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50 and More Essential NMR Experiments

A Detailed Guide

Matthias Findeisen
Stefan Berger

50 and More Essential NMR Experiments

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Matthias Findeisen
Stefan Berger

50 and More Essential NMR Experiments

A Detailed Guide

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Authors

Matthias Findeisen
University of Leipzig
Dept. of Analytical Chemistry
Johannisallee 29
04103 Leipzig
Germany

Stefan Berger
Dept. of Analytical Chemistry
University of Leipzig
Johannisallee 29
04103 Leipzig
Germany

Cover
The cover shows *Strychnos nux vomica*, the tree from which semen strychnine, one of the model compounds used in many of the experiments in this book, is extracted. The picture was taken by the senior author in the botanical garden of Cape town. In addition, the 3D structure of strychnine and the HSQC pulse diagram to observe 2D (^{1}H,^{13}C)-correlation NMR spectra is given.

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Preface

Many of our readers know the earlier books in this series, "*100 and more Basic NMR Experiments*" (**1996**), "*150 and more Basic NMR Experiments*" (**1998**) and "*200 and more NMR Experiments*" (**2004**). Since all books of this highly successful series have been sold out, we have asked ourselves whether it would be worthwhile to further increase the number of NMR experiments in a next edition. This could easily have been done, because NMR is still a very vivid field of science with a steady urge of innovation.

However, in daily discussions with our own co-workers we had to realize that even these people were not able to recall all or even the more important experiments from "*200 and more*". Thus we finally decided on a "*downsizing*" and present in this volume "*50 and more Essential NMR Experiments*", a much smaller collection. We feel that at least these should be known to the experimental NMR operator in a chemical environment. A severe cut concerns solid state NMR and NMR in structural biology, where we provide only 4 examples in this volume, knowing that especially these two fields of NMR are currently the most active ones. However, since this volume is mainly intended for organic and inorganic chemists we feel that this community is better served with a more targetted selection.

By implementing the experiments on your spectrometer, please keep in mind that some of the parameters are strongly machine dependent (e.g. power levels, spectral widths in Hz) and must be modified according to your situation. We reflect the values here as we used them to record the spectra pictured in the book.

Compared with the previous editions there are several changes to note.

(1) Dr. Matthias Findeisen, a physicist, replaces Siegmar Braun who retired several years ago.

(2) Nearly all experiments shown in this volume have been recorded anew, and the cited literature goes to mid 2012. There are a few entirely new experiments not present in the previous volumes. All experiments have been regrouped from a more chemical perspective.

(3) There is a new section **Variants** in which modifications of the actual experiments are mentioned.

(4) There is a new **Question** section at the end of each experiment. We hope that answering these questions will give the reader much more insight. Short answers are provided in the Appendix.

(5) The biggest change is certainly in the layout. Whereas our previous books were solid but rather dry scholarly textbooks, we have this time tried to compose a more modern book with accompanying graphics and photographs, with some historic text clips from celebrities of NMR and theire pictures. We hope that this change will help to raise interest, especially for the young people entering this fascinating field.

From the number of sold copies of our previous books we know that this information is at hand alongside numerous NMR spectrometers in the world, not counting the Chinese edition of "*200 and more*". We certainly hope that the "*50 Essentials*" will be equally successful. We are fairly certain that every experiment works as described, but if not, please complain by email to

Prof. Dr. Stefan Berger
Institut für Analytische Chemie der Universität Leipzig
Linnéstr. 3, D-04103 Leipzig
e-mail: stberger@rz.uni-leipzig.de
Fax: + 49 341-9736115
Internet: http://www.uni-leipzig.de/~nmr/STB

Finally, we have to thank many colleagues for helpful discussions and in particular Mrs. U. Zeller, who provided all the layout ideas and who was responsible for getting all this together. In addition, we thank Dr. P. Tzetkova for recording the data of experiment 3.9 and Dipl. Biochem. A. Beil for recording the data of experiment 6.7.

Leipzig, April 2013

Matthias Findeisen Stefan Berger

Quo innumerabiles libros et bibliothecas, quarum dominus vix tota vita indices perlegit? Onerat discentem turba, non instruit, multoque satius est paucis te auctoribus tradere quam errare per multos.

Lucius Annaeus Seneca (4 bc - 65 ad, De tranquillitate animi, IX, 4.)

What is the use of having countless books and libraries whose titles their owners can scarcely read through in a whole lifetime? The learner is not instructed but burdened by the mass of them, and it is much better to surrender yourself to a few authors than to wander through many.

Translated by J. W. Basore

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Chapter 1

The Organic Set of NMR Spectra

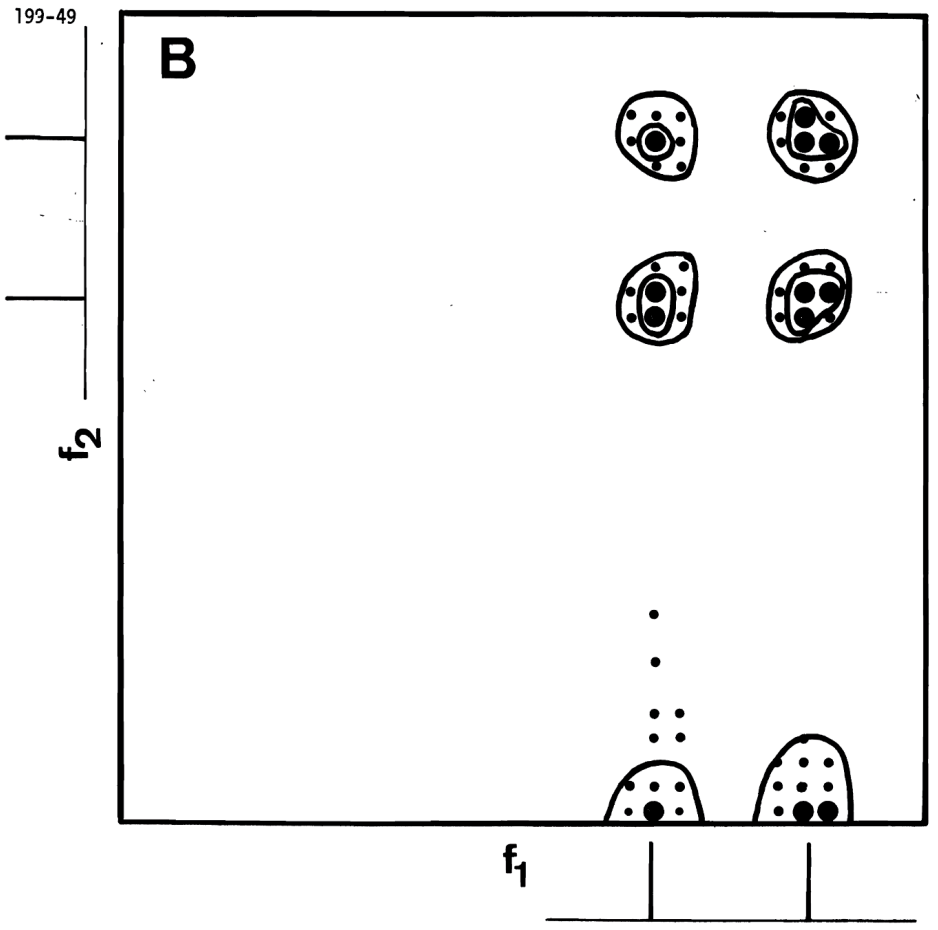
There are six NMR spectral methods, which are usually first measured on a routine basis if an organic chemist has produced a new compound. Usually, this organic set is sufficient for a complete structural elucidation, especially if additional support comes from mass spectrometry and IR- or UV-spectroscopy.

These six methods are:

1.1	^{1}H-NMR		3
1.2	APT-^{13}C-NMR	[**A**ttached **P**roton **T**est]	7
1.3	COSY	[**CO**rrelation **S**pectroscop**Y**]	11
1.4	NOESY	[**N**uclear **O**verhauser **E**ffect Spectroscop**Y**]	17
1.5	HSQC	[**H**eteronuclear **S**ingle **Q**uantum **C**oherence]	23
1.6	HMBC	[**H**eteronuclear **M**ultiple **B**ond **C**orrelation]	29

We describe therefore in this first chapter these six methods in some detail using strychnine as an example. Strychnine with its rather complicated molecular structure provides all the typical problems encountered during spectral assignments in organic chemistry. With concurrent instrumentation and about 20 mg of substance having a molecular weight around 500 Da, the total recording time of these techniques will be about 5 h.

Of course, this book offers much more, but this organic set comprises the most essential of all our essentials.



These spectra are preliminary in several respects. First of all, resolution is severely limited by the available computer memory. A 64x64 data matrix was used. Secondly, the absolute value of the 2D spectrum is plotted disregarding phase information which may be of particular interest. This experiment has several further interesting aspects which we presently are investigating.

Sincerely yours

Richard

Richard R. Ernst

taken from *TAMU NMR Letters*, **1975,** *199*, 49.

