

QFL-1345

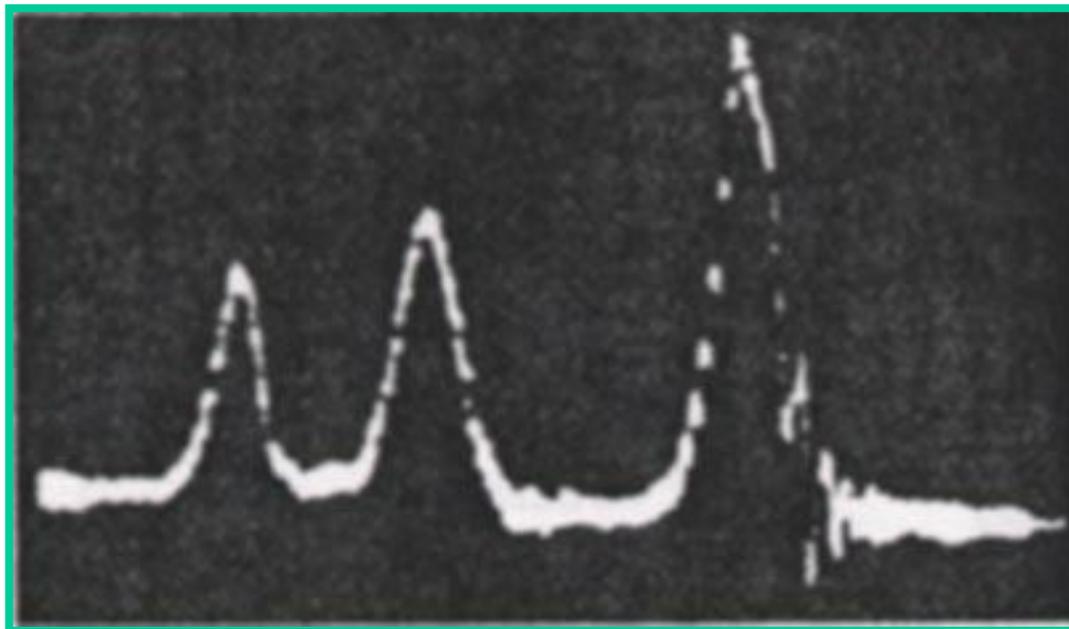
RMN

Introdução

05 de junho - 2023

1951

Primeiro espectro de RMN de ^1H (EtOH)



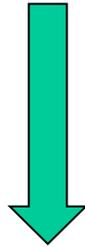
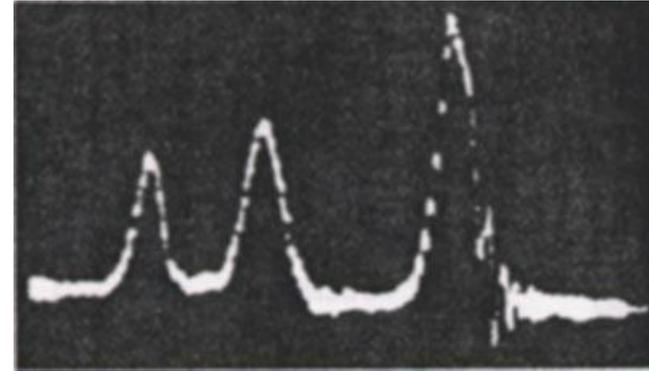
O que se pode dizer desse espectro?

**São 3 tipos de hidrogênios com
intensidades 1H, 2H e 3H.**

Ressonância magnética nuclear

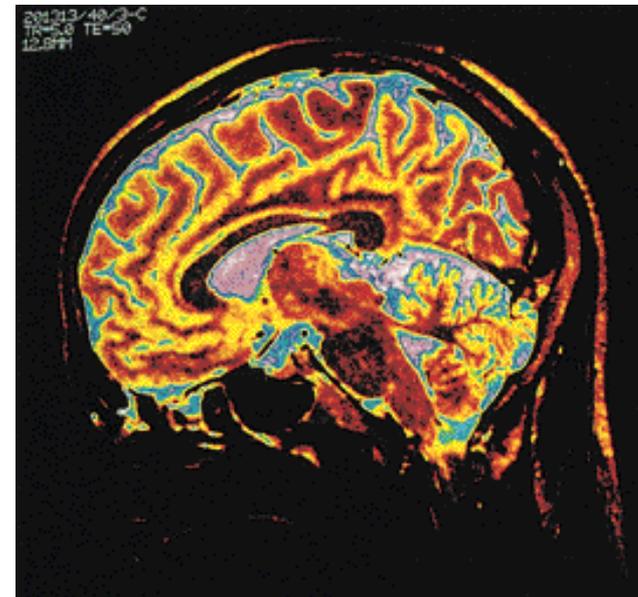
1951

Primeiro espectro de RMN de ^1H (EtOH)



1980

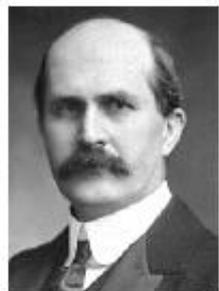
Primeira imagem de órgãos por RMI
(imagem por ressonância magnética)



O hidrogênio é um componente importante da gordura e a água e compreende ~63% do corpo humano.

Laureados por Premio Nobel em análise de estruturas

X-ray Crystallography



William Bragg

Physics, 1915

Ultramicroscope



Lawrence Bragg

Chemistry, 1925



Richard Zsigmondy

Nuclear Magnetic Resonance



Felix Bloch

Physics, 1952



E. M. Purcell

Phase Contrast Microscope



Frits Zernike

Physics, 1953

Large-Array Radio Telescopes



Martin Ryle

Physics, 1974

Electron Microscope



Ernst Ruska

Physics, 1986

Scanning Tunneling Microscope



Gerd Binnig

Physics, 1986



Heinrich Rohrer

Computer Assisted Tomography



Allan Cormack

Physiology or Medicine, 1979



Godfrey Hounsfield

Magnetic Resonance Imaging



Paul Lauterbur

Physiology or Medicine, 2003



Peter Mansfield

The Nobel Prize in Chemistry 2002

John B. Fenn

Virginia Commonwealth University, Richmond, USA

"for the development of methods for identification and structure analyses of biological macromolecules"

"Electrospray Wings for Molecular Elephants"



Koichi Tanaka

Shimadzu Corp., Kyoto, Japan

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

"The Origin of Macromolecule Ionization by Laser Irradiation"



Kurt Wüthrich

Scripps Research Institute, La Jolla, CA, EUA

ETH Zürich, Zürich, Suíça

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution."

"NMR studies of structure and function of biological macromolecules"



Por que aprender RMN?

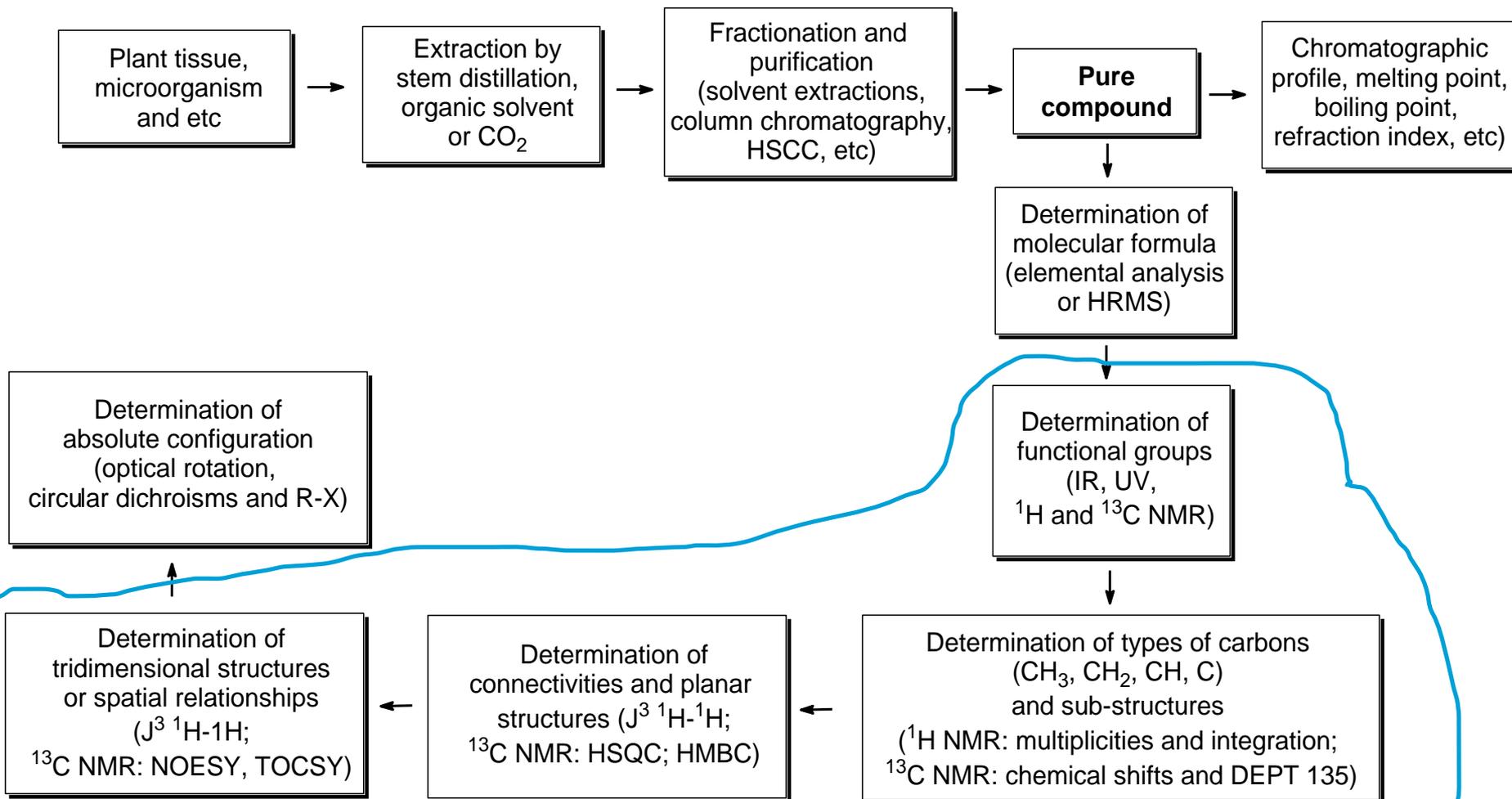
Química orgânica

- Elucidação estrutural de compostos (produtos naturais e sintéticos)
- Estudos estruturais (tridimensionais)
- Estudo de processos dinâmicos e cinética
- Estudo de equilíbrio (químico ou estrutural)

Bioquímica e medicina

- Determinação de estruturas de macromoléculas (proteínas e polissacarídeos)
- Planejamento de medicamentos e estudos de relação estrutura-atividade
- MRI (magnetic resonance imaging)

General scheme for structural elucidation of natural compounds



Advantages of NMR

- ✓ Non-destructive;
- ✓ Quantitative;
- ✓ Identification of new compounds;
- ✓ Not require chemical derivatization;
- ✓ Allows ID of >300 compounds at once;
- ✓ Wide range of compounds.

