# 13 STRUCTURAL DETERMINATION OF MATERIALS

Synthesis and purification are not the final goal for chemists. It is structures of synthesized and purified materials that need to be defined. In fact, this final step is the most difficult.

It must be admitted that until the latter half of the 20th century, chemists were not provided with sufficient means to surmount this difficulty. Some chemists proposed structures that were incorrect even after efforts over many years.

The situation has, however, changed completely since various kinds of spectroscopy have been developed. Nuclear magnetic resonance (NMR) in particular is one superior method among others. For solid samples, X-ray crystallographic analysis has proved extremely useful.

## 13.1 Earlier structural determinations

Before spectroscopic methods were introduced, *i.e.*, until the first half of the 20th century, the determination of the structures of organic compounds mainly based on comparisons with compounds whose structures were already known. If all the physical and chemical properties of a compound are identical with those of a compound already described in the literature, one could believe that the compound examined was identical with the compound compared. This criterion is adopted even today though what comparisons are made may be different.

When the physical and chemical properties of a compound investigated do not coincide with any compound described in the literature, it is likely that this compound is a new compound, never prepared nor reported. In such a case, a new problem arises. How one can determine the structure of a wholly new compound? The method for determining structure changed greatly at the middle of the 20th century. The traditional method, though simple in principle, was tedious and difficult in practice: thus, first a structure for newly produced compound was assumed, and then a route was designed to convert this compound to an already known compound. The conversion might require several steps. As long as the change of structure caused by each step was identified, the successful conversion to that known compound was accepted as a proof of the assumed structure. It must be added that the reactions for conversion were deliberately chosen from those reactions that only involved with functional groups and not the framework of the molecule itself.

Nowadays the determination of the structures is carried out mainly by spectroscopic and diffraction methods. In this chapter, first, the method of structural determination used before modern methods were available will be described and then the modern methods. It must be added that there are now many available methods to obtain information about structures. For instance, quantum chemical calculations may be an important source of information.

### (a) Mixed-meting-point test

This method was already briefly described in the previous chapter (Ch. 12.2). Before the middle of the 20th century, the main procedure in the structural determination of organic compounds was to prove that the compound was identical with one of the known compounds. This proof was mostly achieved by the **mixed-melting-point test (mixed examination)**. This method is based on the principle that the melting point of the solid is highest when it is perfectly pure. When two samples A and B have the same melting points, melting points of A alone, B alone and a mixture of an equal amount of A and B are to be determined. If the three melting points are equal, A and B are proved identical.

In practice, there were several complications. Melting points are not necessarily sharp, and they tend to melt in a range of temperatures. Hence, it is not easy to tell whether two melting points are equal or not. The method and its theory are, however, simple and clear, and had been used as a dependable means for identification for many years.

### (b) Use of solid derivatives

When the sample is a liquid or a gas, the mixed-melting-point method cannot be used. If the

liquid or the gas sample has a reactive functional group, this sample may be able to be converted to a solid which may yield nice crystals. Aldehydes and ketones, which are very important in organic chemistry, tend to be a liquid when the molecular weights are low. In such cases they were usually converted to crystalline derivatives that were easier to handle for structural determinations. The reagents which can react with aldehydes and ketones are, for instance, hydroxylamine $NH_2OH$, hydrazine $NH_2NH_2$ and phenylhydrazine $C_6H_5NHNH_2$. The last compound was famous because the German chemist Emil Fischer (1852-1919) employed this in his successful research on sugars. Some reactions for obtaining crystalline derivatives are shown below.

$$\underset{\text{acetaldehyde}}{CH_3CHO} + \underset{\text{hydroxylamine}}{NH_2OH} \rightarrow \underset{\text{acetaldoxime}}{CH_3CH{=}NOH} + H_2O \qquad (13.1)$$

$$\underset{\text{acetone}}{(CH_3)_2C{=}O} + \underset{\text{phenylhydrazine}}{C_6H_5NHNH_2} \rightarrow \underset{\text{acetone phenylhydrazone}}{(CH_3)_2C{=}NNH\,C_6H_5} + H_2O \qquad (13.2)$$

