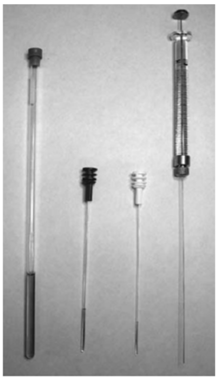

5 mm (600 μL),
1.7 mm (35 μL) and
1 mm tubes (7 μL).

Right, a gas-tight sample syringe fitted with an extra long needle. Image credit: D. S. Dalisay.

Fig. 1 Relative NMR sample tube diameter (OD) and sample volumes. From left to right,

Tadeusz F. Molinski. NMR of natural products at the 'nanomole-scale'. *Nat. Prod. Rep.* **2010**, *27*, 321-329.

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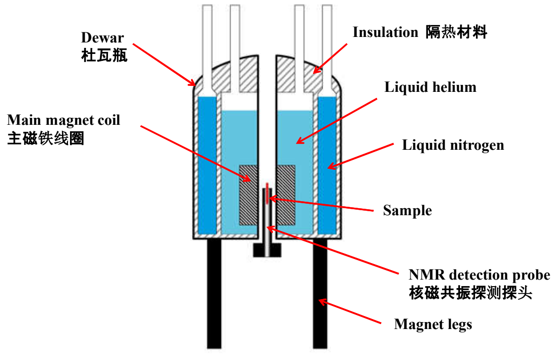
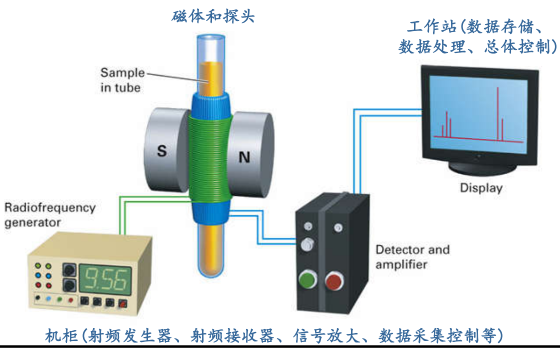
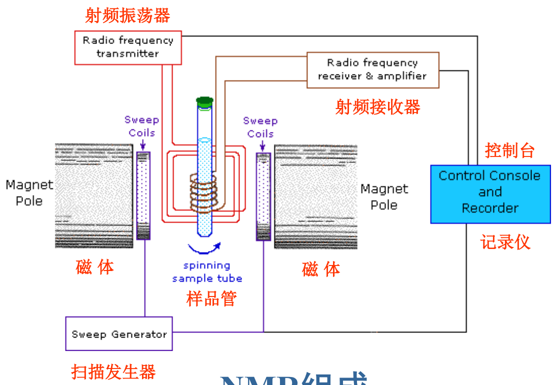
Superconducting Magnet



Schematic operation of a basic NMR spectrometer





NMR组成

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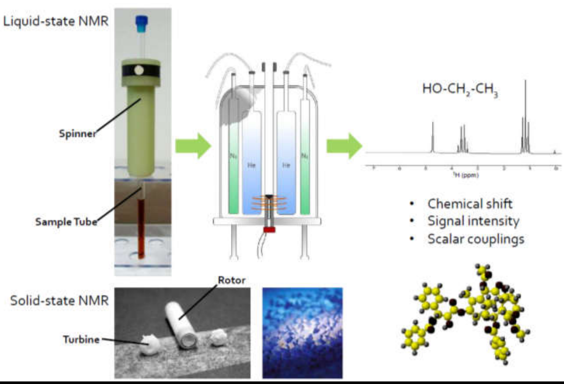
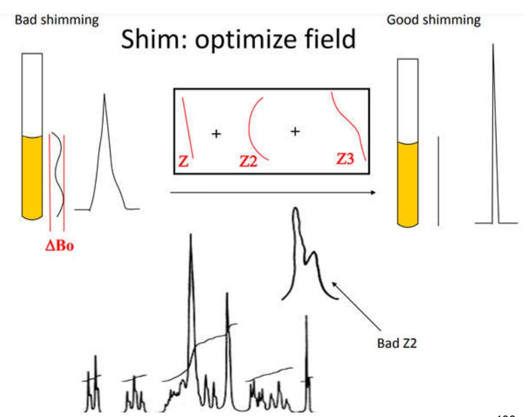
NMR is A Widely Used Structural Tool





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Steps to Acquire Data

- Prepare sample dissolve in deuterated solvent ($CDCl_3$, acetone-d_6 …) filter solution in NMR tube : fill the tube up to ~4 cm of solution
- Insert sample in the magnet
- Lock on deuterium signal from the solvent
- Optimize shimming
- Select parameters ^1H, ^{13}C, ^{31}P, ….
- Set receiver gain
- Acquire the data

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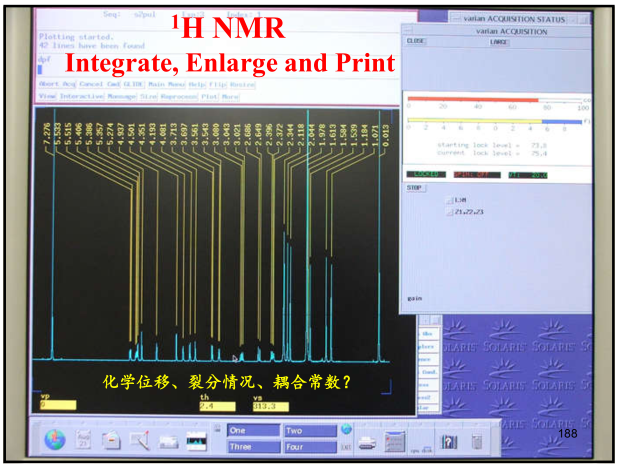

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Applications:

核磁共振已在众多领域中有了广泛的应用.从技术手段上讲，核磁共振的应用主要有两个方面：

Ⅰ核磁共振波谱：实际上是吸收率（纵坐标）对化学位移（横坐标）的关系曲线

Ⅱ核磁共振成象(MRI)：(1) 点成像法

(2) 弛豫时间成像法

Nuclear Magnetic Resonance Imaging also called Spin Imaging

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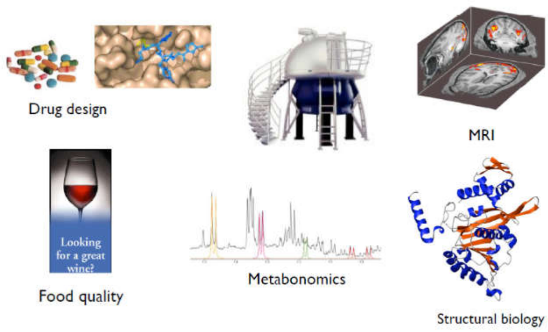
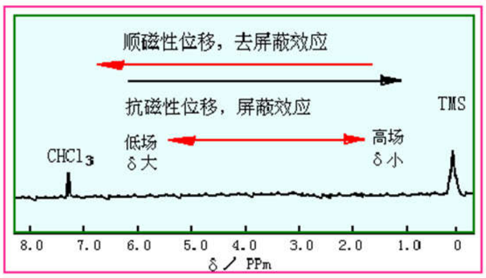
Application of NMR



NMR Spectral Parameters

- Shielding 屏蔽 → Chemical shift
- Scalar interaction 标量相互作用 → *J*-coupling
- Dipolar interaction 偶极相互作用 → Relaxation — Line-width
- Dipolar interaction 偶极相互作用 → Nuclear Overhauser effect — nOe
- Dipolar interaction 偶极相互作用 → Residual dipolar coupling 残余偶极偶合 — RDC

