1
Introduction to NMR Spectroscopy

Tremendous progress has been made in NMR spectroscopy with the introduction of multidimensional NMR spectroscopy and pulse Fourier transform NMR spectroscopy. For a deeper understanding of the experiment, a little knowledge of quantum physics is required. We will summarize the physical foundations of NMR spectroscopy in more detail in the following chapter. In this chapter, we will introduce the novice reader to the field of NMR spectroscopy in a simple way like we ourselves were introduced to it a long time ago. We will show some simple 1D spectra, and briefly describe what kind of information we can extract from these. For the moment we will assume that the spectra have been recorded by "someone," and we will skip the technical aspects. Later in the book we will discuss all aspects of NMR spectroscopy – experimental, technical, and theoretical – to make you an NMR expert, who can run your own spectra and interpret them skillfully. You should then also have obtained the necessary knowledge for troubleshooting problems during data acquisition. Throughout the book we will introduce you to a subject first in a simple way, and then extend the discussion to more specialized topics and provide a more rigorous explanation.

1.1
Our First 1D Spectrum

Let us jump right into cold water and have a first glimpse at the spectrum of a simple organic compound. As an example we will choose an aromatic compound that is a natural product but produced synthetically on a large scale, called vanillin. So, let us have a first look at the proton spectrum (Figure 1.1).

We notice a number of signals at various places. The signals seem to be of different intensity. If we look a bit more closely, we recognize that lines are split into multiplets (see the expansion). Below the spectrum we find a scale which roughly runs from 0 to 10 ppm. The signals indicated by an arrow belong to the solvent (the signal at 2.5 ppm is from residual dimethyl sulfoxide and the signal at 0 ppm is from the tetramethylsilane standard used for referencing). Otherwise we can count six signals, corresponding to six different types of protons in vanillin. The

Applied NMR Spectroscopy for Chemists and Life Scientists, First Edition. Oliver Zerbe and Simon Jurt.

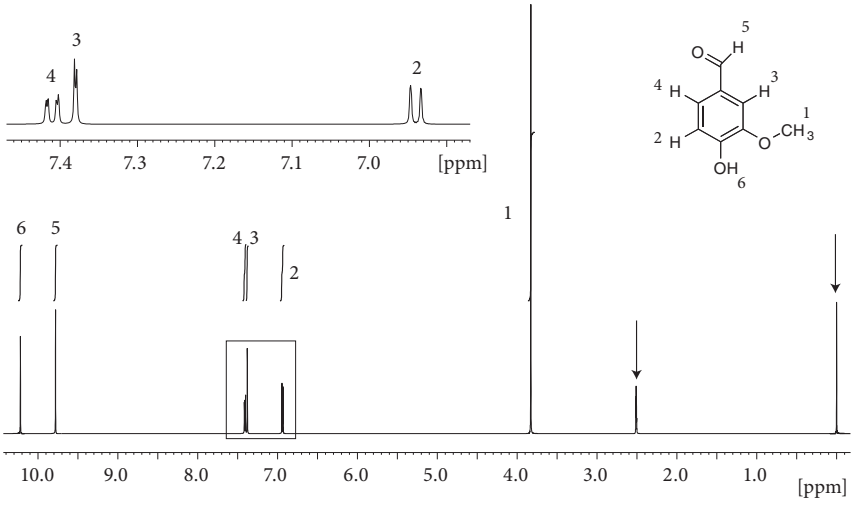

Figure 1.1 Proton NMR spectrum of a simple organic compound. The two arrows point to the standard for referencing (the tetramethylsilane signals) and the solvent line (the dimethyl sulfoxide signal). Integral traces are depicted above the signals. The expansion shows the aromatic protons.

region from 6.9 to 7.5 ppm is expanded in the top panel. To start, let us learn a bit of nomenclature first

1.2
Some Nomenclature: Chemical Shifts, Line Widths, and Scalar Couplings

The phenomenon that the resonance frequency of a nucleus depends on the chemical environment is called *chemical shift*.[1)] The chemical shift is largely determined by the electron density around the nucleus. For practical reasons the chemical shift is given in parts per million relative to a standard. Chemical shifts, in general, are an invaluable source of information for the interpretation of spectra. In principle, they can be computed fairly precisely nowadays using quantum mechanical methods such as density functional theory. What makes chemical shifts really useful is that they are influenced by the presence of functional groups, double bonds, aromatic ring systems, and so on. This has led to elaborate tables of chemical shifts empirically derived from databases. You will find many of these tables in our chapters on proton and heteronuclear NMR, or in textbooks dedicated to that purpose. As a chemist, however, you will need to "memorize" some basic values. If you are

1) The chemical shift was discovered in 1950 by W.G. Proctor and F.C. Yu when they measured the magnetic moment of different types of nuclei. To their surprise they observed two distinct ^{14}N lines for a solution of NH_4NO_3. The same observation was made almost simultaneously by W.C. Dickinson in the case of ^{19}F nuclei.