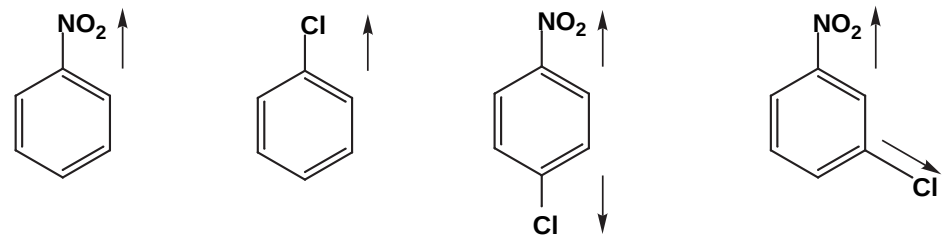
Crystalline derivatives could be used for structural determinations of unknown compounds. The procedure was the same as described above.

(c) **Comparison with physical properties**

Other physical properties such as boiling points, refractive indices, dipole moments and specific rotation for optically active compounds can give useful information. Such data provide information on the properties of the whole molecule. Sometimes, the properties of the whole molecule can be a sum of contributions from various parts of the molecules. In such cases, information on a part of the molecule can be obtained. As an example, the use of the dipole moment μ will be given.

The dipole moments of nitrobenzene (3.98 D) and chlorobenzene (1.58 D) are obtained by experiments, and their directions are determined by the electronic properties of functional groups (such as electronegativity) (Fig. 13.1(a)). When discussing the dipole moments of organic compounds, the C-C and C-H bond moments are assumed to be zero. Hence the dipole moments of these molecules are essentially due to their bond moments of the functional groups.

The dipole moments of two chloronitrobenzene isomers are 2.50 D and 3.40 D. Since the bond moments can be treated as vectors, the dipole moments of chloronitrobenzenes are the vector sum of the dipole moments of nitrobenzene and chlorobenzene. Thus, from the observed dipole moments, two chloronitrobenzenes have been identified as *p*- and *m*-isomers as shown in Fig. 13.1 (b).




| nitrobenzene | chlorobenzene | *p*-chloronitrobenzene | *m*-chloronitrobenzene |
|---|---|---|---|
| μ=3.98 D | μ=1.58 D | μ=2.40 D (calculated)<br>μ=2.50 D (observed) | μ=3.47 D (calculated)<br>μ=3.40 D (observed) |

**(a)** **(b)**

**Fig. 13.1** Dipole moments of substituted benzene derivatives
Comparison between calculated and observed values clearly indicates the relative orientation of the substituents.

**(d) Qualitative reactions**

Structure determinations of organic compounds generally include two approaches. On the one hand, gross structural information about the molecule is collected by determining molecular weights, elemental analysis, *etc*. On the other hand, information on the type and number of functional groups present in the molecule is to be obtained. Thus, information about the molecule as a whole and that of its substituents is gathered side by side.

Before the advent of spectroscopy, the identification of functional groups depended solely on the use of their chemical reactivity. A typical example is the detection of carbonyl groups (aldehydes >CHO and ketones >C=O) by means of the silver mirror reaction and Fehling's test.

Today such a method is never used to detect an aldehyde in any research laboratory. However, such reactions are still very important for educational purposes. Moreover, some classical color reactions are still in use. A good example is the ninhydrin reaction, which even now is very useful to analyze amino acids.

## 13.2 Spectroscopic methods

Use of **spectroscopy** as a means of structural determination of compounds has a long history. The popular **flame reaction** was based on the same principle as spectroscopy. In the middle of the 19$^{th}$ century, the German chemist Robert Wilhelm Bunsen (1811-1899) and the German physicist Gustav Robert Kirchhoff (1824-1887) collaborated in developing the spectrometer (Fig 13.2). With the aid of this new device, they successfully discovered two new elements, rubidium and cesium. Later this device was used by many chemists to discover other new elements such as gallium, indium and the rare earth elements. Spectroscopy played an important role in the discovery of the rare gases.

The method of investigating with the aid of **spectrometer**s is called **spectrometry**. With any type of light source, a spectrometer consists of the light source, a prism, a sample cell, a detector and a recorder. The role of the prism is to separate the panchromatic light from the light source into monochromatic light, and thus plays a key role for the spectrometer.