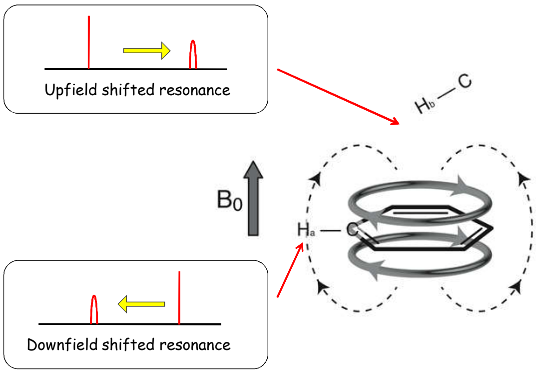
屏蔽效应与去屏蔽效应



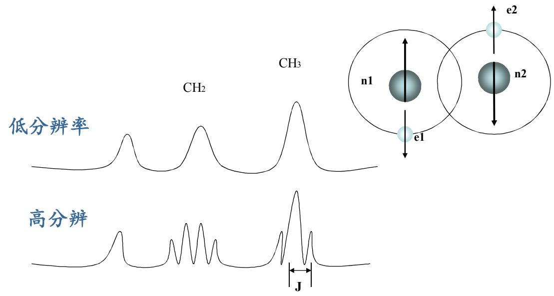
屏蔽作用（屏蔽效应）（shielding effect）
去屏蔽作用（去屏蔽效应）（deshielding effect）

192

Chemical Shift: Ring Current



自旋偶合现象



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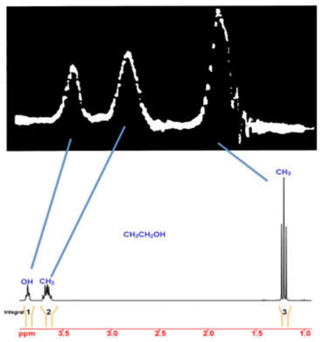
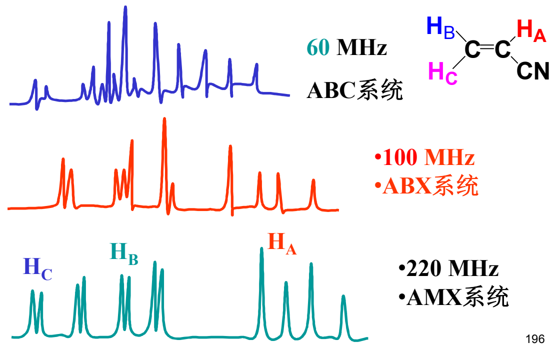
简化谱图的方法-采用高场强仪器



First proton spectrum of ethanol @ 30 MHz

Ethanol proton spectrum @700 MHz

简化谱图的方法-采用高场强仪器



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Chemical shift

化学位移

Chemical shift is defined as *nuclear shielding / applied magnetic field.*

Chemical shift is a function of the nucleus and its environment.

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化学位移的表示法

- **1.** 用赫兹(Hz, CPS)表示化学位移(现已不用)。
- **2.** 用δ值表示　δ值的定义:

$$\delta = \frac{\upsilon_{样} - \upsilon_{标}}{\upsilon_{标}} \times 10^6$$

$$= \frac{\Delta\upsilon}{振荡器射频} \times 10^6$$

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偶合常数 J (Coupling Constants)

- 自旋—自旋偶合产生峰的裂分，而每组峰中峰与峰之间的距离叫偶合常数 (Coupling constants)，用J表示单位Hz.
- 特点：
- (1) J不受外磁场影响，外磁场变化，J值不变；
- (2) 质子之间产生的偶合裂分，J值< 20 Hz；

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偶合常数与分子结构的关系

- 根据相互干扰氢核之间相隔键数的多少，将偶合作用分为：
- 偕偶 (Geminal coupling)也称偕质子偶合，同碳质子偶合。
- 邻偶 (Vicinal coupling)
- 远程偶合 (Long-range coupling)

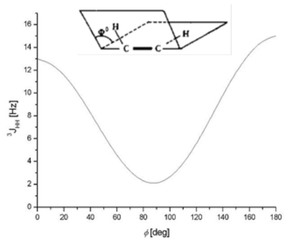
200

Karplus Equation

The Karplus equation, named after Martin Karplus, describes the correlation between ^{3}J-coupling constants and dihedral torsion angles in nuclear magnetic resonance spectroscopy.

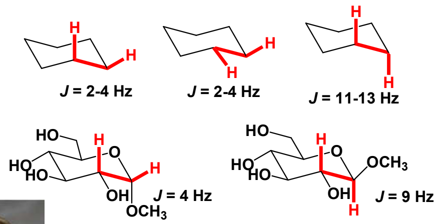
Martin Karplus 2013 NP

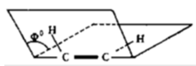
J. Chem. Phys., **30**, 11 (**1959**).



201

偶合常数与分子结构的关系





受电负性氧原子的影响使偶合常数减小

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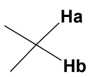
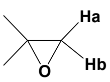
偶合常数与分子结构的关系

偕偶：经过二个化学键的偶合，

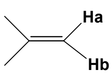
用Jgem，J偕，J同，^{2}J表示



- $^{2}J_{a,b}$ 12-16 Hz



- $^{2}J_{a,b}$ 5.4-6.3 Hz



- $^{2}J_{a,b}$ 0.5-3.5 Hz

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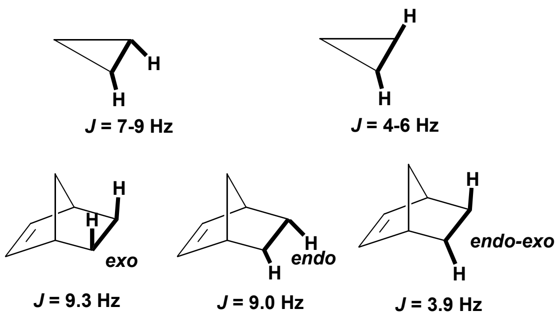
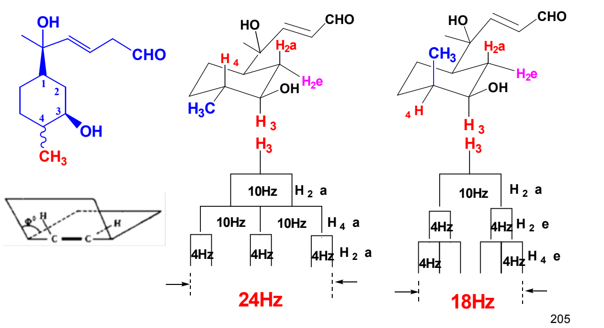
偶合常数与分子结构的关系



204

用于确定六元环中CH_3为a或e键（实测17Hz）



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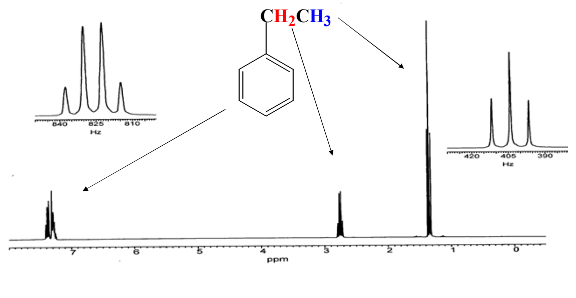
向心原则 Roof effect



