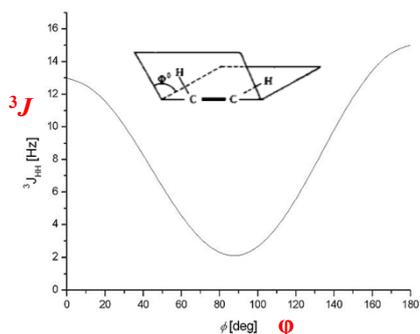


2013年诺贝尔化学奖

Karplus Equation: 耦合常数和二面角之间关系, 为确定分子结构找到了重要规律, 也为后来蓬勃发展的结构生物学奠定了基础。

$${}^3J = J^0 \cos^2\phi - 0.28 \quad (0^\circ \leq \phi \leq 90^\circ) \quad {}^3J = J^{180} \cos^2\phi - 0.28 \quad (90^\circ \leq \phi \leq 180^\circ)$$

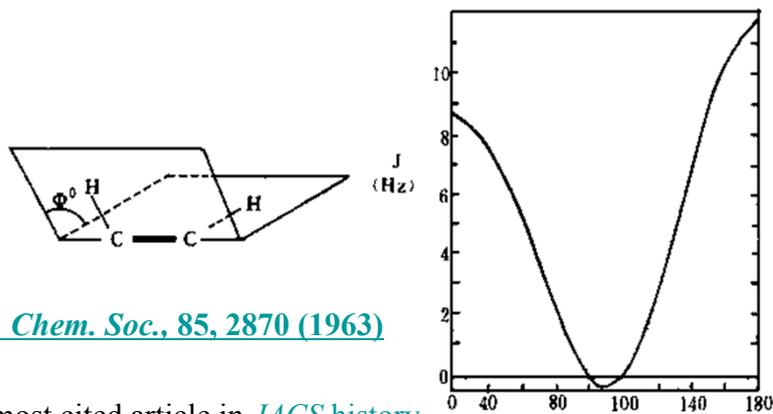


Martin Karplus (1930-)

J. Chem. Phys., **30**, 11-15 (1959).

饱和型化合物 Karplus Equation

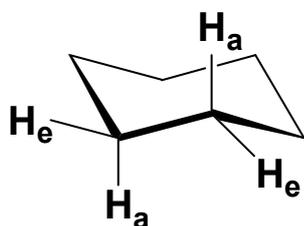
3J 与两面夹角 ϕ 的关系 (Relation)



J. Am. Chem. Soc., **85**, 2870 (1963)

17th most cited article in [JACS history](#).

402

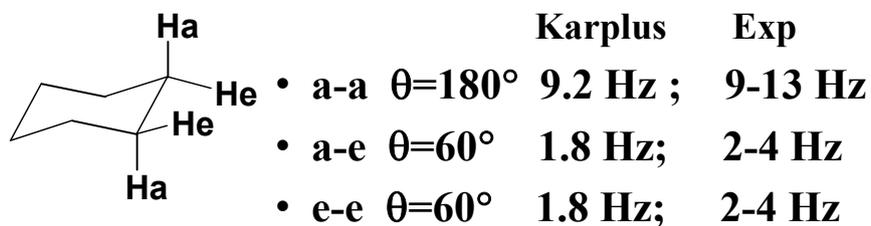


- $H_a \sim H_a$ 耦合, $\Phi_{a \sim a} = 180^\circ$, ${}^3J_{HH} = 8 \sim 12 \text{ Hz}$
- $H_a \sim H_e$ 耦合, $\Phi_{a \sim e} = 60^\circ$, ${}^3J_{HH} = 1 \sim 5 \text{ Hz}$
- $H_e \sim H_e$ 耦合, $\Phi_{e \sim e} = 60^\circ$, ${}^3J_{HH} = 0 \sim 4 \text{ Hz}$

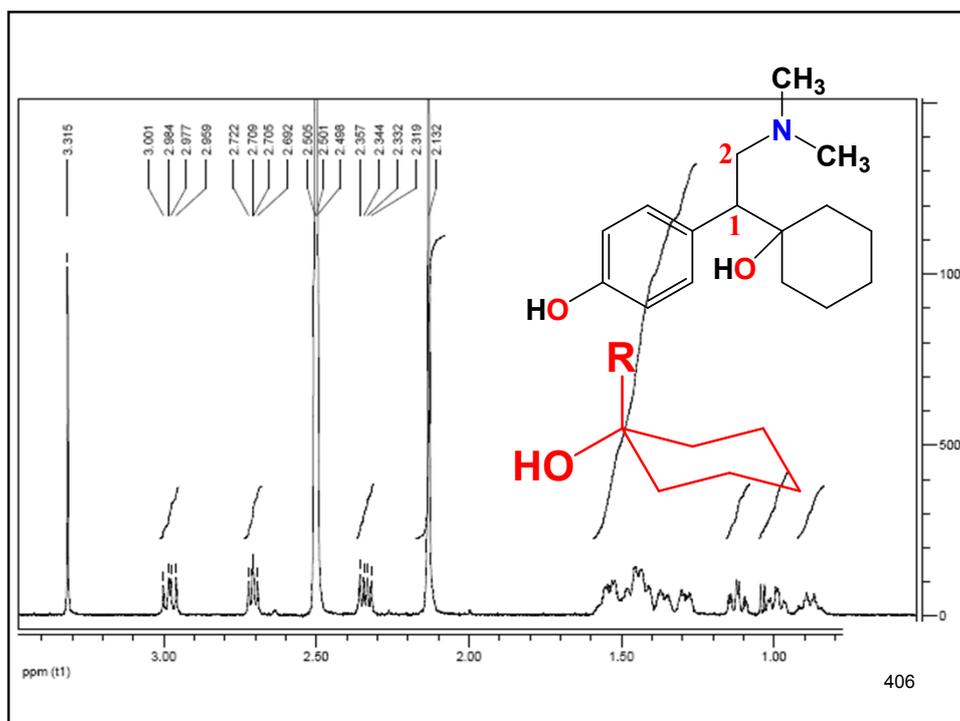
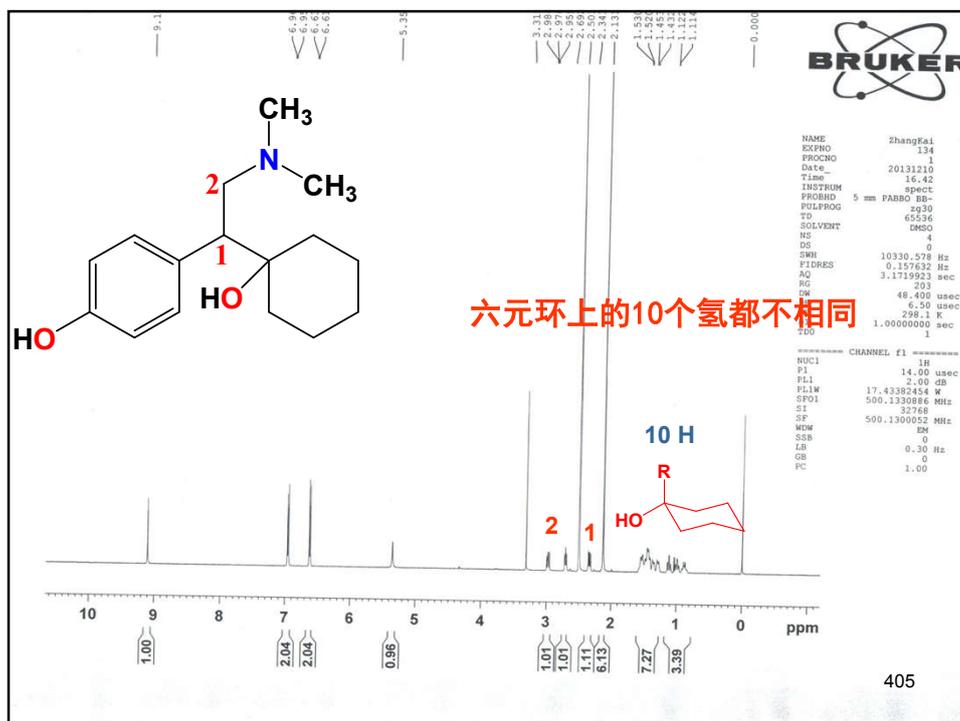
403

Karplus Equation

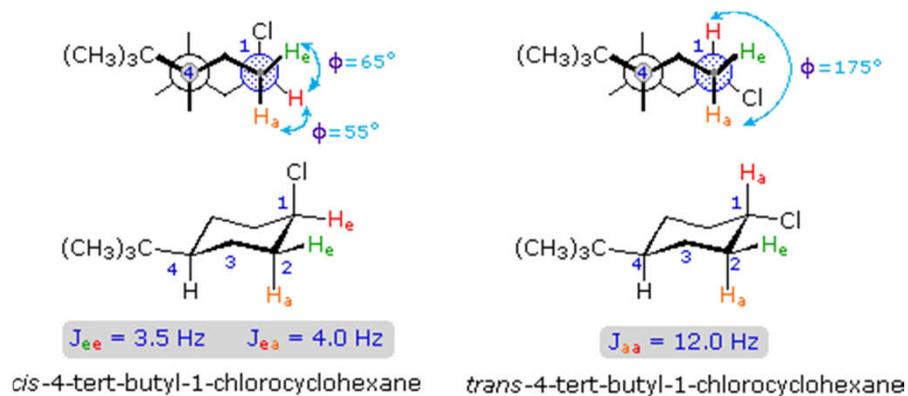
这一公式在计算环戊烷, 环己烷, 糖类, 稠环化合物中很有用。



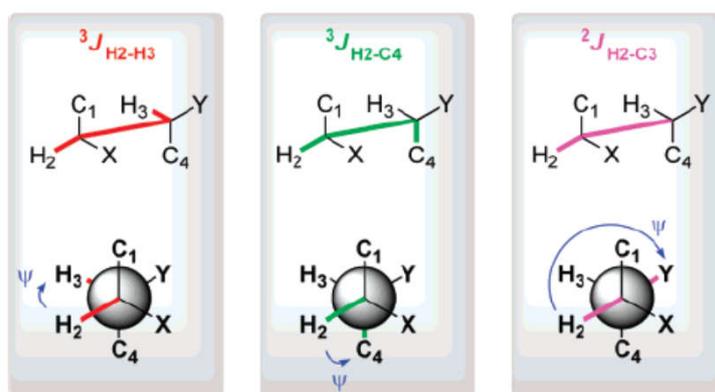
404



Karplus Equation



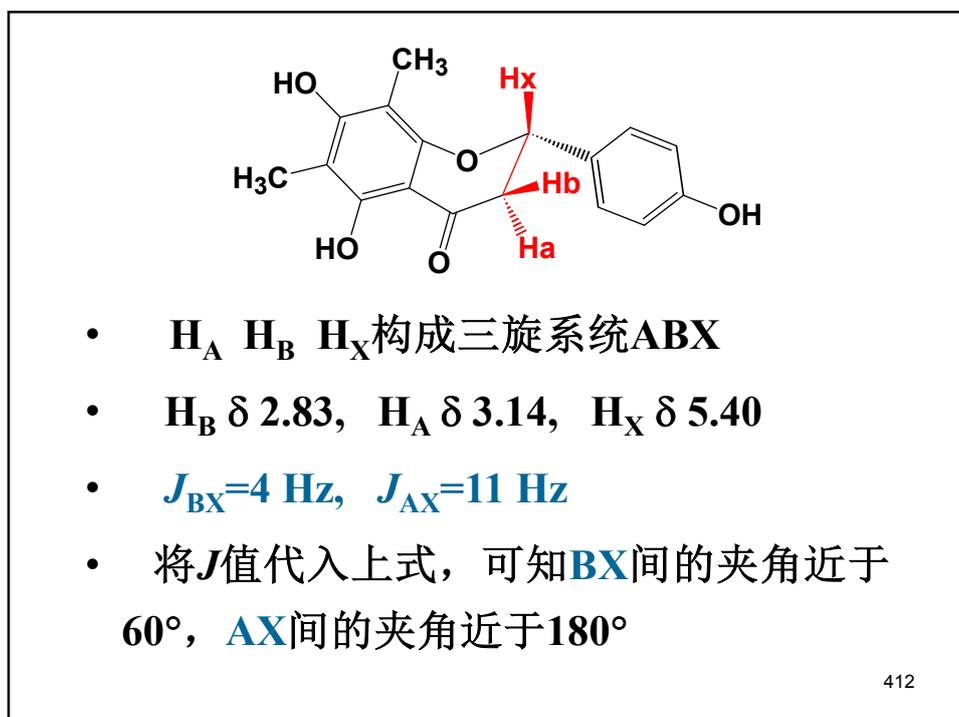
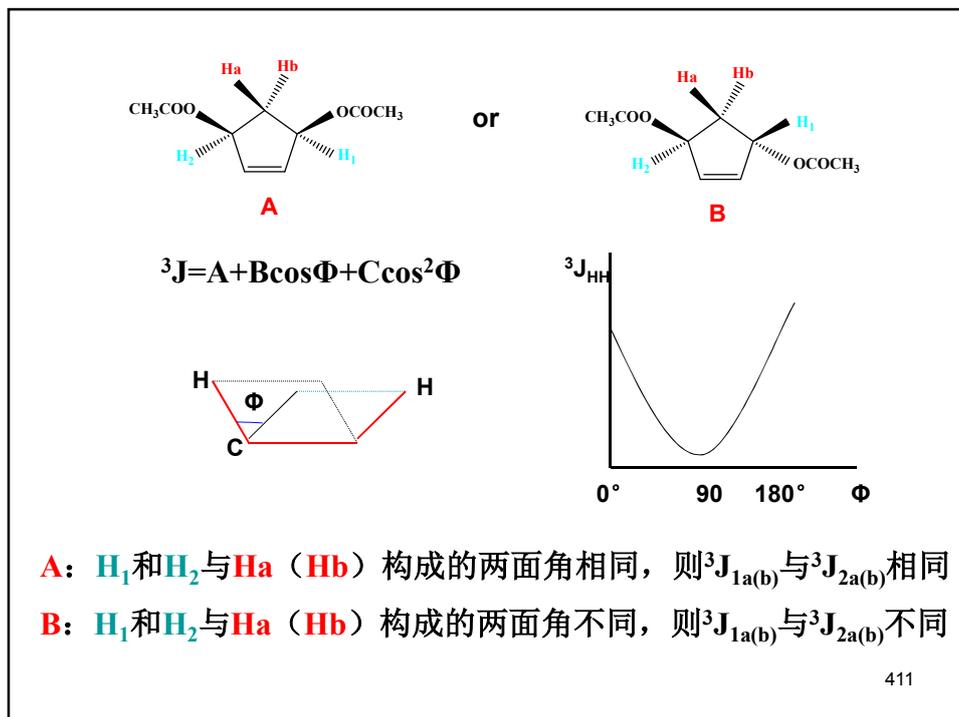
407



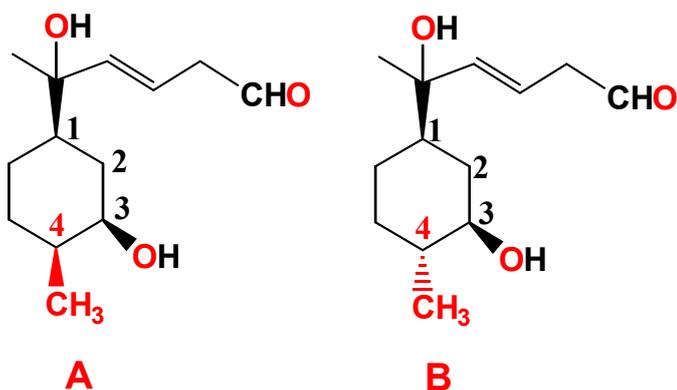
ψ = dihedral angle
 X = generic substituent
 Y = electronegative atom

Figure 1. Three- and two-bond homo- and heteronuclear coupling constants.