Disadvantages of NMR

- ✓ Not sensitive;
- ✓ Lower limit of detection;
- ✓ Large sample size (500 μl – 100 μl);

Advanced techniques offset some of problems:

- Ultra-high magnetic field strength magnets (800 MHz & 900 MHz)
- Cryogenically cooled probe (improve S/N by factor of 3)
- And small volume microprobes (60 μL)

Números quânticos para núcleos comuns

Isótopos magneticamente ativos



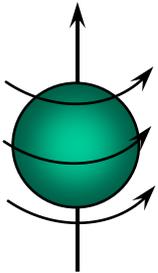
Elemento	¹ H	² H	¹² C	¹³ C	¹⁴ N	¹⁶ O	¹⁷ O	¹⁹ F
Spin Nuclear (I)	1/2	1	0	1/2	1	0	5/2	1/2
Número de estados de spin	2	3	0	2	3	0	6	2
Massa Atômica	1	2	12	13	14	16	17	19
Número atômico	1	1	6	6	7	8	8	9

Nuclear Magnetic Resonance

Nuclear spin

$$\mu = \gamma I \hbar$$

μ - magnetic moment

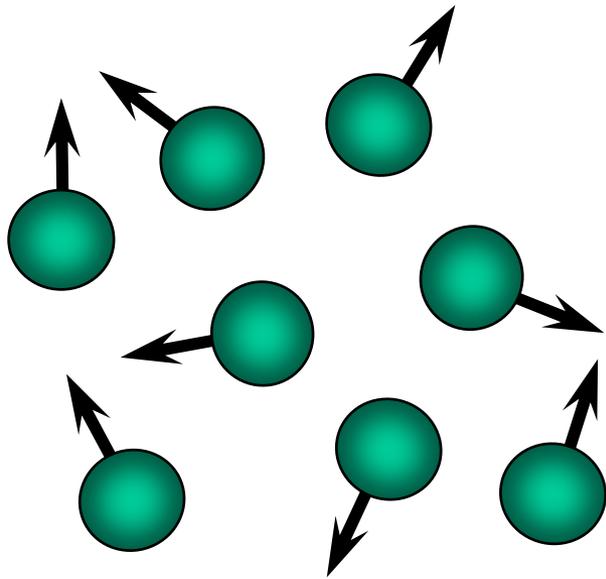


γ - gyromagnetic ratio (Constante dos elementos)

I - spin quantum number

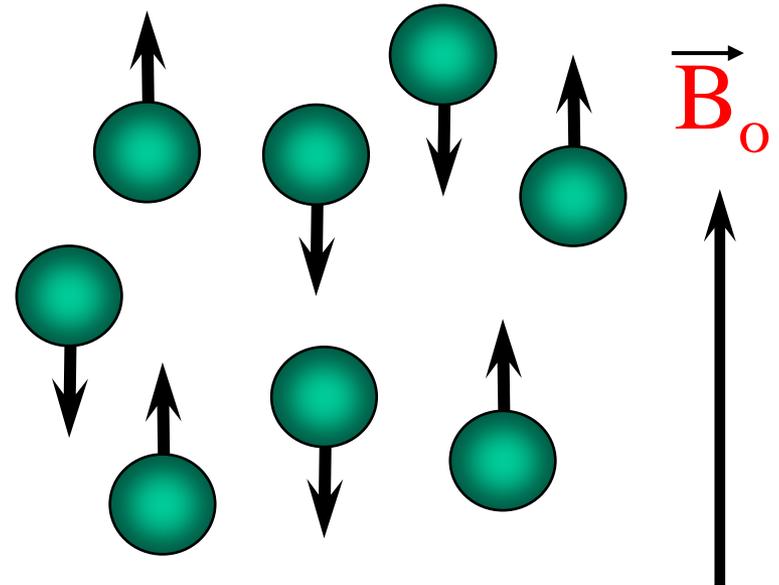
\hbar - Planck's constant

Comportamento de spins nucleares



$$\vec{B}_0 = 0$$

orientados aleatoriamente



$$\vec{B}_0 > 0$$

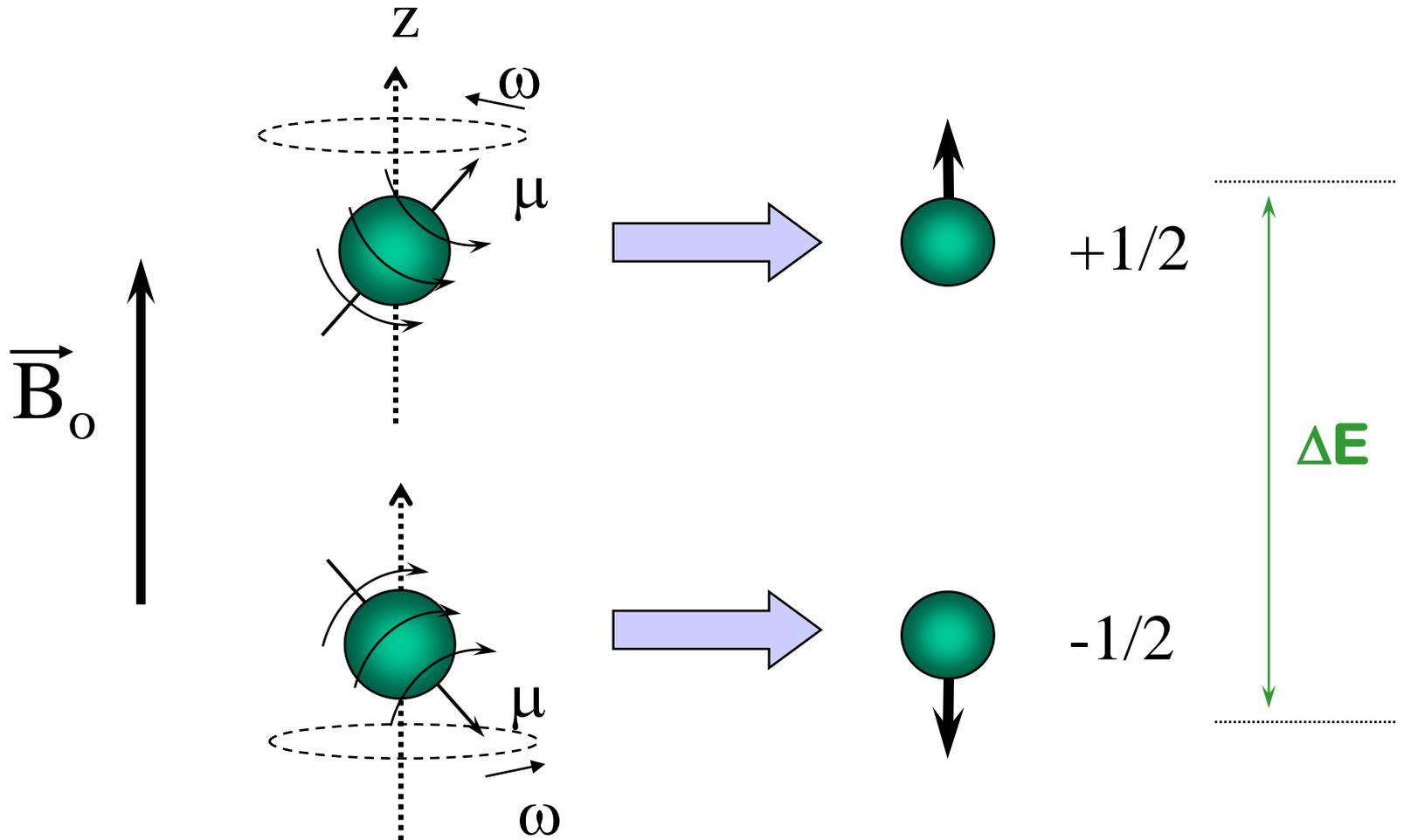
altamente orientados



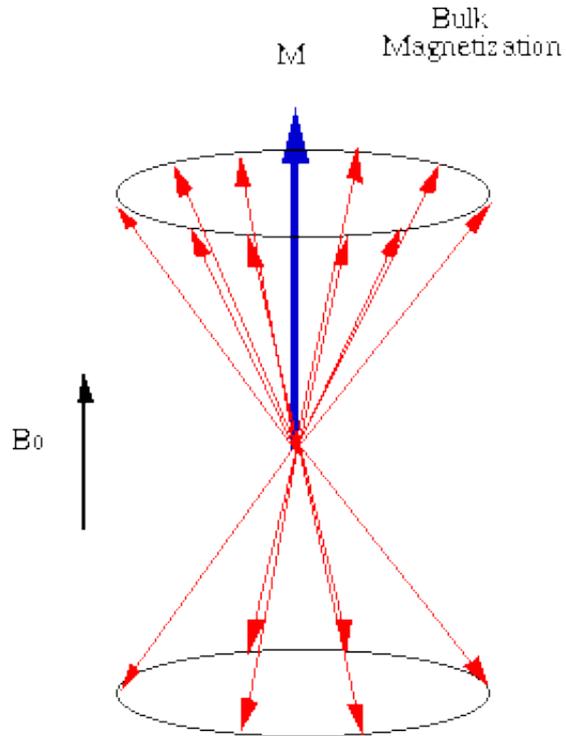
B_0 Campo magnético externo

Cada núcleo se comporta como um bastão magnético.

Momento magnético macroscópico



M: Bulk magnetization (Magnetização macroscópica)



Distribuição de Boltzmann:
Diferença das populações entre os níveis de alto e baixa energia

$$\frac{N_a}{N_b} = e^{\frac{\gamma \hbar B_0}{kT}}$$

A diferença de população é proporcional a intensidade do sinal e a resultante está indicada como M (vetor resultante).

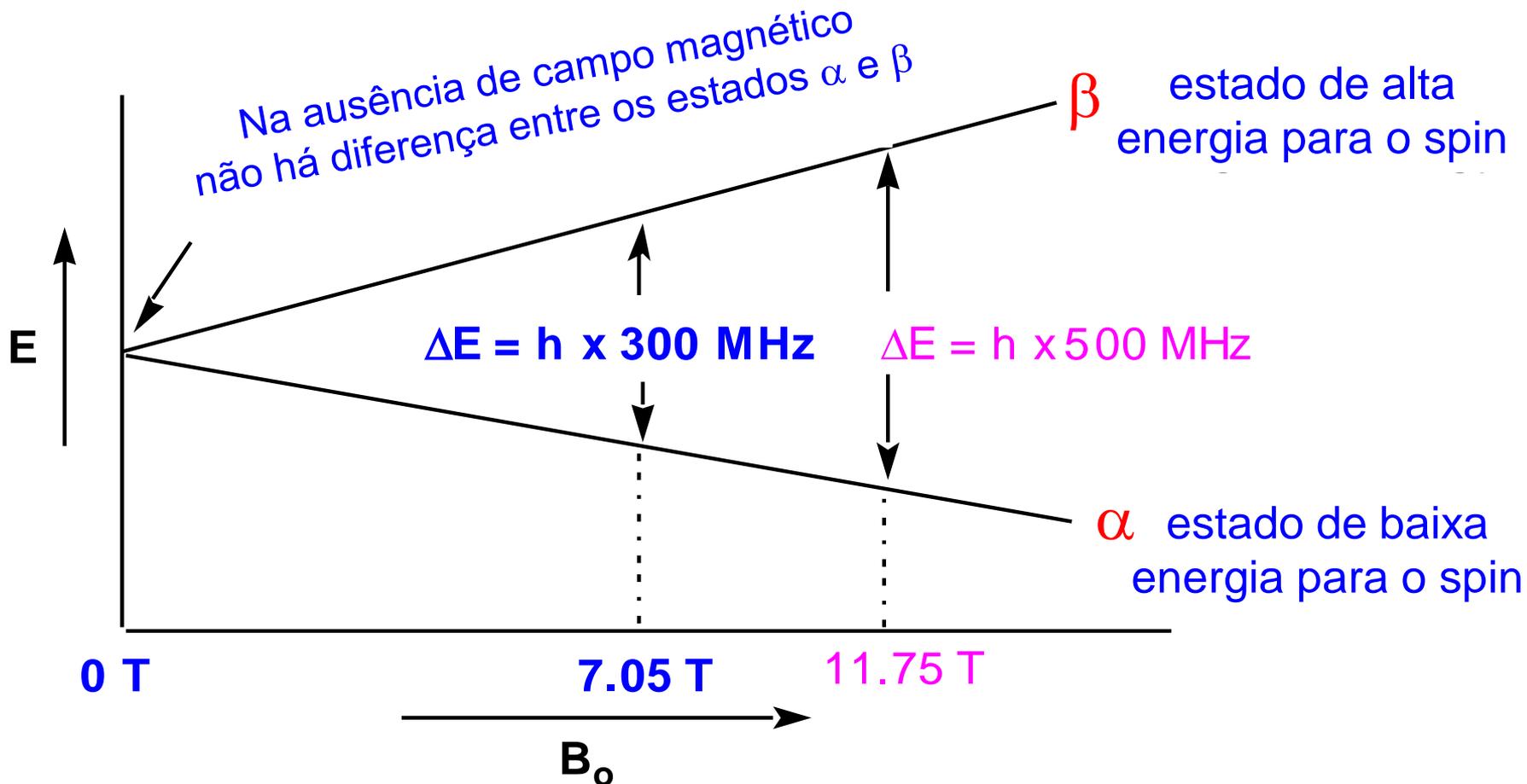
Variação do excesso de núcleos de ^1H no estado fundamental de acordo com a frequência sob um determinado campo magnético

frequência (MHz)	excesso de núcleos
60	9
80	12
100	16
200	32
300	48
600	96

a 60 MHz $N_a = \frac{1\ 000\ 000}{N_b\ 1\ 000\ 009}$

Excesso de 9 em
2 milhões de moléculas!!!

