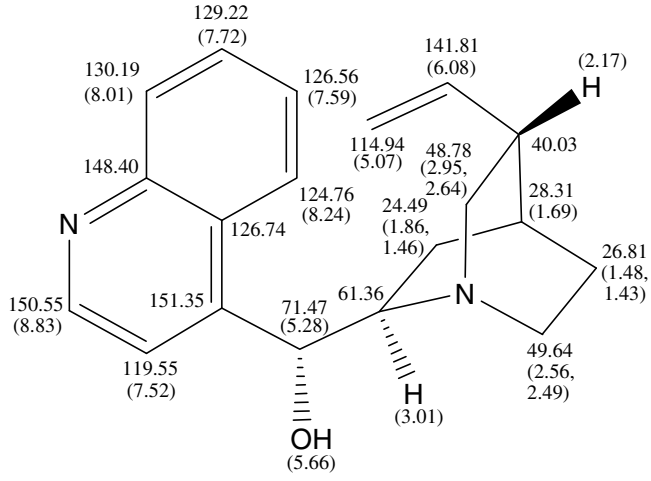


According to Nos. 13, 17 of Table 6.45, that is, the heteroatom long-range couplings of δ_C (126.74)/δ_H (7.59) and δ_C (148.40)/δ_H (7.72, 8.24), the δ_C values of the two quaternary carbon atoms of the substituted benzene ring can be determined.

According to Nos. 17, 13, and 19, that is, the heteroatom long-range couplings of δ_C (148.40)/δ_H (8.83), δ_C (126.74)/δ_H (7.52), and δ_C (151.35)/δ_H (8.83, 8.24) ppm, with the help of the related data of the HMQC spectrum, the assignment of the left part of the anticipated structure is accomplished. That is:



The combination of the three structural units mentioned above forms the whole structure with the assignments of the ^{1}H spectrum and the ^{13}C spectrum.



C6-13

For the above structure, some connections lack direct evidence.

The connection of C ($\delta_C = 151.35$), CH($\delta_C = 71.47$, $\delta_H = 5.28$) lacks related correlated peaks in the HMBC spectrum.

The connection of CH($\delta_C = 40.03$, $\delta_H = 2.17$), CH($\delta_C = 28.31$, $\delta_H = 1.69$) lacks its related correlated peaks in the COSY spectrum and in the HMBC spectrum.

The absence of correlated peaks in the COSY spectrum may be due to the fact that the related dihedral angle is close to 90°. The absence of correlated peaks in the HMBC spectrum may be due to imperfect parameters for the NMR measurement.

In spite of the above-mentioned shortcomings, the assignments prove that the anticipated structure is correct.

Example 6.13 An unknown compound has its molecular weight of 448. Its ^{1}H spectrum, its ^{1}H spectrum measured after the exchange with D_2O, its ^{13}C spectrum, its DEPT spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.129–6.137, respectively. Try to deduce its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated DMSO.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The peak at about 2.50 ppm is the solvent peak. The peak at about 3.30 ppm is the water peak when deuterated DMSO is used as the solvent. From its very small peak area, the peak at 8.29 ppm can be determined as an impurity peak.

From the ^{1}H spectrum, it can be known that the unknown compound contains 20 hydrogen atoms.

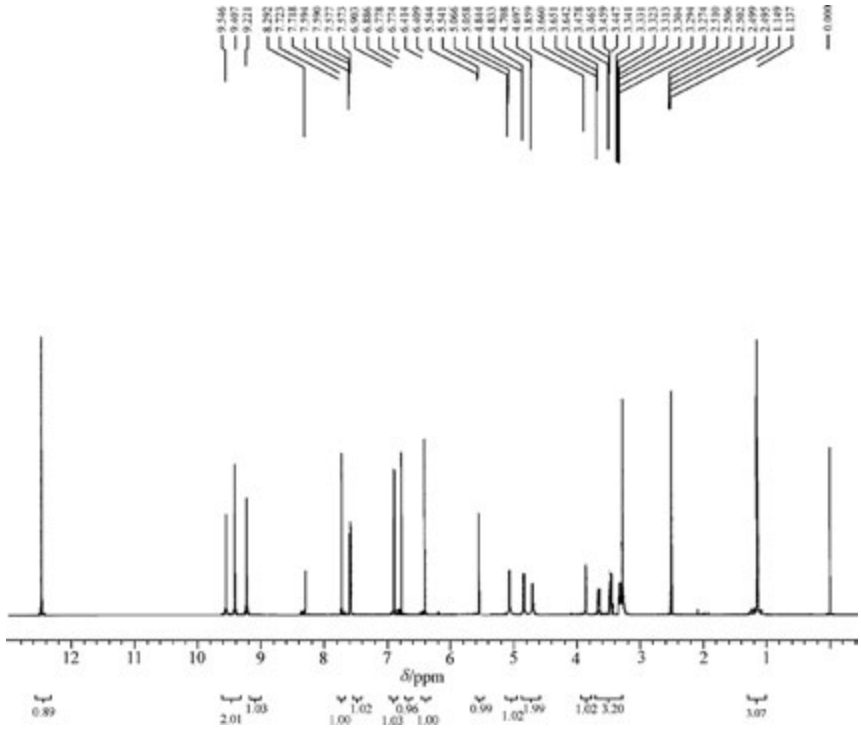


Figure 6.129 The ^{1}H spectrum of the unknown compound

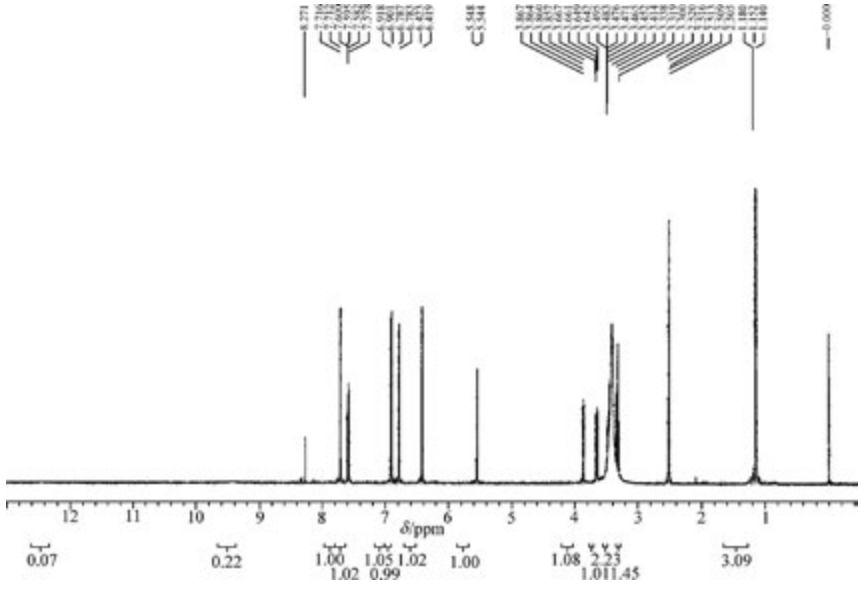


Figure 6.130 The locally enlarged ^{1}H spectrum

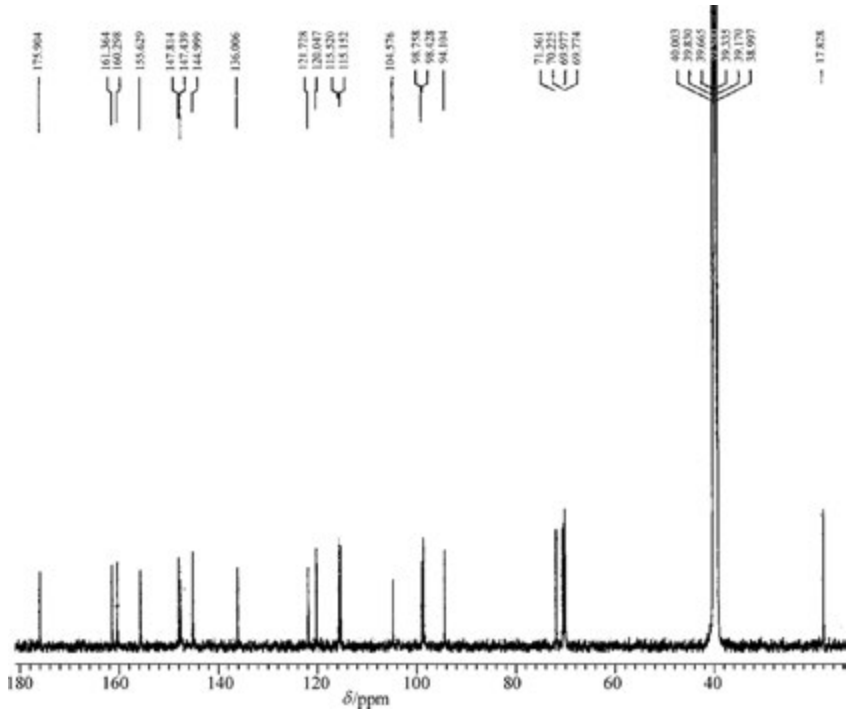


Figure 6.131 The ^{13}C spectrum of the unknown compound

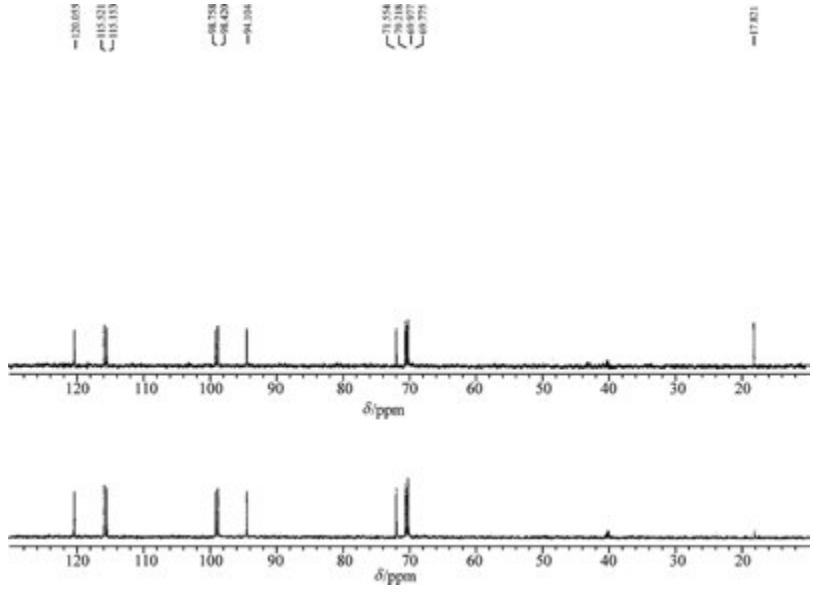


Figure 6.132 The DEPT spectrum of the unknown compound

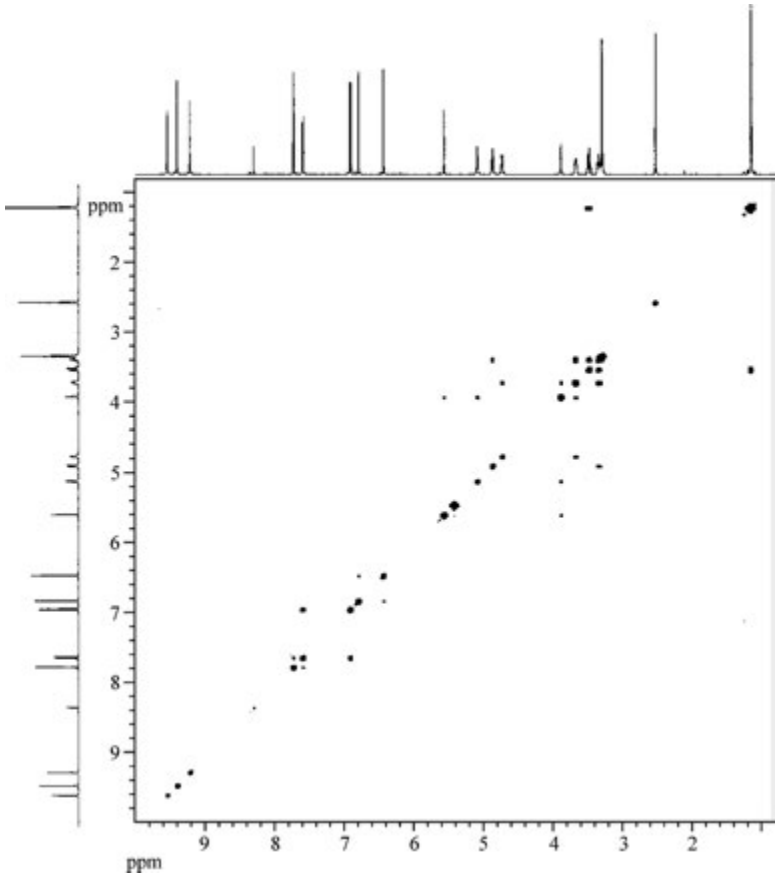


Figure 6.133 The COSY spectrum of the unknown compound

These 20 hydrogen atoms may contain reactive hydrogen atoms, which can be recognized through the exchange with D_2O. In the ^1H spectrum measured after the exchange with D_2O, the peaks at 4.70, 4.84, 5.06, 9.22, 9.41, 9.55, and 12.47 ppm disappear. Therefore, the seven peaks belong to reactive hydrogen atoms. The above-mentioned peaks have no correlated peaks in the HMQC spectrum, which is also a proof of reactive hydrogen atoms. Because their peak shapes are sharp, they should be hydroxyl or phenol groups. The peak shape of amino groups is blunt. It should be noticed that some peaks of the reactive hydrogen atoms are split. This phenomenon comes from the deuterated DMSO as the solvent, in which case the exchange reactions take place at slow rates. Therefore, the peaks of reactive hydrogen atoms are split by their vicinal hydrogen atoms. For the same reason, these reactive hydrogen atoms will produce the splits of their vicinal hydrogen atoms. Their couplings will be shown in the COSY spectrum.

The data summarized from the ^1H spectrum are listed in Table 6.46, in which chemical shift values are taken from the measurement before the exchange with D_2O.

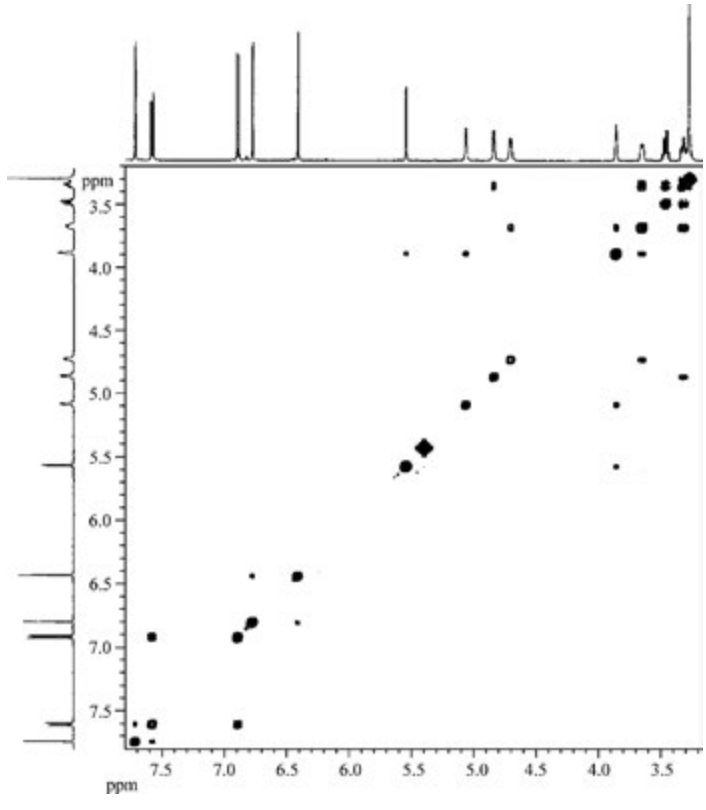


Figure 6.134 The locally enlarged COSY spectrum

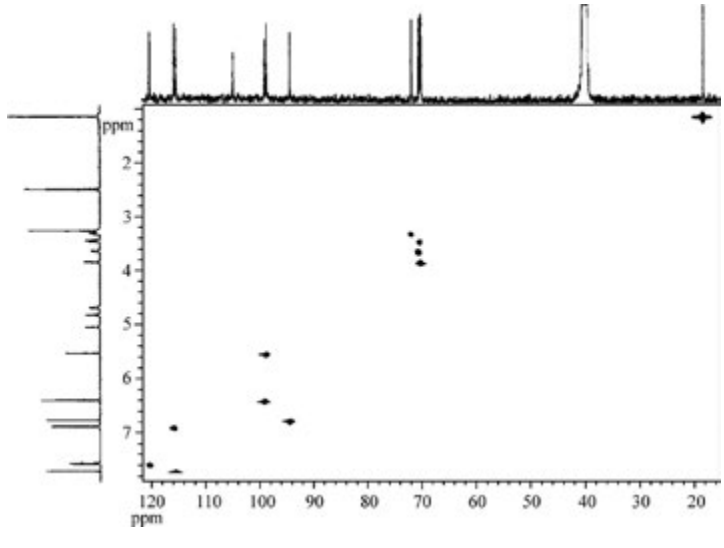


Figure 6.135 The HMQC spectrum of the unknown compound

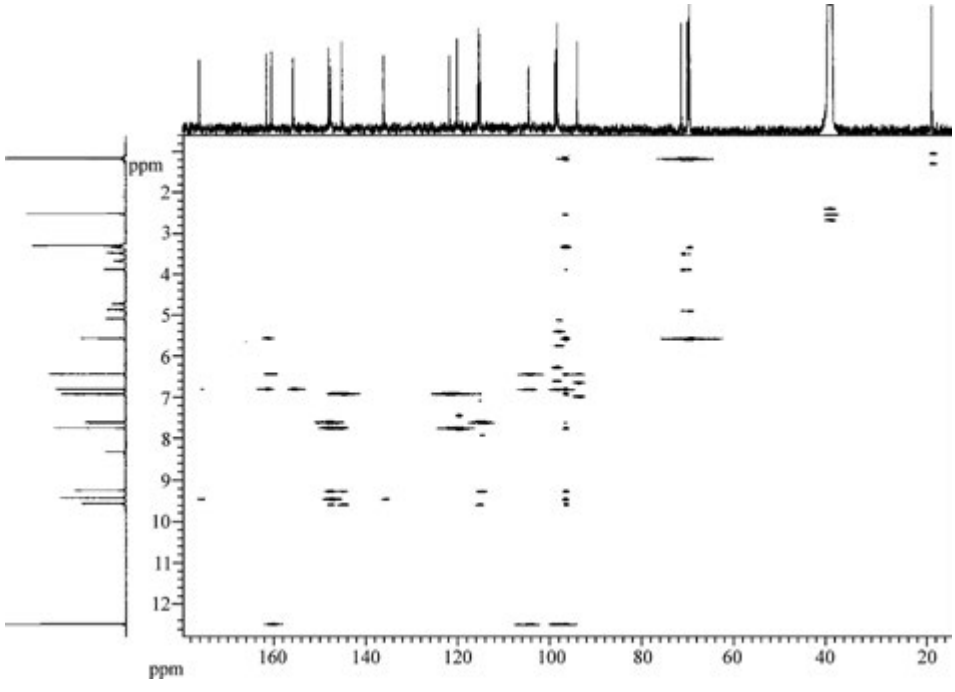


Figure 6.136 The HMBC spectrum of the unknown compound

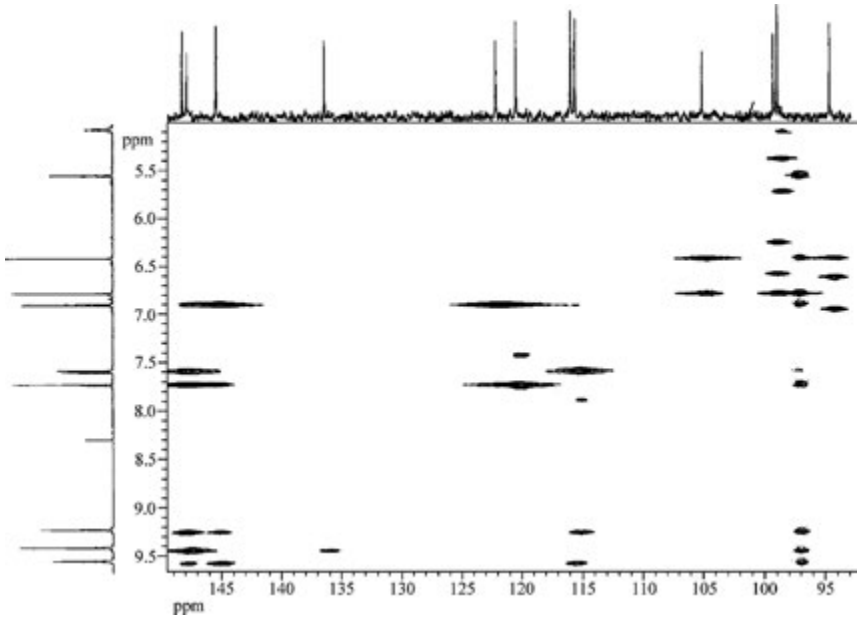


Figure 6.137 The locally enlarged HMBC spectrum

Table 6.46 The data summarized from the ^{1}H spectrum

$\delta_{H(}$ppm)	Number of H atoms	Peak shape	Coupling constant, Hz
1.14	3	d	6
3.31	1	m	
3.46	1	m	
3.65	1	m	
3.86	1	s, blunt	
4.70	1	d	5.5
4.84	1	d	5.5
5.06	1	d	4
5.54	1	d	1.5
6.41	1	d	2.5
6.78	1	d	2
6.89	1	d	8.5
7.58	1	d, d	8.5, 2
7.72	1	d	2.5
9.22	1	s	
9.41	1	s	
9.55	1	s	
12.47	1	s	

Because coupling constants to be read out may suffer from errors, the coupling correlations are determined by the COSY spectrum.

Now we will interpret the ^{13}C spectrum.

The peaks at about 40 ppm are the solvent peaks.

The ^{13}C spectrum shows 21 peaks, which means the unknown compound contains 21 carbon atoms. From the comparison between the ^{13}C spectrum and the DEPT spectrum it can be known that the unknown compound contains 10 quaternary carbon atoms, 10 CH groups, and 1 CH_3 group. The peak at 175.90 ppm should be a carbonyl group, which can be known from its chemical shift value.

The data of the ^{13}C spectrum and the ^{1}H spectrum can be summarized in Table 6.47 by the comprehensive interpretation of the ^{13}C spectrum, the DEPT spectrum, the ^{1}H spectrum, and the HMQC spectrum.

To sum up, the unknown compound contains 21 carbon atoms, 20 hydrogen atoms, and at least 8 oxygen atoms (7 hydroxyl groups and 1 carbonyl group). The addition of the masses of the above-mentioned compositions is 400 (u). The remaining mass of the unknown compound is 48, which corresponds to three (other) oxygen atoms. Therefore, the molecular formula should be $C_2{}^{1}H_{20O_{11}}$, from which its unsaturation number of 12 can be calculated. The value of 12 is a rather great number, which implies the existence of aromatic systems. This estimation coincides with the peaks in the aromatic region of the ^{13}C spectrum.

Now we will find structural units of the unknown compound, and then assemble them. The COSY spectrum plays an important role in the determination of the connection of structural units, especially when the unknown compound contains fewer quaternary carbon atoms and heteroatoms.

The data summarized from the COSY spectrum are listed in Table 6.48.

Table 6.47 The summarized data from the ^{13}C spectrum and the ^{1}H spectrum

δ_C(ppm)	Number of C atoms	Order of C atoms	Directly connected H, δ_H (ppm)
17.83	1	CH_3	1.14
69.77	1	CH	3.86
69.98	1	CH	3.46
70.23	1	CH	3.65
71.56	1	CH	3.31
94.10	1	CH	6.78
98.43	1	CH	5.54
98.76	1	CH	6.41
104.58	1	C	
115.15	1	CH	7.72
115.52	1	CH	6.89
120.05	1	CH	7.58
121.73	1	C	
136.01	1	C	
144.99	1	C	
147.44	1	C	
147.81	1	C	
155.63	1	C	
160.29	1	C	
161.36	1	C	
175.90	1	C	

Table 6.48 The data summarized from the COSY spectrum

δ_H (ppm)	Coupled H, δ_H (ppm)
1.14	3.46
3.31	3.46, 3.65, 4.84
3.46	1.14, 3.31
3.65	3.31, 3.86, 4.70
3.86	3.65, 5.06, 5.54
4.70	3.65
4.84	3.31
5.06	3.86
5.54	3.86
6.41	(6.78)
6.78	(6.41)
6.89	7.58
7.58	6.89, (7.72)
7.72	(7.58)
9.22	isolated
9.41	isolated
9.55	isolated
12.47	isolated

Paenthesis () stands for weak couplings.

Table 6.49 The data summarized from the HMBC spectrum

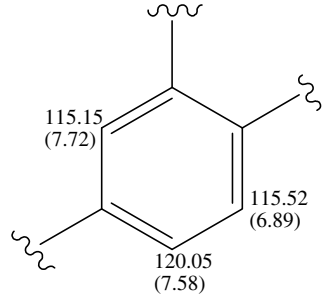
δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
17.83		1.14
69.77, 69.98, 70.23	1.14, 3.31, (3.86), 5.54,	
71.56	1.14, (3.45), (3.86)	
94.10	6.41	6.78
98.43	5.06	5.54
98.76	6.78,	6.41
104.58	6.41, 6.78, 12.47	
115.15	7.58, 9.22	
115.52	9.55	
120.05	7.72	
121.73	6.89	
136.01	(9.41)	
144.99	6.89, 7.72, 9.22, 9.55	
147.44	7.72, 9.41	
147.81	7.58, 7.72, 9.21	
155.63	6.78	
160.29	6.41, 12.47	
161.36	5.54, 6.78	
175.90	9.41	

Parentheis () represents weak correlations.

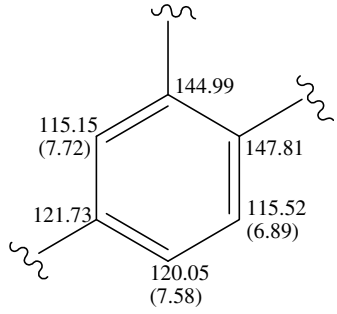
The HMBC spectrum plays an important role in the determination of the connections of structural units separated by quaternary carbon atoms or by heteroatoms. The COSY spectrum can not connect two structural units separated by a quaternary carbon atom or a heteroatom. In this case, the unique solution comes from the HMBC spectrum.

The data summarized from the HMBC spectrum are listed in Table 6.49.

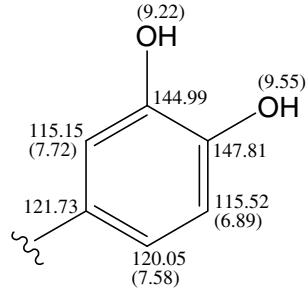
Either from the coupling correlation of δ_H(ppm) 6.89/7.58 and the weak coupling of 7.58/7.72 obtained from the COSY spectrum, or from the analysis of related peak shapes in the ^{1}H spectrum, it can be known that a 1-, 2-, 4- substituted benzene ring exsits. With the help of the HMQC spectrum, we have the structural unit with assignment which follows:



According to the correlations of δ_C (144.99)/δ_H (6.89), δ_C (147.81)/δ_H (7.72, 7.58), and δ_C (121.73)/δ_H (6.89) ppm known from the HMBC spectrum, the chemical shift values of the three quaternary carbon atoms of the substituted benzene ring can be determined as follows:

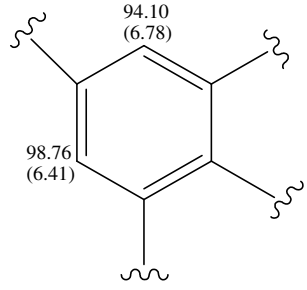


The two quaternary carbon atoms with their chemical shift values of 144.99 and 147.81 ppm, respectively, should connect with an oxygen atom, respectively. And a very important fact is that the correlations of δ_C (144.99)/δ_H (9.55) andδ_C (147.81)/δ_H (9.22) exist. Therefore, the structural unit above can be extended as follows:



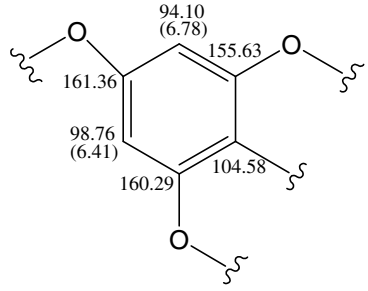
When we extend the above structural unit, we consider that the heteronuclear long-range correlated peak across three chemical bonds has a stronger intensity than that across two or four chemical bonds.

Either from the weak correlation of δ_H (ppm) 6.41/6.78 obtained from the COSY spectrum, or from the analysis of peak shapes in the ^{1}H spectrum, it can be known that a tetra-substituted benzene ring with two separated aromatic hydrogen atoms exists. With the help of the HMQC spectrum, we have the structural unit with assignment which follows:

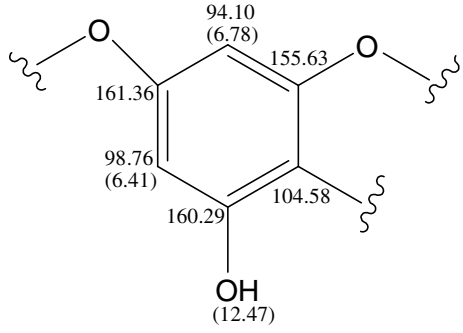


According to the correlations of δ_C (104.58)/δ_H(6.41, 6.78), δ_C (155.63)/δ_H(6.78), δ_C (160.29)/δ_H (6.41), and δ_C (161.36)/δ_H (6.78) ppm known from the HMBC spectrum, the four quaternary carbon atoms of the tetra-substituted benzene ring can be assigned. From

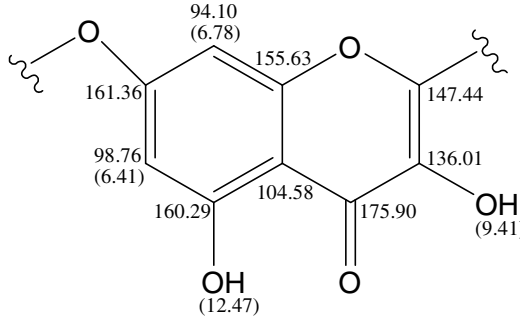
the three chemical shift values greater than 155 ppm, the connection with an oxygen atom, respectively, can be determined.



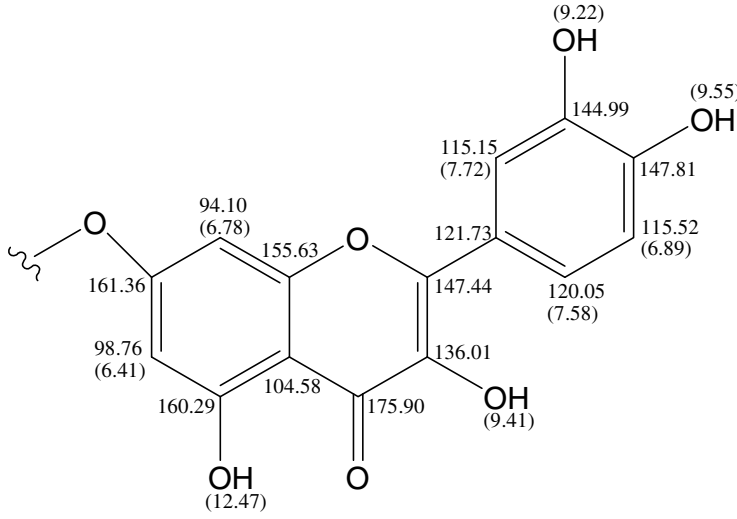
According to the correlations of δ_C (104.58)/δ_H(12.47) and δ_C (160.29)/δ_H(12.47) known from the HMBC spectrum, it can be determined that the functional group connected with the quarternary carbon atom with 160.29 ppm is a hydroxyl group which follows:



In the aromatic-double bond region of the ^{13}C spectrum, there remain two peaks corresponding to quaternary carbon atoms. In the carbonyl region of the ^{13}C spectrum, there remains one peak. According to the correlations of δ_C (147.44)/δ_H (9.41) and δ_C (175.90)/δ_H (9.41) ppm, and the weak correlation of δ_C (136.01)/δ_H (9.41) ppm known from the HMBC spectrum, the above structural unit can be extended as follows:

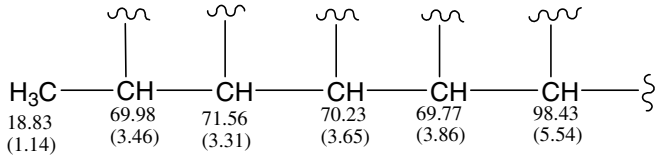


According to the correlation of δ_C (147.44)/δ_H (7.72) known from the HMBC spectrum, these two structural units mentioned above can be connected as follows:

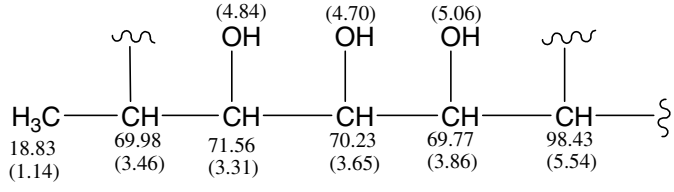


The above structural unit has an unsaturation number of 11. Therefore, this compound should have another ring, because all peaks in the aromatic-double bond region of the ^{13}C spectrum have been set in the structural units mentioned above.

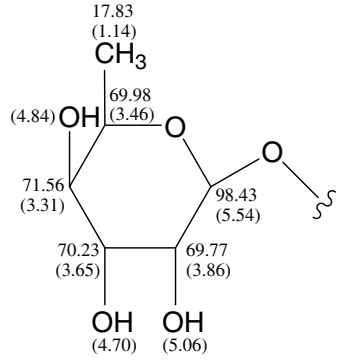
Now we will analyze the peaks in the high field region of the ^{1}H spectrum and the ^{13}C spectrum. From the COSY spectrum, the coupling relationships of δ_H (ppm) 1.14/3.46/3.31/3.65/3.86/5.54 can be found. With the help of the HMQC spectrum, the related δ_C values can be determined. Therefore, we obtain the following structural unit and its assignment:



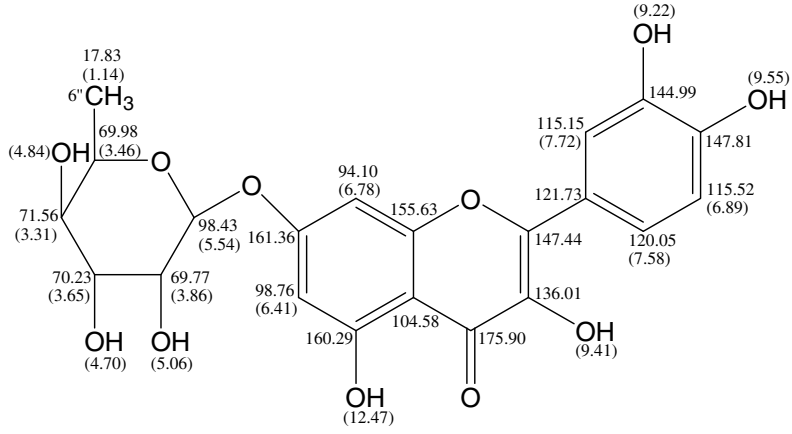
According to the correlations of δ_H (ppm) 4.84/3.31, 4.70/3.65, and 5.06/3.86 known from the COSY spectrum, the connections of three hydroxyl groups with three CH groups can be determined. Therefore, the above structural unit can be extended as follows:



The carbon atom with $\delta_C = 69.98$ ppm should connect with an oxygen atom. The carbon atom with $\delta_C = 98.43$ ppm should connect with two oxygen atoms. From the chemical shift values of these six carbon atoms and from the remaining unsaturation number of 1, it can be postulated that the unknown compound contains a saccharine ring. If the locally enlarged HMBC spectrum around 98.43 ppm is available, the formation of the ring can be proved further. Therefore, we have the following structural unit:



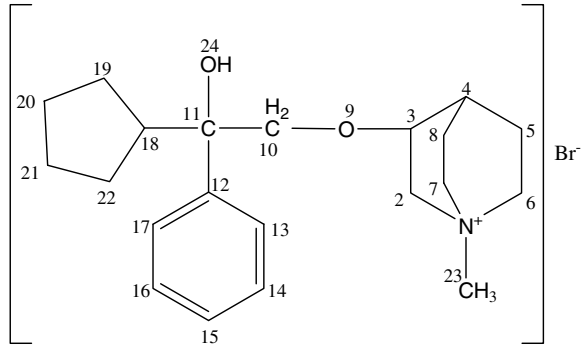
Because of the correlation of δ_C (161.36)/δ_H (5.54) ppm and the weak correlation of δ_C (98.43)/δ_H (6.78) known from the HMBC spectrum, the above two structural units can be connected. Therefore, we obtain the structure of the unknown compound and its assignments which follow:



C6-14

The assignments coincide with all NMR spectra. Therefore, the deduced structure is correct.

Example 6.14 A synthesized compound has an anticipated structure which follows:



C6-15

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum (in the high field region), its ^{13}C spectrum, the locally enlarged ^{13}C spectrum (in the high field region), its DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum (in the high field region and in the low field region), its HMQC spectrum, the locally enlarged HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum (in the high field region) are shown in Figures 6.138–6.153. Try to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is deuterated methanol.

Solution

It should be noticed that the ^{1}H spectrum will change greatly with different solvents. The NMR spectra above were measured in deuterated methanol.

First we will interpret the ^{1}H spectrum.

Because the concentration of the sample in the measured tube is high, the solvent peak in the ^{1}H spectrum almost disappears. However, the water peak is obvious.

The peak shapes of most peak sets in the ^{1}H spectrum are complicated.

The data summarized from the ^{1}H spectrum are listed in Table 6.50. Some explanation will be given later.

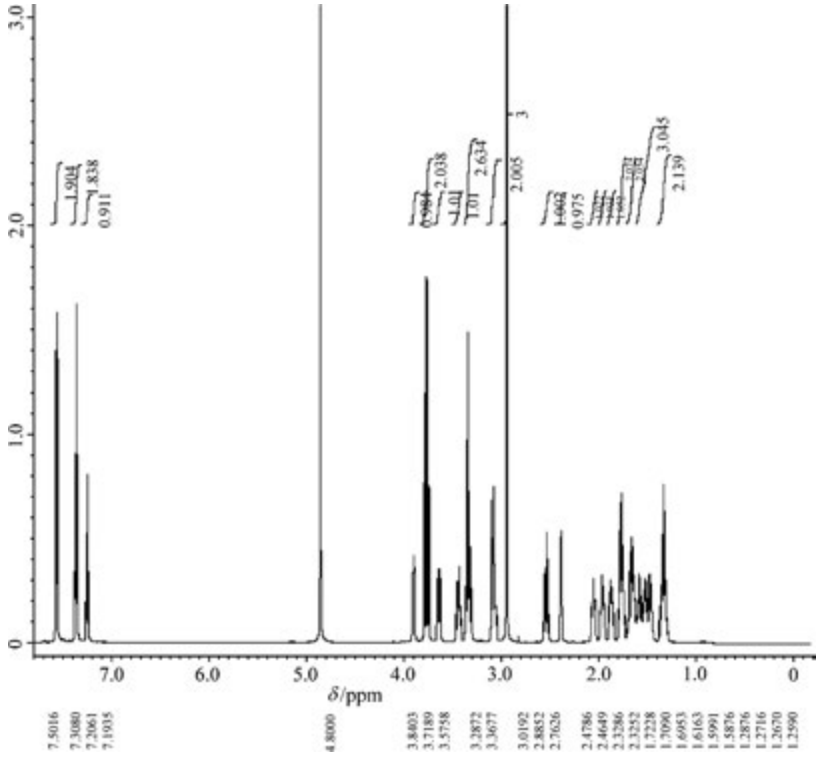


Figure 6.138 The ^{1}H spectrum of the compound

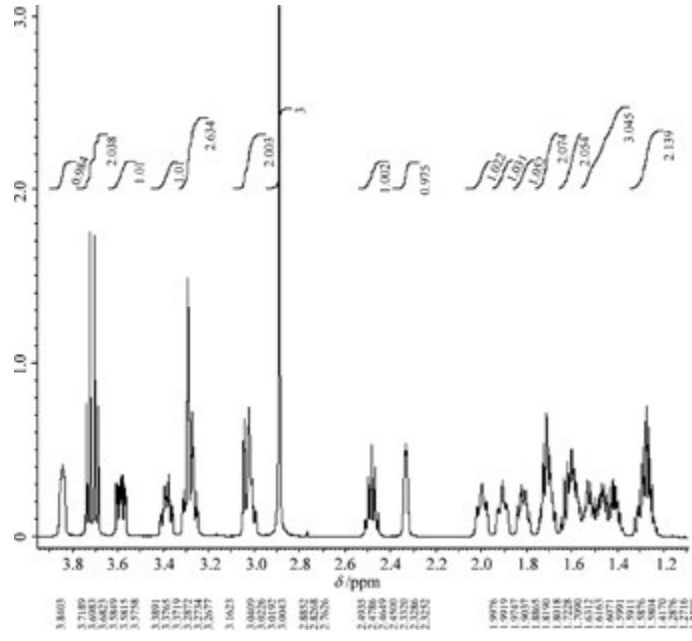


Figure 6.139 The locally enlarged ^{1}H spectrum (in the high field region)

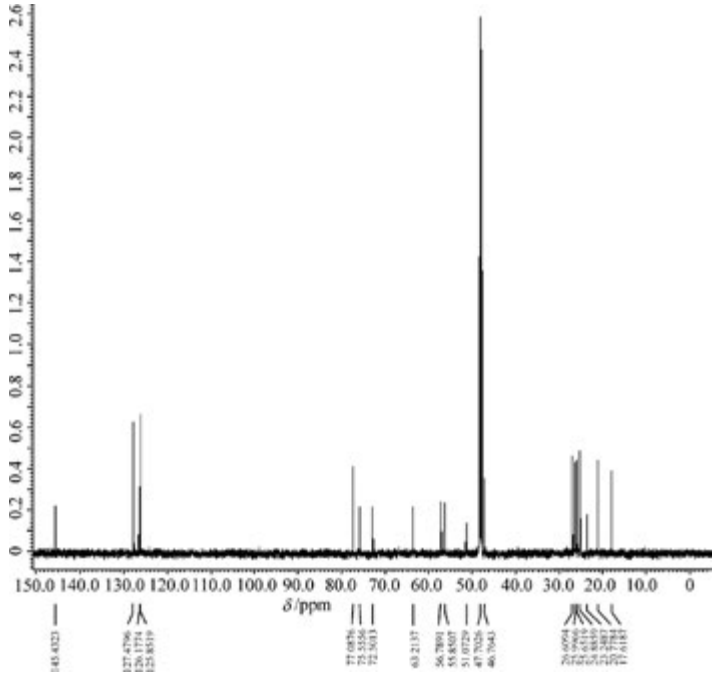


Figure 6.140 The ^{13}C spectrum of the compound

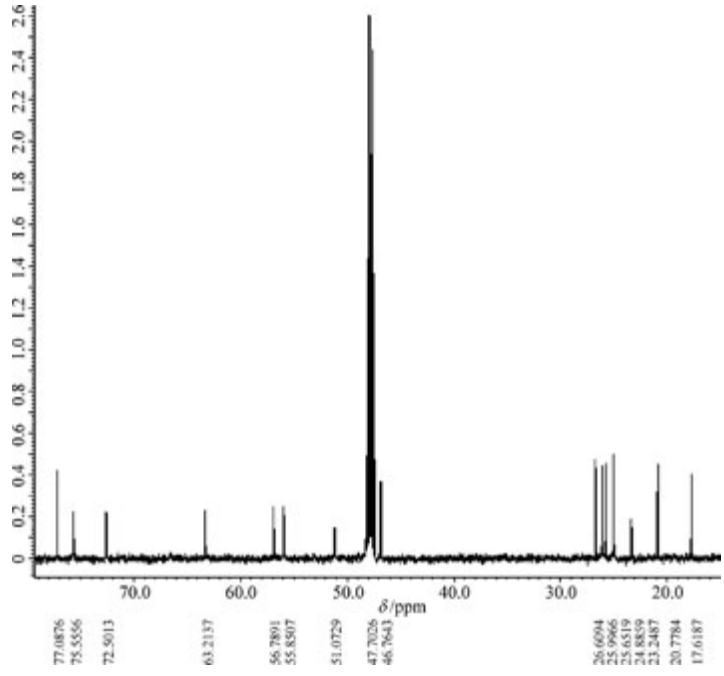


Figure 6.141 The locally enlarged ^{13}C spectrum (in the high field region)

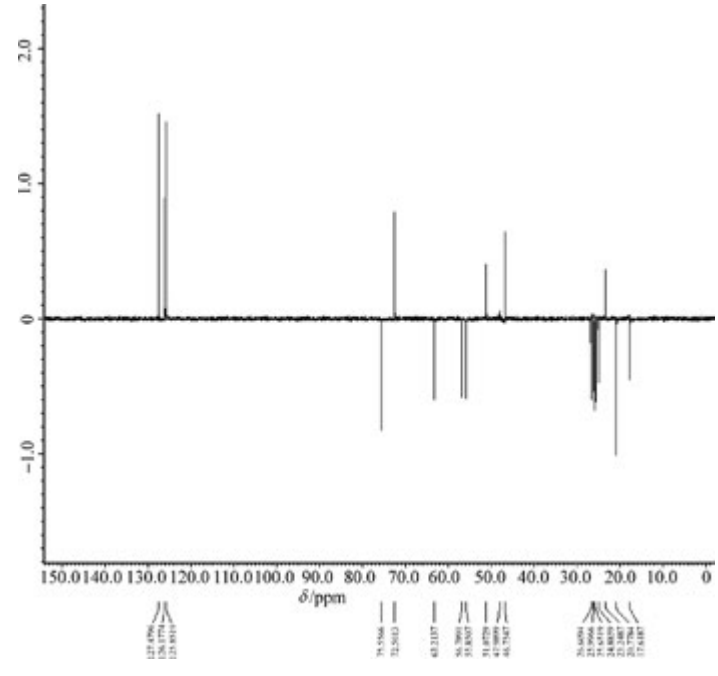


Figure 6.142 The DEPT-135 spectrum of the compound

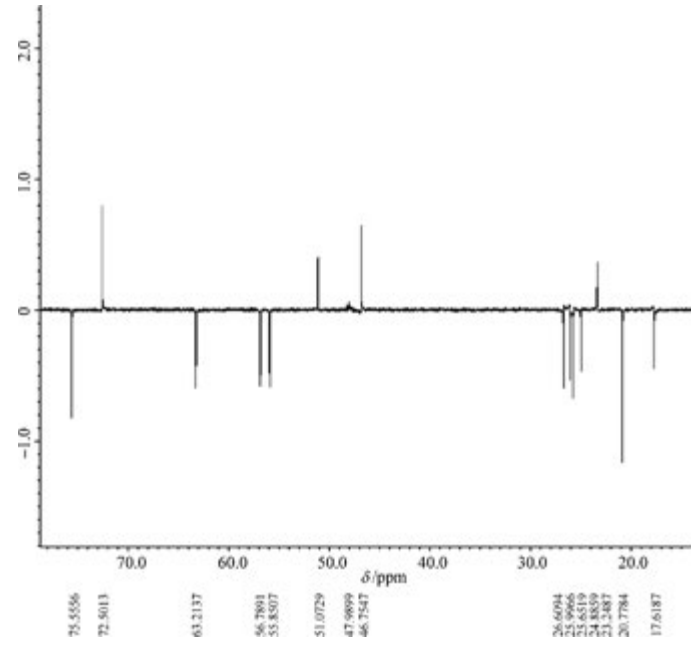


Figure 6.143 The locally enlarged DEPT-135 spectrum (in the high field region)

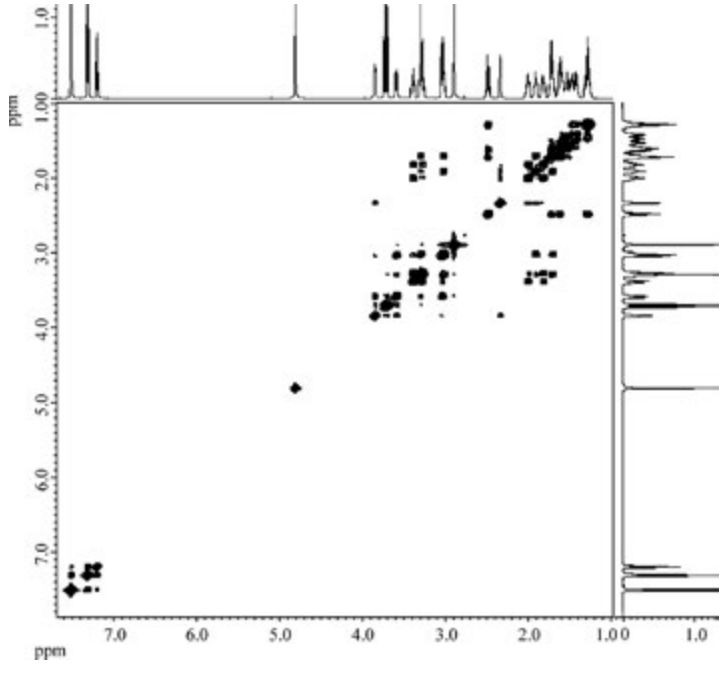


Figure 6.144 The COSY spectrum of the compound

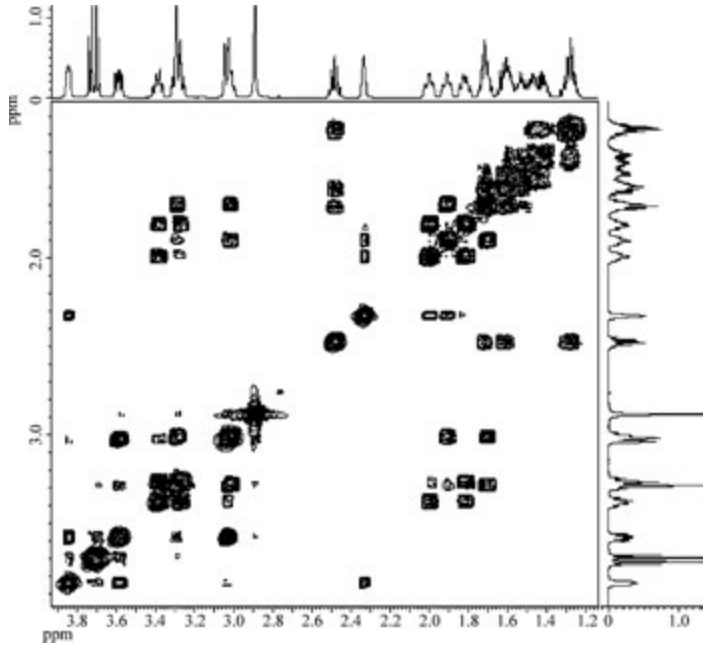


Figure 6.145 The locally enlarged COSY spectrum (in the high field region)