408

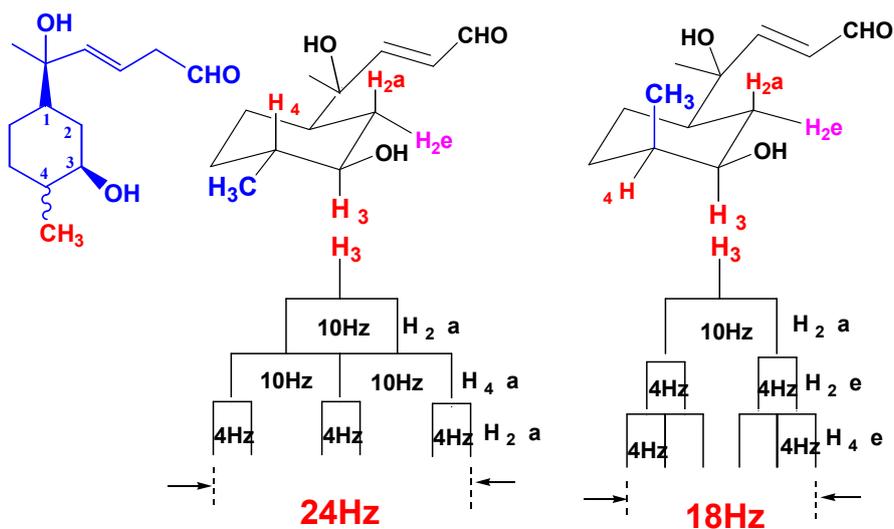


1. How to Decide the Relative Configuration of C-4-Me



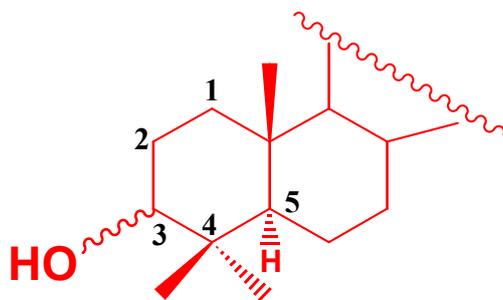
413

用于确定六元环中CH₃为a或e键（实测17Hz）

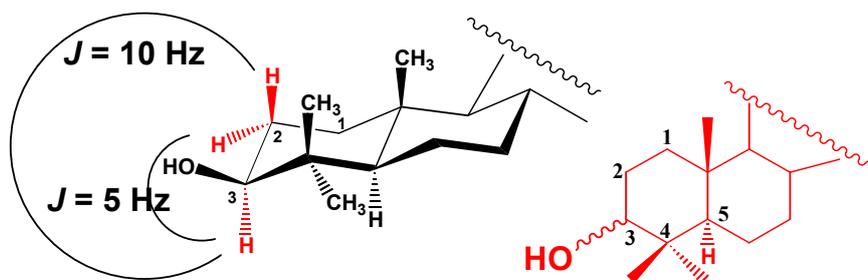


414

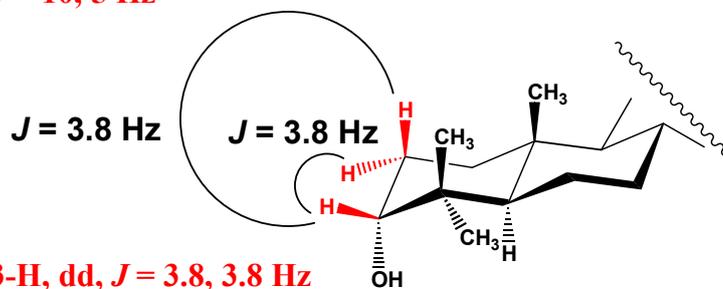
基于 3J 值的构型分析



415

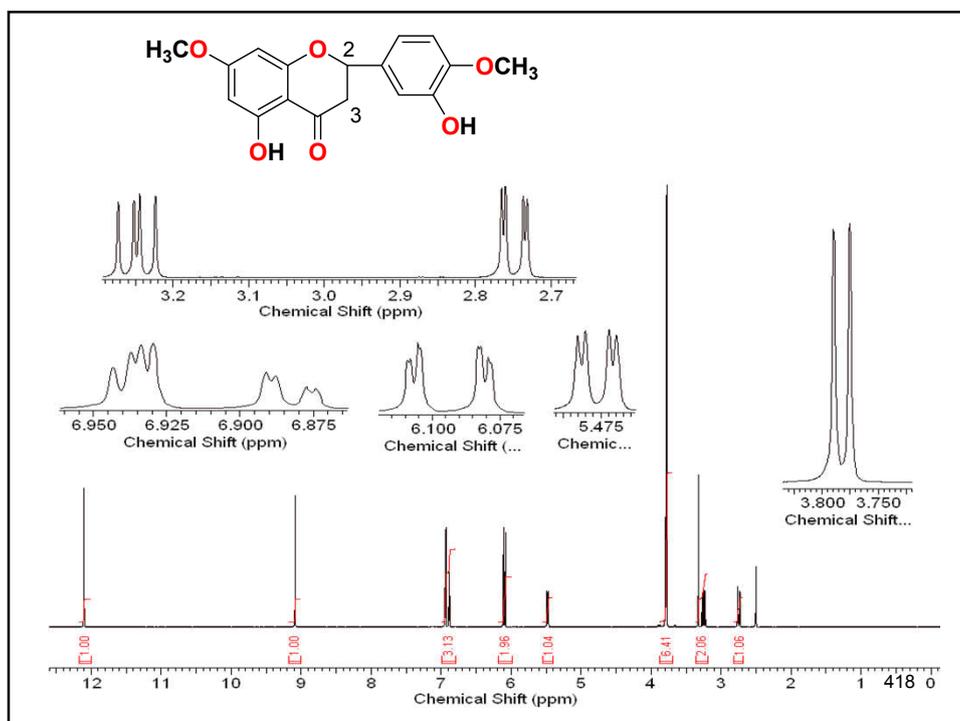
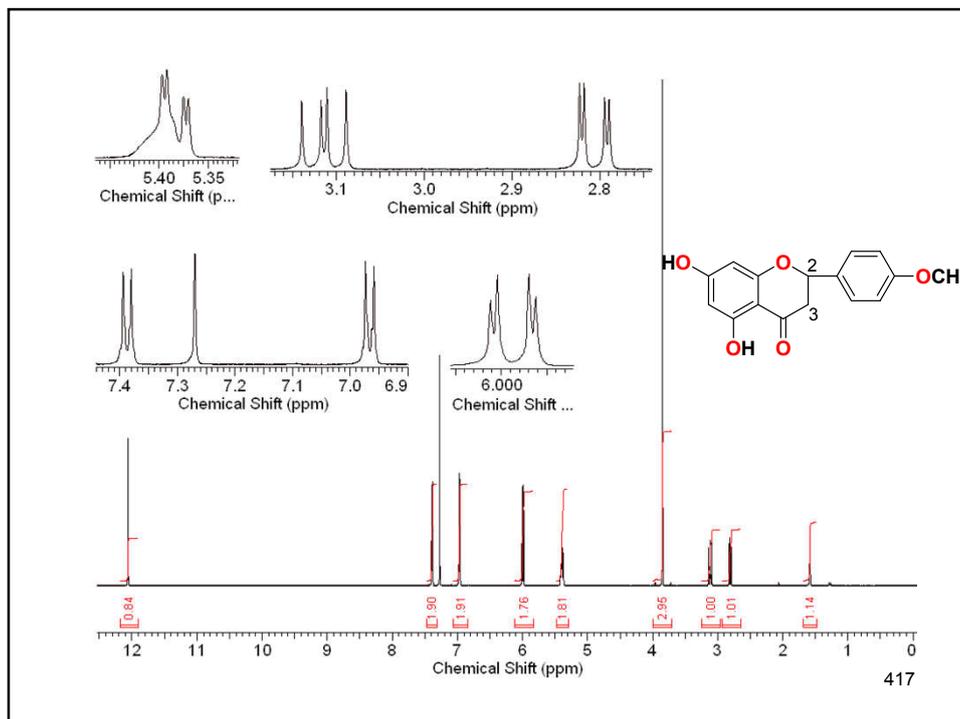


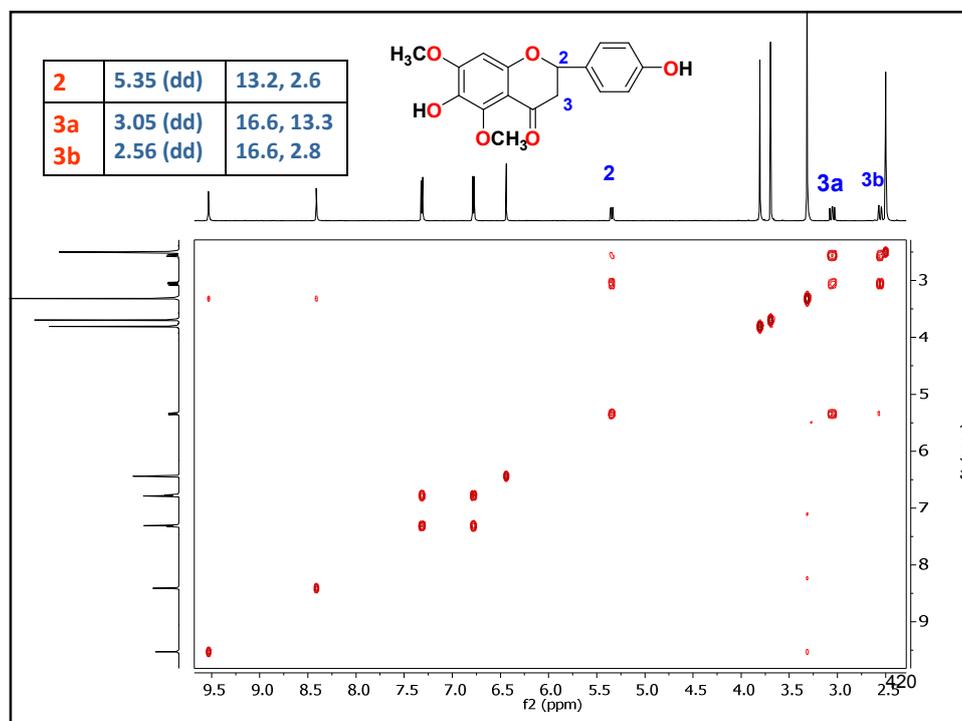
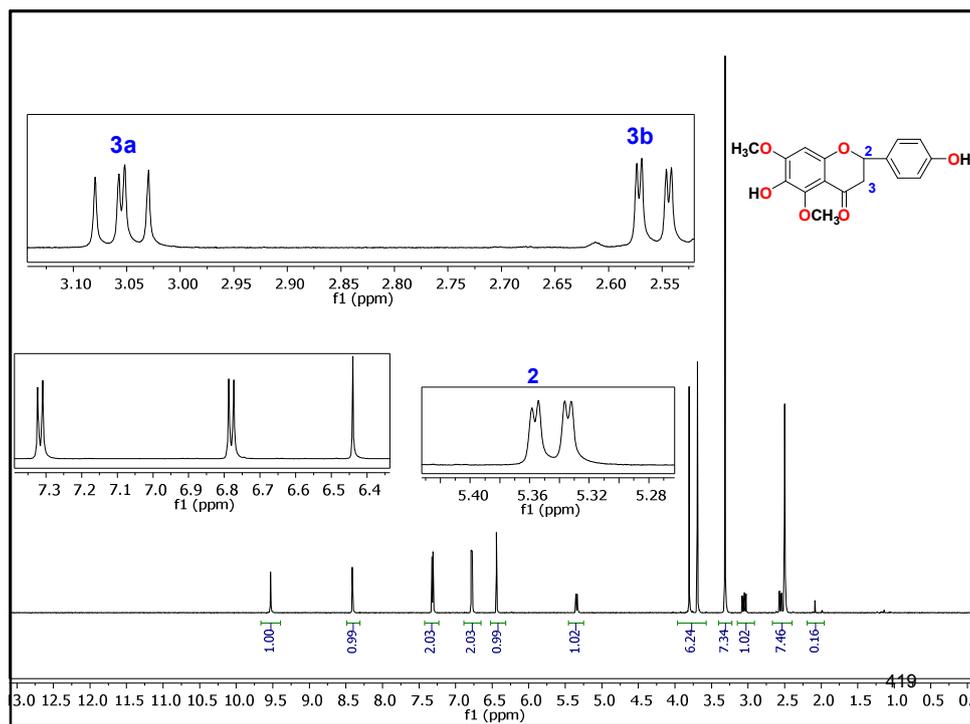
3-H, dd, $J = 10, 5 \text{ Hz}$

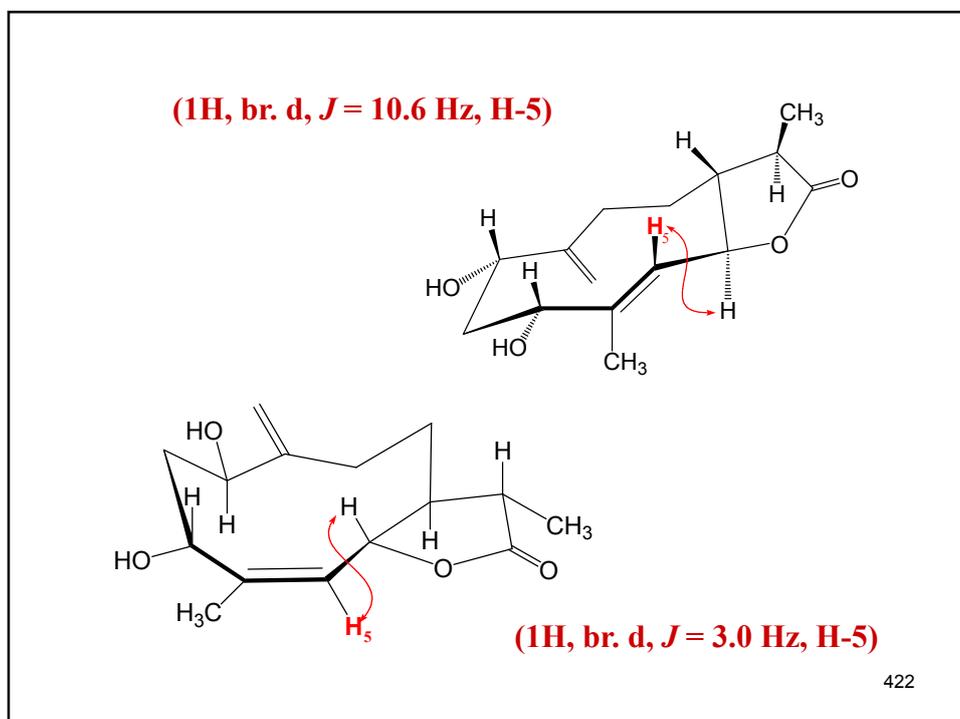
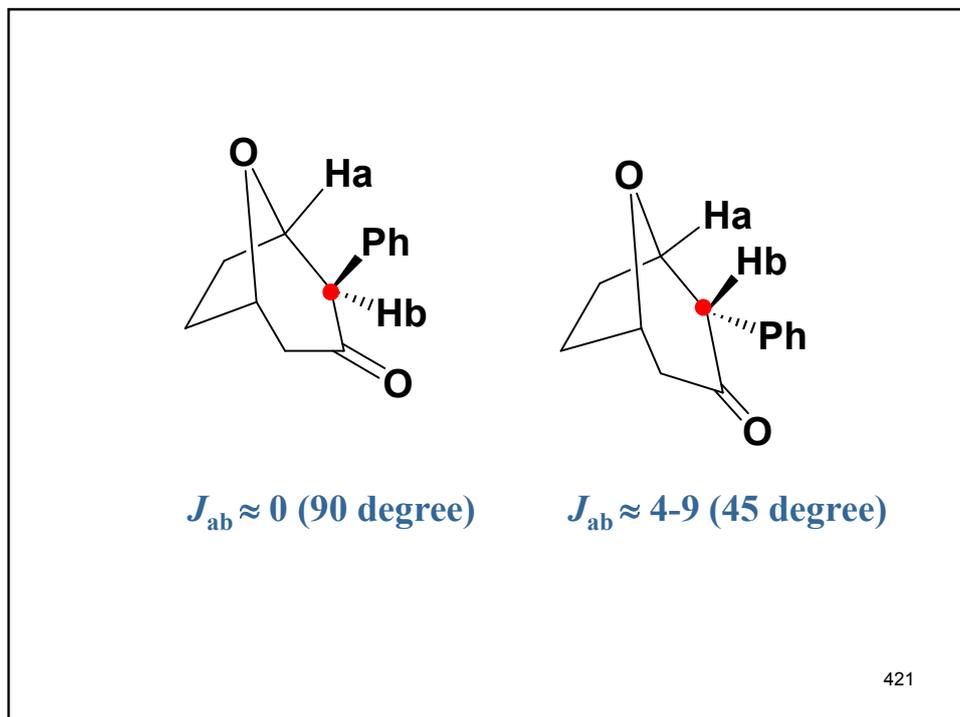