Relação entre campo magnético (B_0) e frequência (ν) para absorções em RMN de ^1H



Porque usar unidade em ppm ao invés de frequência em Hertz

$$\frac{\delta=110}{60 \text{ MHz}} = 1,83 \text{ ppm}$$

60 MHz

$J = 7.5 \text{ Hz}$

$J = 7.5 \text{ Hz}$

$$\frac{\delta=183}{100 \text{ MHz}} = 1,83 \text{ ppm}$$

100 MHz

$J = 7.5 \text{ Hz}$

$J = 7.5 \text{ Hz}$

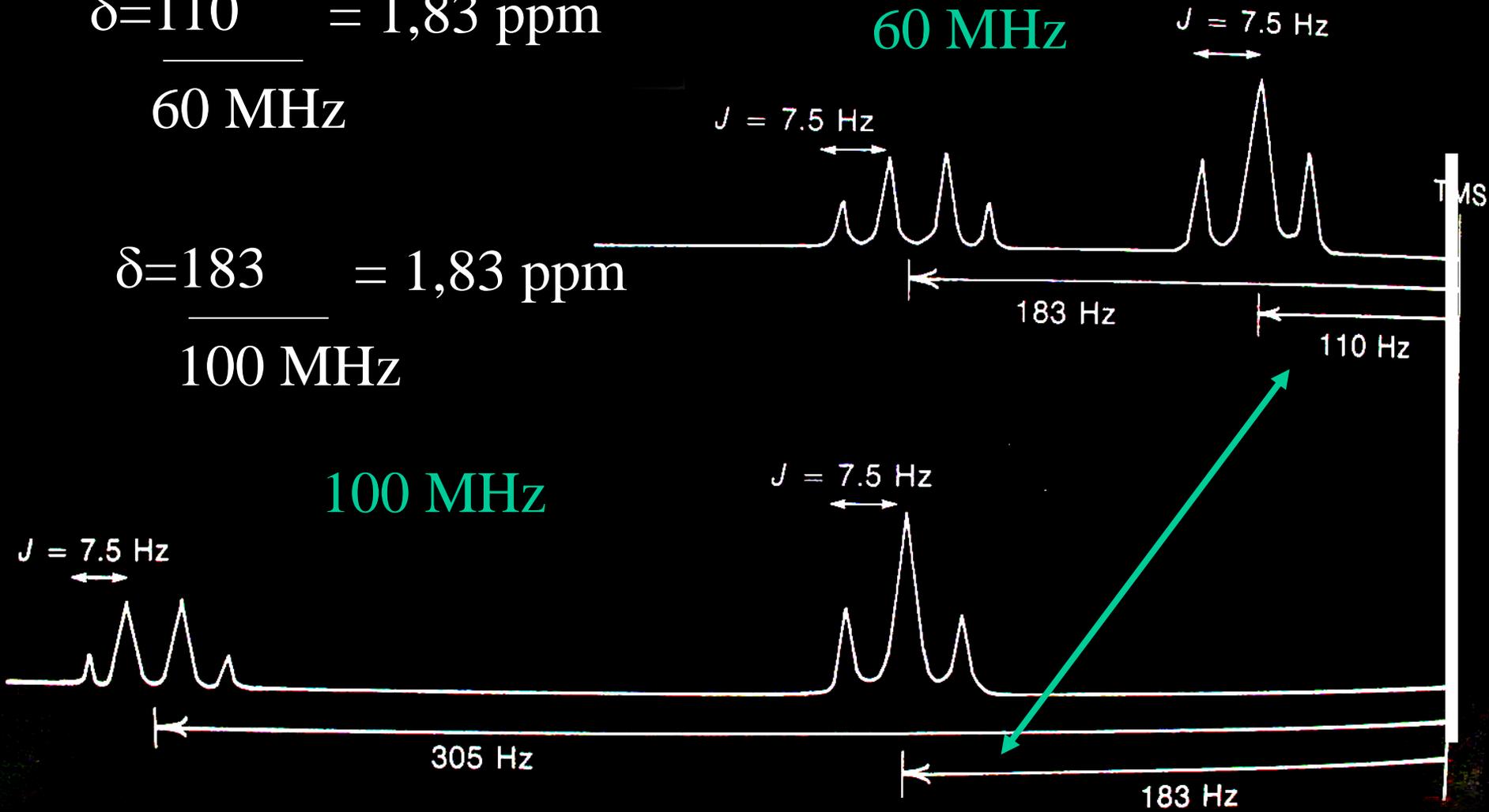
305 Hz

183 Hz

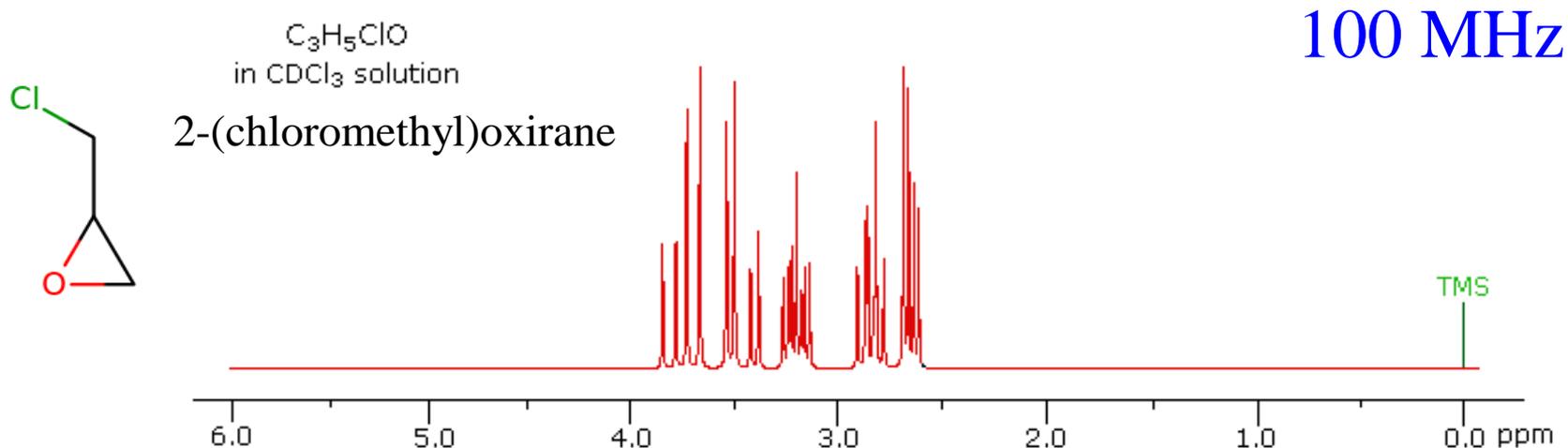
110 Hz

183 Hz

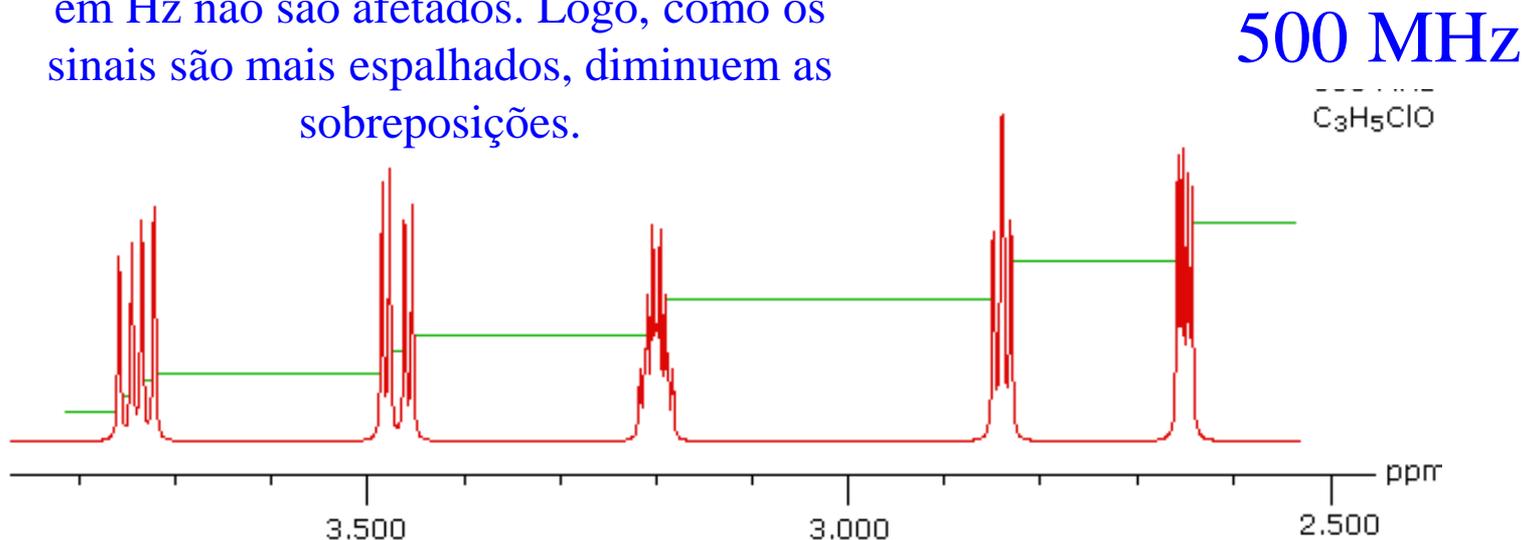
TMS



Efeito do aumento do campo magnético na resolução dos sinais



Os valores das constantes de acoplamento em Hz não são afetados. Logo, como os sinais são mais espalhados, diminuem as sobreposições.



Os espectros de RMN eram obtidos por varredura

Atualmente: por transformada de Fourier

Ondas contínuas



Largura de varredura



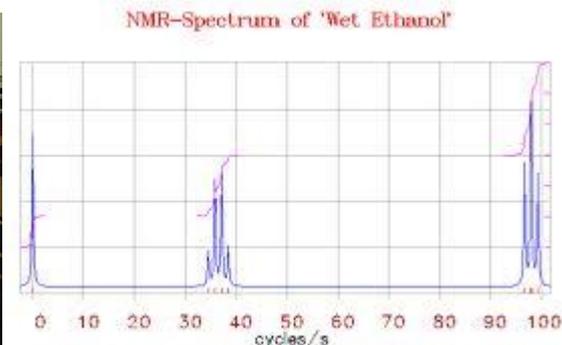
A 15 ppm **sweep width** at 300 MHz takes 4500 s (**75min**) per scan for a resolution of 1 Hz !