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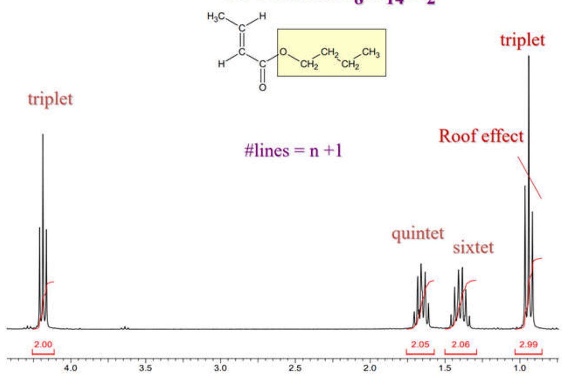
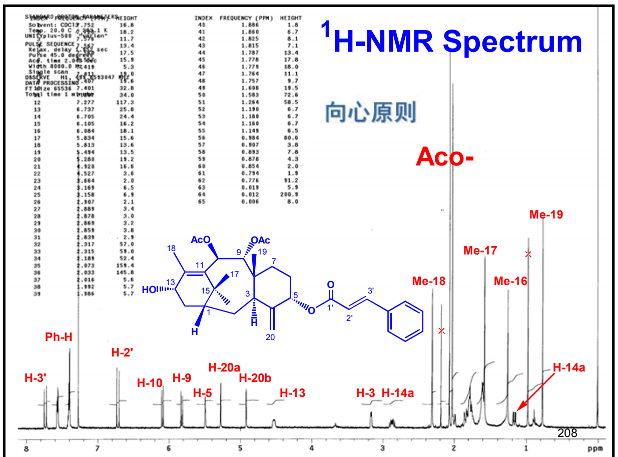
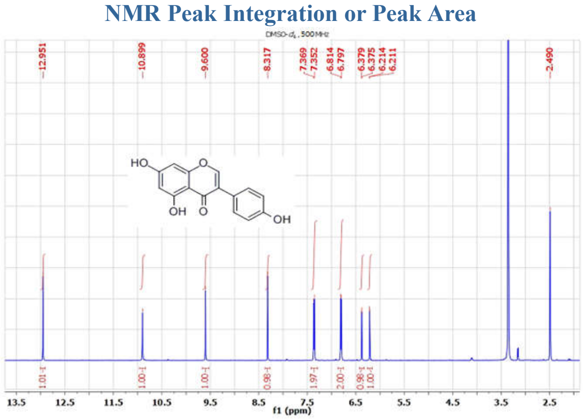
^{1}H NMR:$C_8H_{14}O_2$





NMR Peak Integration or Peak Area



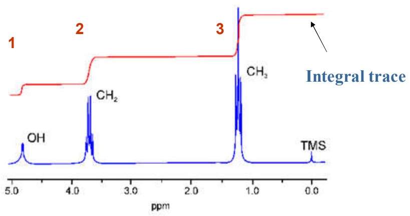
NMR Peak Integration or Peak Area

a) The relative peak intensity or peak area is proportional to the number of protons associated with the observed peak.

b) Means to determine relative concentrations of multiple species present in an NMR sample.

Relative peak areas = Number of protons

HO-CH_2-CH_3



^{1}H-NMR的空间构象分析

- 有机化学的发展要求人们必须在三维空间上了解分子的结构和性能，尤其是与生命过程有关的化学问题。如药物分子的立体构型和受体之间的相互作用，生化反应过程的立体选择性与分子的立体构型之间的关系，各类天然产物的立体构型与它们表现出的生物活性之间的关系。对许多天然产物而言，其生物活性往往只为一种特定的绝对构型所有。

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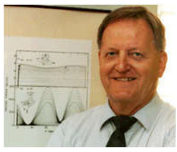
核 Overhauser 效应 (NOE)



Albert W. Overhauser (1926~) Purdue University

- 1953年，Overhauser研究金属钠的液氨（顺磁）溶液，当用一个高频场使电子自旋发生共振并达到饱和时，^{23}Na核自旋能级粒子数的平衡分布被破坏，核自旋有关能级上粒子数差额增加很多，共振信号大为加强。这被称为Overhauser效应。A milestone which increased the signal-to-noise (S/N) ratio was the discovery of the nuclear Overhauser effect, which improves the S/N in less sensitive nuclei by polarization transfer.

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核Overhauser效应

（Nuclear Overhauser Effect）

Albert W. Overhauser (1926~)

- NOE: 若对分子中空间相距较近的两核（<5Å）之一进行辐照，使之达到跃迁的饱和状态，此时记录另一个核的核磁共振峰，可发现较无此辐照时，谱峰强度有所变化，这即是核Overhauser效应（NOE）。

NOE的产生仅以两核空间相近为决定性条件，而与两核间化学键存在与否无关（与有无 *J* 耦合无关）。

National Medal of Science

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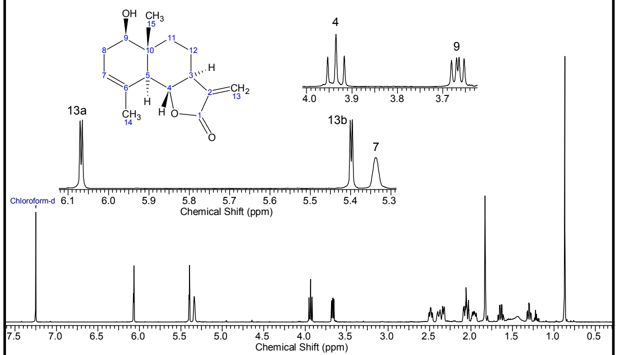
Some examples: Sesquiterpene



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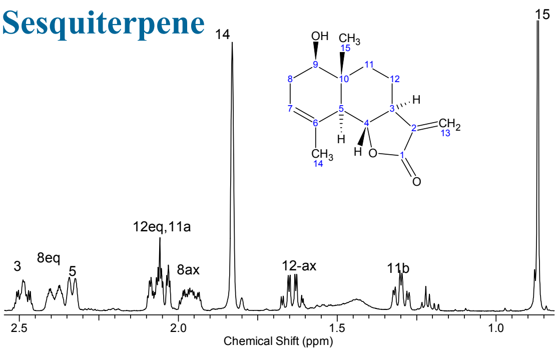
Sesquiterpene



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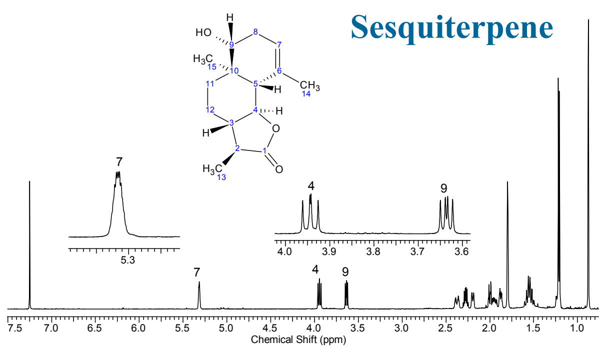
NOE和偶合常数均可说明H-3, H-4, H-5都是反式关系。