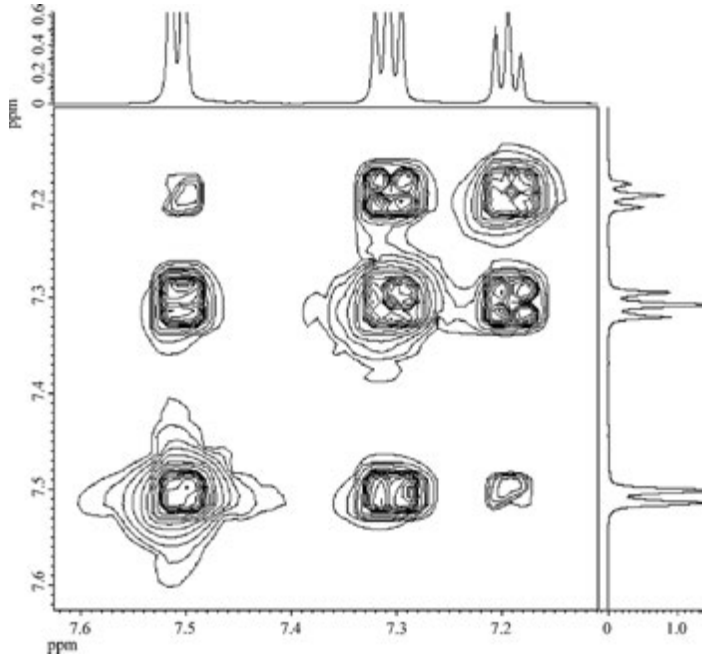


Figure 6.146 The locally enlarged COSY spectrum (in the low field region)

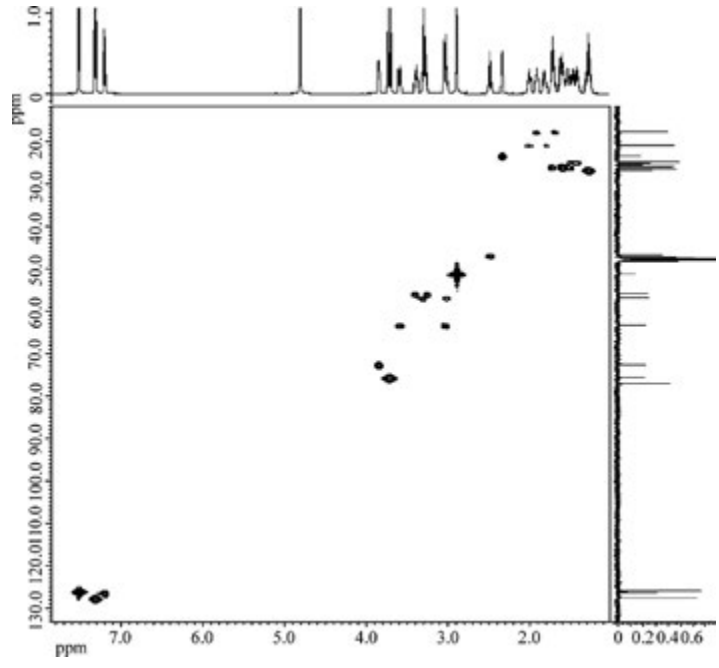


Figure 6.147 The HMQC spectrum of the compound

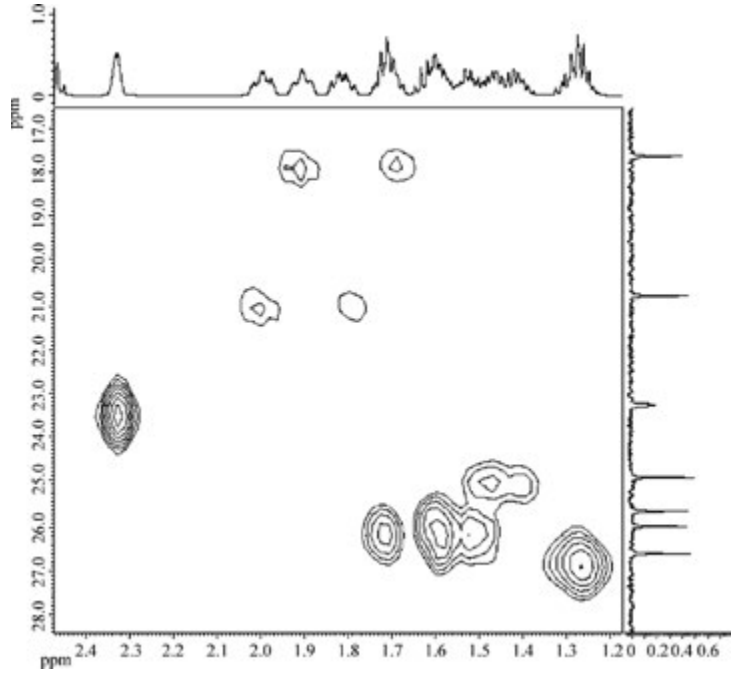


Figure 6.148 The locally enlarged HMQC spectrum (1) (in the high field region)

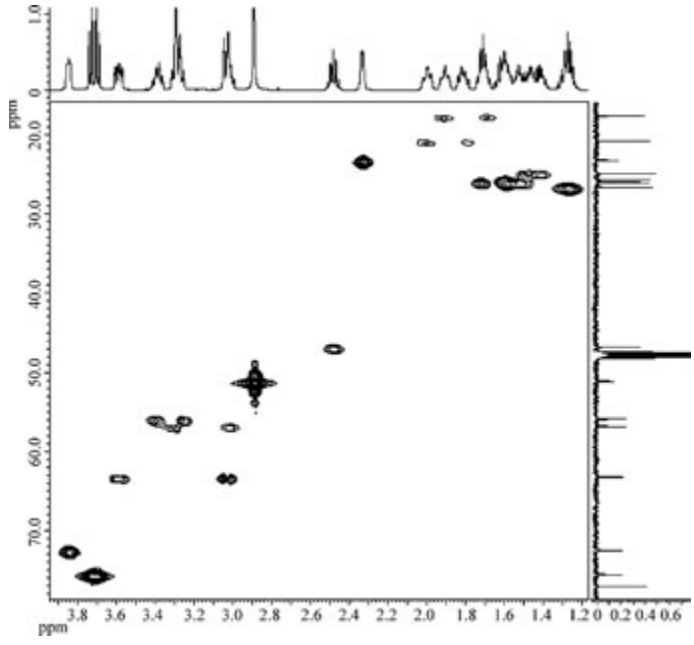


Figure 6.149 The locally enlarged HMQC spectrum (2) (in the high field region)

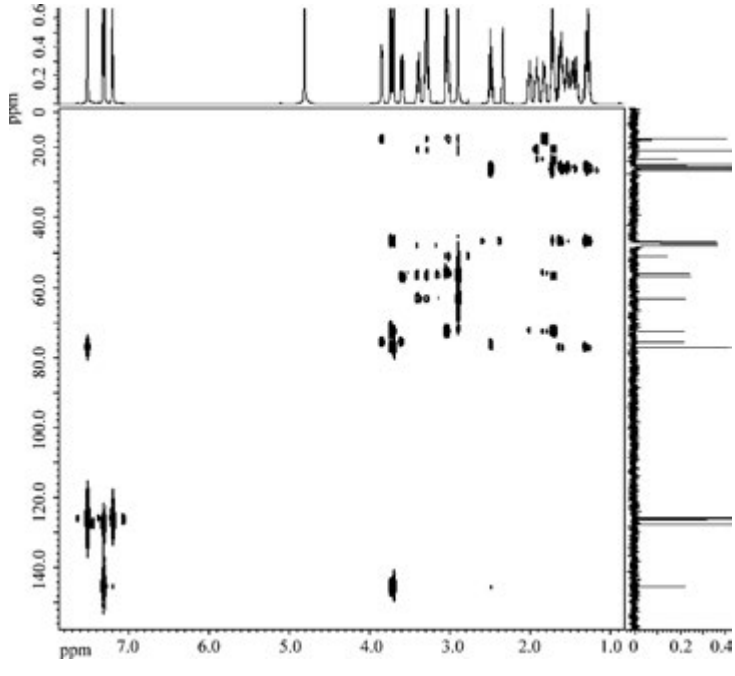


Figure 6.150 The HMBC spectrum of the compound

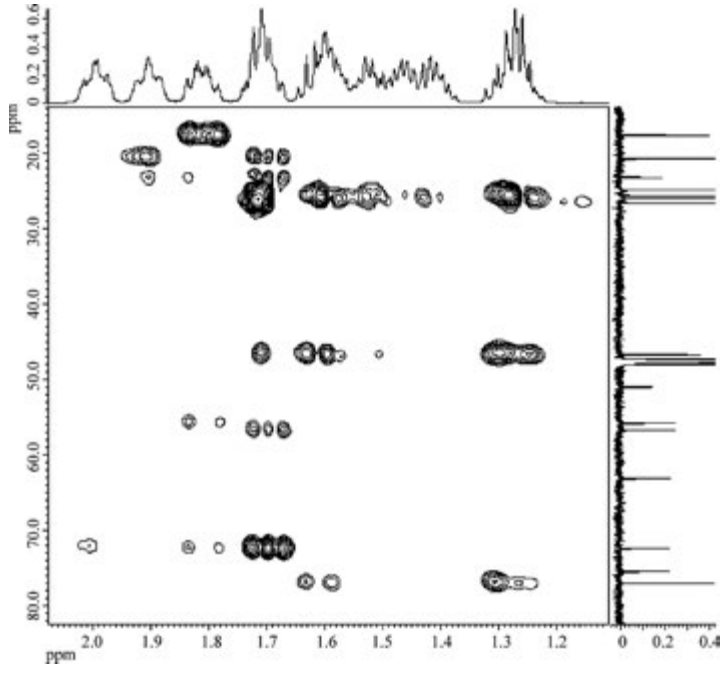


Figure 6.151 The locally enlarged HMBC spectrum (1) (in the high field region)

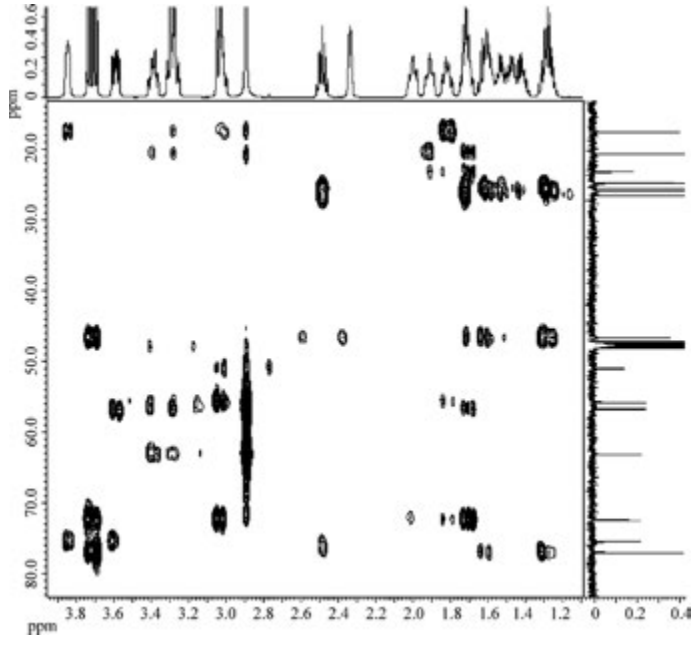


Figure 6.152 The locally enlarged HMBC spectrum (2) (in the high field region)

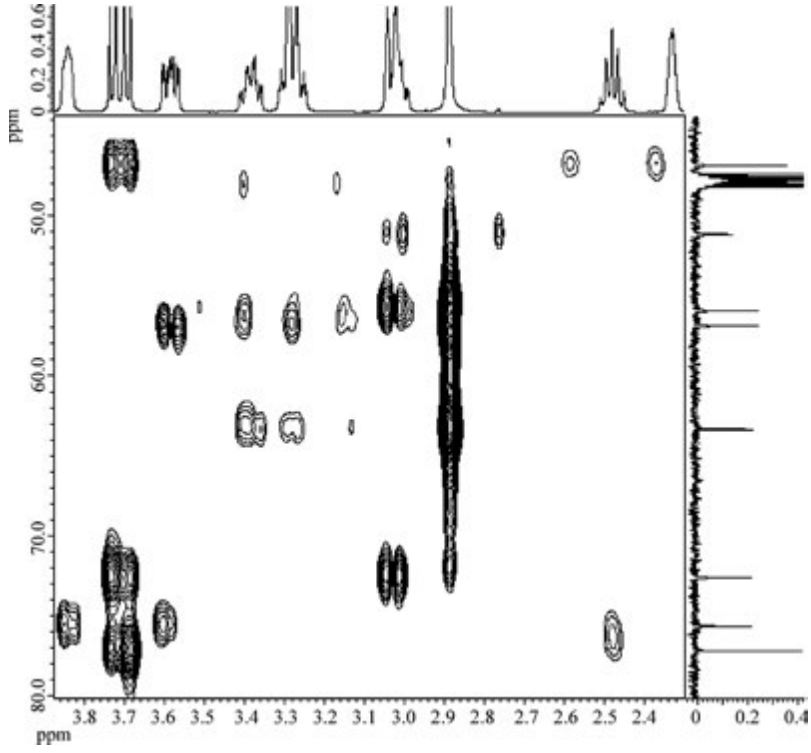


Figure 6.153 The locally enlarged HMBC spectrum (3) (in the high field region)

When we interpret the ^{1}H spectrum, a comprehensive interpretation of the HMQC spectrum and the ^{1}H spectrum is necessary. For example, the integral value of a peak set is not close to an integer, which should be determined by the HMQC spectrum. When several peak sets are close in a narrow region, their discernment needs the help of the HMQC spectrum.

Now we supplement some explanation of Table 6.50.

The peak shapes of the peak sets in the aromatic region are easy to mark.

Because the peak shapes of the two peak sets situated at 3.73 and 3.69 ppm, respectively, show the typical pattern of an AB system, it can be known that they belong to two hydrogen atoms of a CH_2 group. The postulation is proved with the help of the HMQC spectrum.

The integral value of the peak sets in the region of 3.31–3.27 ppm is 2.63, from which the corresponding hydrogen atom number can not be determined as 2 or 3. The most effective method is the interpretation of the HMQC spectrum, from which the overlapped peak sets of two CH groups can be determined. Therefore, the hydrogen atom number is 2.

The integral value of the peak sets at about 3.02 ppm is 2. Because their peak shape is not symmetrical, which implies the two overlapped peak sets with different peak shapes. The HMQC spectrum shows clearly the two overlapped peak sets.

From the HMQC spectrum, it can be known that the peak sets corresponding to two hydrogen atoms at about 1.70 ppm form from two functional groups.

Similarly, from the HMQC spectrum, it can be known that the two hydrogen atoms, whose peak sets are situated at about 1.60 ppm, result from two functional groups.

Table 6.50 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape	Coupling constant *J* (Hz)	Remarks
7.50	2	d	7.6	
7.31	2	t	7.6	
7.19	1	t	7.6	
3.84	1	blunt		
3.73	1	d	9.6	One side of an AB system
3.69	1	d	9.6	One side of an AB system
3.58	1	m		
3.38	1	m		
3.29	1	m		
3.27	1	m		
3.03	1	d		
3.01	1	m		
2.89	3	s		
2.48	1	quintet		
2.33	1	blunt		
1.99	1	m		
1.90	1	m		
1.81	1	m		
1.71	1	m		
1.69	1	m		
1.61	1	m		
1.59	1	m		
1.53	1	m		
1.45	1	m		
1.41	1	m		
1.27	2	m		

All reactive hydrogen atoms have no peaks in the ^{1}H spectrum. Their peaks merge in the water peak because of their rapid exchange reactions.

From Table 6.50 it can be known that the compound contains 31 hydrogen atoms connected directly with carbon atoms, which coincides with the anticipated structure.

Because the peak shapes of the peak sets in the high field region of the ^{1}H spectrum are very complicated, it is difficult to calculate related coupling constants from their splits.

The above explanation is aimed at Table 6.50.

The interpretation of the ^{13}C spectrum should combine the DEPT-135 spectrum, from which the order of the carbon atoms can be determined.

The data summarized from the ^{13}C spectrum and the DEPT-135 spectrum are listed in Table 6.51.

From Table 6.51 it can be known that the compound contains 21 carbon atoms, which coincides with the anticipated structure.

Now we will interpret the HMQC spectrum which correlates the signals of the peaks in the ^{13}C spectrum and the peak sets in the ^{1}H spectrum of the hydrogen atoms, which are connected directly with carbon atoms. Of course, quaternary carbon atoms have no correlated peaks in the HMQC spectrum.

The data summarized from the HMQC spectrum are listed in Table 6.52.

Table 6.51 The data summarized from the ^{13}C spectrum and the DEPT spectrum

δ_C (ppm)	Order of C atoms	Number of C atoms
145.4	C	1
127.5	CH	2
126.2	CH	1
125.9	CH	2
77.1	C	1
75.6	CH_2	1
72.5	CH	1
63.2	CH_2	1
56.8	CH_2	1
55.9	CH_2	1
51.1	CH	1
46.8	CH	1
26.6	CH_2	1
25.9	CH_2	1
25.7	CH_2	1
24.9	CH_2	1
23.2	CH	1
20.8	CH_3	1
17.6	CH_3	1

Note: In the DEPT-135 spectrum, the peaks of CH_3 and CH are upwards, the peaks of CH_2 downwards, and the peaks of quaternary carbon atoms absent. The comparison between the DEPT spectrum and the ^{13}C spectrum can discern the peaks of quaternary carbon atoms.

Table 6.52 The data summarized from the HMQC spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atom	Directly connected H atoms, δ_H (ppm)
1	145.4	1	C	
2	127.5	2	CH	7.31
3	126.2	1	CH	7.19
4	125.9	2	CH	7.50
5	77.1	1	C	
6	75.6	1	CH_2	3.73, 3.69
7	72.5	1	CH	3.84
8	63.2	1	CH_2	3.58, 3.03
9	56.8	1	CH_2	3.29, 3.01
10	55.9	1	CH_2	3.38, 3.27
11	51.1	1	CH_3	2.89
12	46.8	1	CH	2.48
13	26.6	1	CH_2	1.27
14	25.9	1	CH_2	1.61, 1.53
15	25.7	1	CH_2	1.71, 1.59
16	24.9	1	CH_2	1.45, 1.41
17	23.2	1	CH	2.33
18	20.8	1	CH_2	1.99, 1.81
19	17.6	1	CH_2	1.90, 1.69

On the basis of the comprehensive interpretation of the ^{13}C spectrum, the ^{1}H spectrum, the DEPT-135 spectrum, and the HMQC spectrum, the ^{13}C spectrum and the ^{1}H spectrum have been well known. Our aim is the confirmation of the anticipated structure through the assignment of the NMR spectra. In general, we determine structural units first, and then assemble them. For the connections of structural units, the interpretation of the COSY spectrum and the HMBC spectrum is necessary.

The interpretations of different types of NMR spectra are related. When we interpret the COSY spectrum with the help of the HMBC spectrum, two points should be paid attention to.

1. If the two hydrogen atoms of a CH_2 are chemically non-equivalent, they will have two correlated peaks in the HMQC spectrum. Their coupling with ^{2}J will also be shown in the COSY spectrum. Therefore, the correlations with ^{3}J couplings can be differentiated from those with ^{2}J couplings.
2. When two functional groups containing hydrogen atoms have close chemical shift values, their correlated peaks in the COSY spectrum will be close also. Sometimes, the HMQC spectrum can help to resolve their close correlated peaks.

The data summarized from the COSY spectrum with the help of the HMQC spectrum are listed in Table 6.53.

Table 6.53 The data obtained from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)
1	7.50	7.31, (7.19)
2	7.31	7.50, 7.19
3	7.19	(7.50), 7.31
4	3.84	3.58, 2.33
5	3.73	3.69
6	3.69	3.73
7	3.58	3.84, 3.03
8	3.38	3.27, (3.02), 1.99, 1.81
9	3.29–3.27[a]	3.38, 3.02, (1.99), (1.90), 1.81, 1.70
10	3.03–3.01[a]	3.58, (3.38), 3.28, 1.90, 1.71
11	2.89	isolated
12	2.48	1.71, 1.59, 1.27
13	2.33	3.84, 1.99, 1.90
14	1.99	3.38, (3.28), 2.33, 1.81
15	1.90	(3.28), 3.02, 2.33, 1.70
16	1.81	3.38, 3.28, 1.99
17	1.71–1.69[a]	3.28, 3.02, 2.48, 1.91, 1.60, 1.53
18	1.61–1.59[a]	2.48, 1.70, 1.53
19	1.53	1.70, 1.60, 1.45
20	1.45	1.41, 1.27
21	1.41	1.61, 1.45, 1.27
22	1.27	2.48, 1.45, 1.41

Parenthesis () stands for weak coplings.
[a] Although two close peak sets can be discerned by the HMQC spectrum, their close correlated peaks in the COSY spectrum still can not be differentiated. Their chemical shift values are represented by a dash or an averaged value.

We emphasize again that the information obtained from the COSY spectrum is very reliable if related correlated peaks are not too close.

The HMBC spectrum shows long-range couplings between carbon atoms and hydrogen atoms, with the commonest correlation being that across three chemical bonds. Because the correlations of the HMBC spectrum can stride quaternary carbon atoms or heteroatoms, the HMBC spectrum is the unique method to connect two structural units separated by a quaternary carbon atom or a heteroatom.

The data summarized from the HMBC spectrum are listed in Table 6.54.

According to Nos. 1, 2, and 3 of Table 6.53, a coupled system can be found. With the help of the HSQC spectrum, the related δ_C can be assigned. Therefore, we obtain the following structural unit:

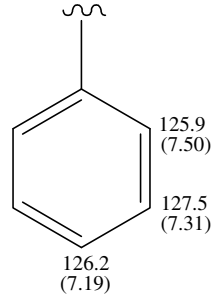
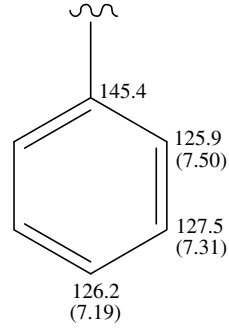


Table 6.54 The data summarized from the HMBC spectrum

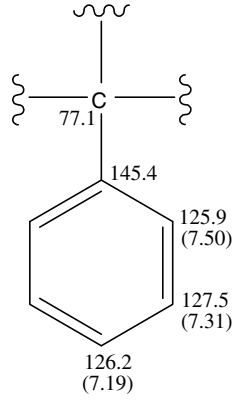
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	145.4	7.31, 3.73, 3.69	
2	127.5		7.31
3	126.2		7.19
4	125.9		7.50
5	77.1	7.50, 3.73, 3.69, 1.59, 1.27	
6	75.6	2.48,	3.73, 3.69
7	72.5	3.73, 3.69, 3.03, 1.69	
8	63.2	3.38, 3.28, 2.89	
9	56.8	3.58, 2.89	
10	55.9	3.02, 2.89	
11	51.1		2.89
12	46.8	3.73, 3.69	
13	26.6[a]		
14	25.9[a]		
15	25.7[a]		
16	24.9[a]		
17	23.2	1.70	
18	20.8	3.38, 3.27, 2.89, 1.90, 1.70	
19	17.6	3.84, 3.29, 3.01, 2.89, 1.81	

[a] These four peaks are very close. It is difficult to read out related data.

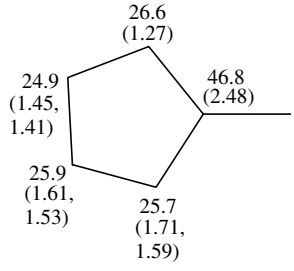
According to No. 1 of Table 6.54, that is, the correlation of δ_C (145.4)/δ_H (7.31), the chemical shift value of the quaternary carbon atom of the substituted benzene ring can be determined.



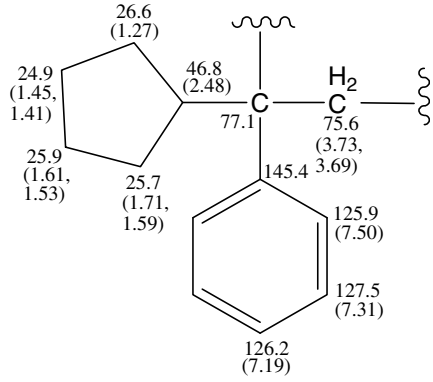
According to No. 5 of Table 6.54, that is, the correlation of δ_C (77.1)/δ_H (7.50), the above structural unit can be extended as follows:



According to Nos. 12, 17–22 of Table 6.53, together with the data of Nos. 12, 13, 16, 14, and 15 of Table 6.52, the following structural unit with its assignment can be obtained:



According to Nos. 6, 5, 12, and 1 of Table 6.54, that is, the correlations of δ_C (75.6)/δ_H (2.48), δ_C (77.1)/δ_H (7.50, 3.73, 3.69, 1.59, 1.27), δ_C (46.8)/δ_H (3.73, 3.69), and δ_C (145.4)/δ_H (3.73, 3.69) ppm, together with the related data of the HMQC spectrum, the above two structural units can be connected as follows:



The two doublets at 3.73 and 3.69 ppm form an AB system pattern, which means they belong to an isolated CH_2 group. From its δ_C and δ_H values it can be known that the CH_2 group should connect with an oxygen atom.

The analysis of the CH group with $\delta_C = 72.5$ and $\delta_H = 3.84$ ppm is an important clue. From its δ_C and δ_H it can be known that the CH group should connect with an oxygen atom. According to No. 4 of Table 6.53, together with the data of Nos. 7, 8, and 17 of Table 6.52, the following structural unit can be obtained:

$$CH(\delta_C = 23.2,\ \delta_H = 2.33)-CH(\delta_C = 72.5,\ \delta_H = 3.84)-CH_2(\delta_C = 63.2;\ \delta_H = 3.58,\ 3.03)$$

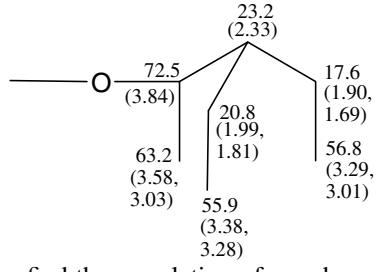
According to Nos. 13, 14, and 16 of Table 6.53, together with the data of Nos. 17, 18, and 10 of Table 6.52, the following structural unit can be obtained:

$$CH(\delta_C = 23.2,\ \delta_H = 2.33)-CH_2(\delta_C = 20.8;\ \delta_H = 1.99,\ 1.81)-CH_2(\delta_C = 55.9;\ \delta_H = 3.38,\ 3.27)$$

According to Nos. 13, 15, and 17 of Table 6.53, together with the data of Nos. 17, 19, and 9 of Table 6.52, the following structural unit can be obtained:

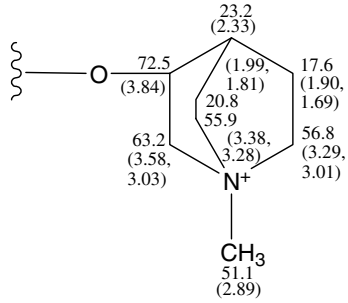
$$CH(\delta_C = 23.2, \delta_H = 2.33)-CH_2(\delta_C = 17.6; \delta_H = 1.90, 1.71)-CH_2(\delta_C = 56.8; \delta_H = 3.29, 3.01)$$

By combining the above three structural units, a large structural unit can be obtained as follows:

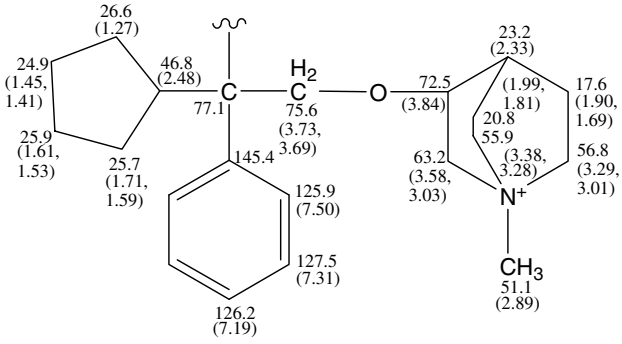


The HMBC spectrum can find the correlation of a carbon atom with a hydrogen atom which strides three chemical bonds. Its most powerful function is the determination of the connection around a quaternary carbon atom or a heteroatom

For this example, the HMBC spectrum plays a very important role in the connections between the (N)CH_3 and the three CH_2 groups (at 2-, 6, and 7- positions) on the basis of Nos. 8, 9, and 10 of Table 6.54. Therefore, we obtain the following structural unit:



According to No. 7 of Table 6.54, that is, the correlation of δ_C (72.5)/δ_H (3.73, 3.69) ppm, the above two structural units can be connected as follows:

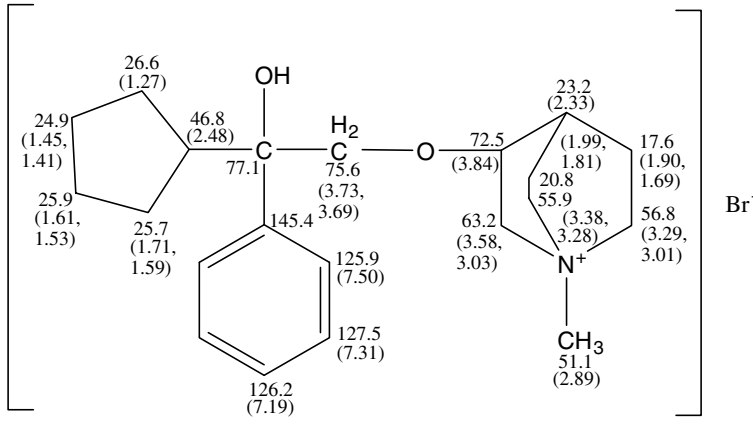


The connection of the CH_2 group at $\delta_C = 75.6$ and $\delta_H = 3.69$, 7.73 ppm with an oxygen atom is determined in the assemblage of the two large structural units.

The peak of the hydroxyl group merges in the water peak.

The above structure is a quaternary salt.

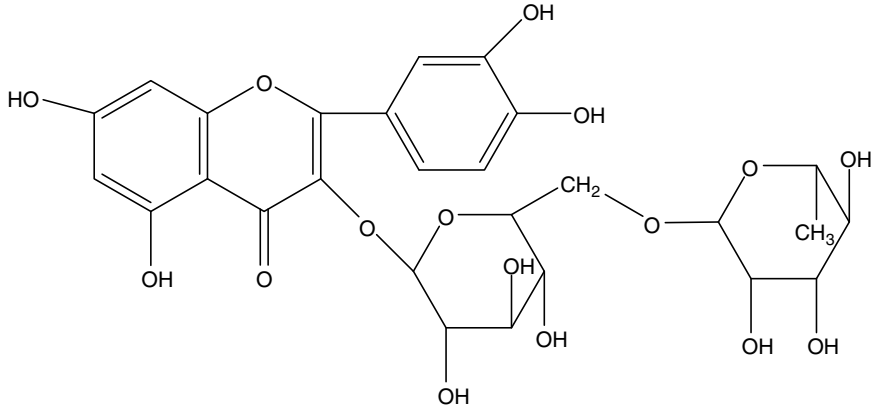
To sum up, the anticipated structure is confirmed, and its complete assignments can be obtained as follows:



C6-15

From this example, the importance of the symmetrical plane rule is shown again. Because this compound has no symmetrical plane, the two hydrogen atoms of all CH_2 groups are chemically non-equivalent.

Example 6.15 A synthesized compound has an anticipated structure which follows:



C6-16

Its ^1H spectrum, the locally enlarged ^1H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its DEPT-135 spectrum, the locally enlarged DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HMQC spectrum, the locally enlarged HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.154–6.170. Try to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is deuterated methanol.

Solution

The ^1H spectrum of the compound is complicated, especially in the region of 3.0–4.0 ppm. Therefore, it is necessary to inspect carefully its locally enlarged ^1H spectrum.

The peak at 3.21 ppm is the solvent peak, and that at 4.75 ppm the water peak. The peaks with very small areas are the peaks of impurities.

The data summarized from the ^1H spectrum and its locally enlarged spectra are listed in Table 6.55.

Except for the regions of 3.39–3.28 and 3.19–3.17 ppm, the areas of all peak sets are close to integers. Therefore, their hydrogen atom numbers can be easily determined. From the HMQC spectrum, the hydrogen atom numbers corresponding to the two regions mentioned above can be determined as 4 and 2, respectively.

From Table 6.55, the hydrogen atom number of the compound is 20, which coincides with the anticipated structure.

Now we will interpret the ^{13}C spectrum.

The peak at 47.7 ppm is the solvent peak. All peaks of impurities have low intensities.

The data summarized from the ^{13}C spectrum and the DEPT spectrum are listed in Table 6.56.

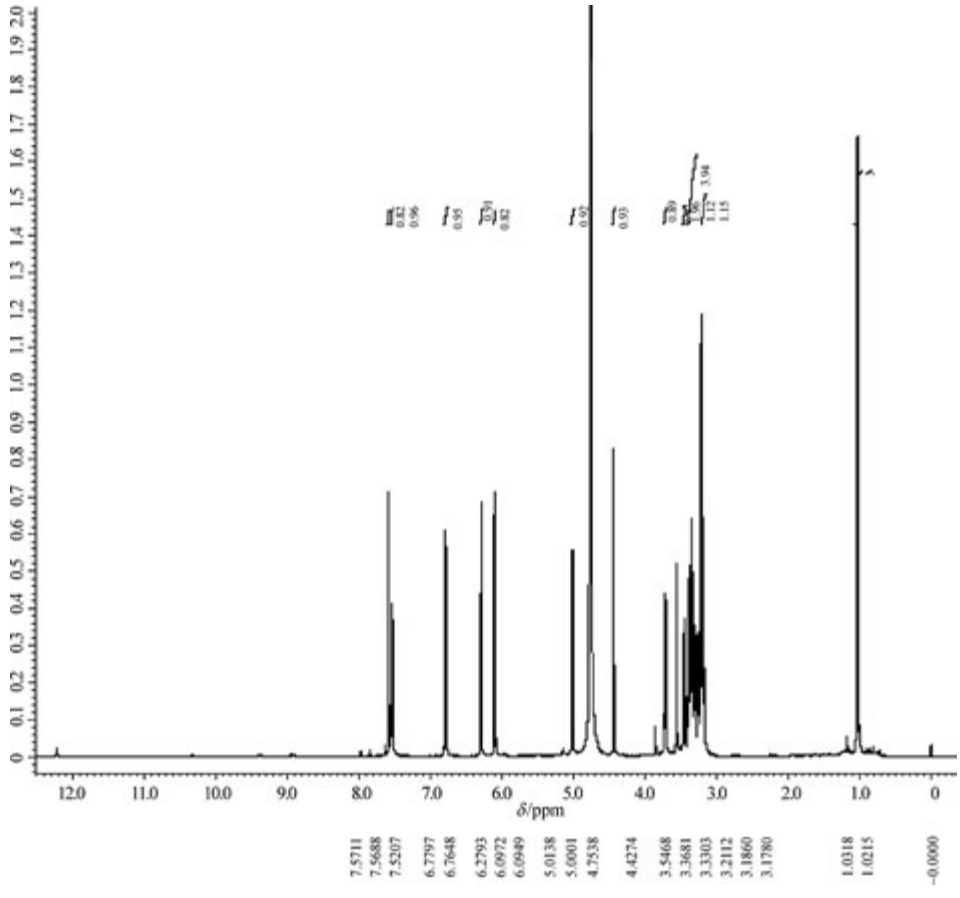


Figure 6.154 The ^{1}H spectrum of the compound

Now we will interpret the HMQC spectrum. The related data are summarized in Table 6.57.

Even with the help of the HMQC spectrum, it is still difficult to resolve some overlapped peak sets in the ^{1}H spectrum. In this case, the use of the HMBC spectrum can help, because the correlated peaks in the HMBC spectrum have rather small areas.

The data summarized from the HMBC spectrum are listed in Table 6.58.

The locally enlarged HMBC spectrum can resolve well the overlapped correlated peaks in the region of 3.17–3.19 ppm. Other overlapped correlated peaks in the ^{1}H spectrum can be resolved better, because their differences in chemical shift value are greater than 0.02 ppm.

The data summarized from the COSY spectrum are listed in Table 6.59.

The above five tables generalize the information on all NMR spectra. On the basis of the five tables, we can assign and confirm the anticipated structure.

We start from Nos. 16 and 17 of Table 6.57, that is, the two CH groups in an aromatic system. From their weak coupling known from the COSY spectrum, they should be two CH

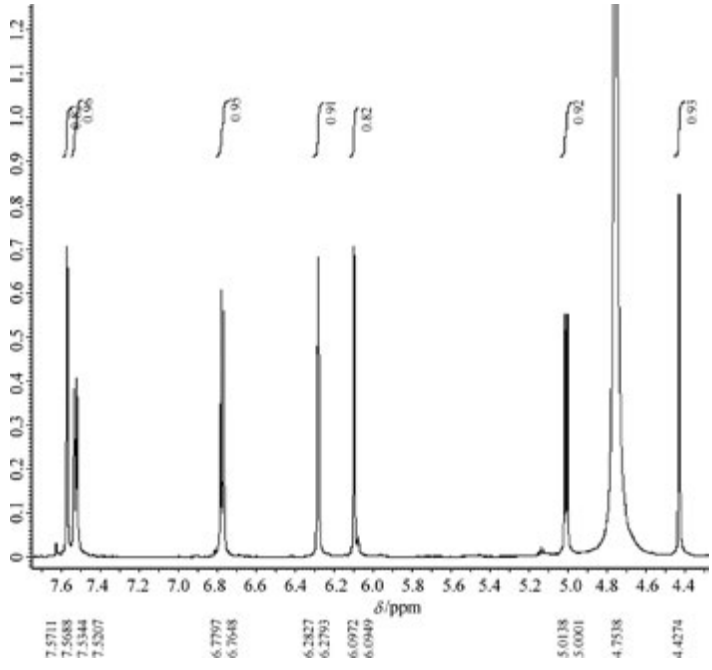


Figure 6.155 The locally enlarged ^{1}H spectrum (1)

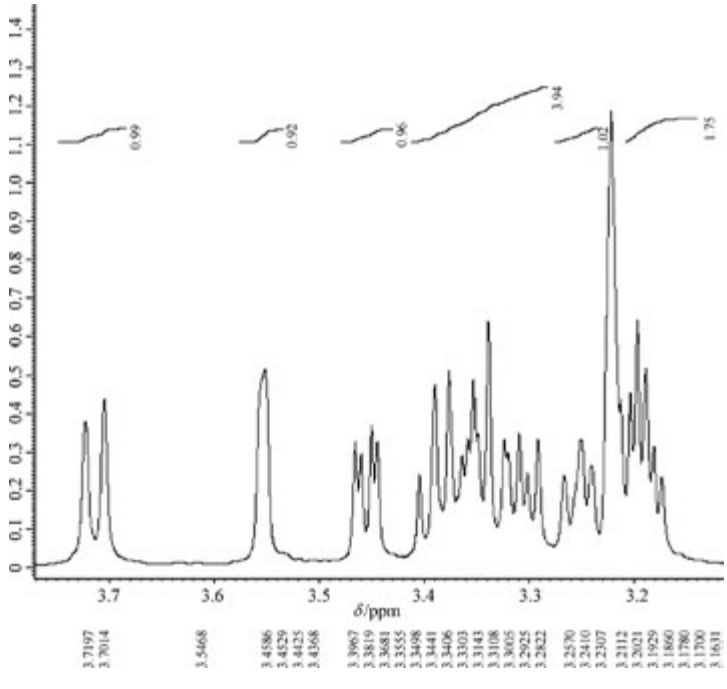


Figure 6.156 The locally enlarged ^{1}H spectrum (2)

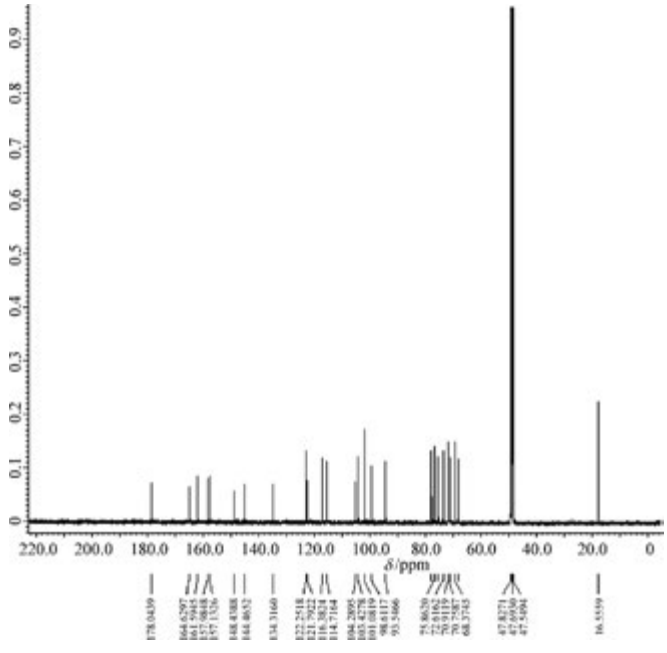


Figure 6.157 The ^{13}C spectrum of the compound

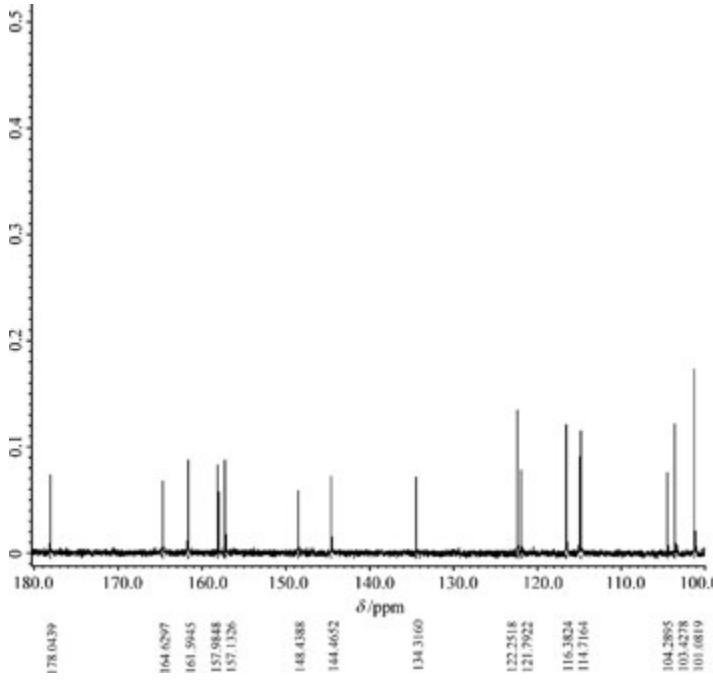


Figure 6.158 The locally enlarged ^{13}C spectrum (1)

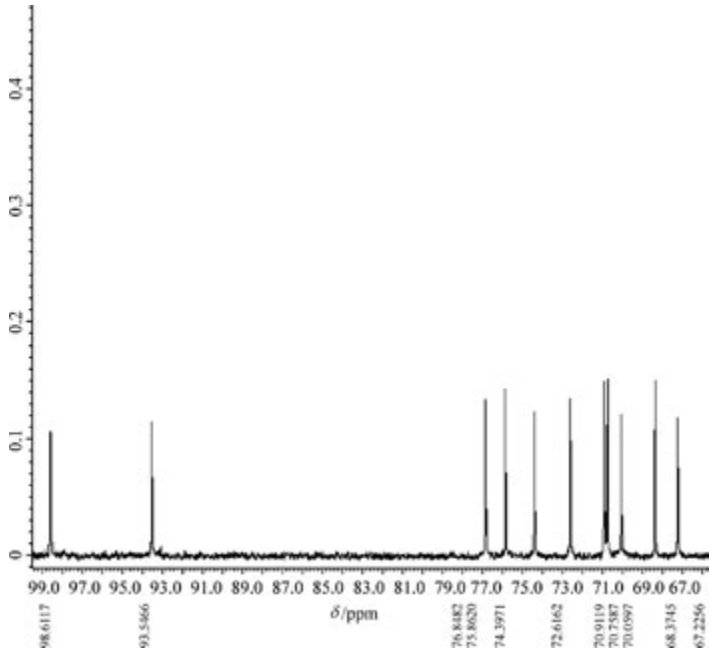


Figure 6.159 The locally enlarged ^{13}C spectrum (2)