3-H, dd, $J = 3.8, 3.8 \text{ Hz}$

416





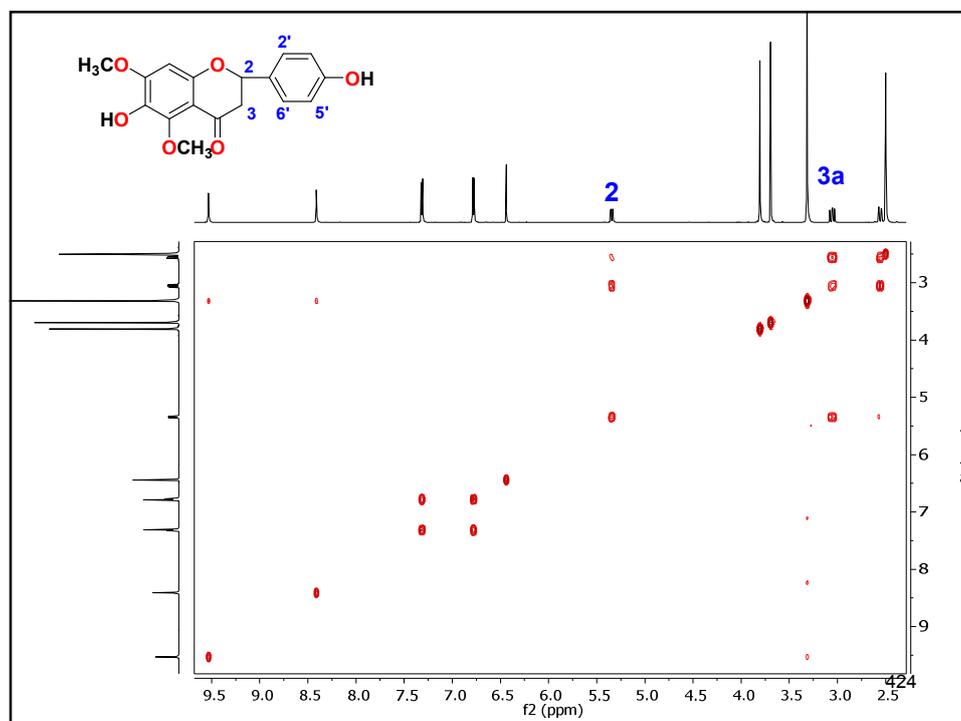


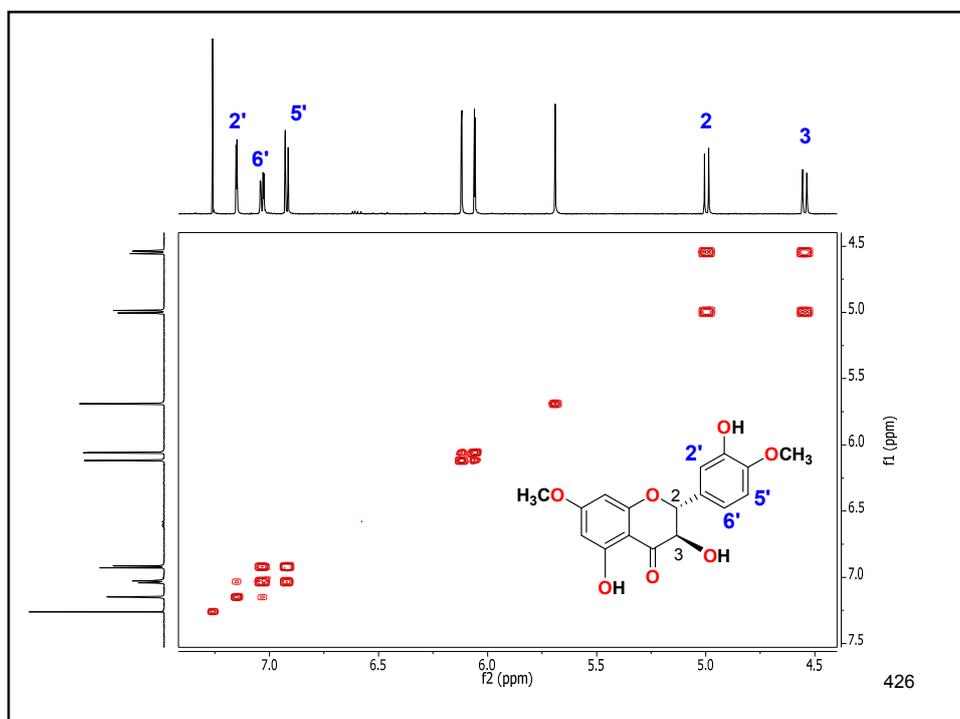
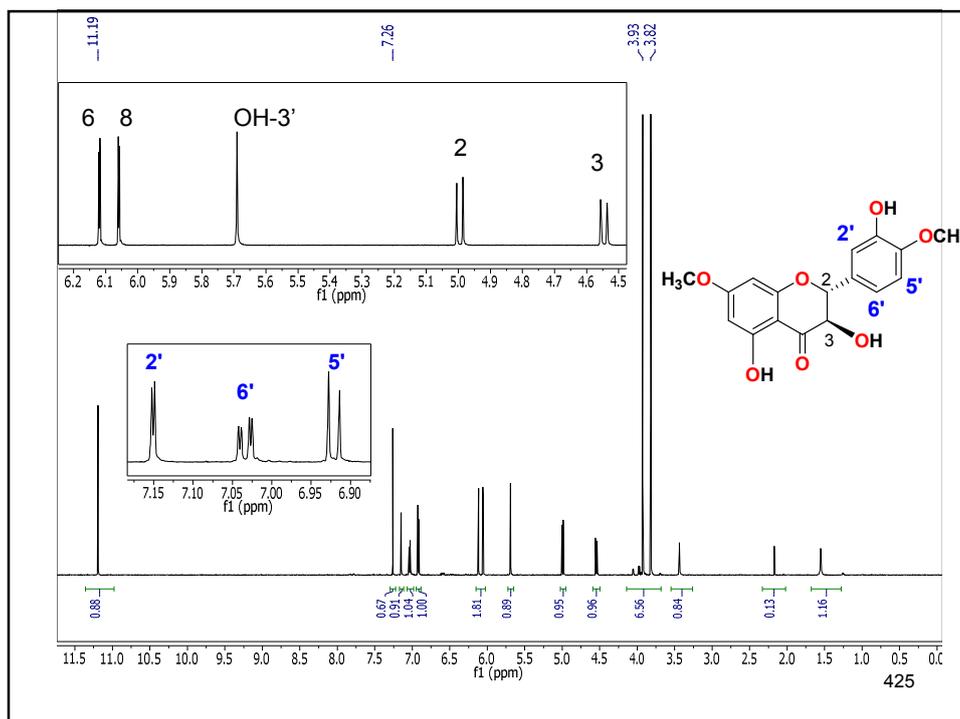
如何计算偶合常数

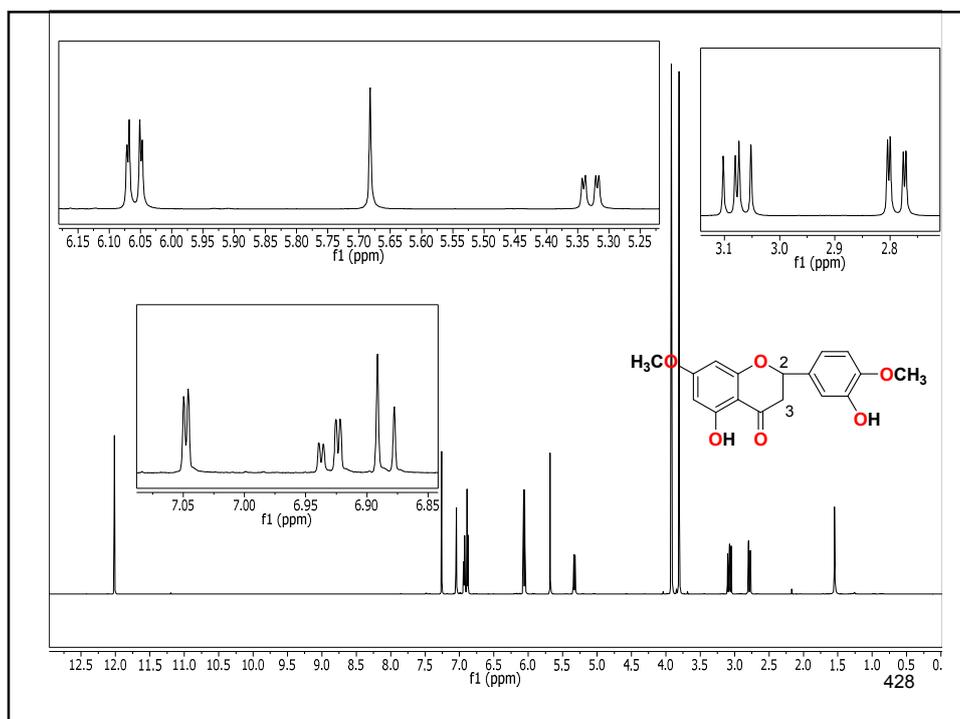
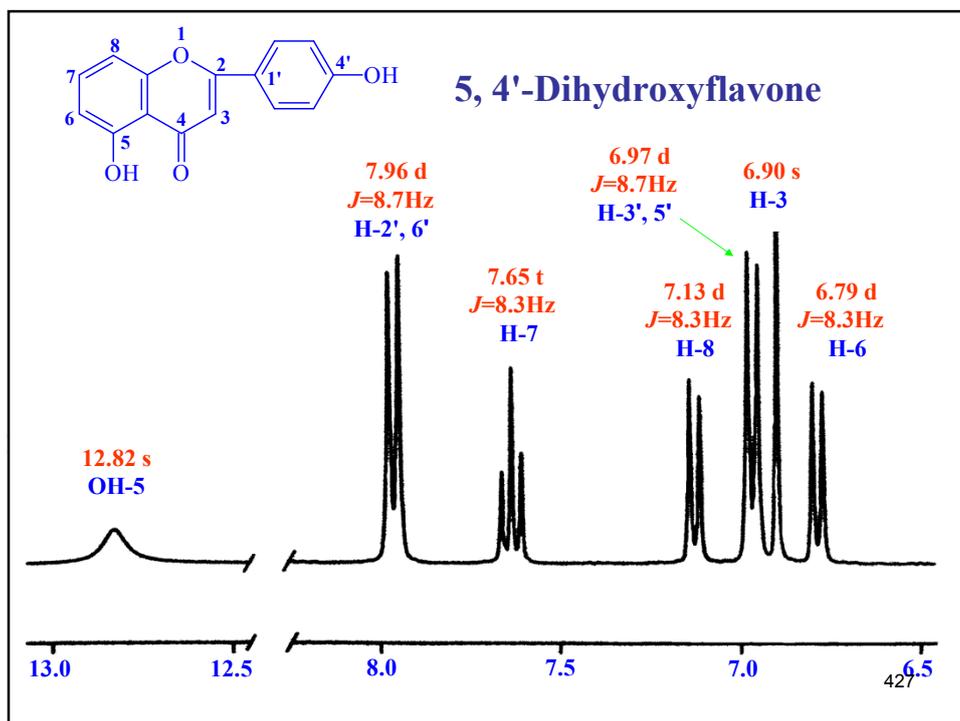
The spacing between the lines of a doublet, triplet or quartet is called the coupling constant. It is given the symbol J and is measured in units of Hertz (cycles per second). The magnitude of the coupling constant can be calculated by multiplying the separation of the lines in units (ppm) by the resonance frequency of the spectrometer in mega Hertz.

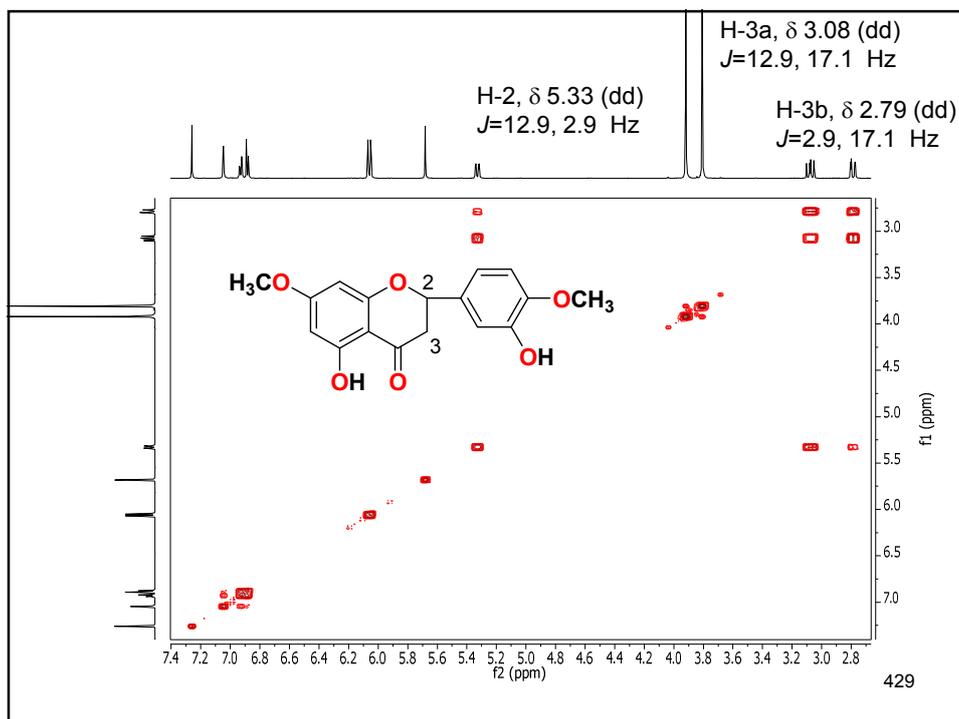
$$J \text{ Hz} = \text{ppm} \times \text{MHz (typically 300, 400, or 500 MHz)}$$

423







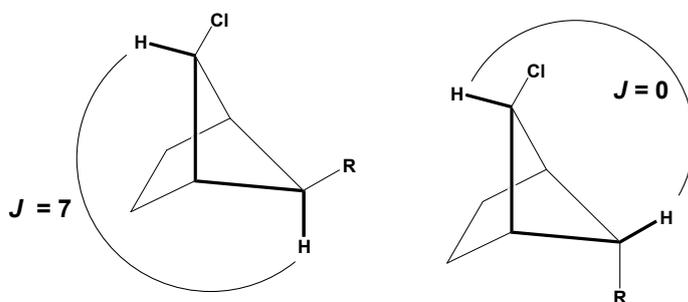


W 型 偶 合

当两个氢核正好位于英文字母“w”的两端时，由于空间上很接近，而可发生远程偶合，但 J 值很小，仅约为1Hz，称W型偶合。

430

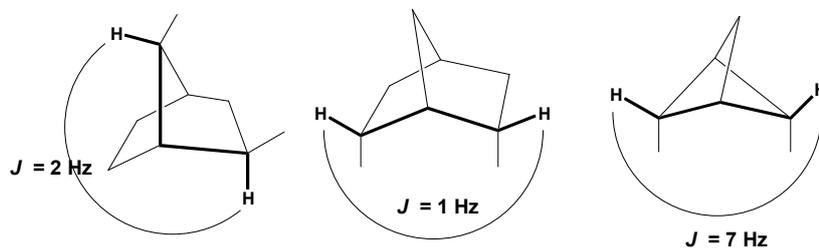
"W" coupling



四个单键共处于同一个平面并构成W折线时，两端的质子有远程耦合，耦合常数一般1-2 Hz。

431

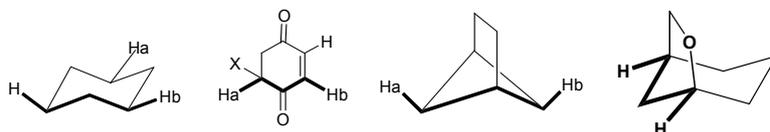
"W" coupling



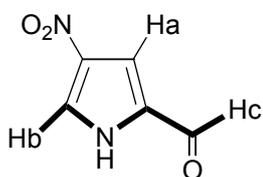
耦合常数随着环的缩小，环张力的增加而增大。

432

跨四个单键的折线型体系(W型)