Experiment 1.1

^1H NMR Experiment

1. Purpose

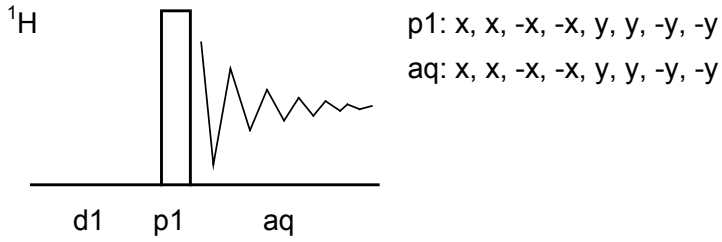
The aim of the standard ^1H NMR experiment is to record a routine proton NMR spectrum in order to get structure-related information for the protons of the sample, i. e. chemical shifts, spin–spin couplings, and intensities. Here we apply this standard procedure to strychnine and discuss different weighting functions and problems of integration.

2. Variants

Variants of this form of NMR spectroscopy include first of all excitation with different pulse angles.

However, since recent NMR instruments are sensitive enough, usually one 90° scan is sufficient to obtain a spectrum. Therefore no considerations about reduced pulse angles are necessary. Second, if a strong solvent signal is present, different forms of signal suppression are available. These are discussed in chapter 3.

3. Pulse Scheme and Phase Cycle



Scheme 1.1-1

4. Acquisition

Special values used for the spectrum shown:

Sample: 3% strychnine in $CDCl_3$.
Time requirement: 1 min
Spectrometer: Bruker DRX-600 with 5-mm-TBI-probe

td:	64K
sw:	15 ppm
aq:	3.6 s
o1:	middle of ^1H NMR spectrum
d1:	2 s
ns:	1

These data will lead to an FID digital resolution of 0.28 Hz/point for the real or imaginary part of the FID.

The prospect of measuring very rapid reaction rates by NMR provided the inspiration for getting an affirmative response from Linus Pauling [then chairman of the division of Chemistry and Chemical Engineering at California Institute of Technology] that "with NMR, we could investigate the borderline between resonance and tautomerism". For example, investigating the NMR spectrum of cycloheptatriene with temperature to see if it existed as a rapidly equilibrating mixture of cycloheptatriene and norcaradiene, or was what later would be called a "monohomobenzene". The argument was persuasive and we soon ordered a 30-MHz Varian proton and fluorine spectrometer.

J. D. Roberts, * 1918 "A Personal NMR Odyssey" *Enyclopedia of NMR*, **1996**, *1*, 590–598.

Common values:

p1: 90° ^1H transmitter pulse
d1: relaxation delay

The appearance of an unsplit methyl signal in a CH_3CH moiety, where the chemical shift difference was large compared to the vicinal coupling constant, expected to be about 7 Hz, subsequently impressed on me the importance of MR spectral analysis. When I understood what was going on, I wrote a paper which was published in 1961 on the nature of the signals of C-methyl groups, and this explained many anomalous "coupling constants" involving methyl groups in steroids and other compounds; it anticipated later research by others on virtual coupling. Although spectral analysis is becoming almost a lost art in the midst of so-called "modern NMR", it is in fact just as useful and necessary as it has ever been, in my own experience.

F. A. L. Anet, * 1926 "A lapsed organic chemist in the wonderland of NMR" *Enyclopedia of NMR*, **1996**, *1*, 187–190.

5. Processing

Use zero filling to si = 64K and exponential weighting with lb = 0.1 Hz, phase correction and referencing to internal TMS, which is the only acceptable reference scheme. The digital resolution of the spectrum will be with these data sw/si = 0.14 Hz/point. Before integration, perform phase and baseline correction on the spectrum accurately. A comparison of the spectra in Fig. 1.1-3 and 1.1-4 shows how a Gaussian weighting function with lb = –1 Hz and gb = 0.3 makes additional small spin couplings visible.

6. Result

The figures show two expansions of the 600 MHz ^{1}H NMR spectrum of strychnine.

A closer inspection of the integrals reveals that the integral of H-4 is too small as compared to all other integrals of the compound. Although the spectrum was recorded with only one scan, the waiting time after the receiver gain adjust command before and the actual measurement was appartently too short (see Question A).

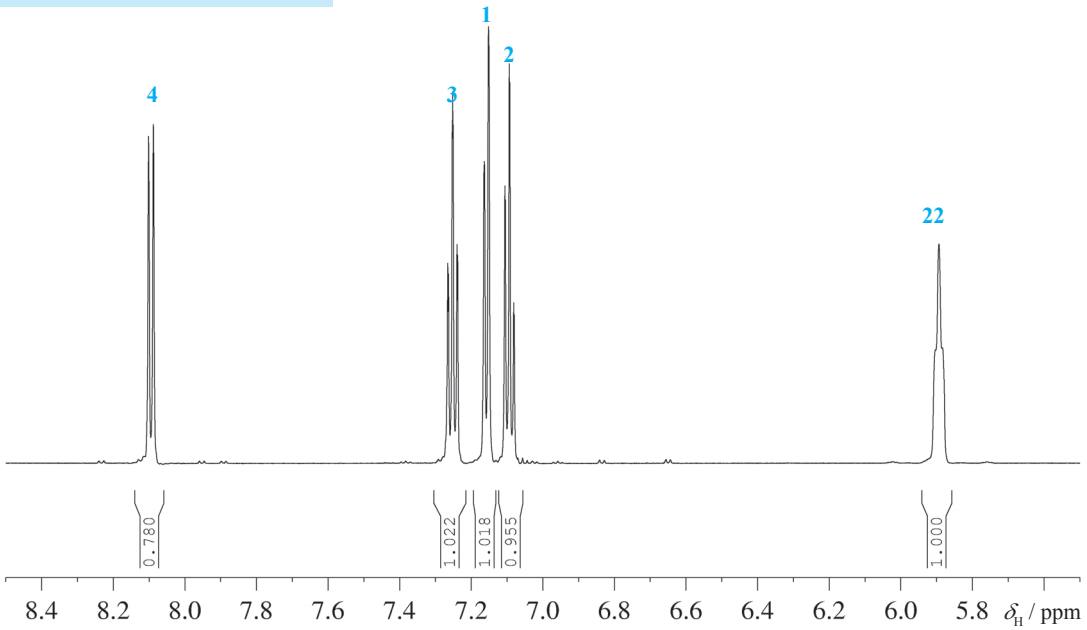
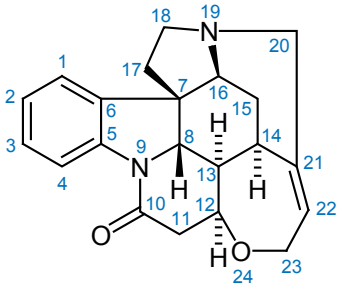