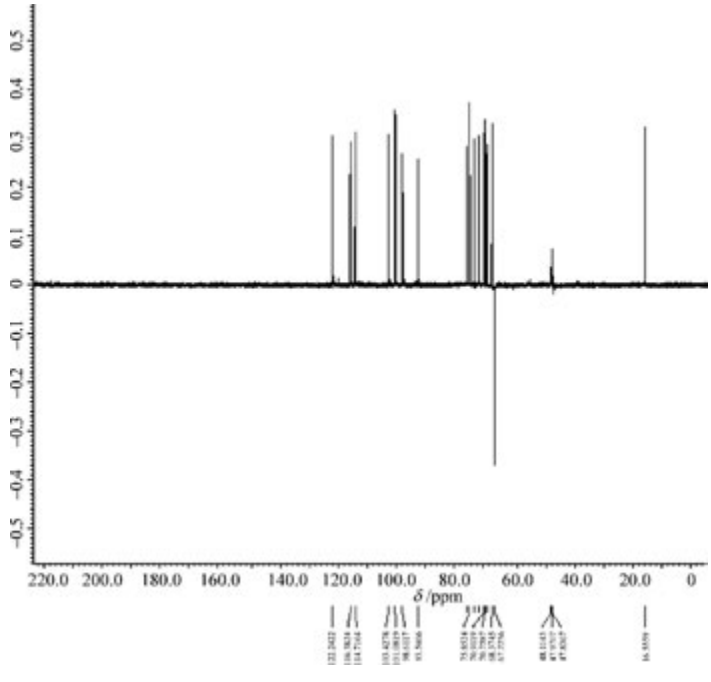


Figure 6.160 The DEPT-135 spectrum of the compound

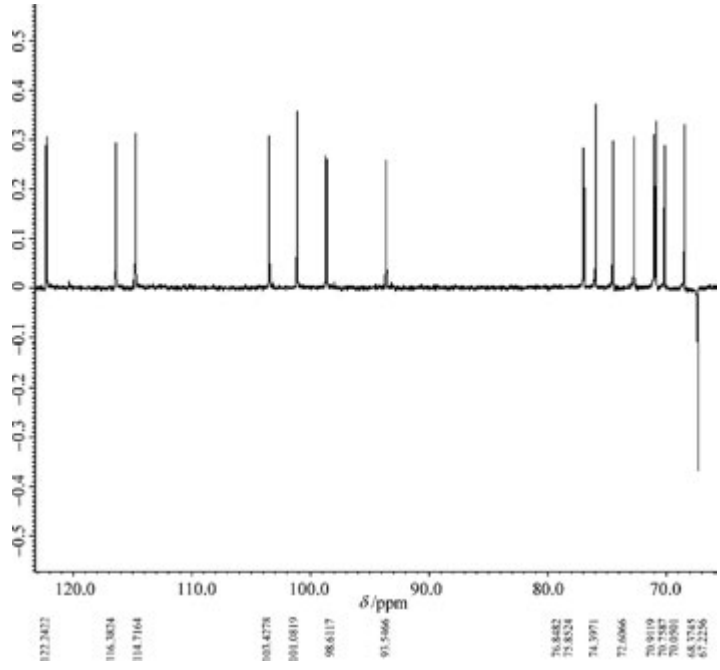


Figure 6.161 The locally enlarged DEPT-135 spectrum

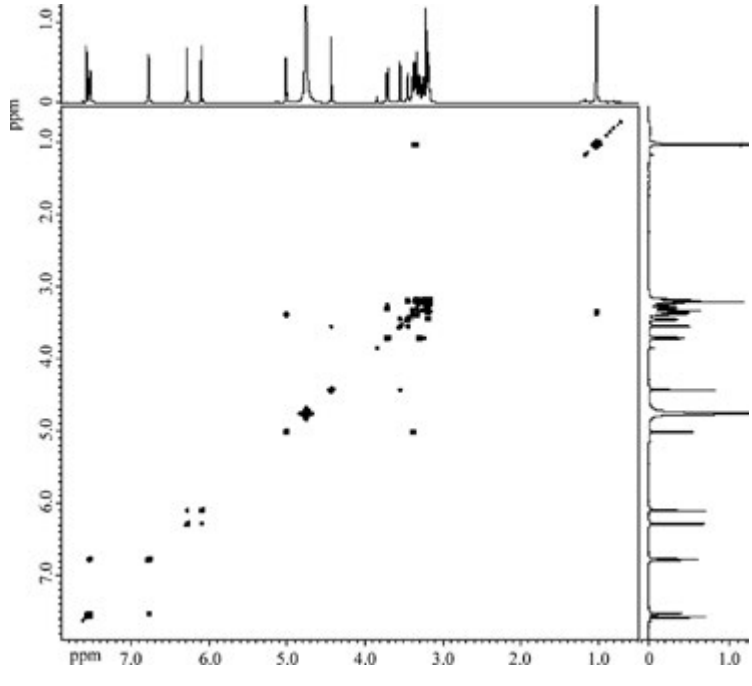


Figure 6.162 The COSY spectrum of the compound

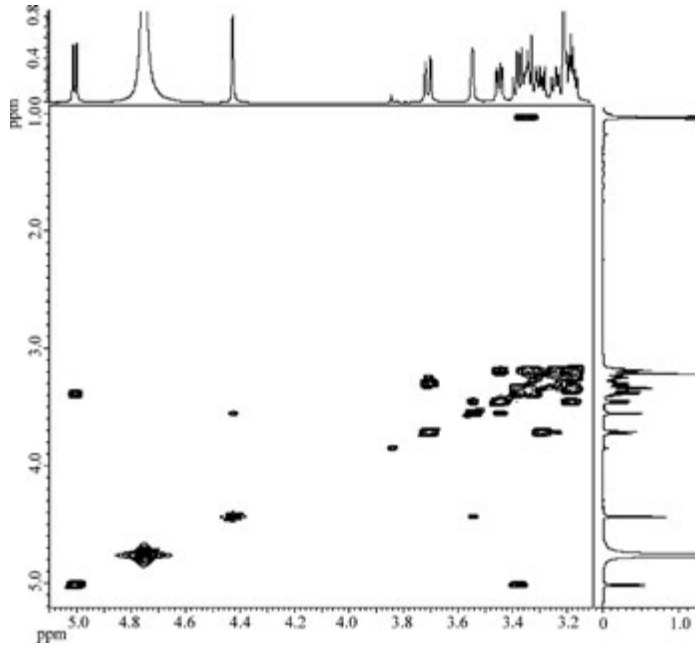


Figure 6.163 The locally enlarged COSY spectrum

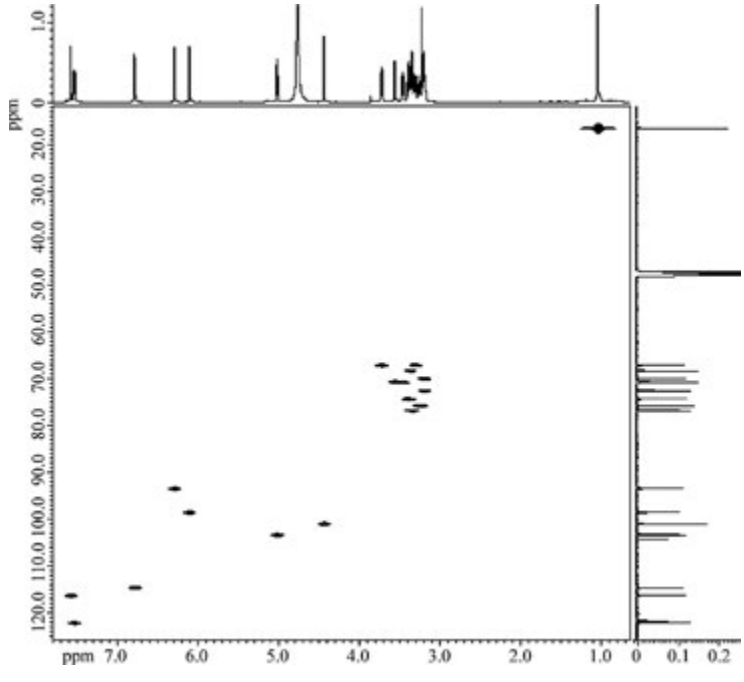


Figure 6.164 The HMQC spectrum of the compound

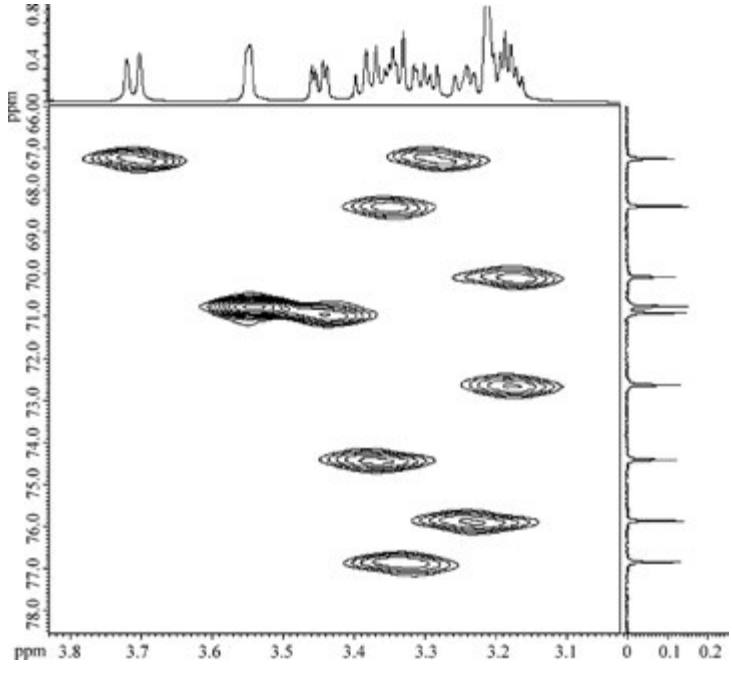


Figure 6.165 The locally enlarged HMQC spectrum (1)

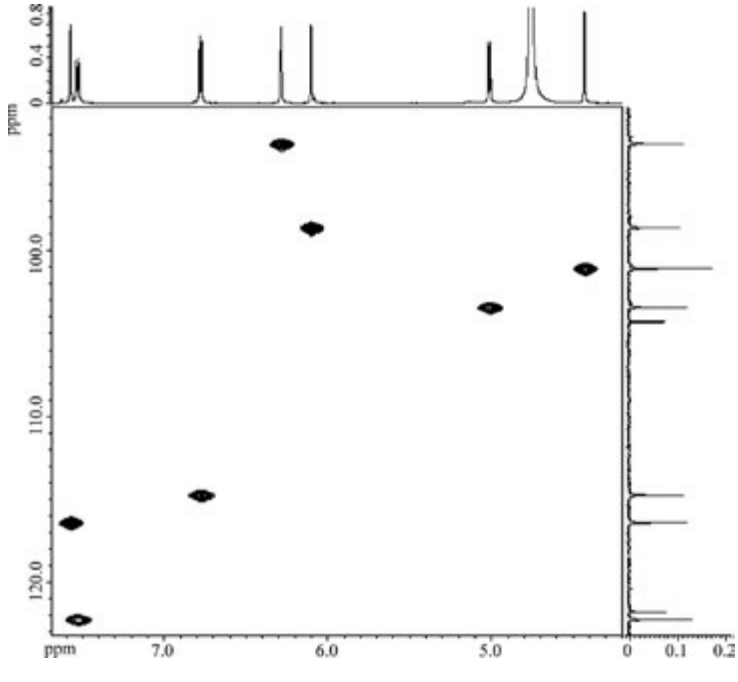


Figure 6.166 The locally enlarged HMQC spectrum (2)

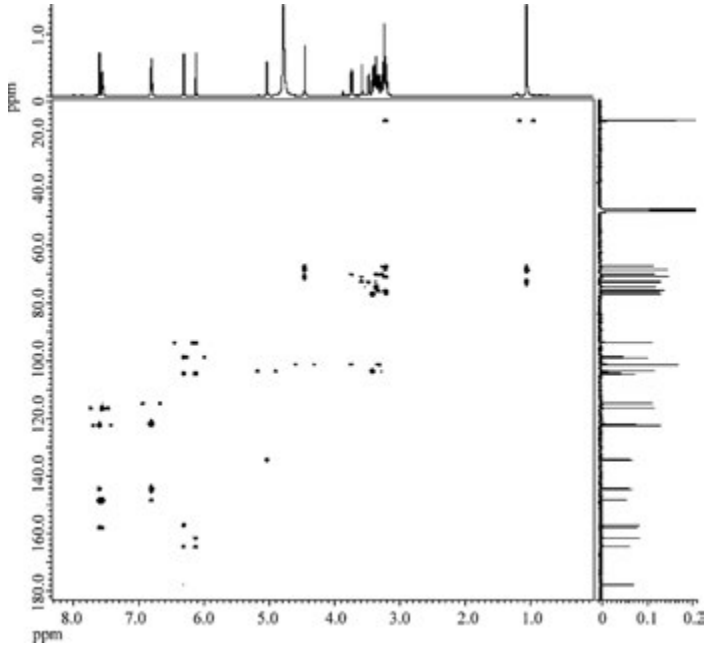


Figure 6.167 The HMBC spectrum of the compound

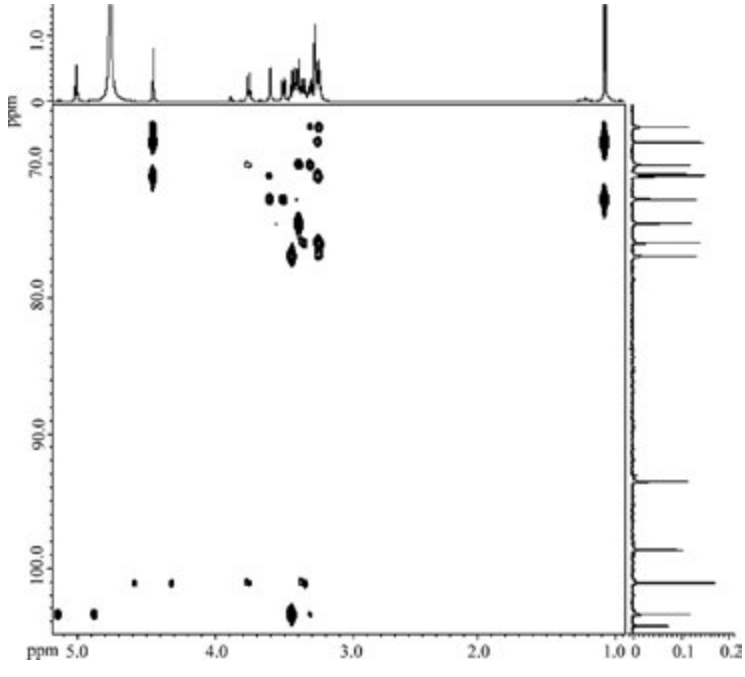


Figure 6.168 The locally enlarged HMBC spectrum (1)

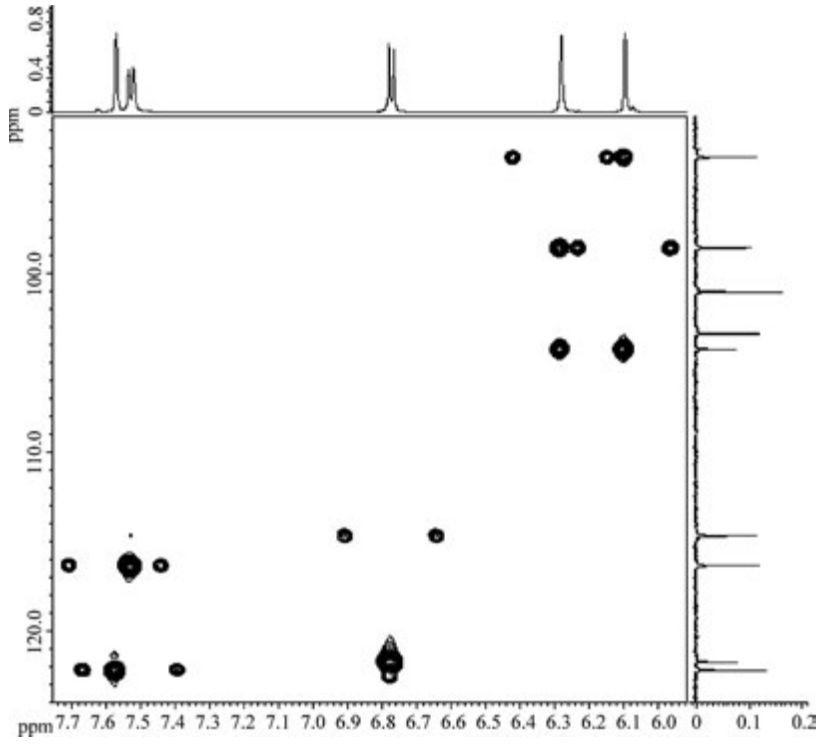
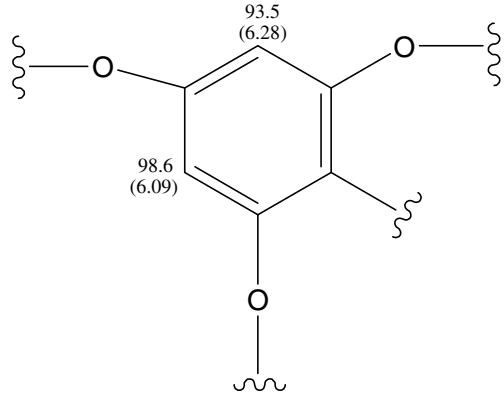


Figure 6.169 The locally enlarged HMBC spectrum (2)

of the benzene ring with a meta-position coupling. Their coupling constant of 2 Hz, which is known from the ^{1}H spectrum, coincides with this postulation. Because their δ_C and δ_H values are rather small, they should be situated between two substituted functional groups containing oxygen atom (the secondary type of substituents for a benzene ring). To sum up, the compound should contain the following structural unit:



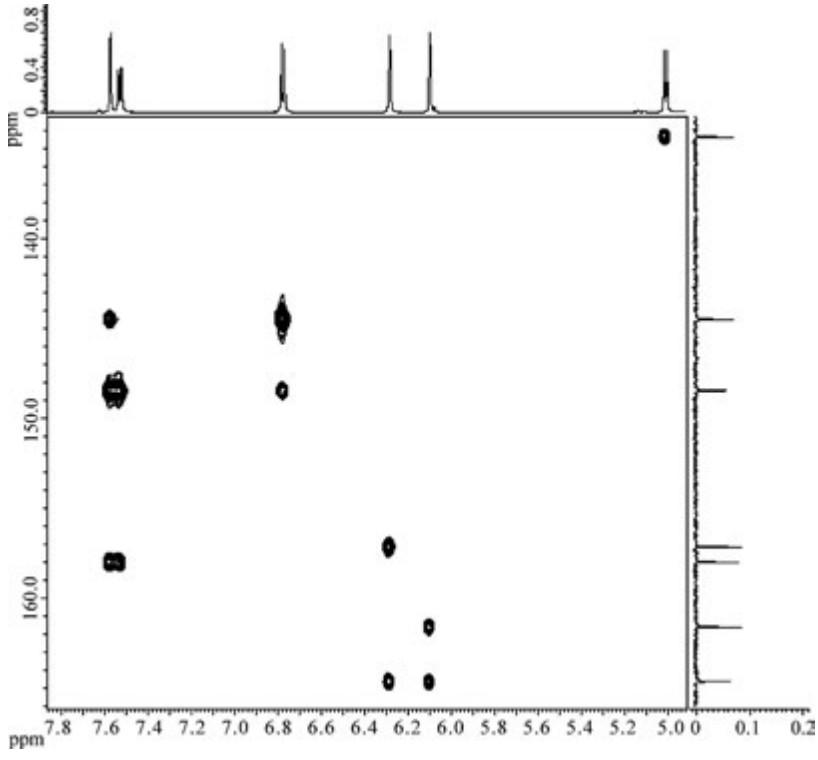


Figure 6.170 The locally enlarged HMBC spectrum (3)

Table 6.55 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Peak area	Peak shape	*J* (Hz)	Remarks
7.57	0.82	d	1.38	
7.53	0.96	d	8.22	
6.77	0.95	d	8.94	
6.28	0.91	d	2.04	
6.09	0.84	d	1.38	
5.01	0.92	d	8.22	
4.43	0.93	s		
3.71	0.99	d	10.98	
3.55	0.92	s, blunt		
3.45	0.96	d, d	9.66, 3.42	
3.39–3.28	3.94			
3.24	1.02	m		
3.19–3.16	1.75			
1.03	3	d	6.18	

Table 6.56 The data summarized from the ^{13}C spectrum and the DEPT spectrum

No.	δ_C (ppm)	Number of C atoms	Order of C atoms
1	178.0	1	C
2	164.6	1	C
3	161.6	1	C
4	157.9	1	C
5	157.1	1	C
6	148.4	1	C
7	144.5	1	C
8	134.3	1	C
9	122.3	1	CH
10	121.8	1	C
11	116.4	1	CH
12	114.7	1	CH
13	104.3	1	C
14	103.4	1	CH
15	101.1	1	CH
16	98.6	1	CH
17	93.5	1	CH
18	76.8	1	CH
19	75.9	1	CH
20	74.4	1	CH
21	72.6	1	CH
22	70.9	1	CH
23	70.6	1	CH
24	70.1	1	CH
25	68.4	1	CH
26	67.2	1	CH_2
27	16.6	1	CH_3

Because of the substitutions of the three oxygen atoms, all the three substituted quaternary carbon atoms should have a rather large chemical shift value, respectively. According to Nos. 2, 3, 5, and 13 of Table 6.58, the chemical shift values of the three substituted carbon atoms and the other substituted carbon atom can be determined as follows:

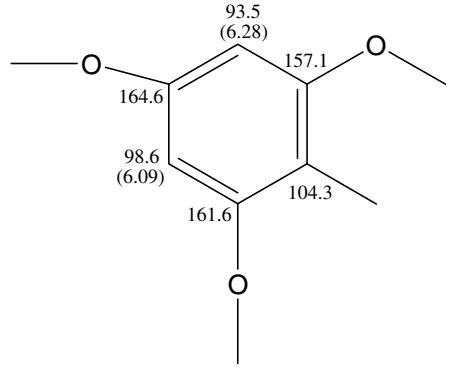
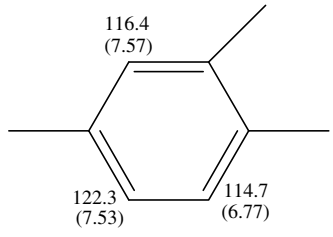


Table 6.57 The data summarized from the HMQC spectrum

No.	δ_C (ppm)	Order of C atoms	Directly connected H, δ_H (ppm)
1	178.0	C	
2	164.6	C	
3	161.6	C	
4	157.9	C	
5	157.1	C	
6	148.4	C	
7	144.5	C	
8	134.3	C	
9	122.3	CH	7.53
10	121.8	C	
11	116.4	CH	7.57
12	114.7	CH	6.77
13	104.3	C	
14	103.4	CH	5.01
15	101.1	CH	4.43
16	98.6	CH	6.09
17	93.5	CH	6.28
18	76.8	CH	3.33
19	75.9	CH	3.24
20	74.4	CH	3.38
21	72.6	CH	3.19
22	70.9	CH	3.45
23	70.6	CH	3.55
24	70.1	CH	3.17
25	68.4	CH	3.35
26	67.2	CH_2	3.71, 3.29
27	16.6	CH_3	1.03

Then according to Nos. 9, 11, and 12 of Table 6.57, three aromatic CH groups can be known. From the correlation of δ_H (ppm) 6.77/7.53 known from the COSY spectrum, and according to Nos. 9 and 11 of Table 6.58, that is, the correlations of δ_C (122.3)/δ_H (7.57) and δ_C (116.4)/δ_H (7.53), a 1-, 2-, 4-substituted benzene ring can be determined as follows:

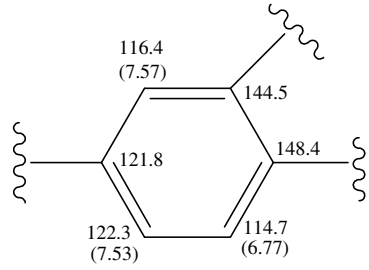


According to Nos. 6, 7, and 10 of Table 6.58, that is, the correlations of δ_C (148.4)/δ_H (7.57, 7.53), δ_C (144.5)/δ_H (7.57, 6.77), and δ_C (121.8)/δ_H (6.77) ppm, the chemical shift values of the three quaternary carbon atoms of the substituted benzene ring can be determined as follows:

Table 6.58 The data summarized from the HMBC spectrum

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	178.0	(6.28)	
2	164.6	6.28, 6.09	
3	161.6	6.09	
4	157.9	7.57, 7.53	
5	157.1	6.28	
6	148.4	7.57, 7.53, (6.77)	
7	144.5	7.57, 6.77	
8	134.3	5.01	
9	122.3	7.57	7.53
10	121.8	6.77	
11	116.4	7.53	7.57
12	114.7		6.77
13	104.3	6.28, 6.09	
14	103.4	3.38, (3.33)	5.01
15	101.1	3.71, 3.29	4.43
16	98.6	6.28	6.09
17	93.5	6.09	6.28
18	76.8	3.38, 3.17	
19	75.9	3.29, 3.17	
20	74.4	3.33	
21	72.6	3.55, 3.45, (3.35), 1.03	
22	70.9	4.43, 3.55, (3.35), 3.19	
23	70.6	4.43	
24	70.1	(3.71), 3.33, 3.24	
25	68.4	4.43, 3.19, 1.03	
26	67.2	4.43, 3.24, 3.17	
27	16.6	3.19	1.03

Parenthesis () stands for weak couplings.

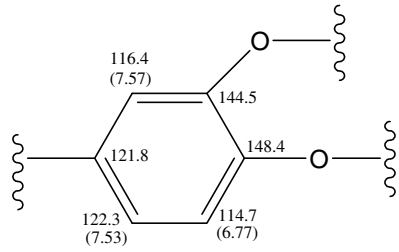


When we use the data of the HMBC spectrum, the intensities of correlated peaks should be paid attention to. For example, the intensity of the correlated peak of δ_C (144.5)/δ_H (6.77) ppm is rather strong, which implies that there exists a correlated coupling across three chemical bonds. The intensity of the correlated peak of δ_C (144.5)/δ_H (7.57) ppm is rather weak, which implies that there exists a correlated coupling across two chemical bonds.

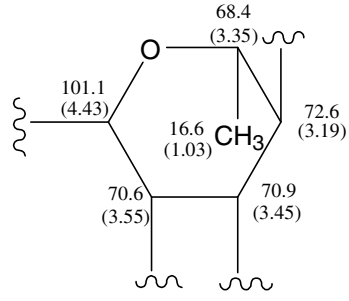
In the above structural unit, the two quaternary atoms with $\delta_C = 144.5$ and 148.4 ppm, respectively, should connect with an oxygen atom. Therefore, we have

Table 6.59 The data summarized from the COSY spectrum

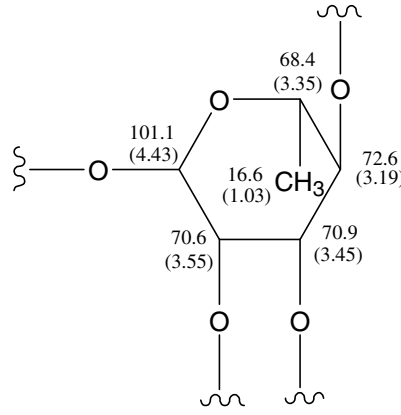
δ_H (ppm)	Coupled H, δ_H (ppm)	Remarks
7.57	7.53	
7.53	7.57, 6.77	
6.77	7.53	
6.28	6.09	
6.09	6.28	
5.01	3.38	
4.43	3.55	
3.71	3.29, (3.24)	With the help of the HMBC spectrum
3.55	4.43, 3.45	With the help of the HMBC spectrum
3.45	3.55, 3.19	With the help of the HMBC spectrum
3.38	5.01, 3.33	
3.35	3.19, 1.03	
3.33	3.38, 3.17	With the help of the HMBC spectrum
3.29	3.71	
3.24	(3.71), 3.17	
3.19	3.45, 3.35	
3.17	3.33, 3.24	With the help of the HMBC spectrum
1.03	3.35	



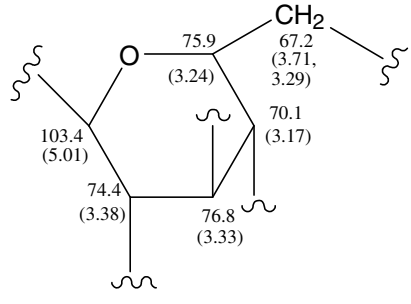
Then we start from No. 27 of Table 6.57, that is, the methyl group with $\delta_C = 16.6$ ppm and $\delta_H = 1.03$ ppm. According to the correlations of δ_H (ppm): 1.03/3.35, 3.19/(3.45, 3.35), 3.45/3.55, and 3.55/(4.43, 3.45) known from the COSY spectrum, and according to Nos. 27, 25, 23, 22, and 21 of Table 6.58, that is, the correlations of δ_C (16.6)/δ_H (3.19), δ_C (68.4)/δ_H (4.43, 3.19, 1.03), δ_C (70.6)/δ_H (4.43), δ_C (70.9)/δ_H (4.43, 3.55, 3.35, 3.19), and δ_C (72.6)/δ_H (3.55, 3.45, 3.35, 1.03) ppm, together with the related data of the HMQC spectrum, the following structural unit can be deduced:



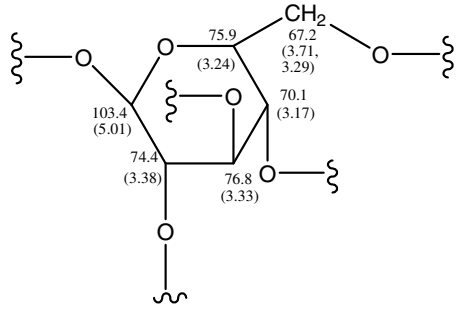
In the above structural unit, the four substituted carbon atoms have chemical shift values close to 70 ppm or even greater than 70 ppm, which means they should connect with an oxygen atom, as shown below:



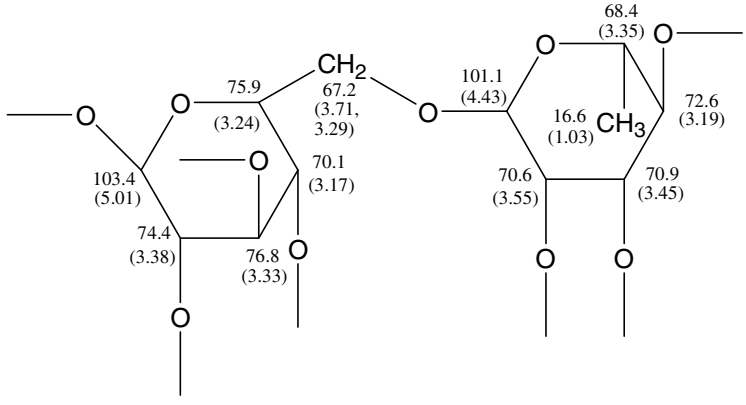
Then we start from No. 14 of Table 6.57, that is, the CH group ($\delta_C = 103.4$, $\delta_H = 5.01$). According to the correlations of δ_H (ppm):5.01/3.38, 3.17/(3.33, 3.24), 3.71/(3.29, 3.24) known from the COSY spectrum, and according to Nos. 14, 18, 19, 20, 24, and 26 of Table 6.58, that is, the correlations of δ_C (103.4)/δ_H (3.38, 3.33), δ_C (76.8)/δ_H (3.38, 3.17), δ_C (75.9)/δ_H (3.29, 3.17), δ_C (74.4)/δ_H (3.33), δ_C (70.1)/δ_H (3.71, 3.33, 3.24), and δ_C (67.2)/δ_H (3.24, 3.17), together with the related data of the HMQC spectrum, the following structural unit can be deduced:



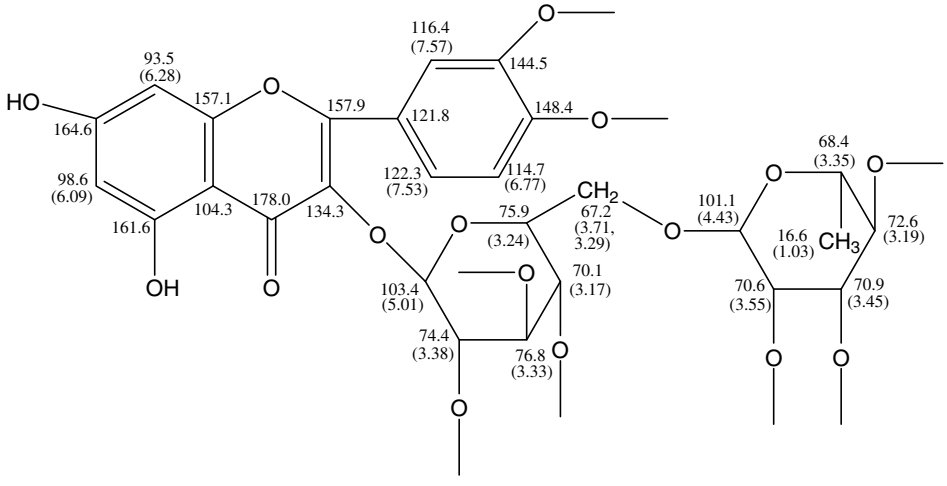
In the above structural unit, the four substituted carbon atoms have chemical shift values close to 70 ppm or even greater than 70 ppm, which means they should connect with an oxygen atom, as shown below:



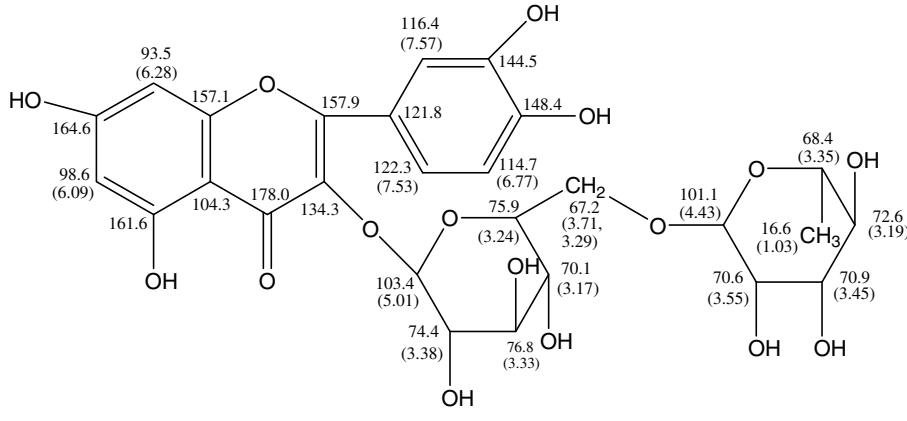
According to No. 26 of Table 6.59, that is, the correlation of δ_C (67.2)/δ_H (4.43) ppm, the above two saccharine rings can be connected as follows:



So far we have obtained two separated benzene rings and a structural unit containing two saccharine rings. Compared with Table 6.57, the remaining composition only contains two unsaturated carbon atoms at $\delta_C = 157.9$ and $\delta_C = 134.3$, respectively, and a carbonyl group. Consider Nos. 4 and 8 of Table 6.59, that is, the correlations of δ_C (157.8)/δ_H (7.57, 7.53) and δ_C (134.3)/δ_H (5.01) ppm, which implies that the two unsaturated carbon atoms should form a double bond. The carbonyl group has no correlated peak in the HMBC spectrum. To sum up, the following structure is reasonable:



Because there are remaining peaks in the ^{1}H spectrum, the oxygen atoms in the above structure should be situated in hydroxyl groups. Therefore, we finally obtain the following structure and assignment:

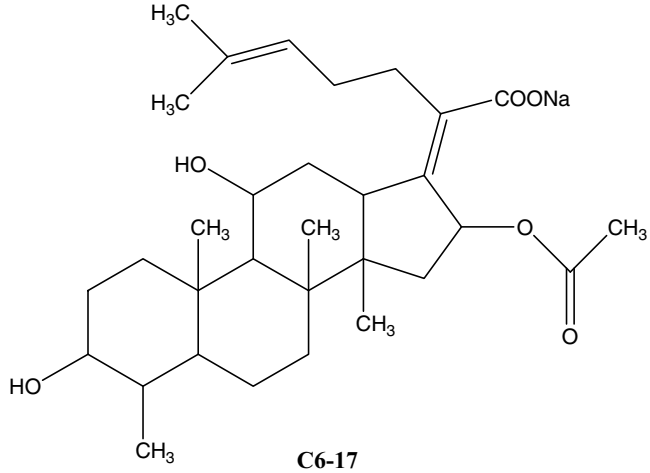


C6-16

For this example, we have completed the assignment by way of the deduction of an unknown structure. Therefore, the anticipated structure is correct.

From this example, we can understand the important function of the HMBC spectrum. The resolution of the COSY spectrum is similar to that of the ^{1}H spectrum. Because some peak sets overlap in the ^{1}H spectrum, the correlated peaks in the COSY spectrum can not be distinguished clearly. Since the correlated peaks in the HMQC spectrum have rather large areas, it is not easy to determine the chemical shift values of some peak sets in the ^{1}H spectrum. Fortunately, the correlated peaks in the HMBC spectrum have small areas, so that the connections and the chemical shift values can be determined.

Example 6.16 A synthesized compound has the following anticipated structure:



C6-17

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum, its ^{13}C spectrum, its DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HMQC spectrum,

the locally enlarged HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.171–6.185. Try to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated DMSO.

Solution

Our strategy follows this order: interpretation of NMR spectra, generalization of related data in tables to find structural units, assemblage of the structural units, and finally comparison of the assembled structure with the anticipated structure.

We will start from the interpretation of the ^{1}H spectrum.

The peak at about 2.50 ppm is the solvent peak. The peak at 3.28 ppm with a high intensity is the water peak.

We can see that many peak sets overlap in the ^{1}H spectrum, which can be encountered in general for the compounds with a large molecular weight. As we have said many times that the interpretation of the ^{1}H spectrum should be completed satisfactorily with the help of its 2D NMR spectra. When we interpret the ^{1}H spectrum, we inspect the HMQC spectrum first, so that we can know how some peak sets overlap to form a complicated peak set. The HMBC spectrum can also help to resolve the overlapped peak sets in the ^{1}H spectrum.

The data summarized from the ^{1}H spectrum and its locally enlarged spectrum is listed in Table 6.60.

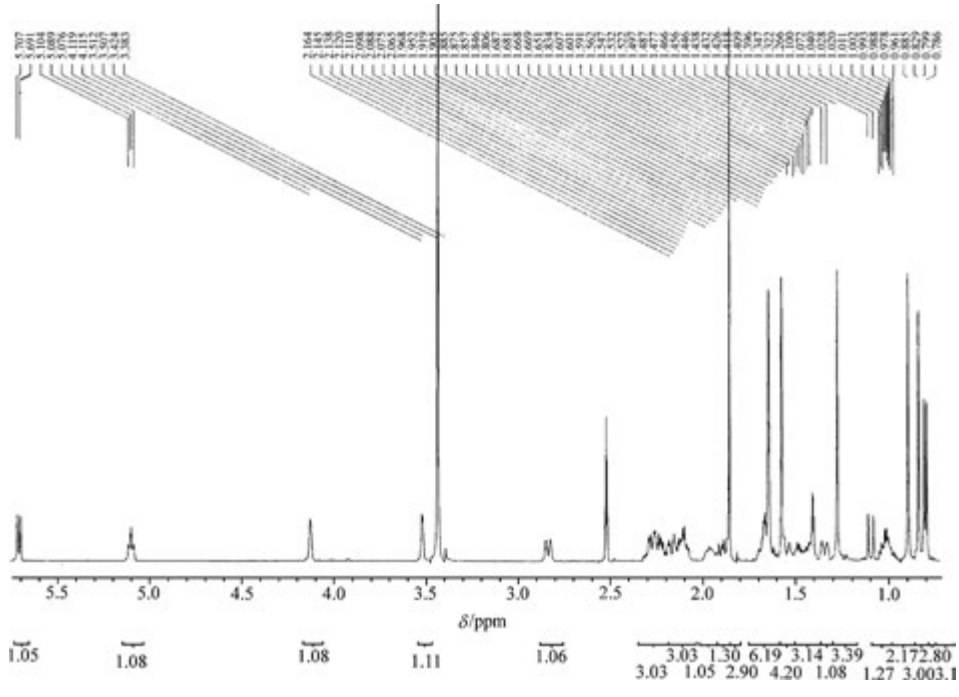


Figure 6.171 The ^{1}H spectrum of the compound

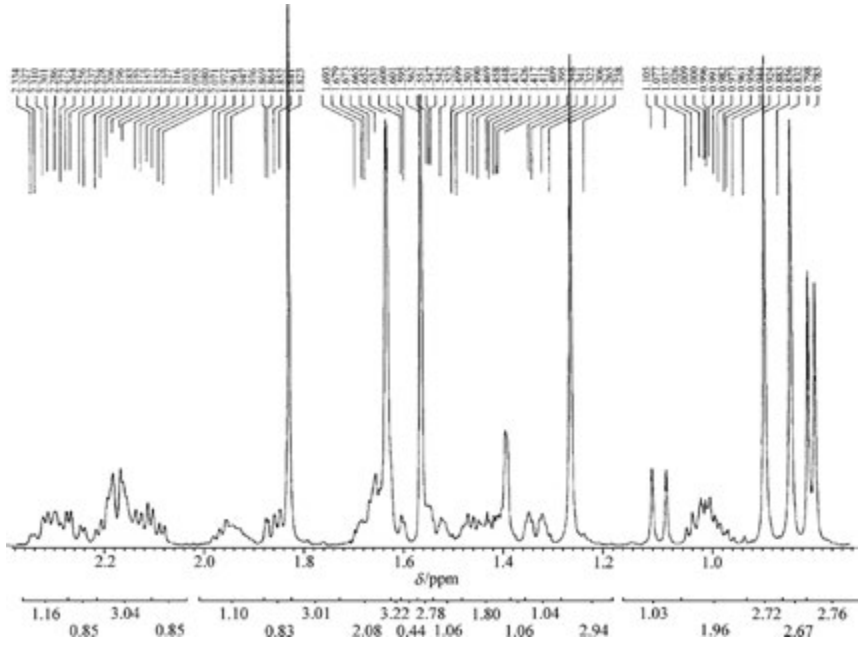


Figure 6.172 The locally enlarged ^{1}H spectrum (1)

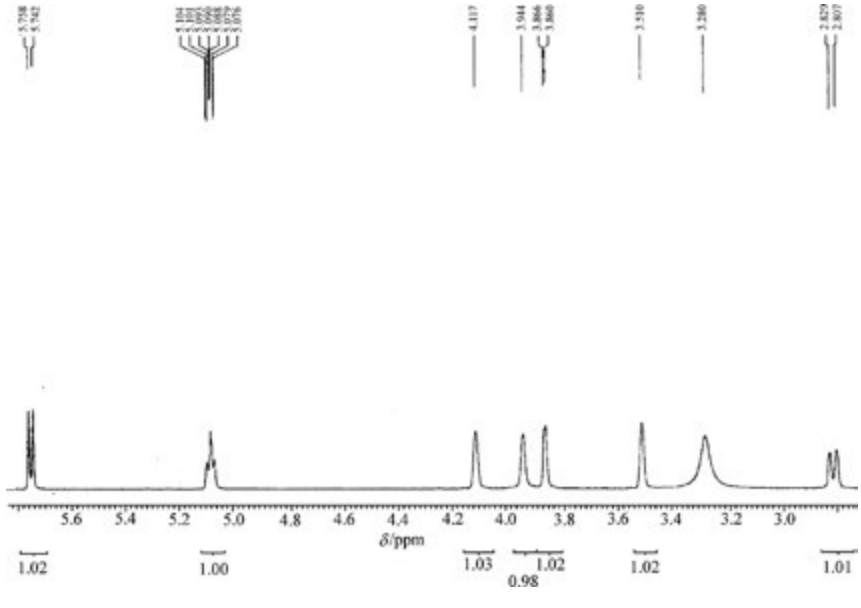


Figure 6.173 The locally enlarged ^{1}H spectrum (2)

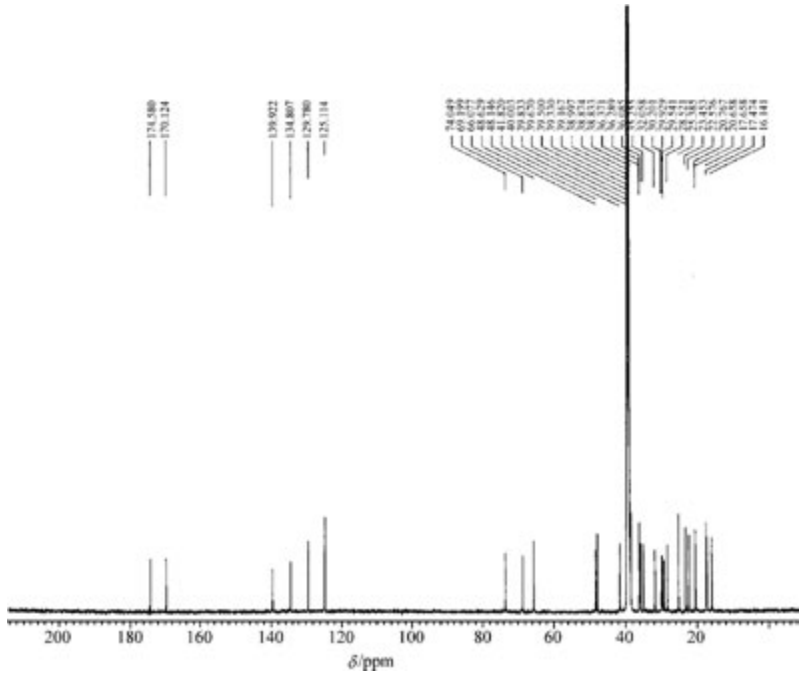


Figure 6.174 The ^{13}C spectrum of the compound

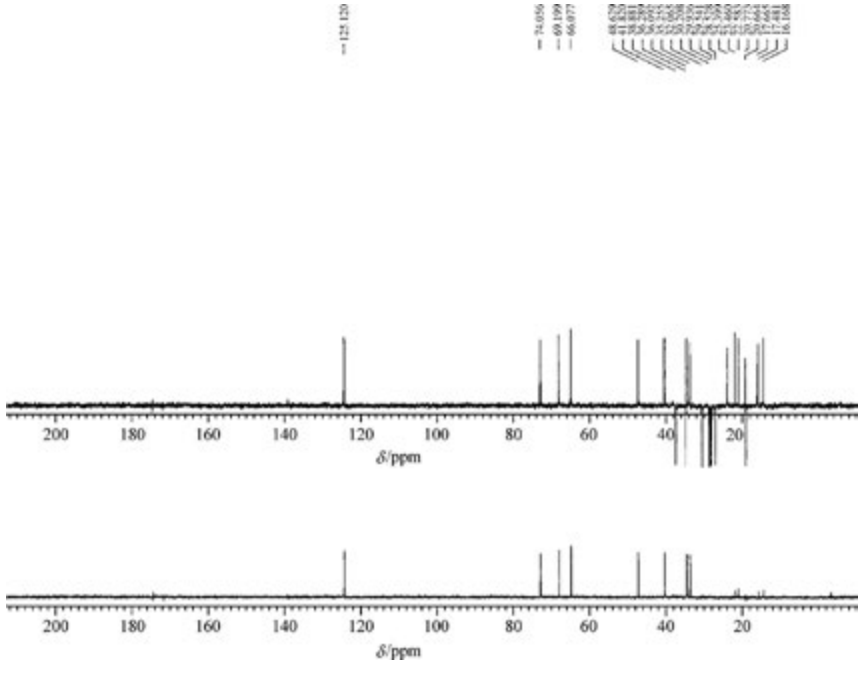


Figure 6.175 The DEPT-135 spectrum of the compound

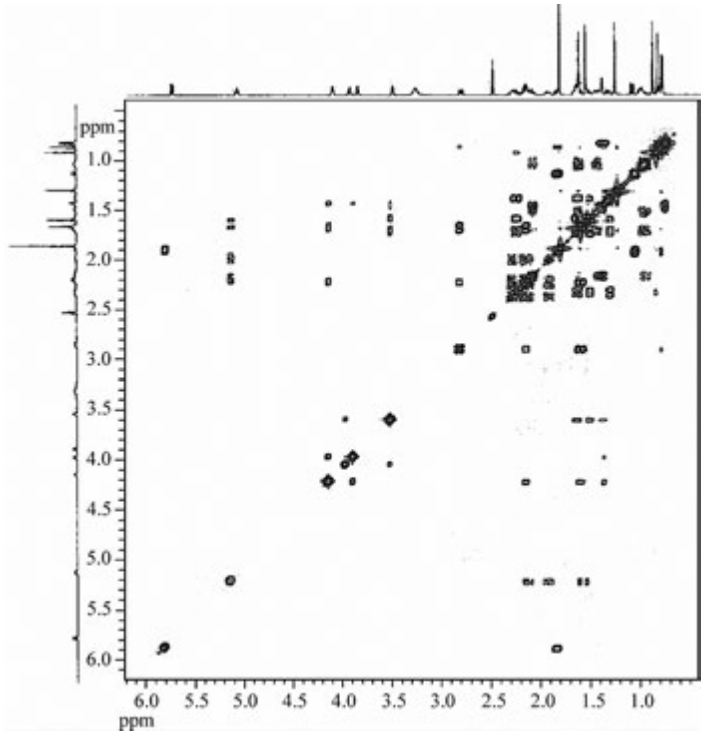


Figure 6.176 The COSY spectrum of the compound

Peak sets overlap seriously in the region of 2.06–2.30 ppm. It can be known that the overlapped peak sets come from six hydrogen atoms, which belong to five functional groups, as shown below:

CH, δ_C (ppm) 35.3, δ_H (ppm) 2.10;
CH_2, δ_C (ppm) 36.3, δ_H (ppm) 2.17 (another hydrogen atom at 1.62 ppm);
CH_2, δ_C (ppm) 29.9, δ_H (ppm) 2.15, 2.30;
CH_2, δ_C (ppm) 28.5, δ_H (ppm) 2.19 (another hydrogen atom at 1.96 ppm);
CH_2, δ_C (ppm) 29.5, δ_H (ppm) 2.27 (another hydrogen atom at 1.34 ppm).

Similarly, other overlapped peak sets, which are overlapped not so seriously, can be discerned.

With the help of the HMQC spectrum and the DEPT spectrum, the data of the ^{13}C spectrum and the ^{1}H spectrum can be summarized in Table 6.61.

From Table 6.61, it can be known that the compound contains 7 CH_3, 8 CH_2, 8 CH, and 8 quaternary carbon atoms, of which two are carbonyl groups.

Besides the hydrogen atoms listed in Table 6.61, two peaks at 3.51 and 4.12 ppm, respectively, remain in the ^{1}H spectrum. Since they do not connect with carbon atoms, they should be reactive hydrogen atoms. According to the molecular formula, they are in hydroxyl groups, because the compound contains only the heteroatoms as oxygen atoms.

