ORGANIC SPECTROSCOPY

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Summary

A primer about the use of nuclear magnetic resonance and mass spectrometry in organic chemistry is presented with the aim of giving the reader the fundamental notions for investigating the structure of organic molecules.

1. Introduction

With the term “spectroscopy” one usually refers to an ensemble of techniques that exploit some kind of interaction between a compound under investigation and an external perturbation – namely, electromagnetic radiation - and provide information about its chemical composition and/or structure. In its simpler form a “spectrum” (from *specere*, Latin for “look at”) is a chart that shows a certain physical quantity on the horizontal axis (such as the wavelength or the frequency of a transition) and its intensity on the vertical axis. (*See: Chapter A History of Chemistry*). While the word

spectroscopy is normally used when interactions between matter and electromagnetic radiation are involved, the term spectrometry is used in a broader context. One thus says nuclear magnetic resonance *spectroscopy* and mass *spectrometry*, as the latter technique does not require interactions with light of any wavelength.

Three complementary techniques are usually covered by modern textbooks dealing with the application of physical methods in organic chemistry, namely, nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and mass spectrometry (MS). Older books used to add ultraviolet-visible (UV-VIS) spectroscopy to this list. While all of these techniques may be successfully applied to the investigation of some particular aspect of a structural study, in these authors' opinion NMR and MS stand out because of the of amount of clear-cut information they provide. In this article NMR and MS will be described from the point of view of the organic chemist. Simulated and real life examples will be presented with the aim of helping learners to understand which information can be gathered from each of these structural tools.

2. Nuclear Magnetic Resonance

2.1. The Resonance Phenomenon

Many subatomic particles are characterized by a quantum mechanical property called spin. Although there is no exact classic analogy, it is helpful to visualize spin as if a particle could rotate about its axis. Differently from this simplified view, the angular momentum that originates from this "rotation" is quantized and its modulus amounts to $\hbar\sqrt{S(S+1)}$, where $\hbar$ is the Planck constant *h* divided by 2π and *S* is the spin quantum number. Atomic nuclei contain a number of protons and neutrons, both of which have *S* = ½. If a nuclide contains an even number of protons and neutrons its spin is zero and it cannot be observed by NMR. Nuclei with spin different from zero are characterized by a nuclear spin vector **I** with modulus $\hbar\sqrt{I(I+1)}$, *I* being a non-negative nuclear spin number ranging from ½ onwards in steps of ½. Nuclides with $I \neq 0$ possess an intrinsic magnetic dipole moment $\mu = \gamma \mathbf{I}$, where γ is the magnetogyric ratio, a constant characteristic of each nucleus.

If an external magnetic field (B_0) is applied, magnetic moments align in $2I + 1$ quantum mechanically allowed orientations. In the case of I = ½ (typical of ^{1}H, ^{13}C, ^{31}P and ^{19}F, just to name a few common nuclei in organic chemistry) magnetic moments can align in only two ways indicated by the magnetic quantum numbers m_I = + ½ and m_I = - ½, corresponding respectively (for $\gamma > 0$), to the parallel and antiparallel orientation with respect to B_0. While in the absence of B_0 all orientations have the same energy, the presence of the magnetic field removes the degeneracy and two energy levels arise, their energy difference being $\Delta E = \hbar\gamma B_0$. When electromagnetic radiation of a frequency ν such that $\Delta E = h\nu = \hbar\gamma B_0$ is delivered to the sample, the so-called resonance condition is fulfilled and absorption of energy takes place, producing a line in the NMR spectrum. At the magnetic fields available in modern commercial high-resolution spectrometers (4.7 – 21 T) the frequency of the incident radiation (for protons) is in the range 200-900

MHz, *i.e.* in the radiofrequency region of the electromagnetic spectrum. The resonance frequency for protons at a given B_0 is called the operating frequency of the spectrometer.