Fig. 1.1-1 Expansion of the ^{1}H-NMR spectrum in the aromatic region



Scheme 1.1-2

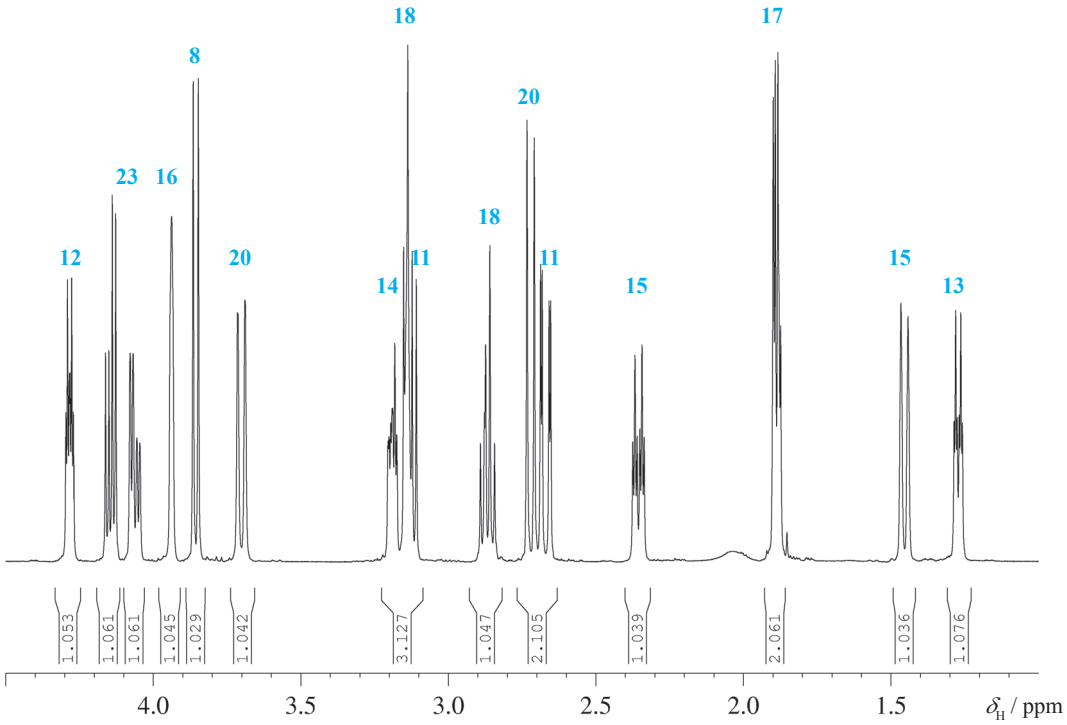


Fig. 1.1-2 Expansion of the ^{1}H-NMR spectrum in the aliphatic region

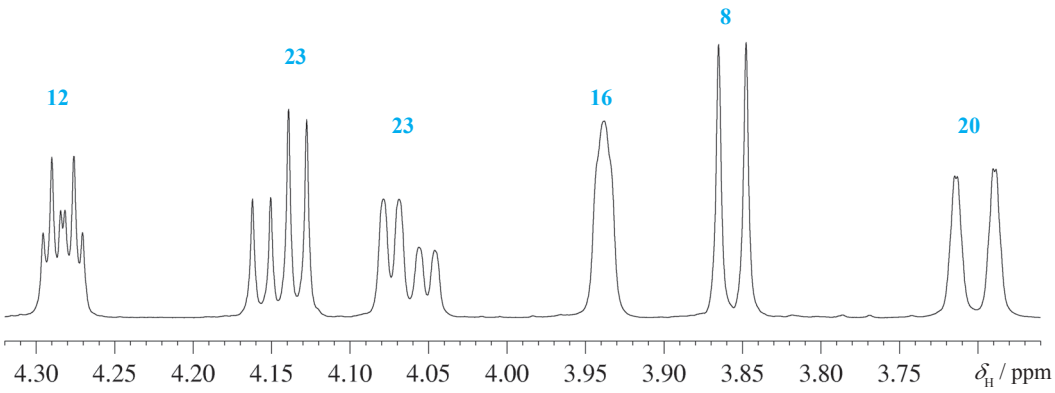


Fig. 1.1-3 Edited with lb = 0.1 Hz (zoom of Fig. 1.1-2)

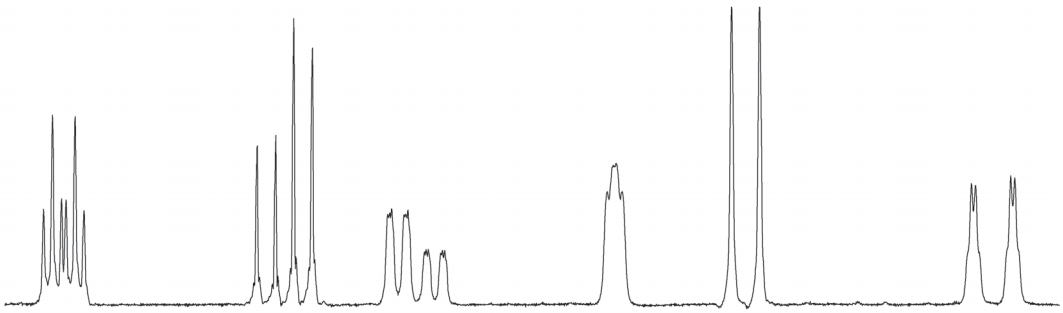


Fig. 1.1-4 Edited with gb = 0.3, lb = –1

7. Comments

The excitation pulse p1 converts the equilibrium magnetization of the ^1H nuclei into a transverse magnetization as shown in Equation (1). During the acquisition time chemical shifts and spin–spin couplings develop in the *x,y* plane, as shown separately in Equations (2) and (3), and are detected by the receiver in the *x,y* plane in quadrature mode.

(1)

$$I_{H_z} \xrightarrow{90^\circ I_x} -I_{H_y}$$

(2)

$$-I_{H_y} \xrightarrow{\Omega I_z t} -I_{H_y}\cos\Omega t + I_{H_x}\sin\Omega t$$

(3)

$$-I_{H_y} \xrightarrow{\pi J 2I_{1z}I_{2z} t} -I_{H_y}\cos\pi Jt + 2I_{H_x}I_{H_z}\sin\pi J t$$

[1] T. D. W. Claridge, "High-resolution NMR techniques in organic chemistry", Pergamon, Oxford, **1999**.

[2] I. K. M. Sanders, B. K. Hunter, "Modern NMR spectroscopy", 2nd Edition, Oxford University Press, Oxford, **1993**.

[3] H. Friebolin, "Basic one- and two-dimensional NMR spectroscopy", 3rd Edition, Wiley-VCH, Weinheim, **1998**.

[4] H. Günther, "NMR Spectroscopy", 2nd Edition, Wiley, Chichester, **1995**.

8. Questions

A. Suggest a reason why the integral of H-4 is considerable smaller than the others.

B. How would one classify the aromatic spin system?

C. The intensity pattern of the signals between 4.2 and 4.0 ppm has a special name?

D. The signals at 2.35 and 1.27 ppm both belong to the methylene group of C-15 and are spin coupled to each other; however, only one has an additional spin coupling. Why?

9. Own Observations

Experiment 1.2

ATP-^{13}C NMR

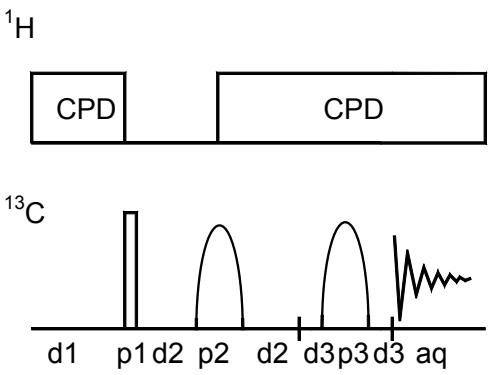
1. Purpose

The aim of a routine ^{13}C NMR experiment is to record a ^{13}C NMR spectrum with proton broad-band decoupling and data accumulation in order to get chemical shift information for structure determination. At the same time one wants to have multiplicity information. From the many schemes proposed we feel the APT (**A**ttached **P**roton **T**est) technique is the most useful and convenient method available, especially if carried out with a chirp 180° pulse on the carbon channel to avoid phase problems at high field spectrometers.

2. Variants

Alternative methods that give information about the multiplicities are INEPT, DEPT, DEPTQ, and PENDANT, and the historic off-resonance ^{1}H-decoupling technique. Unlike INEPT or DEPT, the APT method yields ^{13}C NMR spectra that are only enhanced by the NOE. However, APT also gives information about quaternary carbon atoms. Improved modifications of APT are known [2-4].

3. Pulse Scheme and Phase Cycle



p1: $(x)_4$, $(y)_4$, $(-x)_4$, $(-y)_4$

p2: x, y, -x, -y, y, -x, -y, x, -x, -y, x, y, -y, x, y, -x

p3: x, y, y, x, $(y, x, x, y)_2$, x, y, y, x

aq: x, x, -x, -x, y, y, -y, -y

Scheme 1.2-1

4. Acquisition

Special values used for the spectrum shown:

Sample:	3 % strychnine in $CDCl_3$.
Time requirement:	1 h
Spectrometer:	Bruker DRX-600 with 5-mm TBI probe

Fig. 1.2-1 P. J. Lauterbur (1929-2007)

Common values:

p1: 45° ^{13}C transmitter pulse
p2: 180° ^{13}C CHIRP pulse
d1: relaxation delay
d2: $1/J_{CH}$
d3: switching delay
CPD composite pulse decoupling

Fig. 1.2-2 J. D. Roberts (*1918)

Natural-abundance ^{13}C NMR was not really routine until the introduction of broadband noise proton decoupling. Such decoupling removes the proton splittings from the ^{13}C resonances and, in addition, gives a favorable nuclear Overhauser effect (NOE). Thus, a proton-coupled doublet ^{13}C resonance, such as exhibited by trichloromethane, with broadband decoupling produces a singlet peak with a sixfold increase over the intensity of each of the individual doublets. There is hardly a better early example of the utility of broadband proton decoupling for ^{13}C spectra than for cholesterol. Weigert had found it impossible to make sense out of the 15-MHz coupled spectrum of cholesterol, because of the jumble of resonances and the generally poor signal-to-noise ratio, even after hours of signal averaging. In contrast, with broadband proton decoupling, 25 rather well-separated resonances were observed. A challenge was thus presented of spectral assignments and was met by H. Reich and M. Jautelat in about six months. An important element in the unraveling of the resonances was D. M. Grant's work on the steric influence of axial methyl groups on ^{13}C shifts in cyclohexane rings.

J. D. Roberts, * 1918 "A personal NMR odyssey" *Enyclopedia of NMR*, **1996**, *1*, 590-598.

td: 64K
sw: 200 ppm
aq: 1.0 s
p1: 45° ^{13}C transmitter pulse 6 µs
o1: middle of ^{13}C NMR spectrum
o2: middle of ^{1}H NMR spectrum
p2: adiabatic chirped 180° ^{13}C pulse [crp 60, 0.5, 20.1; 500 µs, 5.5 dB]
d1: 2 s
d2: 6.9 ms corresponding to a J_{CH} = 145 Hz
d3: 100 µs
CPD: WALTZ16 sequence, individual 90° ^{1}H pulse:100 µs at 12 dB
ns = 1024

5. Processing

Use zero filling to si = 64K and exponential weighting with lb = 2 Hz, phase correction and referencing to either internal TMS or via the Ξ scale using the proton spectrum of the same sample.