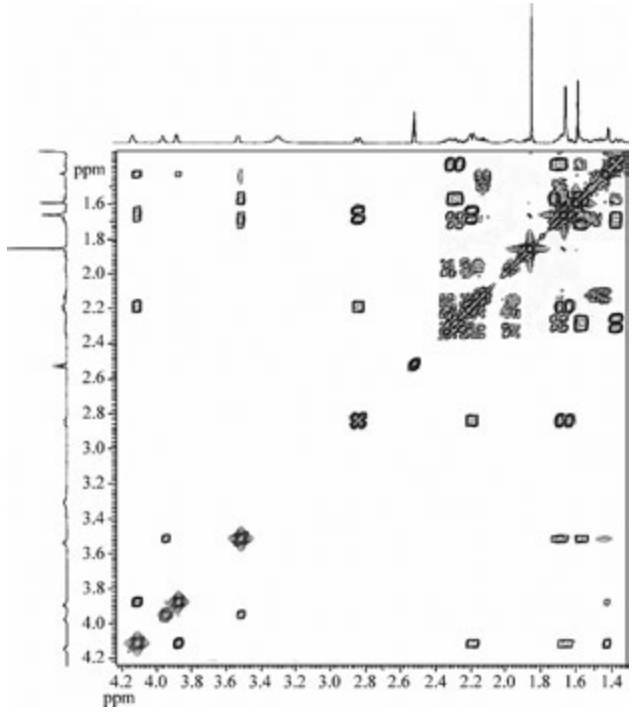


Figure 6.177 The locally enlarged COSY spectrum (1)

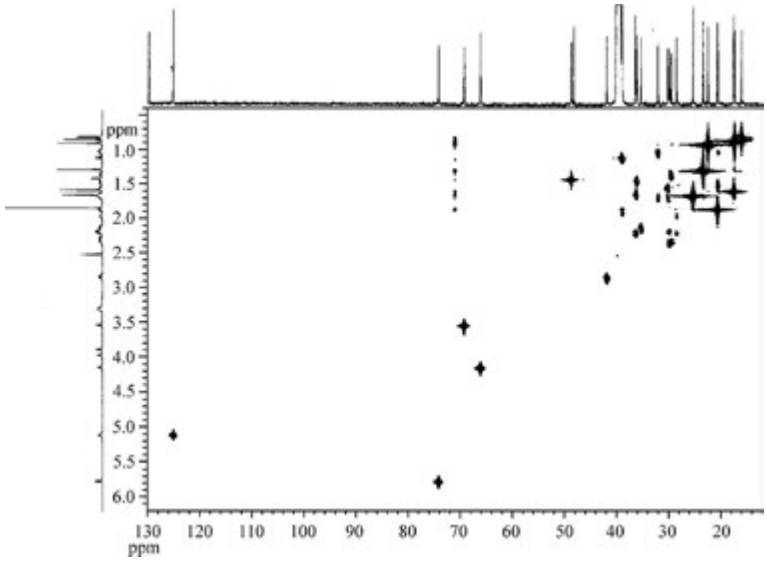


Figure 6.178 The locally enlarged COSY spectrum (2)

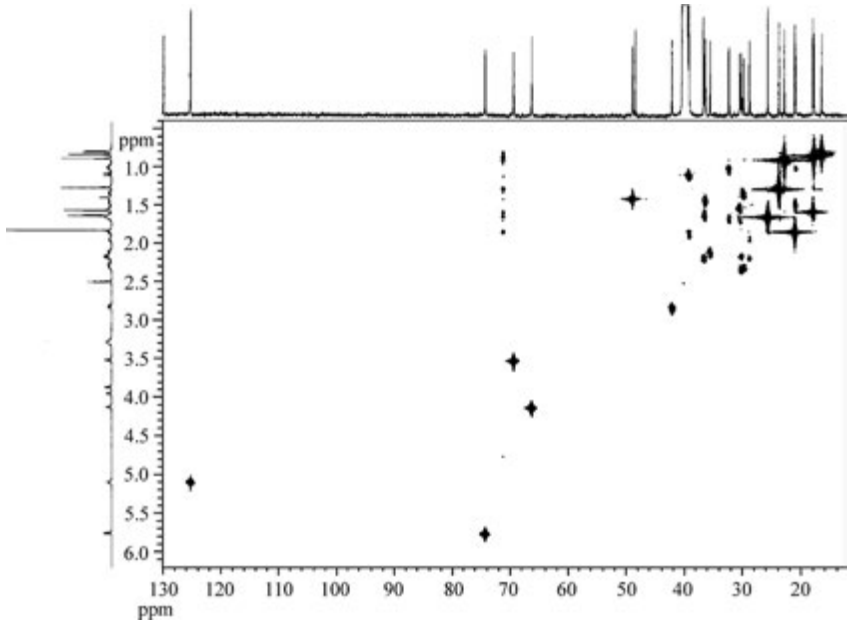


Figure 6.179 The HMQC spectrum of the compound

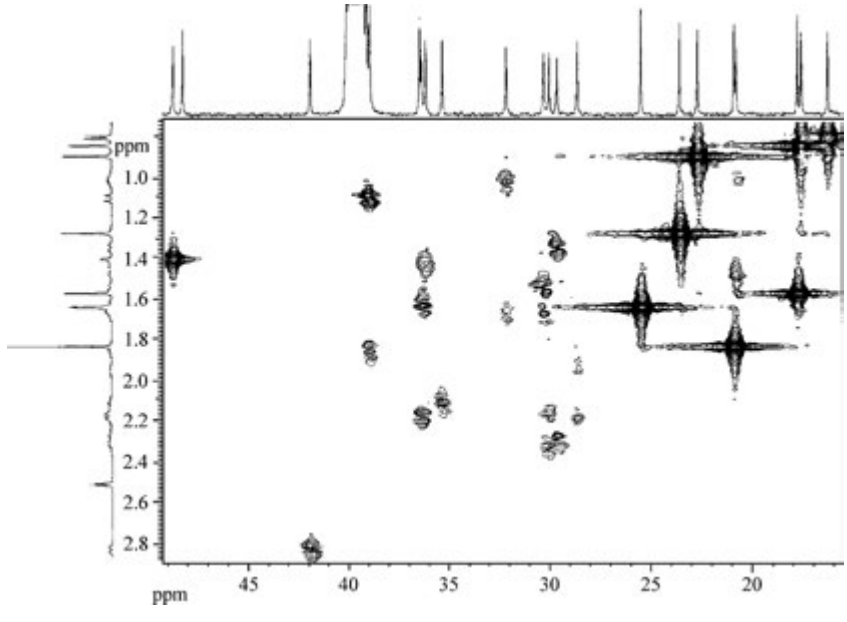


Figure 6.180 The locally enlarged HMQC spectrum (1)

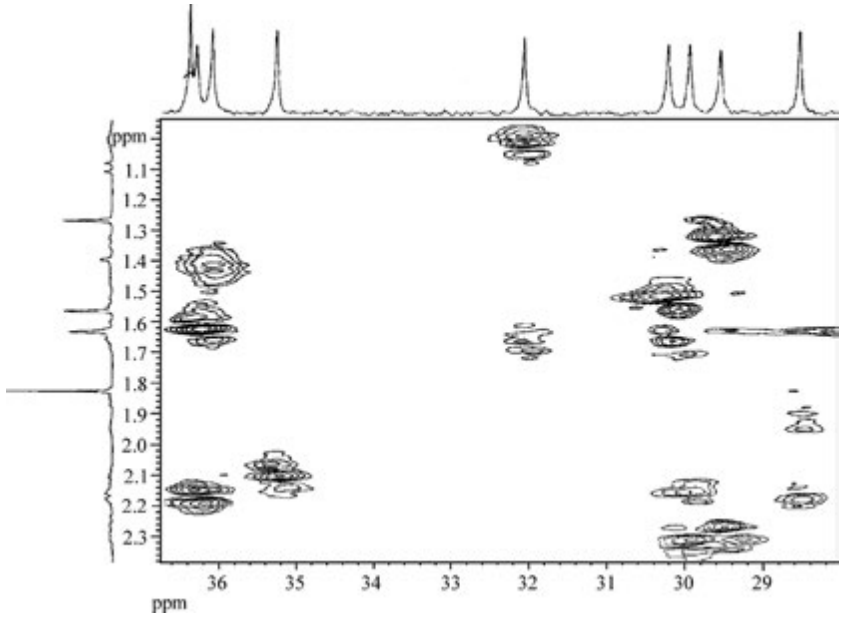


Figure 6.181 The locally enlarged HMQC spectrum (2)

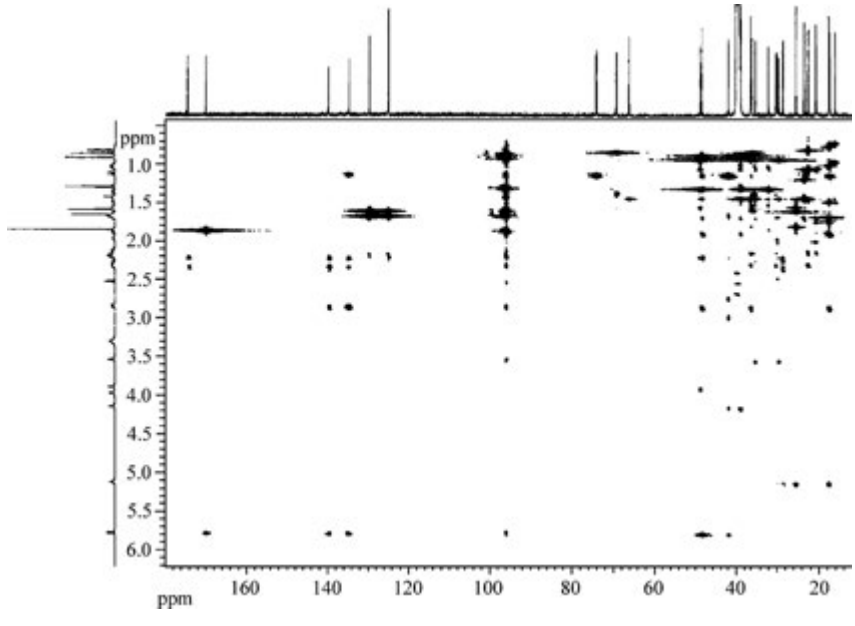


Figure 6.182 The HMBC spectrum of the compound

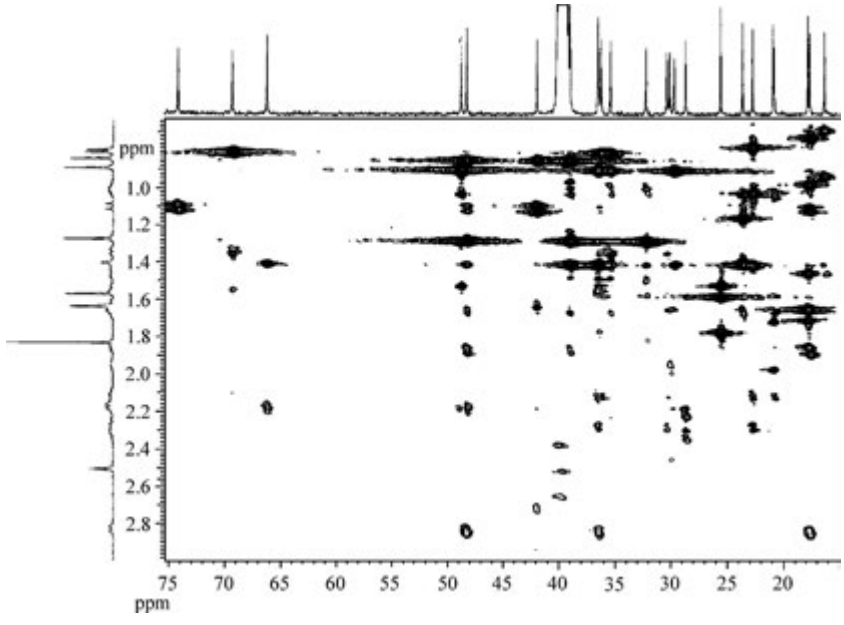


Figure 6.183 The locally enlarged HMBC spectrum (1)

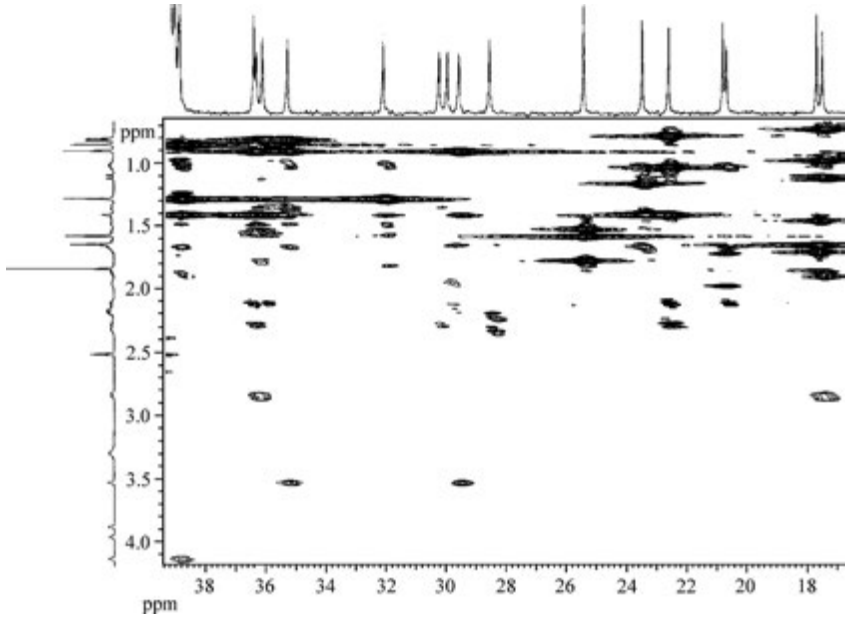


Figure 6.184 The locally enlarged HMBC spectrum (2)

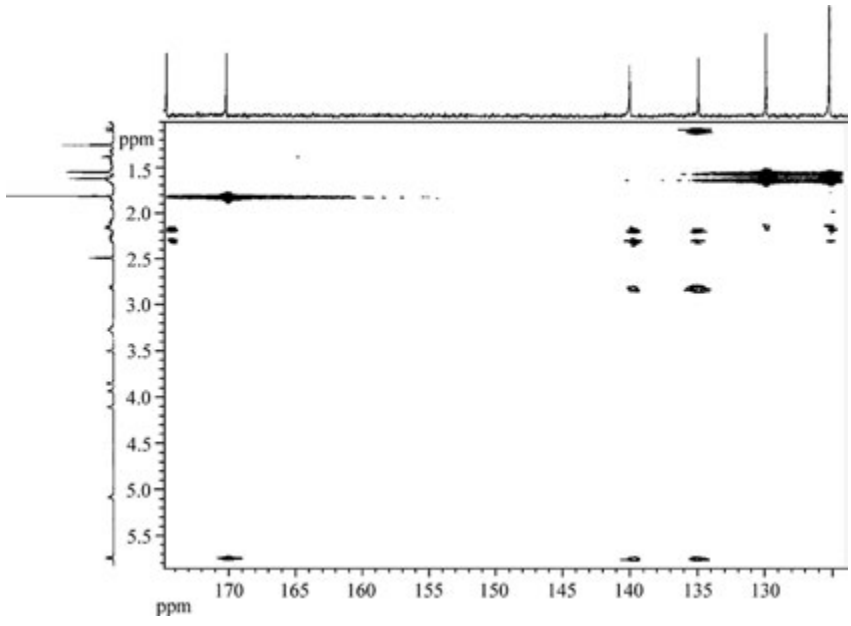


Figure 6.185 The locally enlarged HMBC spectrum (3)

From the COSY spectrum, the H-H correlations can be found. The related data are listed in Table 6.62.

From Table 6.62 it can be known that hydroxyl groups have correlated peaks in the COSY spectrum, because their exchange rates are slow when deuterated DMSO is used as the solvent.

The data summarized from the HMBC spectrum including its locally enlarged spectra are listed in Table 6.63.

The above four tables contain the information on all NMR spectrum.

We will find structural units and then assemble them by use of the four tables.

According to Nos. 8, 17, 28, 10, 21, and 11 of Table 6.62, together with the related data of the HMQC spectrum, the following structural unit can be deduced:

Unit 1.

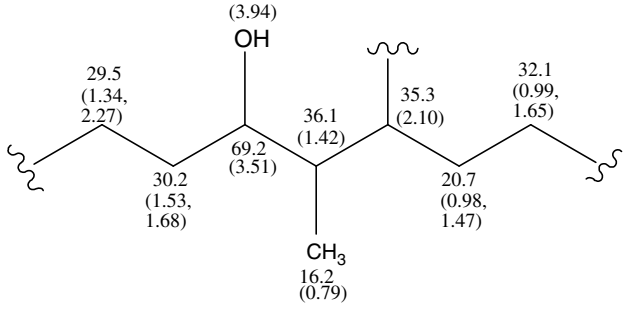


Table 6.60 The data summarized from the ^{1}H spectrum and its locally enlarged spectrum

δ_H (ppm)	Integral value (number of H atoms)	Peak shape	Coupling constant J (Hz)	Remarks
0.79	2.76 (3)	d	6.5	
0.83	2.67 (3)	s		
0.88	2.72 (3)	s		
0.99	1.96 (1 + 1)	m, m		two partially overlapped peak sets[a]
1.10	1.03 (1)	d	14.0	
1.27	2.94 (3)	s		
1.34	1.04 (1)	m		
1.40	1.06 (1)			
1.45	1.80 (2)	m		two partially overlapped peak sets[a]
1.53	1.06 (1)	m		
1.56	2.78 (3)	s		
1.63	3.22 (3)	s		
1.66	2.52 (3)	m		three partially overlapped peak sets[a]
1.82	3.01 (3)	s		
1.85	0.83 (1)	m		
1.95	1.10 (1)	m		
2.06–2.34	5.90 (6)	m		See the discussion later.
2.82	1.01 (1)	d	11	
3.51	1.02 (1)	s, blunt		
3.86	1.02 (1)	s		
3.94	0.98 (1)	s		
4.12	1.03 (1)	s		
5.09	1.00 (1)	t		
5.75	1.02 (1)	d	7	

[a] Known from the HMQC spectrum.

Then, according to Nos. 9, 31, 23, and 14 of Table 6.62, together with the related data of the HMQC spectrum, the following structural unit can be deduced:

Unit 2.

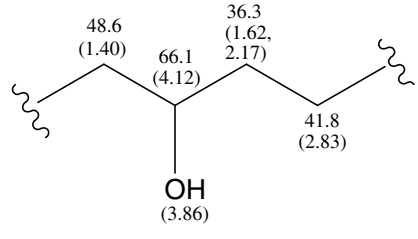


Table 6.61 The summarized data of the ^{13}C spectrum and the ^{1}H spectrum

No.	δ_C (ppm)	Order of C atom	Directly connected H, δ_H (ppm)
1	16.2	CH_3	0.79
2	17.5	CH_3	0.83
3	17.7	CH_3	1.56
4	20.7	CH_2	0.98, 1.47
5	20.8	CH_3	1.82
6	22.6	CH_3	0.88
7	23.5	CH_3	1.27
8	25.4	CH_3	1.63
9	28.5	CH_2	1.97, 2.18
10	29.5	CH_2	1.34, 2.27
11	29.9	CH_2	2.15, 2.30
12	30.2	CH_2	1.53, 1.68
13	32.1	CH_2	0.99, 1.65
14	35.3	CH	2.10
15	36.1	CH	1.42
16	36.3	CH_2	1.62, 2.17
17	36.4	C	
18	38.8	C	
19	38.9	CH_2	1.10, 1.86
20	41.8	CH	2.83
21	48.1	C	
22	48.6	CH	1.40
23	66.1	CH	4.12
24	69.2	CH	3.51
25	74.0	CH	5.75
26	125.1	CH	5.09
27	129.8	C	
28	134.8	C	
29	139.9	C	
30	170.1	CO	
31	174.6	CO	

Then according to Nos. 26 and 32 of Table 6.62, together with the related data of the HMQC spectrum, the following structural unit can be deduced:

Unit 3

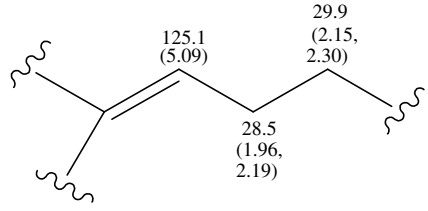


Table 6.62 The H-H correlations known from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)
1	0.79	1.42
2	0.83	1.10, (1.86)
3	0.88	
4	0.98	1.47*, 2.10, 1.65
5	0.99	1.65*
6	1.10	1.86*
7	1.27	
8	1.34	2.27*, 1.68, 1.53
9	1.40	4.12,
10	1.42	3.51, 2.10, 0.79
11	1.47	0.98*, 1.65, 2.10
12	1.53	1.68*, 3.51, 2.27, 1.34
13	1.56	5.09
14	1.62	2.17*, 4.12, 2.83
15	1.63	5.09
16	1.65	0.99*, 1.47
17	1.68	1.53*, 3.51, 2.27, 1.34
18	1.82	
19	1.86	1.10*, 5.75, (0.83)
20	1.96	2.19*, 2.30, 2.15
21	2.10	1.47, 1.42, 0.98
22	2.15	2.30*,
23	2.17	1.62*, 4.12, 2.83,
24	2.19	1.96*, 2.30, 2.15
25	2.27	1.34*, 1.68, 1.53, (0.88)
26	2.30	2.15*, 2.19, 1.96
27	2.83	2.17, 1.62
28	3.51	3.94, 1.68, 1.53, 1.42
29	3.86	4.12
30	3.94	3.51,
31	4.12	3.86, 2.17, 1.62, 1.40
32	5.09	2.19, 1.96, 1.63, 1.56
33	5.75	1.86

Notes:
1. Parenthesis stands for weak couplings, produced from the long-range coupling with the W type configuration.
2. * represents ^2J couplings, which can be recognized by the HMQC spectrum.

Then according to Nos. 19 and 33 of Table 6.62, together with the related data of the HMQC spectrum, the following structural unit can be deduced:

Unit 4

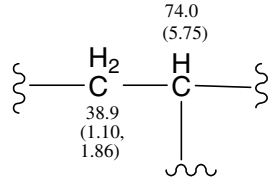
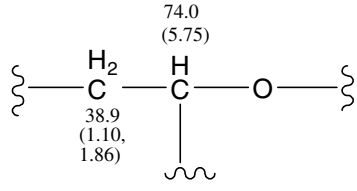


Table 6.63 The data summarized from the HMBC spectra

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	16.2	(1.42)	0.79
2	17.5	1.10, 1.86, 2.83	0.83
3	17.7	1.63, 5.09	1.56
4, 5	20.7, 20.8	2.10	1.82
6	22.6	1.40	0.88
7	23.5	1.40, 1.65	1.27
8	25.4	1.56, 5.09	1.63
9	28.5	2.15, 2.30, 5.09	
10	29.5	0.88, 1.40, 3.51	
11	29.9	1.96, 5.09	
12	30.2	1.34, 2.27	
13	32.1	0.98, 1.27, 1.40, 1.47	
14	35.3	0.79, 0.88, 1.37, 1.40, 1.47, 1.65, 3.51	
15	36.1	0.79, 2.10	
16	36.3	2.83, 1.40	
17	36.4	0.88, 1.47, 1.53, 2.10	
18	38.8	0.83, 0.98, 1.27, 1.40, 1.47, 1.65, 1.86, 4.12	
19	38.9	5.75	
20	41.8	0.83, 1.10, 4.12, 5.75	2.83
21	48.1	0.83, 1.10, 1.27, 1.40, 1.65, 1.86, 2.83, 5.75	
22	48.6	0.88, 0.99, 1.47, 3.86	
23	66.1	1.40, 2.17	
24	69.2	0.79, 1.34, 1.53	
25	74.0	1.10	
26	125.1	1.56, 1.63, 2.15, 2.30	
27	129.8	1.56, 1.63, 2.15	
28	134.8	1.10, 2.15, 2.30, 2.83, 5.75	
29	139.9	2.15, 2.30, 2.83, 5.75	
30	170.1	1.82, 5.75	
31	174.6	2.15, 2.30	

From the chemical shift value of 74.0 ppm, it can be known that this CH group should connect with an oxygen atom:



The connections of the four structural units deduced will be completed by use of the HMBC spectrum, and so will the connections of the remaining methyl groups and quaternary carbon atoms.

Besides the determination of the above-mentioned connections, the correlated peaks of the HMBC spectrum can be used to verify correlated peaks in the COSY spectrum, especially when the correlated peaks in the COSY spectrum are close.

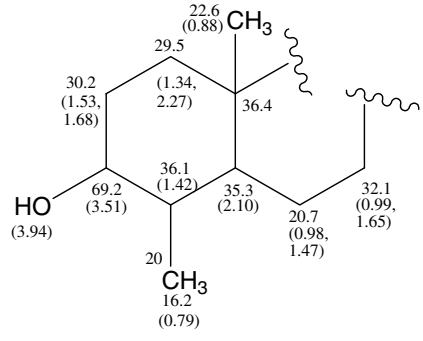
It should be noted that the correlated peaks of methyl groups in the HMBC spectrum have high intensities, so that this information should be used well.

Because the correlated peaks in the HMBC spectrum can stride two to four chemical bonds, the interpretation of the HMBC spectrum should be done very carefully.

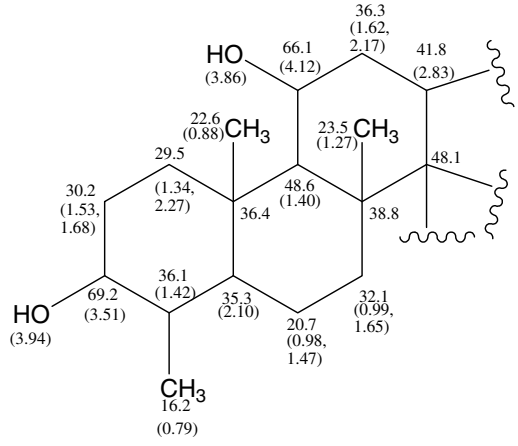
We now start from Unit 1. It should be noted whether some groups of Unit 1 have correlated peaks in the HMBC spectrum. From No. 17 of Table 6.63, that is, the correlations of δ_C (36.4)/δ_H (0.88, 1.47, 1.53, 2.10), together with the related data of the HMQC spectrum, their connection with the quaternary carbon atom ($\delta_C = 36.4$ ppm) can be determined.

According to Nos. 10, 14, and 17 of Table 6.63, the correlations of δ_C (29.5, 35.3, 36.4)/δ_H (0.88) exist. From No. 6 of Table 6.61, $\delta_H = 0.88$ ppm belongs to a methyl group.

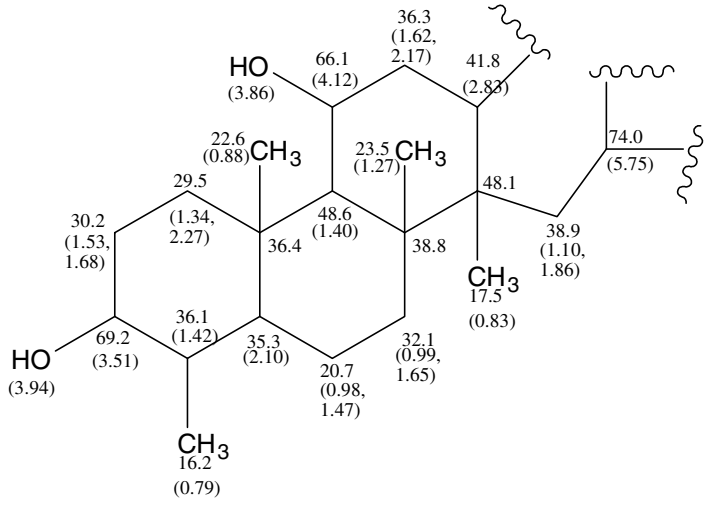
By combining the above analyses, the Unit 1 can be extended as follows:



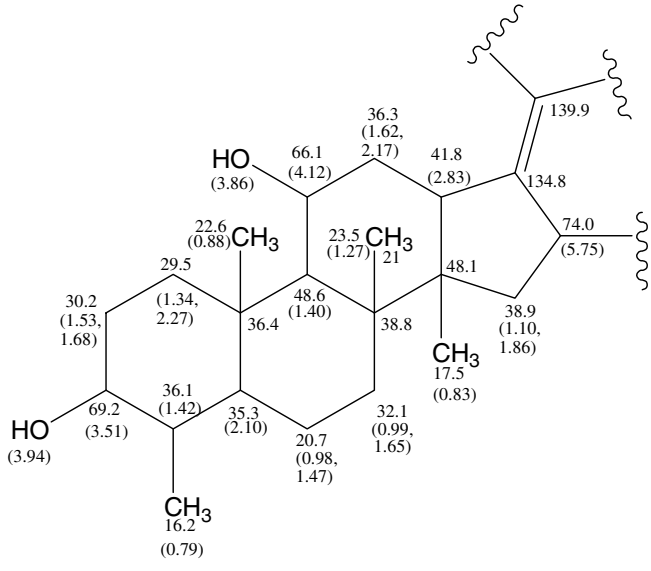
From No. 2 of Table 6.63, the correlation between CH_3 ($\delta_H = 0.88$) and CH ($\delta_C = 48.6$) exists. From the HMQC spectrum, δ_H of the CH can be found as 1.40 ppm. Then from Nos. 7, 10, 13, 14, 16, 18, 21, and 23 of Table 6.63, the CH ($\delta_H = 1.40$) has heteronulear long-range couplings with the following carbon atoms: CH_3 (23.5 ppm), CH_2 (29.5 ppm), CH_2 (32.1 ppm), CH (35.3 ppm), CH_2 (36.3 ppm), C (38.8), C (48.1 ppm), and CH (66.1 ppm). Compared with the structure of Unit 2, the above structural unit can be extended as follows:



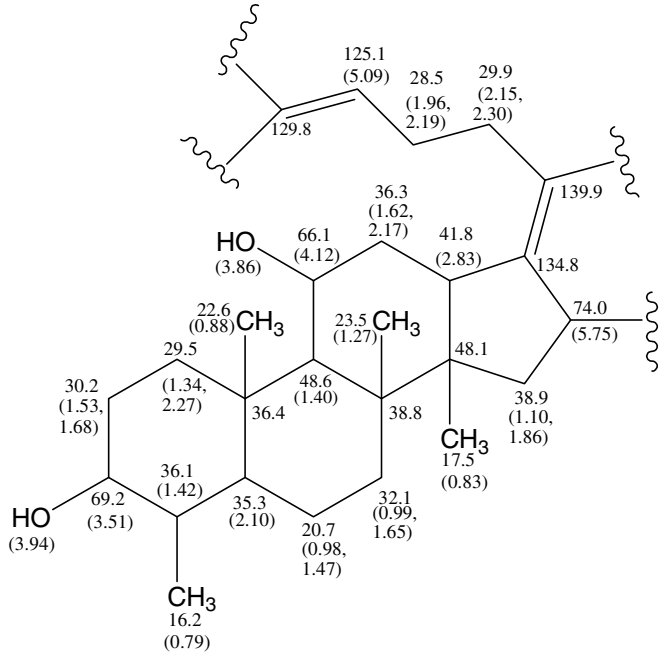
According to Nos. 20 and 21 of Table 6.63, the correlations of δ_C (41.8, 48.1)/δ_H (0.83) exist. From No. 2 of Table 6.61, δ_H (0.83) belongs to a methyl group ($\delta_C = 17.5$). From No. 2 of Table 6.63, the correlation of δ_C (17.5)/δ_H (2.83) exists. The above-mentioned correlations represent the connection between the quaternary carbon atom with $\delta_C = 48.1$ ppm and the methyl group with $\delta_C = 17.5$ and $\delta_H = 0.83$ ppm. According to No. 2 of Table 6.63, the correlations of δ_C (17.5)/δ_H (1.10, 1.86) exist. Compared with the structure of Unit 4, the above structure can be extended as follows:



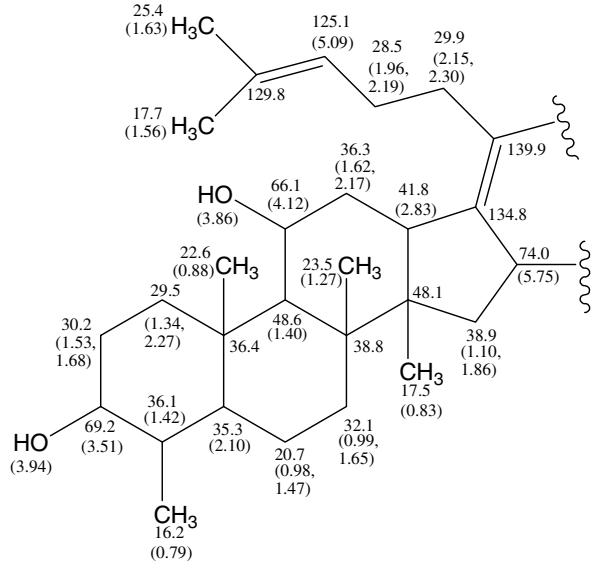
From Nos. 19, 20, 28 and 29 of Table 6.63, it can be known that the peak at 5.75 ppm couples with the carbon atoms at 38.9, 41.8, 48.1, 134.8, and 139.9 ppm, respectively. From their chemical shift values of 134.8 and 139.9, they should be alkene carbon atoms. Therefore, the above structure can be further extended as follows:



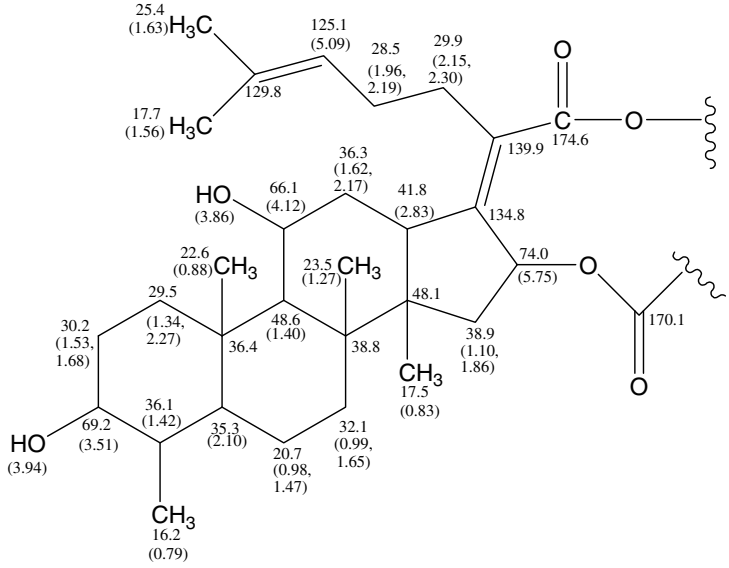
From Nos. 28 and 29 of Table 6.63, the correlations of δ_C (139.9)/δ_H (2.15, 2.30) and δ_C (134.8)/δ_H (1.10, 2.15, 2.30) exist. Compared with the structure of Unit 3, the above structure can be further extended as follows:



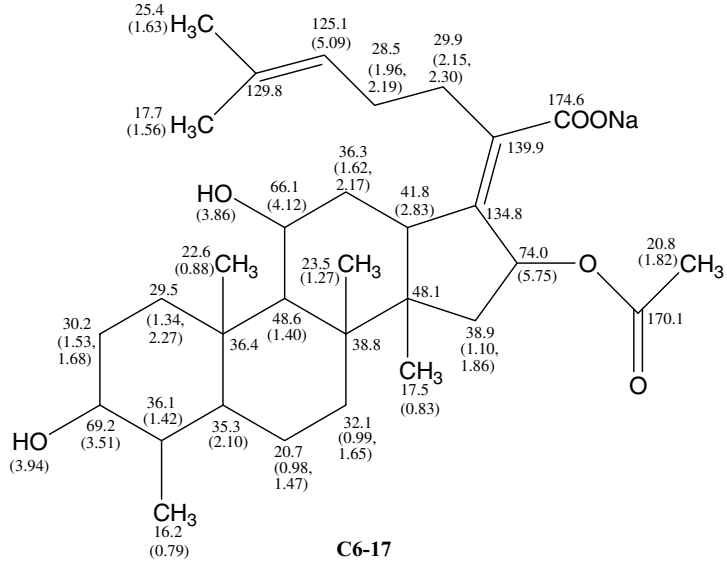
According to Nos. 26 and 27 of Table 6.63, that is, the correlations of δ_C (125.1)/δ_H (1.56, 1.63) and δ_C (129.8)/δ_H (1.56, 1.63), where $\delta_H = 1.56$ and $\delta_H = 1.63$ correspond to two methyl groups, the above structural unit can be extended as follows:



From No. 31 of Table 6.63, the correlation of δ_C (174.6)/δ_H (2.15, 2.30) exists. From No. 30 of Table 6.63, the correlation of δ_C (170.1)/δ_H (5.75) exists. From the chemical shift values of 174.6 and 170.1 ppm it can be known that these two carbonyl groups should connect with an oxygen atom, respectively. On the other hand, the CH with $\delta_C = 74.0$ and $\delta_H = 5.75$ ppm should connect also with an oxygen atom. Therefore, the above structure can be further extended as follows:



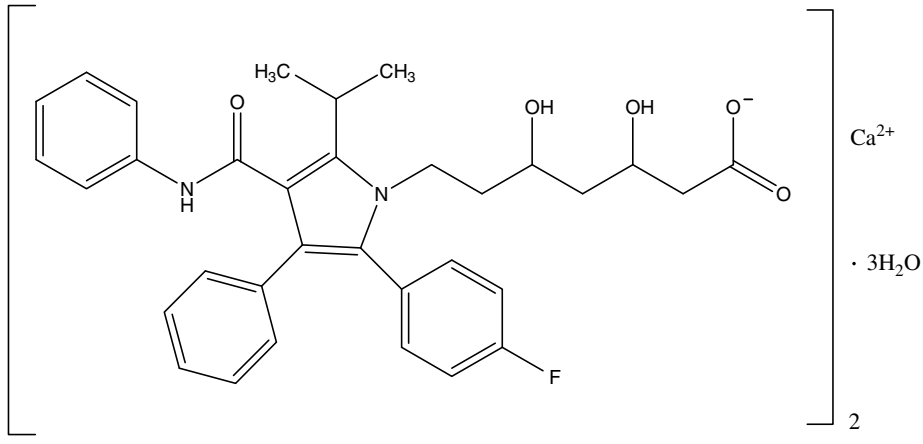
According to No. 30 of Table 6.63, that is, the correlation of δ_C (170.1)/δ_H (1.82), the carbonyl group should connect with the CH_3 ($\delta_C = 20.8$ and $\delta_H = 1.82$ ppm). In addition, this compound is a salt of sodium, the structure of the compound can be completed as follows:



C6-17

The above detailed assignment shows that the anticipated structure is correct.

Example 6.17 A synthesized compound has the anticipated structure which follows:



C6-18

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its DEPT-135 spectrum, the locally enlarged DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HMQC spectrum, the locally enlarged HMQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.186–6.203. Try to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 600 MHz. The solvent used is deuterated methanol.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The peak at 3.29 ppm is the solvent peak. The peak at 4.86 ppm is the water peak.

The peak at 1.91 ppm, whose correlated peak in the HMQC spectrum has a low intensity, can be determined as a peak of impurities.

The peaks of NH and OH will merge in the water peak.

Because peak sets in the ^{1}H spectrum overlap seriously, we comprehensively interpret the ^{1}H spectrum, the ^{13}C spectrum, the HMQC spectrum, and the DEPT-135 spectrum. The data of the ^{1}H spectrum and the ^{13}C spectrum can be generalized in Table 6.64.

The peaks at 168.1 and 180.7 ppm can be assigned as two carbonyl groups. If a carbonyl group lacks electrons, it will have a rather large chemical shift value. Therefore, the peak at 180.7 ppm can be assigned as the ion of a carboxyl and that at 168.1 ppm the amide, which can also be proved with the help of the HMBC spectrum.

The peaks at 163.3 and 161.6 ppm can be assigned as the peaks of the quaternary carbon atom which are split by the fluorine atom, because of the direct connection with the fluorine atom. The reason for this assignment is based on two facts. First, their peaks have a similar height. Second, the distance between the two peaks corresponds to the $^{1}J_{\text{F-C}}$ coupling constant (247 Hz), whose standard value is 245 Hz. Similarly, the peaks at 114.9 and 115.1 ppm can be assigned as the peaks of the two carbon atoms split by the fluorine atom

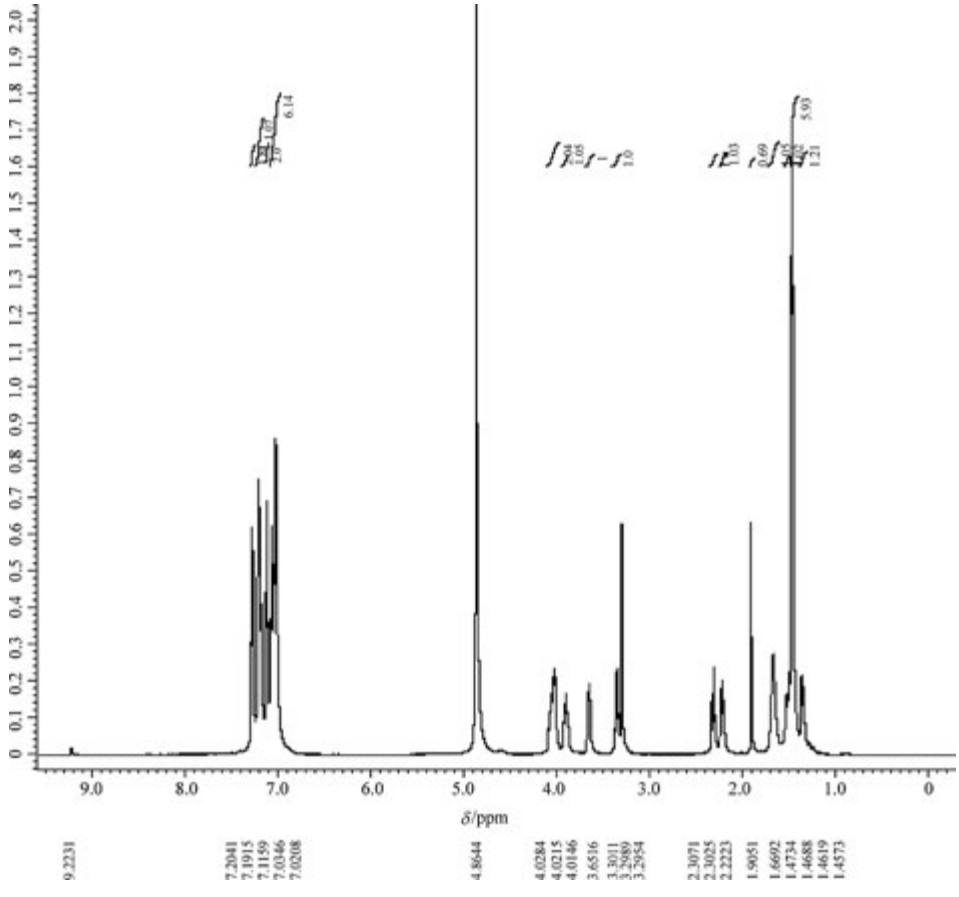


Figure 6.186 The ^{1}H spectrum of the compound

with a $^{2}J_{F\text{-}C}$ coupling constant of 21.5 Hz, whose standard value is 21 Hz. And the peaks at 133.4 and 133.5 ppm can be assigned as the peaks of the two carbon atoms split by the fluorine atom with a $^{3}J_{F\text{-}C}$ coupling constant of 7.2 Hz, whose standard value is 7.8 Hz.

From Table 6.64, the total number of carbon atoms is 32. If we compare it with the structural formula, one carbon atom is missing. We will discuss it later.

From the correlated peaks in the high field region of the COSY spectrum, the correlations of aliphatic functional groups can be known. Related results are listed in Table 6.65.

The correlated peaks in the aromatic region of the locally enlarged COSY spectrum are generalized in Table 6.66.

The data summarized from the HMBC spectrum including its locally enlarged spectra are listed in Table 6.67.

The above four tables contain the information on all NMR spectra.

Now we will assign the spectra.

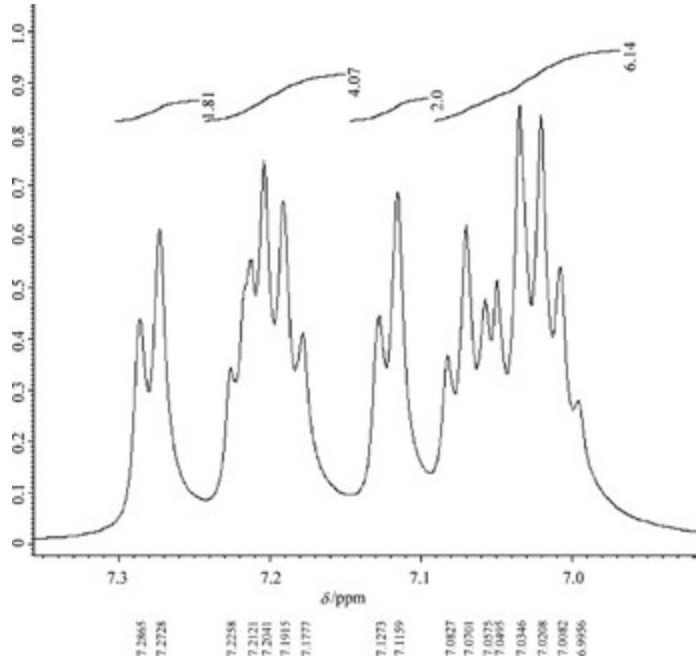


Figure 6.187 The locally enlarged ^{1}H spectrum (1)

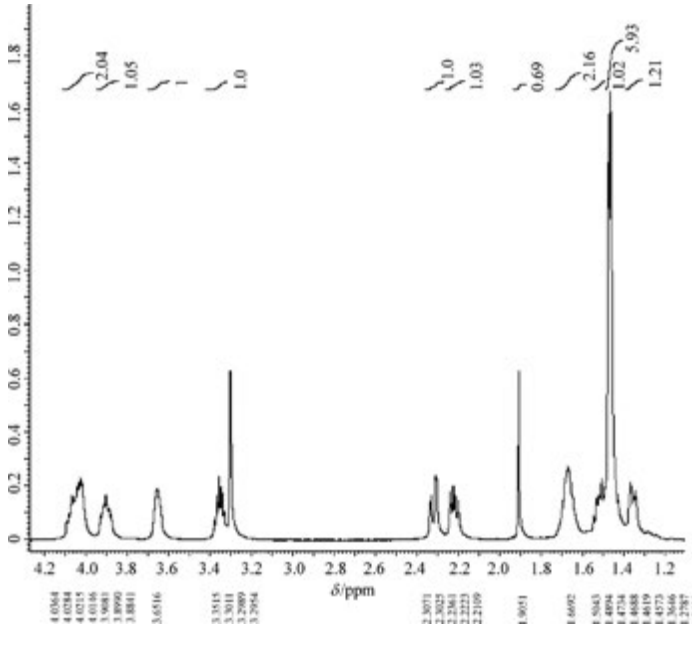


Figure 6.188 The locally enlarged ^{1}H spectrum (2)

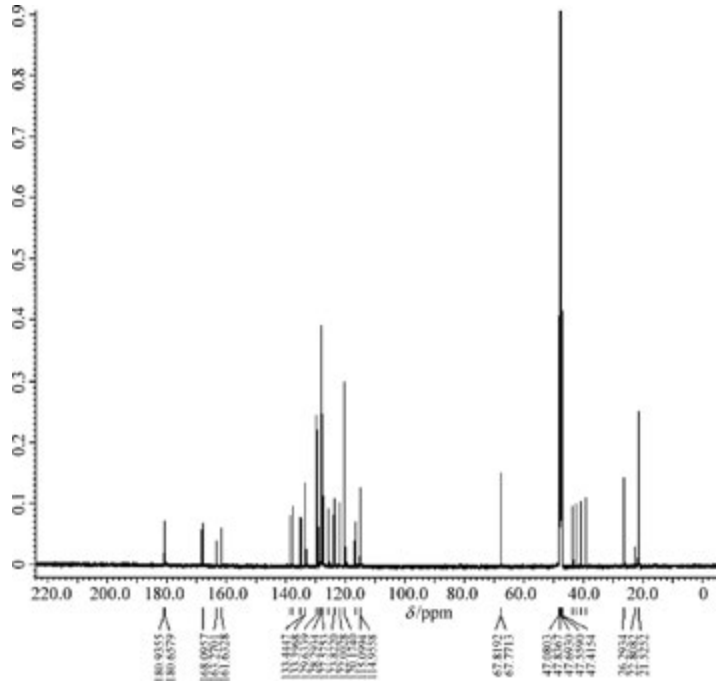


Figure 6.189 The ^{13}C spectrum of the compound

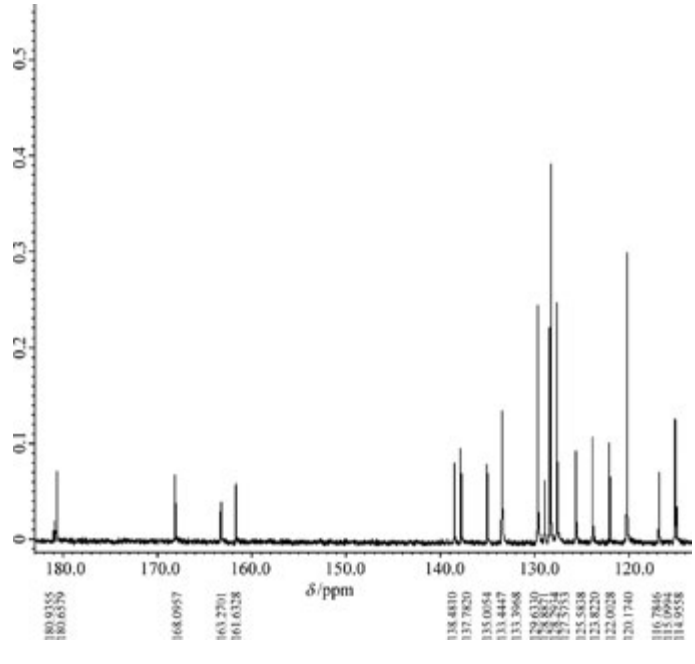


Figure 6.190 The locally enlarged ^{13}C spectrum

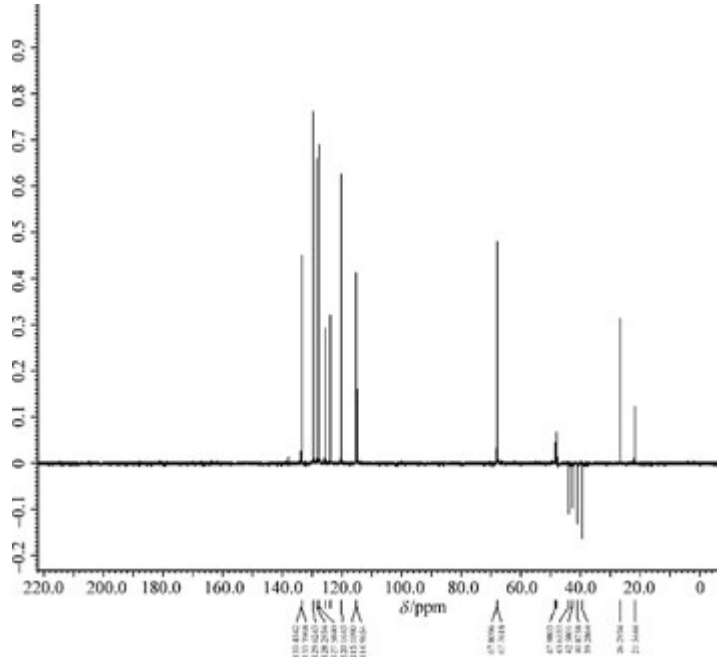


Figure 6.191 The DEPT-135 spectrum of the compound

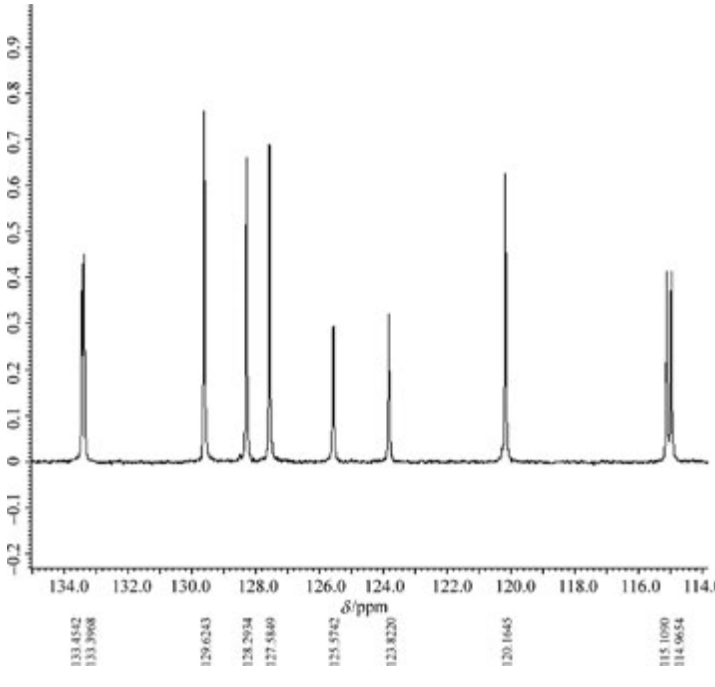


Figure 6.192 The locally enlarged DEPT-135 spectrum

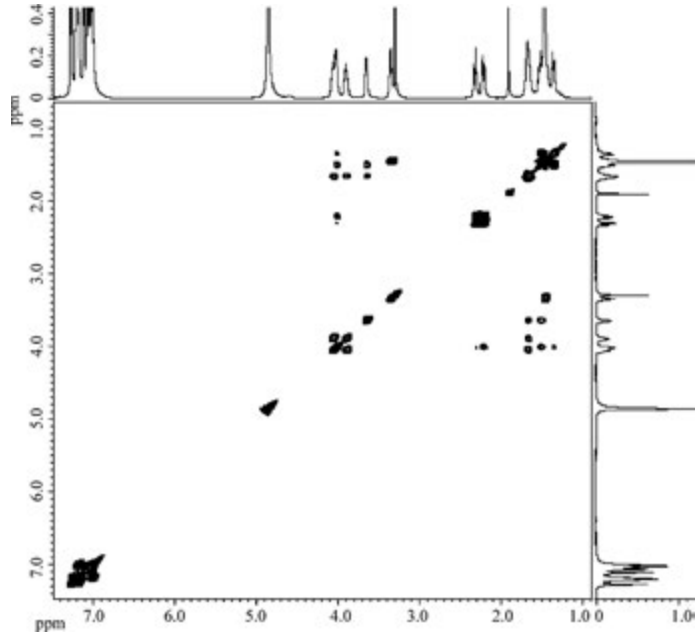


Figure 6.193 The COSY spectrum of the compound

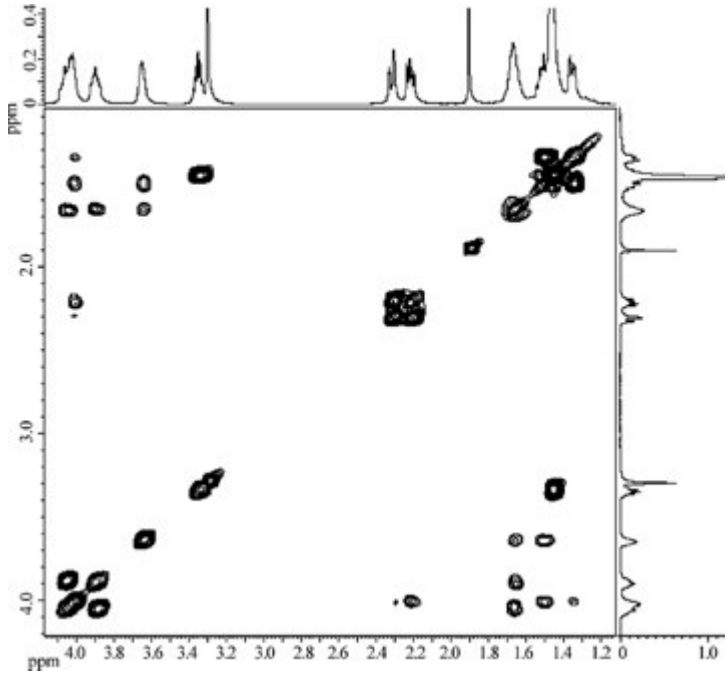


Figure 6.194 The locally enlarged COSY spectrum (1)