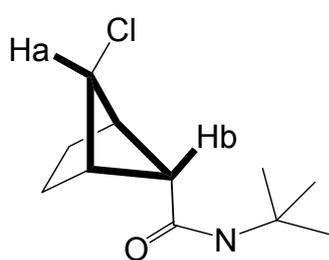


跨五个单键的折线型体系

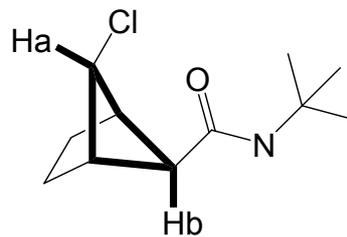


433

"W" coupling



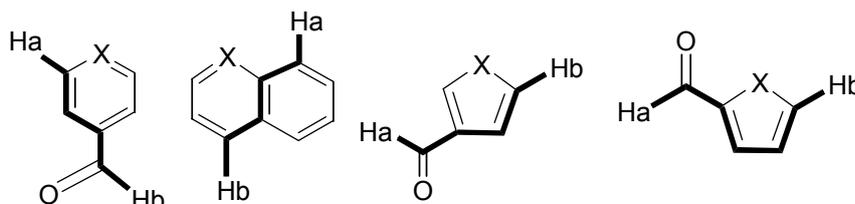
$${}^4J_{a,b} = 0 \text{ Hz}$$



$${}^4J_{a,b} = 7 \text{ Hz}$$

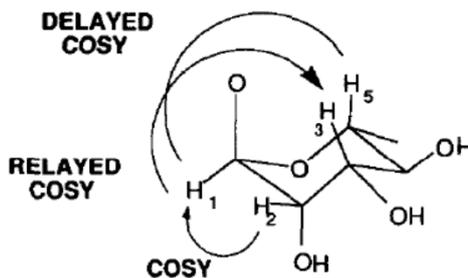
434

其它体系的远程偶合



435

Delayed COSY



Delayed COSY allows linking of signals which are very weakly coupled (down to 0.1 Hz)

Journal of Ethnopharm. 1991, 32, 103-110_{#36}

JBCA, *J*-based Configuration Analysis

- NMR is the most useful method to elucidate the structures of natural products with a molecular weight over 1000 since these compounds can hardly be crystalized. Murata had developed the method called "**JBCA, *J*-based Configuration Analysis**" which can be used to determine the stereochemistry of asymmetric carbon atoms residing in acyclic structures of natural products.
- Murata, M., Matsuoka, S., Matsumori, N., Paul, G. K. and Tachibana, K. *J. Am. Chem. Soc.* **121**, 870-871 (1999).
- Matsumori, N., Kaneno, D., Murata, M., Nakamura, H. and Tachibana, K. *J. Org. Chem.* **64**, 866-876 (1999).

437

固相核磁共振新技术

- 固相核磁共振技术可以实现对固体天然产物样品的直接测定,但是所需样品量要大,所以在天然产物分析中较少使用。

438

2 D ^1H - ^1H 相关谱 (^1H - ^1H COSY)

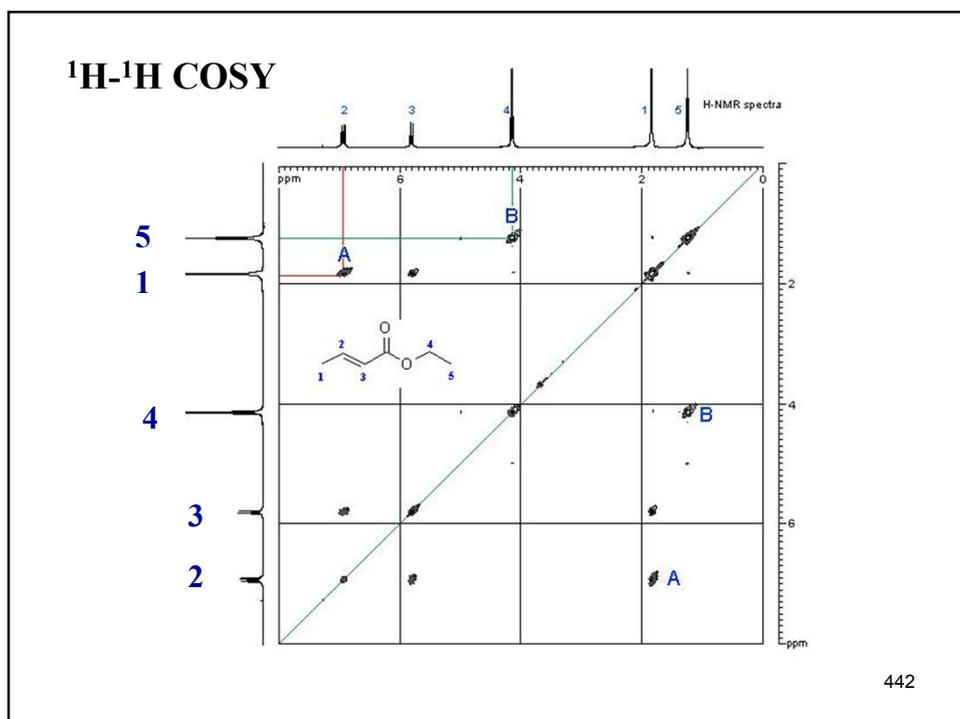
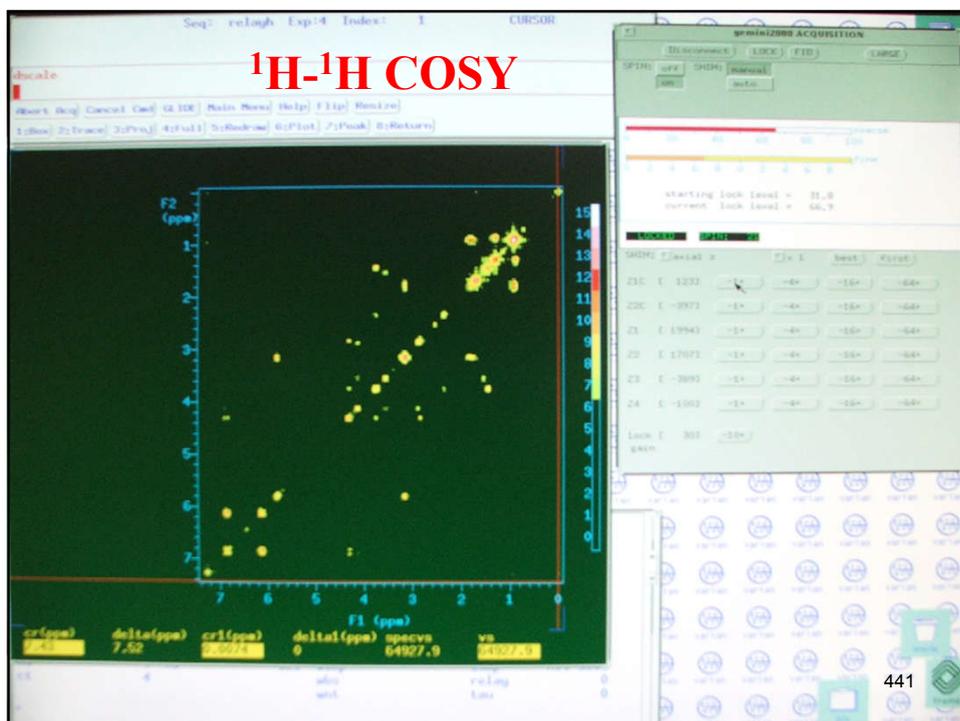
- COSY 谱本身为正方形，当 F1和 F2谱宽不等时则为矩形。正方形中有一条对角线（一般为左下--右上）。对角线上的峰称为对角峰（diagonal peak）。对角线外的峰称为交叉峰（cross peaks）或相关峰（correlated peaks）。每个相关峰或交叉峰反映两个峰组间的耦合关系。COSY 主要反映 3J 耦合关系。

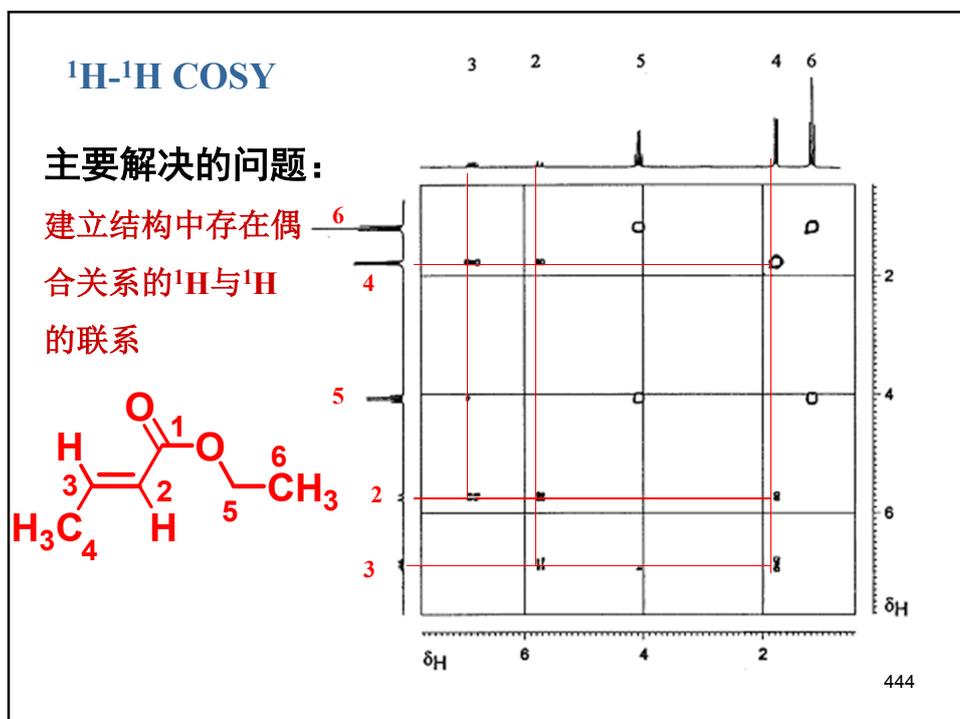
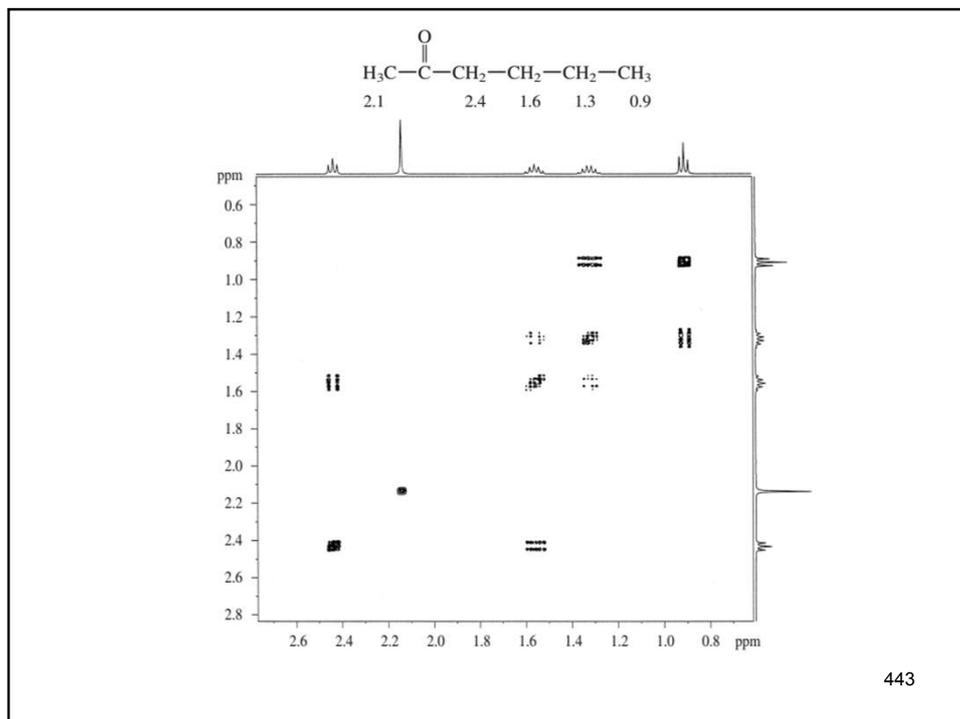
439

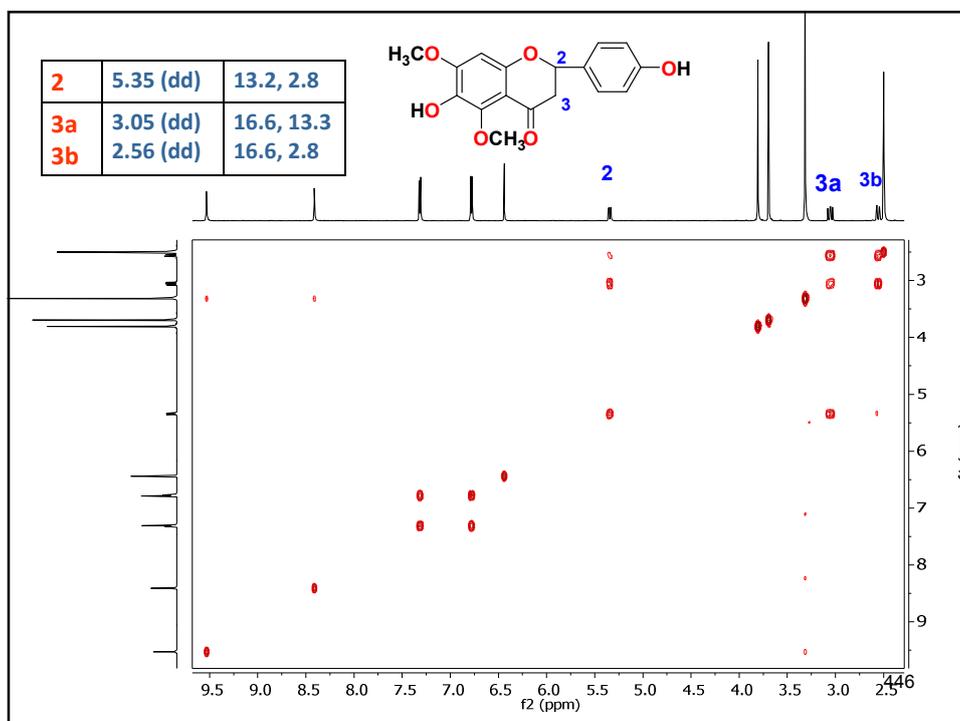
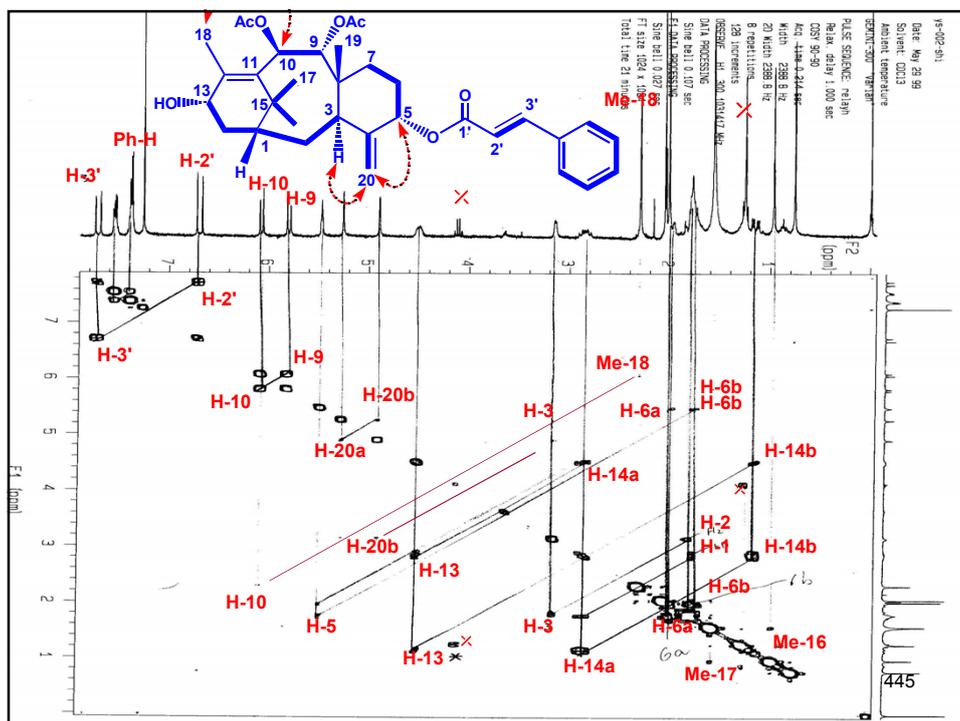
2 D ^1H - ^1H 相关谱 (^1H - ^1H COSY)