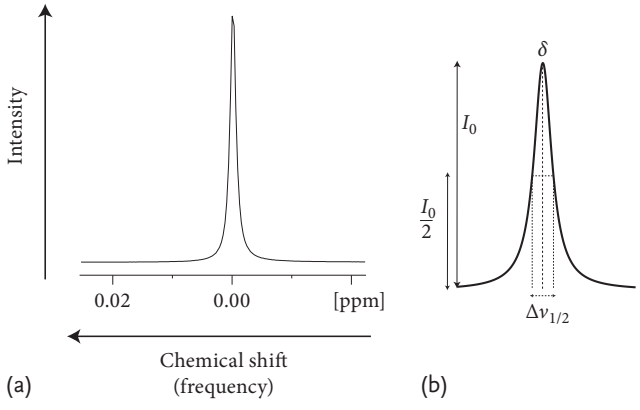

Figure 1.2 (a) A single resonance line. The frequency scale runs from the right to the left. A line with typical Lorentzian shape is depicted in (b).

working on a certain class of compounds, you will become an expert on chemical shifts for these molecules.

Let us now look more closely at a single line (Figure 1.2).

The line has a certain shape, a *Lorentzian* lineform. The signal is symmetric, and the highest intensity denotes the chemical shift position δ. The *line width* of the signal usually refers to the width at half height. Increasing values of chemical shift or frequency are plotted to the left for traditional reasons (note this is different from how it is usually done in physics or mathematics). Although the signals occur at certain frequencies, the frequency scale itself is not drawn, because it depends on the strength of the magnet. Instead, the values are presented in parts per million, which is the difference in frequency from a standard normalized by the frequency of the standard (do not worry, we will see how this scale is derived in more detail later).

Often signals are split into a number of lines (Figure 1.3), sometimes as many as nine or even more. These splittings are called *scalar couplings*, and originate from an interaction of the corresponding proton with neighboring protons, either on the same carbon or on the adjacent carbon(s) or heteroatom.

The center of the multiplet corresponds to the chemical shift δ of that signal. The separation of adjacent lines is called the *scalar coupling constant*, often abbreviated as J. Depending on whether the neighboring carbons are separated by rotatable bonds or whether the bond is sterically fixed, the number of lines due to scalar coupling is $N + 1$ (free rotation about the C–C bond) or 2^N (defined dihedral angle), where N denotes the number of neighboring protons. J is independent of the magnetic field strength and is specified in hertz. The individual lines often have different intensities (see Figure 1.3). Shown on the right of Figure 1.3 is a singlet, a doublet, a triplet, and a quartet. In the case of the quartet, the line intensities are 1 : 3 : 3 : 1. Since the number of lines follows simple rules, it helps us to establish the environment of the proton.

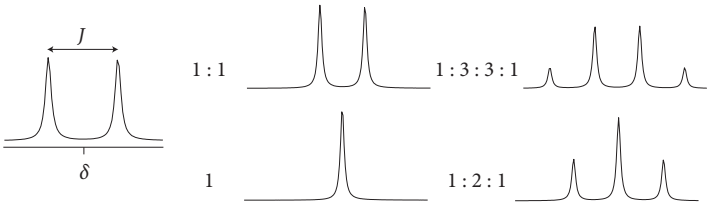

Figure 1.3 Scalar *J* couplings. Typical multiplet patterns for doublets, triplets and quartets are shown.