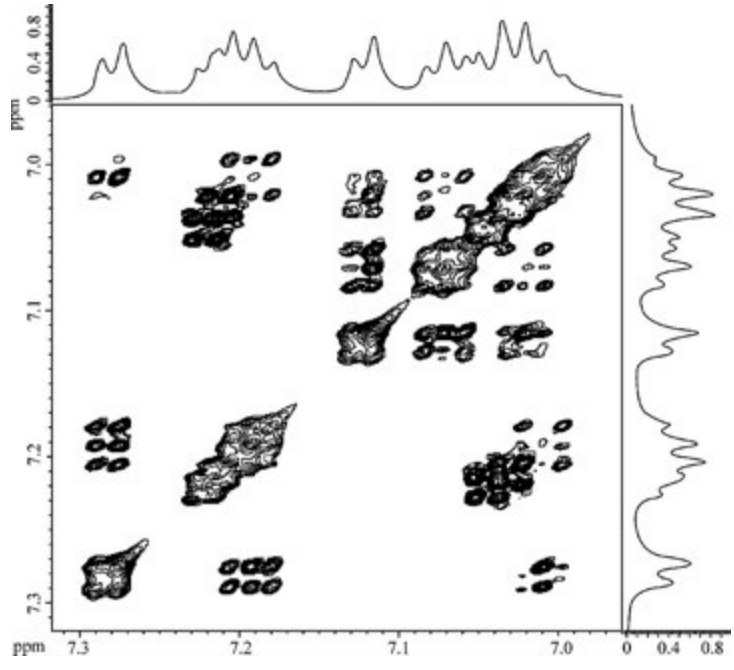


Figure 6.195 The locally enlarged COSY spectrum (2)

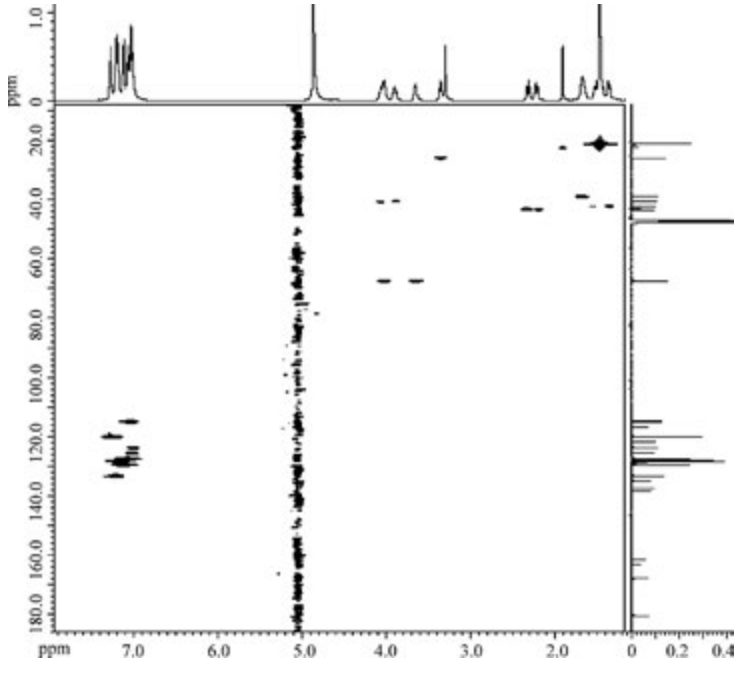


Figure 6.196 The HMQC spectrum of the compound

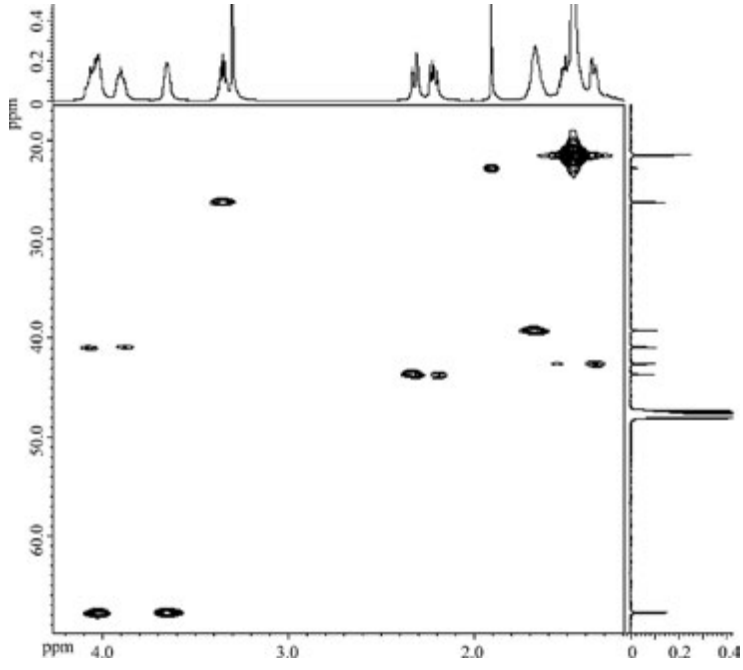


Figure 6.197 The locally enlarged HMQC spectrum (1)

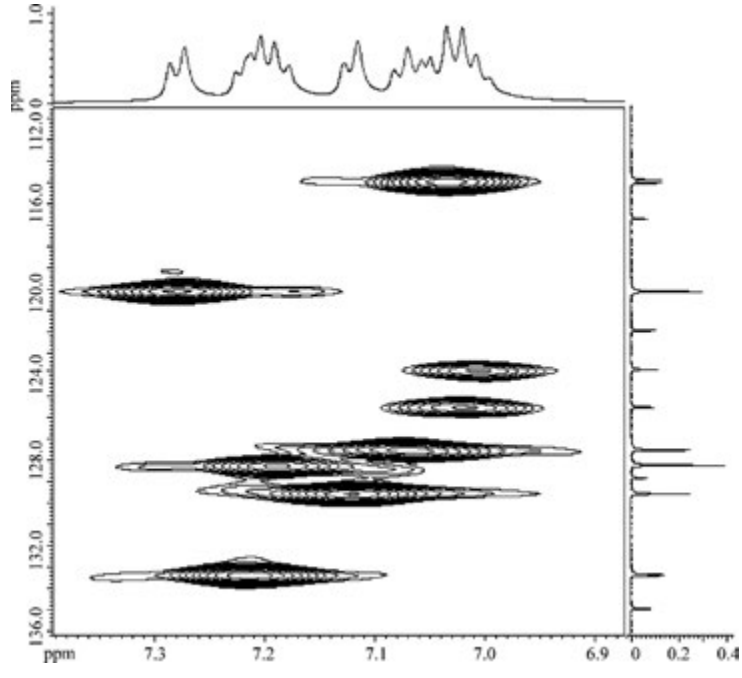


Figure 6.198 The locally enlarged HMQC spectrum (2)

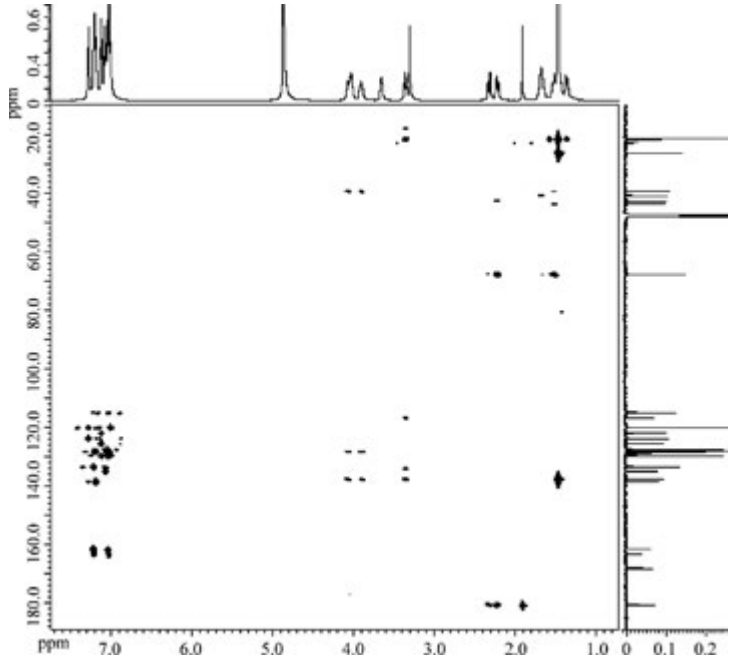


Figure 6.199 The HMBC spectrum of the compound

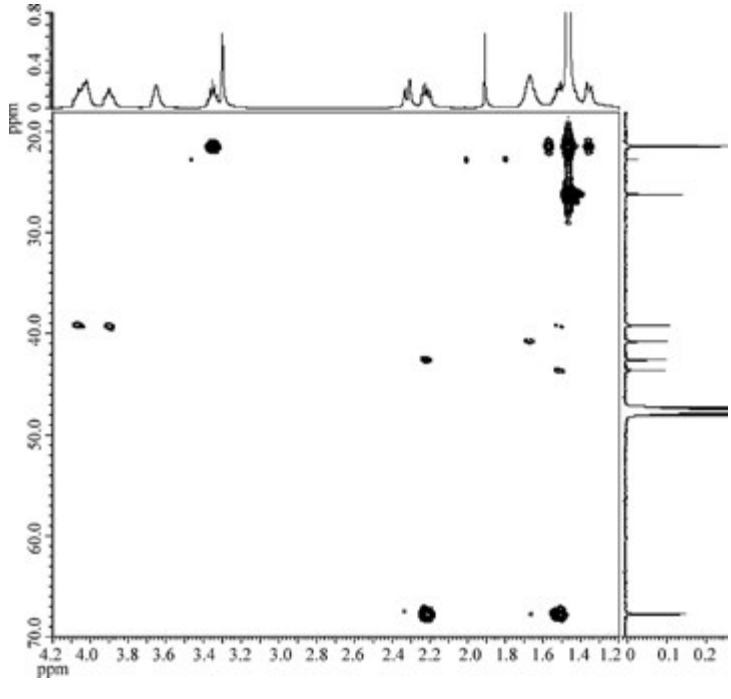


Figure 6.200 The locally enlarged HMBC spectrum (1)

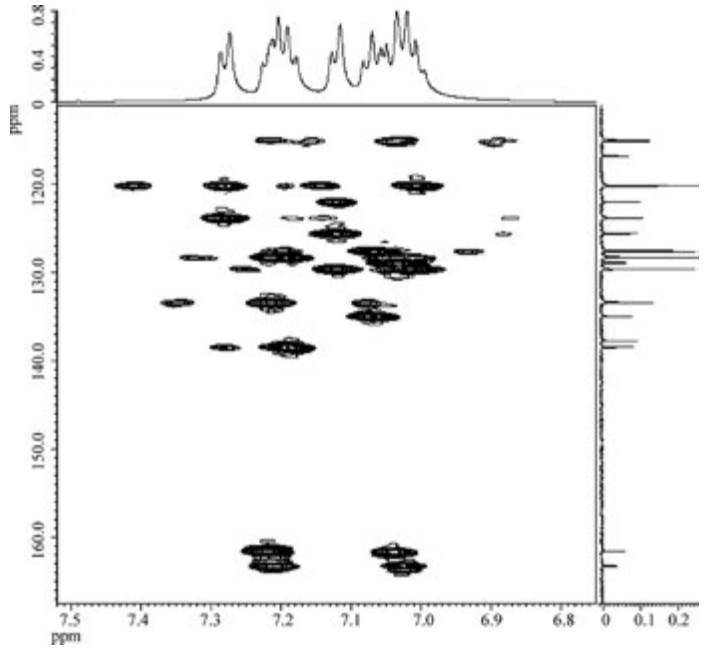


Figure 6.201 The locally enlarged HMBC spectrum (2)

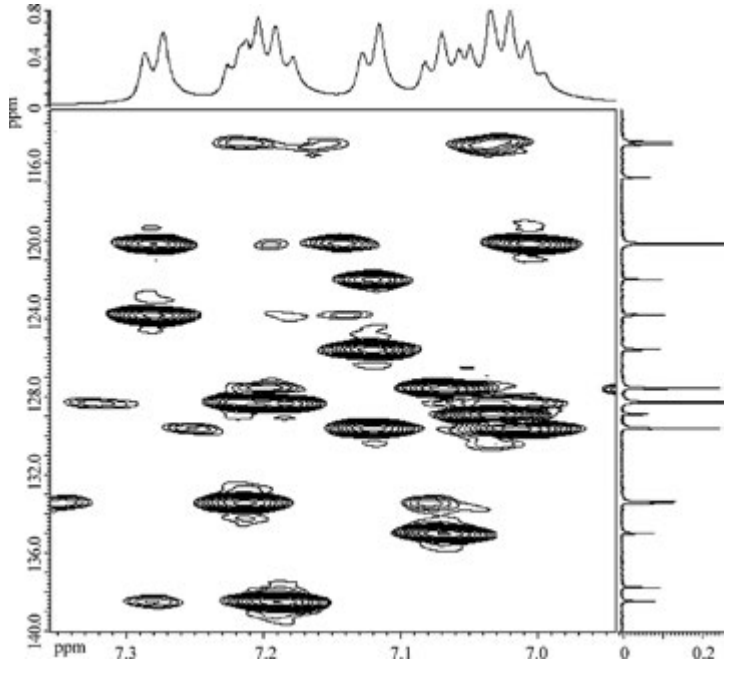


Figure 6.202 The locally enlarged HMBC spectrum (3)

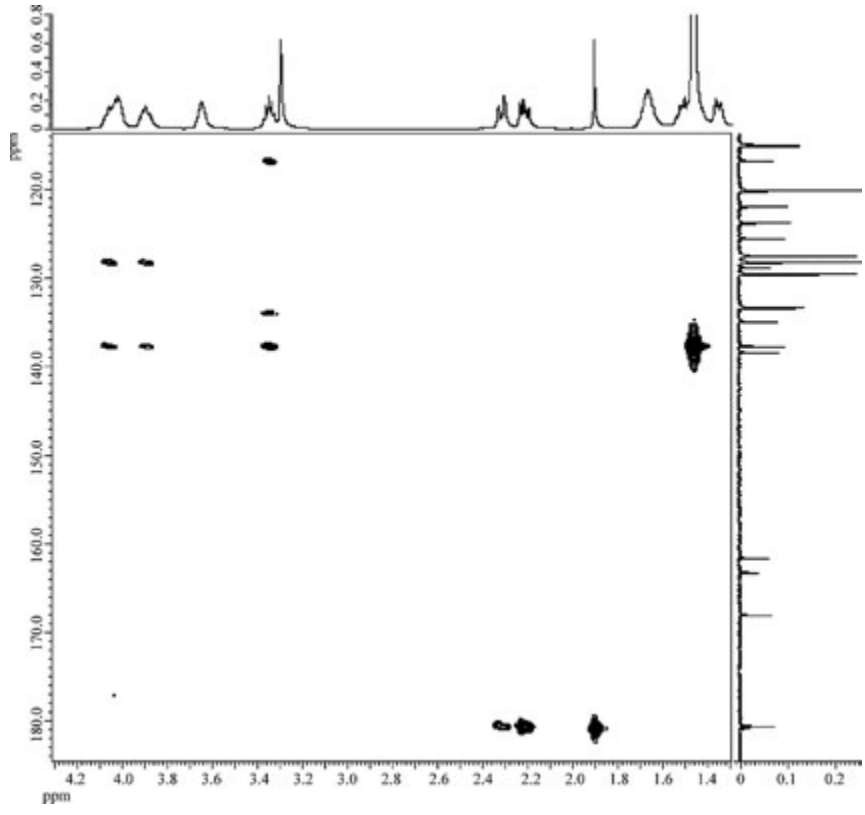


Figure 6.203 The locally enlarged HMBC spectrum (4)

According to Nos. 2, 11, 9, 4, 8, 3, and 10 of Table 6.65, together with the related data of the HMQC spectrum, and from Nos. 1, 10, and 16 of Table 6.67, we obtain the assignments of all aliphatic functional groups and partial aromatic carbon atoms which follow:

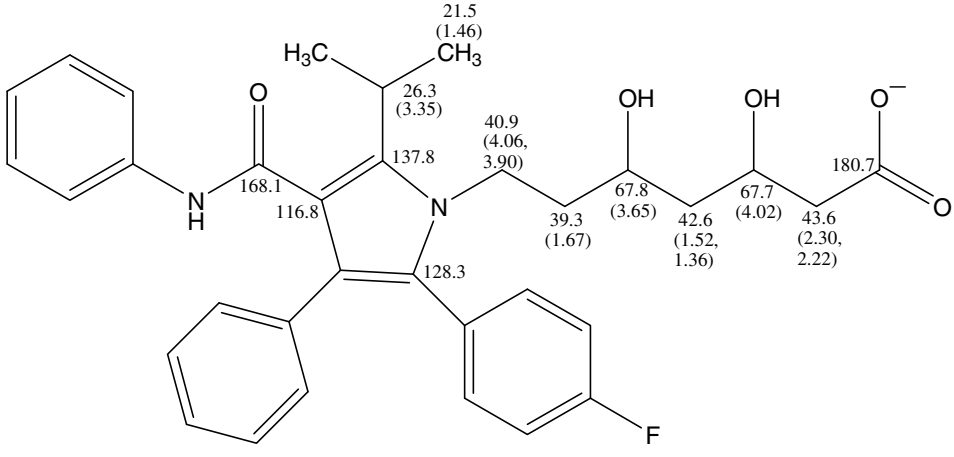


Table 6.64 The generalized data of the ^{1}H spectrum and the ^{13}C spectrum

δ_C (ppm)	δ_H (ppm)	Order of C atoms	Number of C atoms	Remarks
180.7		C=O	1	
168.1		C=O	1	
163.3		C	0.5	^{1}J split by F
161.6		C	0.5	^{1}J split by F
138.5		C	1	
137.8		C	1	
135.0		C	1	
133.5	7.21	CH	1	^{3}J split by F
133.4	7.21	CH	1	^{3}J split by F
129.6	7.12	CH	2	
128.9		C	1	
128.3	7.19	CH	2	
127.6	7.07	CH	2	
125.6	7.02	CH	1	
123.8	7.01	CH	1	
122.0		C	1	
120.2	7.28	CH	2	
116.8		C	1	
115.1	7.04	CH	1	^{2}J split by F
114.9	7.04	CH	1	^{2}J split by F
67.8	3.65	CH	1	
67.7	4.02	CH	1	
43.6	2.30, 2.22	CH_2	1	
42.6	1.52, 1.36	CH_2	1	
40.9	4.06, 3.90	CH_2	1	
39.3	1.67	CH_2	1	
26.3	3.35	CH	1	
21.5	1.46	CH_3	2	

Table 6.65 The correlated peaks in the high field region of the COSY spectrum

No.	δ(H) ppm	Coupled H, δ(H) ppm
1	1.36	4.02, 1.52
2	1.46	3.35
3	1.52	4.02, 3.65, 1.36
4	1.67	4.06, 3.90, 3.65
5	2.22	4.02, 2.30
6	2.30	(4.02), 2.22
7	3.35	1.46
8	3.65	1.67, 1.52
9	3.90	4.06, 1.67
10	4.02	2.22, 1.52, 1.36
11	4.06	3.90,

Parenthesis () stands for weak correlations.

Table 6.66 The generalized correlations in the aromatic region

No.	δ_H (ppm)	Coupled H, δ(H) ppm
1	7.01	7.19
2	7.02	7.07
3	7.04	7.21
4	7.07	7.12, 7.02
5	7.12	7.07, 7.02
6	7.19	7.28, 7.01
7	7.21	7.04,
8	7.28	7.19, (7.01)

Parenthesis () stands for weak correlations.

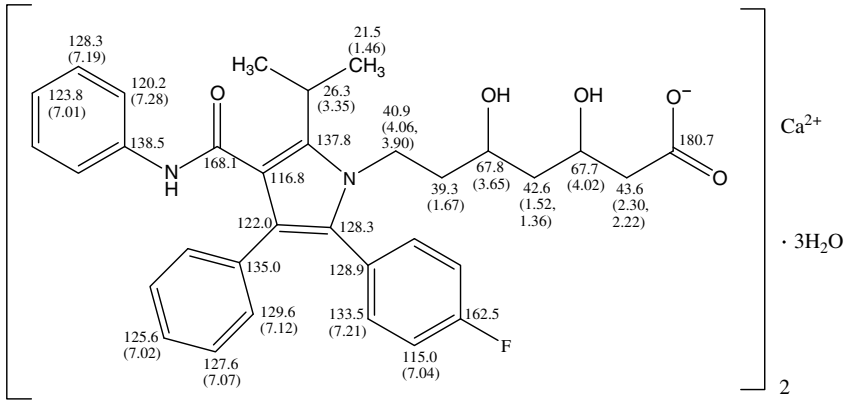
Table 6.67 The data summarized from the HMBC spectra

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	180.7	2.22, 2.30	
2	168.1		
3	162.5	7.04, 7.21	
4	138.5	7.19, 7.28	
5	137.8	1.46, 3.35, 3.90, 4.06	
6	135.0	7.07	
7	133.5		7.21
8	129.6	7.02	7.12
9	128.9	7.04	
10	128.3	3.90[a], 4.06[a], 7.21	7.19
11	127.6		7.07
12	125.6	7.12	
13	123.8	7.28	
14	122.0	7.12	
15	120.2	7.01	7.28
16	116.8	3.35	
17	115.0	7.21	
18	67.8	1.52, 1.67, 2.22	
19	67.7	1.52,	
20	43.6	1.52	
21	42.6	2.22	
22	40.9	1.67	
23	39.3	1.52, 3.90, 4.06	
24	26.3	1.46	
25	21.5	3.35	1.46

The averaged value is used in Table 6.67 to replace split peaks, which is different from that in Table 6.64.
[a] From these two correlated peaks, it can be known that the missing peak in the ^{13}C spectrum is right at 128.3 ppm. It is a quaternary carbon atom, whose peak overlaps accidentally with the peak of a CH at 128.3 ppm. The prominent height of the peak at 128.3 ppm coincides with this postulation.

According to the locally enlarged COSY spectrum (in the aromatic region), and according to Nos. 3, 4, 6, 8, 9, 10, 12, 13, 14, 15, and 17 of Table 6.67, together with the related data of the HMQC spectrum, we can obtain the assignment of the remaining aromatic carbon atoms.

All the assignments are then accomplished. The final result is shown below:

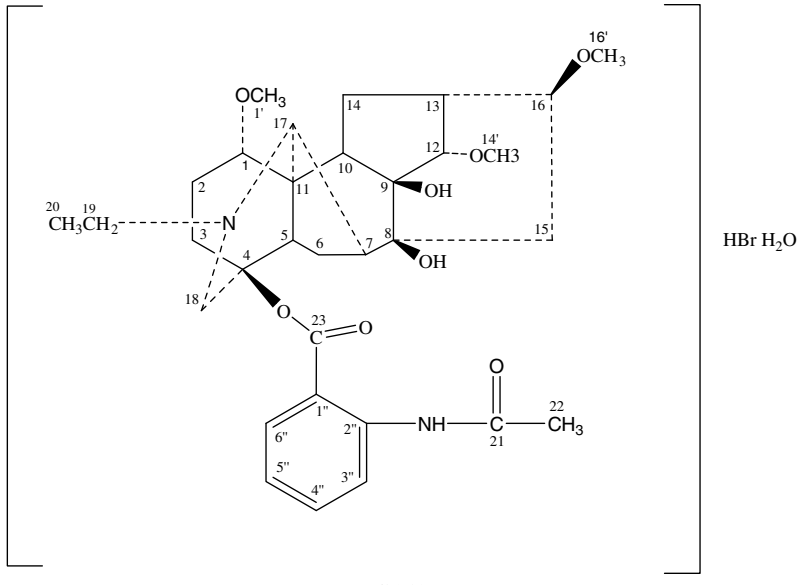


C6-18

Because all assignments are reasonable, the anticipated structure of the compound is confirmed by its NMR spectra.

For the proof of the correctness of the result mentioned above, the solvent was changed from deuterated methanol to deuterated DMSO. In the ^{13}C spectrum measured with the deuterated DMSO, a new peak appears in the aromatic region, which further proves the result.

Example 6.18 A natural product is extracted, separated, and purified from a certain plant. From the measurement of its accurate mass, its molecular formula of $C_{32}H_{44}O_8N_2$ •HBr •H_2O is determined. By comparison with the NMR spectra of the compounds in the same category, a possible structure is proposed as follows:



C6-19

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HSQC spectrum, the locally enlarged HSQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.204–6.222. Try to interpret all NMR spectra to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 500 MHz. The solvent used is deuterated methanol.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The peak at 4.78 ppm is the water peak. From the ^{1}H spectrum it can be known that the purity of the sample is satisfactory.

From the integral values of the peak sets of the ^{1}H spectrum, the triplet at 1.12 ppm and the quartet at 3.55 ppm can be determined as the peaks of impurities.

Because there are overlapped peak sets, for example, in the region of 2.18–2.36 ppm, the use of the HSQC spectrum is necessary for the interpretation of the ^{1}H spectrum. The related data are summarized in Table 6.68.

Because of the existence of impurities and the errors of the integral curve, the hydrogen atom numbers of some peak sets can not be determined directly from the integral curve. However, if we use the HSQC spectrum, this difficulty can be removed.

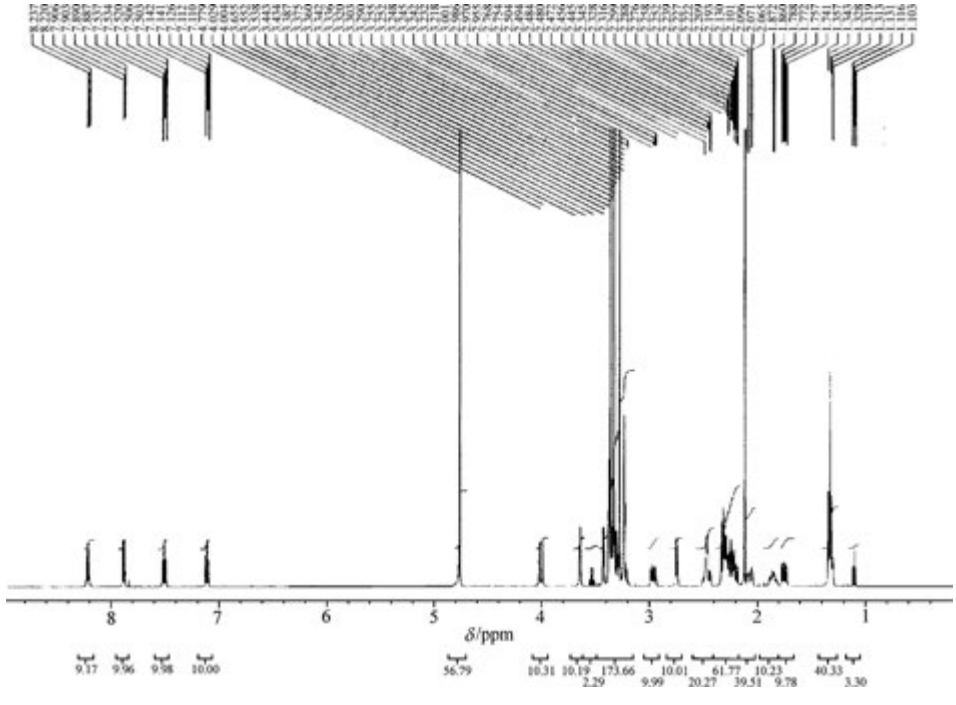


Figure 6.204 The ^{1}H spectrum of the compound

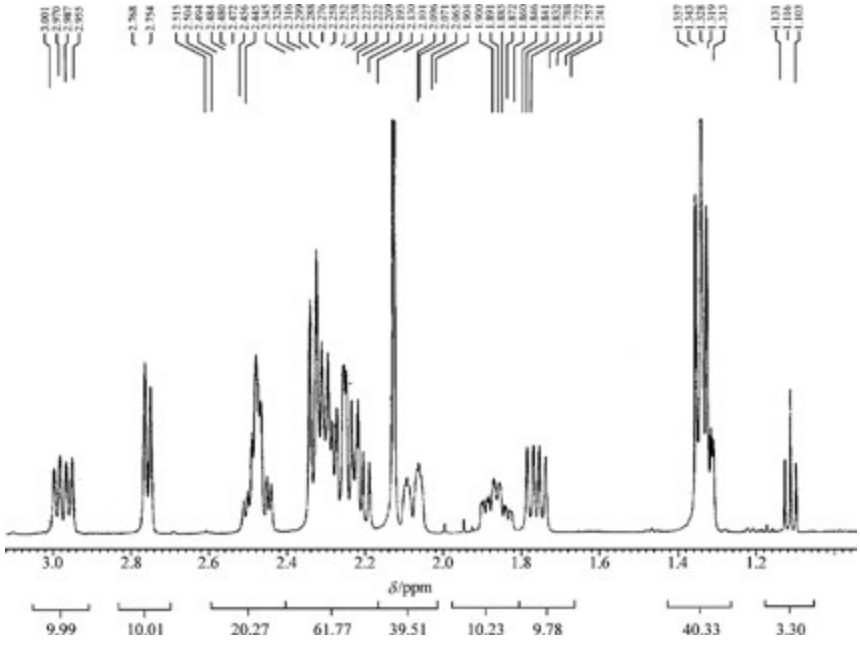


Figure 6.205 The locally enlarged ^{1}H spectrum (1)

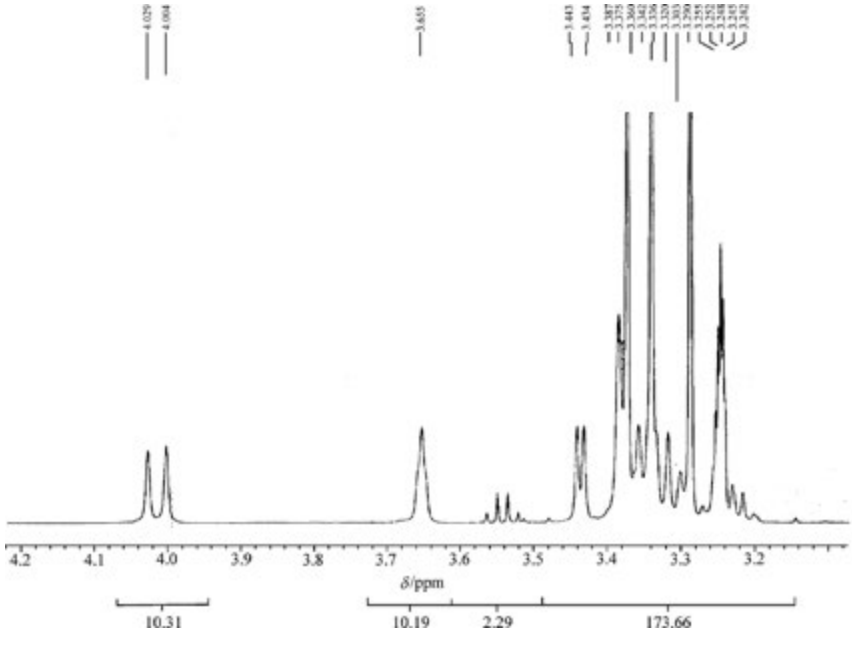


Figure 6.206 The locally enlarged ^{1}H spectrum (2)

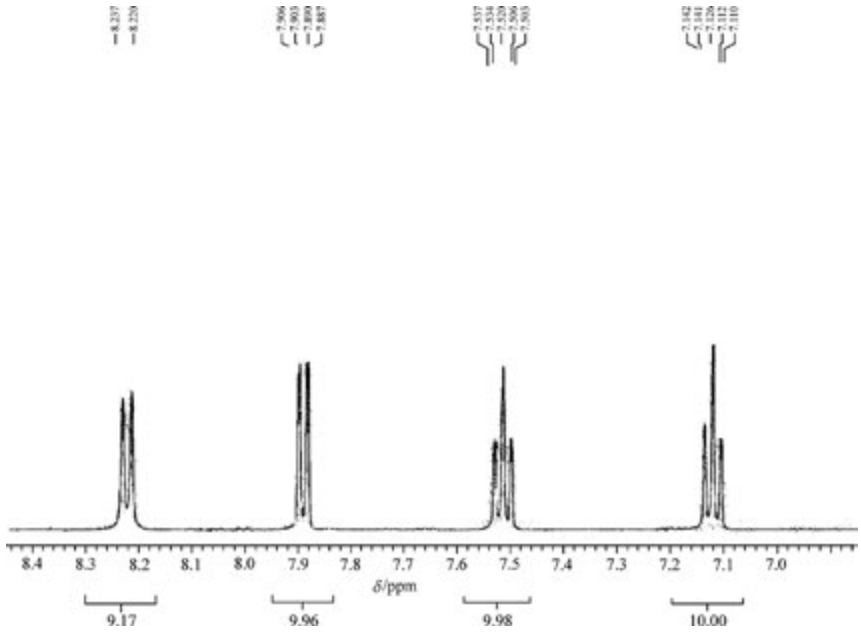


Figure 6.207 The locally enlarged ^{1}H spectrum (3)

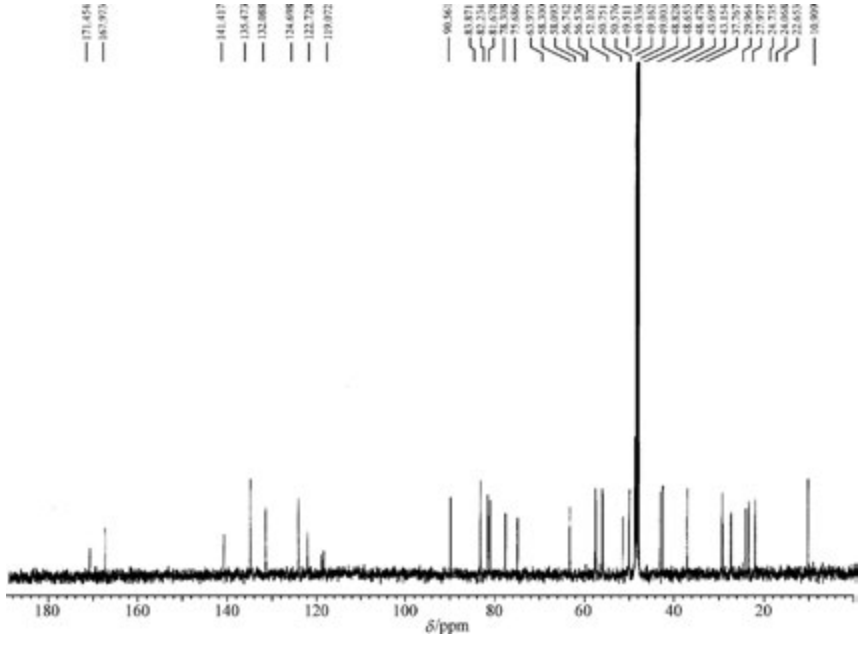


Figure 6.208 The ^{13}C spectrum of the compound

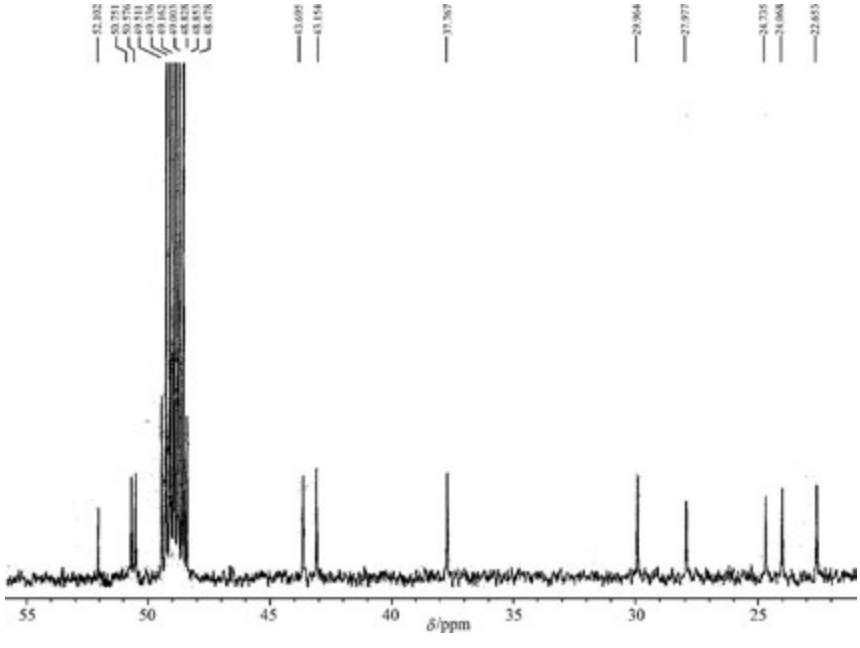


Figure 6.209 The locally enlarged ^{13}C spectrum (1)

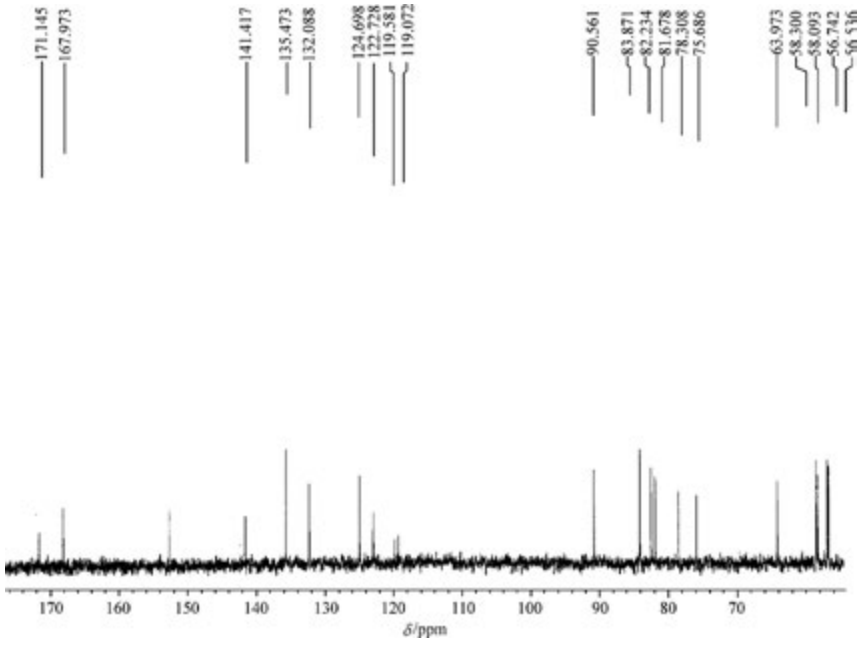


Figure 6.210 The locally enlarged ^{13}C spectrum (2)

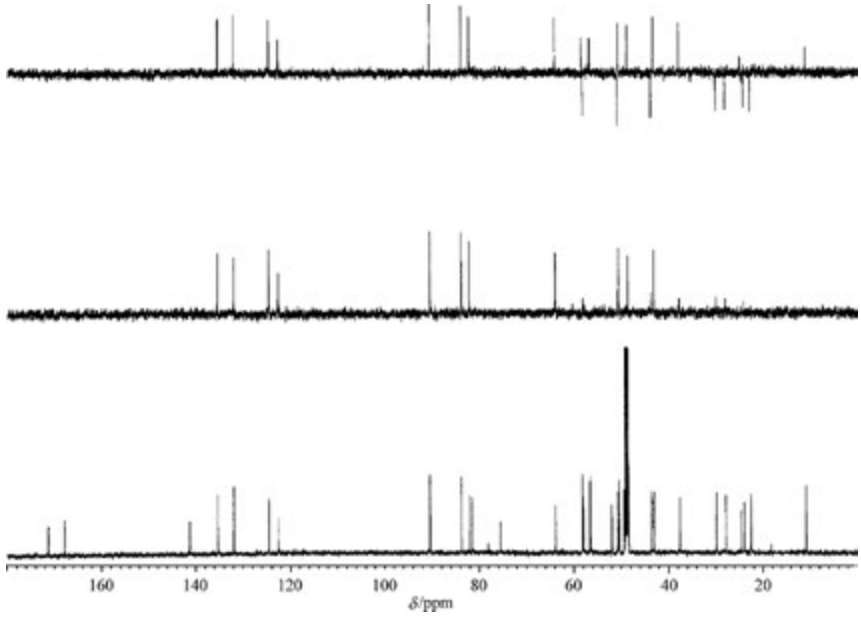


Figure 6.211 The DEPT-135 spectrum of the compound

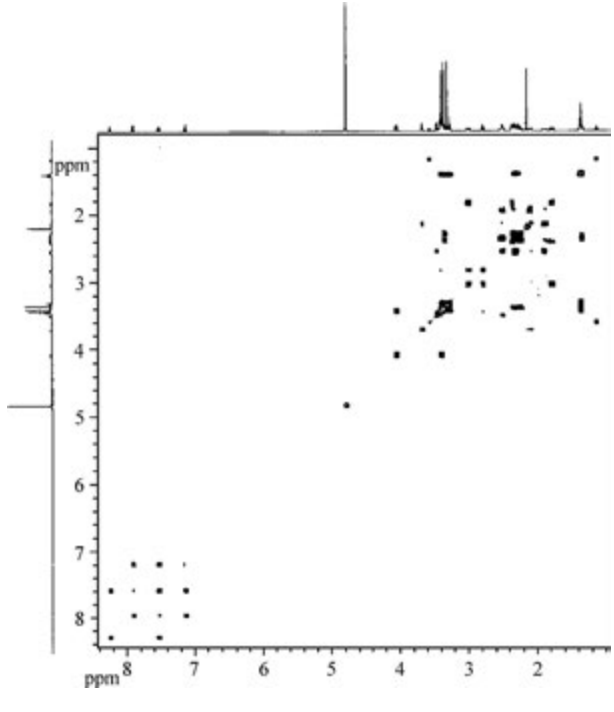


Figure 6.212 The COSY spectrum of the compound

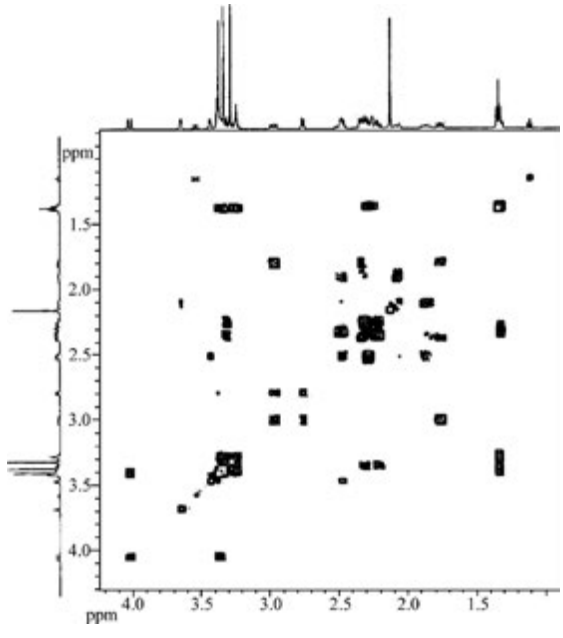


Figure 6.213 The locally enlarged COSY spectrum (1)

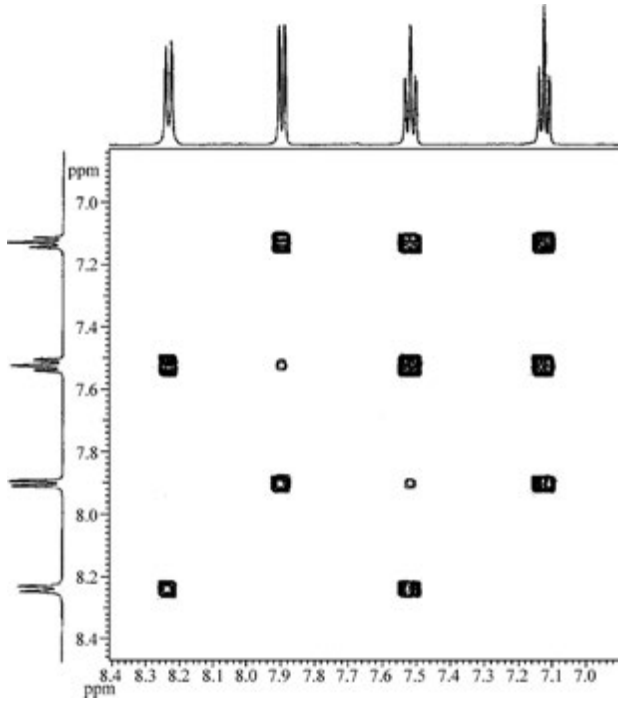


Figure 6.214 The locally enlarged COSY spectrum (2)