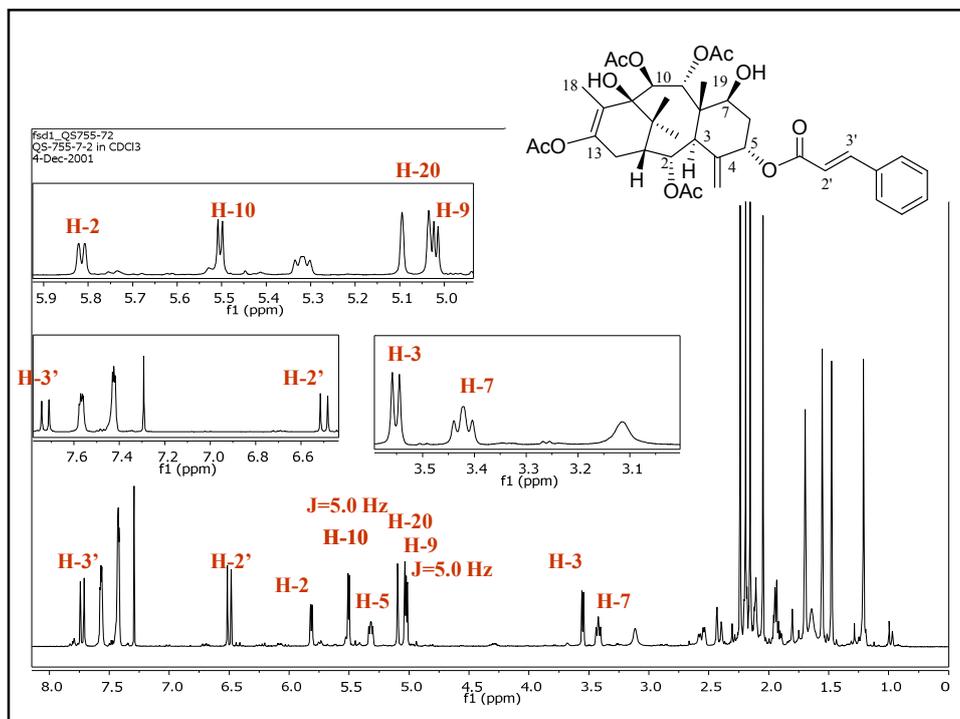
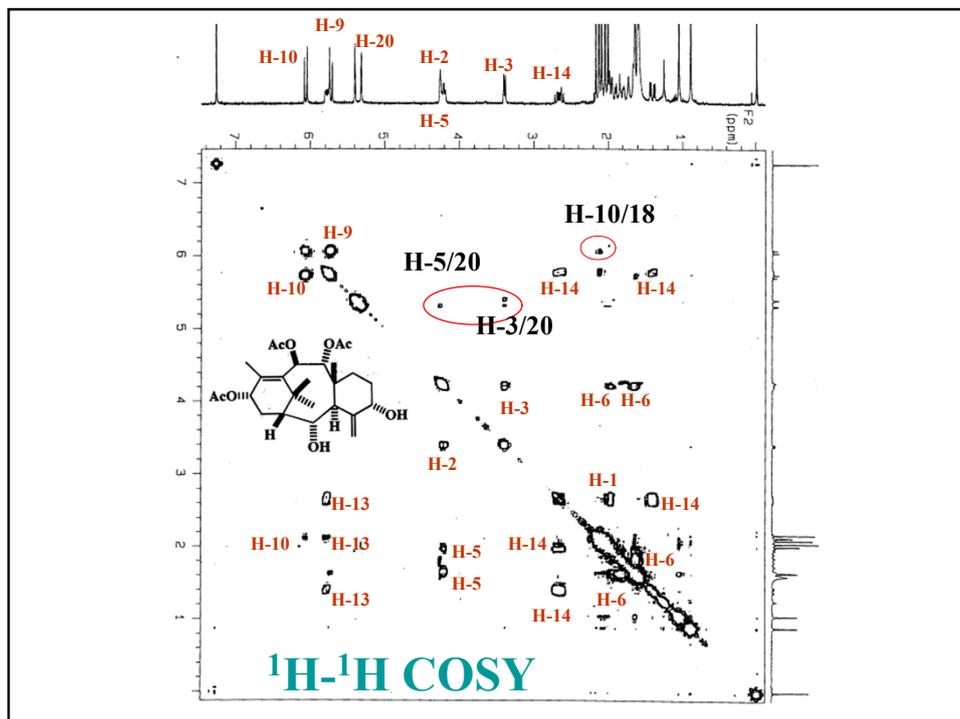
- 它的解谱方法是：取任一交叉峰作为出发点，通过它作垂线，会与某对角线峰及上方的氢谱中的某峰组相交，它们即是构成此交叉峰的一个峰组。再通过该交叉峰作水平线，与另一对角线峰相交，再通过该对角线峰作垂线，又会与氢谱中的另一峰组相交，此即构成该交叉峰的另一峰组。
- 要注意的是 COSY 一般反映的是 3J 耦合关系，但有时也会出现少数反映长程耦合的相关峰，另一方面，当 3J 小时(如两面角接近 90, 使 3J 很小)，也可能没有相应的交叉峰。

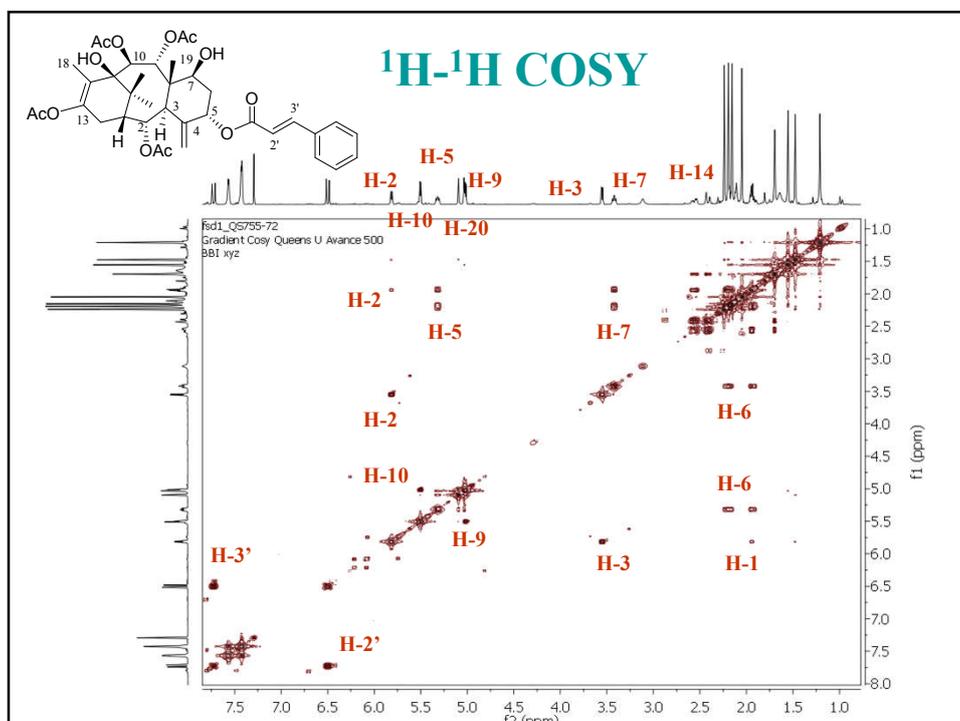
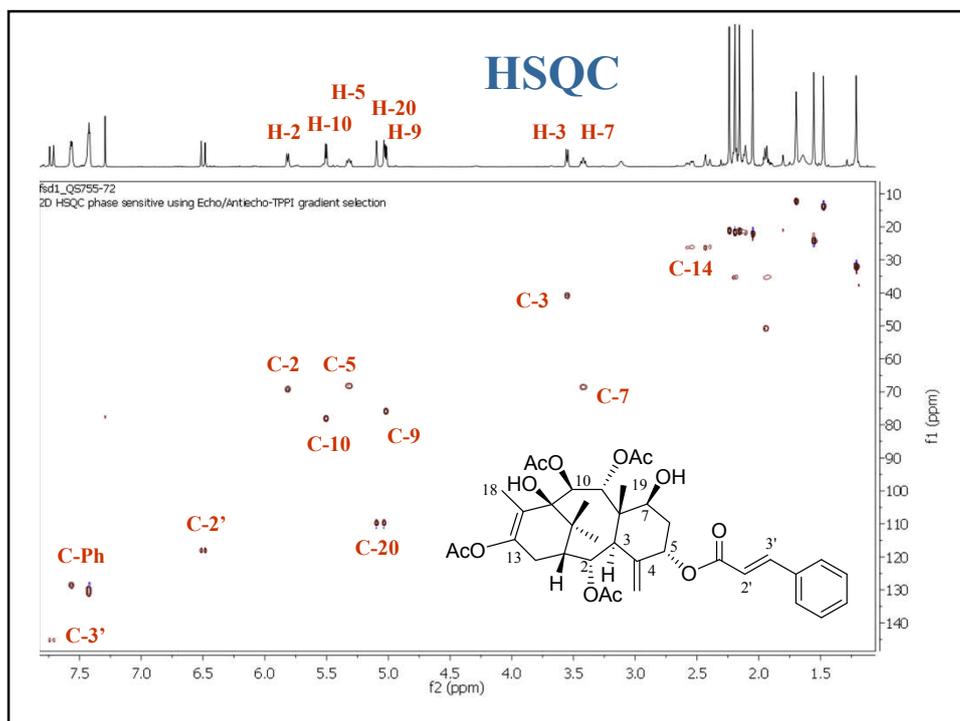
440

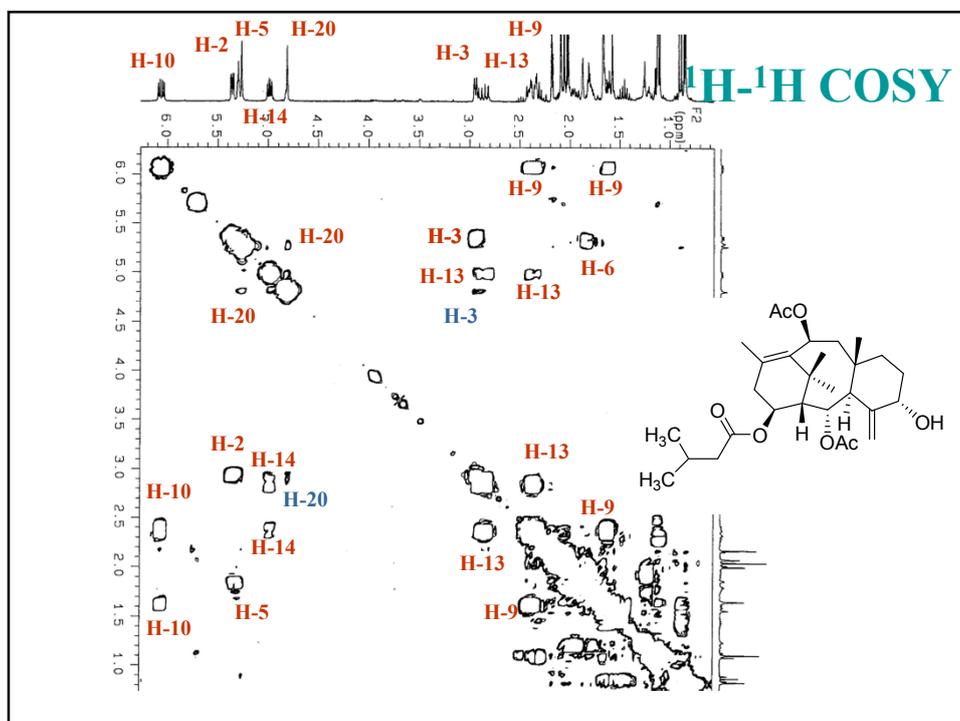
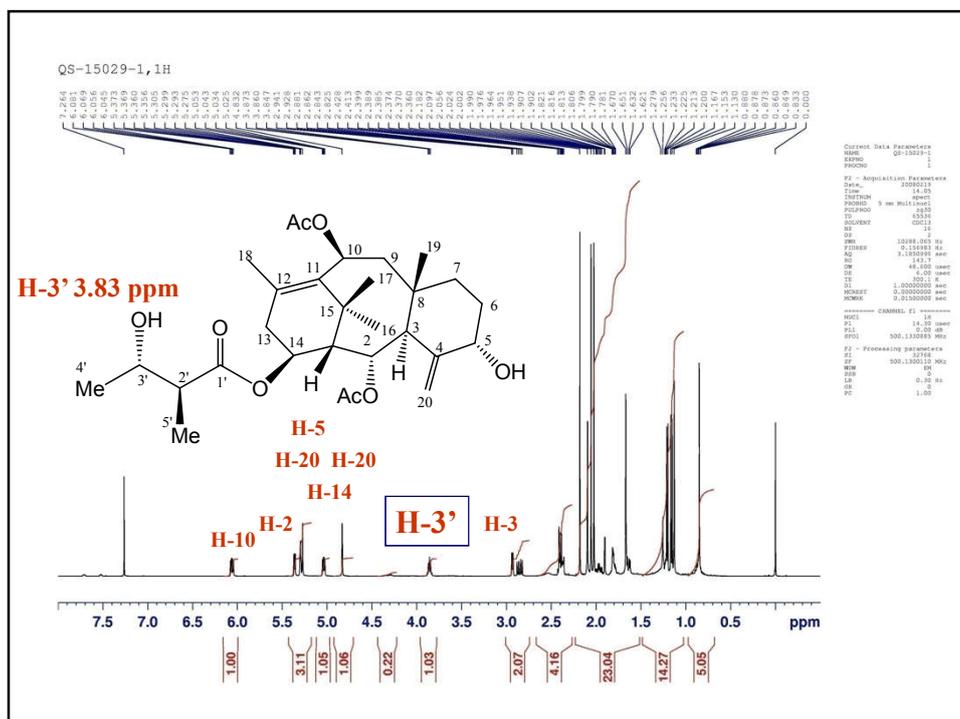