6. Result

The figure shows the ^{1}H broad-band decoupled APT ^{13}C NMR spectrum of strychnine as obtained on an DRX-600 spectrometer using a TBI probe head. Because of the inverse probe a certain number of scans had to be accumulated. Note that as usual no integration is performed, since under routine conditions the signal areas are not necessarily proportional to the number of ^{13}C nuclei giving rise to that signal. Furthermore, since the d2 delay hits exactly one $J_{C,H}$ value, the others will be scaled in intensity.

The signal of the solvent $CDCl_3$ was adjusted to be negative like the other signals of carbon atoms carrying no protons. Signals of CH and CH_3 groups are positive and signals of CH_2 groups negative.

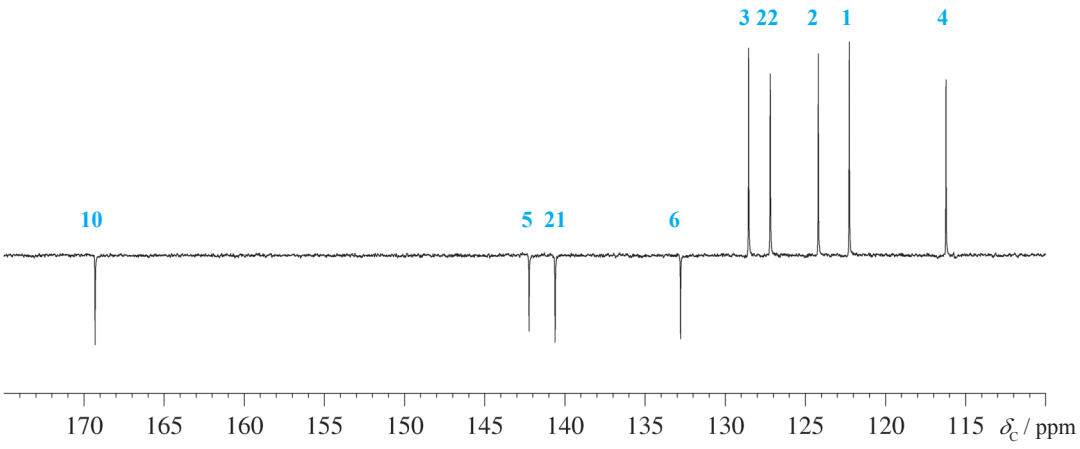


Fig. 1.2-3 Expansion of the ^{13}C NMR spectrum in the aromatic and carbonyl region

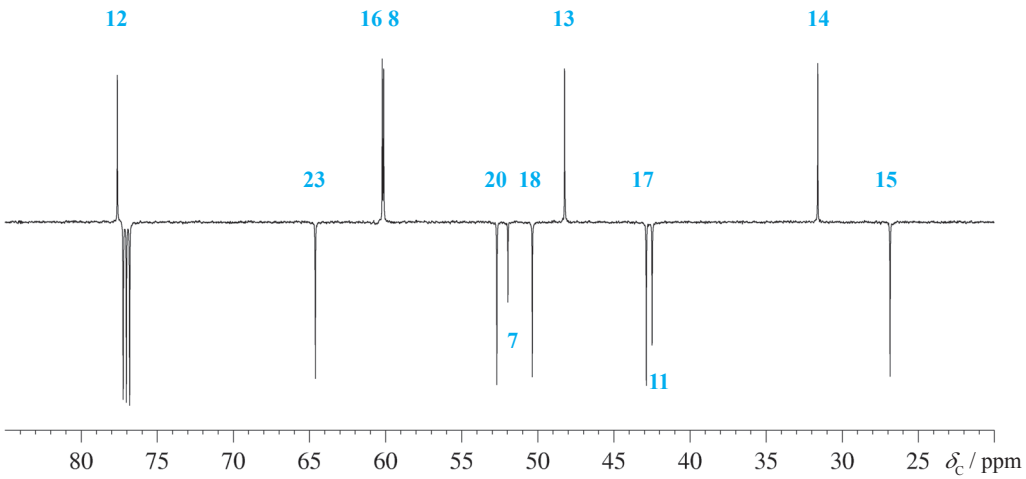
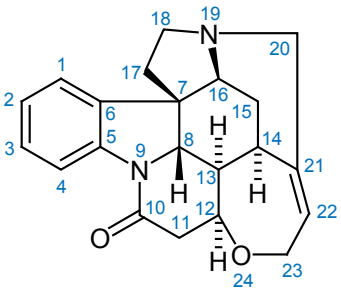


Fig. 1.2-4 Expansion of the ^{13}C NMR spectrum in the aliphatic region

7. Comments

The APT sequence is in principle a double spin-echo experiment. In the first echo period d2 the evolution of *J*-coupling modulates the phases of the signals according to C_q or CH_2 groups respective CH and CH_3 groups. By using a 45° or shorter excitation pulse a part of the initial magnetization remains in the *z*-direction and is inverted to $-z$ by the first 180° pulse. This could lead to a canceling of signals with long spin-lattice relaxation times, but in the second spin-echo period the 180° pulse reinverts the *z*-magnetization, thus eliminating this problem. In comparison with all other editing techniques APT still seems to be the most simple and efficient method, since it gives in one experiment all the necessary information on *all* sorts of carbon atoms. The lower sensitivity compared with polarization transfer methods such as DEPT is in practice not important for the C,H spin pair. See, however, the new DEPTQ experiment where the shortcomings of the traditional DEPT are overcome.

Scheme 1.2-2



It was the time of the Korean War, however, and my deferments ran out. I was drafted and eventually ended up at the Army Chemical Center. where my 'experience' with NMR got me a transfer and assignment to help set up the Varian NMR machine (40 MHz proton and ^{19}F, 17 MHz ^{31}P) which they purchased to support chemical warfare research. There I actually learned something about NMR and even published a few papers. After Army service two options developed: go to Illinois as a graduate student with Gutowsky, or persuade the Mellon Institute to buy an NMR spectrometer. The institute itself declined, but the Dow Corning group took the plunge, at least partly because I claimed that useful ^{29}Si spectra would be possible. I chose that option, visited Varian, and confirmed that ^{29}Si spectra could be seen (with an 8.5 Mc s^{-1} rf unit) and immediately decided that ^{13}C would be even more interesting. I soon published the first paper on ^{13}C NMR spectra. The rest is another branch of personal and scientific history, except that ^{13}C led to interest in spin decoupling and biological polymers, which were both to become essential links in the chain of events leading to 'zeugmatography', as I originally called magnetic resonance imaging.

Paul Lauterbur (1929-2007)
"One Path out of Many – How MRI actually began" *Enyclopedia of NMR*, **1996**, *1*, 445–449.

[1] H.-O. Kalinowski, S. Berger, S. Braun, "Carbon-13 NMR spectroscopy", Wiley, Chichester, **1988**.

[2] T. D. W. Claridge, "High-resolution NMR techniques in organic chemistry", Pergamon, Oxford, **1999**.

[3] K. M. Sanders, B. K. Hunter, "Modern NMR spectroscopy", 2nd Edition, Oxford University Press, Oxford, **1993**.

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[5] J. C. Madsen, H. Bildsøe, H. J. Jakobsen, O. W. Sørensen "ESCORT editing. An update of the APT experiment" *J. Magn. Reson*. **1986**, *67*, 243–257.

[6] A. M. Torres, T. T. Nakashima, R. E. D. McClung "Improved *J*-compensated APT experiments" *J. Magn. Reson*. Ser. A **1993**, *101*, 285–294.

[7] U. Beckmann, W. Dietrich, R. Radeglia "ORSAT and modifications of SEFT and APT" *J. Magn. Reson.* **1999**, *137*, 132–137.

8. Questions

A. What is understood by the "Ernst angle"?

B. Which parameter controls the correct phases obtained in this experiment?

C. What is meant with a WALTZ16 sequence?

D. The signals of $CDCl_3$ show a 1:1:1 triplet with a spin coupling of 31.7 Hz. Explain and calculate from this the spin coupling found in $CHCl_3$.

E. Looking at the spectrum and the chemical formula of strychnine, you should be able to assign at once at least two carbon atoms. Which ones?

9. Own Observations

Experiment 1.3

COSY

1. Purpose

The COSY (**CO**rrelation **S**pectroscop**Y**) pulse sequence generates a 2D NMR spectrum in which the signals of a normal ^{1}H NMR spectrum are correlated with each other. Cross-peaks appear if homonuclear spin coupling is present; thus the COSY sequence detects coupled pairs of protons (or pairs of other nuclei such as ^{19}F, ^{31}P or even ^{13}C in the case of labelled proteins). Since coupled protons are usually separated by two or three bonds, the connectivity and very often a chemical structure can be derived from the COSY spectrum; however, one must be also aware of long-range spin couplings. The COSY sequence is the most important and most frequently used 2D NMR experiment.