Sesquiterpene



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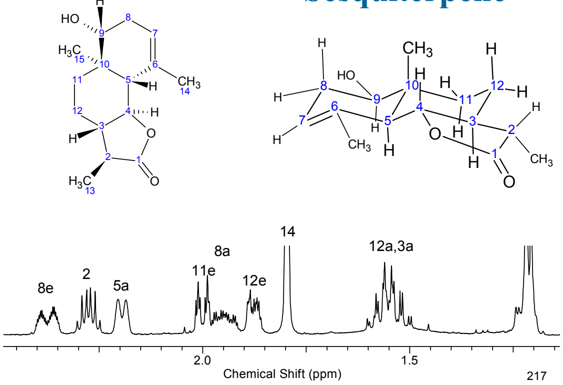
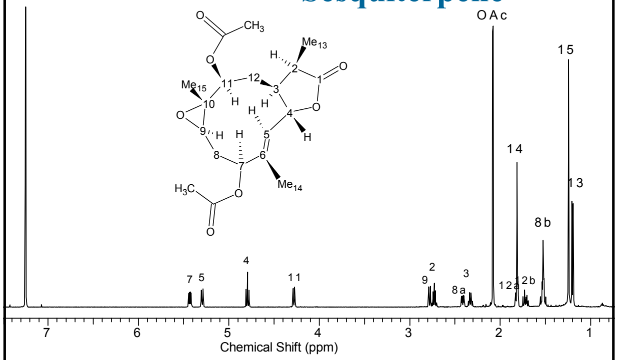
Sesquiterpene



217

Sesquiterpene



这种裂分是如何产生的？ 哪些质子存在相互耦合关系？

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2 D ^{1}H-^{1}H相关谱 (^{1}H-^{1}H COSY)

- COSY 谱本身为正方形，当 F1和 F2谱宽不等时则为矩形。正方形中有一条对角线（一般为左下---右上）。对角线上的峰称为对角峰（diagonal peak）。对角线外的峰称为交叉峰（cross peaks）或相关峰（correlated peaks）。每个相关峰或交叉峰反映两个峰组间的耦合关系。COSY 主要反映^3J耦合关系。

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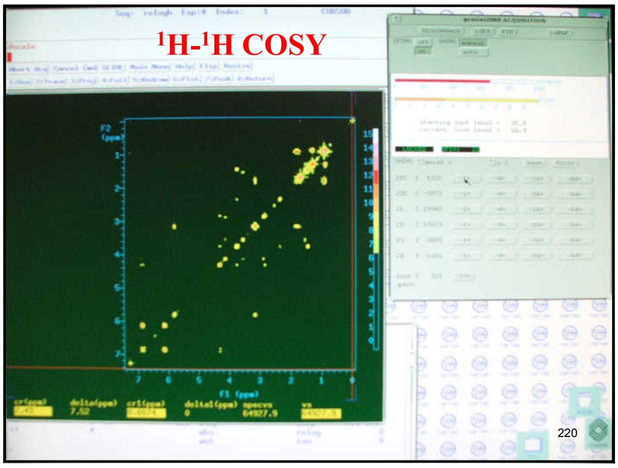
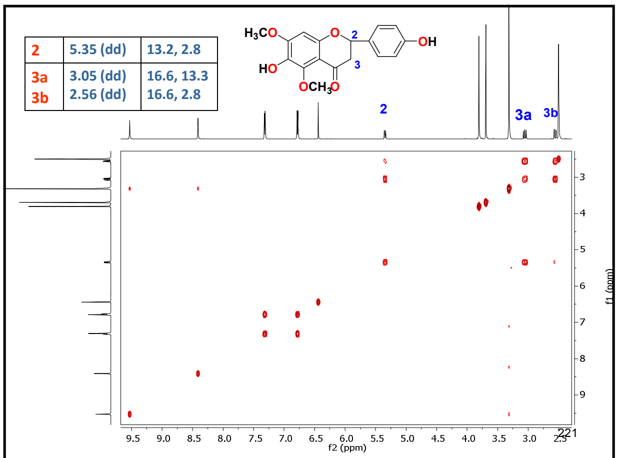
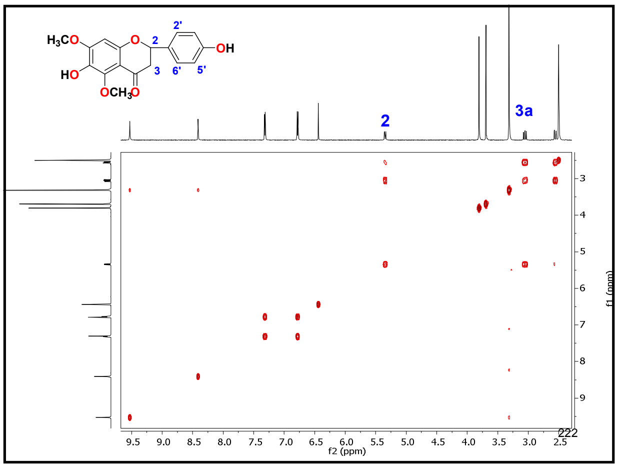


220

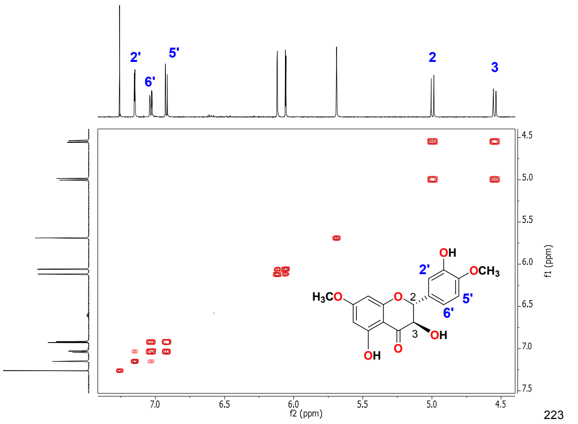


2	5.35 (dd)	13.2, 2.8
3a	3.05 (dd)	16.6, 13.3
3b	2.56 (dd)	16.6, 2.8

221



222



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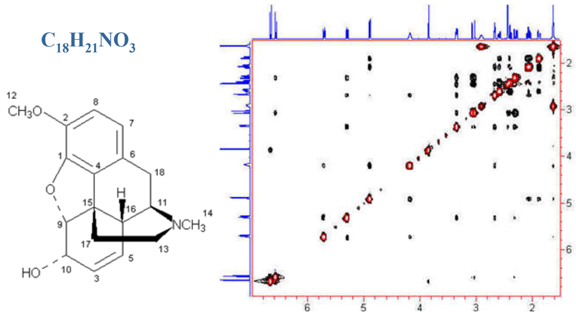
From Formula to Structure

$C_{18}H_{21}NO_3$



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核磁共振碳谱

^{13}C-NMR Spectrum

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核磁共振碳谱

在决定有机化合物结构时，与^{1}H-NMR相比^{13}C-NMR在某种程度上起着更为重要的作用，两者相辅相成。

自然界存在的碳同位素中，^{12}C虽然丰度比最大(98.9%)但因没有磁性而无法测定，^{13}C虽有磁性 (I = 1/2)，但因观测灵敏度只有^{1}H核的1/64，且丰度甚小，仅为1.1%，故总的信号灵敏度仅为^{1}H核的1/6000，致使^{13}C-NMR长期不能投入实际应用。近30年来电脉冲-傅丽叶变换(PFT) 核磁共振装置的出现，及计算机的引入，^{13}C-NMR才得到真正应用。