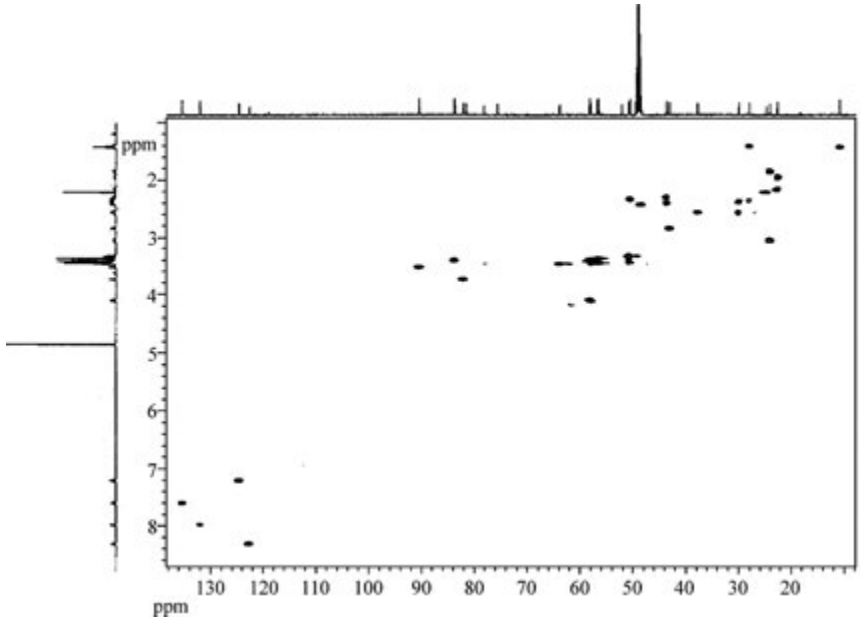


Figure 6.215 The HSQC spectrum of the compound

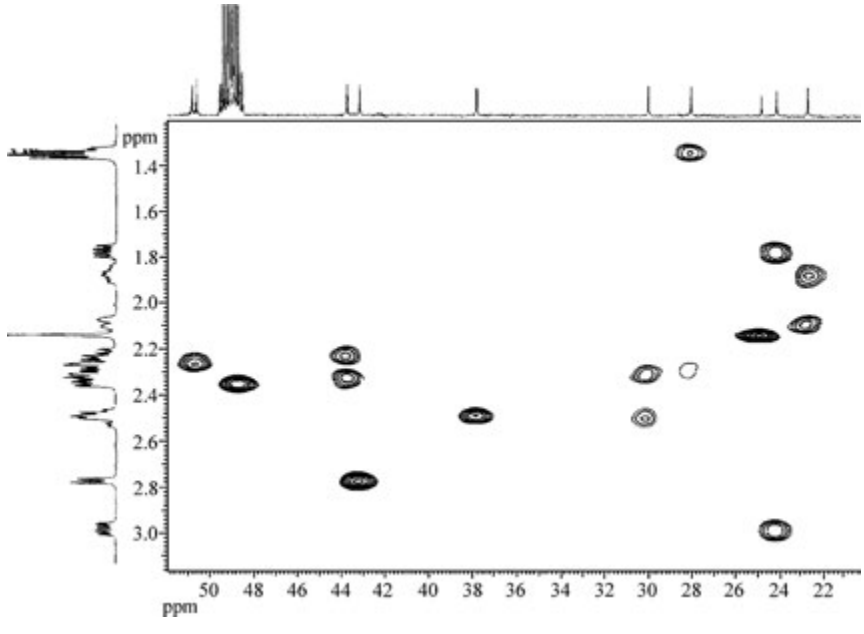


Figure 6.216 The locally enlarged HSQC spectrum (1)

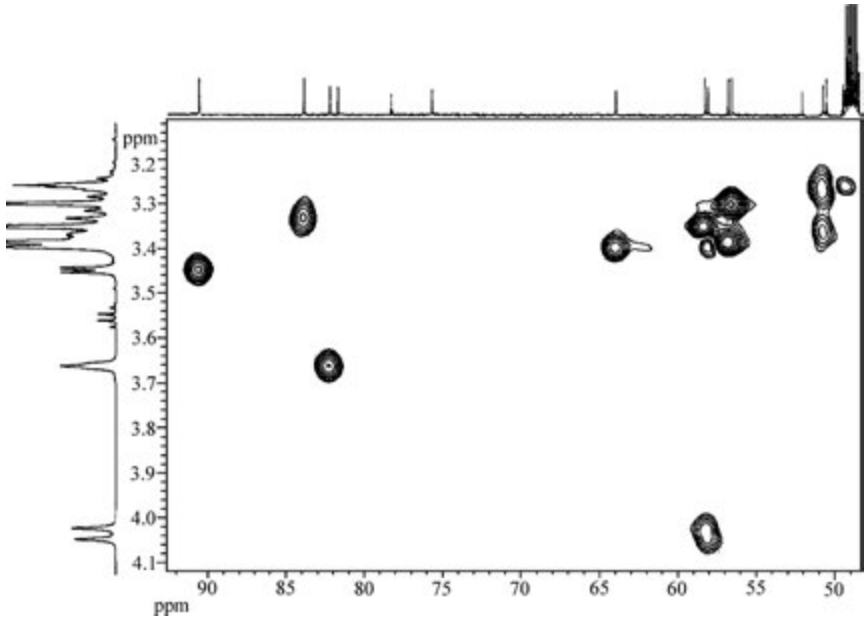


Figure 6.217 The locally enlarged HSQC spectrum (2)

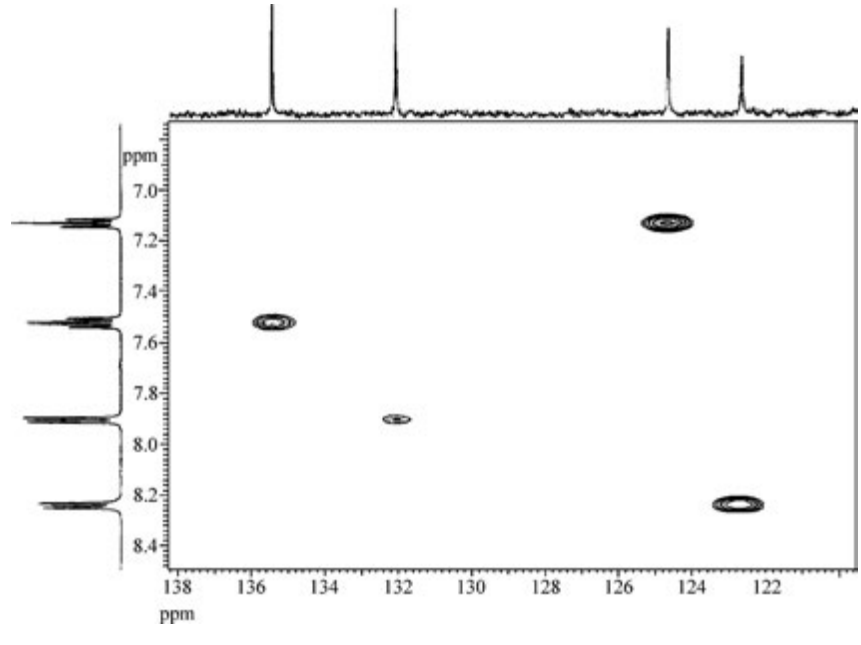


Figure 6.218 The locally enlarged HSQC spectrum (3)

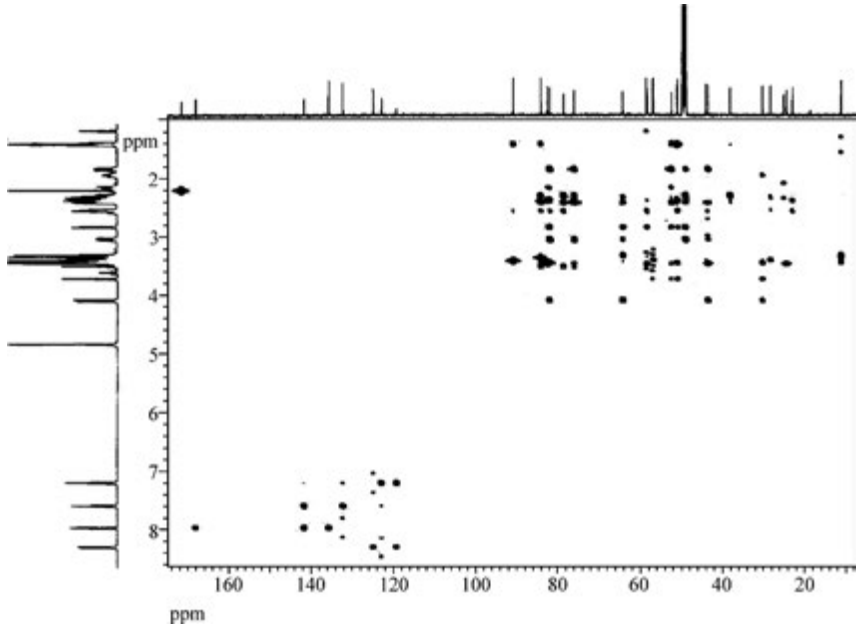


Figure 6.219 The HMBC spectrum of the compound

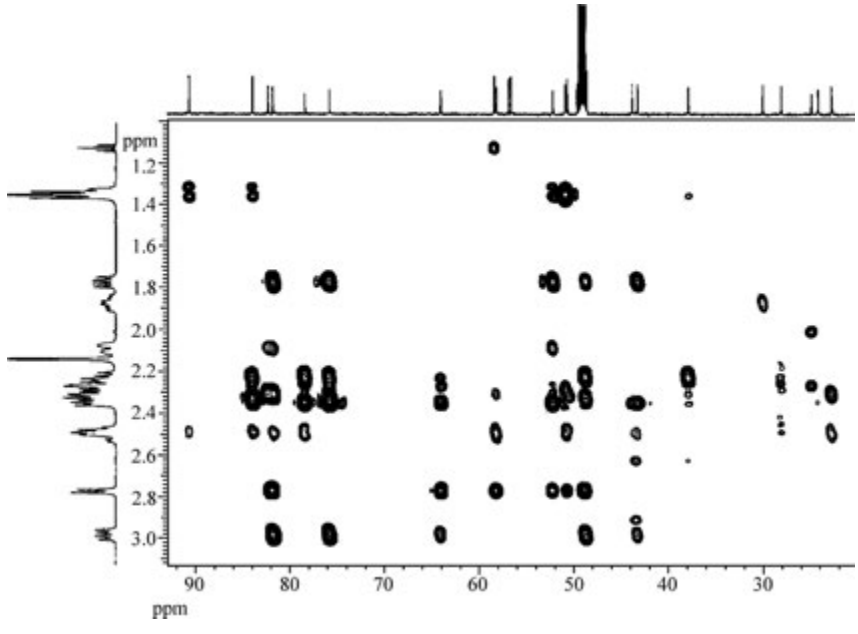


Figure 6.220 The locally enlarged HMBC spectrum (1)

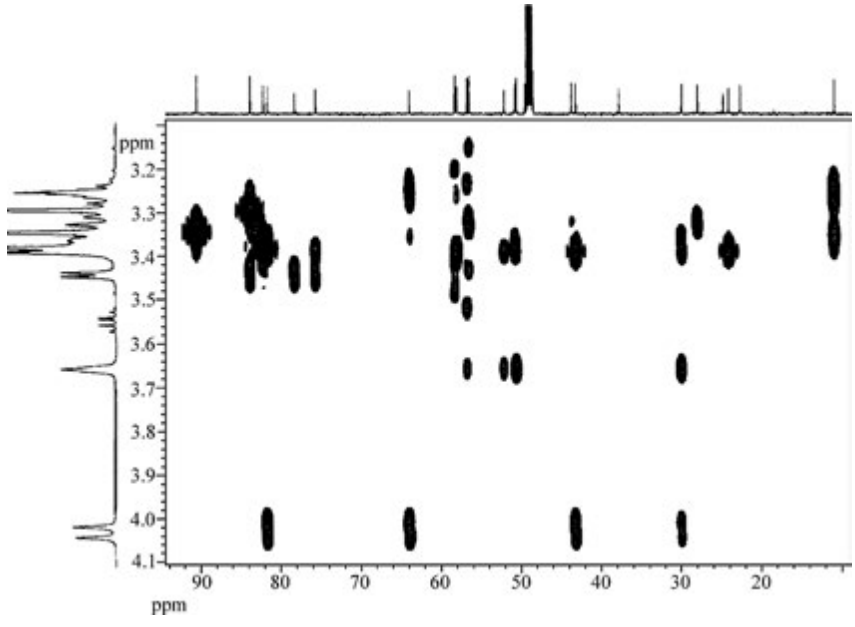


Figure 6.221 The locally enlarged HMBC spectrum (2)

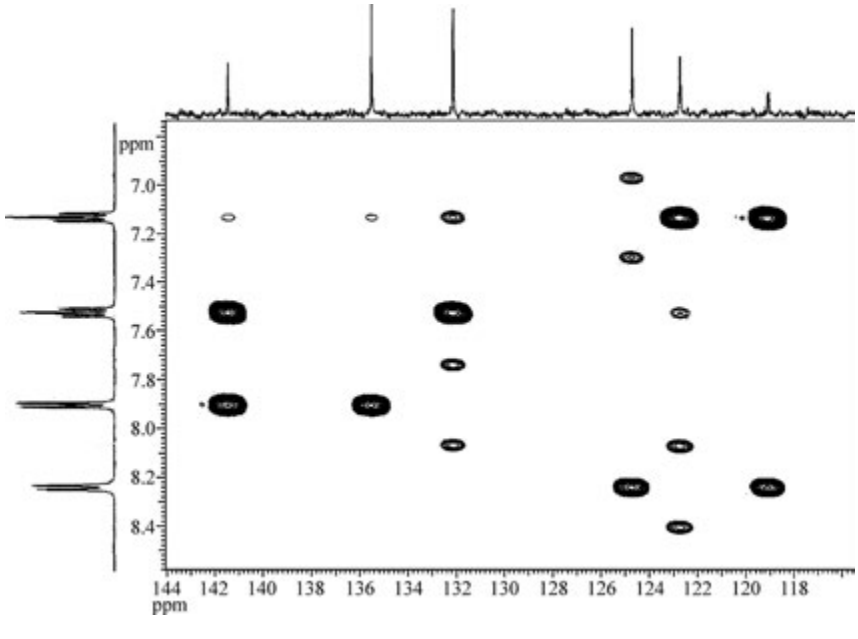


Figure 6.222 The locally enlarged HMBC spectrum (3)

Table 6.68 The generalized data of the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape	Remarks (*J*, Hz)
1.32	1	m	
1.34	3	t	7.0
1.76	1	d, d	8.0, 15.5
1.87	1	m	15.5, and so on.
2.08	1	d, m	15.5, and so on.
2.13	3	s	
2.21	1	m	
2.22	1	m	
2.27	1	m	
2.29	1	m	
2.32	1	m	
2.33	1	m	
2.48	1	m	
2.49	1	m	
2.76	1	d	7.5
2.98	1	d, d	7.5, 15.5
3.25	1	m	
3.29	3	s	
3.31	1	m	
3.34	3	s	
3.36	1	m	
3.38	3	s	
3.39	2	m	
3.44	1	d	4.5
3.65	1	s	
4.02	1	d	10.0
7.12	1	t	8.0
7.52	1	t, d	8.5, 1.5
7.89	1	d, d	8.0, 1.5
8.22	1	d	8.5

From Table 6.68, the hydrogen atom number of the compound is 41, which coincides with the structural formula, because the peaks of the three reactive hydrogen atoms (two OH and one NH) merge in the water peak.

From the HSQC spectrum, it can be known that the overlapped peak sets in the region of 2.18–2.36 ppm come from six H atoms of five peak sets, including three H atoms of three peak sets of three chemically non-equivalent CH_2 (one H atom from each CH_2), two H atoms of one chemically non-equivalent CH_2, and one H atom of one CH. These six chemical shift values can be read from the HSQC spectrum.

The overlapped peak sets in the region of 3.20–3.40 ppm can be interpreted similarly, but with the help of the DEPT spectrum.

Now we will interpret the ^{13}C spectrum.

The peak at about 49.0 ppm is the solvent peak.

At first glance, the ^{13}C spectrum shows 31 peaks with the lack of one peak. However, we can see one upwards peak in the region of the solvent in the DEPT-135 spectrum. Also we

Table 6.69 The attribution of the ^{13}C spectrum and the ^{1}H spectrum

No.	δ_C (ppm)	Order of C atoms	Directly connected H, δ_H (ppm)
1	10.9	CH_3	1.34
2	22.7	CH_2	1.87, 2.08
3	24.1	CH_2	1.76, 2.98
4	24.7	CH_3	2.13
5	27.9	CH_2	1.32, 2.27
6	29.9	CH_2	2.29, 2.49
7	37.8	CH	2.48
8	43.2	CH	2.76
9	43.7	CH_2	2.21, 2.32
10	48.6[a]	CH	2.33
11	50.6	CH	2.22
12	50.8	CH_2	3.25, 3.36
13	52.1	C	
14	56.5	OCH_3	3.29
15	56.7	OCH_3	3.38
16	58.1	CH_2	3.39, 4.02
17	58.3	OCH_3	3.34
18	63.9	CH	3.39
19	75.7	C	
20	78.3	C	
21	81.7	C	
22	82.2	CH	3.65
23	83.9	CH	3.31
24	90.6	CH	3.44
25	119.1	C	
26	122.7	CH	8.22
27	124.7	CH	7.12
28	132.1	CH	7.89
29	135.5	CH	7.52
30	141.4	C	
31	167.9	CO	
32	171.5	CO	

[a] Because the peak at 48.6 ppm is upwards in the DEPT spectrum and it has a correlated peak in the HSQC spectrum, this peak can be differentiated from the solvent peaks.

can see one correlated peak in this position of the HSQC spectrum. Therefore, there is a peak of one CH group.

According to Table 6.68, with the help of the HSQC spectrum and the DEPT spectrum, the order of the carbon atoms and the chemical shift values of the hydrogen atoms which are directly connected with a carbon atom can be determined. The related data are listed in Table 6.69.

The data summarized from the COSY spectrum are listed in Table 6.70.

The data summarized from the HMBC spectrum are listed in Table 6.71.

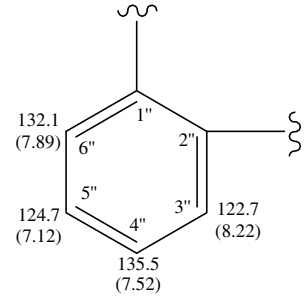
Now we will find structural units of the compound.

From Nos. 25, 23, and 22 of Table 6.70, an ortho-substituted benzene ring can be determined. With the help of the related data of the HSQC spectrum, the following structural unit with its assignment can be obtained as follows:

Table 6.70 The H-H correlations obtained from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H ppm
1	1.32	2.27[a]
2	1.34	3.25, 3.36
3	1.76	2.33, 2.98[a]
4	1.87	2.08[a], (2.29), (2.49)
5	2.08	1.87[a], (2.49), (3.65)
6	2.13	isolated
7	2.21–2.22	2.32[a]
8	2.27–2.29	1.32[a], 1.87
9	2.32–2.33	1.76, 3.31
10	2.48–2.49	1.87, (2.08), 2.29[a], 3.44
11	2.76	2.98, (3.39)
12	2.98	1.76[a], 2.76
13	3.25	
14	3.29	
15	3.31	2.21, 2.32
16	3.34	
17	3.36	
18	3.38–3.39	(2.76), 4.02[a]
19	3.44	2.48
20	3.65	(2.08)
21	4.02	3.39[a]
22	7.12	7.52, 7.89
23	7.52	7.12, (7.89), 8.22
24	7.89	7.12, (7.52)
25	8.22	7.52

Parenthesis () stands for weak couplings.
[a] Represents ^2J couplings, which can also be known from the HSQC spectrum.

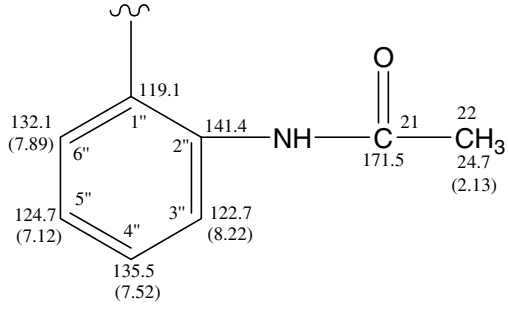


The above structural unit just corresponds to the part marked 6″, 5″, 4″, and 3″ in the anticipated structural formula. According to Nos. 30 and 25 of Table 6.71, that is, the correlations of δ_C (141.4)/δ_H (7.52, 7.89) and δ_C (119.1)/δ_H (7.12, 8.22) ppm, the chemical shift values of the two quaternary carbon atoms at 2″ and 1″ can be assigned. Notice that the correlated peaks across three chemical bonds have rather strong intensities. The assignment also coincides with their chemical environment.

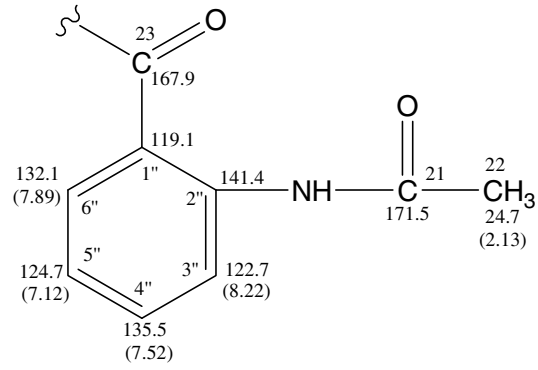
Then according to No. 32 of Table 6.71, that is, δ_C (171.5)/δ_H (2.13) ppm, in which $\delta_H = 2.13$ ppm belongs to a methyl group, the following assignment can be obtained:

Table 6.71 The data summarized from the HMBC spectrum

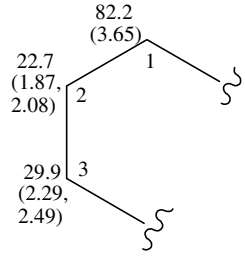
No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	10.9	3.25, 3.36	1.34
2	22.7	2.29, 2.49	
3	24.1	3.39	
4	24.7		2.13
5	27.9	3.31	
6	29.9	1.87, 3.39, 3.65, 4.02	
7	37.8	2.22	
8	43.2	1.76, 2.32, 2.49, 2.98, 3.39, 4.02	
9	43.7		
10	48.6	1.76, 2.21, 2.32, 2.76, 2.98	
11, 12	50.6, 50.8	1.32, (2.32), 2.48, 2.76, 3.39, 3.65	
13	52.1	1.32, 1.76, 2.08, 2.33, 2.76, 3.39, 3.65	
14, 15	56.5, 56.7	3.31, 3.44, (3.65)	
16, 17	58.1, 58.3	2.49, 2.76	3.39
18	63.9	2.33, 2.76, 2.98, 3.25, 4.02	
19	75.7	1.76, 2.21, 2.32, 2.98, 3.39, 3.44	
20	78.3	2.21, 2.32, 2.48, 3.44	
21	81.7	1.76, 2.08, 2.29, 2.49, 2.76, 2.98, 4.02	
22	82.2	2.29, 3.38	
23	83.9	1.32, 2.21, 2.32, 2.48, 3.29, 3.44	
24	90.6	1.32, (2.48), 3.34	
25	119.1	7.12, 8.22	
26	122.7	7.12, (7.52)	8.22
27	124.7	8.22	7.12
28	132.1	(7.12), 7.52,	7.89
29	135.5	(7.12), 7.89	
30	141.4	(7.12), 7.52, 7.89	
31	167.9	7.89	
32	171.5	2.13	



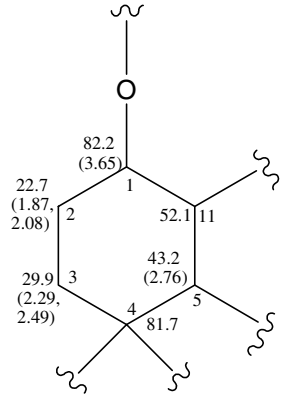
According to Nos. 31 of Table 6.71, that is, the correlation of δ_C (167.9)/δ_H (7.89) ppm, the assignment can be extended as follows:



From Nos. 20, 5, and 4 of Table 6.70, according to Nos. 22, 2, and 6 of Table 6.71, together with the related data of the HSQC spectrum, we can see the following structural unit:

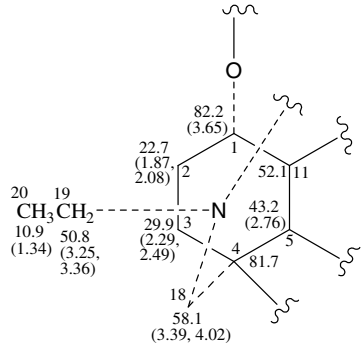


According to Nos. 13, 8, and 21 of Table 6.71, that is, the correlations of δ_C (52.1)/δ_H (2.08, 3.65), δ_C (43.2)/δ_H (2.49), and δ_C (81.7)/δ_H (2.08, 2.29, 2.49) ppm, together with the related data of the HSQC spectrum, the above assignment can be extended as follows:



The connection between the CH (δ_C, 82.2, $\delta_H = 3.65$ ppm) and an oxygen atom is determined by its chemical shift values.

Similarly, according to No. 1 of Table 6.70, that is, the correlations of δ_H (ppm) 1.34/3.25, 3.36 ppm, and according to Nos. 6, 21 and 12 of Table 6.71, that is, the correlations of δ_C (29.9)/δ_H (3.39, 3.65, 4.02), δ_C (81.7)/δ_H (4.02), and δ_C (50.8)/δ_H (3.39) ppm, together with the related data of the HSQC spectrum, the above assignment can be extended as follows:



From the three singlets corresponding to three hydrogen atoms, respectively, in the ^{1}H spectrum, together with the related data of the HSQC spectrum, their chemical shift values can be determined as follows:

δ_C, 56.5, $\delta_H = 3.29$ ppm; δ_C, 58.3, $\delta_H = 3.34$ ppm; δ_C, 56.7, $\delta_H = 3.38$ ppm.

The above discussions about structural units are definite. However, the other parts of the possible structure are difficult to assign by the method we use commonly. The difficulties come from the following facts:

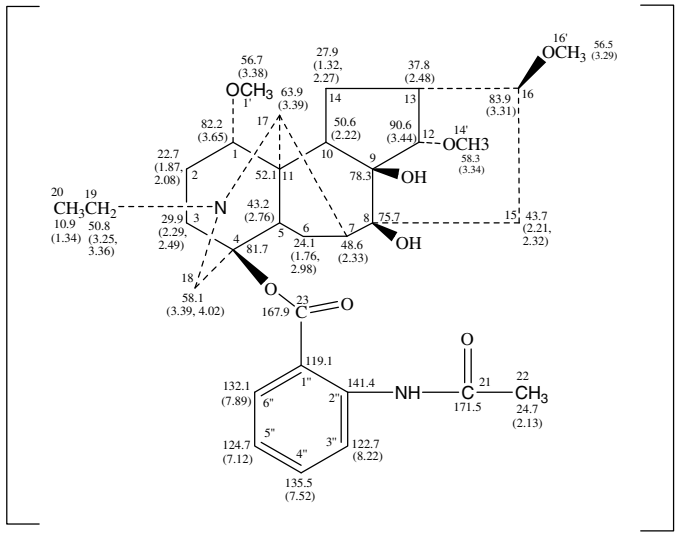
The structure is complicated. It contains several aliphatic rings.

Many peak sets overlap in the ^{1}H spectrum, which leads to the difficulty in interpreting the COSY spectrum.

Many peaks are close in the ^{13}C spectrum. They are difficult to resolve.

To sum up, we can not assign the spectra step by step as we did in the deduction of an unknown structure for other examples.

After careful consideration from several aspects, we accomplished the following assignments. Objectively speaking, we can confirm the rationality of the result about the structure only through the coincidence between the final assignments and the NMR spectra.

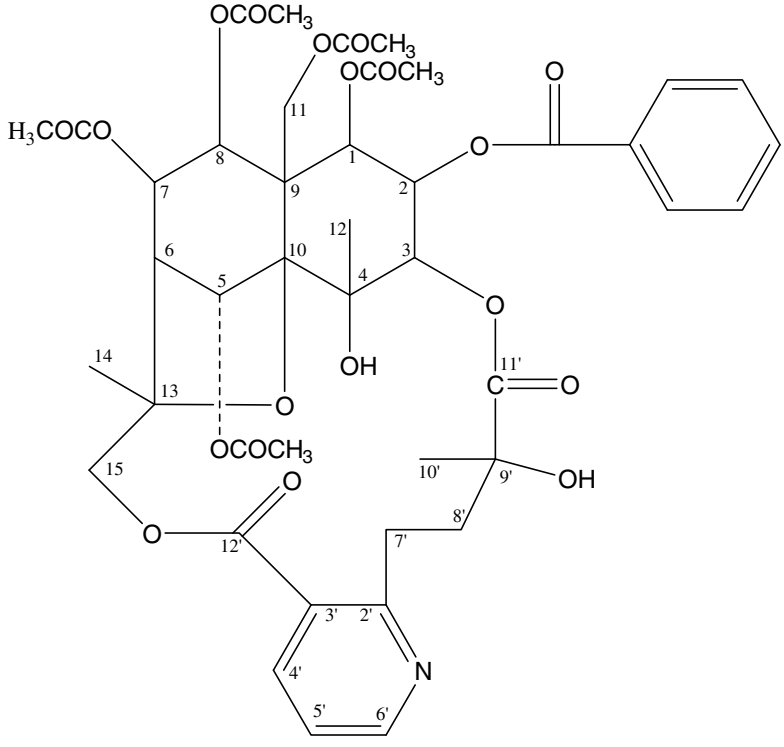


HBr H_2O

C6-19

The above assignments coincide with its NMR spectra. Especially, the long-range couplings in the HMBC spectrum can be explained reasonably.

Example 6.19 A natural product is extracted, separated, and purified from a certain plant. From the measurement of its accurate mass, its molecular formula of $C_{43}H_{49}O_{19}N$ is determined. By comparison with the NMR spectra of the compounds in the same category, a possible structure is proposed below:



C6-20

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its HSQC spectrum, the locally enlarged HSQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.223–6.241. Try to interpret all NMR spectra to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 400 MHz. The solvent used is deuterated $CDCl_3$.

Solution

We now start from the interpretation of the ^{1}H spectrum.

The ^{1}H spectrum shows clearly eight sharp singlets. The three singlets at the right edge of the ^{1}H spectrum should be three isolated methyl groups according to their chemical shift values and their peak area. The other five singlets should be five methyl groups of five acetyl groups according to their chemical shift values and their peak area.

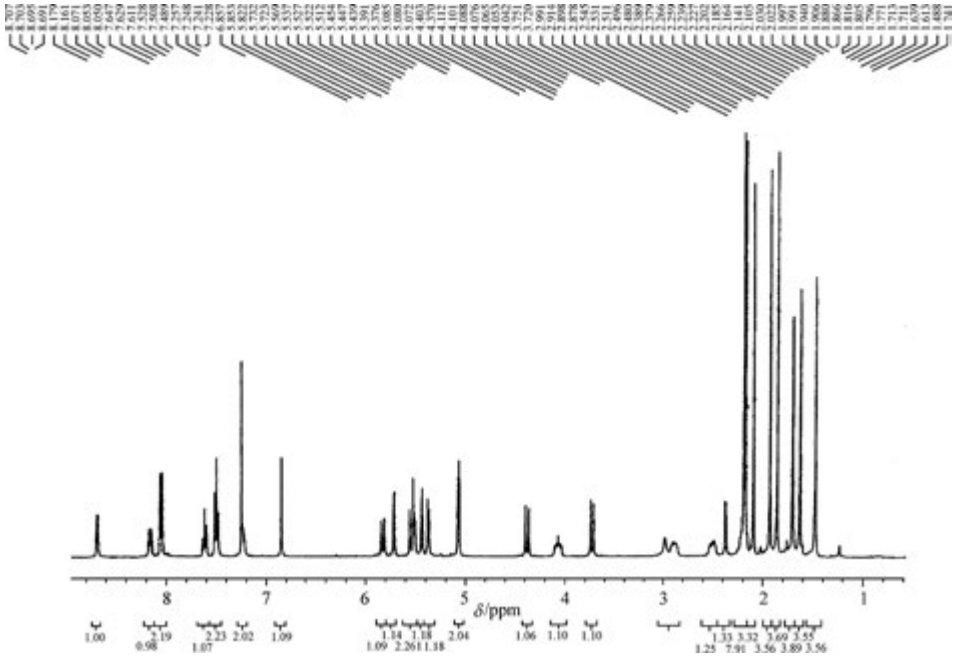


Figure 6.223 The ^{1}H spectrum of the compound

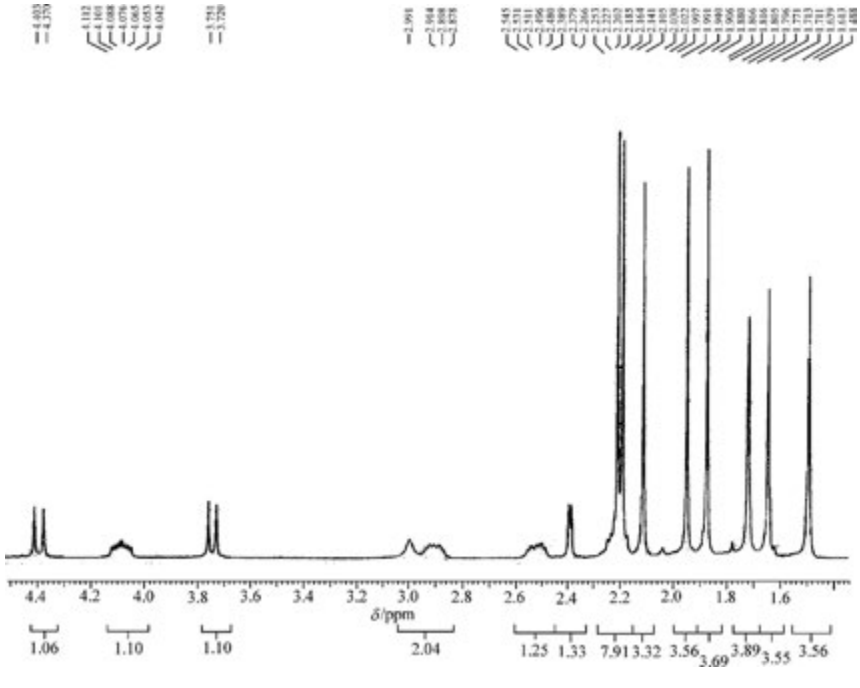


Figure 6.224 The locally enlarged ^{1}H spectrum (1)

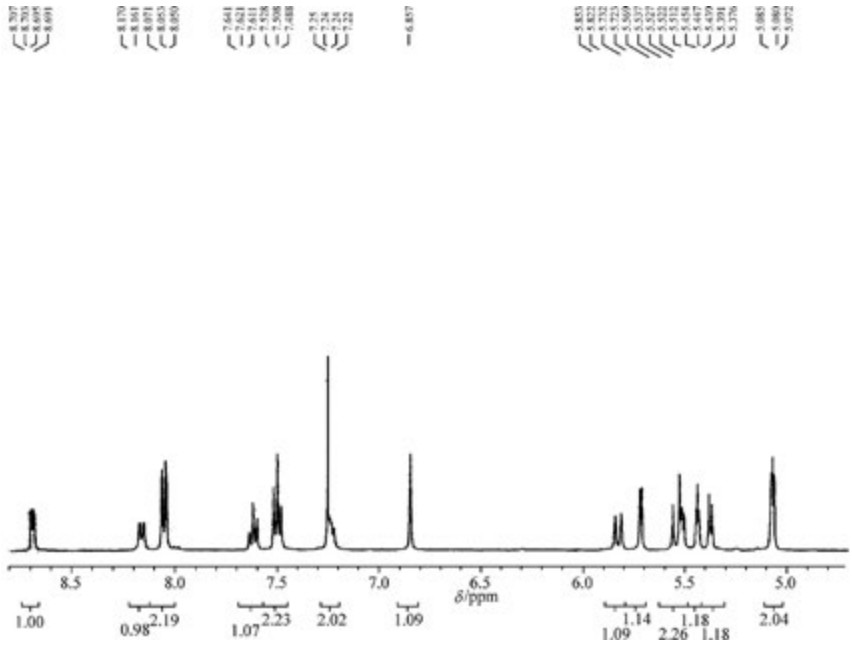


Figure 6.225 The locally enlarged ^{1}H spectrum (2)

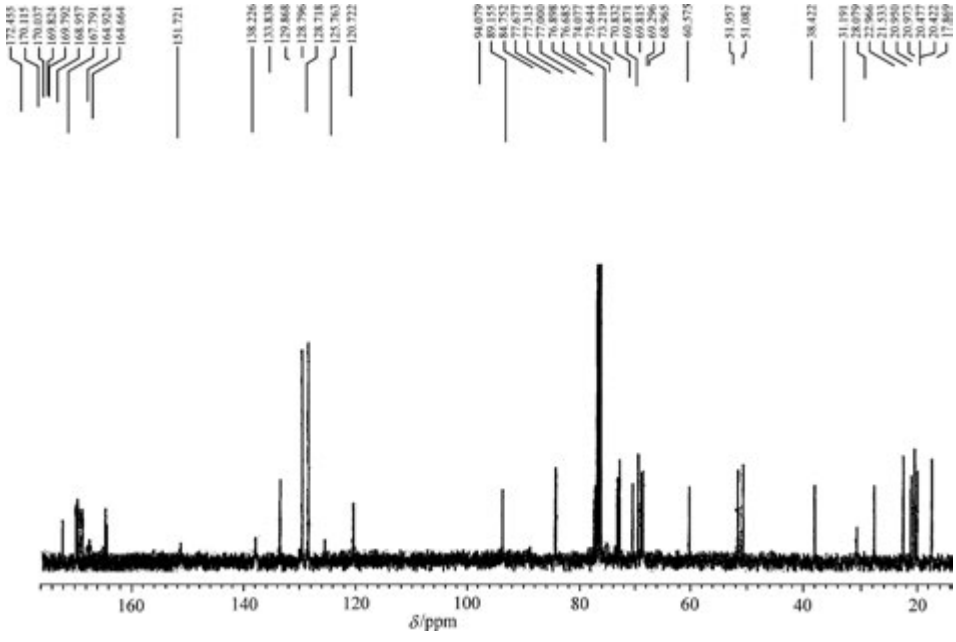


Figure 6.226 The ^{13}C spectrum of the compound

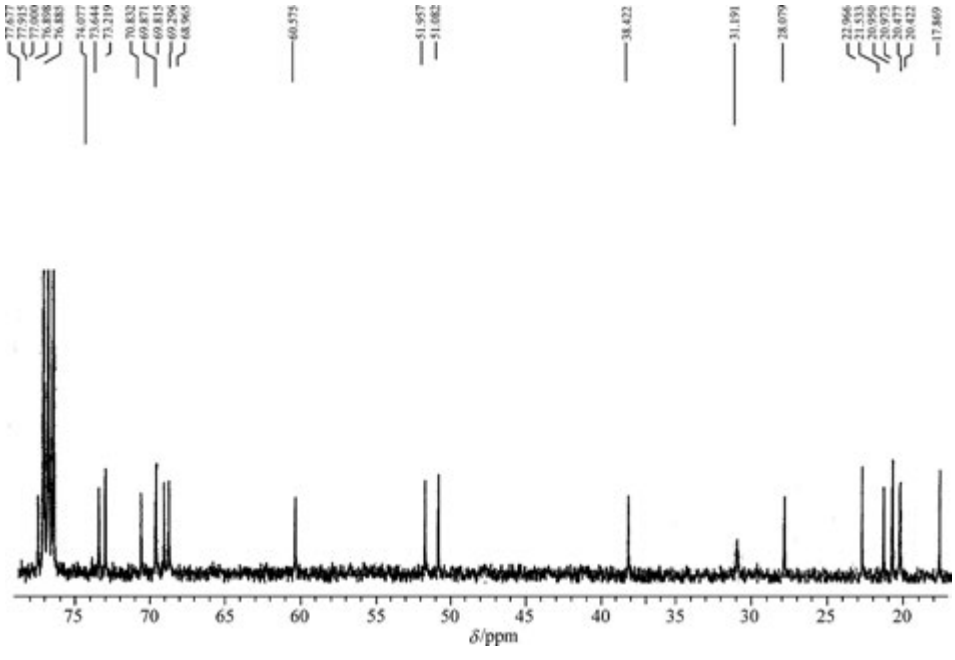


Figure 6.227 The locally enlarged ^{13}C spectrum

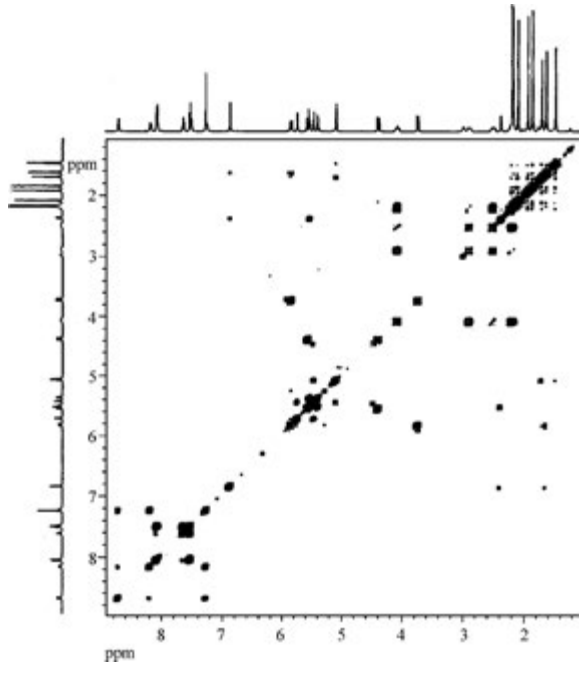


Figure 6.228 The COSY spectrum of the compound

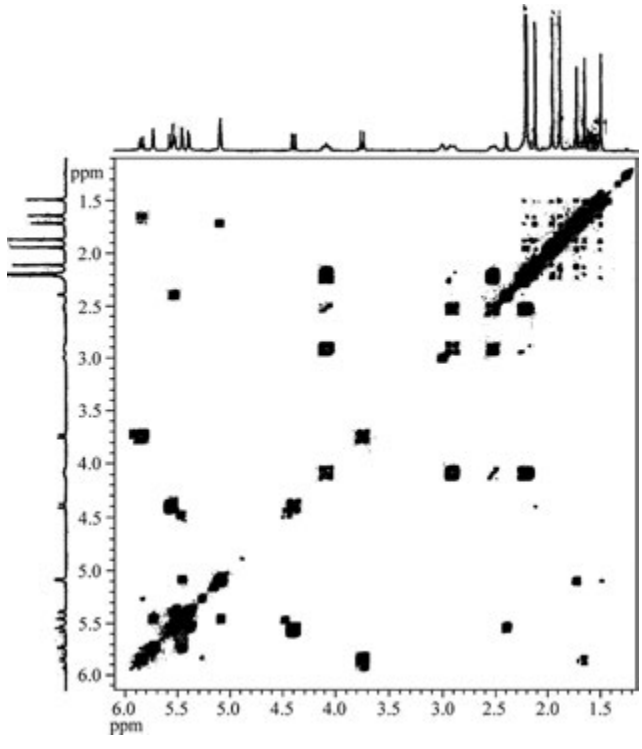


Figure 6.229 The locally enlarged COSY spectrum

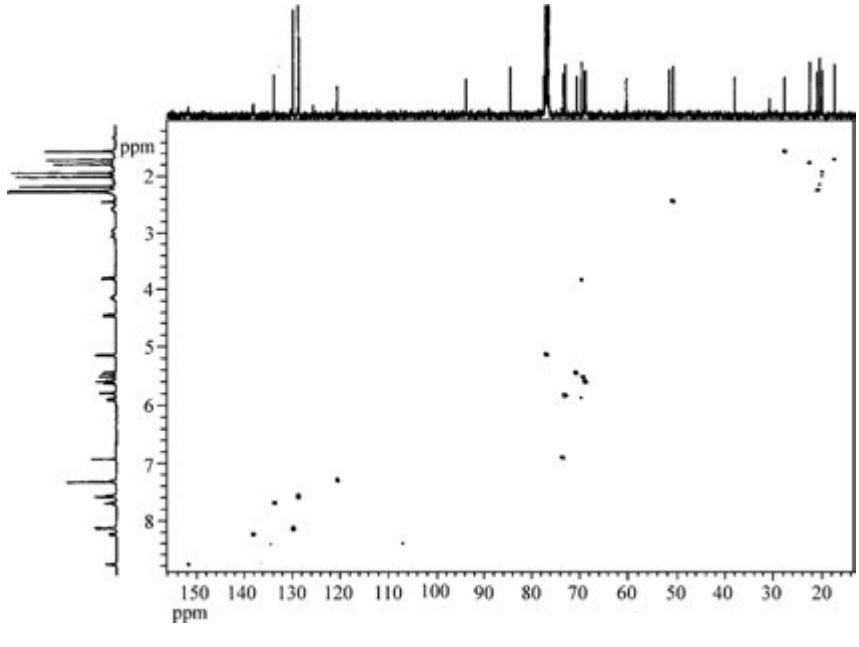


Figure 6.230 The HSQC spectrum of the compound

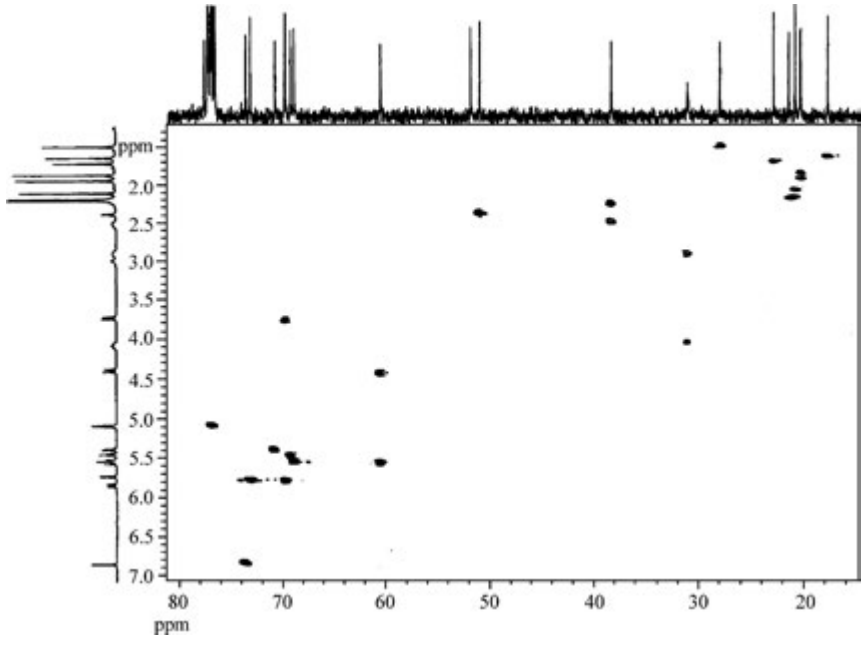


Figure 6.231 The locally enlarged HSQC spectrum (1)

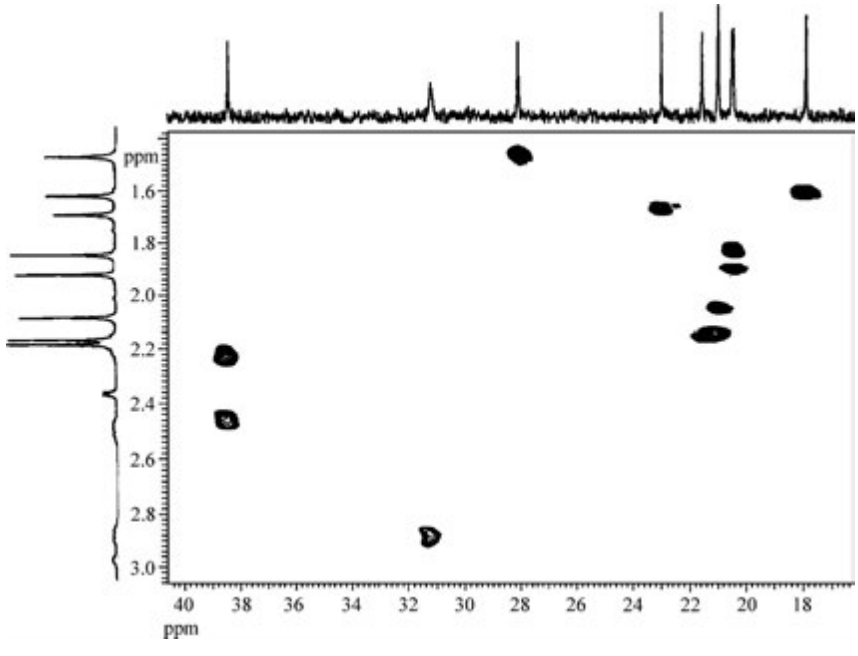


Figure 6.232 The locally enlarged HSQC spectrum (2)

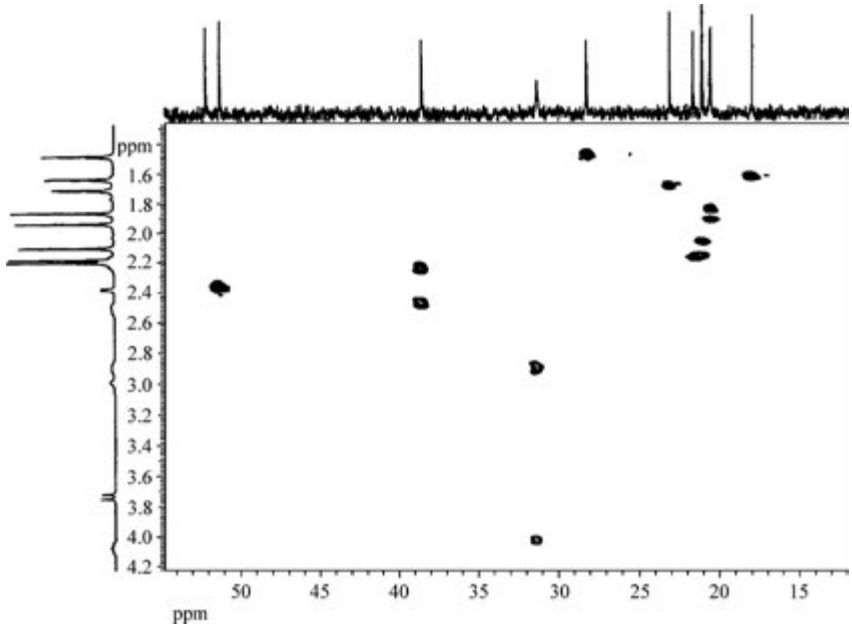


Figure 6.233 The locally enlarged HSQC spectrum (3)