A 15 ppm sweep width at 300 MHz takes **1 s** per scan for a resolution of 1 Hz !

1961 Shoolery introduces the Varian A-60 high-resolution spectrometer.

RMN no IQUSP



1970: EM 360 Varian; T-60 Varian

1980: AC 80 e AC 200 Bruker

1990: DPX 300 Bruker

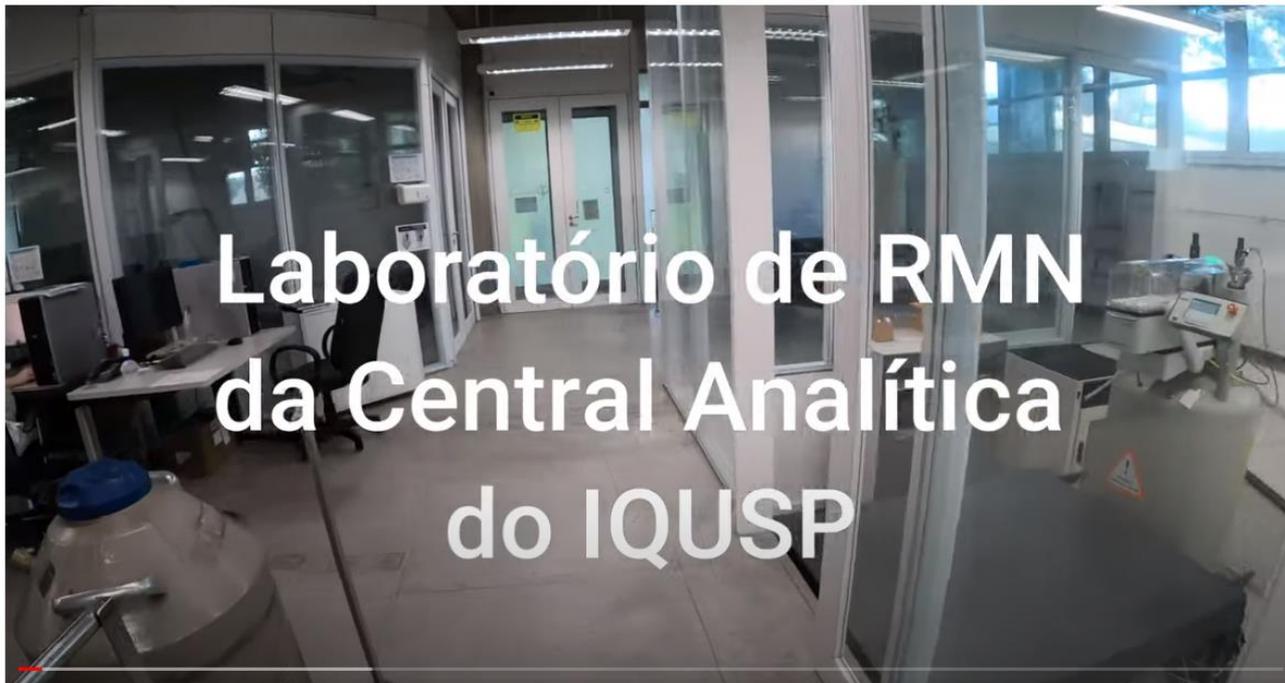
1995: Bruker DRX 500

2013: Bruker 800 MHz

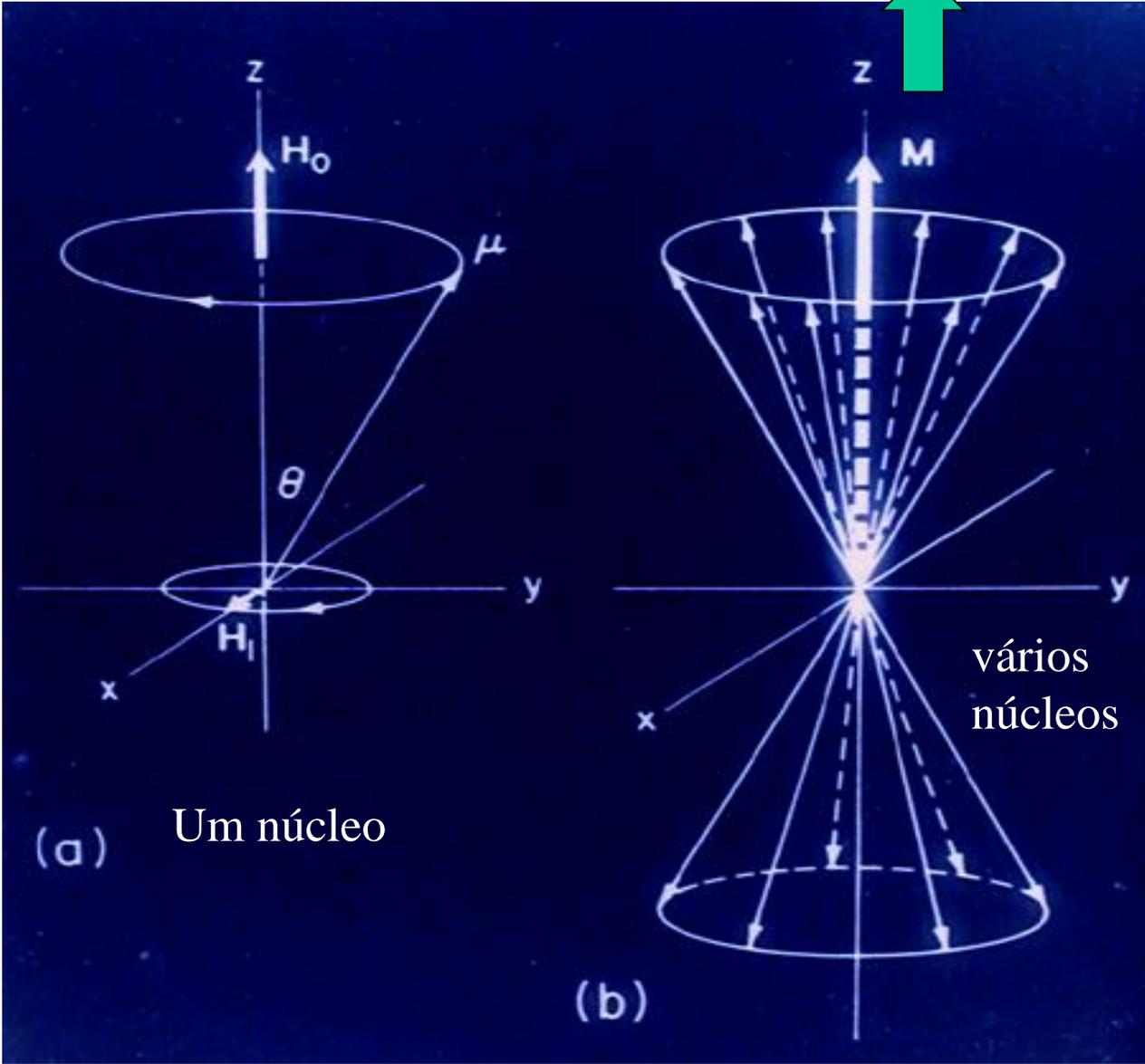


Espectrômetros de RMN da Central Analítica do IQUSP

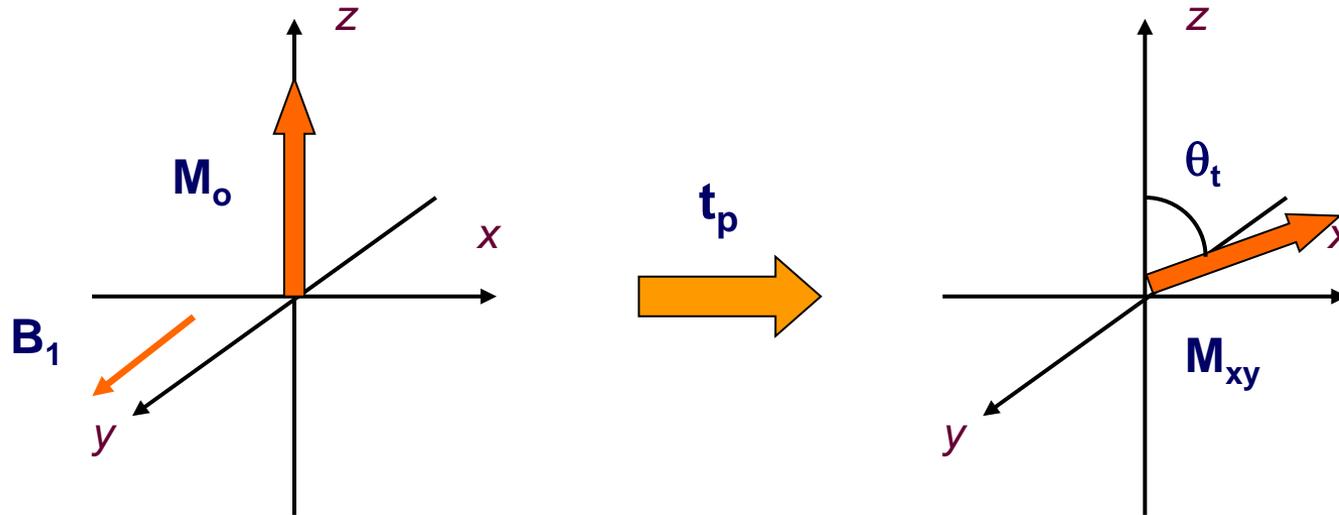
<https://www.youtube.com/watch?v=p7ad5bC-HS8>



M: Bulk magnetization (Magnetização macroscópica)



Largura do pulso de rf e desequilíbrio do vetor magnetização

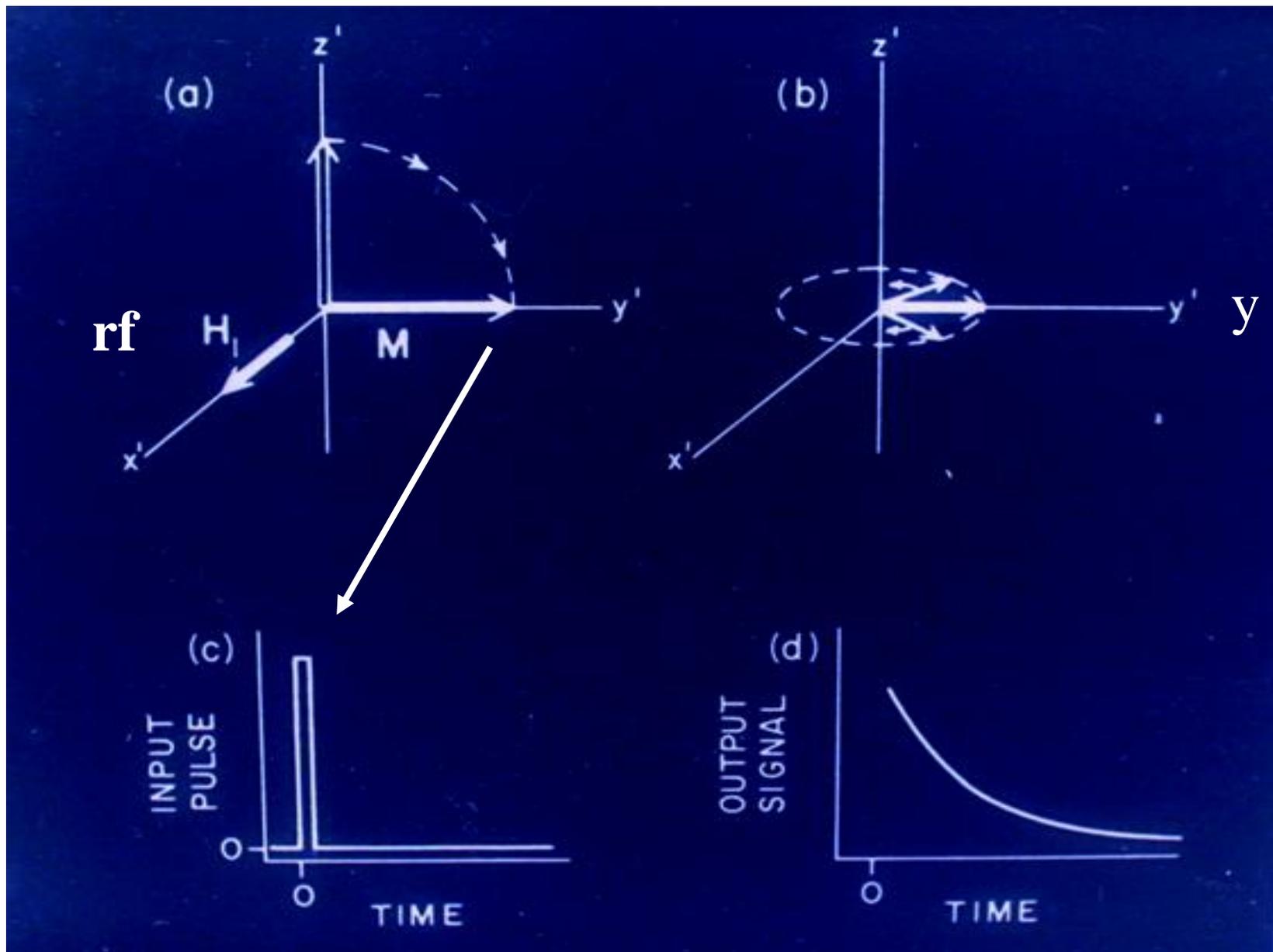


B_1 : pulso de
Radiofrequencia (rf)