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核磁共振碳谱

^{13}C-NMR的化学位移是最重要的参数，它直接反映了所观察核周围的基团电子分布的情况，即核所受的屏蔽作用的大小。

d_C 对核所处的化学环境很敏感，d_C的范围远比d_H宽。分子中有不同的构型和构象时，d_C比 d_H更敏感。因此，很少重合。

对于季碳，碳谱比氢谱更具优势。

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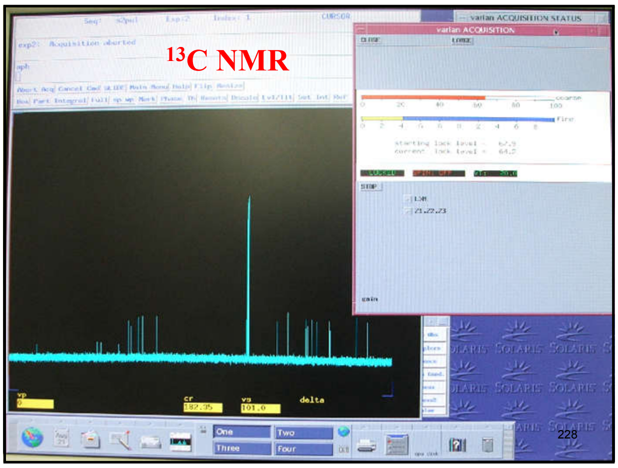

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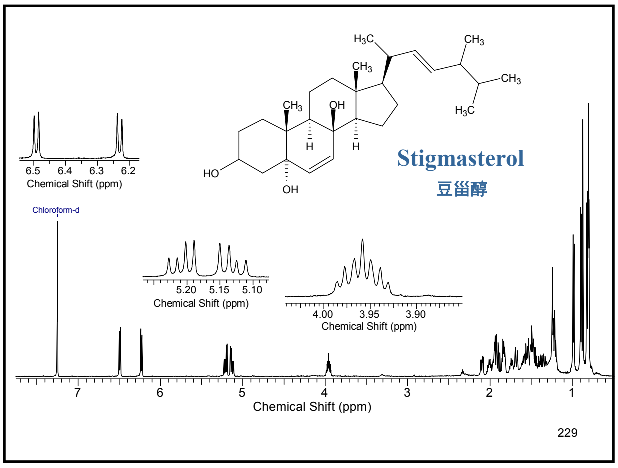
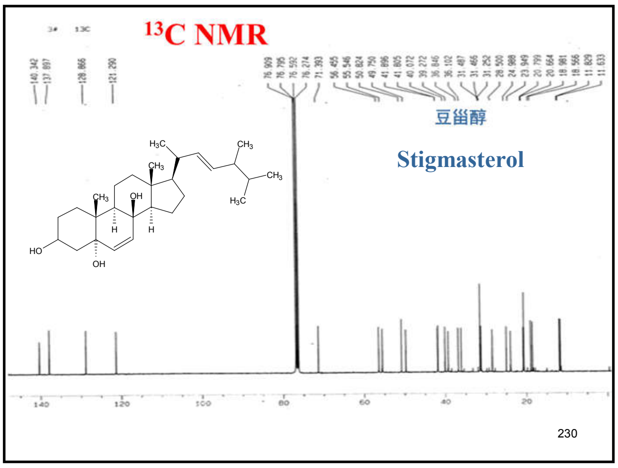
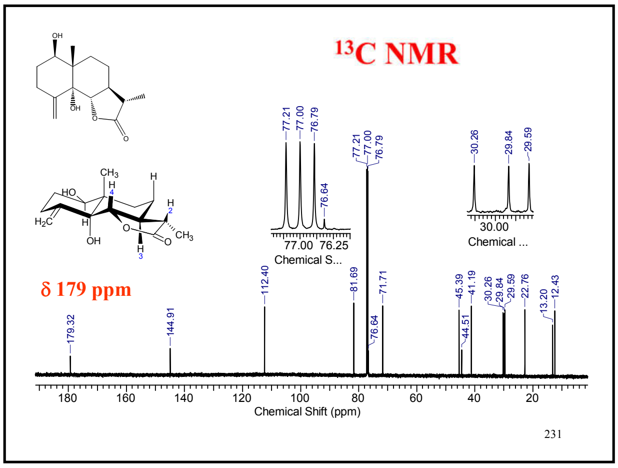
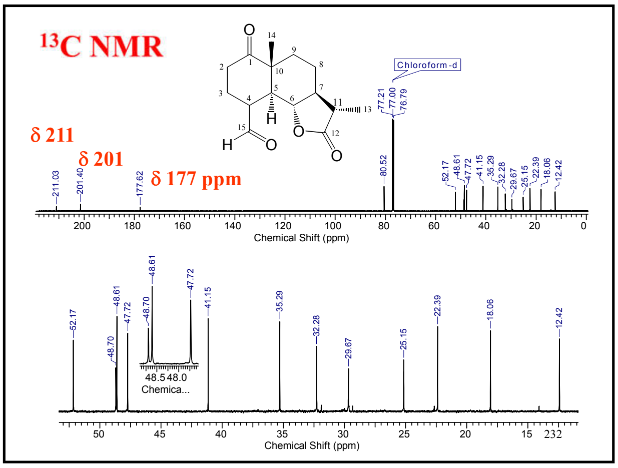
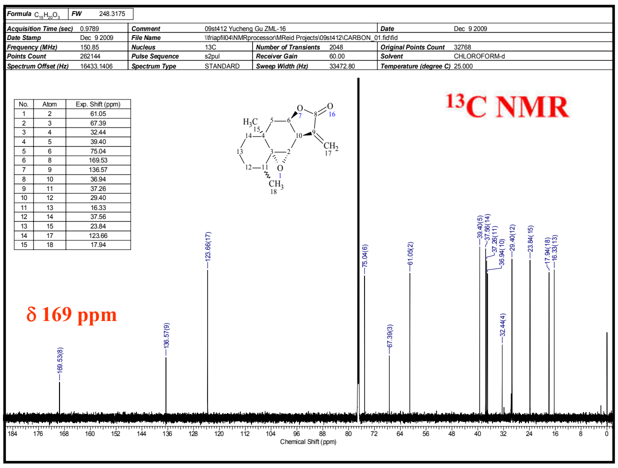


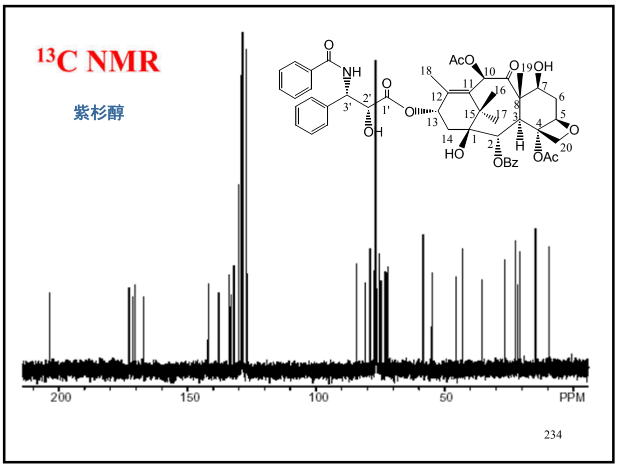








No.	Atom	Exp. Shift (ppm)
1	2	61.05
2	3	67.39
3	4	32.44
4	5	39.40
5	6	75.04
6	8	169.53
7	9	136.57
8	10	36.94
9	11	37.26
10	12	29.40
11	13	16.33
12	14	37.56
13	15	23.84
14	17	123.66
15	18	17.94



核磁共振二维谱

2D NMR Spectrum

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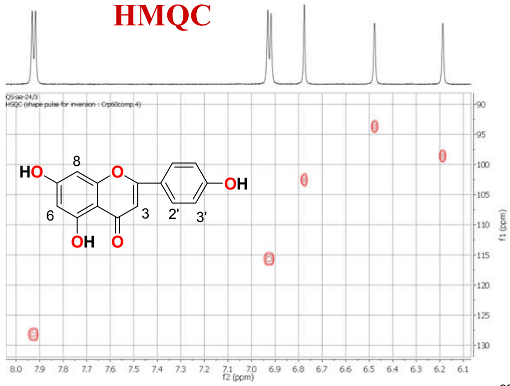
HMQC