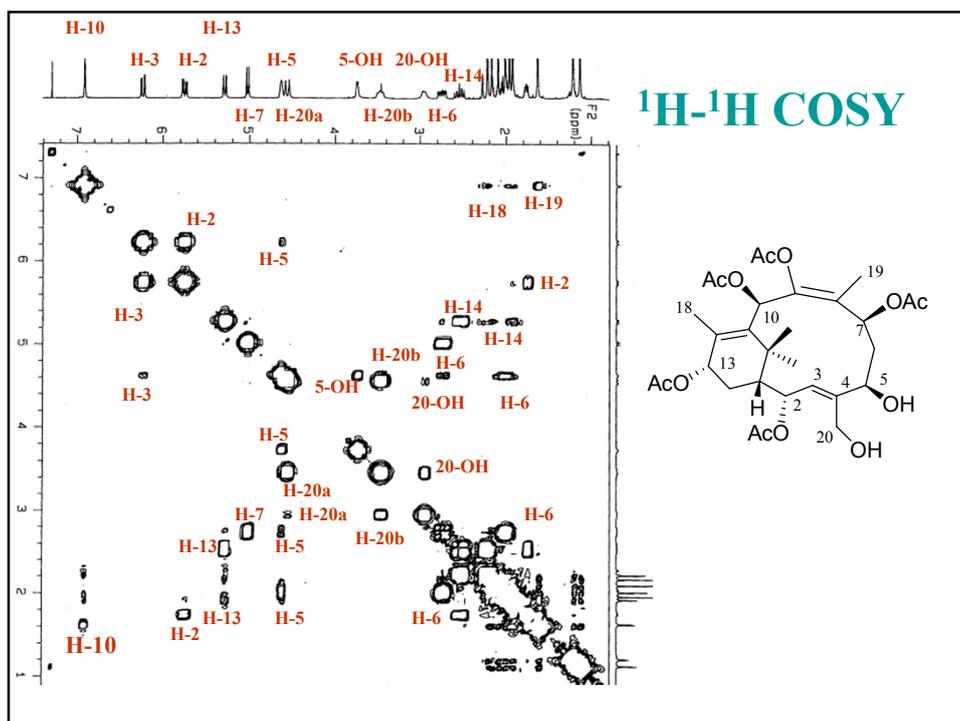
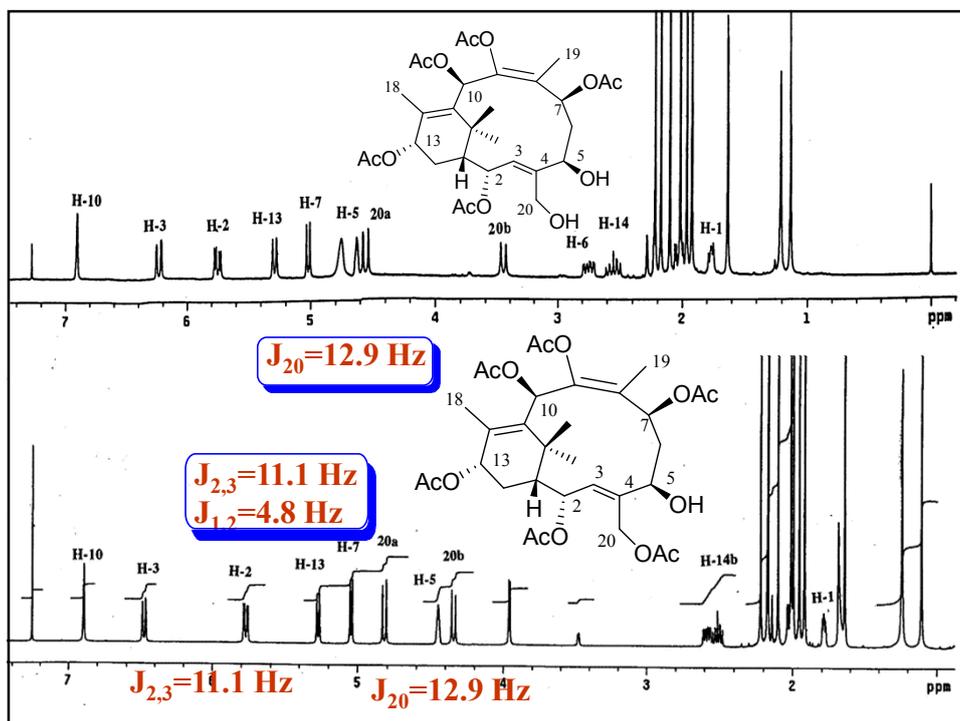


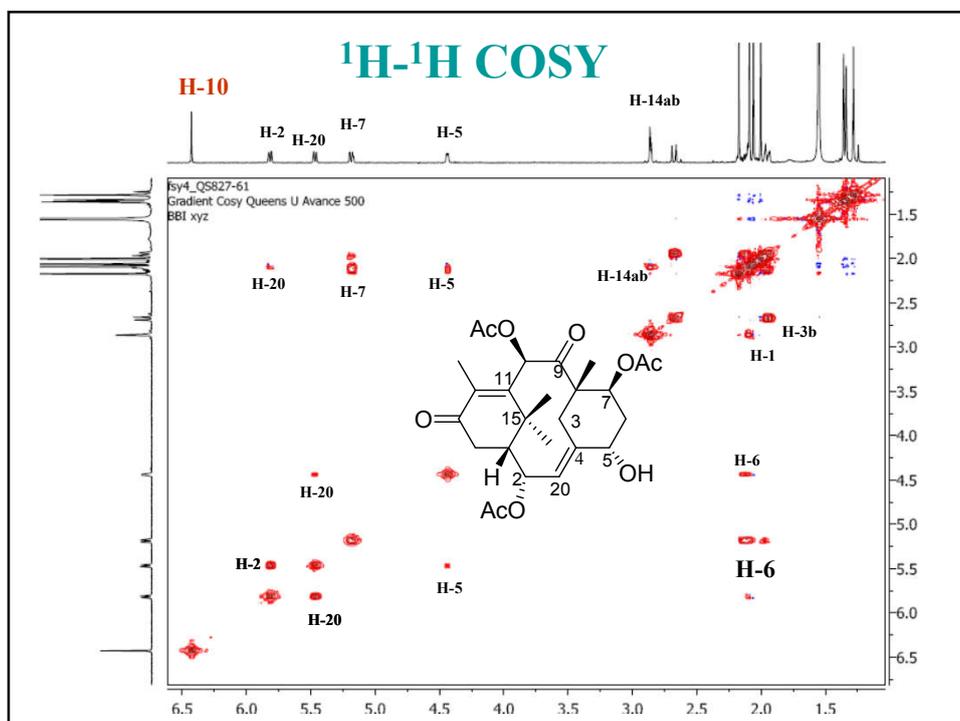
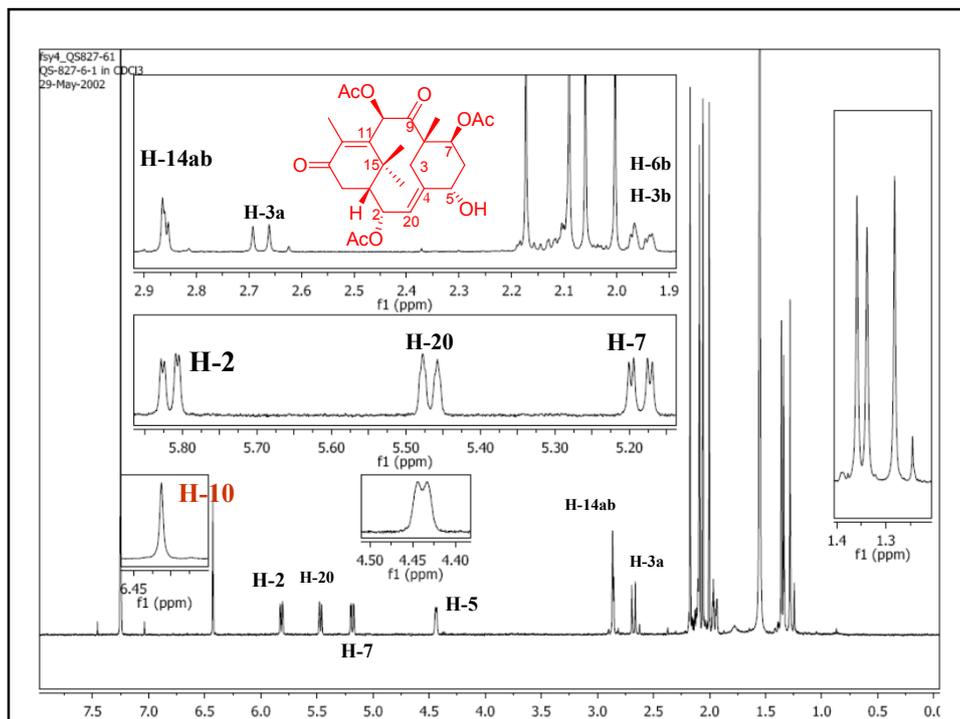


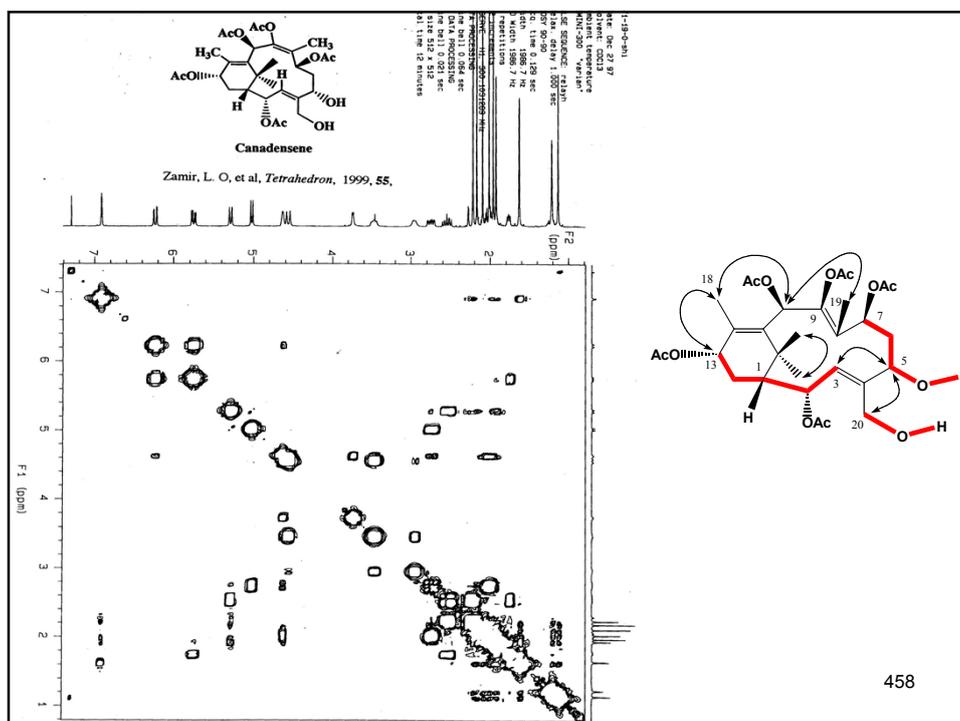
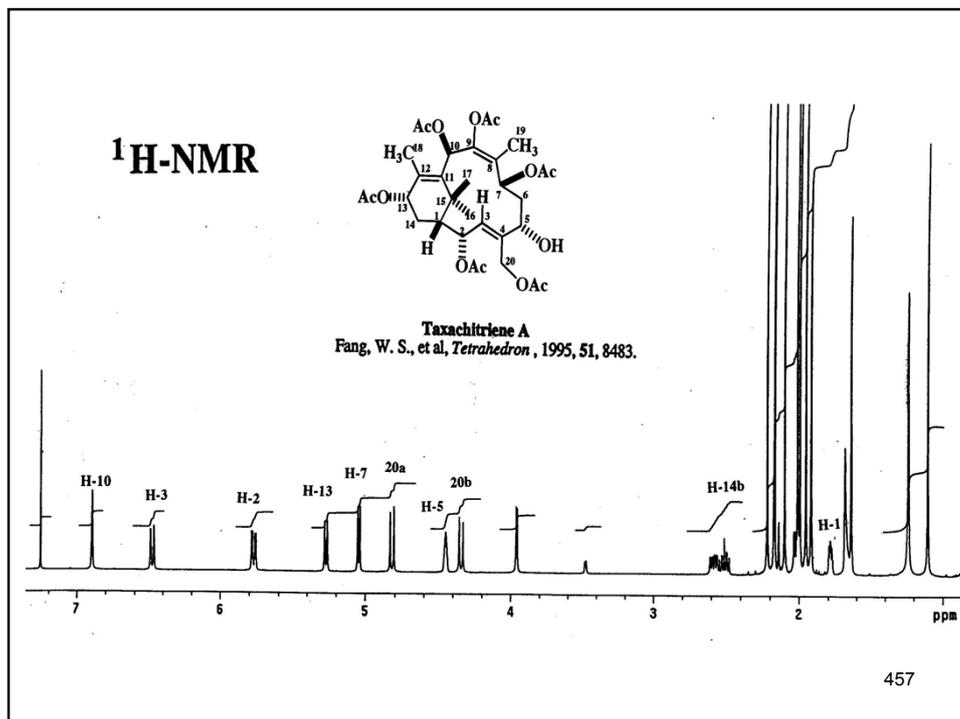


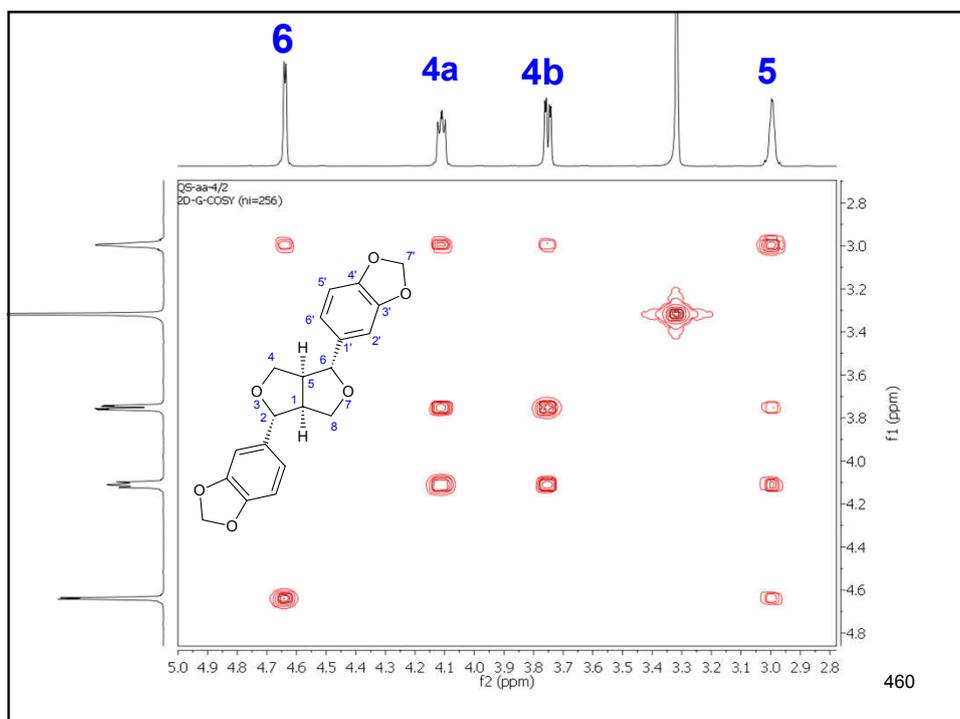
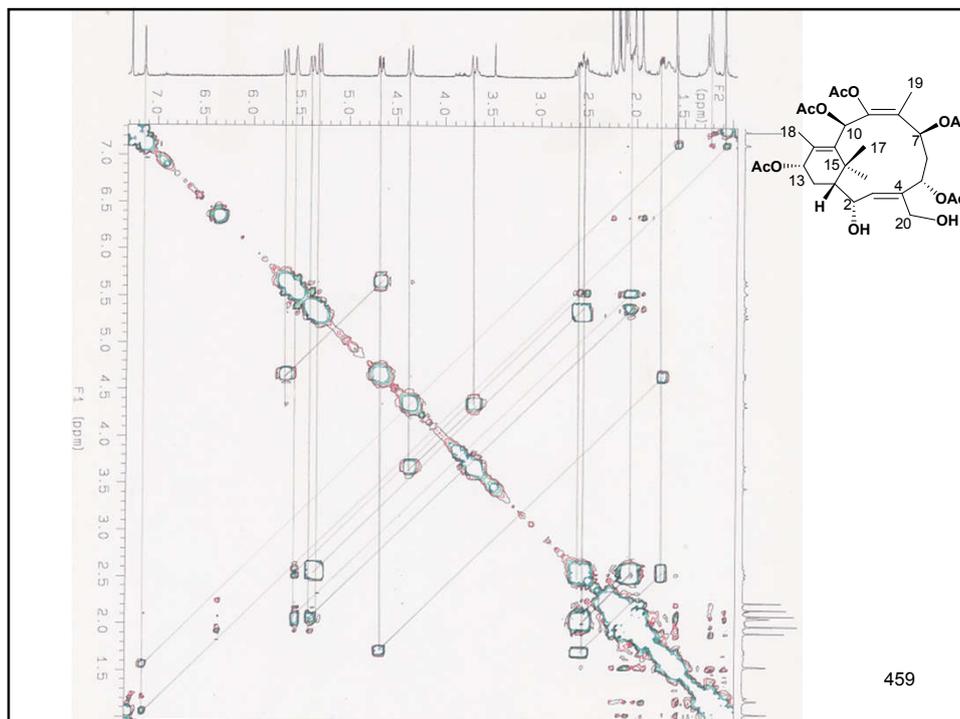


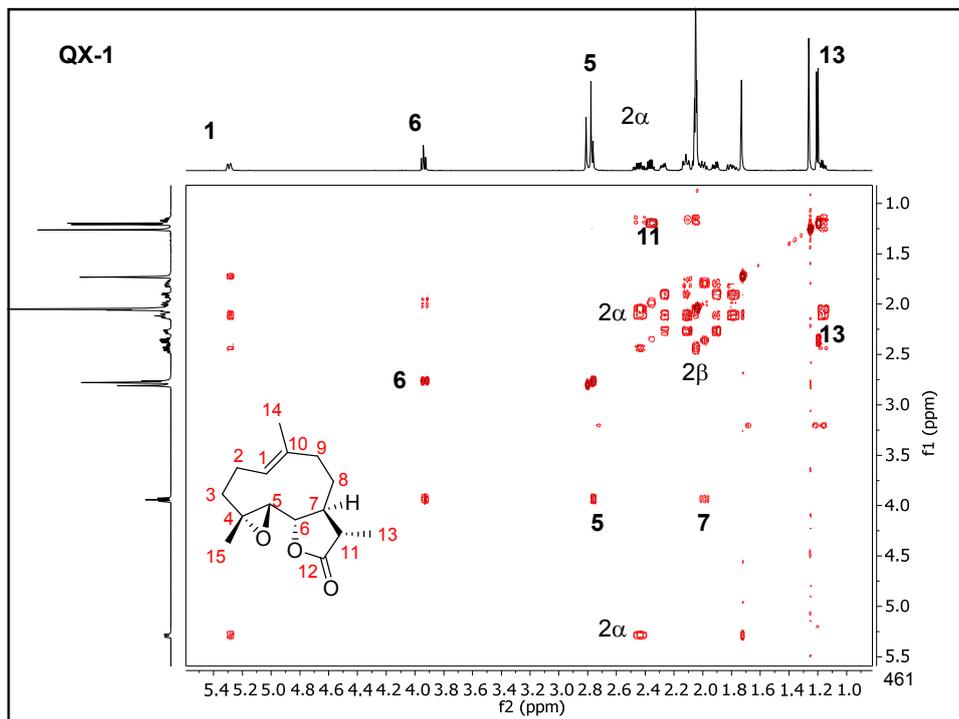












Facts about chemical shifts and coupling constants:

Chemical shifts in δ ppm are independent of the strength of the applied field B_0 and correspondingly, the instrument operating frequency.