In the modern spectrometer, the incident light on the sample changes its wavelength continuously. The results of experiment are expressed in a **spectrum** where the abscissa gives the wavelength (or wavenumber or frequency) of the incident light and the ordinate gives the amount of energy absorbed by the sample.

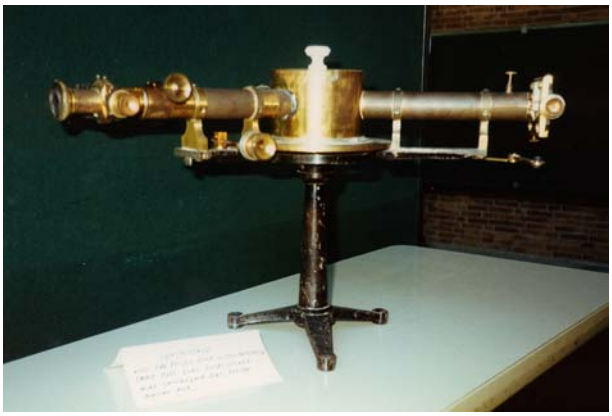


**Fig. 13.2** The spectrometer made by Bunsen and Kirchhoff
At first the detector was extremely simple (human eyes).
Later photographic plates were extensively used.

**(a) UV-VIS spectroscopy**

Generally spectroscopy with ultraviolet light (UV) and that with visible light (VIS) are treated together since sometimes the two measurements are carried out at the same time. Since the UV-VIS

spectroscopy handles with such a high-energy process as the transition of electrons in the molecules, what is obtained tends to be information about the molecule as a whole rather than of its parts. On the other hand, this method is very sensitive and hence very suitable for analytical purposes. Moreover, UV-VIS spectroscopy is very quantitative and the amount of light absorbed by the sample is given by the Lambert-Beer law. According to this law, the absorbance of the sample solution is proportional to the length of the optical path *d* and the concentration of the solution *c*.

**The Lambert-Beer law**

$$\log_{10} (I_0/I) = \varepsilon cd \qquad (13.3)$$

where ε is the molar extinction coefficient, which is characteristic of the solute in question given identical conditions for the measurement. $I_0$and $I$ are the intensity of light after passing through the same thickness of pure solvent and of the solution. $I/I_0$is called the transmittance $T$.

**Sample exercise 13.1** The Lambert-Beer law

An aqueous solution of compound X is placed in a cell of 1 cm thickness and its absorption of light (λ = 366 nm) was determined at four different concentrations. The observed transmittances are listed in the following table.

| $c$ ($10^{-4}$ mol dm$^{-3}$) | 0.80 | 1.20 | 1.60 | 2.00 |
|---|---|---|---|---|
| $T$ | 0.420 | 0.275 | 0.175 | 0.110 |

Calculate the molar extinction coefficient of X.

**Answer**

$A = \log_{10} (I_0/I)$ values at each concentration are given below.

| $c$ ($10^{-4}$ mol dm$^{-3}$) | 0.80 | 1.20 | 1.60 | 2.00 |
|---|---|---|---|---|
| $A$ | 0.377 | 0.561 | 0.757 | 0.959 |

A linear relation is obtained when $A$ is plotted against $c$, which indicates that the Lambert-Beer law is applicable in this case. The slope of the plot is *ca*. 4.9 x $10^3$ dm$^3$ mol$^{-1}$. Hence ε = (slope)/$d$ = 490 dm$^3$ mol$^{-1}$.

By measuring the transmittance of the sample solution, it is possible to determine its concentration with the aid of the Lambert-Beer law. Since UV-VIS spectroscopy is very sensitive and the spectrometer can be made in a very small size, this method is particularly useful in environmental analysis, and particularly suitable for fieldwork.