2.2. Chemical Shift

In molecules nuclei appear inside atoms and as such they are surrounded by electrons that react to B_0 and give rise to an additional induced magnetic field (B_{ind}) opposite to B_0. Each nucleus senses an actual magnetic field equal to $B_0 – B_{ind}$ or, as it is usually expressed, $(1-\sigma)B_0$, where σ is called the "shielding constant". Since the value of the shielding constant depends largely on the variation in electron density in the neighborhood of each nucleus, NMR-active atoms placed in different molecular locations may thus attain resonance at a slightly different frequency as compared to "naked" nuclei of the same kind. This phenomenon is at the basis of the great success of NMR because the resonance frequency of a nucleus is characteristic of a specific chemical environment in which it is located. For example, an electron-withdrawing group decreases the electron density (hence the shielding constant) at a nearby nucleus. This "deshielded" nucleus resonates at a higher frequency than the one necessary for the same nucleus in a not electron withdrawing environment. Likewise, a proton bound to an sp^2 carbon is expected to be more deshielded than a proton bound to an sp^3 carbon, owing to the increased electronegativity of the former carbon resulting from bond orbitals hybridization with an higher s character.

However, a problem arises because the resonance frequency also depends linearly on the applied B_0. For this reason a "chemical shift" (δ) scale in units of part per million (ppm) is used in which the position of each line is given by difference (measured in Hz) from the resonance line of an internal standard divided by the operating frequency of the spectrometer expressed in MHz. The chemical shift scale is independent of the external magnetic field and it is thus convenient for comparing data obtained with different spectrometers. The reference chemical shift standard ($\delta = 0$ ppm) for ^1H and ^{13}C NMR spectroscopy is tetramethysilane (TMS), $(CH_3)_4Si$. TMS has been chosen because it is unreactive, readily soluble in most common solvents and easily removed owing to its low boiling point (28 °C). More importantly, it yields an NMR line that does not overlap with the NMR signals of most organic compounds. In fact, since silicon is less electronegative than carbon, it produces an electron density *increase* at carbon and hydrogen and makes both nuclei more shielded than any other common organic compound. As a result, the ^1H and ^{13}C TMS lines appear at one edge of the corresponding NMR spectrum. A chart summarizing the chemical shifts of carbon and protons associated with the most common functional groups is provided in Figure 1.

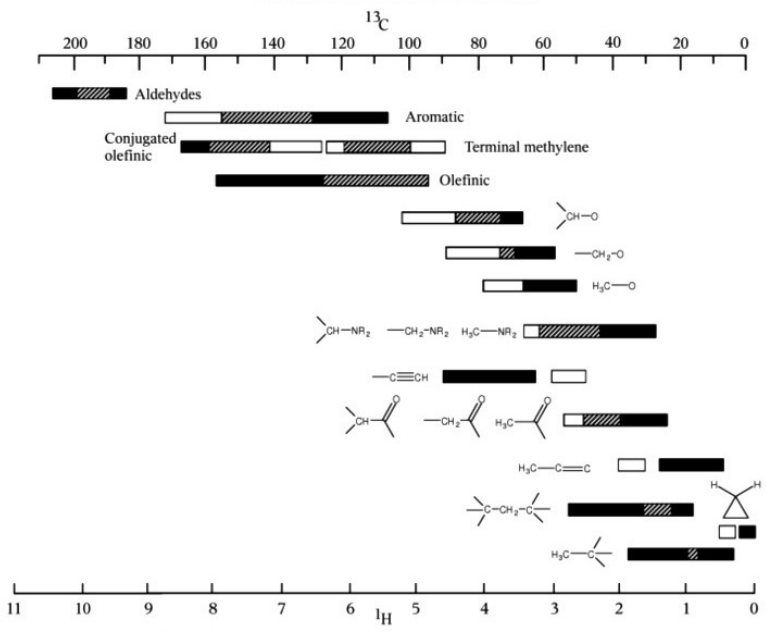

Figure 1: ^{1}H and ^{13}C chemical shift ranges for common proton (white fill) and carbon (black fill) environments in organic molecules. Hatched fill indicates overlap.

By looking at Figure 1 it is clear that the simple reasoning that relates shielding and deshielding only to the inductive effect of the substituents does not hold when groups containing π electrons are present. In particular, one may find quite intriguing the fact that alkynes are more shielded than alkenes, and that (at least for protons) aromatics are on the average more deshielded than alkenes. Indeed, in the first case one would expect this pattern to be reversed, since sp carbons are more electronegative than sp^2 carbons (because of their higher s character) and in the second case one would not expect any difference because of the like hybridization of the carbons. The explanation relies on the so-called anisotropy of the chemical bonds. In fact, as already seen for electrons surrounding a nucleus, also chemical bonds electrons react to the presence of B_0 and give rise to an additional induced magnetic field opposite to the external magnetic field. However, due to electronic circulation in π bonds, the induced fields in one direction are stronger than those in another and therefore their effect on the chemical shift depends on the relative position between the π bonds and nearby nuclei. For example in alkenes electronic circulation induced by B_0 creates a deshielding zone in the plane of the double bond (the induced magnetic field sums to B_0) and a shielding zone above and below the double bond (Figure 2, left). The same effect, albeit reversed in orientation, takes place in alkynes: here the circulating electrons produce an induced magnetic field opposite to B_0 along the axis of the triple bond that shields the acetylenic protons (Figure 2, center). As a result the chemical shift is higher for alkenes than for alkynes protons. Chemical bond anisotropy and deshielding effects due to electronegativity of the substituents may also sum up and give rise to dramatic effects like the huge deshielding observed for aldehydes (Figure 1).