Chemical shifts in Hz are field and frequency dependent.

Coupling constants J in Hz are field and frequency independent.

Coupling constants J in δ ppm are field and frequency dependent.

462

NMR is more robust and the time of analysis is shorter because the NMR instruments are always ready for measurement, without any preceding conditioning or calibration procedure being necessary.

463

Preparation of NMR Samples

NMR instruments are quite versatile; with the proper design, they can accommodate solid, liquid or gas phase samples; even living organisms can be measured. You will be making liquid samples.

464

· Firstly we want to use a **Deuterated** solvent. Although deuterium is an NMR-active nucleus, its signal comes at a very different signal than do the ^1H nuclei you want to observe. We can prevent the solvent from "swamping" the sample signal by using solvents that have had the protons replaced with deuterium. Normally, about 98-99% of the H atoms are replaced, leaving a residual proton signal. This is actually quite useful as a chemical shift reference.

465

Sample filtration----It is important that the solution be free of precipitate, dust, fibers, *etc.* This can be achieved by filtering the sample. Make up slightly more sample than is required. Pack a small piece of cotton or glass wool tightly into a Pasteur pipette and wash with a small amount of solvent. Filter the solution into the NMR tube using a teat if necessary to force the sample through.

0.4-0.5 mL in a 5 mm NMR tube

466

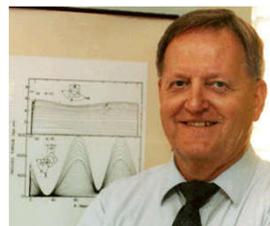
$^1\text{H-NMR}$ 的空间构象分析

- 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。

467

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.

468

NOE



National Medal of Science

- 后来发现这种效应也会发生在分子内空间位置相近的两个磁核之间。当分子内有空间位置靠近的两个质子 H_A 和 H_B ，如果用双共振法照射 H_B ，且使干扰场 B_2 的强度正好达到使被干扰的 H_B 谱线饱和，这时 H_A 的共振信号就会增加。这种现象称为核 Overhauser 效应 (NOE)。

469

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 ($<5\text{\AA}$) 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)。

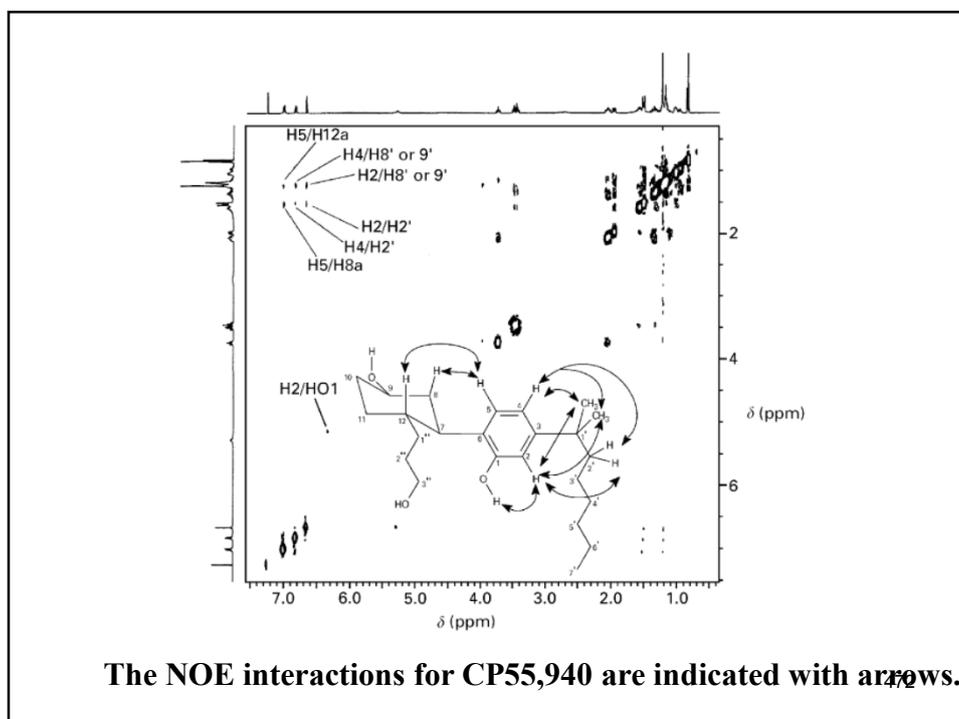
NOE的产生仅以两核空间相近为决定性条件，而与两核间化学键存在与否无关（与有无 J 耦合无关）。

470

核Overhauser效应

The nuclear Overhauser effect (NOE) is another important NMR parameter used in conformational analysis because the magnitude of the NOE is inversely proportional to the sixth power of the interproton distance in space ($I_{\text{NOE}} \propto r^{-6}$). NOE spectroscopy (NOESY) is a twodimensional experiment that may be run routinely in which the NOE is manifested as a crosspeak between two resonances indicating that the two protons are near in space.

471

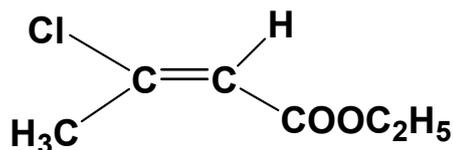
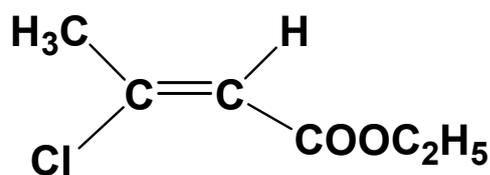


用NOESY方法对异构体的鉴别

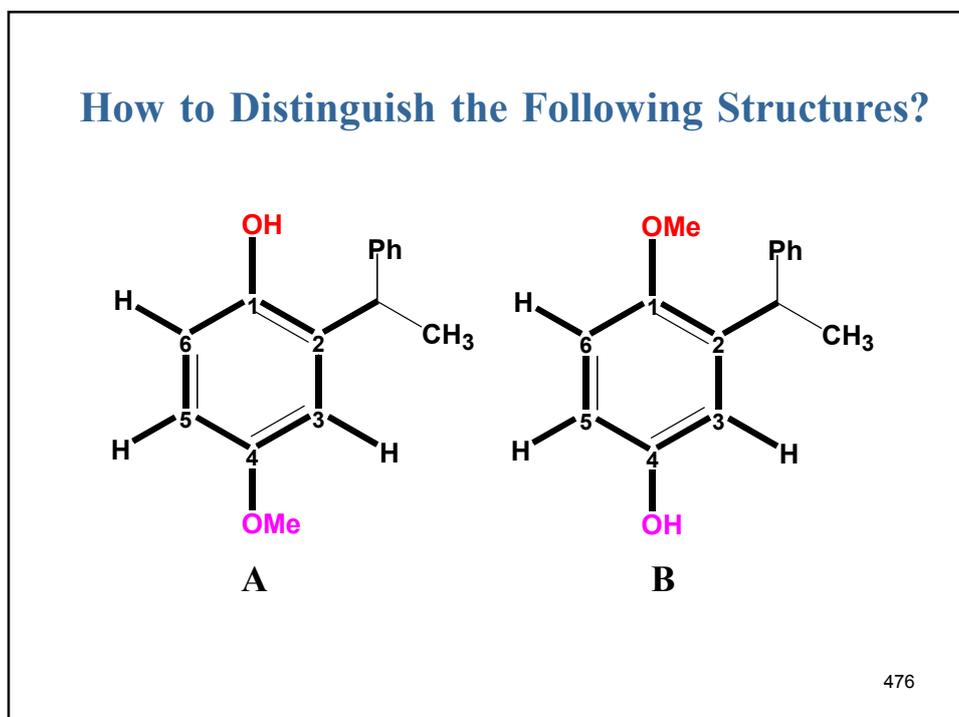
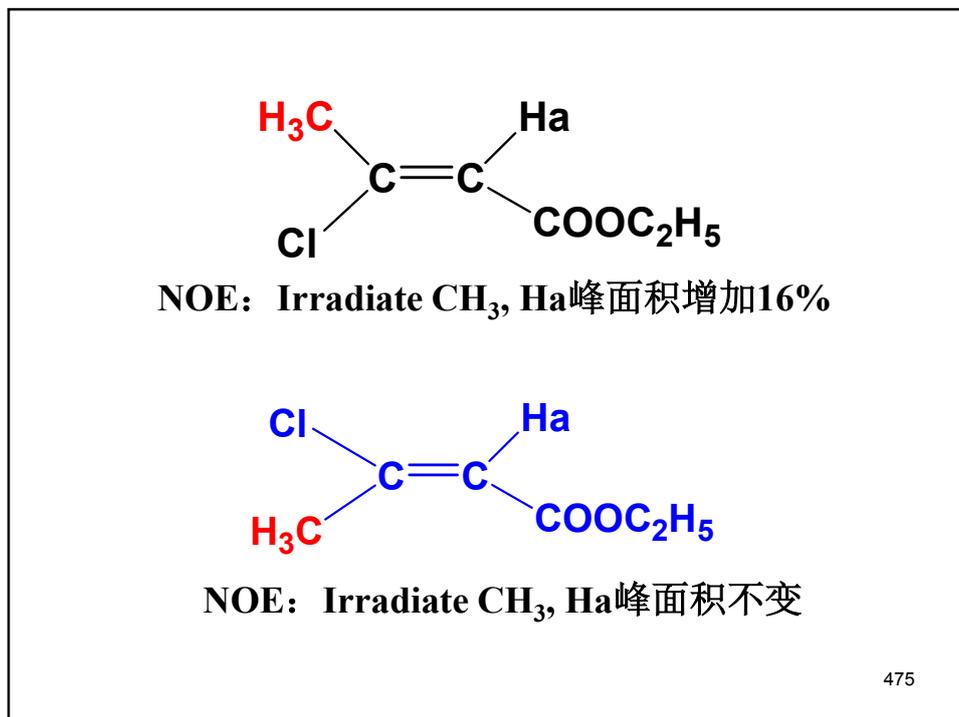
- ◆ 在有机合成反应中会经常出现异构体，在异构体构型的鉴别中，NOE是一种非常有效的手段。NOE谱对有机化合物结构、构型、构象的鉴定能够提供重要信息。NOE谱可以采用一维方式或二维方式，我们通常都采用二维谱图的方式，因为二维谱方便快捷，可观察的信息全。
- ◆ **NOE主要用来确定两种质子在分子立体空间结构中是否距离相近。**要求两种质子的空间距离小于5Å。从以上可以看出NOE和空间因素很有关系，和相隔的化学键数无关，所以在分析NOE谱图时候，一定要能画出结构的立体构型以便解析。下面是用NOE方法来鉴别异构体的简单例子。

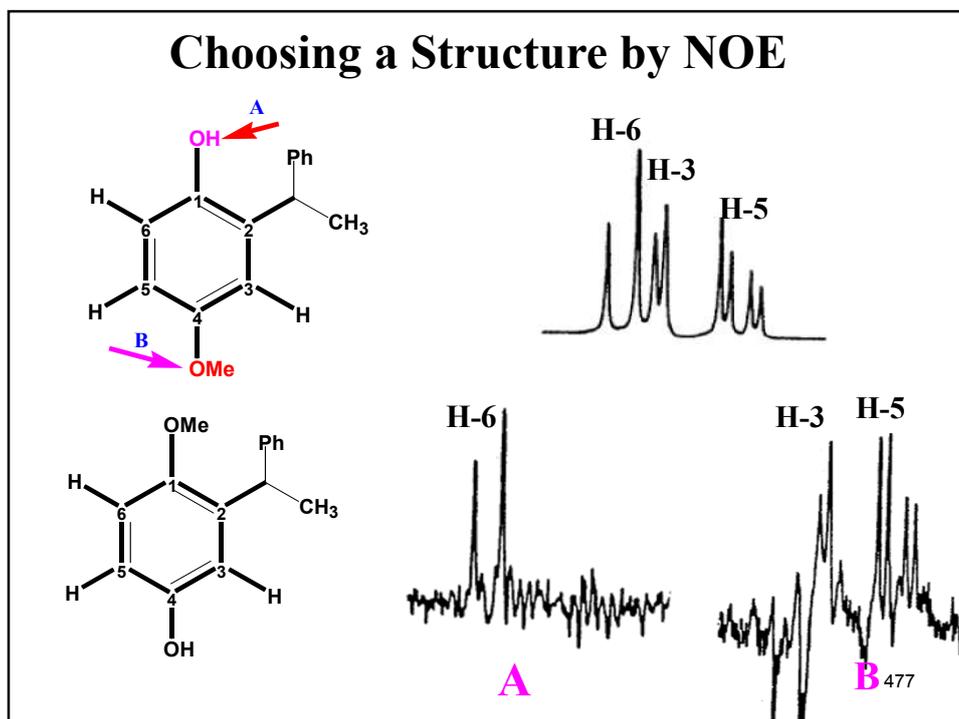
473

How to distinguish following two structures?