The Lambert-Beer law is valid regardless of the wavelength of the light absorbed by the sample. On the other hand, the wavelength absorbed by the sample depends on the structure of the sample. Hence UV-VIS spectrometry can be used as a means of structural determination. As early as 1876, the Swiss-German chemist Otto Nikolaus Witt (1853-1915) proposed an empirical theory on the color of substances (which is determined by the wavelength of the absorbed light) and the structures of their component parts. According to this theory, all colored compounds have some unsaturated functional groups such as those given in Fig. 13.3. Such functional groups are called **chromophores**. All well-known dyes and pigments have some chromophores.

There are some other factors to be considered with regard to the color of compounds. The length of linear conjugation is one of the important factors. For instance, the red color of β–carotene (Fig. 13.4) originates from the conjugated system, and its color agrees well with the results of quantum chemical calculations.

There are some functional groups, such as –$NR_2$, -NHR, -$NH_2$, -OH and –$OCH_3$, which have the effect of deepening the color due to the chromophore. These are called **auxochromes**.

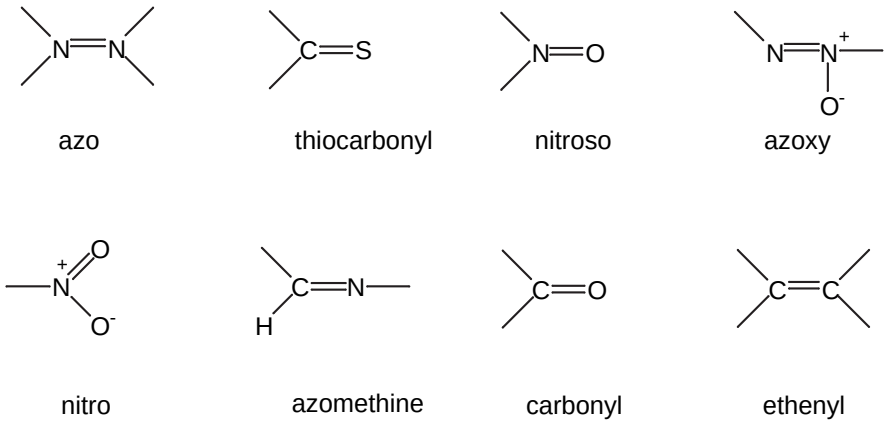



**Fig. 13. 3** Chromophores

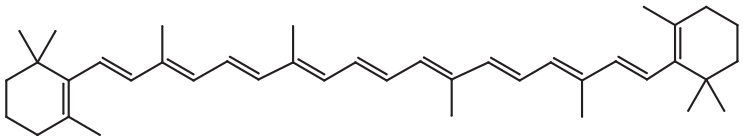


**Fig. 13.4** The structure of β–carotene.
The red color of carrots and tomatoes is due to the long conjugated system.

It is, however, impossible to infer the structure of compounds from their color or the wavelength of the light absorbed by them.

**(b) Infrared (IR) spectroscopy**

As compared with the wavelengths of ultraviolet and visible light, those of infrared light are longer and hence have lower energy, which correlates with molecular vibration. The molecule is excited in a manner that depends on the wavelength of the absorbed light. Stretching and bending vibrations are the important modes of excitation and these correspond to the infrared light with wave numbers (the number of waves contained in a unit length) in the range 1200-4000 $cm^{-1}$.

Almost all organic functional groups have characteristic absorptions at almost constant wave numbers in the indicated region. Hence the region is called the functional group region, and the absorption the **characteristic absorption**. Fig. 13. 5 shows IR spectra of three compounds with a carbonyl group. All of them have a strong absorption within the range of 1700-1750 $cm^{-1}$.

The wave number of carbonyl stretching is different to some extent among aldehydes, ketones and caroboxylic acids, which indicates that careful analyses of the wave number of these characteristic absorptions may provide some information of the structures of component parts. In Table 13.1 representative characteristic absorptions are tabulated. Characteristic absorptions are indeed rich sources of information, but you have to remember that the strength of absorption does not yield any quantitative information. In this regard, IR spectroscopy is qualitative in nature, which is different from UV-VIS and NMR spectroscopy.