2. Variants

Due to the importance of the COSY technique an impressive number of variants has been developed in the last 40 years. The current Bruker pulse sequence library, for example, contains more than 30 different applications, and it is impossible to discuss them all in this book. We show here a COSY sequence which includes a gradient-selected double quantum filter and the echo-antiecho scheme for the phase sensitive frequency generation in the indirect dimension. The reason for this selection is that in COSY one usually wants to see and interpret the interaction of two protons with each other. The double quantum filter suppresses singlets and reduces multiplets to AX patterns; the echo-antiecho scheme provides phase sensitive spectra.

Other important variants of COSY which should be mentionend are:

(1) The long-range COSY, which emphasizes connectivities caused by small spin-coupling constants. This is important in the case of allylic or W-spin couplings < 2 Hz.

(2) The COSY-45 or the E.COSY methods, which lead to slim diagonals and allow the determination of the sign of spin-coupling constants.

(3) All COSY variants may be combined with different schemes of water suppression.

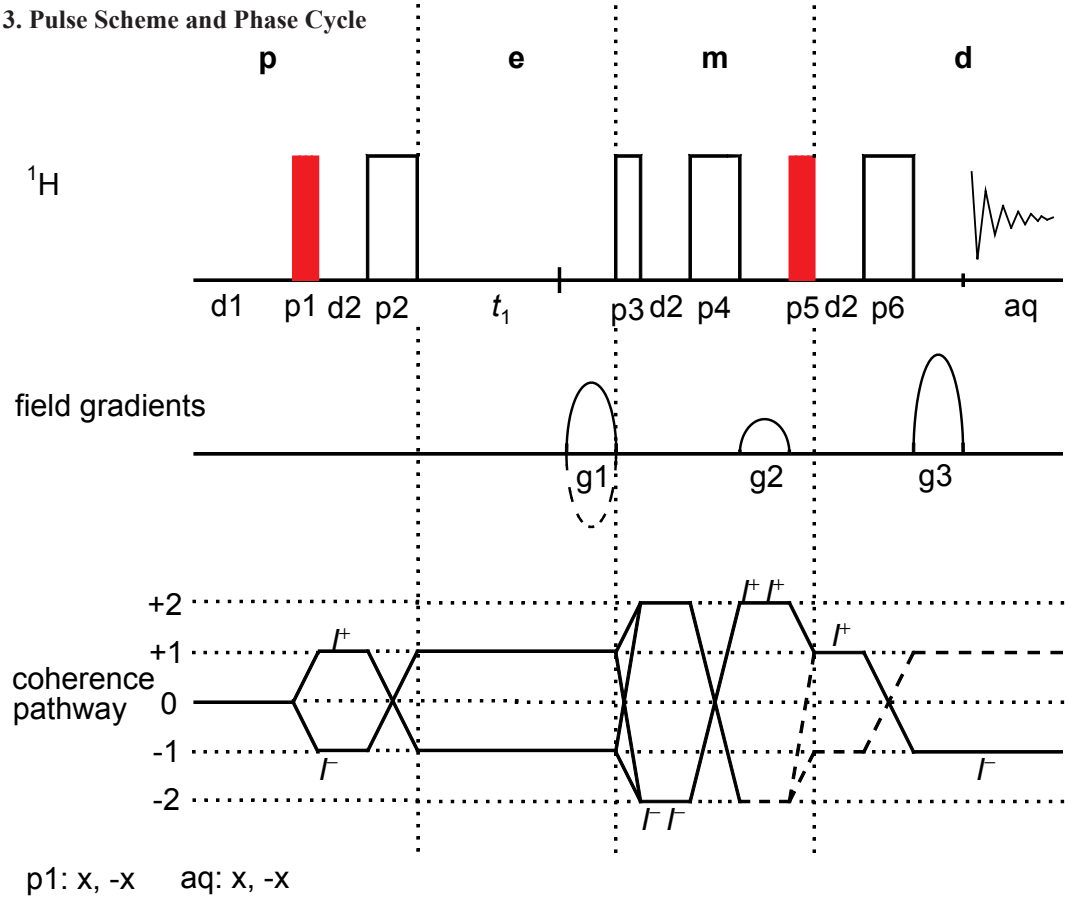
(4) The phase-sensitive COSY variants can be recorded in high resolution in both dimensions which enables the extraction of digital correct spin-coupling values in the direct dimension.

Fig. 1.3-1 J. Jeener *1931

Almost in despair, I started investigating this latter problem with the help of Gerit Alewaelers, initially on the simple case of the standard AB spectrum in liquids. This soon led to the general proposal of 2D FT NMR spectroscopy in the simple case of homonuclear COSY without phase cycling. We tried to observe the effect experimentally on ethylbenzene, spending weekends working with the rather primitive FT spectrometer available in organic chemistry at our university. Due to the bad phase stability of this spectrometer and to our total lack of practice of high-resolution NMR, Gerit Alewaeters could only observe some of the strongest cross peaks of the 2D spectrum with a signal-to-noise ratio high enough to confirm that our quantum mechanical predictions were correct (not really a surprise.....), but too low to make the new technique look very usable. Formal publication was deferred until cleaner experimental confirmation would be available, but I kept spreading the idea by personal contacts, lectures (probably for the first time at the AMPERE Summer School in Basko Polje in 1971) and by circulating 'unpublished' notes written in November 1971. Soon, Richard Ernst let me know that his co-worker Baumann had brought the 2D FT idea back from Basko Polje to Zürich, and that the Zürich group intended to work on it. As it turned out, one of our recurrent delights in Brussels for a number of years has been receiving news from Zürich about the progress of 2D NMR, both experimental and theoretical. It was also a pleasure to learn about the new 2D FT ideas developing in many other groups.

Jean Jeener, *1931, "Reminiscences about the early days of 2D NMR" *Enyclopedia of NMR*, **1996**, *1*, 409–410.

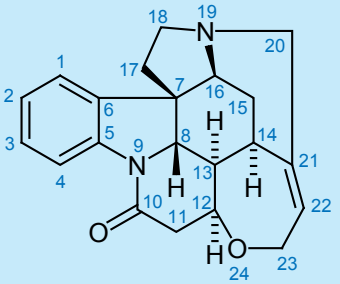
3. Pulse Scheme and Phase Cycle



Scheme 1.3-1

Common values:

p1, p3, p5: 90° ^{1}H transmitter pulse
p2, p4, p6: 180° ^{1}H transmitter pulse
d1: relaxation delay
d2: effective length of gradient
t1: evolution increment
g1, g3: gradients for Echo/Antiecho selection
g2: gradient for double quantum selection



Scheme 1.3-2

4. Acquisition

Special values used for the spectrum shown:

Sample: 3% strychnine in $CDCl_3$.
Time requirement: 20 min
Spectrometer: Bruker DRX-600 with 5-mm-TBI probe

td2:	2K data points in F_2
td1:	256 data points in F_1
sw2:	10 ppm
sw1:	10 ppm
aq1:	0.023 s
aq2:	0.19 s
o1:	middle of ^{1}H NMR spectrum
d1:	2 s
d2:	equal to effective duration of gradient used, here 1.05 ms
g1, g2, g3:	sinusoidal-shaped field gradients, 1 ms duration gradient ratio 30:10:50 with 0.56 T/m = 100%
rg:	One must be very careful in setting the receiver gain for this experiment. The gradient filter allows only the

desired coherences to pass into the receiver; however, the double-quantum coherences develop only at higher t_1 increments because of modulation with sin ($\pi J t_1$), cf. Equ. 2 see Question C. The receiver gain must therefore be set using a high t_1 increment to avoid overloading.

ds: 2
ns: 2

5. Processing

Apply zero-filling in F_1 to 1K words in order to have a symmetrical matrix of 1024×1024 data points. Use an exponential window with lb = 3 Hz in the F_2 dimension and a squared π/2 shifted sinusoidal window in the indirect dimension. Apply complex Fourier transformation in both dimensions. Phase correction in both dimensions can be performed after the 2D transformation in order to get clean up/down patterns of the cross-peaks. Zero order phase correction of 90° is a good starting point for the F_1 dimension.

6. Result

The overview spectrum displays all relevant connectivities for strychnine. In the aliphatic expansion clearly the AX pattern can be observed, even though more complicated multipletts are coupled to each other. In the aromatic expansion this is also demonstrated.