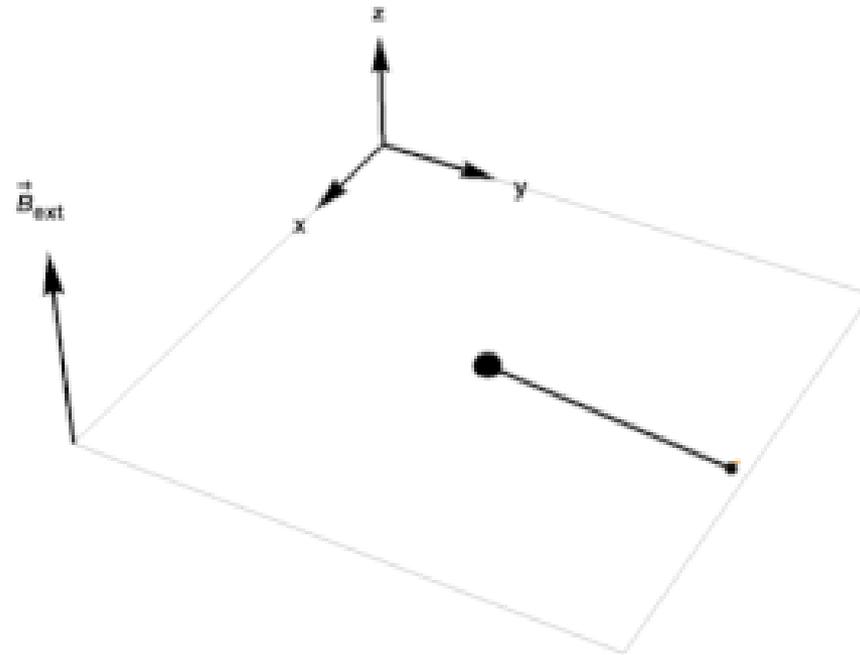
$$\theta_t = \gamma * t_p * B_1$$

- Os pulsos variam de acordo com os instrumentos (B_1), e as rotações variam de acordo com a rotação que se deseja: pulsos de $\pi / 4$ (45), $\pi / 2$ (90), π (180), etc.

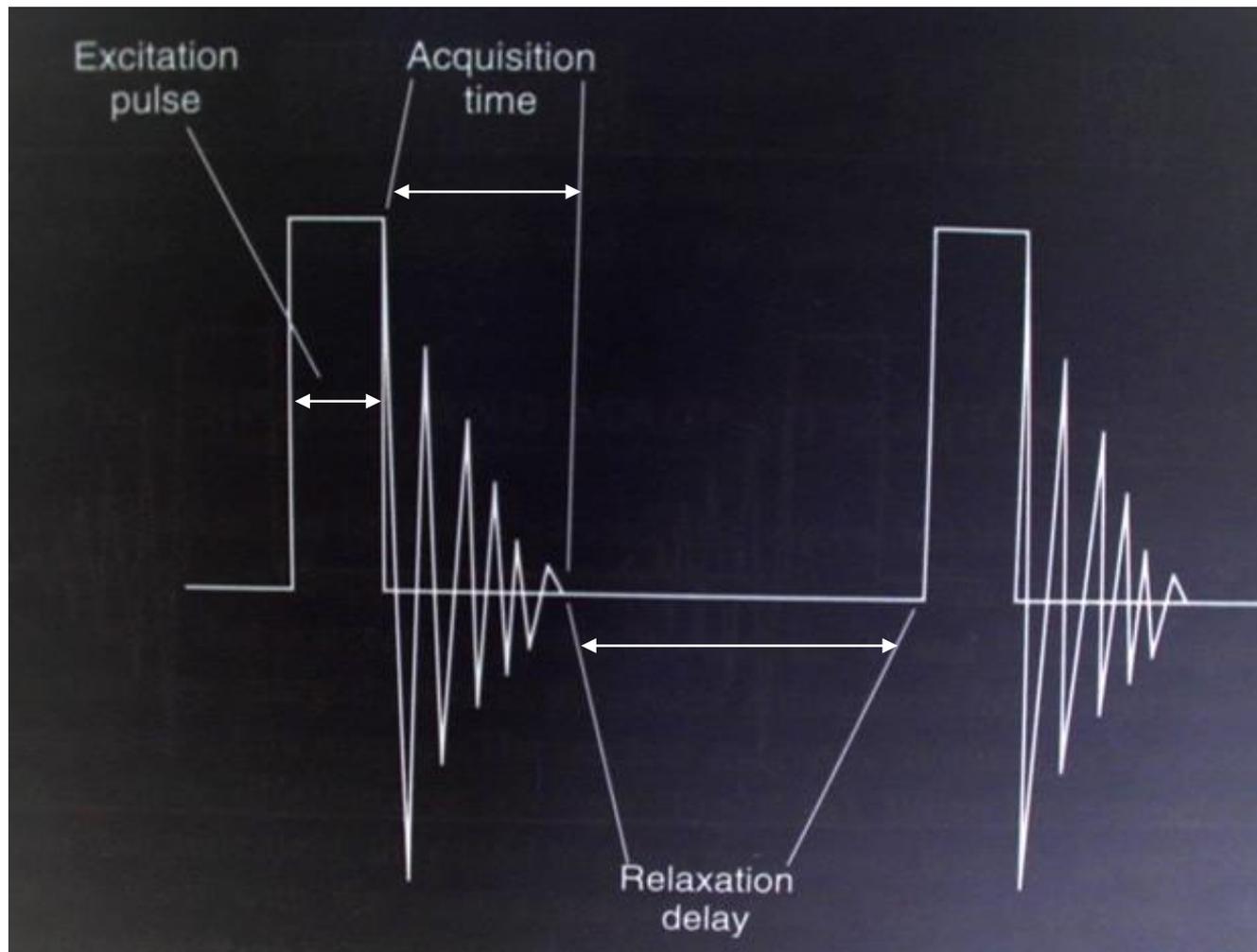
Pulso de 90° e intensidade do sinal no detector (em y)



Relaxamento de um núcleo após pulso de 90°



Representação de um pulso de excitação e geração do FID



Repete-se **n** vezes o pulso e a coleta dos dados do FID

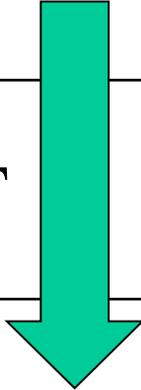
FID: free induced decay
(decaimento livre de indução)



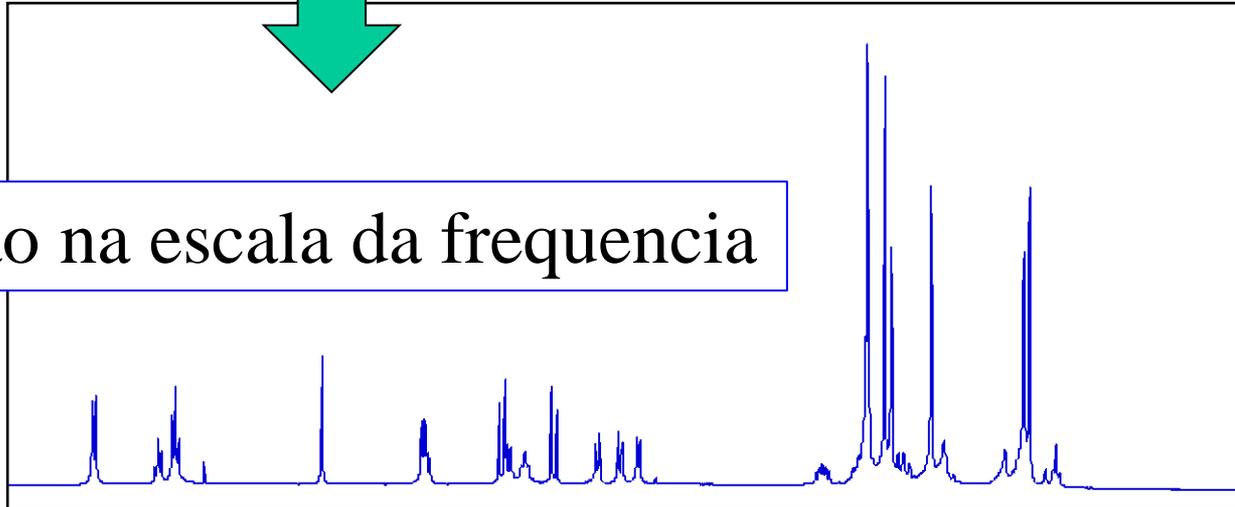
Informação na escala do tempo

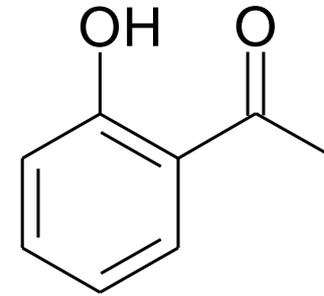
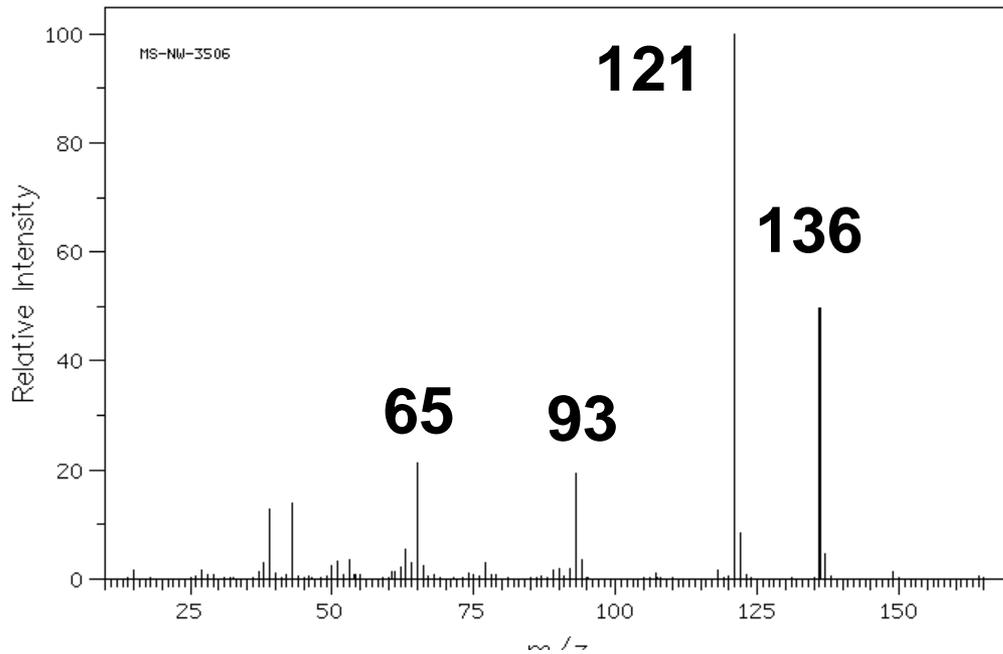
FT

$$\mathbf{S}(\omega) = \int_{-\infty}^{\infty} \mathbf{S}(t) \mathbf{e}^{-i\omega t} dt$$