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NOE

- ◆ 为测NOE，需对样品的¹H NMR有准确的指认；
- ◆ NOE信息的价值与两个相关的磁核跨越的化学键的数目有关。当两核越是跨越了多根化学键还显示NOE时，这越能排除相当多的（构型、构象）可能性，因而提供较重要的立体化学信息。
- ◆ 在应用NOE时，常有某些预定的分子模型，**根据NOE的结果可以从中作出明确的抉择。**
- ◆ NOE的具体数值除和研究的分子相关外，也和仪器、实验条件等有关，因而准确性和相互可比性不够好；

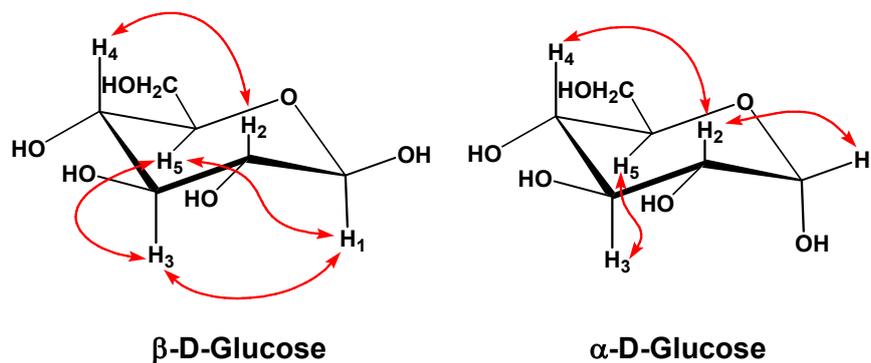
478

NOE

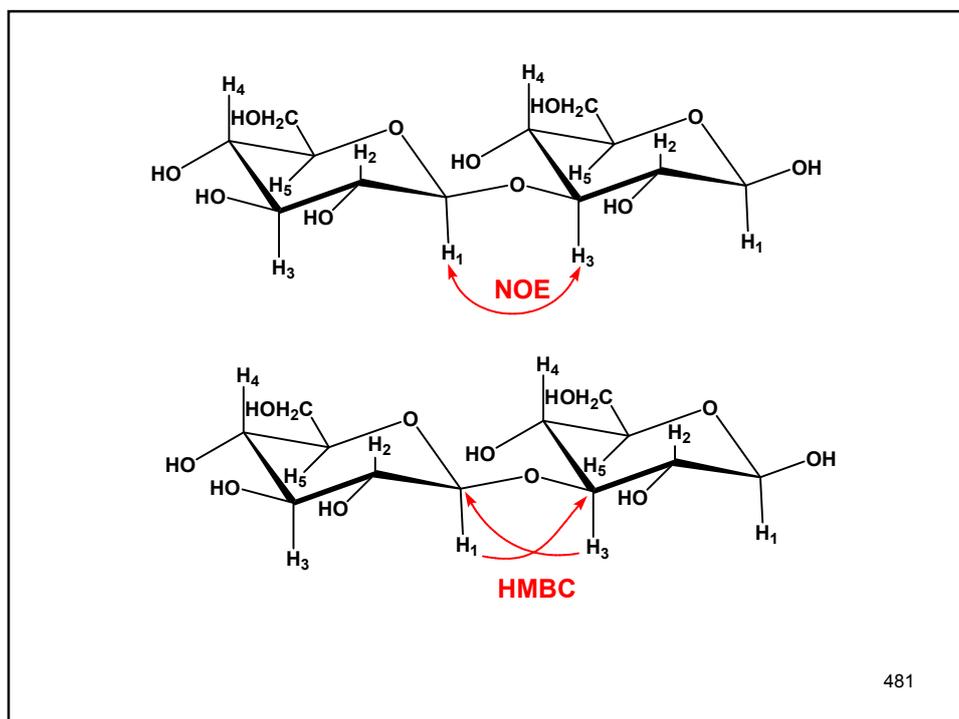
- ◆ NOE最适合应用于刚性分子。在这种情况下，核组之间具有确定的距离。根据NOE可以得到分子的立体化学信息。
- ◆ 若样品为柔性分子，相对于核磁共振的时标，这样的分子在溶液中存在着较快的构象互变，NOE测定的是个平均的结果，因而无法得到具体的构象信息。
 - 变温实验
 - 加入使溶液变稠的物质，使构象转换的速率变低
 - 将样品分子进行化学修饰，以便测得NOE

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NOE



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NOE类二维核磁共振谱

检测NOE可以采用一维方式或二维方式。如采用一维式,需选定某峰组,进行选择性辐照,然后纪录此时的谱图,由扣去未辐照时的常规氢谱而得的差谱,得到NOE信息(差谱中某些谱峰的区域呈正/负峰)。由于预先的选择性辐照以使该跃迁达到饱和,是一种稳定态下的实验,故灵敏度高。但若要对有兴趣的基团或谱峰均进行选择性辐照,不仅是费时费力的,还有可能遗漏,因而若以二维谱的方式,用一张二维谱表示出所有基团间的NOE作用,纵然灵敏度稍差,也很具有吸引力的方法。

由于NOE对确定有机化合物结构、构型和构象的作用及对生物分子能提供重要信息,故NOE类二维谱在二维谱中占有重要的地位。

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NOESY

Nuclear Overhauser Effect Spectroscopy

二维 NOE 谱简称为 NOESY，它反映了有机化合物结构中核与核之间**空间距离**的关系，而**与二者间相距多少根化学键无关**。因此对确定有机化合物结构、构型和构象以及生物大分子（如蛋白质分子在溶液中的二级结构等）有着重要意义。

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NOESY

- NOESY 的谱图与 ^1H - ^1H COSY 非常相似，它的 F2维和F1维上的投影均是氢谱，也有对角峰和交叉峰，图谱解析的方法也和 COSY 相同，唯一不同的是图中的交叉峰并非表示两个氢核之间有耦合关系，而是表示两个氢核之间的空间位置接近。
- **交叉峰的大小决定于核间距离。**

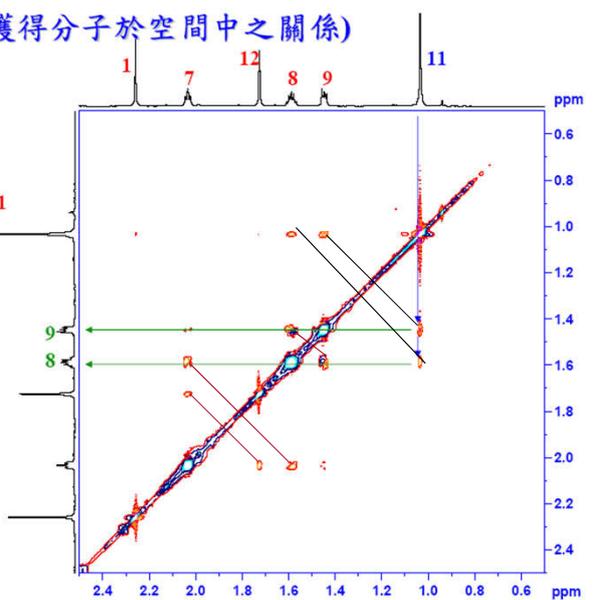
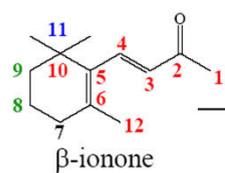
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NOESY

- 由于 NOESY 实验是由 COSY 实验发展而来的，因此在图谱中往往出现 COSY 峰，即 J 耦合交叉峰，故在解析时需对照它的 ^1H - ^1H COSY 谱将 J 耦合交叉峰扣除。

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7.4.1 NOESY (獲得分子於空間中之關係) ($<5\text{\AA}$)



• Aphanamol-I的结构和NOESY谱为:

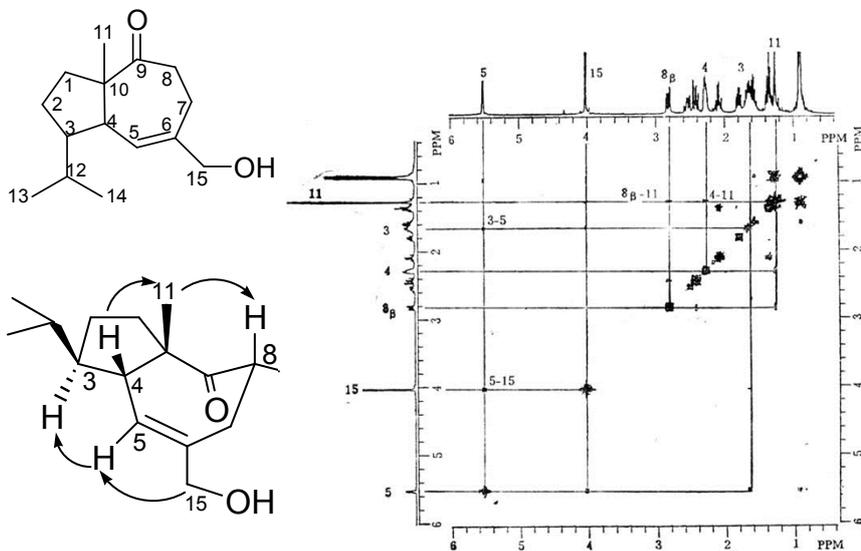
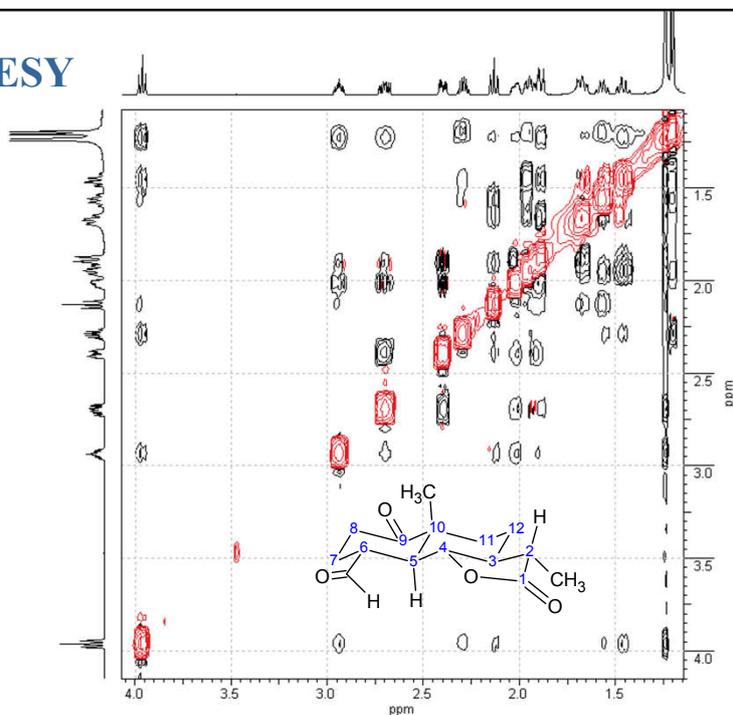


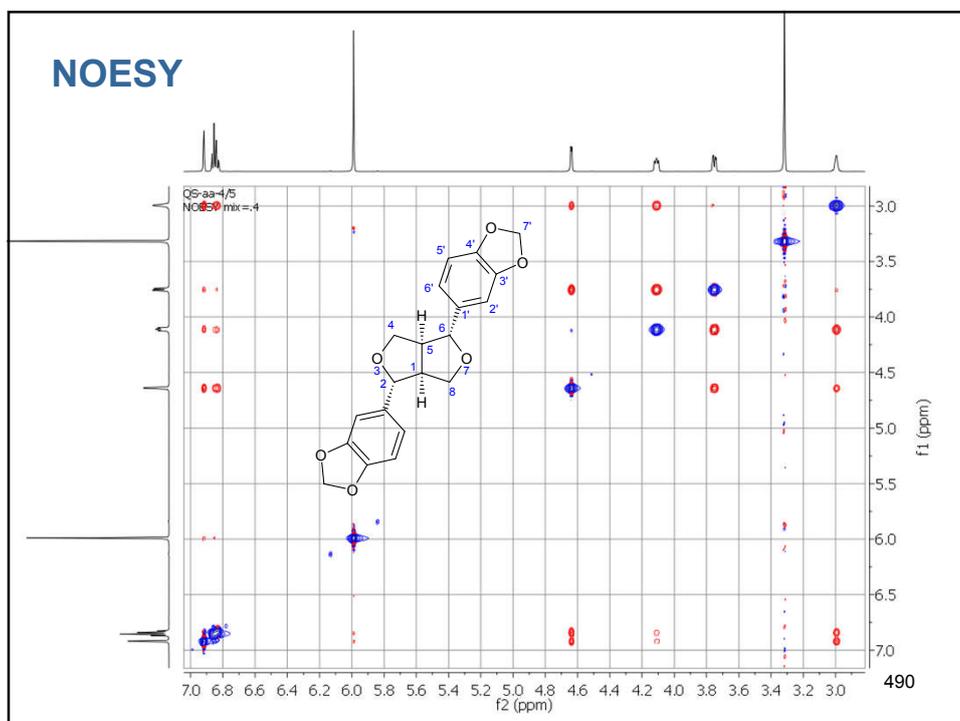
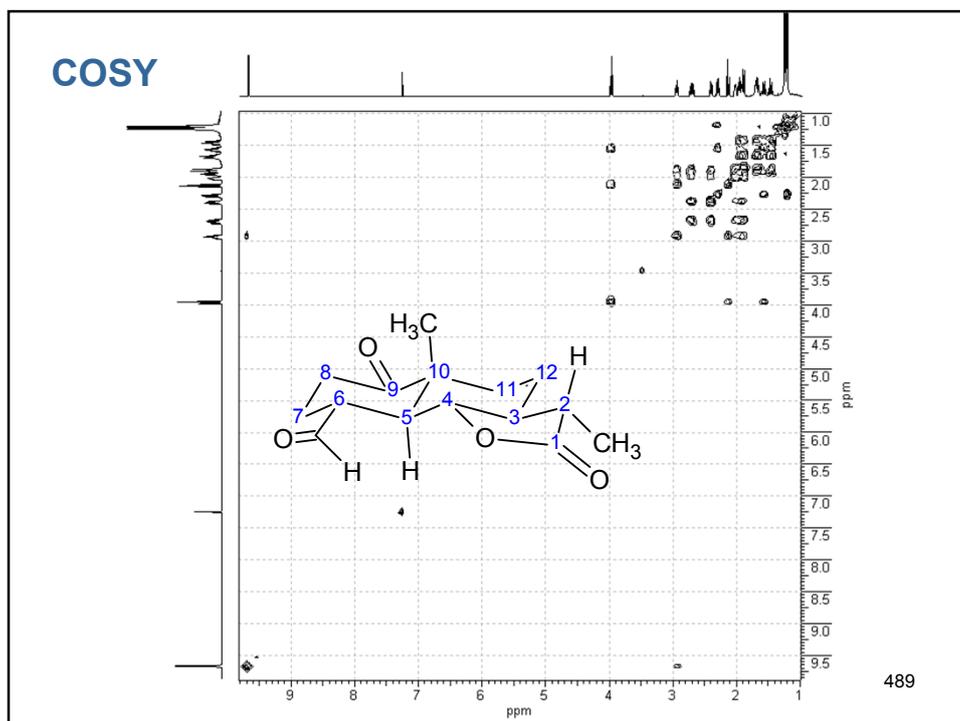
图 2-30 aphanamol-I 的NOESY谱 (360MHz, CDCl₃)

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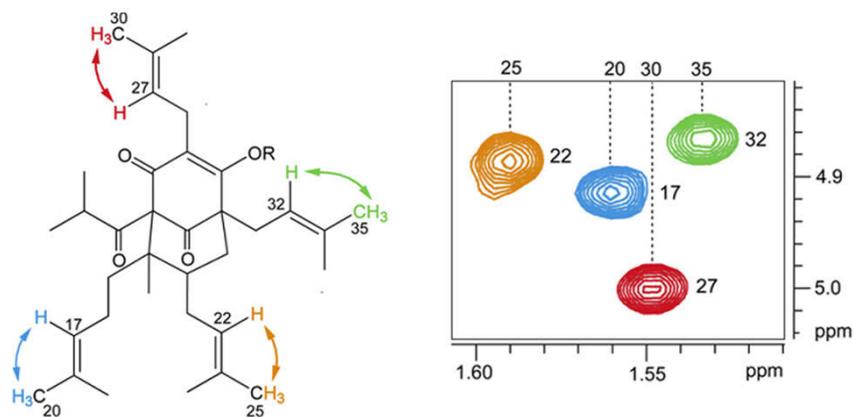
NOESY



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NOESY



The right panel displays a part of a NOESY spectrum showing correlations through space between a methyl group and a methine hydrogen atom on the same prenyl moiety in the molecule hyperforin from *Hypericum perforatum*

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ROESY

- 当遇到中等大小的分子时(分子量约为 1000-3000)，由于此时 NOE 的增益约为零，无法测到NOESY 谱中的相关峰(交叉峰)，此时测定旋转坐标系中的 NOESY 则是一种理想的解决方法，这种方法称为 ROESY (Rotating frame Overhauser Effect Spectroscopy)，由此测得的图谱称为 ROESY谱。
- ROESY 谱的解析方法与 NOESY 相似，同样 ROESY 谱中的交叉峰并不全都表示空间相邻的关系，有一部分则是反映了耦合关系，因此在解谱时需注意。

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