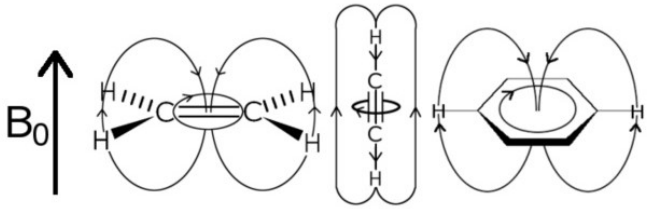

Figure 2: Induced fields produced by chemical bond anisotropy in alkenes (left), alkynes (center) and aromatics (right)

A pronounced effect of chemical bond anisotropy is encountered in aromatics. Here, the electron circulation takes place in the whole π system and creates the so-called ring current which - in turn - produces an induced field with a toroidal shape around the aromatic ring (Figure 2, right).

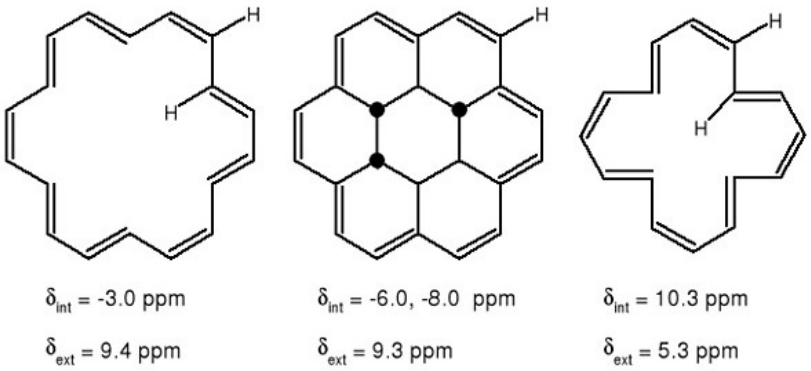

Figure 3: Shielding and deshielding effects produced by the ring current in aromatic compounds (left, center) as compared to a non aromatic ring compound bearing a conjugated π system (right).

The presence of the ring current can be proved by measuring the chemical shifts of complex aromatic compounds like the ones depicted in Figure 3. In these molecules protons exist that are located both in the shielding and deshielding region of space inside and outside the aromatic ring.

For example, [18]-annulene (Figure 3, left) shows two groups of protons (external and internal) with chemical shifts of 9.3 and -3.0 ppm, respectively. While the chemical shift for the external protons is quite typical for aromatic compounds, that of the internal ones is extremely low. This is actually a clear indication for the existence of the ring current. The induced field due to the ring current adds to B_0 in the outer region of the ring and it is opposite to it in the center of the molecule. Therefore, their resonance frequency is shifted upwards or downwards making them appear as deshielded and highly shielded, respectively. The compound in Figure 3 (center) shows the same behavior, but for the fact that now the protons of the saturated central core display a greater low frequency shift (-6 to -8 ppm) because of a more effective conjugation due to an enhanced rigidity of the retained [18]-annulene system. It is interesting to note that non-aromatic compounds like [16]-annulene (Figure 3, right) behave like normal alkenes and show only local anisotropy of the double bonds. Here the induced fields add to B_0 in the plane of the double bonds and, accordingly, all protons appear deshielded.