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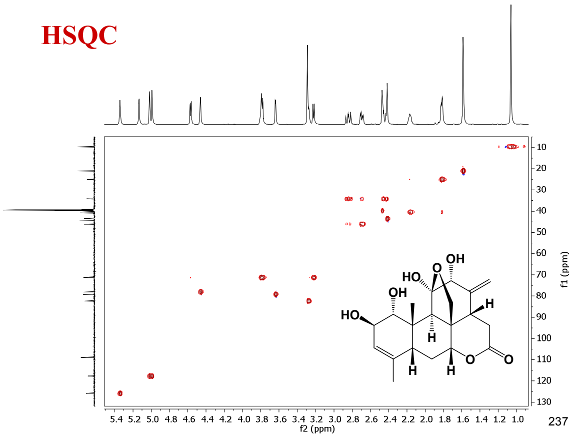
HSQC



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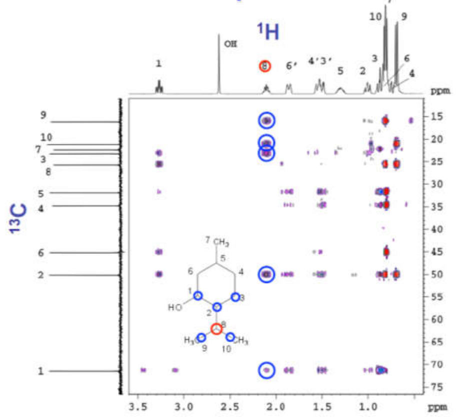
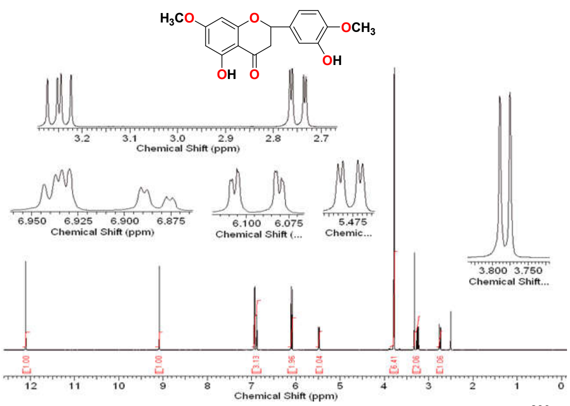
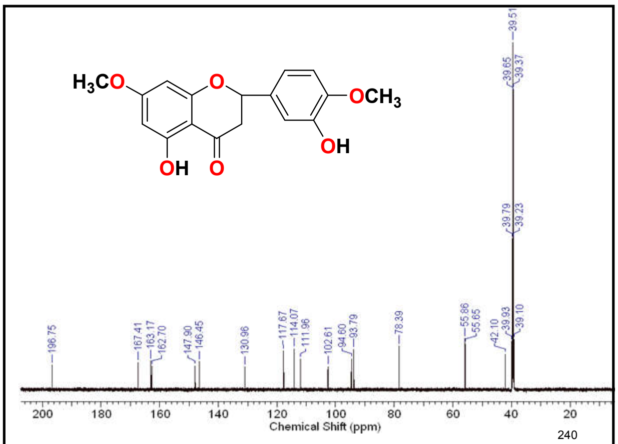
HMBC spectrum



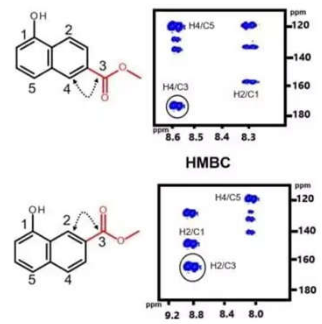


239

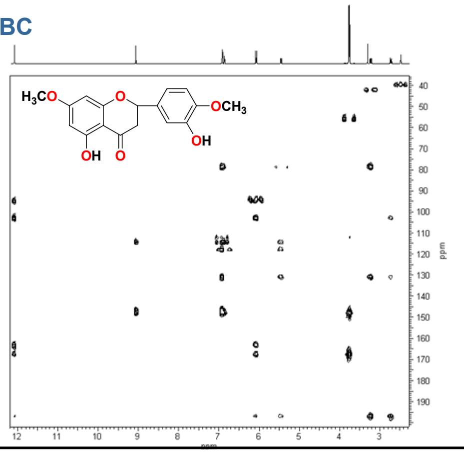


240

HMBC区别区域异构(Regioisomerism)

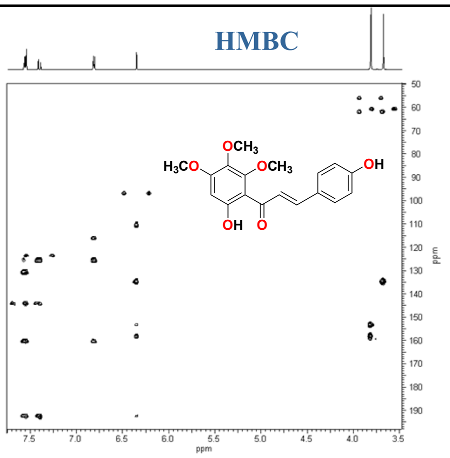


HMBC



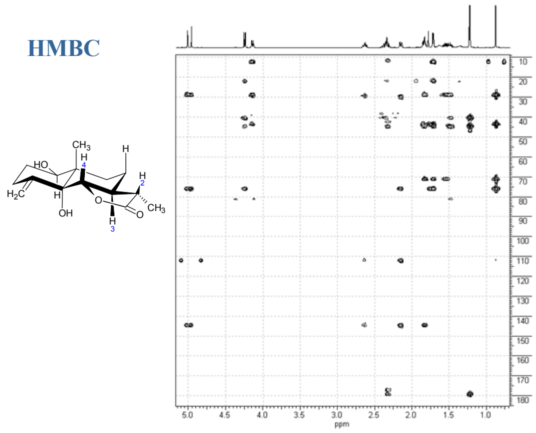
242

HMBC



243

HMBC



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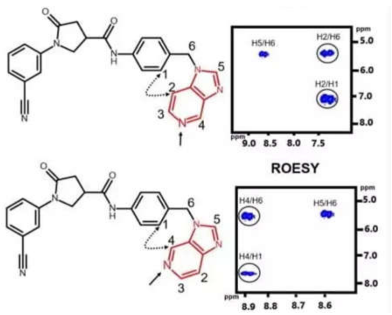
ROESY区别区域异构(Regioisomerism)



波谱法特点

- (1) 样品用量少，一般来说2-3 mg即可（最低可少到<1 mg）；
- (2) 除质谱外，其它方法无样品消耗，可回收再使用；
- (3) 省时、简便
- (4) 配合元素分析（或高分辨质谱），可以准确地确定化合物的结构

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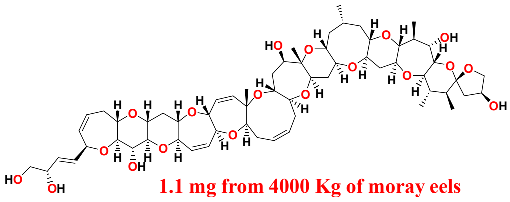
Ciguatoxin CTX



1.1 mg from 4000 Kg of moray eels

A C55 fatty acid coiled into one terminal spiro and twelve contiguous transfused ether rings ranging in size from oxolane to oxonane. The remaining structural features were unremarkable-five olefins, five methyls, and six hydroxyls, two as a terminal vicinal diol.