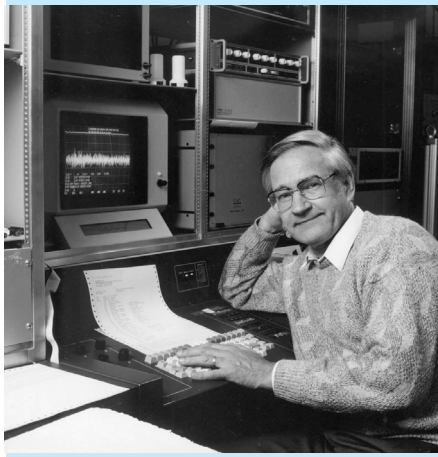


Fig. 1.3-2 R. R. Ernst *1933

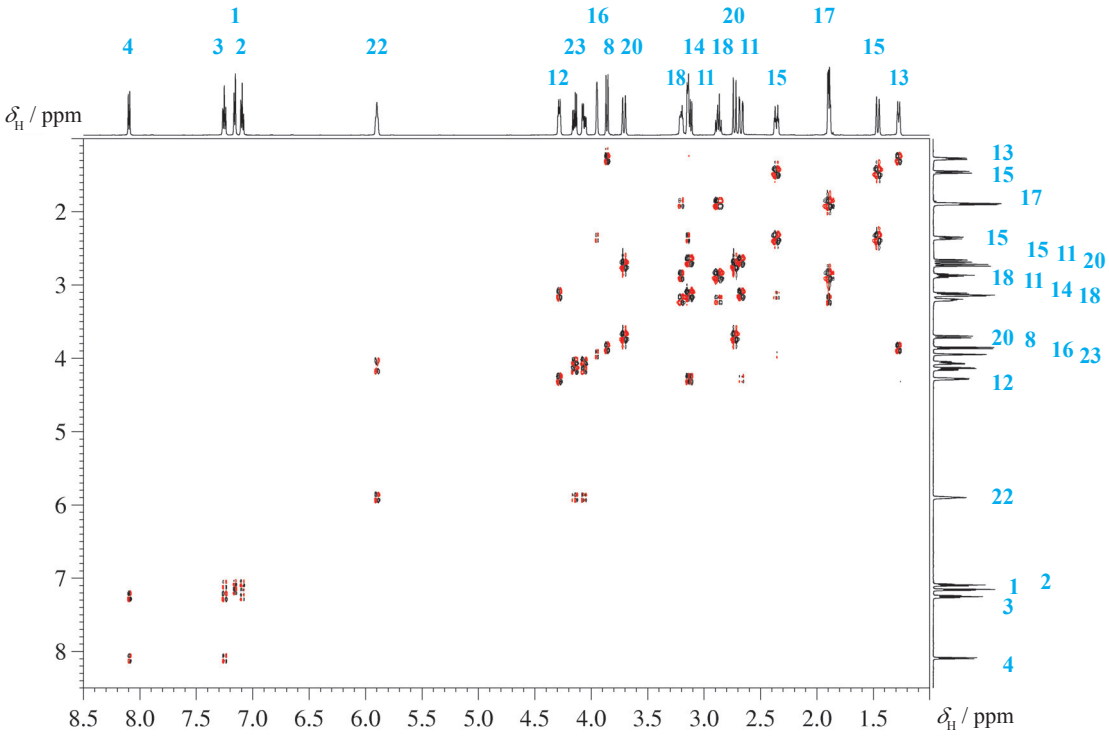


Fig. 1.3-3 COSY spectrum of strychnine

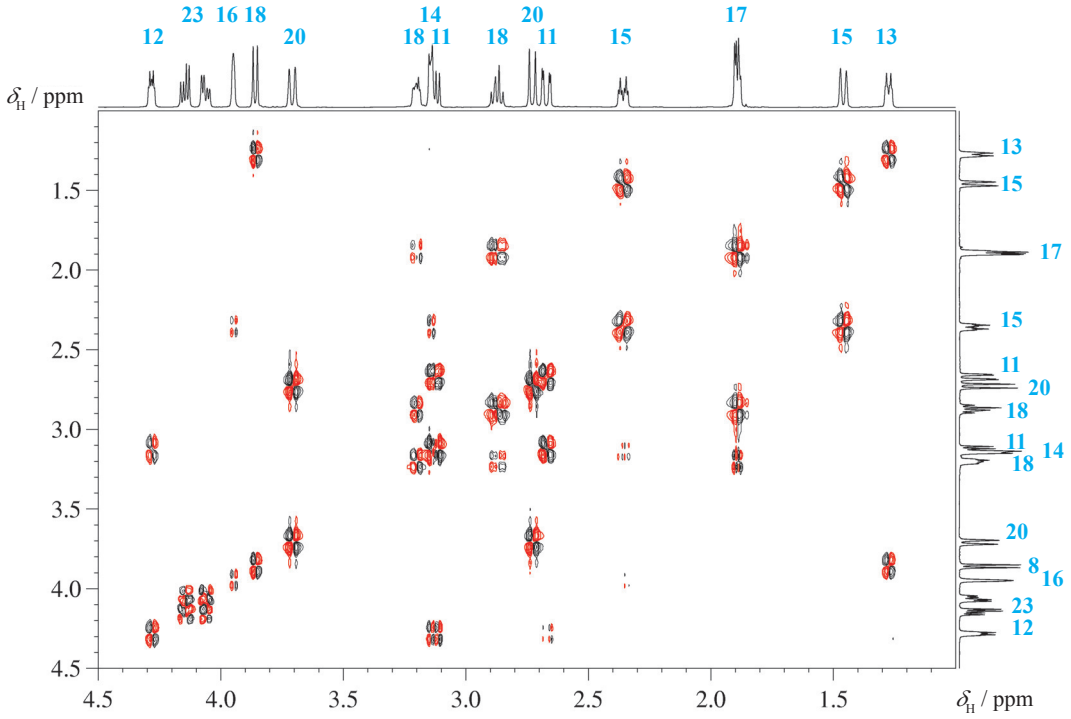


Fig.1.3-4 Expansion of the COSY spectrum in the aliphatic region

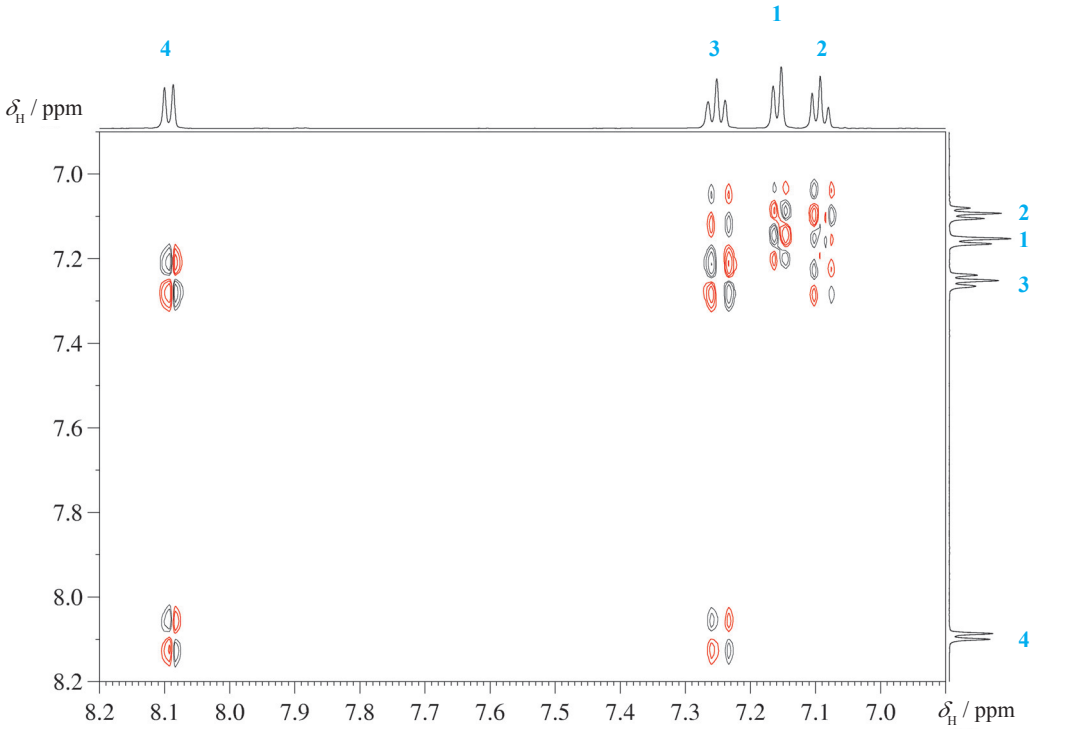
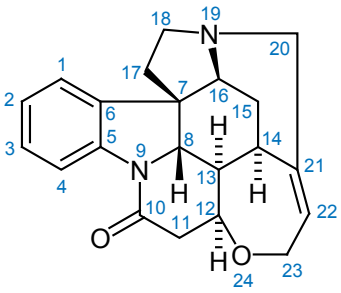


Fig. 1.3-5 Expansion of the COSY spectrum in the aromatic region



Scheme 1.3-3 Strychnine

The participation of Thomas W. Bauman at the AMPERE summer School in Basko Polje, Yugoslavia, in September 1971 was an extremely fortunate event. Being a meticulous scientist, he brought home a careful script of the lectures, among them one by Jean Jeener that attracted my attention immediately: a simple two-pulse experiment that produced revealing 2D spectra by 2D Fourier transformation of a 2D set of response signals. This was exactly the technique I had been waiting for. I had been thinking for some time about systematic computer-controlled double resonance experiments, but appreciated the complexity of the resulting 2D spectra should they follow the shape of the famous Anderson-Freeman plots.