2.3. Chemical Equivalence and Signal Intensity

All nuclei of the same element that are related by a symmetry element are chemically equivalent and isochronous, i.e. display the same chemical shift. When constitutionally equivalent nuclei (or groups) are considered, three cases can be distinguished:

a) the nuclei are related by a proper rotation axis C_n. In this case they are termed homotopic. Homotopic nuclei are isochronous in both chiral and achiral media. The three protons of chloromethane are an example of homotopic nuclei. Conformationally averaged methyl protons are also homotopic;
b) the nuclei are related only by a mirror plane. For example, methyls 1 and 3 in propan-2-ol are enantiotopic. They are isochronous in achiral media and show anisochrony in chiral media;
c) the nuclei are not related by any symmetry element. They are diastereotopic and anisochronous in any medium. For example, methylene protons (or isopropilic methyls) are diastereotopic in chiral molecules. Note that the same rule applies also in achiral molecules if the molecular symmetry plane is not a local mirror plane. In this case the groups are not related by any symmetry element and are thus diastereotopic. Methylene protons at C_1 and C_3 in glycerol (propan-1,2,3-triol) are diastereotopic for this very reason (Figure 4).

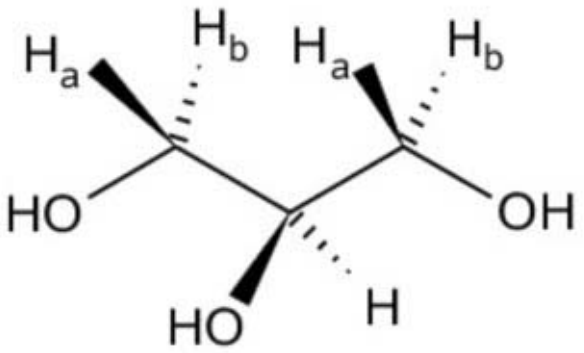

Figure 4: Structure of glycerol. The molecular plane of symmetry passing through C_2 and its bound oxygen and hydrogen is not a local symmetry plane for methylene protons at C_1 and C_3. H_a and H_b are therefore diastereotopic.

The intensity of an NMR resonance is given by the area under each peak. All programs for the analysis of NMR spectra provide integrals of the NMR lines. Ratios among integrals correspond to ratios among the number of isochronous nuclei that give rise to the signals.

2.4. A Simple ^{1}H-NMR Spectrum

In an NMR spectrum frequencies (hence chemical shifts) run from right to left. The TMS line in ^{1}H and ^{13}C spectra is thus at the right edge of the spectrum. In Figure 5 is reported the ^{1}H-NMR spectrum of a simple molecule: 2,4,4-trimethyl-pentan-2-ol. In this molecule four groups of chemically equivalent hydrogens exist, indicated by letters

a-d (note that protons a are homotopic and protons b and c are both enantiotopic). While the chemical shift of protons a is typical for methyls in an alkylic chain (see Figure 1), the protons belonging to methyls c and methylenes b are slightly deshielded, owing to the electron withdrawing effect of the nearby -OH group.

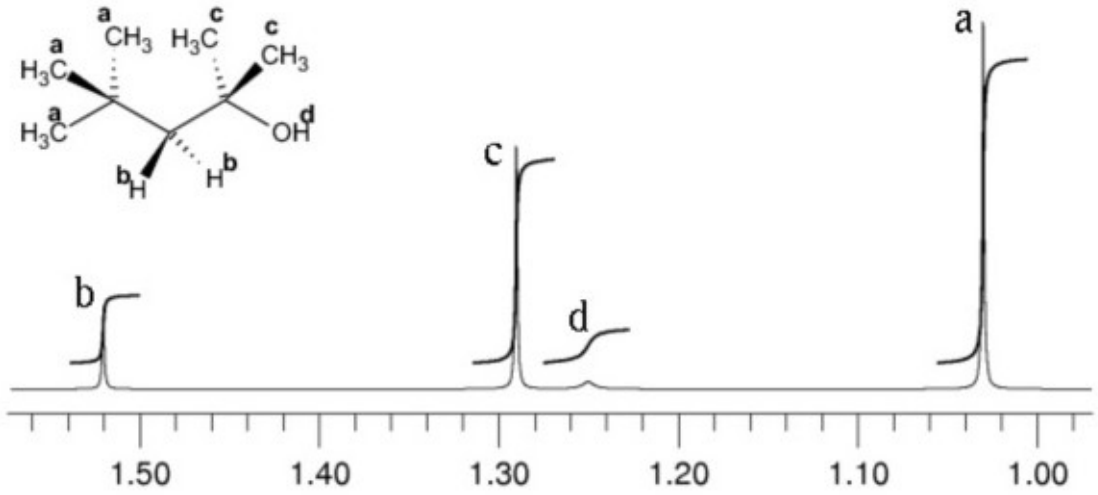

Figure 5: ^{1}H-NMR spectrum of 2,4,4-trimethylpentan-1-ol.