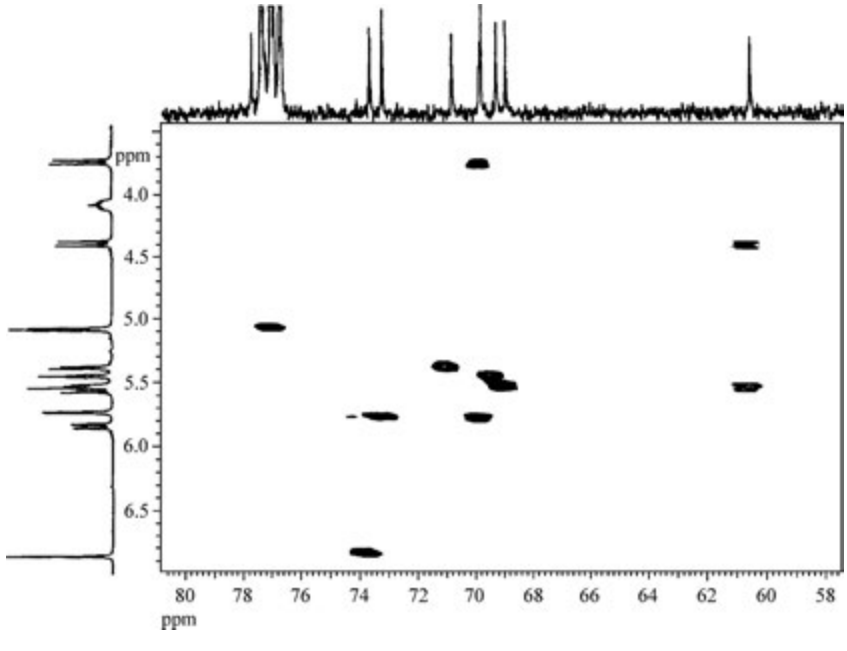


Figure 6.234 The locally enlarged HSQC spectrum (4)

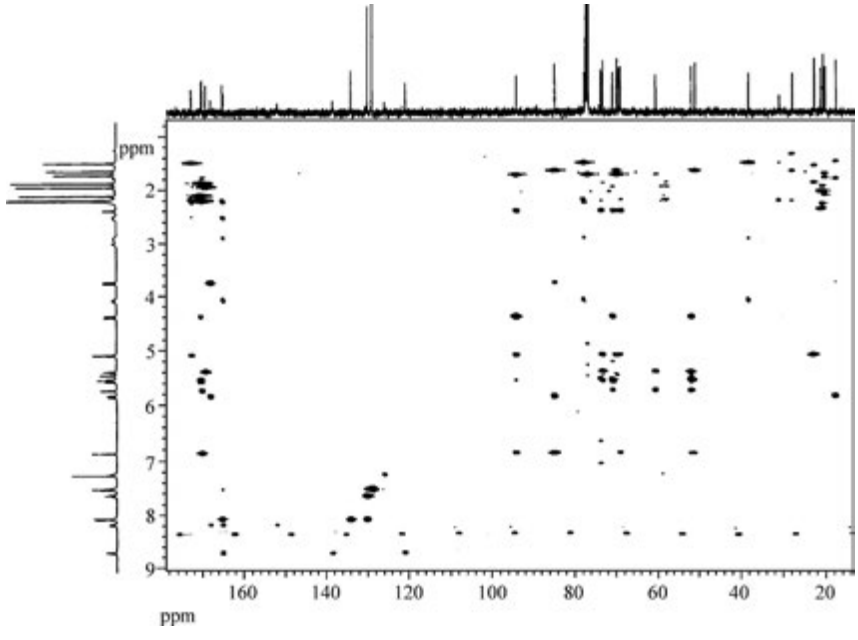


Figure 6.235 The HMBC spectrum of the compound

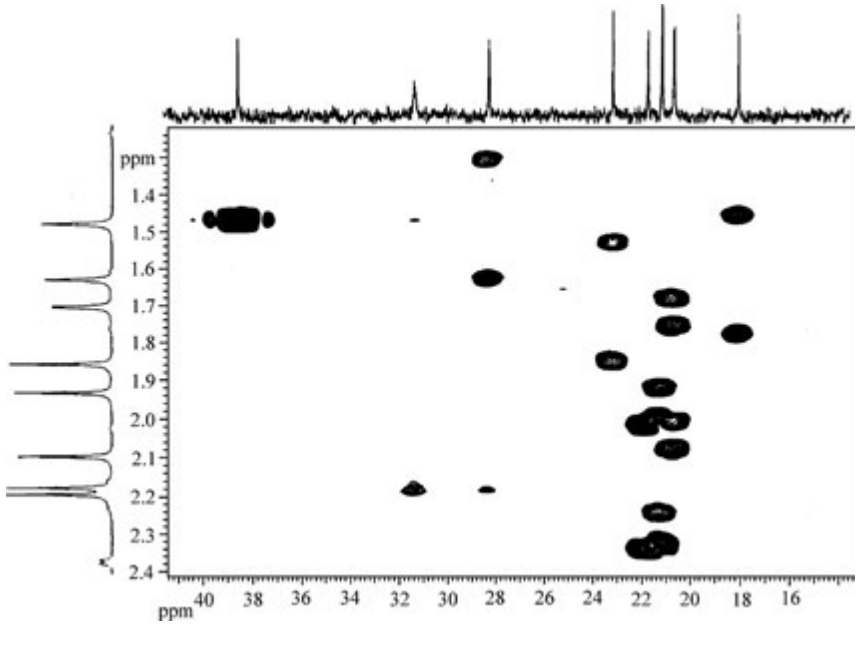


Figure 6.236 The locally enlarged HMBC spectrum (1)

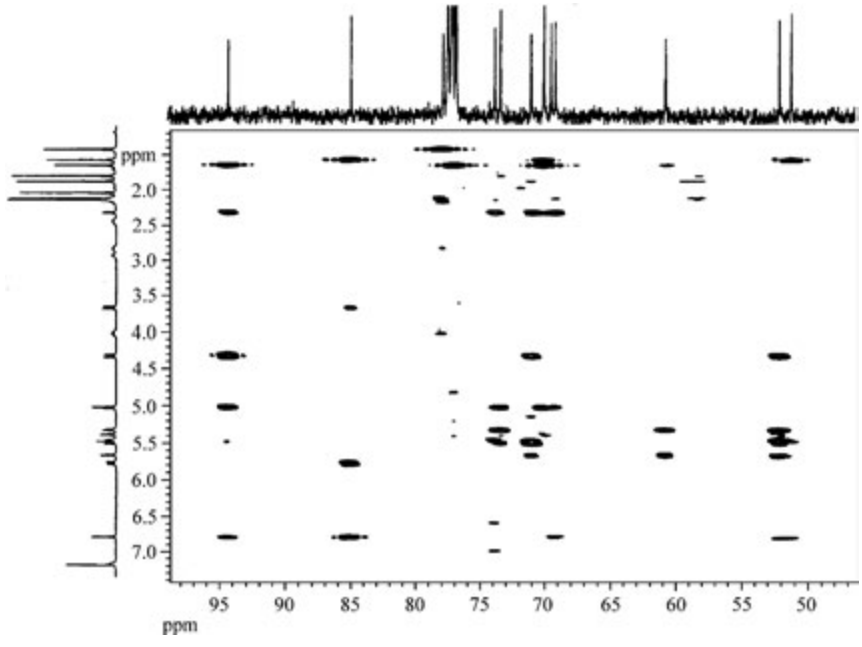


Figure 6.237 The locally enlarged HMBC spectrum (2)

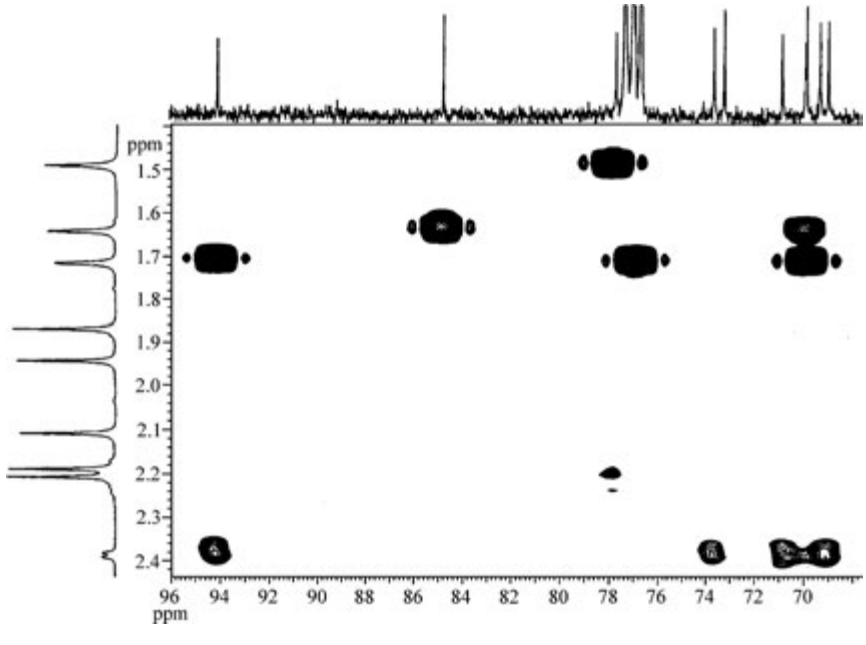


Figure 6.238 The locally enlarged HMBC spectrum (3)

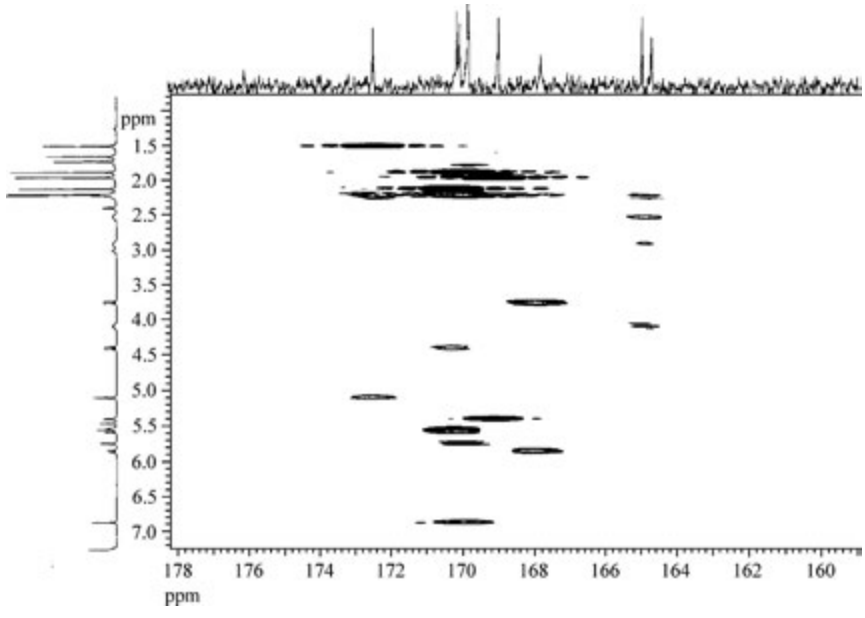


Figure 6.239 The locally enlarged HMBC spectrum (4)

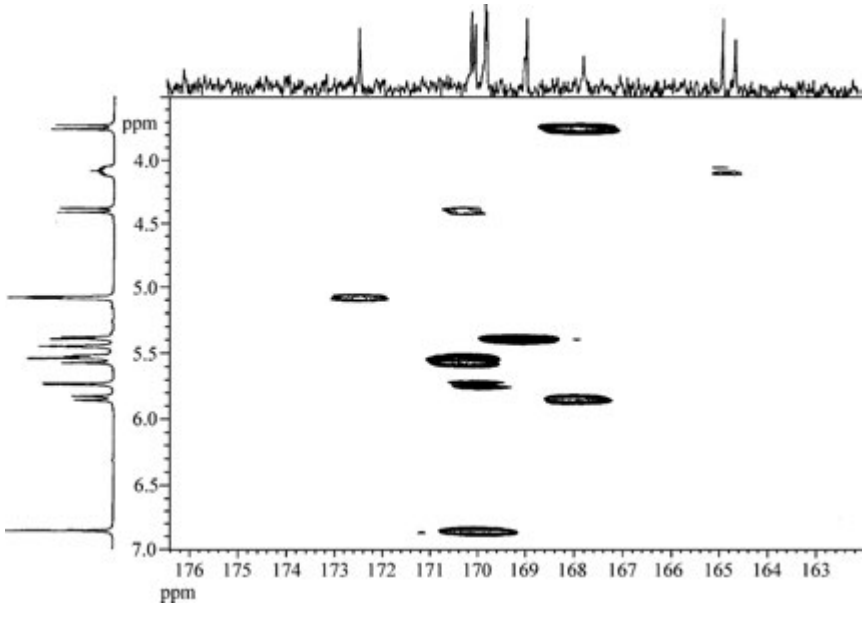


Figure 6.240 The locally enlarged HMBC spectrum (5)

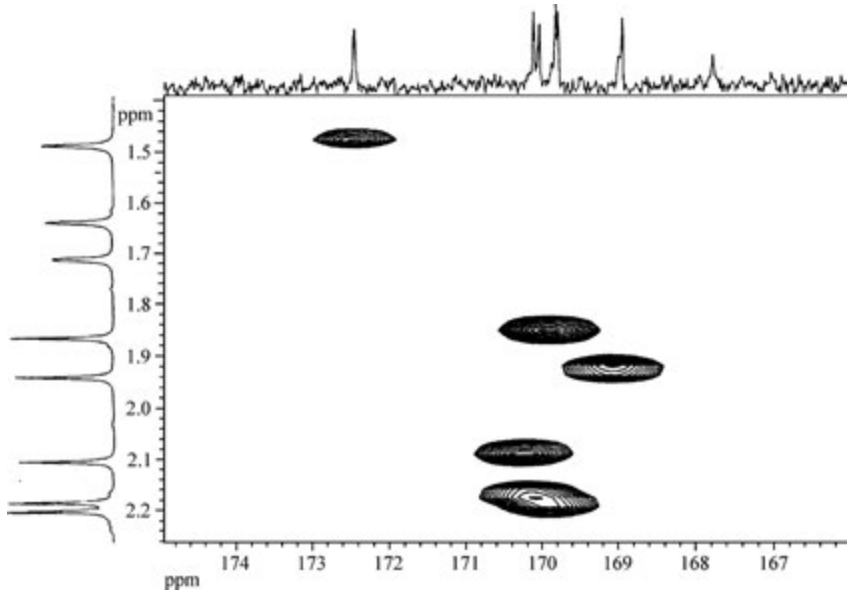


Figure 6.241 The locally enlarged HMBC spectrum (6)

Because of overlapped peak sets in the ^{1}H spectrum, the interpretation of the spectrum is done with the help of the HSQC spectrum. The related data are listed in Table 6.72.

Although 2D NMR spectra can help the interpretation of the ^{1}H spectrum, the analysis of peak shapes is still an important method for the interpretation. For example, the peak sets at about 5.53 ppm correspond to two hydrogen atoms, which have close correlated peaks in the HSQC spectrum. A doublet, from which a coupling constant can be calculated as 12.8 Hz, can be found in the region of the ^{1}H spectrum. Another doublet at 4.39 ppm, from which a coupling constant can be calculated as 12.4 Hz, can be found. Although these two values are not the same, their difference is tolerable. Therefore, the analysis of peak shapes can help the interpretation of the HSQC spectrum to distinguish the overlapped peak sets in the ^{1}H spectrum. Finally, we can determine the overlapped peak sets at about 5.53 ppm which come from one hydrogen atom of a CH_2, and one hydrogen atom of a CH, which is known from the HSQC spectrum.

By the combination of the ^{1}H spectrum, the ^{13}C spectrum, and the HSQC spectrum, the order of carbon atoms, the number of carbon atoms, and δ_H of the hydrogen atoms, which are connected directly with a carbon atom, can be determined. The data are listed in Table 6.73.

Now we will interpret the COSY spectrum. The related data are listed in Table 6.74.

Now we will interpret the HMBC spectrum. It should be noted that the spots on a horizontal line at about $F_1 \cong 8.36$ ppm are artificial peaks.

The data summarized from the HMBC spectrum are listed in Table 6.75.

The above four tables contain the information on all NMR spectra.

Now we will find the structural units of this compound. We will start from the COSY spectrum, and then use the HMBC spectrum.

Table 6.72 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Number of H atoms	Peak shape	Remarks (^3J, Hz)
1.49	3	s	
1.64	3	s	
1.71	3	s	
1.87	3	s	
1.94	3	s	
2.11	3	s	
2.19	3	s	
2.20	3	s	
2.22	1	m	
2.38	1	d	$^3J = 4$
2.51	1	m	$^3J = 6.4$
2.89	1	m	$^3J = 6.4$
2.99	1	s, blunt	[a]
3.74	1	d	$^3J = 12.4$
4.08	1	m	$^3J = 4.4$
4.39	1	d	$^3J = 13.2$
5.08	2		[b]
5.38	1	d	$^3J = 6$
5.45	1	m	$^3J = 3.2$
5.52	1	m	
5.54	1	d	12.8
5.72	1	d	$^3J = 3.6$
5.84	1	d	$^3J = 12.4$
6.86	1	s	
7.24	1	m	Besides the solvent peak
7.51	2	t	$J = 8$
7.62	1	t	$J = 8$
8.06	2	d	$J = 8$
8.16	1	d	$J = 8$
8.70	1	d	$J = 4.8$

[a] The peak has no correlated peaks in the HSQC spectrum. It should be the peak of a hydroxyl group.
[b] Indicates that the two hydrogen atoms have just one correlated peak in the HSQC spectrum. Therefore, one of them should be the peak of a hydroxyl peak.

From Nos. 18, 20, and 10 of Table 6.74, that is, the correlations of δ_H (ppm) 5.38/5.52/2.38/6.86, together with the related data of the HSQC spectrum, the following structural unit can be assigned:

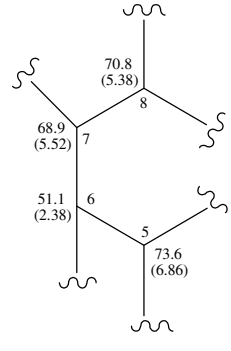


Table 6.73 The attribution of the data of the ^{1}H spectrum and the ^{13}C spectrum

No.	δ_C (ppm)	Order of C atoms	Number of C atoms	Directly connected H, δ_H (ppm)
1	17.9	CH_3	1	1.64
2	20.4	CH_3	1	1.94
3	20.5	CH_3	1	1.87
4	20.9	CH_3	1	2.11
5	20.9	CH_3	1	2.19
6	21.5	CH_3	1	2.20
7	22.9	CH_3	1	1.71
8	28.1	CH_3	1	1.49
9	31.2	CH_2	1	2.89, 4.08
10	38.4	CH_2	1	2.22, 2.51
11	51.1	CH	1	2.38
12	51.9	C		
13	60.6	CH_2	1	4.39, 5.54
14	68.9	CH	1	5.52
15	69.3	CH	1	5.45
16	69.8	CH_2	1	3.74, 5.84
17	69.9	C	1	
18	70.8	CH	1	5.38
19	73.2	CH	1	5.72
20	73.6	CH	1	6.86
21	76.9	CH	1	5.08
22	77.7	C	1	
23	84.8	C	1	
24	94.1	C	1	
25	120.7	CH	1	7.24
26	125.8	C	1	
27	128.7	CH	2	7.51
28	128.8	C	1	
29	129.9	CH	2	8.06
30	133.8	CH	1	7.62
31	138.2	CH	1	8.16
32	151.7	CH	1	8.70
33	164.7	C	1	
34	164.9	C	1	
35	167.8	C	1	
36	168.9	C	1	
37	169.7	C	1	
38	169.8	C	1	
39	170.0	C	1	
40	170.1	C	1	
41	172.5	C	1	

According to Nos. 12 and 14 of Table 6.75, that is, the correlations of δ_C (51.9)/δ_H (5.38, 5.52), and δ_C (94.1)/δ_H (2.38, 6.86) ppm, together with the related data of the HSQC spectrum, the assignment of the above structural unit can be extended as follows:

Table 6.74 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)
1	1.49	
2	1.64	
3	1.71	
4	1.87	
5	1.94	
6	2.11	
7	2.19	
8	2.20	
9	2.22	2.51, (2.89), 4.08
10	2.38	5.52, 6.86
11	2.51	2.22, 2.89, (4.08)
12	2.89	(2.22), 2.51, 4.08
13	2.99	
14	3.74	5.84
15	4.08	2.22, (2.51), 2.89
16	4.39	5.54
17	5.08	5.45
18	5.38	5.52
19	5.45	5.08, 5.72
20	5.52	2.38, 5.38
21	5.54	4.39
22	5.72	5.45
23	5.84	3.74
24	6.86	2.38
25	7.24	8.16, 8.70
26	7.51	7.62, 8.06
27	7.62	7.51, (8.06)
28	8.06	7.51, (7.62)
29	8.16	7.24, (8.70)
30	8.70	7.24, (8.16)

Parenthesis () stands for weak couplings.

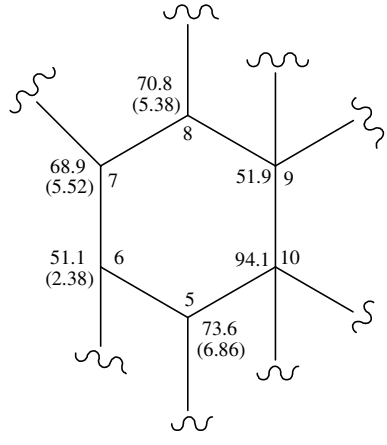
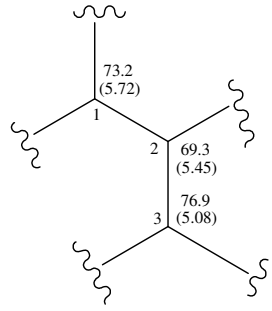


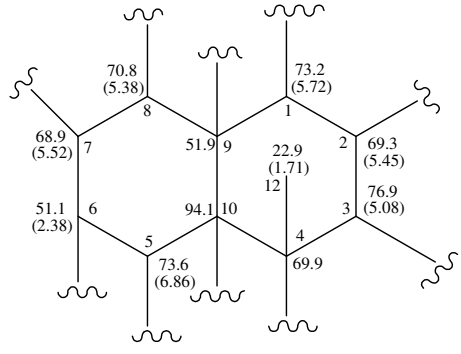
Table 6.75 The data summarized from the HMBC spectrum

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)
1	17.9	(3.74), 5.84	1.64
2	20.4		1.94
3	20.5		1.87
4	20.9		2.11
5	20.9		2.19
6	21.5		2.20
7	22.9	5.08	1.71
8	28.1	(2.22)	1.49
9	31.2	(1.49), 2.22	
10	38.4	1.49	
11	51.1	1.64, 5.52	
12	51.9	4.39, 5.38, 5.52, 5.72, 6.86	
13	60.6	5.38, 5.72	
14	68.9	2.38, 6.86	
15	69.3	5.08	
16, 17	69.8, 69.9	1.64, 1.71, 5.08	
18	70.8	2.38, 4.39, 5.52, 5.54, 5.72	
19	73.2	5.08, 5.38	
20	73.6	2.38, 5.08, 5.38, 5.52	6.86
21	76.9	1.71	
22	77.7	1.49, 2.22, (2.89), (4.08)	
23	84.8	1.64, 3.74, 5.84, 6.86	
24	94.1	1.71, 2.38, 4.39, 5.08, 6.86	
25	120.7	8.70	
26	125.8	7.24	
27	128.7		
28	128.8		
29	129.9		
30	133.8	8.06	
31	138.2	8.70	
32	151.7		
33	164.7	8.06	
34	164.9	2.22, 2.51, 2.89, 4.08, 7.24, 8.16, 8.70	
35	167.8	3.74, 5.84, 8.16	
36	168.9	1.94, 5.38	
37	169.7	2.20, 6.86	
38	169.8	1.87, 5.72	
39	170.0	2.19, 5.52	
40	170.1	2.11, 5.54	
41	172.5	1.49, 5.08	

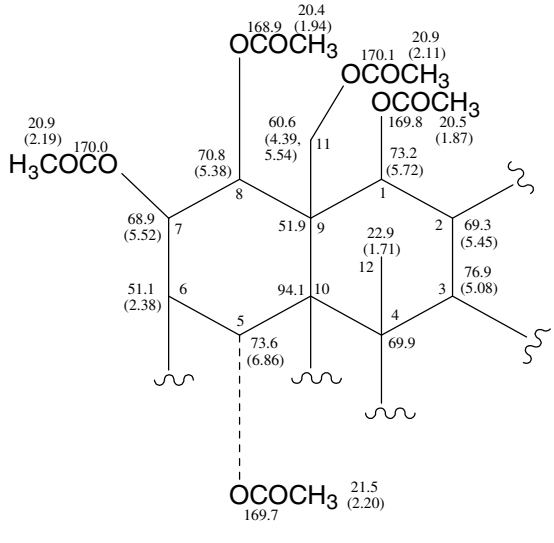
From Nos. 22 and 19 of Table 6.74, that is, the correlations of δ_H (ppm) 5.72/5.45/5.08, together with the related data of the HSQC spectrum, the following structural unit can be assigned:



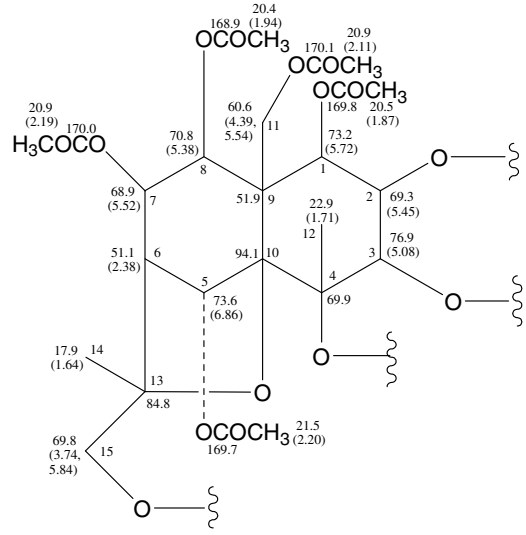
According to Nos. 12, 17, 19, 24, and 7 of Table 6.75, that is, the correlations of δ_C (51.9)/δ_H (5.72), δ_C (69.9)/δ_H (1.71), δ_C (73.2)/δ_H (5.08, 5.38), δ_C (94.1)/δ_H (1.71, 5.08), and δ_C (22.9)/δ_H (5.08) ppm, together with the related data of the HSQC spectrum, the above two assigned structural units can be connected and the assignment can be extended as follows:



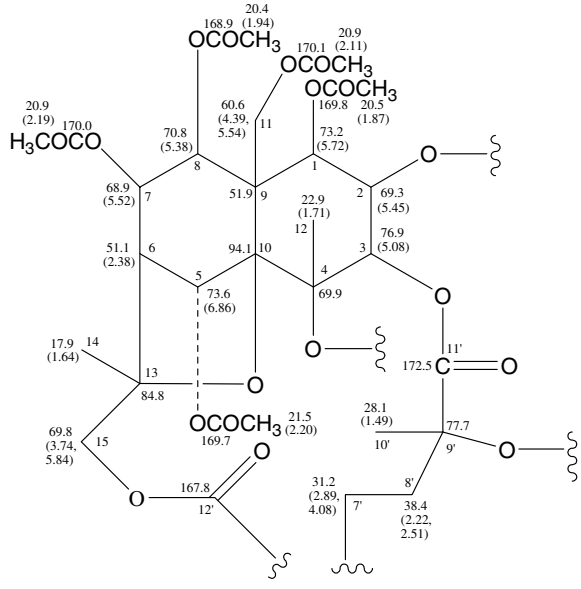
From Nos. 39, 36, 13, 40, 38, and 37 of Table 6.75, that is, the correlations of δ_C (170.0)/δ_H (2.19, 5.52), δ_C (168.9)/δ_H (1.94, 5.38), δ_C (60.6)/δ_H (5.38, 5.72), δ_C (170.1)/δ_H (2.11, 5.54), δ_C (169.8)/δ_H (1.87, 5.72), and δ_C (169.7)/δ_H (2.20, 6.86) ppm, together with the related data of the HSQC spectrum, the above assignment can be further extended as follows:



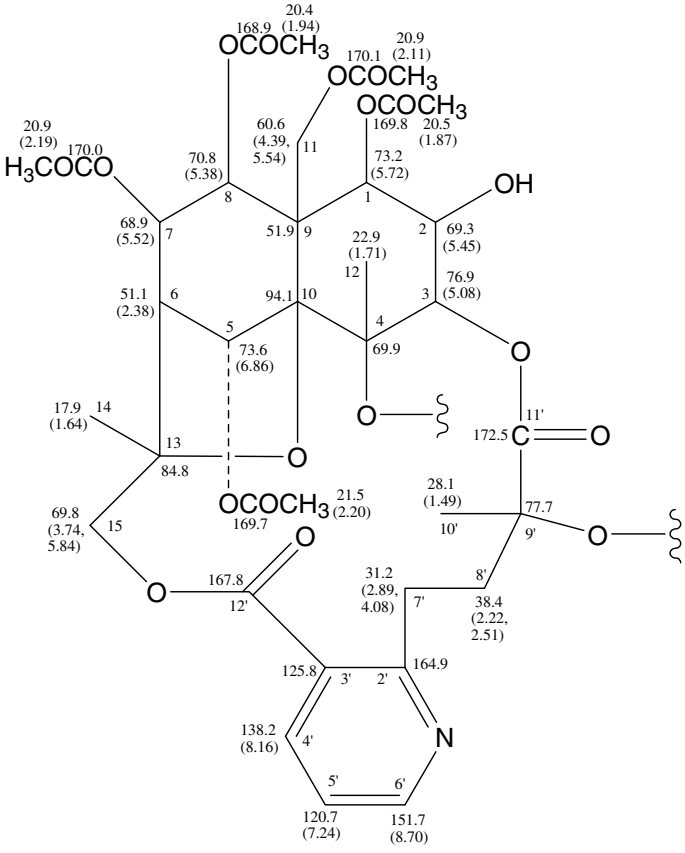
From Nos. 11, 1, 23, and 16 of Table 6.75, that is, the correlations of δ_C (51.1)/δ_H (1.64), δ_C (17.9)/δ_H (3.74, 5.84), δ_C (84.8)/δ_H (1.64, 3.74, 5.84, 6.86), and δ_C (69.8)/δ_H (1.64) ppm, together with the related data of the HSQC spectrum, considering the related chemical shift values, the above assignment can be further extended as follows:



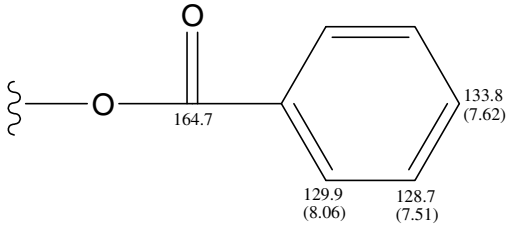
From Nos. 35, 41, 22, 8, 19, and 9 of Table 6.75, that is, the correlations of δ_C (167.8)/δ_H (3.74, 5.84), δ_C (172.5)/δ_H (5.08, 1.49), δ_C (77.7)/δ_H (1.49, 2.22, 2.89, 4.08), δ_C (28.1)/δ_H (2.22), δ_C (38.4)/δ_H (1.49), and δ_C (31.2)/δ_H (1.49, 2.22) ppm, together with the related data of the HSQC spectrum, considering the related chemical shift values, the above assignment can be further extended as follows:



According to Nos. 29, 25, and 30 of Table 6.74, that is, the correlations of δ_H (ppm) 8.16/7.24/8.70, and according to Nos. 35, 26, and 34 of Table 6.75, that is, the correlations of δ_C (167.8)/δ_H (3.74, 5.84, 8.16), δ_C (125.8)/δ_H (7.24), and δ_C (164.9)/δ_H (2.22, 2.51, 2.89, 4.08, 8.70) ppm, together with the related data of the HSQC spectrum, the above assignment can be further extended as follows:



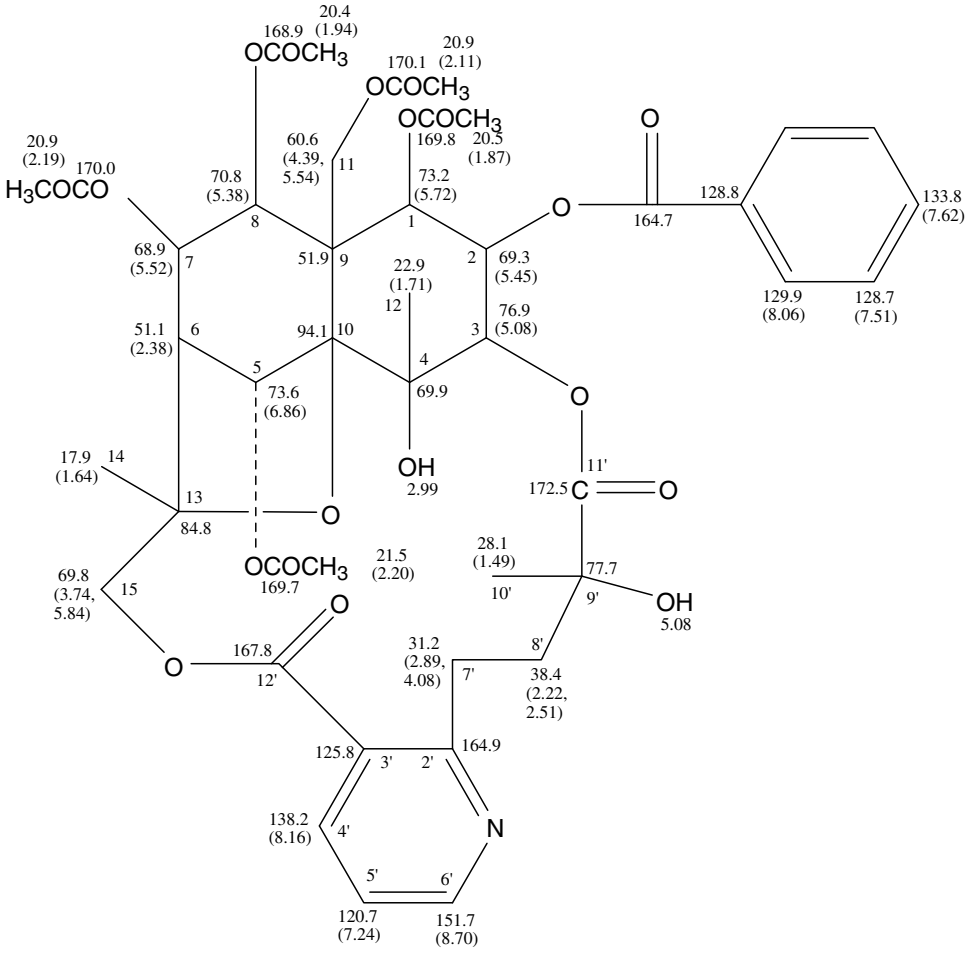
In addition, from Nos. 27, 26, and 28 of Table 6.74, that is, the correlations of δ_H (ppm) 7.62/7.51/8.06, according to No. 33 of Table 6.75, that is, the correlation of δ_C (164.7)/δ_H (8.06), with the related data of the HSQC spectrum, considering $\delta_C = 164.7$, the following structural unit can be assigned:



We have two large assigned structural units so far. The quaternary carbon atom in the above structural unit has no correlated peaks in the HMBC spectrum. However, only the peak at 128.8 ppm is not assigned in the ^{13}C spectrum yet. Therefore, the peak at 128.8 ppm can be assigned as the quaternary carbon atom.

Two peaks at 2.99 and 5.08 ppm in the ^{1}H spectrum are not assigned yet. They belong to two hydroxyl groups. We add these two chemical shift values in the assignment (in the assigned structural unit, their oxygen atoms have been connected).

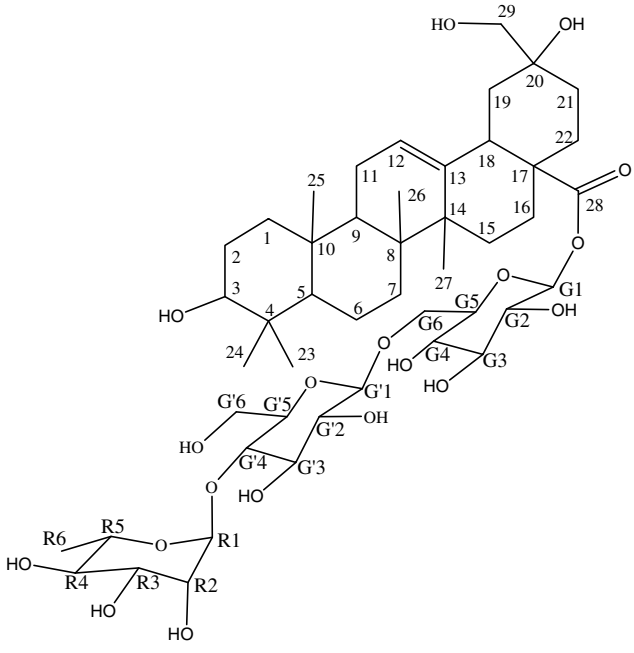
To sum up, we finally obtain the whole assignment of the compound which follows:



C6-20

Through the detailed assignment, we can come to the conclusion that the possible structural formula coincides with the NMR spectra. Therefore, the anticipated structure is confirmed.

Example 6.20 [1] A natural product is extracted, separated, and purified from a certain plant. From the measurement of its accurate mass, its molecular formula of $C_{47}H_{76}O_{19}$ is determined. By comparison with the NMR spectra of the compounds in the same category, a possible structure is proposed as follows:



C6-21

Its ^{1}H spectrum, the locally enlarged ^{1}H spectrum, its ^{13}C spectrum, the locally enlarged ^{13}C spectrum, its DEPT-135 spectrum, the locally enlarged DEPT-135 spectrum, its COSY spectrum, the locally enlarged COSY spectrum, its TOCSY spectrum, the locally enlarged TOCSY spectrum, its HSQC spectrum, the locally enlarged HSQC spectrum, its HMBC spectrum, and the locally enlarged HMBC spectrum are shown in Figures 6.242–6.265. Try to interpret all NMR spectra to confirm its structure. All NMR spectra were measured by an NMR spectrometer with a frequency of 400 MHz. The solvent used is deuterated pyridine.

Solution

This example shows that even an NMR spectrometer with a frequency of 400 MHz can be used to deal with a complicated structure.

We now start from the interpretation of the ^{1}H spectrum.

The peak sets at 7.19, 7.57, and 8.71 ppm are the solvent peaks when deuterated pyridine is used as the solvent.

The peak at about 4.9 ppm with a blunt shape is the water peak.

Because the compound has a complicated structure, peak sets overlap seriously in the ^{1}H spectrum.

Because peak sets have a certain width and they overlap, the central positions of peak sets can not be read directly sometimes. The chemical shift values are determined mainly by the HSQC spectrum.

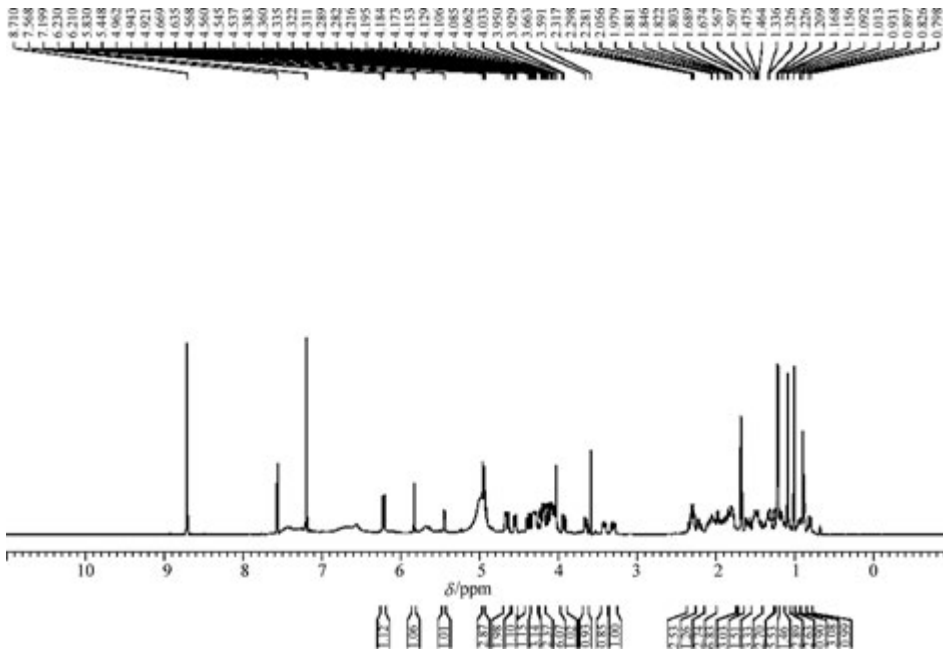


Figure 6.242 The ^{1}H spectrum of the compound

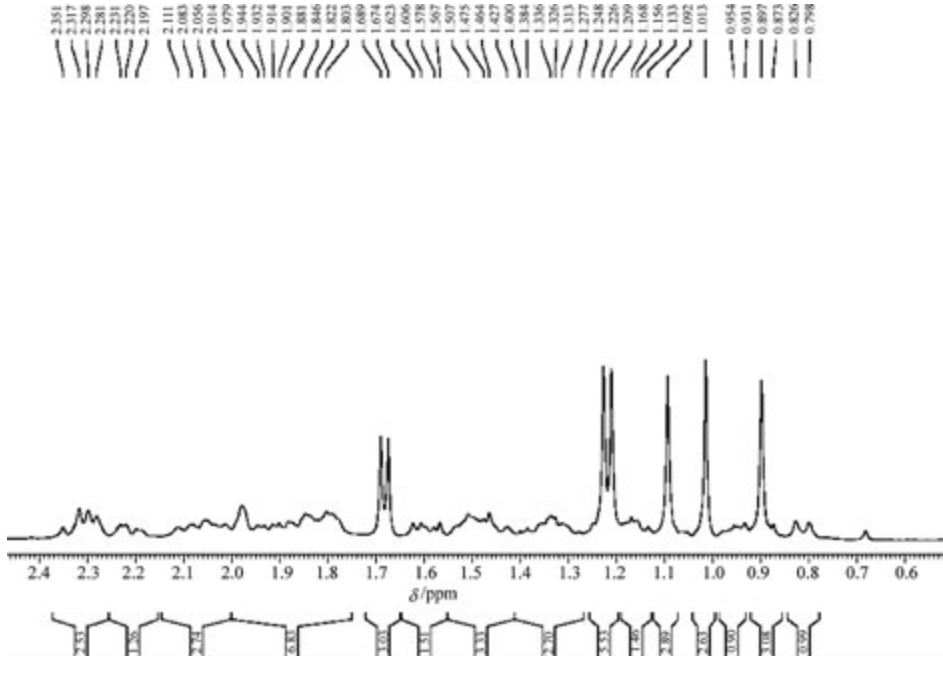


Figure 6.243 The locally enlarged ^{1}H spectrum (1)

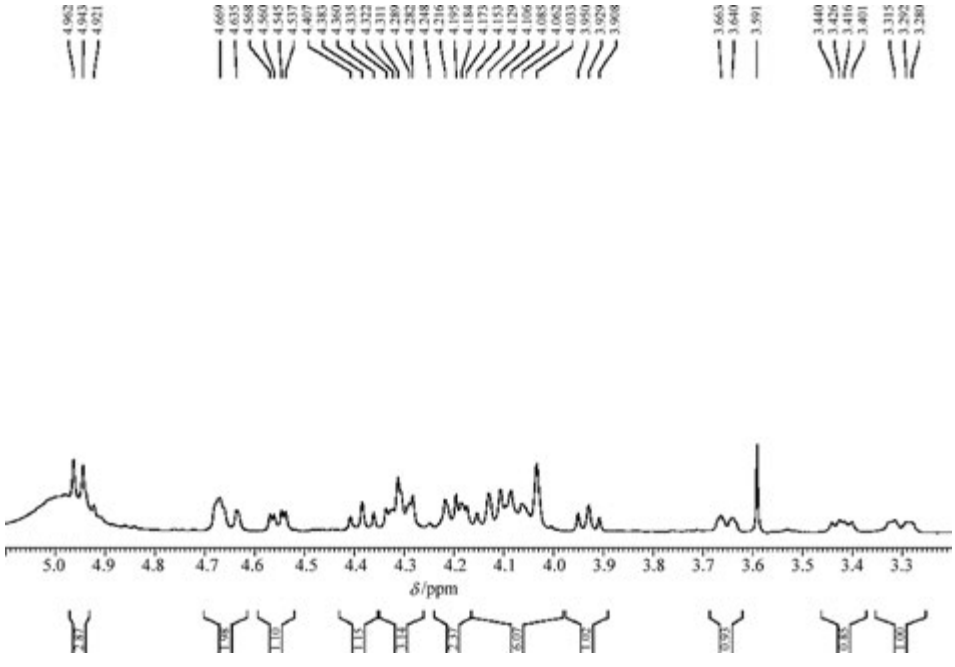


Figure 6.244 The locally enlarged ^{1}H spectrum (2)

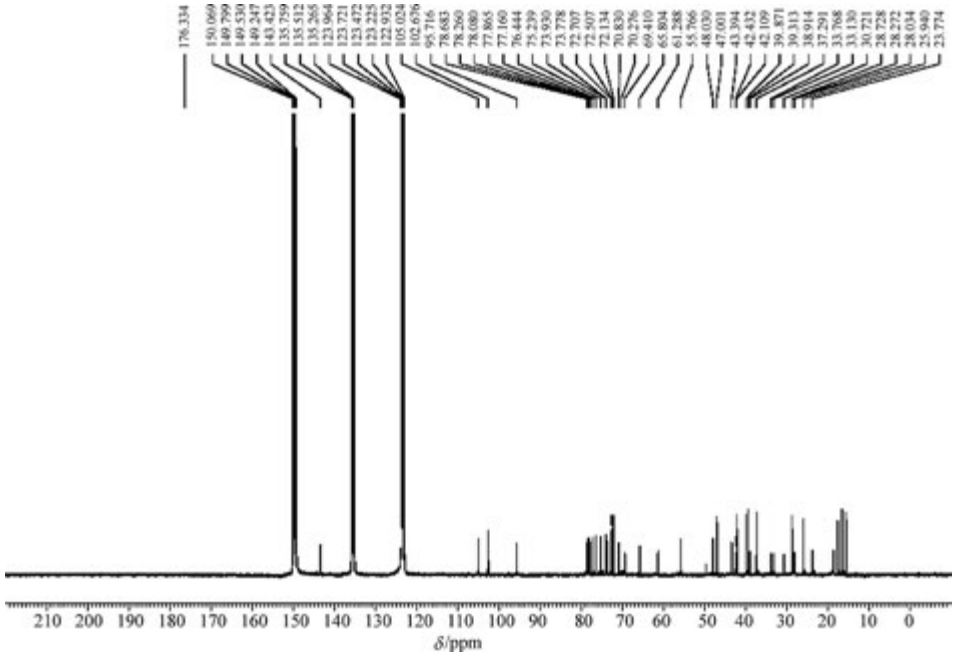


Figure 6.245 The ^{13}C spectrum of the compound

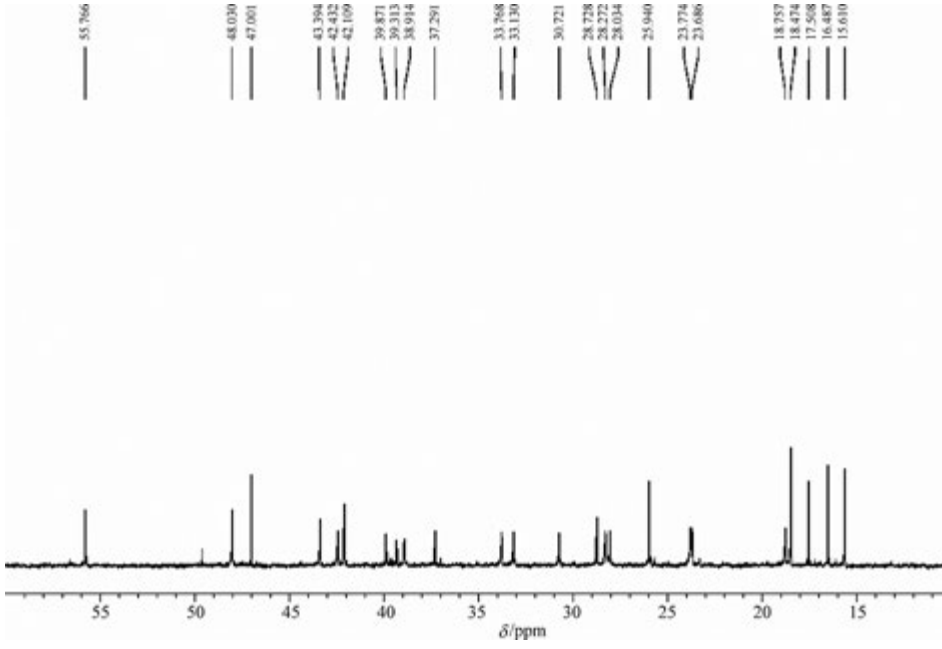


Figure 6.246 The locally enlarged ^{13}C spectrum (1)

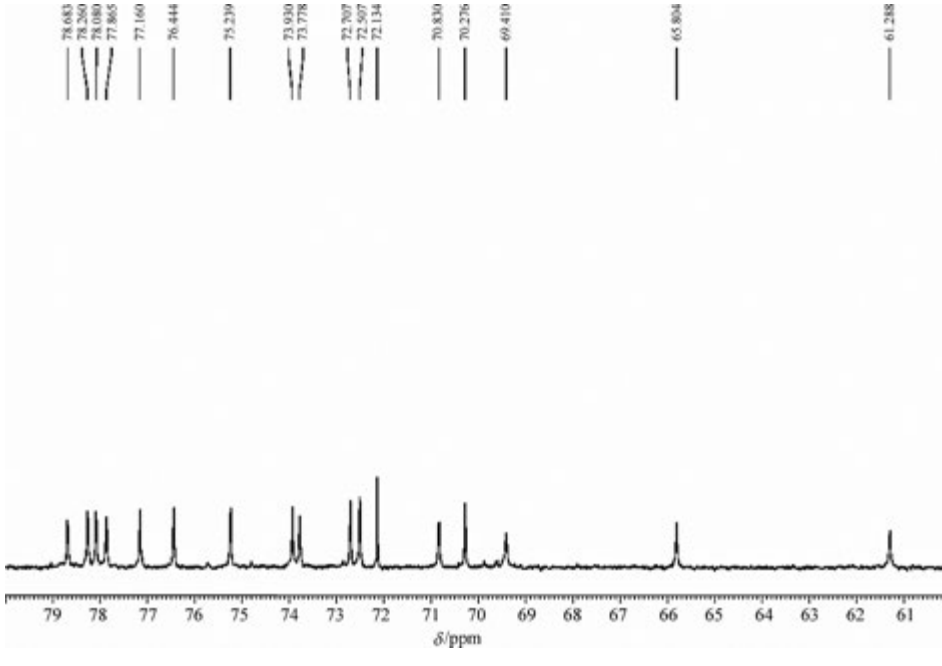


Figure 6.247 The locally enlarged ^{13}C spectrum (2)

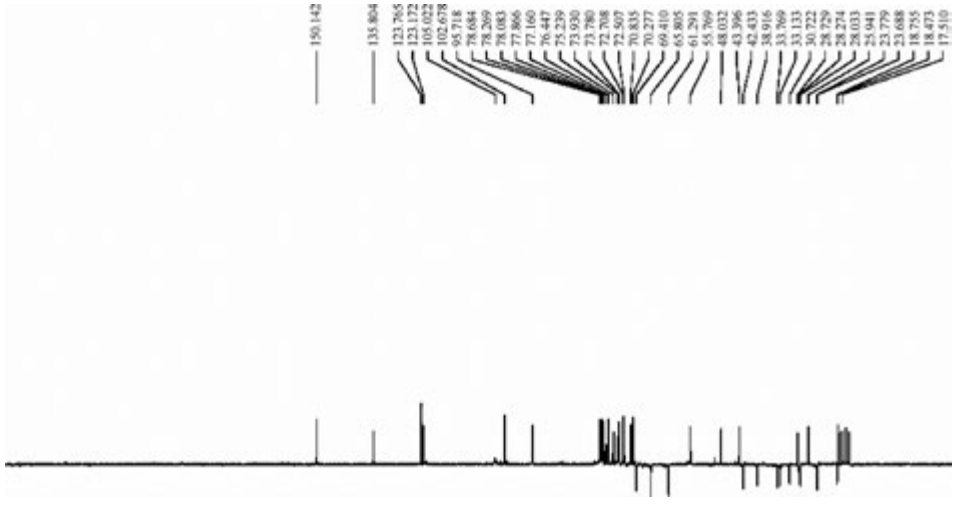


Figure 6.248 The DEPT-135 spectrum

The data summarized from the ^{1}H spectrum are listed in Table 6.76. Because many peak sets overlap, integral is performed region by region. If an integral value is not close to an integer, the hydrogen atom number is determined with the help of the HSQC spectrum, even further of other 2D NMR spectra.