(LD_{50} 0.25-4 μg/kg)

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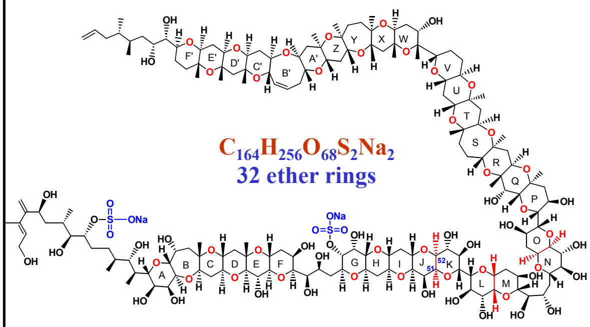
Maitotoxin MTX

Polyether ladder 刺尾鱼毒素



Murata, M.; Iwashita, T.; Yokoyama, A.; Sasaki, M.; Yasumoto, T. *J. Am. Chem. Soc.* 1992, 114, 6594

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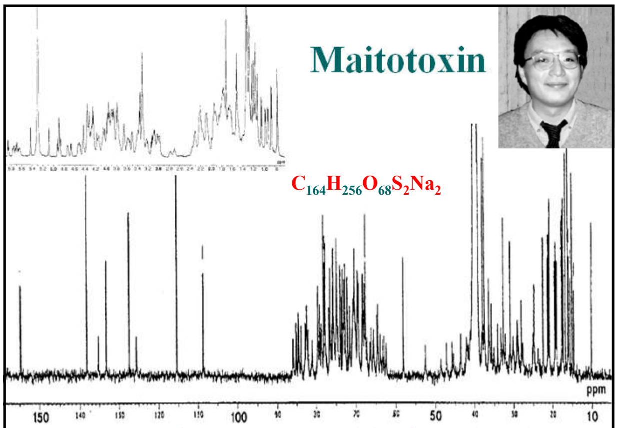

MTX代表着现代鉴定技术NMR-MS在天然产物化学结构研究中的应用水平

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小 结

- 化学位移：NMR 信号组的数目代表了分子中的等价质子的数目。
- NMR 信号组的位置告诉我们分子中每一种等价质子的化学环境。
- 峰强度：峰面积积分比对应于分子中各等价质子的数目比。
- 裂分：裂分的峰数与相邻的另一组等价质子的数目。
- 根据偶合常数可判别哪两组等价质子为相邻关系。
- 氘-氢交换：判别活泼氢的常用方法。

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核磁共振碳谱的解析

解析步骤：

1）区分出杂质峰、溶剂峰，不要遗漏季碳的谱线；
2）计算不饱和度
3）分子对称性的分析：若谱线数目少于元素组成式中碳原子的数目，说明分子有一定的对称性；
4）碳原子级数的确定（活泼氢数目的确定）；
5）碳原子δ值的分区：饱和区、不饱和区、杂原子区、羰基区
6）推出结构单元，组合可能的结构式；
7）对推出的结构进行碳谱指认。

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Kurt Wüthrich and NMR of Biological Macromolecules

1. 确定测出足够数量与结构相关的参数，从而给出准确、唯一的可能结构。
2. 可发出一种计算技术，它能把结构参数转化为唯一的大分子三维结构。

The Nobel Prize in Chemistry 2002

The Nobel Prize Laureates in 2002


Kurt Wüthrich 1938-


The Nobel Prize in Chemistry 2002


Richard R. Ernst
1933-　1991 NP

1985年,利用Kurt Wuthrich 的方法确定了第一个蛋白质的结构。

2017年12月， Kurt Wuthrich成为首批来沪工作并拥有"中国绿卡"的诺奖得主。

Wüthrich collaborated with, among others, Nobel laureate Richard R. Ernst on developing the first two-dimensional NMR experiments, and established the NOE as a convenient way of measuring distances within proteins.

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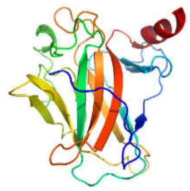
Experimentally Derived Structural Restraints


p53 WT core domain

Overhauser effect)
- structure determined from short-range H-H interactions (<6Å)

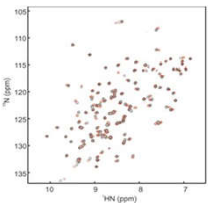
Residual Dipolar Coupling
- bond orientation

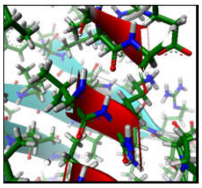
Chemical Shift Index (TALOS)
- bond dihedral angles

J-coupling constants
- bond dihedral angles

Hydrogen bonds
- from hydrogen exchange protection factors

How to Interpret Spectra?

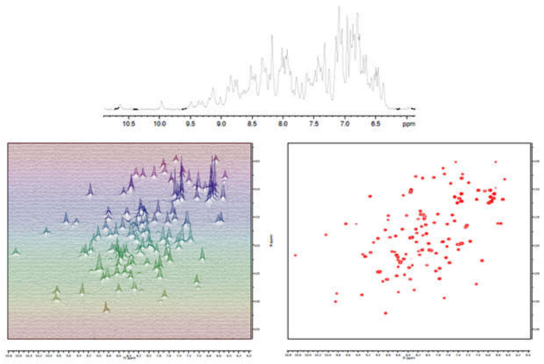




Spectral parameters
Resonance frequency
Modulation of frequency
Correlation via dipolar field
Correlation indirectly via electrons (scalar coupling)
Relaxation

Structural implications
Atom type (and near neighbours)
Spatially near neighbours
Chemically bonded neighbours
Dynamic consequences
Fluctuating magnetic environment ?

Multi-Dimensional NMR: Reducing Peak Overlap



2003年诺贝尔医学或生理奖

核磁共振在医学上的应用——核磁共振成像


Paul C. Lauterbur
(1929-2007) USA


Peter Mansfield
(1933-) English

核磁共振成像（Nuclear Magnetic Resonance Imaging, MRI）

Magnetic Resonance Imaging (MRI)

全球各大公司所生产的医用核磁共振成像仪中，价格最高的要达到1900万元，最便宜的，也要360万元。

患者接受一次核磁共振成像检查，最少要花费1400元左右。

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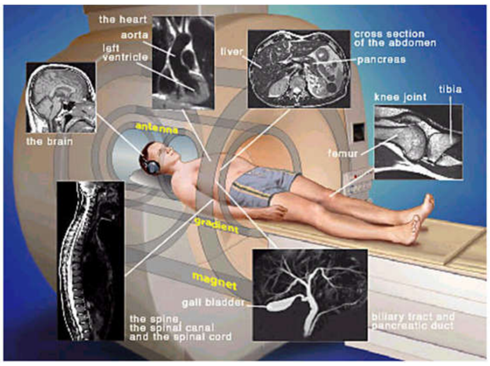


MRI is used for imaging of all organs in the body 259

核磁共振成像—磁共振成像
Nuclear Magnetic Resonance Imaging, MRI

日本广岛原子弹爆炸
1945年8月6日上午8时

切尔诺贝利核泄漏
1986年4月26日

Magnetic Resonance Imaging

- What is MRI?
- How does MRI work?
- What is MRI used for?
- Are there risks?