The broad signal of the proton directly bound to the oxygen appears at about 1.25 ppm. Protons bound to oxygen or nitrogen yield usually broader lines than any other line in the NMR spectrum, depending on the extent to which they are involved in hydrogen bonding. This phenomenon also affects chemical shift, which becomes both unpredictable and concentration dependent. Protons of this type are also termed "exchangeable" in that they undergo chemical exchange with other pools of mobile protons (or deuterons) available in the system. An easy experiment for proving the presence of exchangeable protons is that of adding a small amount of deuterated water (D_2O) into the organic solution containing the molecule under study. Owing to chemical exchange, -OH groups (or $–NH_2$ or –SH) are transformed to –OD (or $–ND_2$ or –SD) and disappear from the ^{1}H-spectrum. Deuteration experiments of the same type are often carried out when studying the exposition to the solvent of aminoacids in proteins. Usually a lyophilized protein is dissolved in D_2O and the disappearance of the exchangeable proton signals is monitored as a function of time. Signals belonging to exchangeable protons inside the protein are more involved in hydrogen bond and will take much more time to be substituted by deuterium.

In Figure 5 integrals are also reported. One can easily obtain the number of chemical equivalent protons contributing to each line by measuring the heights of the integrals (for example with a ruler) and dividing each by the smallest one, recalling that in this molecule it amounts to one proton.

2.5. Coupling Constant

The spectrum of 1,2-dichloroethane (CH_3-$CHCl_2$) is shown in Figure 6. This molecule contains two groups of chemically equivalent hydrogens, i.e. the methyl and the methine hydrogens. According to paragraph 2.4 one would thus expect two lines in the

^{1}H-NMR spectrum with a 3:1 integral ratio, whereas six lines are visible instead. However, at a closer look it is clear that two lines are indeed present in the spectrum, each featuring a fine structure which is centered at about the reasonable chemical shifts of 2.05 ppm (the methyl hydrogens are deshielded by the nearby chlorines) and 5.90 ppm (the methine hydrogen is bound to a carbon to which two chlorine are directly attached and it is thus much more deshielded than the methyl hydrogens).

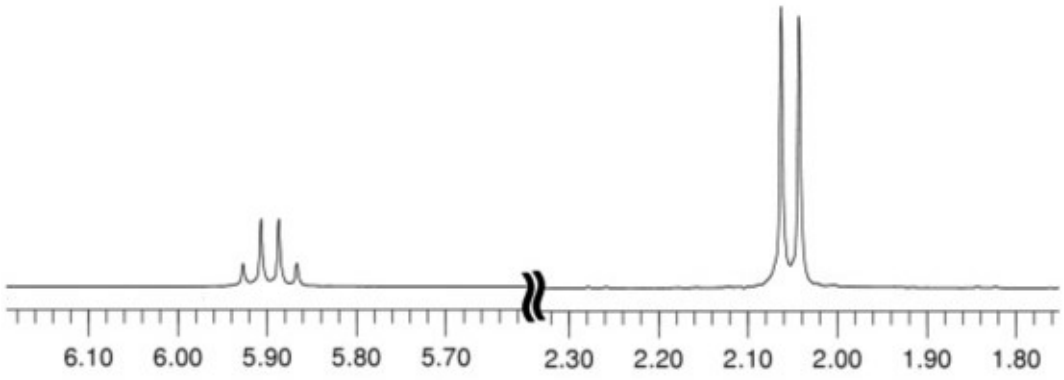

Figure 6: ^{1}H-NMR spectrum of 1,1-dichloroethane

What has not been considered yet is that the magnetic field experienced by each group of chemically equivalent protons depends also on how the surrounding protons align with respect to B_0 and how this phenomenon is transmitted to the sensing protons *via* the chemical bonds. Since protons are I = ½ nuclei, the small magnetic fields corresponding to the two allowed orientation ($m_I = \pm ½$) of the nuclear magnetic moment of the methine proton with respect to B_0 adds to the field experienced by the methyl protons and modifies their resonance frequency by a small positive or negative amount, respectively. Therefore, the methyl signal appears in the form of a doublet. The methyl protons are then said to be *coupled* to the methine; the distance in Hz between the two lines of the doublet is called *spin-spin coupling constant* and it is a measure of the strength of the magnetic interaction between the protons. At variance with resonance frequency, coupling constants do not depend on the magnetic field of the spectrometer. Coupling constants are indicated by the symbol ${}^nJ_{XY}$ where n is the number of bonds between the coupled nuclei *X* and *Y*. From the doublet at δ = 2.5 ppm one measures ${}^3J_{H_1H_2} = 6\ Hz$.