As is clear in Fig. 13.5, in the region below 1600 $cm^{-1}$, there are in general many peaks that correspond to the overtones and combination tones of some absorption, in addition to the stretching and bending frequencies of some single bonds. Though it is difficult to assign each peak, the general pattern is characteristic of that compound like human fingerprint. Thus, this region is called the **fingerprint region**. You must remember that agreement of the IR spectra of two compounds is unequivocal evidence that the two compounds are identical. Since the comparison can be made with the spectrum already recorded, there is no necessity for a standard sample that is essential for the mixed-melting-point test. The mixed-melting-point test has been seldom used since the advent of the IR spectroscopy.

As for the richness of information on the structure of organic compounds, it must be admitted

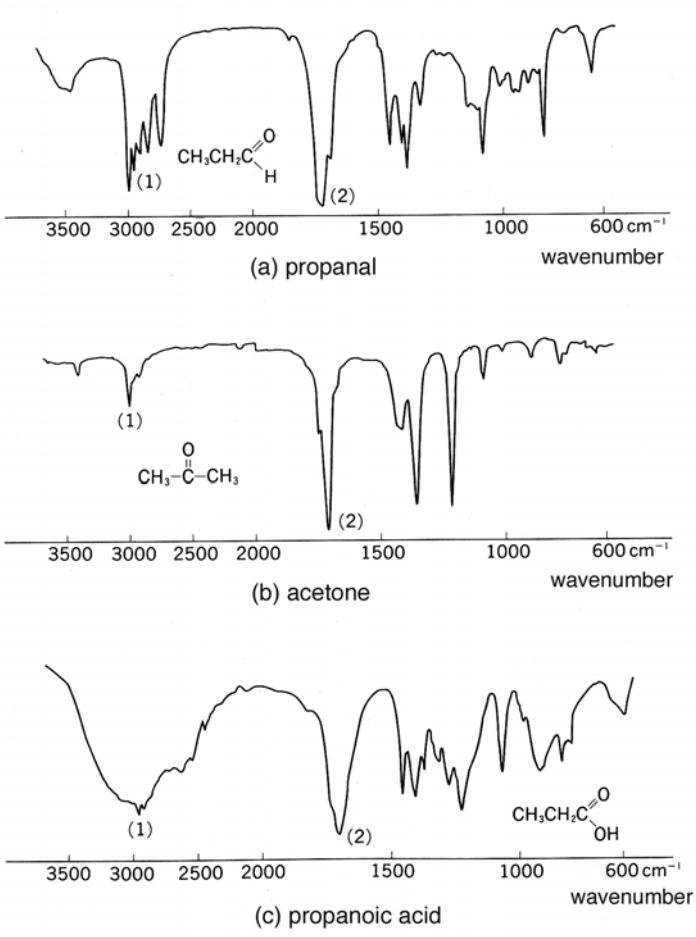



**Fig. 13. 5** IR spectra of three carbonyl compounds.
(a) propanal $CH_3CH_2CHO$; (b) acetone $CH_3COCH_3$; (c) propanoic acid $CH_3CH_2COOH$

**Table 13.1** Representative characteristic absorption

| assignments | type of compounds | region of absorption($cm^{-1}$) |
|---|---|---|
| C-H | alkane | 2850-2960, 1350-1470 |
| C-H | alkene | 3020-3080, 675-1000 |
| C-H | aromatic | 3000-3100, 675-870 |
| C-H | alkyne | 3300 |
| C=C | alkene | 1640-1680 |
| C≡C | alkyne | 2100-2260 |
| C=C | aromatic ring | 1500-1600 |
| C-H | alkane | 2850-2960, 1350-1470 |
| C-O | alcohol, ether, carboxylic acid, ester | 1080-1300 |
| C=O | aldehyde, ketone, carboxylic acid, ester | 1690-1760 |
| O-H | alcohol, phenol(monomer) | 3610-3640 |
| O-H | alcohol, phenol (H-bonding) | 3200-3600(broad) |
| O-H | carboxylic acid | 2500-3000(broad) |
| N-H | amine | 3300-3500 |
| C-N | amine | 1180-1360 |
| C≡N | nitrile | 2210-2260 |
| $-NO_2$ | nitro group | 1515-1560, 1345-1385 |

that IR spectra are inferior to NMR spectra. Nevertheless, IR spectroscopy has remained, and will

remain as one of the most frequently used means to obtain structural information of various types of compounds. The advantage of IR spectroscopy over NMR spectroscopy is that the measurement is so simple and easy, and IR spectra are not as much influenced by the conditions of the measurements.