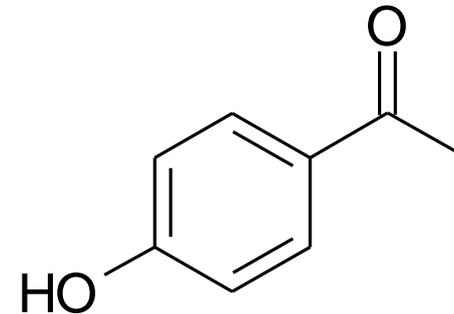
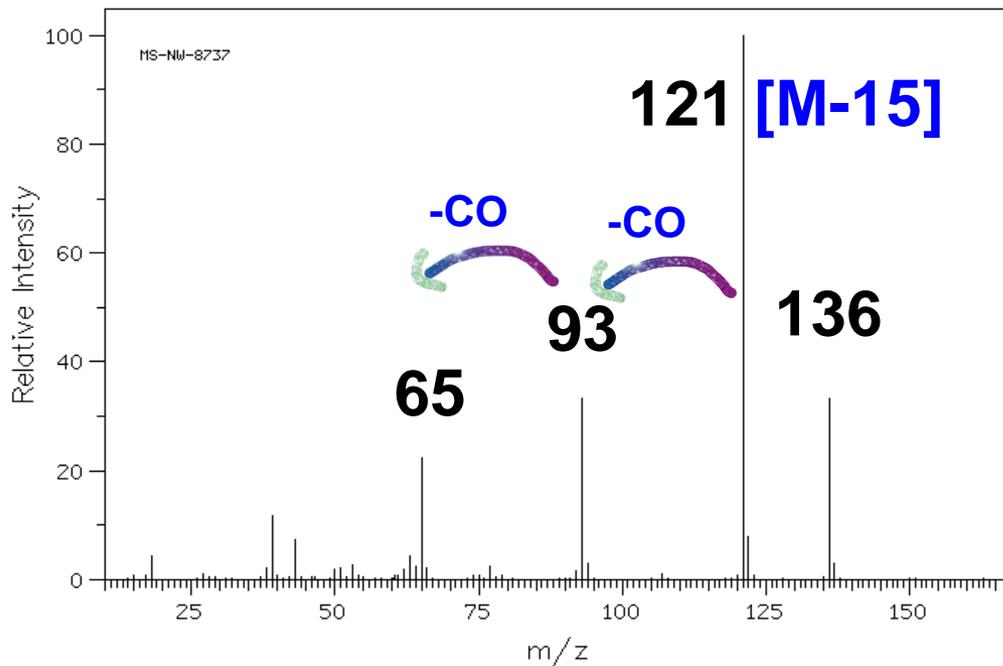


Informação na escala da frequencia

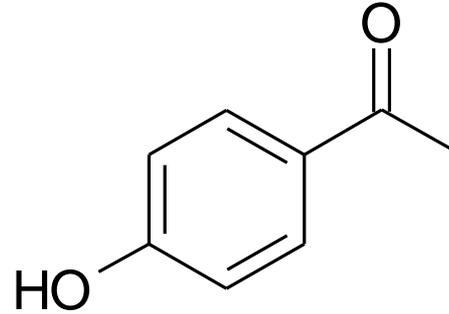
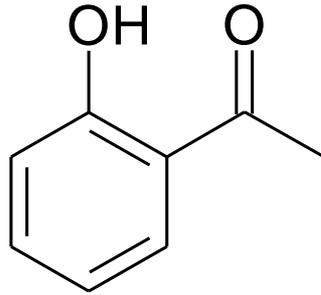




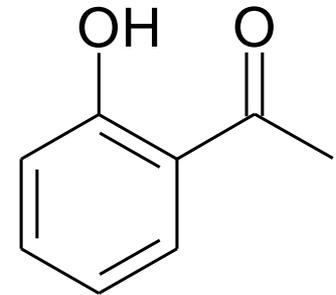
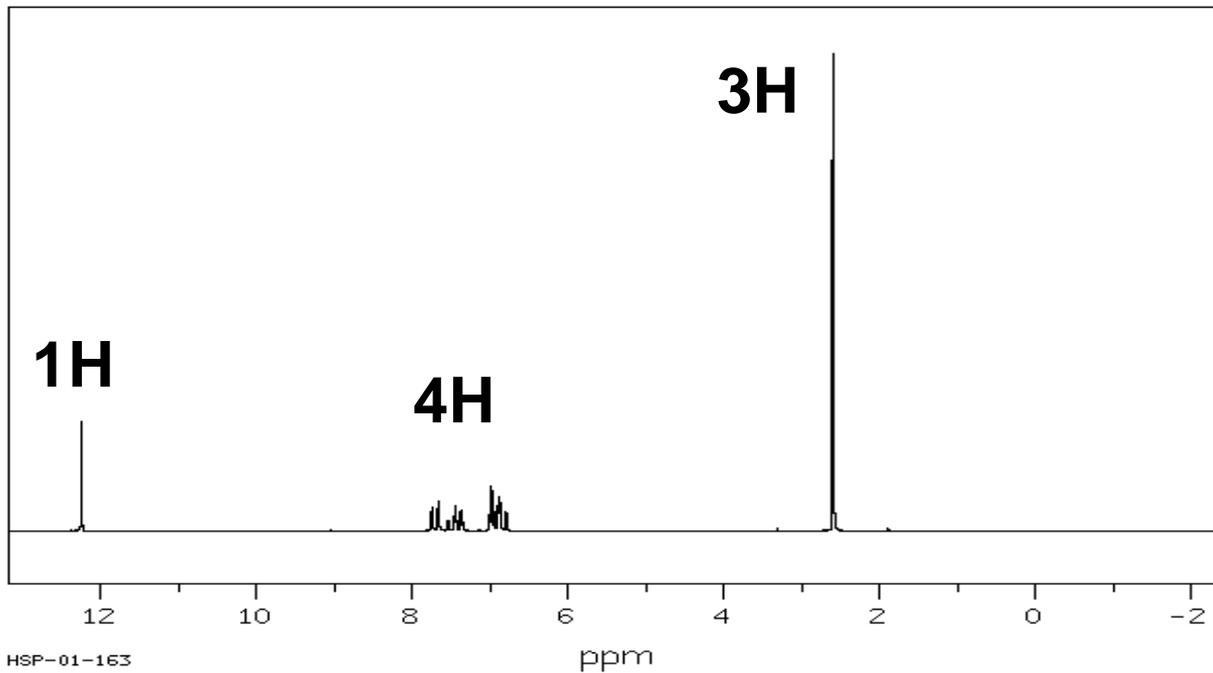
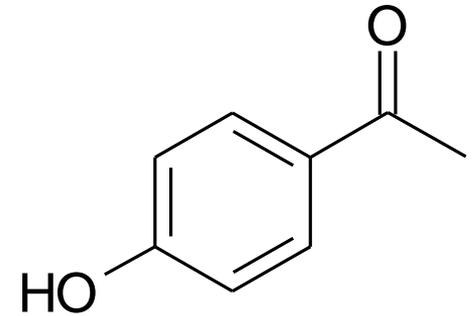
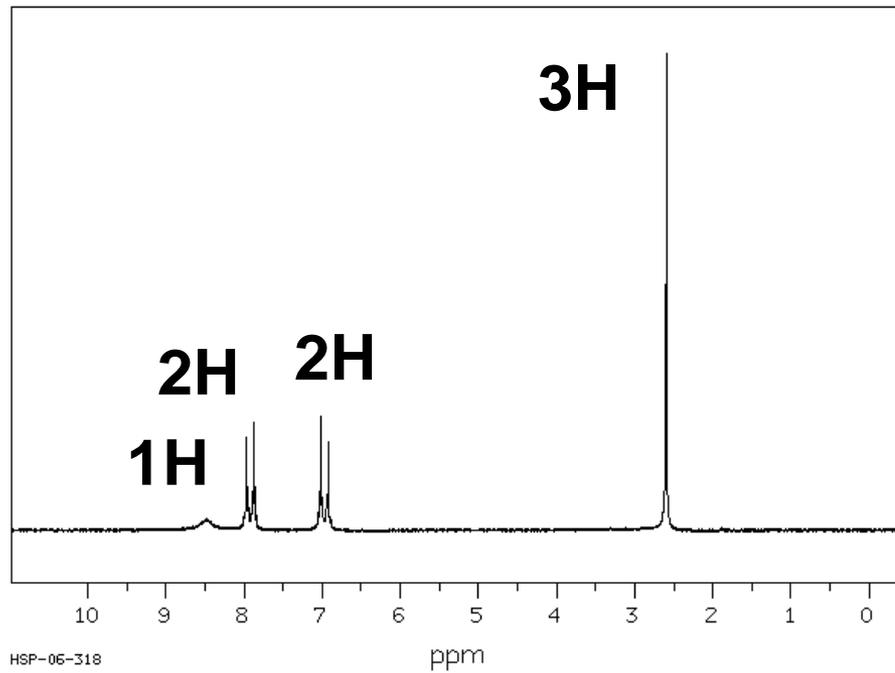
O EM não possibilita
diferenciar
esses isômeros !!!



Seria possível usar os dados de RMN de ^1H para diferenciar os dois isômeros?