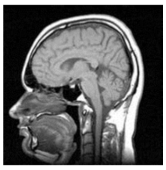
Paul C. Lauterbur

Peter Mansfiel

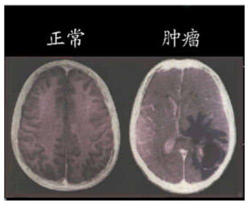
物质是由原子组成的，而原子的主要部分是原子核。根据物体的原子核的位置和种类，绘制成物体内部的精确立体图像。把这种技术用于人体内部结构的成像，就可获得一种非常重要的诊断工具——核磁共振成像。

Peter

2003年诺贝尔医学奖:美国科学家保罗·劳特布尔 (Paul Lauterbur)和英国科学家彼得·曼斯菲尔德(Peter Mansfield)



人脑纵切面的核磁共振成像



用核磁共振层析“拍摄”的脑截面图象 262

Science 1971, 171, 1151–1153

MRI技术的最大受益者—医生和患者

Invention of the MRI scanner revolutionised medicine

欧洲磁共振奖
1986

Paul C. Lauterbur和Peter Mansfield在颁奖仪式上从瑞典国王手里接过荣誉证书。但也有学者认为这是NMR在医学上的应用而已，不足以获得诺贝尔奖。

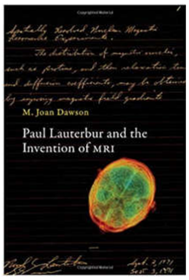
“核磁共振成像技术之父”-Paul C. Lauterbur

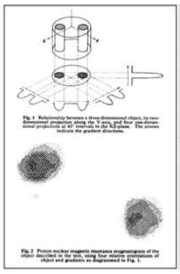
保罗·劳特布尔(Paul Lauterbur)，‘the father of MRI’，美国科学家。上世纪70年代初，执教于纽约州立大学石溪分校期间，他致力于核磁共振光谱学及其应用的研究。他还把核磁共振成像技术推广应用到生物化学和生物物理学领域。National Inventors Hall of Fame, class of 2007。

Studing Intact Tissues Using NMR

Lauterbur's wife and scientific partner

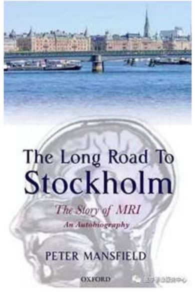
M. Joan Dawson
1944-2017





Dr. Dawson was a respected member of the biomedical NMR community, helping to pioneer the use of NMR to study the linkage between metabolism and function in living systems. M. Joan Dawson, the wife of Paul C. Lauterbur

Sir Peter Mansfield



《通往斯德哥尔摩的漫长道路》

曼斯菲尔德(1933-2017) 为了更精确地显示共振中的差异，使用了磁场梯度。他发现了被探测的信号是如何迅速而有效的被分析转换成图像。这是获得实用方法的关键一步。然后通过改进数学算法并发明了能够在数秒内进行成像的回波平面成像 (EPI)，这项发明奠定了磁共振快速成像的基础，将扫描速度提升了成百上千倍，使得扫描人体真正具有实用性。

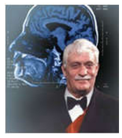
Sir Peter Mansfield at an MRI scanner with Margaret Thatcher and Sir Colin Campbell

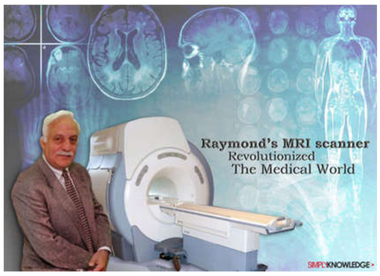
The images were presented at a special meeting in 1976

Raymond Damadian

Changed the face of medicine in 1977 with the world's first MRI device

2003年诺贝尔生理学或医学奖-历史上最明显的遗漏?





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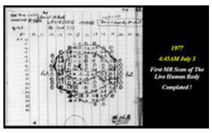
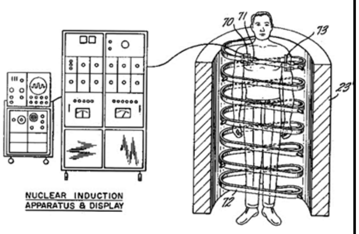
Raymond Damadian - First Patent in the Field of MRI





"Apparatus and Method for Detecting Cancer in Tissue." A patent was granted in 1974, it was the world's first patent issued in the field of MRI. By 1977, Dr. Damadian completed construction of the first whole-body MRI scanner, which he dubbed the "Indomitable."

Denied Nobel for M.R.I., He Wins Another Prize

President Ronald Reagan presents the National Medal of Technology to Damadian, 1988.

Raymond V. Damadian, MD, 1999H

Inventor of magnetic resonance imaging

Paul Dreizen, MD, 2001H

As a faculty member in the Department of Medicine and Biophysics Graduate Program at Downstate from 1967 to 1978, Raymond Damadian made fundamental contributions to the discovery and development of magnetic resonance imaging. These contributions include the original conception of magnetic resonance imaging for whole-body scanning of living humans; the discovery of differences of proton T1 and T2 relaxations among normal tissues and between normal and cancerous tissues that provides the biological basis for MRI; and devising a scanning method used in construction of the first full-body human MRI machine in his laboratory at Downstate.

The first MRI scanner, The Indomitable, has been on permanent display in the Hall of Medical Sciences at the Smithsonian Institution since 1986. The National Medal of Technology was awarded jointly to Raymond Damadian and Paul Lauterbur by President Reagan in 1988 with the citation, "For their independent contributions in conceiving and developing the application of magnetic resonance technology to medical uses including whole body scanning and diagnostic imaging."

Damadian was inducted into the National Inventors Hall of Fame in 1989. His 1972 patent for magnetic resonance imaging based upon T1 and T2 proton relaxations was affirmed by the U.S. Supreme Court in 1997. Among other honors, he has received honorary doctorates from the University of Wisconsin, Albert Einstein College of Medicine, and Downstate.

Early Scientific Career (1960 to 1969)

Damadian was born and grew up in New York City and attended the Juilliard School of Music. Forsaking a promising career as a concert violinist, he became a Ford Foundation Scholar at University of Wisconsin, received his MD degree at Albert Einstein College of Medicine, and entered residency training in medicine at Kings County Hospital in 1960. With strong encouragement from Ludwig Eichna, the recently appointed chairman of medicine at Downstate [See Eichna monograph. Ed.], Damadian began postdoctoral research in renal physiology with Neil Bricker at Washington University School of Medicine (1962), followed by a fellowship (1963-1965) in Arthur Solomon's biophysical laboratory at Harvard Medical School, a main center for biophysical studies of membrane transport. While there, Damadian identified and isolated an *E. coli* mutant deficient in the active transport of potassium ions (Damadian and Solomon, *Science* 1964). This project was continued during military service at the U.S. Air Force School of Aerospace Medicine (1965-67).

Damadian returned to Downstate as assistant professor of medicine and biophysics in 1967. In research supported by grants from the NIH and the Health Research Council of New York City, the relation between intracellular ionic composition and transmembrane potential of *E. coli* was studied in native and mutant forms (Damadian, *J. Bacteriology* 1968; *Science* 1969). This work led to the first measurements, in collaboration with Freeman Cope, of potassium (K^{39}) NMR relaxations in bacteria, intended to provide information about the physical states of intracellular K^+. Using *Halobacter halobium*, a microbe from the Dead Sea with high intracellular K^+ concentration, and

Downstate at 150: A Celebration of Achievement 77