**Sample exercise 13.2** IR spectra

In Fig. 13.5, the IR spectra of (a) propanal $CH_3CH_2CHO$, (b) acetone $(CH_3)_2CO$ and (c) propanoic acid $CH_3CH_2COOH$ are shown. Assign the peaks indicated as (1) and (2) for each compound.

**Answer**

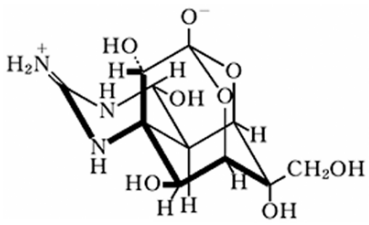
(a) (1) C-H (aldehyde) stretching; (2) C=O stretching
(b) (1) C-H stretching; (2) C=O stretching
(c) (1) O-H stretching; (2) C=O stretching

**Coffee break**

**The structural determination of tetrodotoxin, the poison of puffer fish**

The year 1964 is an unforgettable year in the history of Japanese organic chemistry. The structural determination of tetrodotoxin, the poison of puffer fish, was the most attractive but difficult theme of organic chemistry at that time. Many organic chemists, including Woodward, wrestled with this problem. In that year, the International Conference on Natural Products Chemistry was held in Japan, and three researchers--Woodward, Yoshimasa Hirata (1915-2000) and Kyosuke Tsuda (1907-1999), reported the structure of tetrodotoxin that they had determined. The three structures were identical!

Their success indicated that Japanese organic chemistry merited a top-rating. Woodward used X-ray crystallography extensively, and Tsuda used NMR spectroscopy effectively.




Structure of tetrodotoxin

Yoshimasa Hirata
www.sci.nagoya-u.ac.jp/ pub/rigaku/rigaku-j.html

Kyosuke Tsuda
www.pref.saitama.lg.jp/ A02/BP00/ijin/06.h

## 13.3 NMR spectroscopy

### (a) Principle

Many nuclei (strictly speaking, nuclei in which at least one of the number of protons or that of the neutrons is odd) may be regarded as tiny magnets. The representative nuclei of this kind are the proton ($^{1}$H or H-1) and the nuclei of carbon-13 ($^{13}$C or C-13; natural abundance is *ca*. 1%). Carbon –12 ($^{12}$C), which is the standard for atomic weight, is not a magnet.

When a sample that contains $^{1}$H or $^{13}$C (in fact almost all organic compounds) is placed in a magnetic field, there will arise interaction between the magnetic field and these tiny magnets (*i.e*., nuclei). Due to this interaction, the tiny magnets (*i.e*., nuclei) will be divided into two different states (a slightly more stable (+) state and a slightly less stable (-) state) whose energies are slightly different. Since the world of nuclei is microscopic, the energies associated with the nuclei are quantized, *i.e*., discontinuous. The energy difference between two states is given by the following equation.

$$\Delta E = \gamma hH/2\pi \tag{13.4}$$

where $H$ is the strength of the external magnetic field (*i.e*., of the magnet of the spectrometer), $h$ the Planck constant, $\gamma$ a constant characteristic of the type of nuclei. The last constant is called the **gyromagnetic ratio** and for a proton, 2.6752 x $10^{8}$ $kg^{-1}$ s A (A= ampere).

If the sample is irradiated with an electromagnetic wave $\nu$ that corresponds to the energy difference $\Delta E$, *i.e*.,

$$\Delta E = h\nu \tag{13.5}$$

nuclei in the (+) state absorb that energy and are excited to the high energy (-) state. The process in which nuclei in a magnetic field absorb energy (resonate) is called **nuclear magnetic resonance** (**NMR**).

The frequency of the absorbed electromagnetic wave is expressed in terms of $H$.

$$\nu = \gamma H/2\pi \tag{13.6}$$

When the strength of the external magnetic field, *i.e*., of the magnet of the spectrometer, is 2.3490 $T$ (tesla; 1$T$ = 23490 Gauss), $\nu$ is *ca*.1 x $10^{8}$ Hz = 100 MHz. This is in the range of low energy microwaves.