Espectros de
RMN de ^1H dos isômeros
da hidroxiacetofenona



Informações obtidas de um espectro de RMN de ^1H :

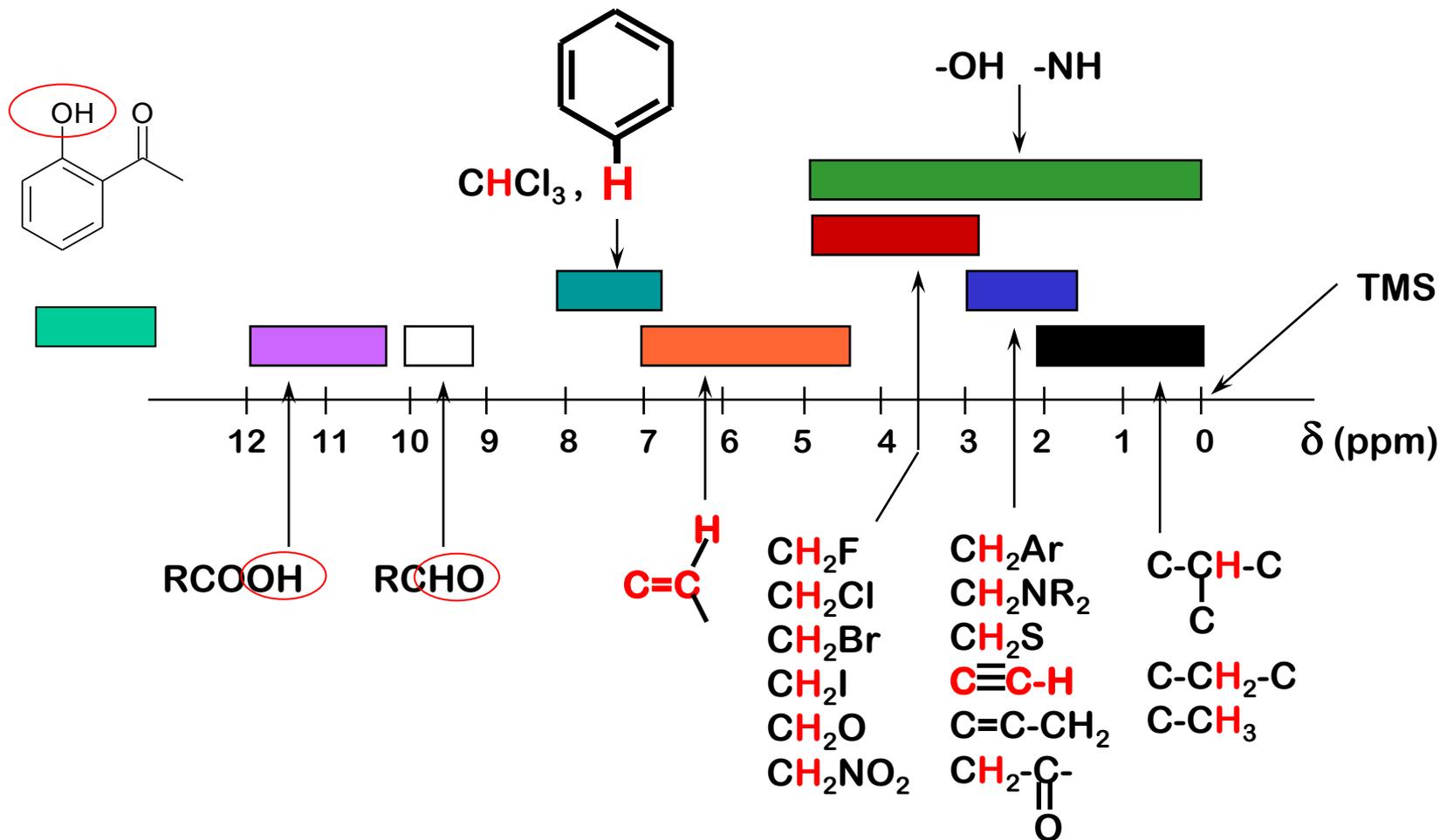
- 1) Deslocamentos químicos (δ - ppm);
- 2) Integrações (n^{os} relativo de Hs);
- 3) Constantes de acoplamento (J em Hertz);

Informações obtidas de um espectro de RMN de ^1H :

1) Deslocamentos químicos (δ - ppm):

A posição dos sinais no espectro estão associados à densidade de elétrons em torno dos núcleos de ^1H , de ^{13}C e etc.

δ em RMN para diversos Hs



núcleos
com menor
densidade de
elétrons



núcleos
com maior
densidade de
elétrons

núcleos
desblindados
(ou desprotegidos)
por menor
densidade de
elétrons



núcleos
blindados
(ou protegidos)
por maior
densidade de
elétrons

campo baixo



campo alto

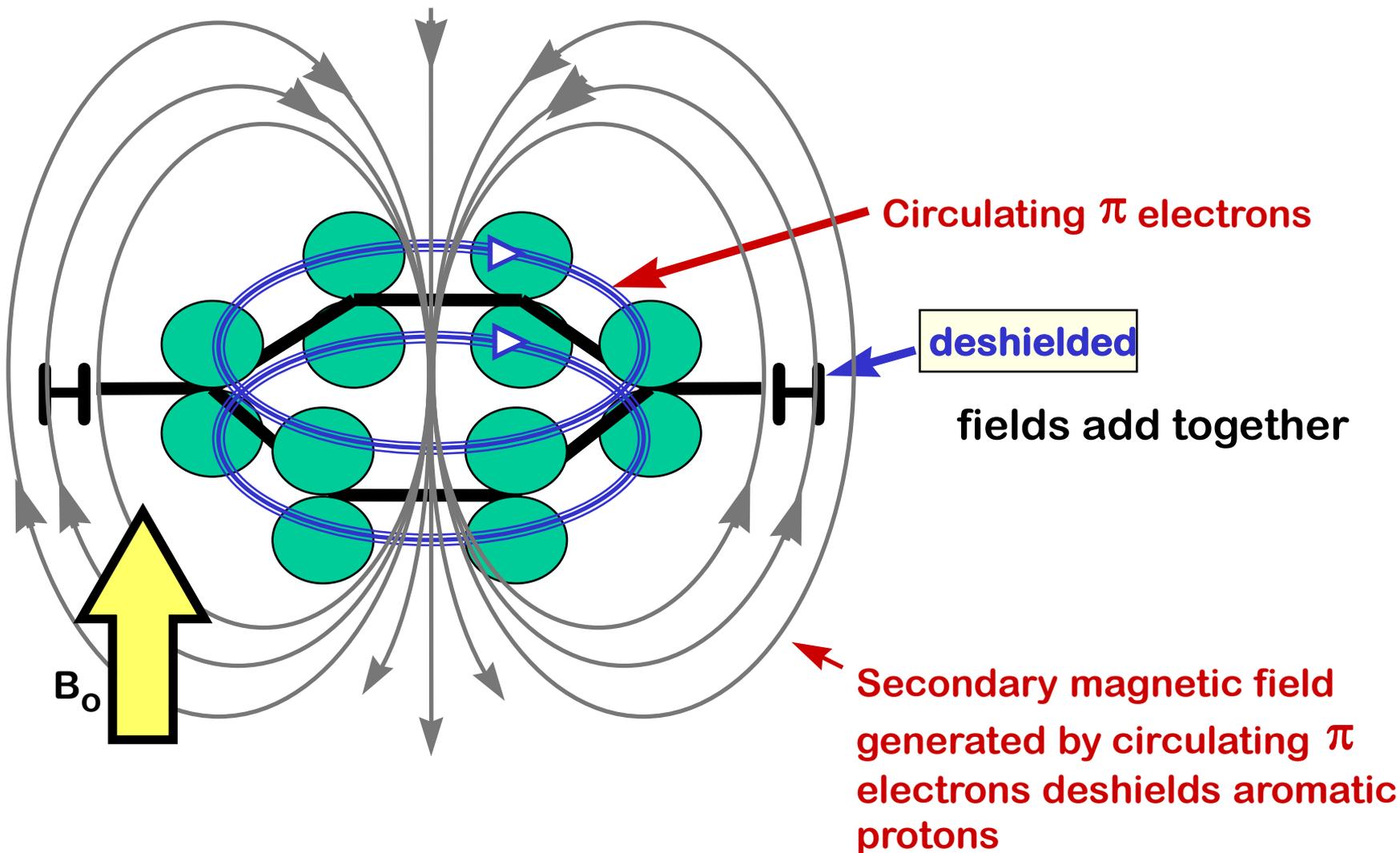
"downfield from TMS"



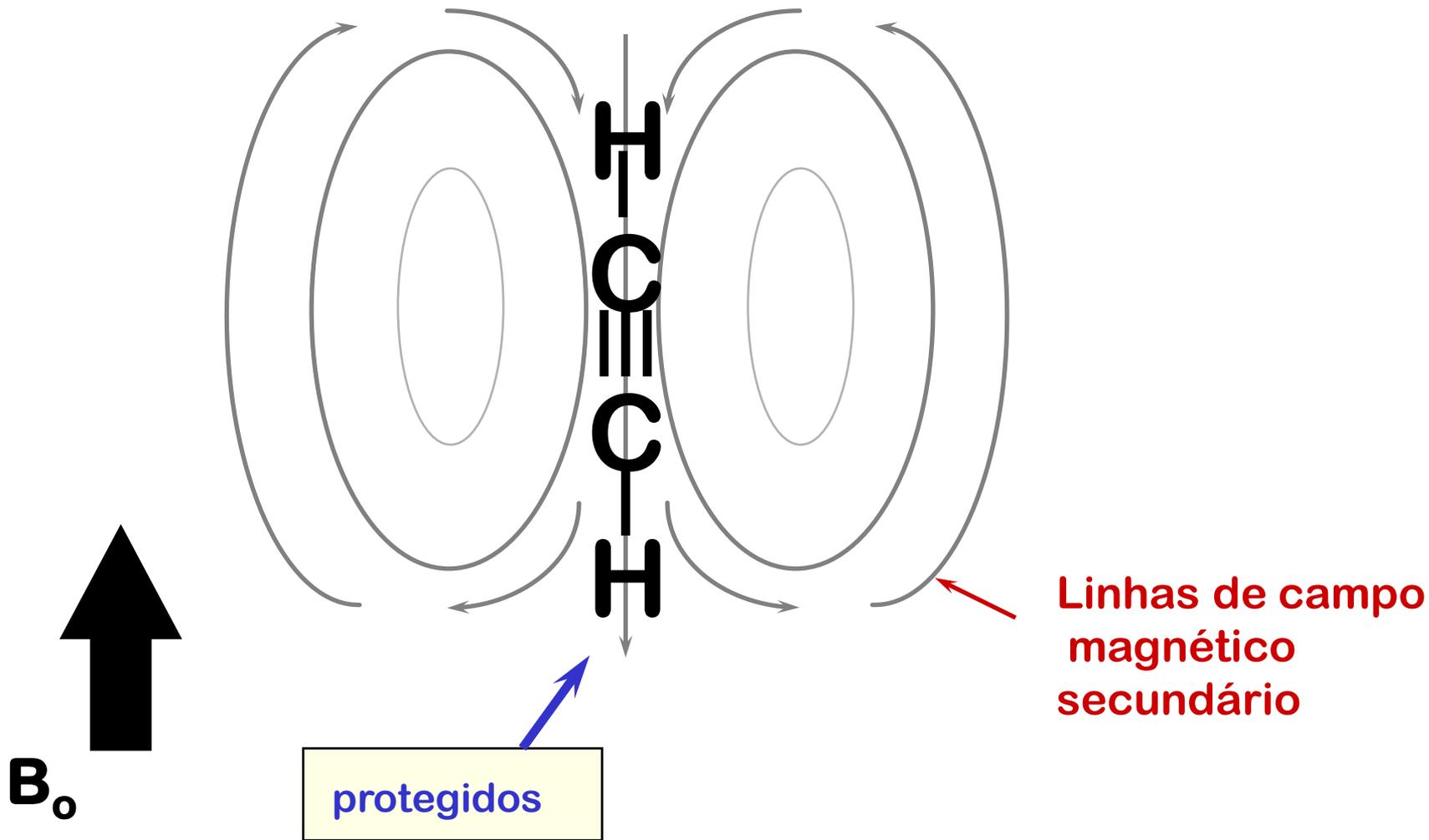
Chemical shift listed in parts per million (ppm)

(TMS
standard)