R.R. Ernst "The success story of fourier transformation in NMR" *Enyclopedia of NMR*, **1996**, *1*, 297.

7. Comments

The two pulses given in red color are the essential COSY pulses used in the original pulse sequence. The additional pulses are used for the double-quantum filter (p3) and for phase correction due to the finite length of the gradient pulses (p2, p4, p6)

In the *preparation period* **p** of the pulse sequence we find the relaxation delay d1 and the first r.f. pulse p1 which transforms *z*-magnetization into transverse magnetization. The delay d2 and the 180° pulse p2 only serve to alleviate the finite length of the gradient pulse g1, which would otherwise cause a problem in phasing the 2D spectrum. Then the chemical shift develops in the *evolution period* **e** during t_1, which is written here only for proton 1, giving Equation (1). In addition, spin–spin coupling develops; thus each of the two terms with a modulation by the chemical shift will create two more terms including the spin–spin coupling.

$$I_{1_z} + I_{2_z} \xrightarrow{I_x} -I_{1_y} - I_{2_y} \xrightarrow{\Omega_1 t_1 I_{1_z}} -I_{1_y}\cos\Omega_1 t_1 + I_{1_x}\sin\Omega_1 t_1 \qquad (1)$$

$$\xrightarrow{\pi J t_1 2I_{1_z} I_{2_z}} -I_{1_y}\cos\Omega_1 t_1\cos\pi J t_1 + 2I_{1_x} I_{2_z}\cos\Omega_1 t_1\sin\pi J t_1$$
$$+ I_{1_x}\sin\Omega_1 t_1\cos\pi J t_1 + 2I_{1_y} I_{2_z}\sin\Omega_1 t_1\sin\pi J t_1 \qquad (2)$$

In the *mixing period* **m** of the pulse sequence the r.f. pulse p3 creates double-quantum magnetization $-2I_{1_x} I_{2_y}$, as in Equation (3). Again the delay d2 and the 180° pulse p4 are only for phase correction and correct for the finite length of the gradient pulse g2, which encodes the double-quantum magnetization.

$$2I_{1_x} I_{2_z}\cos\Omega_1 t_1\sin\pi J t_1 \xrightarrow{I_x} -2I_{1_x} I_{2_y}\cos\Omega_1 t_1\sin\pi J t_1 \qquad (3)$$

The 90° pulse p5 creates antiphase magnetization from the double-quantum term as given in eq. (4)

(4)

$$-2I_{1_x}I_{2_y}\cos\Omega_1 t_1 \sin\pi J t_1 \xrightarrow{I_x} -2I_{1_x}I_{2_z}\cos\Omega_1 t_1 \sin\pi J t_1$$

In the *detection period* **d** chemical shift and spin–spin coupling develop once again during the acquisition time t_2, giving Equation (5).

(5)

$$\xrightarrow{\Omega_2 t_2 I_{1_z}} \xrightarrow{\pi J t_2 2I_{1_z}I_{2_z}} I_{2_y}\cos\Omega_1 t_1 \sin\pi J t_1 \sin\Omega_2 t_2 \sin\pi J t_2$$

The last expression describes a cross-peak in the COSY matrix.

With the pulse sequence and the echo-antiecho scheme of the gradients used, the sign of the frequencies in F_1 is determined by keeping the sine and cosine terms separate, and thus allows phase-sensitive processing.

As can be seen from the coherence pathway diagram above, the first gradient g1 acts during a period when single-quantum magnetization I^+ is present (coherence level +1), whereas the second acts during a period when double-quantum coherence I^+I^+ is present. The final gradient pulse acts on I^-. Therefore the gradient ratios 30:10:50 and -30:10:50 will be successful to obtain the desired signals. All other coherences are further dephased and are not observable.

[1] J. Jeener, *Ampère International Summer School*, Basko Polje, **1971** (proposal).

[2] W. P. Aue, E. Bartholdi, R. R. Ernst "Two-dimensional spectroscopy. Application to nuclear magnetic resonance" *J. Chem. Phys.* **1975**, *64*, 2229–2246.

[3] T. D. W. Claridge, "High-Resolution NMR techniques in organic chemistry", Pergamon, Oxford, **1999**, 155–159.

[4] J. Keeler, "Understanding NMR spectroscopy", Wiley, Chichester, 2nd Ed. 2010.

8. Questions

A. Why does the standard COSY not reveal strong cross peaks for long-range spin couplings and what is the trick, whereby the long-range COSY is functioning?

B. In which dimension one would extract spin coupling values from a high-resolution COSY and why?

C. Why are double quantum coherences only developing at higher t_1 increments?

D. In the aromatic expansion of the strychnine spectrum one observes only an AX pattern from the triplet. Why?

E. Only one of the protons H-15 displays a cross peak to H-16. Why?

9. Own Observations

Experiment 1.4

NOESY

1. Purpose

The NOESY (**N**uclear **O**verhauser **E**nhancement **S**pectroscop**Y**) experiment is the two-dimensional equivalent of the NOE difference experiment (see chapter 3.6) and yields correlation signals that are caused by dipolar cross-relaxation between nuclei in a close spatial relationship.

The intensities of the cross-peaks under certain conditions are proportional to the sixth power of the proton–proton distances. Quantitatively, the results differ from 1D NOE difference spectroscopy, since the latter is a steady-state experiment obtained from a saturation of the energy levels, whereas NOESY is a transient experiment obtained after population inversion of the energy levels. In a qualitative way, the NOESY technique gives answers to many stereochemical problems such as *exo/endo*, *E/Z* and similar assignment questions. In NMR studies of peptides and proteins NOESY is the essential method for determining peptide conformations or tertiary structure of proteins. We show here a phase-sensitive version with two spoiling gradients during the mixing time.

Fig. 1.4-1 A. W. Overhauser (1925-2011)

2. Variants

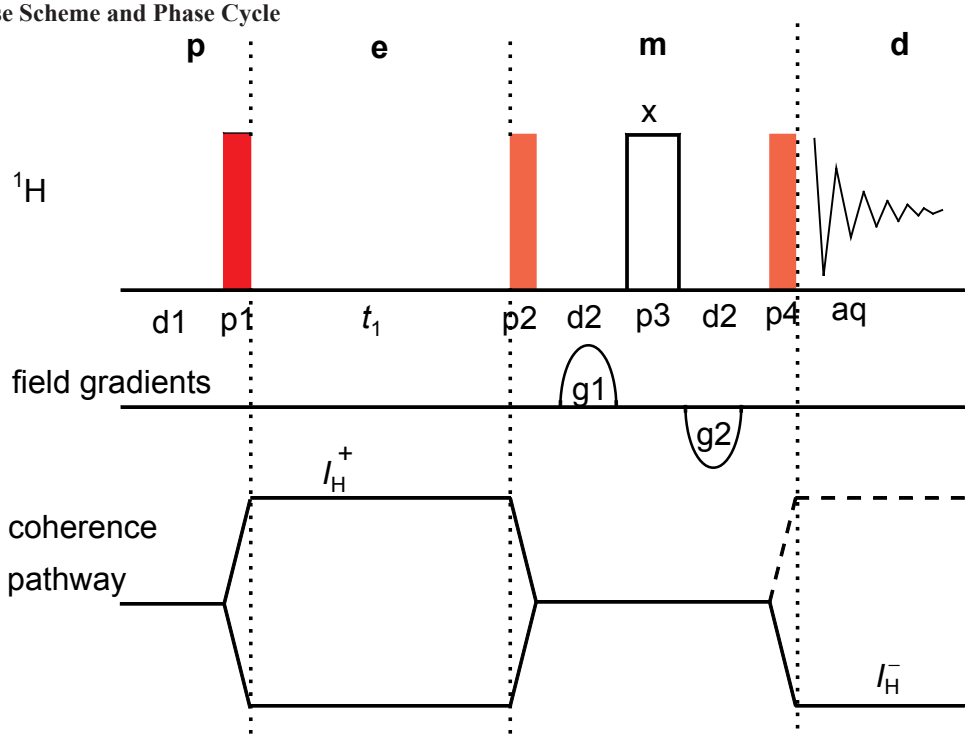
The gradient pulses during the mixing time destroy most of the COSY artefacts present in NOESY, however, not the zero-quantum coherences. Further improvements of a gradient-supported NOESY technique have been described recently [4]. The NOESY technique has been combined with different schemes of water suppression, if biological samples have to be measured. Also, in protein NMR a 3D version with ^{15}N editing is the standard technique. Selective 1D NOESY sequences are known if only the answer of a particular spin is needed. An echo-antiecho method as shown in chapter 1.3 for COSY is not advisable because of diffusion effects during the mixing time (encoding gradients before and after the mixing time).

A considerable drawback of the NOESY technique is the dependence of the NOE effect on molar mass and viscosity, which can change its sign and may cause it to disappear for certain conditions. The ROESY technique as described in Experiment 2.2 may be more effective in this case.

In both, chemical exchange of nuclei may yield cross peaks, too.