In principle, the frequency of the absorbed electromagnetic wave is determined by the strength of the magnet and the type of nuclei observed. However, a small change in the frequency is induced due to the difference in the chemical environment in which the nucleus in question is located. This change is called the **chemical shift**.

In $^{1}$H NMR spectroscopy, the chemical shift is expressed as the value relative to the absorption frequency (0 Hz) of the standard material tetramethylsilane (TMS) $(CH_3)_4Si$. The chemical shifts of the three kinds of protons of ethanol $CH_3CH_2OH$ are *ca*. 105, 325 and 490 Hz if recorded by a spectrometer with a magnet of 2.1140 $T$ (90 MHz) (Fig. 13.6(a)). Since the absorption frequency of a proton is 0.9 x $10^{8}$Hz (90 MHz), the chemical shifts involved are very minute variations.

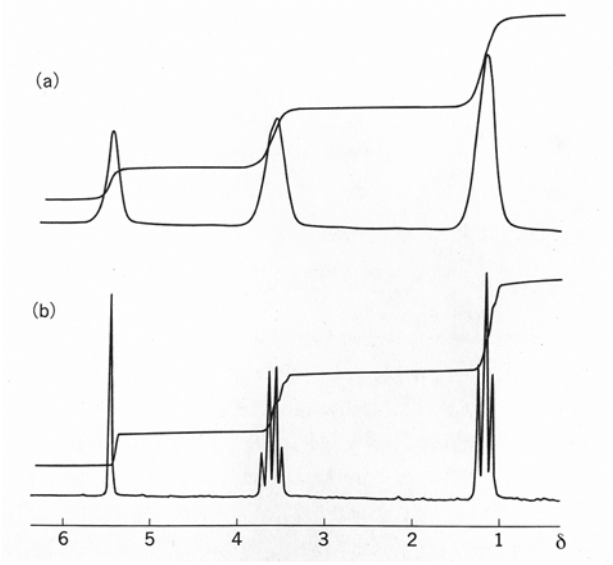



**Fig. 13.6** $^{1}$H NMR spectra of ethanol $CH_3CH_2OH$
(a) low resolution spectrum, (b) high resolution spectrum.
Stepwise lines are integral absorption intensity

The resonance frequency (absorption frequency) of a proton (or any other nuclei) is proportional to the strength of the magnets of the spectrometer. Comparisons of data are complicated when the data is obtained by spectrometers with magnets of different strength. To circumvent this difficulty, the δ scale, which does not depend on the strength of the magnetic field, was introduced. The δ values are defined as follows.

$$\delta = (\Delta\nu/\nu) \times 10^6 \text{ (ppm)} \qquad (13.7)$$

where Δν is the difference in the resonance frequency (in Hz) of the nucleus in question from that of the standard sample TMS (in most cases) and ν the frequency (in Hz) of protons determined by the same spectrometer. You should be aware that *H* appeared in both the denominator and the numerator of the above equation and therefore cancelled. Since Δν/ν values are so small, they are multiplied by $10^6$. Hence δ values are expressed in terms of ppm.

For most of organic compounds δ values of protons are in the range of 0-10 ppm. The δ values of the three peaks of ethanol in Fig. 13. 6 are *ca*. 1.15, 3.6 and 5.4.

The discovery of chemical shifts brought a great advantage for chemistry. Since then NMR spectroscopy has become the most effective means for the structural determination of all types of compounds. Chemical shifts may be regarded as characteristic of the structural component parts. For instance, the proton chemical shifts of methyl groups are *ca.* 1 ppm regardless of the structure of other parts. Furthermore, as shown in Fig. 13.6, in the case of $^{1}$H NMR spectra, the integrated intensity of signals is proportional to the number of nuclei relevant to the signal. This is a great help for the structural determination of organic compounds.