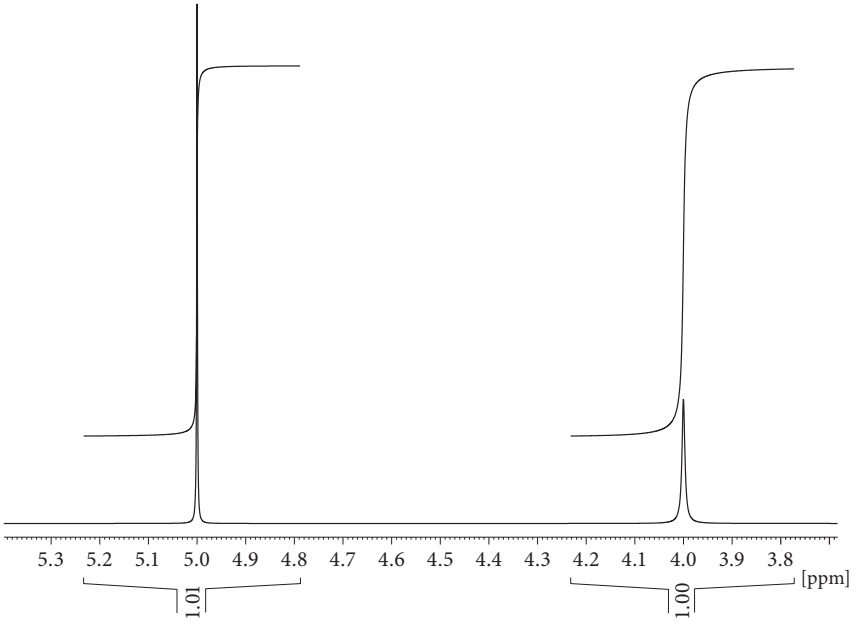

Figure 1.4 The effect of variable line widths. Two lines of very different intensity but the same integral are shown.

The intensity of the signals can be determined by integrating the spectra, and the *integrals* will tell us whether a certain signal is due to one, two, three, or more protons (Figure 1.4).

Integrals can be drawn as integral trails (usually directly on top of the signal) or their value can be plotted below the signal. Figure 1.4 displays two signals of identical integral but very different line width, with the signal at the lower frequency (the one on the right) being less intense. The line width has diagnostic value that is often underappreciated. Some lines become broader than others because the lifetime of the proton in a certain environment is short, a phenomenon due to either chemical or conformational exchange.

Spectra often also contain lines that do not belong to the molecule under study; some of them are referred to as *artifacts*. Such signals can belong to the solvent. In

Fourier transform NMR spectroscopy deuterated solvents are mandatory, but the degree of deuteration is never 100% and residual signal from the nondeuterated form is present. Another signal that is almost always present in proton spectra is the signal due to water, either from residual water in the solvent or because the compound has not been dried completely. Thirdly, a standard is often added for calibrating spectra. In most organic solvents tetramethylsilane is used because the signal usually occurs at one end of the spectrum and does not overlap with the signals of interest. Two-dimensional spectra contain other artifacts that are due to incomplete removal of unwanted coherence pathways, and we will deal with them later.

1.3
Interpretation of Spectra: A Simple Example

To get used to interpreting spectra, and to illustrate the strength of NMR spectroscopy, let us try to elucidate the structure of a small organic molecule. Its ^{1}H spectrum is shown in Figure 1.5.

The spectrum displays a number of signals, and the particular location of the signals, the chemical shift, already tells us a lot about the chemical nature of this molecule. For example, the signals at 7 ppm appear in a range that is typical for aromatic protons. Or, the signal around 3.6 ppm is most likely from a proton in the vicinity of some heteroatom. The signals around 1 ppm are most likely from methyl protons, which is also supported by the integral values of 3 and 6, respectively. Even