The appearance of the methine quartet at 5.90 ppm can be explained by recognizing that three methyl protons may align in 2^3 different ways (↑↑↑, ↑↑↓, ↑↓↑, ↓↑↑, ↓↓↑, ↓↑↓, ↑↓↓, ↓↓↓), each yielding an effect proportional to its total magnetic quantum number ($\sum m_I$). Accordingly, the resonance line of the methine is split into four lines, whose intensities are proportional to 1:3:3:1 because there are three times more combinations for the two central lines with $\sum m_I = \pm 1/2$ then there are for the two external lines with $\sum m_I = \pm 3/2$. A simple method for predicting the pattern of lines that is produced when a group of equivalent NMR-active nuclei "sees" *n* equivalent *I* = ½ nuclei is shown in Figure 7 (left). Here each line is repeatedly split into two lines and by the same amount (the coupling constant) by each of the *n* equivalent nuclei: *n*+1 lines are

obtained whose intensities are given by the coefficients of the terms of the expansion $(x+1)^n$, also known as the Tartaglia triangle. The same method can be used when a nucleus is coupled to n non chemically equivalent I = ½ nuclei, where in principle n different J's are present (Figure 7, right): in this case a multiplet is formed comprising 2^n lines.

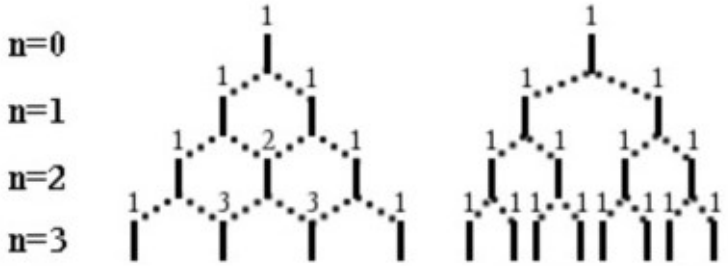

Figure 7: Graphical method for constructing NMR multiplets produced by spin-spin coupling between an NMR active nucleus and up to three I=½ nuclei. If all coupled nuclei have the same coupling constant, the multiplet comprises (n+1) lines whose intensities follow the so-called "Tartaglia triangle". If each coupling constant is different, the multiplet comprises 2^n lines.

In general, coupling constants between isochronous nuclei do not show up in a NMR spectrum but some special cases exist in which two chemically equivalent nuclei do couple with each other and give rise to complex patterns (see paragraph 2.7 for further discussion).

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Biographical Sketches

Mauro Andrea Cremonini was born in 1965 in Bologna (Italy). In 1988 he graduated in Industrial Chemistry cum laude at the University of Bologna. He obtained his PhD in Chemical Sciences in 1992 from the University of Bologna under the supervision of Professor Lodovico Lunazzi and Professor Giuseppe Placucci for work on Electron Spin Resonance studies of organic radicals. From 1993 to 1996 he worked in the group of Professor Claudio Luchinat at the Faculty of Agriculture of the University of Bologna as a Research Associate, dealing with the development of new methods for the determination of protein structures by means of NMR. Since 1999 he is a Researcher at the Faculty of Agriculture of the University of Bologna where he teaches Organic Chemistry and Physical Methods in Organic Chemistry. His work is focused on the application of low and high field NMR for the development of new quality parameters of foods. He is a member of the Italian Chemical Society and secretary of the GIDRM, the Italian Group of NMR Discussions.

Giorgio Bonaga was born in 1945 in Bologna (Italy) and graduated in Agricultural Science (1969) and Chemistry (1971) at the University of Bologna. He is Associated Professor of "Food Toxicology" at the Faculty of Agriculture of the University of Bologna where he teaches "Organic Chemistry of Natural Compounds with Analytical Methods". From 1980 to 1985 he was the Head of the “Interfaculty Center Of Gas Chromatography/Mass Spectrometry” of the University of Bologna. His main research activity is the study of the chemical composition of foods, food contaminants and food additives by analytical methods like GC, HPLC, GC/MS, LC/MS.