It is recommended that the reader ignore the marks of the following tables at first. He or she should read the marks at the end of this example so that they can obtain a clear conclusion.

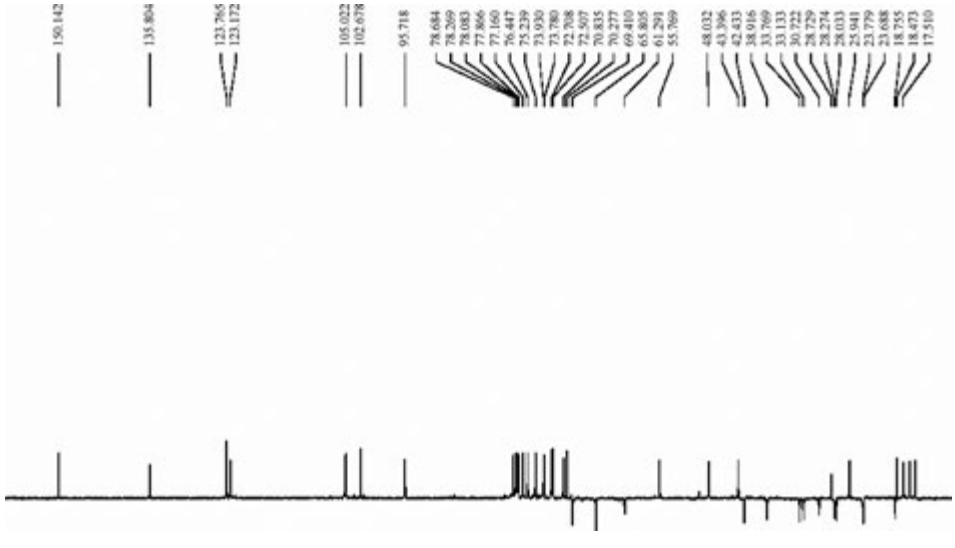


Figure 6.249 The locally enlarged DEPT-135 spectrum

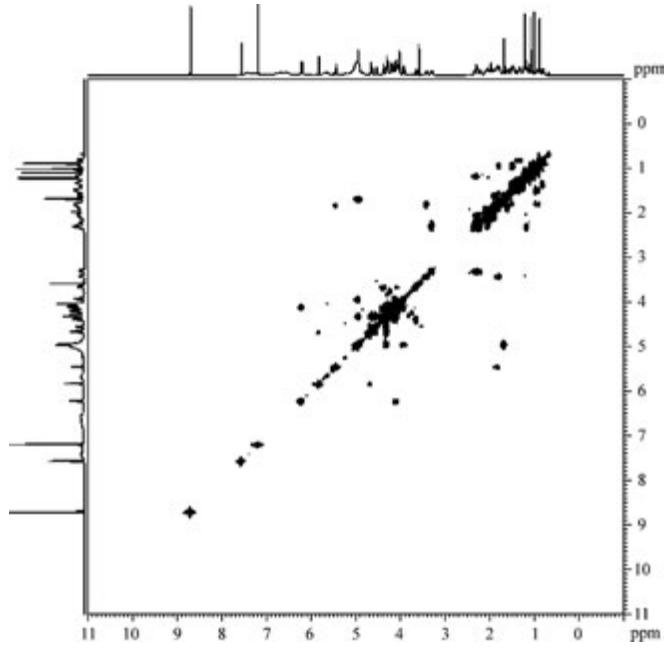


Figure 6.250 The COSY spectrum of the compound

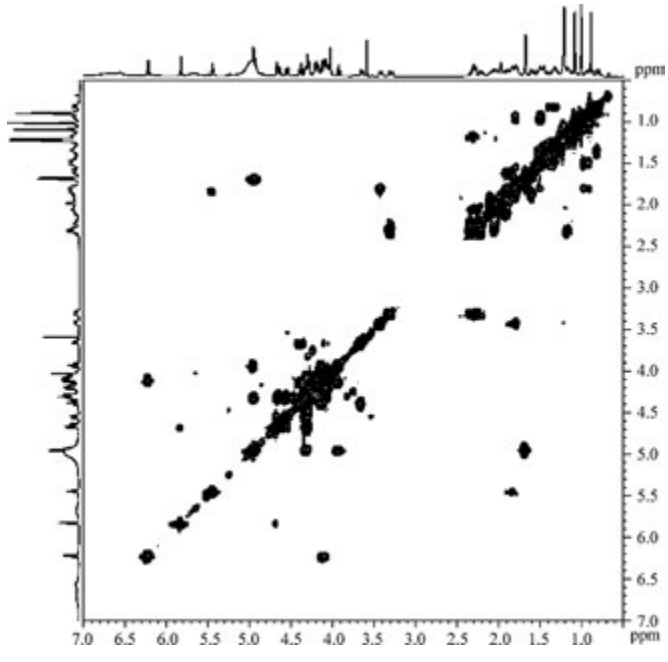


Figure 6.251 The locally enlarged COSY spectrum (1)

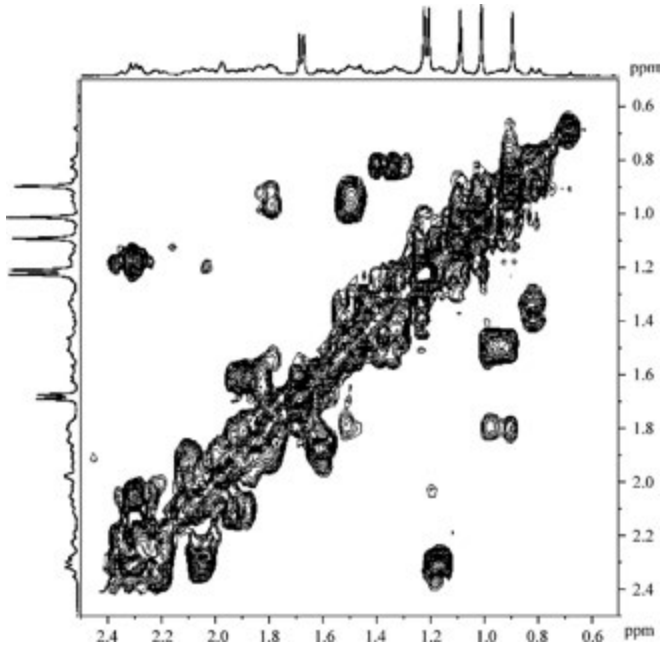


Figure 6.252 The locally enlarged COSY spectrum (2)

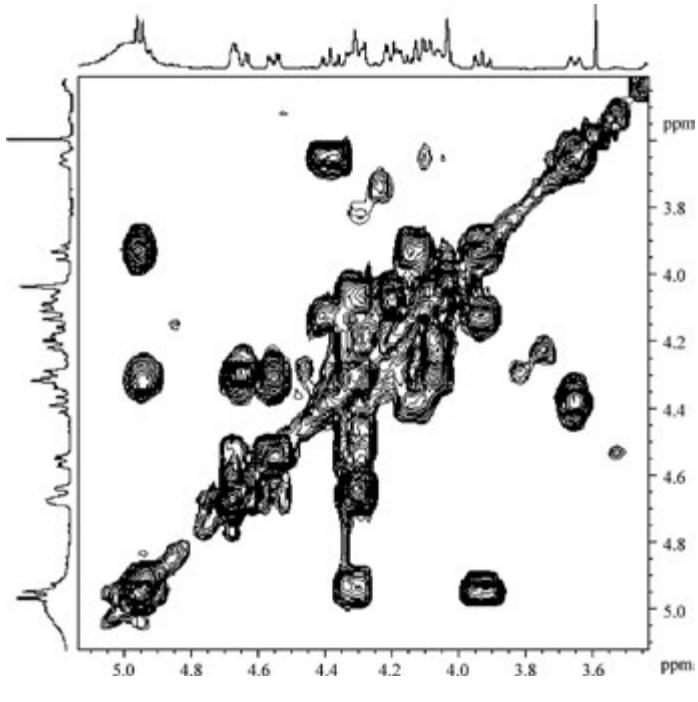


Figure 6.253 The locally enlarged COSY spectrum (3)

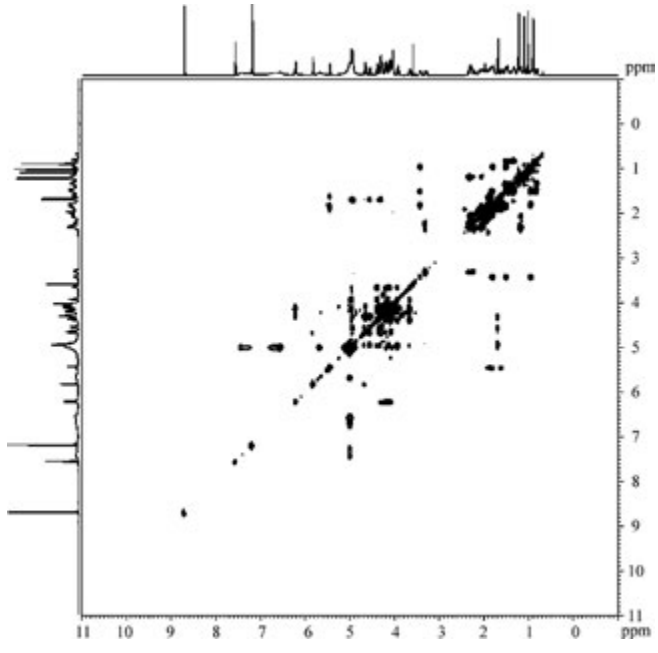


Figure 6.254 The TOCSY spectrum of the compound

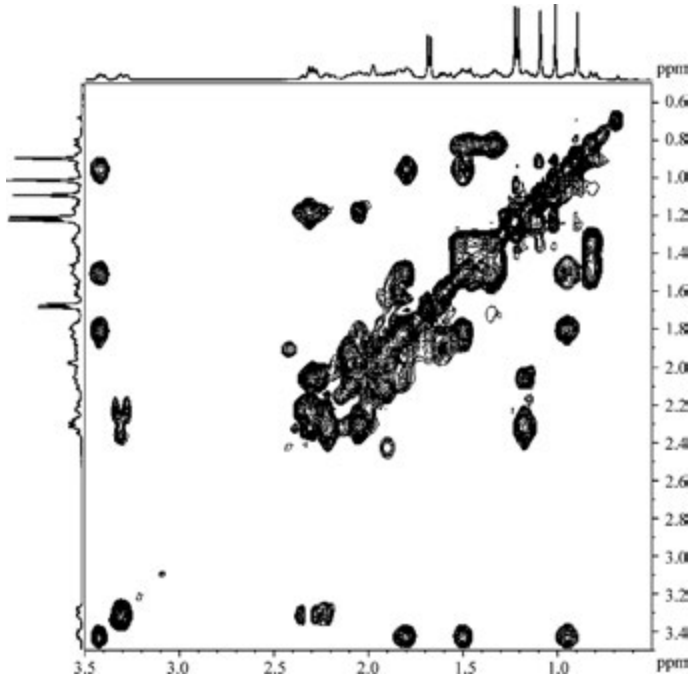


Figure 6.255 The locally enlarged TOCSY spectrum (1)

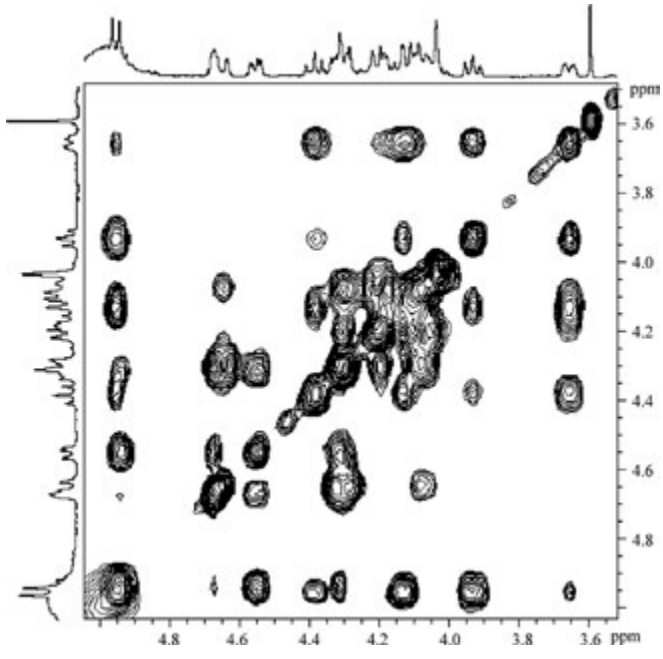


Figure 6.256 The locally enlarged TOCSY spectrum (2)

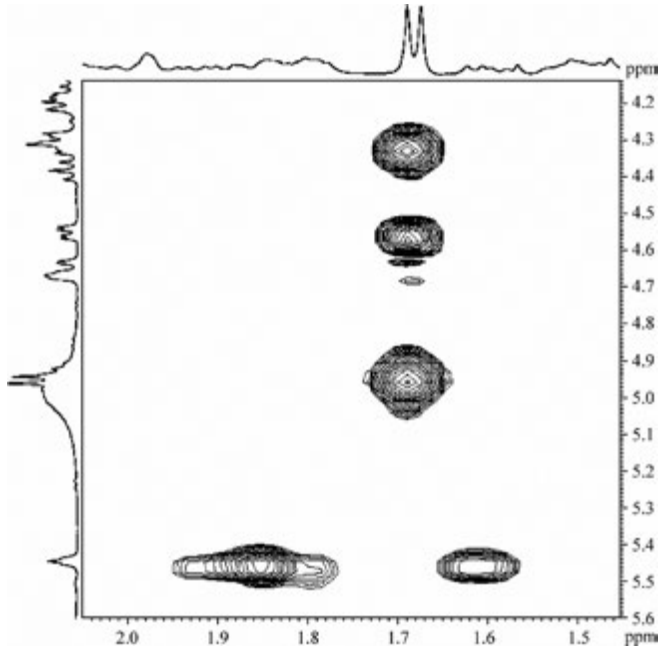


Figure 6.257 The locally enlarged TOCSY spectrum (3)

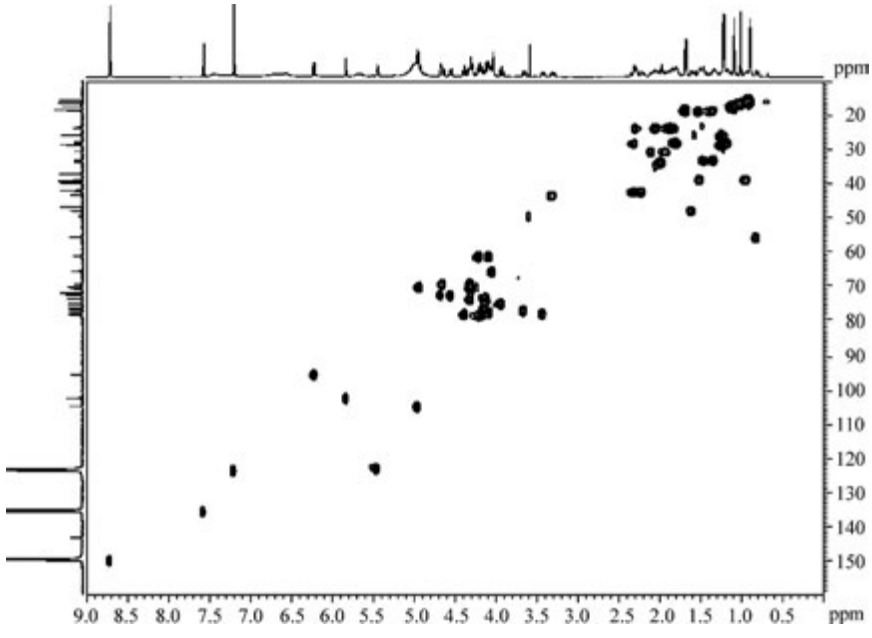


Figure 6.258 The HSQC spectrum of the compound

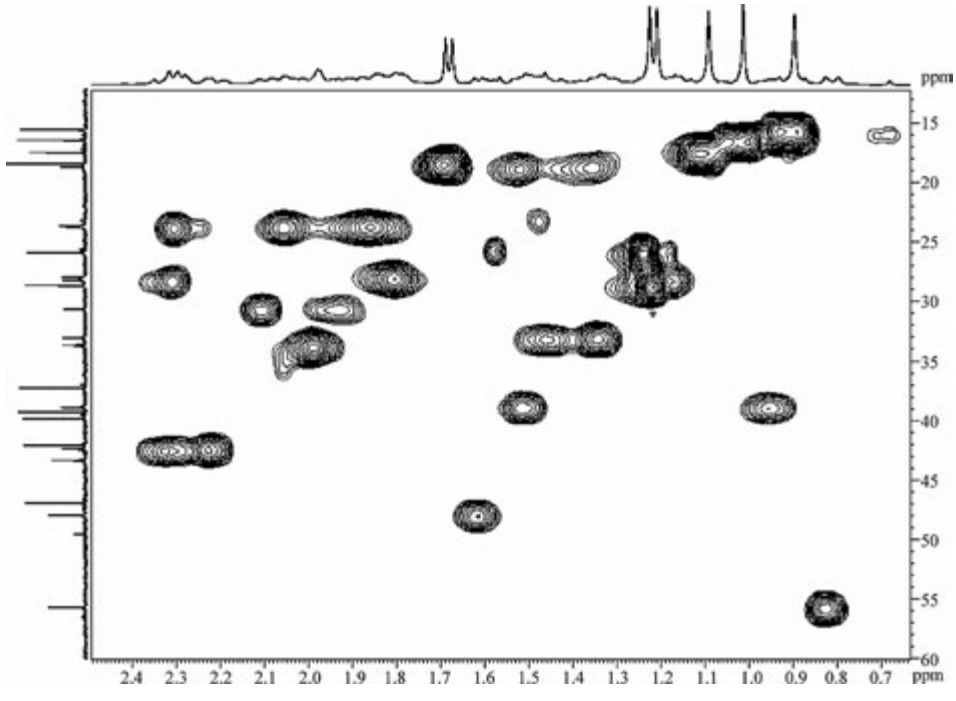


Figure 6.259 The locally enlarged HSQC spectrum (1)

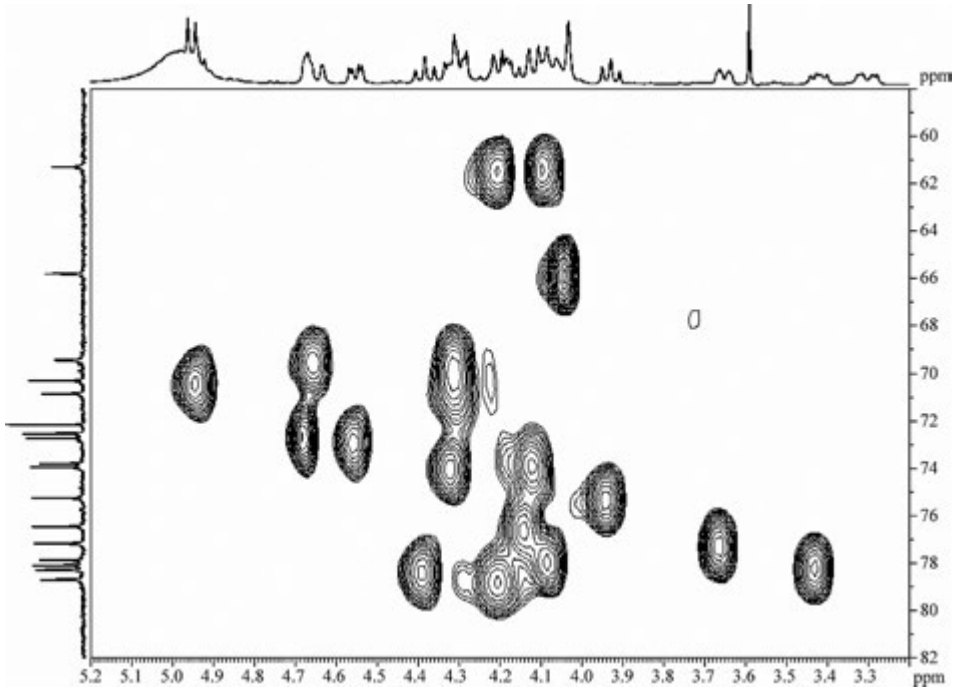


Figure 6.260 The locally enlarged HSQC spectrum (2)

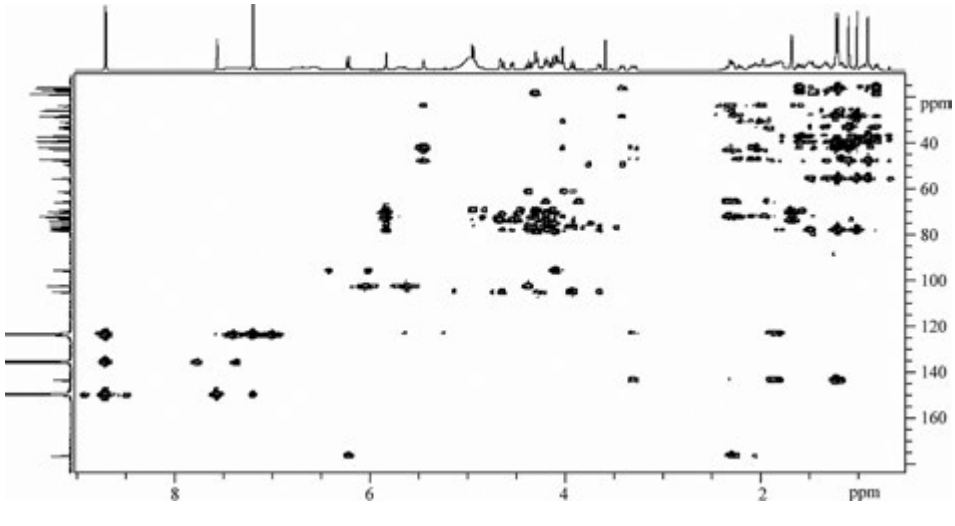


Figure 6.261 The HMBC spectrum of the compound

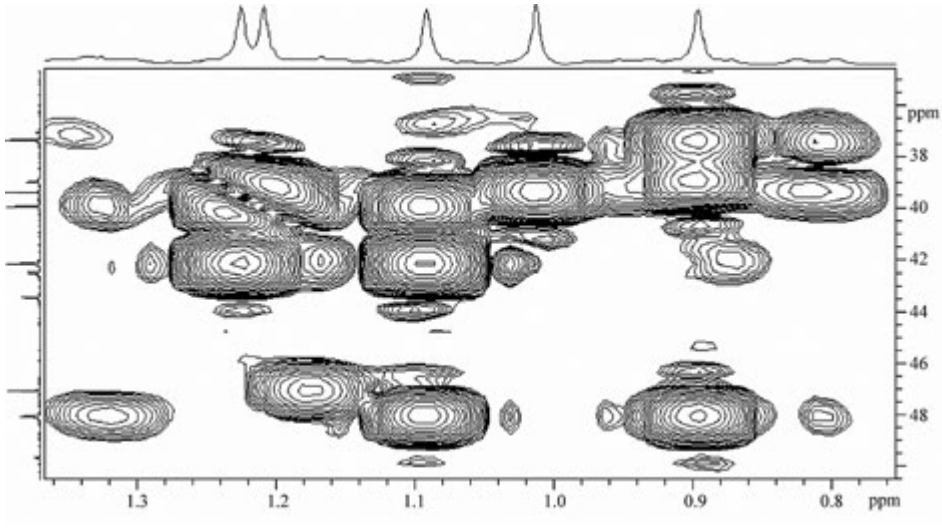


Figure 6.262 The locally enlarged HMBC spectrum (1)

From Table 6.76 it can be known that the hydrogen atom numbers in several regions can not be determined, and that central positions of many peak sets can not be determined. These two problems need the help from the interpretation of the HSQC spectrum. Before this interpretation however, the interpretation of the ^{13}C spectrum and the DEPT-135 spectrum is necessary.

The three peak sets at about 123, 135, and 150 ppm, respectively, in the ^{13}C spectrum are the solvent peak sets.

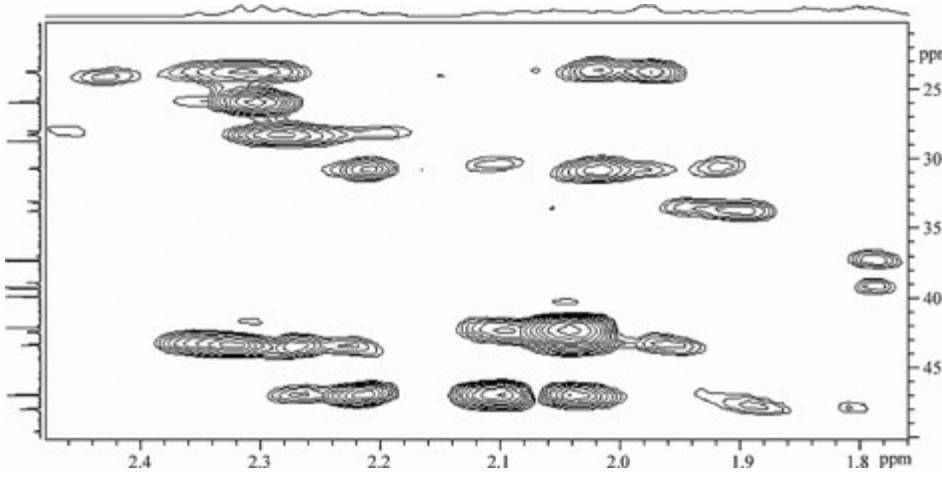


Figure 6.263 The locally enlarged HMBC spectrum (2)

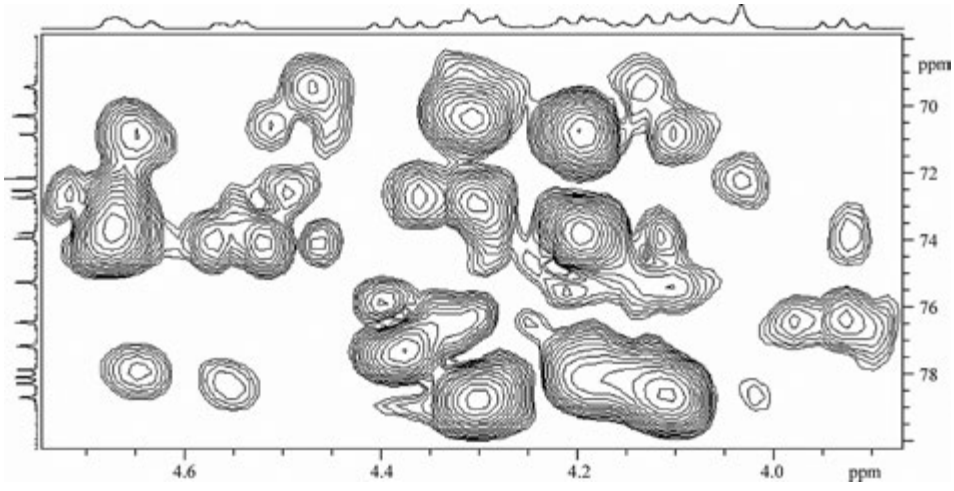


Figure 6.264 The locally enlarged HMBC spectrum (3)

Because the structure is complicated, some peaks in the ^{13}C spectrum are close. A comprehensive interpretation of the ^{13}C spectrum, the DEPT-135 spectrum, and the HSQC spectrum is necessary to obtain accurate chemical shift values. For example, the peak at 123.17 ppm overlaps on the solvent peak sets at about 123 ppm. Therefore, this signal can be omitted. In the HSQC spectrum, we can see correlated peaks at about 123, 135, and 150 ppm, respectively, which are produced from the correlations of the solvent. They can be recognized from their positions of the abscissa at $F_2 = 7.19$, 7.57, and 8.71 ppm. However,

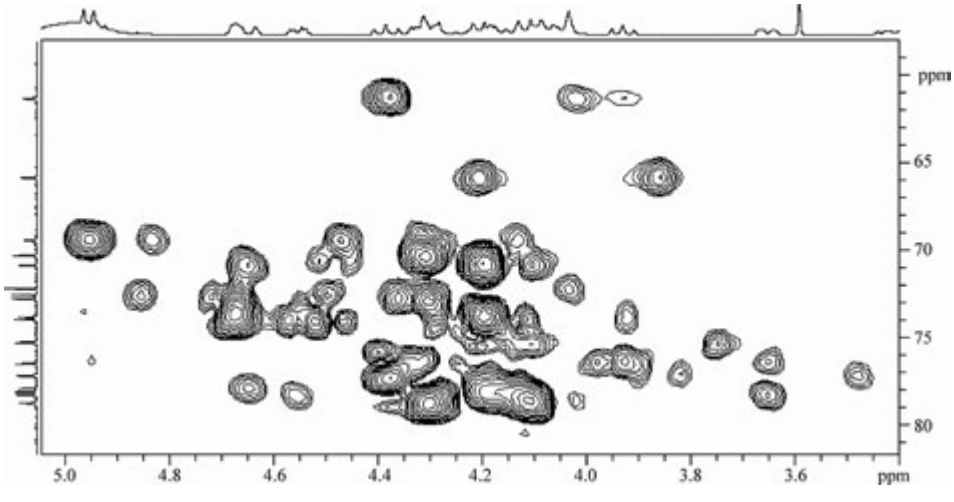


Figure 6.265 The locally enlarged HMBC spectrum (4)

Table 6.76 The data summarized from the ^{1}H spectrum

δ_H (ppm)	Integral value	Number of H atoms	Peak shape	Remarks	Mark
0.81	0.99	1	d		5
0.90	3.08	3	s		25
0.93–0.98	0.90	1	m		1
1.01	2.63	3	s		24
1.09	2.89	3	s		26
1.13–1.21	1.46	1?	m	[a]	15
1.21	[b]	3	s		23
1.23	[b]	3	s		27
1.25–1.41	2.70	2? 3?	m	[a]	6, 7
1.41–1.55	3.33	3	m		1, 6, 7
1.55–1.65	1.51	1?	m	[a]	9
1.68	3.03	3	d		R6
1.76–2.00	6.83	7	m		2, 11, 21, 22
2.00–2.15	2.74	2? 3?	m	[a]	16, 21,
2.15–2.25	1.26	1	m		19
2.25–2.36	2.53	2? 3?	m	[a]	15, 16, 19
3.30	1.00	1	d, d		18
3.42	0.85	1	d, d		3
3.66	0.93	1	d (br.)		G′5
3.93	1.02	1	t		G′2,
4.03	[c]		s		29
3.98–4.17	[c]		m		G2, G5, G′3, G′6
4.17–4.24	2.37	2	m		G3, G′6
4.26–4.35	3.14	3	m		G4, G6, R4
4.38	1.15	1	t		G′4
4.55	1.10	1	d, d		R3
4.62–4.69	1.98	2	m, m		G6, R2
4.92–4.97	2.87	2?	m		G′1, R5
5.45	1.01	1	t		12
5.83	1.06	1	s		R1
6.22	1.17	1	d		G1

[a] The hydrogen atom number is determined with the help of other spectra.
[b] These two peak sets share the integral value of 5.53.
[c] These two peak sets share the integral value of 6.02.

we can see another correlated peak at $F_1 \approx 123$ and $F_2 = 5.45$ ppm in the HSQC spectrum. This is the correlated peak produced from the sample. Therefore, the overlapped signal of the sample can be discerned by the HSQC spectrum.

The central positions of the peak sets in the ^{1}H spectrum are determined mainly by the HSQC spectrum.

From the combination of the ^{13}C spectrum, the DEPT-135 spectrum, the HSQC spectrum, and the ^{1}H spectrum, the data of the ^{13}C spectrum and the ^{1}H spectrum can be summarized.

The solvent peak sets are not listed.

If the central positions of the peak sets in the ^{1}H spectrum are determined, their data are used in Table 6.78, which lists the data summarized from the COSY spectrum. If the chemical shift values have not been determined so far, a range of chemical shift values is used in Table 6.78.

Table 6.77 The generalized data of the ^{13}C spectrum and the ^{1}H spectrum

δ_C (ppm)	Number of C atoms	Order of C atomos	Directly connected H, δ_H (ppm)	Mark
15.61	1	CH_3	0.90	25
16.49	1	CH_3	1.01	24
17.51	1	CH_3	1.09	26
18.47	1	CH_3	1.68	R6
18.76	1	CH_2	1.36, 1.52	6
23.69[a]	1	CH_2	1.86	11
23.77[a]	1	CH_2	2.06, 2.30	16
25.94	1	CH_3	1.23	27
28.03	1	CH_2	1.80	2
28.27	1	CH_2	1.17, 2.31	15
28.73	1	CH_3	1.21	23
30.72	1	CH_2	1.93, 2.11	21
33.13	1	CH_2	1.34, 1.46	7
33.77	1	CH_2	1.97	22
37.29	1	C		10
38.91	1	CH_2	0.95, 1.51	1
39.31	1	C		4
39.87	1	C		8
42.11	1	C		14
42.43	1	CH_2	2.22, 2.33	19
43.39	1	CH	3.30	18
47.00	1	C		17
48.03	1	CH	1.61	9
55.77	1	CH	0.81	5
61.29	1	CH_2	4.09, 4.20	G′6
65.80	1	CH_2	4.03	29
69.41	1	CH_2	4.32, 4.66	G6
70.28	1	CH	4.94	R5
70.83	1	CH	4.31	G4
72.13	1	C		20
72.51	1	CH	4.68	R2
72.71	1	CH	4.55	R3
73.78	1	CH	4.13	G2
73.93	1	CH	4.32	R4
75.24	1	CH	3.93	G′2
76.44	1	CH	4.14	G′3
77.16	1	CH	3.66	G′5
77.87[a]	1	CH	4.08	G5
78.08[a]	1	CH	3.42	3
78.26[a]	1	CH	4.38	G′4
78.68	1	CH	4.20	G3
95.72	1	CH	6.22	G1
102.68	1	CH	5.83	R1
105.02	1	CH	4.95	G′1
123.17	1	CH	5.45	12
143.42	1	C		13
176.33	1	C		28

[a] Read from the enlarged HSQC spectrum.

Table 6.78 The data summarized from the COSY spectrum

No.	δ_H (ppm)	Coupled H, δ_H (ppm)	Mark
1	0.81	1.36	5/6
2	0.90	isolated	25
3	0.93–0.98	1.51, 1.80	1/1, 2
4	1.01	isolated	24
5	1.09	isolated	26
6	1.17	2.31	15/15, 16
7	1.21	isolated	23
8	1.23	isolated	27
9	1.36	0.81, 1.46	6/5, 7
10	1.51	0.95	1/1
11	1.61	1.86	9/11
12	1.68	4.94	R6/R5
13	1.80	0.95	2/1
14	1.86	1.61, 5.45	11/9, 12
15	1.93	2.11	21/21
16	2.06	2.30	16/16
17	2.11	1.93	21/21
18	2.22	2.33	19/19
19	2.30	2.06	16/16
20	3.30[a]	2.22, 2.33	18/19
21	3.42	1.80	3/2
22	3.66	(4.09), 4.38	G′5/G′6,G′4
23	3.93	4.14, 4.95	G′2/G′3,G′1
24	4.03	isolated	29
25	4.08–4.09	4.20, 4.31	G′6/G′6,G5/G4,G6
26	4.13–4.14	3.93, 6.22	G′3/G′2,G2/G1
27	4.20	4.09	G′6/G′6
28	4.31–4.32	4.08, 4.55, 4.66, 4.94	G4/G5,R4/R3,G6/G6,R4/R5
29	4.38	3.66, 4.14	G′4/G′5, G′4/G′3,
30	4.55	4.32	R3/R4
31	4.66–4.68	4.32, 5.83	G6/G6, R2/R1
32	4.94–4.95	1.68, 3.93, 4.32	R5/R6, G′1/G′2, R5/R4
33	5.45	1.86	12/11
34	5.83	4.68	R1/R2
35	6.22	4.13	G1/G2

Parenthesis () stands for weak couplings.
[a] Obtained with the help of the TOCSY spectrum.

The data summarized from the TOCSY spectrum are listed in Table 6.79.

It should be emphasized that the TOCSY spectrum plays an important role in this example. Because all couplings in a coupled system can be illustrated by the TOCSY spectrum, this is very useful for the deduction of an unknown structure or the confirmation of an anticipated structure. The compound contains three saccharine rings, which can be differentiated from each other by use of the TOCSY spectrum. Other 2D NMR spectra can not be used to do it.

The data summarized from the HMBC spectrum are listed in Table 6.80.

Table 6.79 The data summarized from the TOCSY spectrum

δ_H (ppm)	Coupled H, δ_H (ppm)	Marks
0.81	1.36, 1.51	5/6,6
0.90		
0.95	1.51, 1.80, 3.42	1/1,2,3
1.01		
1.09		
1.17	2.06, 2.30	15/16
1.21		
1.23		
1.34–1.36	0.81, 1.52	6,7/5,6
1.46	0.81, 1.52	7/5,6
1.51–1.52	0.81, 0.95, 1.36, 1.80	6/5,1/1, 6/6,1/2
1.61	1.86	9/11
1.68	4.32, 4.55, 4.94	R6/R4,R3,R5
1.80	0.95, 1.51, 3.42	2/1,1,3
1.86	1.61	11/9
1.93	2.11	21/21
2.06	1.17, 2.30	16/15,16
2.11	1.93, 1.97	21/21,22
2.22	2.33	19/19
2.30–2.31	1.17, 2.06	15,16/15,16
2.33	2.22	19/19
3.30	2.22, 2.33	18/19,19
3.42	0.95, 1.51, 1.80,	3/1,1,2
3.66	3.93, 4.15, 4.38, 4.95	G′5/G′2,G′3,G′4
3.93	3.66, 4.15, 4.38, 4.95	G′2/G′5,G′3,G′4
4.03		
4.08–4.09	4.32, 4.66	G5/G6,G6
4.13–4.14	3.66, 3.93, 4.38, 4.95, 6.22	G′3/G′5,G′2,G′4,G′1,G2/G1
4.31–4.32	4.20, 4.55, 4.66, 4.94	G4/G3,R4/R3,G6/G6,R4/R5
4.20	4.09, 4.31	G′6/G′6,G3/G4
4.38	3.66, 3.93, 4.14, 4.95	G′4/G′5,G′2,G′3,G′1
4.55	4.32, 4.68, 4.94	R3/R4,R2,R5
4.66–4.68	4.08, 4.32, 4.55	G6/G5,G4, R2/R4,R3
4.94–4.95	3.66, 3.93, 4.14, 4.32, 4.38, 4.55, 4.68, 5.83	G′1/G′5,G′2,G′3,G′4,R5/R4,R3,R2,R1
5.45	1.61, 1.86	12/9,11
5.83	4.68	R1/R6
6.22	4.13, 4.20, 4.31	G1/G2,G3,G4

Note:
The chemical shift values, each of which differs from the listed value by less than 0.02 ppm, are not listed in the second column. For example, "2.30" includes 2.31–2.32 ppm.

It can be seen that the HMBC spectrum plays an important role in determining the central positions of the peak sets in the ^{1}H spectrum, because its ordinate, that is, the F_1 axis, which is δ_C, has a rather high resolution.

The above five tables contain the information on tens of NMR spectra.

The reader is familiar with the commonly used method already. In addition, the structure of this compound is very complicated. Therefore, we simplify the following description. We present only some key points.

Table 6.80 The data summarized from the HMBC spectrum

No.	δ_C (ppm)	H atoms with a long-range coupling, δ_H (ppm)	H atoms with the ^1J coupling, δ_H (ppm)	Mark
1	15.61	1.61		25/9
2	16.49	1.21, 3.42		24/23, 3
3	17.51	1.61		26/9
4	18.47	4.32		R6/R4
5	18.76			6
6, 7	23.69, 23.77	1.97, 2.31		11, 16/22, 15
8	25.94	2.31, (5.45)		27/15, 12
9	28.03			2
10	28.27	1.23, 2.30		15/27, 16
11	28.73	1.01, 3.42		23/24, 3
12	30.72	2.01, 2.22, 4.03		21/22, 19, 29
13	33.13	1.09		7/26
14	33.77	1.90		22/21
15	37.29	0.90, 1.51, 1.60, 1.79		10/25, 6, 9, 2
16	38.91	0.90, 1.79		1/25,2
17	39.31	1.01, 1.21		4/23, 24
18	39.87	1.09, 1.23, 1.32, 1.51, 1.60		8/26, 27, 6, 6, 9
19	42.11	1.09, 1.23, 2.08, 5.45		14/26, 27, 16, 12
20	42.43	1.46, 4.03		19/29
21	43.39	2.32		18/19
22	47.00	1.17, 2.04, 2.10, 2.21, 5.45		17/15, 16, 21, 19, 13
23	48.03	0.90, 1.09, 1.32		9/25, 26, 7
24	55.77	0.90, 1.01, 1.21		5/25, 24, 23
25	61.29	4.38		G6/G4
26	65.80	2.32	4.03	29/19
27	69.41	4.95	4.66	G6/G′1
28	70.28	1.68, 4.30, 5.83		R5/R6, R4, R1
29	70.83	4.19, 4.66		G4/G3, G6
30	72.13	2.32		20/19
31	72.51	5.83		R2/R1
32	72.71			
33	73.78	4.19, (5.45)		G2/G3,
34	73.93	1.68		R4/R6
35	75.24	4.38		G′2/G′4
36	76.44	3.66, 3.93		G′3/G′5, G2
37	77.16			
38	77.87			
39	78.08	1.01, 1.21, 1.50		3/24, 23, 1
40	78.26	3.66, 4.19, 5.83		G′4/G′5, G′6, R1
41	78.68	4.11, 4.30	4.20	G3/G5, G4
42	95.72	4.20		G1/G3
43	102.68	4.38		R1/G′4
44	105.02	3.66, 3.93, 4.66		G′1/G′5, G′2, G6
45	123.17	1.86, 3.30		12/11, 18
46	143.42	1.17, 1.23, 1.86, 3.30		13/15, 27, 11, 18
47	176.33	2.06, 2.30, 6.22		28/16, G1

Table 6.81 The final assignment

Note	Order of C atoms	δ_C (ppm)	δ_H (ppm)	Peak shape of the ^1H spectrum
1	CH_2	38.91	0.95, 1.51	m, m
2	CH_2	28.03	1.80	m
3	CH	78.08	3.42	d, d
4	C	39.31		
5	CH	55.77	0.81	d
6	CH_2	18.76	1.36, 1.52	m, m
7	CH_2	33.13	1.34, 1.46	m, m
8	C	39.87		
9	CH	48.03	1.61	m
10	C	37.29		
11	CH_2	23.69	1.86	m
12	CH	123.17	5.45	t
13	C	143.42		
14	C	42.11		
15	CH_2	28.27	1.17, 2.31	m, m
16	CH_2	23.77	2.06, 2.30	m, m
17	C	47.00		
18	CH	43.39	3.30	d, d
19	CH_2	42.43	2.22, 2.33	m, m
20	C	72.13		
21	CH_2	30.72	1.93, 2.11	m, m
22	CH_2	33.77	1.97	m
23	CH_3	28.73	1.21	s
24	CH_3	16.49	1.01	s
25	CH_3	15.61	0.90	s
26	CH_3	17.51	1.09	s
27	CH_3	25.94	1.23	s
28	C	176.33		
29	CH_2	65.80	4.03	s
G1	CH	95.72	6.22	d
G2	CH	73.78	4.13	m
G3	CH	78.68	4.20	m
G4	CH	70.83	4.31	m
G5	CH	77.87	4.08	m
G6	CH_2	69.41	4.32, 4.66	m, m
G′1	CH	105.02	4.95	d
G′2	CH	75.24	3.93	t
G′3	CH	76.44	4.14	m
G′4	CH	78.26	4.38	t
G′5	CH	77.16	3.66	br. d
G′6	CH_2	61.29	4.09, 4.20	m, m
R1	CH	102.68	5.83	br. s
R2	CH	72.51	4.68	m
R3	CH	72.71	4.55	d, d
R4	CH	73.93	4.32	m
R5	CH	70.28	4.94	m
R6	CH_3	18.47	1.68	d

The connection between two saccharine rings needs careful consideration, which is special for this example.

The δ_C value of the C-1 atom of the saccharine ring is greater than 100 ppm in general. Its related δ_H value is close to 5.0 ppm or even greater than 5.0 ppm. Therefore, the signals of the C-1 atom can be differentiated easily from those of other carbon atoms. From the correlated peaks of C-1 atoms of saccharine rings in the HMBC spectrum, the connections between two saccharine rings can be determined.

We take the connection between the R and G′ as an example. According to the correlations of δ_C (78.26, G′4)/δ_H(5.83, R1), and δ_C (102.68, R1)/δ_H(4.38, G′4), the connection between R1 and G′4 via an oxygen atom can be known.

Similarly, according to the correlations of δ_C (69.41, G6)/δ_H(4.95, G′1), and δ_C (105.02, G′1)/δ_H(4.66, G6), the connection of G′1 with G6 via an oxygen atom can be determined.

A very important clue is to find the correlation of δ_C (176.33)/δ_H(6.22), from which the connection between saccharine ring G and the carbonyl group (situated at 28- position) can be determined.

The final assignment is shown in Table 6.81.

The assignment is completed through the interpretation of tens of NMR spectra, including one dimensional NMR spectra and two dimensional NMR spectra. Because the assignment is reasonable, the anticipated structure is correct.

We would like to supplement the mass spectrum data of the compound here.

The accurate mass of $[M + NH_4]$ was determined as 962.5323 (u) by the mass spectrometry. From the value, the elemental composition of $C_{47}H_{80}O_{19}N$, whose calculated accurate mass is 962.5325, was obtained. The subtraction of NH_4 from $C_{47}H_{80}O_{19}N$ gives the molecular formula of $C_{47}H_{76}O_{19}$.

The MS^2 spectrum of the ion at *m/z* 967 ($[M + Na]^+$) gave a positive ion at *m/z* 493 ($[470 + Na]^+$). The MS^2 spectrum of the ion at *m/z* 943 ($[M-H]^-$) gave a negative ion at *m/z* 493 ($[M-H-470]^-$) and 469 ($[470-H]^-$). The sum of masses of the three saccharine rings is 470.

Therefore, the data of the tandem mass spectrometry show the existence of the three saccharine rings in a simple way.

Reference

[1] Gao, H., Wang, Z. et al. (2008) *Helvetica Chimica Acta*, **91**, 451–459.

List of Abbreviations

APCI	atmosphere pressure chemical ionization
CI	chemical ionization
COSY	correlation spectroscopy
DEPT	distortionless enhancement by polarization transfer
EI	electron (impact) ionization
ESI	electrospray ionization
FAB	fast atom bombardment
HMBC	(^{1}H-detected) heteronuclear multiple bond coherence
HMQC	(^{1}H-detected) heteronuclear multiple-quantum coherence
HSQC	(^{1}H-detected) heteronuclear single-quantum coherence
LC-MS	liquid chromatography- mass spectrometry
MALDI	matrix-assisted laser desorption-ionization
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
ROESY	rotating frame Overhauser effect spectroscopy
TOCSY	total correlation spectroscopy
TOF	time of flight

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