Fig. 1.4-2 R. R. Ernst *1933

3. Pulse Scheme and Phase Cycle



p1: x, -x p2: $(x)_8$, $(-x)_8$ p4: x, x, -x, -x, -y, -y, y, y

aq: x, -x, -x, x, y, -y, -y, y, -x, x, x, -x, -y, y, y, -y

phase cycle for p1 incremented according to States-TPPI

Scheme 1.4-1

Common values:

p1, p2, p4: 90° ^{1}H transmitter pulse
p3: 180° ^{1}H transmitter pulse
d1: relaxation delay
d2: mixing time 2, in the order of ^{1}H relaxation time
t1: evolution increment
g1, g2: gradients for artifact suppression

4. Acquisition

Special values used for the spectrum shown:

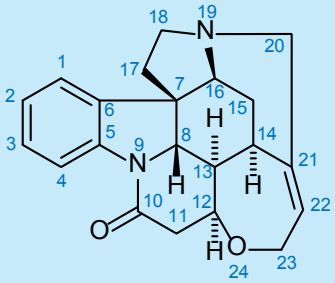
Sample: 3% strychnine in $CDCl_3$.
Time requirement: 5 h
Spectrometer: Bruker DRX-600 with 5-mm-TBI-probe

td2:	2K data points in F_2
td1:	256 data points in F_1
sw2:	10 ppm
sw1:	10 ppm
aq2:	0.17 s
aq1:	0.021 s
o1:	middle of ^{1}H NMR spectrum
d1:	2 s
d2:	1 s
ds:	4
ns:	8
g1, g2:	40 : (-40) with 0.6 T/m = 100 %

5. Processing

Apply zero-filling in F_1 to 1K real data points to obtain a symmetrical matrix of 1K×1K real data points. Use an exponential window in F_2 with lb = 5 Hz and a π/2-shifted squared sine bell in F_1.

Apply complex Fourier transformation corresponding to the States-TPPI mode of data acquisition in F_1. Adjust the phase of the diagonal signals so that they are negative. The NOESY correlation signals will then be positive if the compound has a molar mass below 1000 (positive NOE effect). Correlation signals caused by chemical exchange will have the same phase as the diagonal signals.



Scheme 1.4-2

6. Result

The figures show the result obtained on a DRX-600 spectrometer. Note that the phase of the diagonal signals is opposite to that of the cross-peaks as can be seen from the dotted contours. There is a wealth of information to be taken from the spectrum, which can best be studied using a molecular model or an electronic 3D file. Notice, for instance, that only one of the H-20 protons has an NOE contact with one of the H-15 protons, from which a relative assignment of the protons in these methylene groups can be derived.

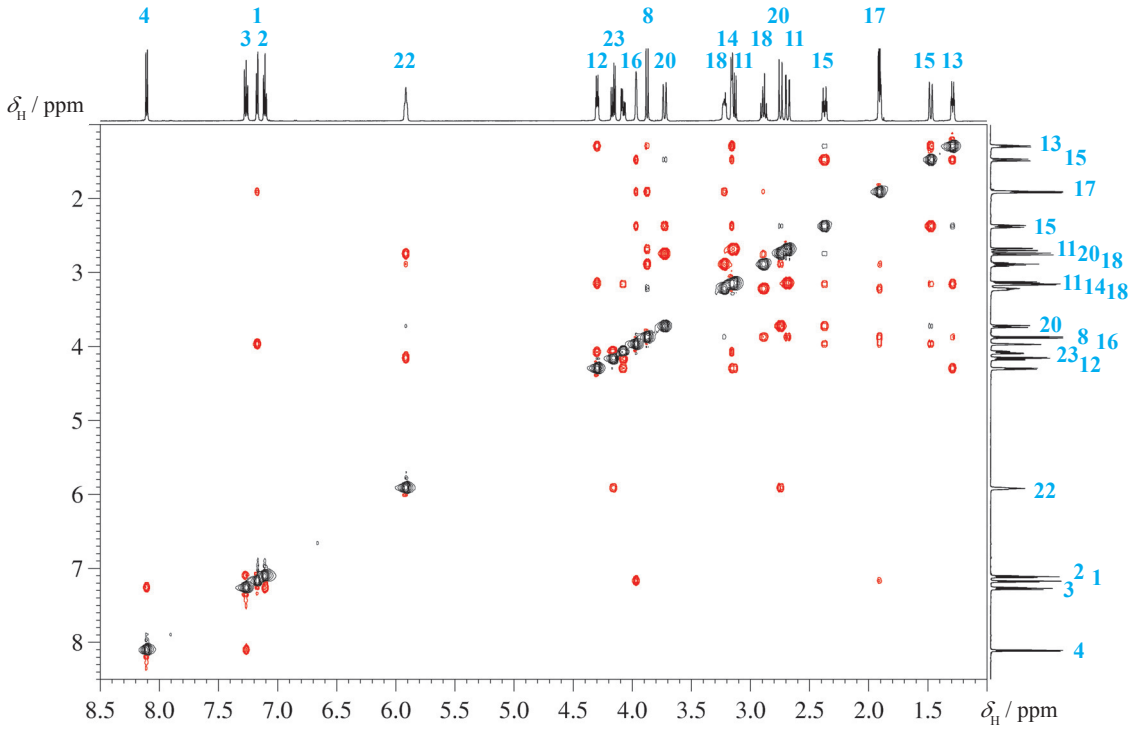


Fig. 1.4-3 NOESY spectrum of strychnine

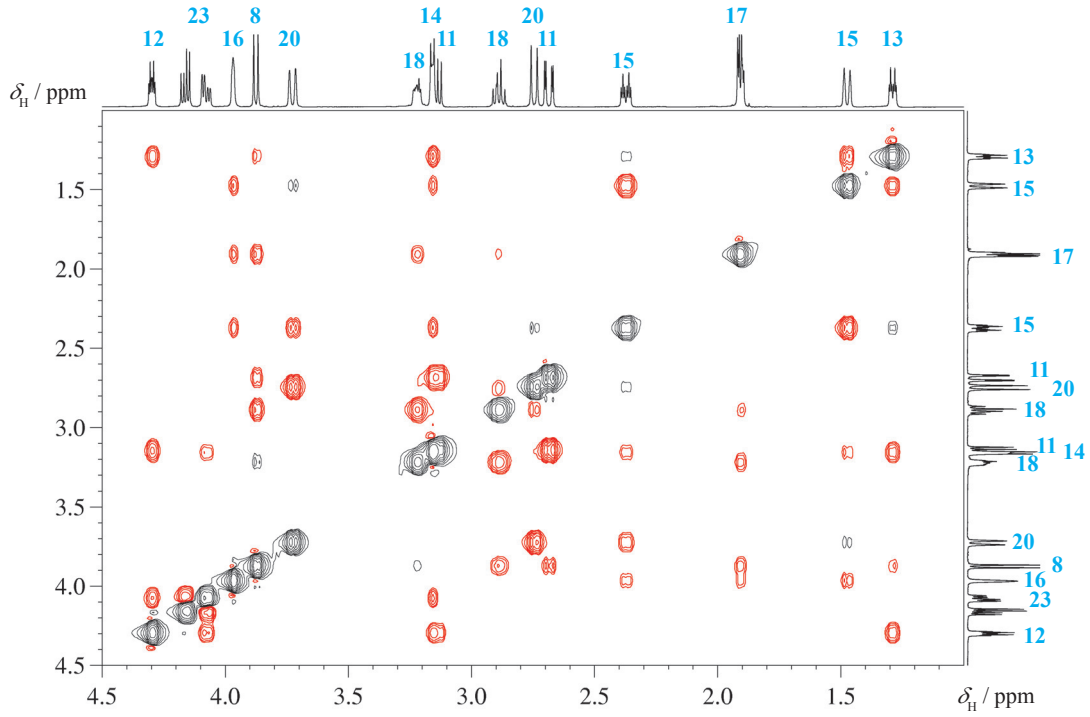
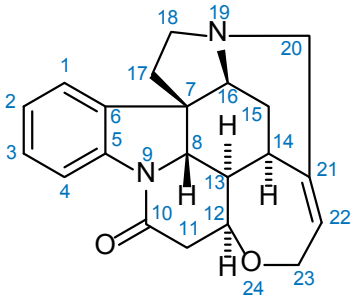


Fig. 1.4-4 Expansion of the spectrum in the aliphatic region

Albert Overhauser, a young theoretician from the University of Illinois, had made the following prediction: in a metal, where the conduction electrons are known to be responsible for the nuclear relaxation, the saturation of the ESR resonance of these electrons should lead to an enormous increase in the nuclear polarization. [...] Secondly, that Overhauser's audience at the meeting of the American Physical Society − where he had (in ten minutes) presented the calculations which had led to his amazing conclusion − was immediately split into two parts, which, however, overlapped: those who did not understand a single word of his demonstration, and those who did not believe a single word of his conclusions. In the first row of the sceptics who did not believe his conclusions shone all the stars of magnetic resonance: Bloch and Purcell, Rabi and Ramsey. Bloembergen was of two minds and so was I. As for the presentation itself, 1 will repeat what Van de



Scheme 1.4-3