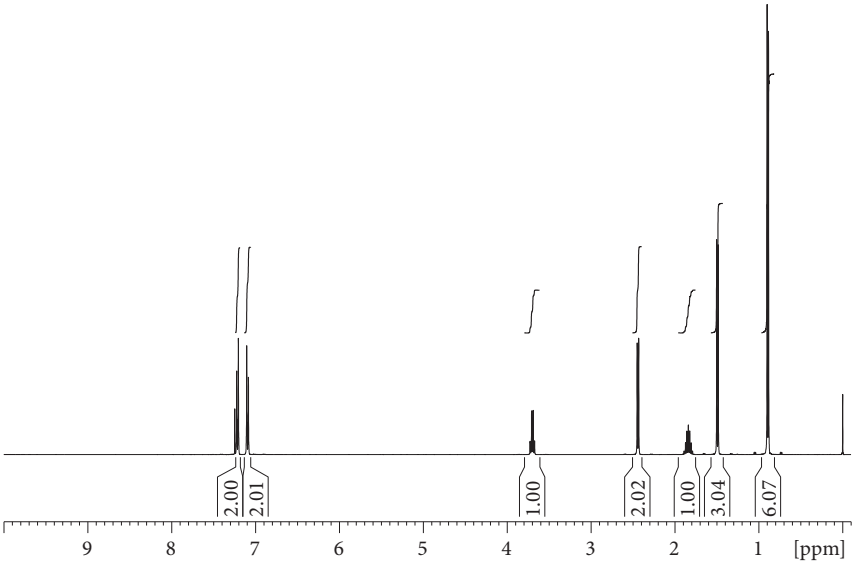

Figure 1.5 Proton NMR spectrum of ibuprofen.

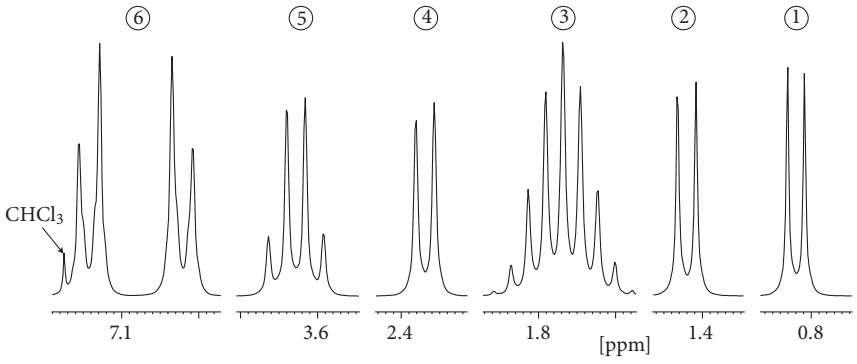

Figure 1.6 Expansions of the proton NMR spectrum revealing the multiplet fine structure of the signals.

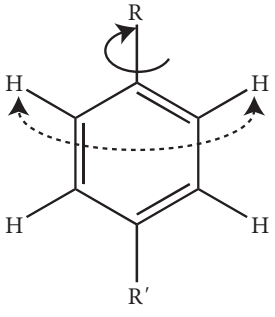

Figure 1.7 Our first fragment. Due to the symmetry of a para distributed benzene only two signals are observed for the four protons.

more helpful is the fine structure of the signals. To see that, let us zoom in a bit on the spectrum (Figure 1.6).

Most of the signals display the usual $(N + 1)$ multiplet pattern expected for protons in freely rotatable chains. The signal group labeled with *6* in Figure 1.6 consists of two doublets, which however, for reasons which will be explained in Section 3.4.2, are somewhat skewed. So let us begin building up the molecule.

We start with the signal group *6* in the range from 7–7.2 ppm. As mentioned before, this is the range typically observed for aromatic protons. The integral of these signals corresponds to 4. Although we do not know much about the chemical nature of the aromatic ring, we assume that it does not contain a heteroatom for the moment, and therefore is most likely derived from benzene. Four aromatic protons (instead of six) therefore indicates that the compound is a *disubstituted* benzene. The next question is whether the π system is 1,2-, 1,3-, or 1,4-disubstituted. In our case it is easy to determine this. We see only two peaks (two doublets). Since we have four aromatic protons, this is only possible if the substitution is such that two protons each become identical because of symmetry (see Figure 1.7). The aromatic ring therefore must be para disubstituted.

We will now try to identify the structure of the two substituents. Let us start with signal *1* at 0.8 ppm. It corresponds to six protons, likely two methyl groups. The

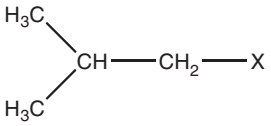

Figure 1.8 Our second fragment, an isobutyl group.

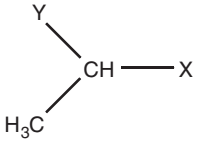

Figure 1.9 Our third fragment.

signal is due to either two distinct methyl groups at quaternary carbons (hence two singlets) or two identical methyl groups bound to a common carbon possessing *one* additional proton (hence two doublets with identical chemical shift). The latter case corresponds to an isopropyl group, for which we expect at least a septet ($6 + 1$ lines) for the CH proton. We say "at least" because the isopropyl group is connected to the remainder of the molecule, and other couplings may be due to the protons from the connecting carbon. In addition, the signal must integrate for *one* proton.