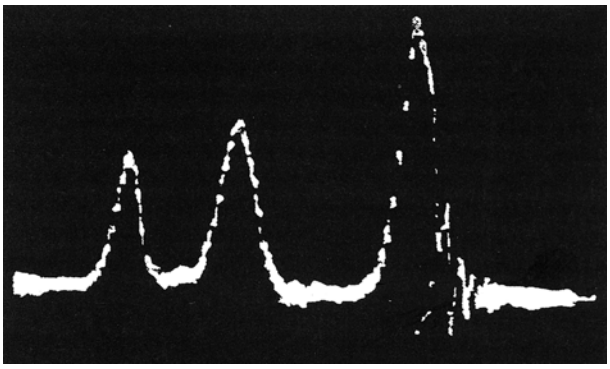
**Coffee break**

**Discovery of chemical shifts**

NMR spectroscopy was initially investigated by physicists who were interested in the magnetic behavior of nuclei. The first observation of NMR signals was made independently and nearly simultaneously by two American physicists Felix Bloch (1905-1983) and Edward Mills Purcell (1912-1987). They jointly received the Nobel prize in 1952.

According to the theory, the resonance frequencies of the proton in water and that in paraffin (hydrocarbon) should be identical as long as the same nuclei, protons, were measured. However, some differences were observed between the values of resonance frequencies of the two samples. A severe question arose as to whether the phenomenon was an essential feature of nature, or was due to some experimental ineptness.

By chance a chemist knew that physicists had this question to be solved, and he advised them to measure the spectrum of ethanol, saying that ethanol has two types of protons, one is water-like and the other paraffin-like. The advice was accepted, and the result was marvelous. Thus, the chemical shift was discovered by the cooperation of physics and chemistry.



Discovery of chemical shifts. $^{1}$H NMR spectrum of ethanol (1951)

**Sample exercise 13.3** $^{1}$H NMR spectrum

Sketch an approximate $^{1}$H NMR spectrum of 1-propanol $CH_3CH_2CH_2OH$, and identify the origin of each signal. This procedure is called an **assignment**.

**Answer**

The pattern of the spectrum is close to that of ethanol except that there is one additional signal due to $CH_3C\mathbf{H}_2$. This signal is expected to appear somewhere between δ 1 and δ 5 in Fig. 13.5. You should notice that protons close to the oxygen atom will resonate at the low field side (*i.e.*, left side of the spectrum).

**(c) Spin-spin coupling**

Even if chemical shifts are the only information NMR spectroscopy can provide, its value as a means of the structural determination of organic compounds is superb. In addition, NMR spectroscopy can provide additional information, that is, information related to the **spin-spin coupling**.

As you have learned, the energy level of nuclei (*e.g.*, proton) splits into high-energy and low-energy states. In addition, these energy states further split due to the interaction with neighboring nuclei (these are also very small magnets). The splitting is very small but has an important feature, namely, that the splitting is not influenced by the strength of the magnet of the spectrometer. It depends only on the magnitude of nuclear-nuclear interaction.

If the $^{1}$H NMR spectrum of ethanol is taken under better conditions (*i.e.*, with a better resolution), the $CH_3$- and $CH_2$-signals split into multiplets (Fig. 13.6(b)). This splitting is due to the spin-spin coupling among protons. Spectra that exhibit splittings due to the spin-spin coupling are called **high resolution** spectra. Spectra that fail to exhibit splittings (Fig. 13.6(a)) are called **low-resolution** spectra

## Exercise

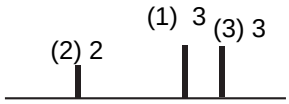
**Question 13.1 Prediction of $^{1}$H NMR spectra**

Draw rough sketches of the low resolution $^{1}$H NMR spectra of the following compounds as bar

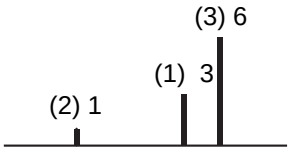
graphs.
(a) ethyl acetate $CH_3COOCH_2CH_3$, (b) isopropyl acetate $CH_3COOCH(CH_3)_2$

**Answer 13.1**

The figures beside parentheses indicate the number of relevant protons.




**(a)** $CH_3(1)COOCH_2(2)CH_3(3)$




**(b)** $CH_3(1)COOCH(2)(CH_3)_2(3)$