Indeed, if we look very carefully, we see that signal *3* at 1.8 ppm is split into nine lines (the outer lines are fairly weak and can easily escape our attention). This greatly supports the presence of an isopropyl group. Nine lines corresponds to eight protons on neighboring carbons. Since we have identified six already, the isopropyl group must be connected to a methylene (CH_2) group. The methylene signal must display an integral equal to 2, and the only signal that is left with such an integral is the one at 2.4 ppm (*4*). Since this signal is a doublet, and one of the connected carbons is a CH (from the isopropyl group), there cannot be any other CH carbons attached. Maybe this isobutyl fragment (Figure 1.8) is directly linked to the aromatic ring, a guess that must be verified later.

So far we have "explained" the presence of signals *1*, *3*, *4*, and *6*, and there remain two more signals (*2* and *5*). Obviously signal *2*, which integrates for three protons, corresponds to a methyl group. Again, the doublet nature tells us that the methyl group is connected to a CH carbon. That proton signal must have at least four lines and an integral of 1, establishing the quartet *5* at 3.6 ppm as the neighbor. Since the signal has a multiplicity of four, no other CH is connected to that carbon.

If we again assume that this is the other fragment (Figure 1.9) linked to the aromatic ring, we are however missing one substituent, because one carbon has so far only three neighbors. The chemical shift of the proton at that carbon is 3.6 ppm, fairly low and indicating that a heteroatom is close. The full spectrum in addition displays a very broad signal around 10 ppm (we do not see it in Figure 1.5 because it is too broad), possibly from a hydroxyl proton. However, it could also be from a carboxyl group, and we will not be able to distinguish the two possibilities on the basis of the proton NMR spectrum. To resolve this ambiguity, let us have a look at the ^{13}C spectrum (Figure 1.10).

The signal at 180 ppm is due to a carboxyl group. The four signals in the range 125–142 ppm are due to the aromatic ring (two carbons each correspond to one sig-

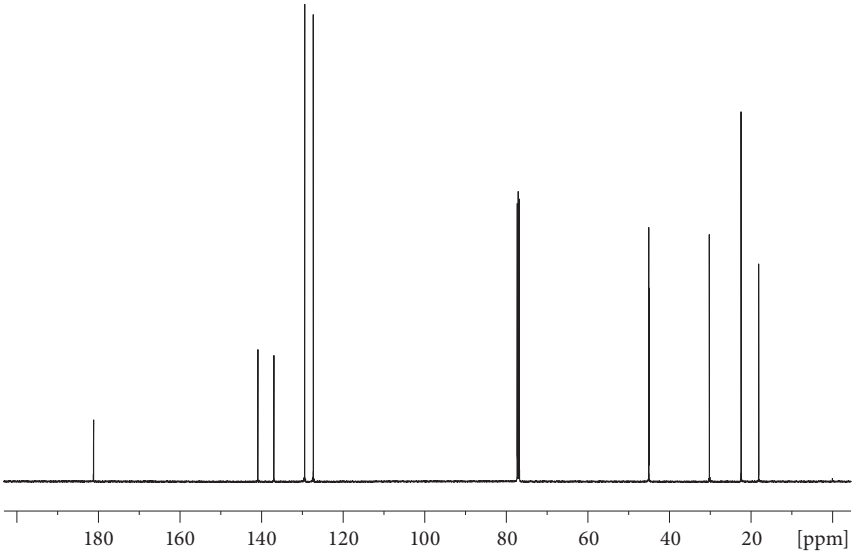

Figure 1.10 The ^{13}C NMR spectrum of ibuprofen.

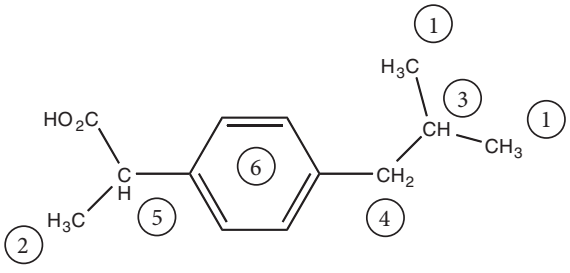

Figure 1.11 The molecular structure of ibuprofen and assignments of the proton signals.

nal due to the symmetry of the para-disubstituted ring). The signal around 77 ppm is from the chloroform solvent, and the four lines are from the other five carbons (the two isopropyl methyl carbons give rise to one signal). The missing fragment is therefore a carboxyl group and the structure of the compound is therefore unambiguously established as 2-[4-(2-methylpropyl)phenyl]propanoic acid (Figure 1.11), also known as ibuprofen, a painkiller that is produced on a multiton scale worldwide.

Of course, this is a very simple case, without any signal overlap. Moreover, the information on couplings and integrals always made the assignments unambiguous, and this is mostly not the case. However, we will see later that with the help of modern methods, in particular 2D NMR spectroscopy, fairly complicated molecules can still be identified unambiguously. However, we need to learn a few things before then so that we can exploit the power of NMR methods fully.

1.4
Two-Dimensional NMR Spectroscopy: An Introduction

The success of modern Fourier transform NMR spectroscopy is intimately linked to the development of multidimensional NMR spectroscopy. Protein structure determination by solution NMR spectroscopy or the elucidation of the structure of complex natural products is impossible without resorting to such methods. In the example of ibuprofen described above, the assignment was only possible in a straightforward fashion using 1D spectra, because at each point only a single resonance could be connected that had the right number of couplings and the correct integral. As soon as the molecules become larger, many ambiguous cases will arise, so further connectivities become unclear. The power of 2D shift-correlation spectroscopy is that the correct correlations can be directly extracted from the spectrum.

Two-dimensional spectra contain two frequency dimensions, and usually these correspond to chemical shifts. In the case of homonuclear spectra (the two frequency axes belong to the same type of nucleus, e.g., two proton frequencies), a diagonal runs through the 2D map, where the frequencies are the same in both dimensions. The really interesting information, however, resides in the off-diagonal, the so-called *cross peaks*. These peaks correspond to different chemical shifts and directly connect coupled nuclei. The exact type of experiment will determine which type of couplings (scalar or dipolar) have been used to establish the correlation. The

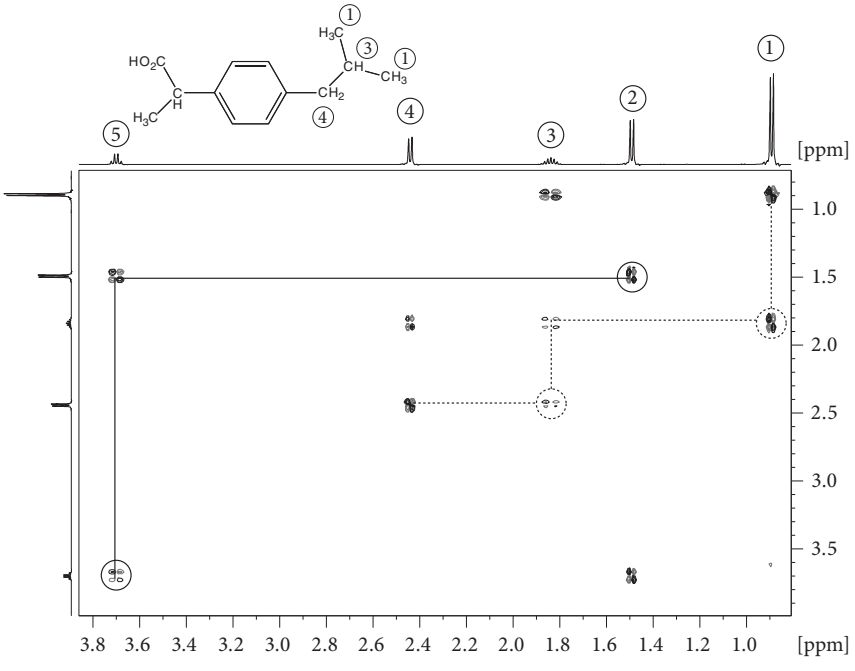

Figure 1.12 Two-dimensional correlation spectroscopy (COSY) spectrum of ibuprofen.

2D spectra are 3D objects, with two frequency dimensions, and the third dimension corresponding to the intensity of the signals. Usually, 2D spectra are displayed in the form of *contour plots*, quite similar to topographic maps, in which different heights (mountains) are indicated by contour lines that connect places of similar height. One of the simplest 2D experiment is the *COSY* experiment, a shift–shift correlation experiment in which correlations occur through scalar (usually vicinal) couplings. In the COSY spectrum in Figure 1.12 we have traced through correlations of the substituents in ibuprofen; the cross peaks are encircled, and the path for the isopropyl fragment is shown by dotted lines.