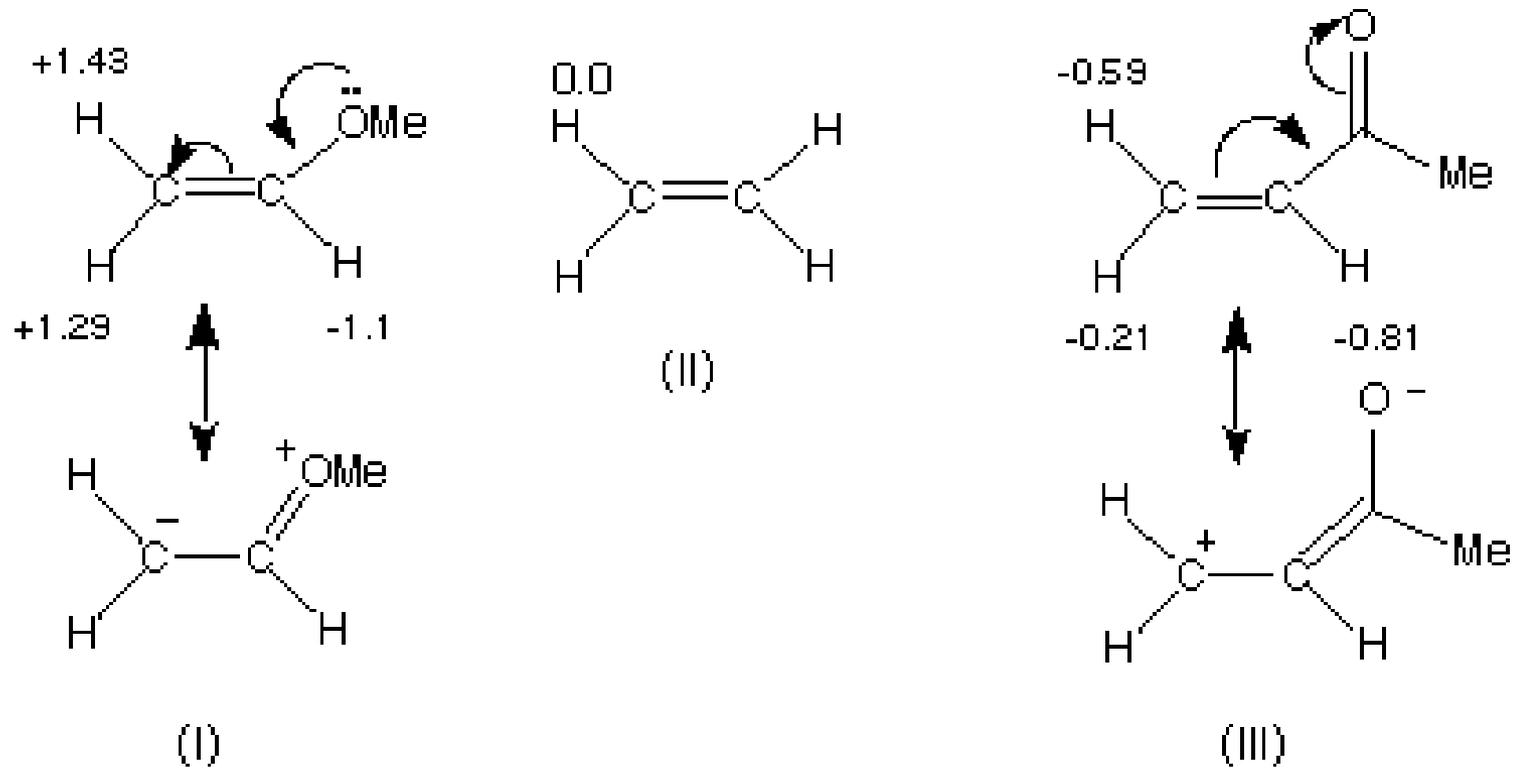
Campo anisotrópico de elétrons no Benzeno



CAMPO ANISOTRÓPICO NO ALCINO



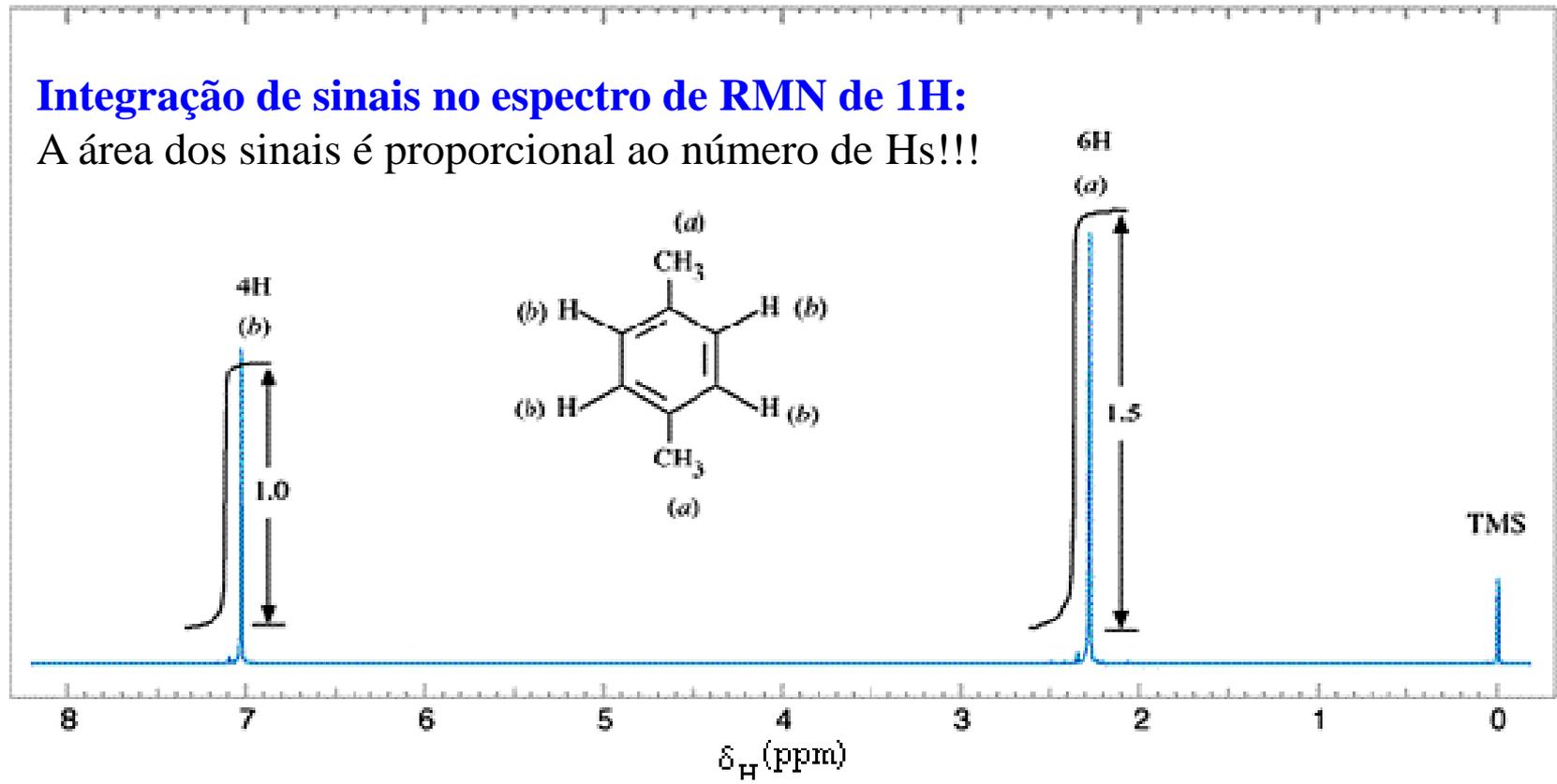
Efeito de ressonância com grupos conjugados



Equivalência magnética e deslocamentos químicos

Integração de sinais no espectro de RMN de ^1H :

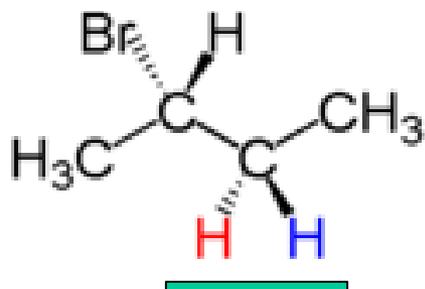
A área dos sinais é proporcional ao número de Hs!!!



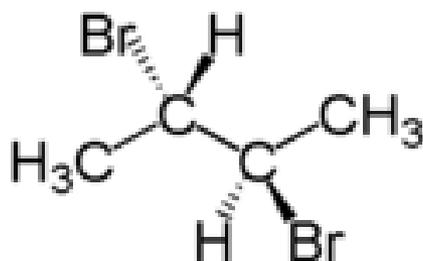
Hidrogênios diastereotópicos:

Hidrogênios com diferentes ambientes, logo com diferentes deslocamentos químicos e acoplam entre sí.

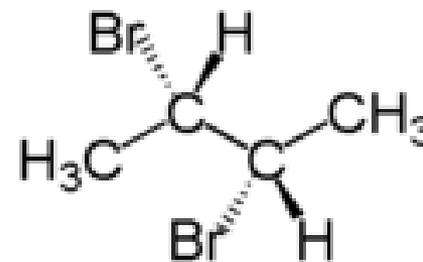
diastereoisômeros



(*S*)-2-bromobutane



(*2S,3R*)-2,3-dibromobutane



(*2S,3S*)-2,3-dibromobutane

Hidrogênios diastereotópicos
(dois sinais distintos)

Informações obtidas de um espectro de RMN de ^1H :

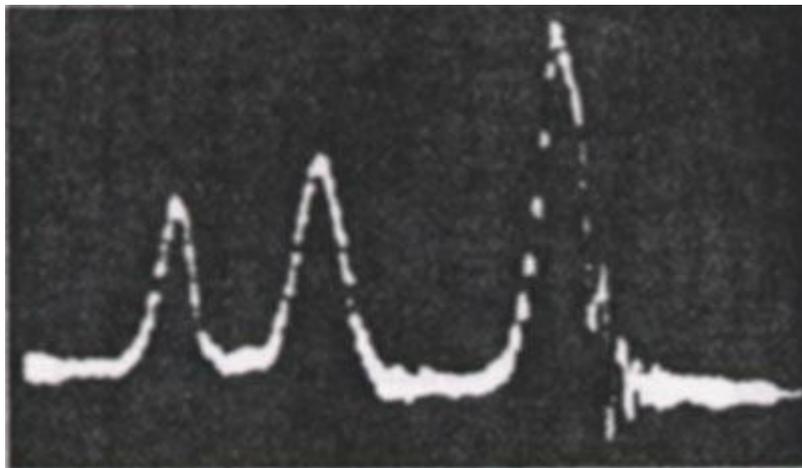
1) Deslocamentos químicos (δ - ppm);

2) Integrações (n^{os} relativo de Hs);

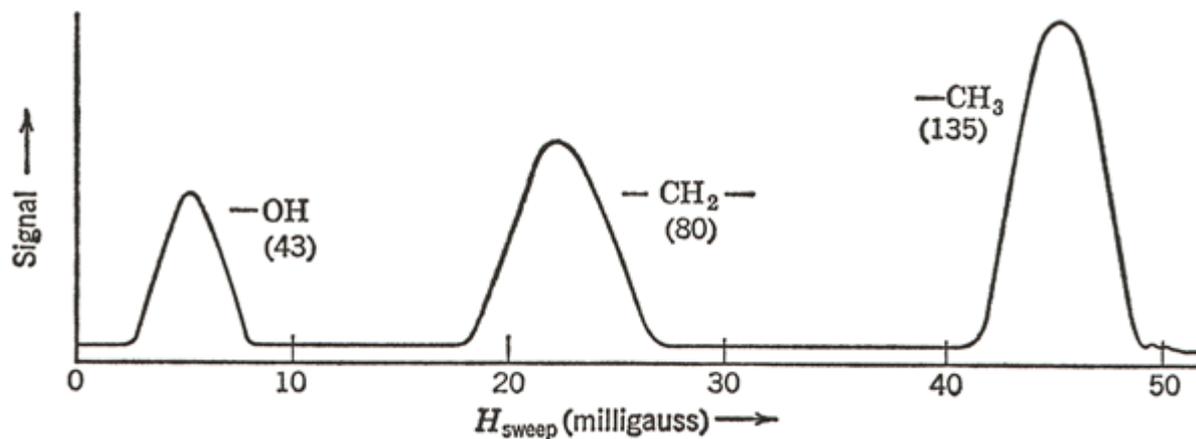
3) Constantes de acoplamento (J em Hertz);

Espectro de RMN de ^1H do EtOH

Integração dos sinais



The first NMR spectrum of ethanol, taken at Stanford University in 1951 (*Varian Associates, Inc.*)

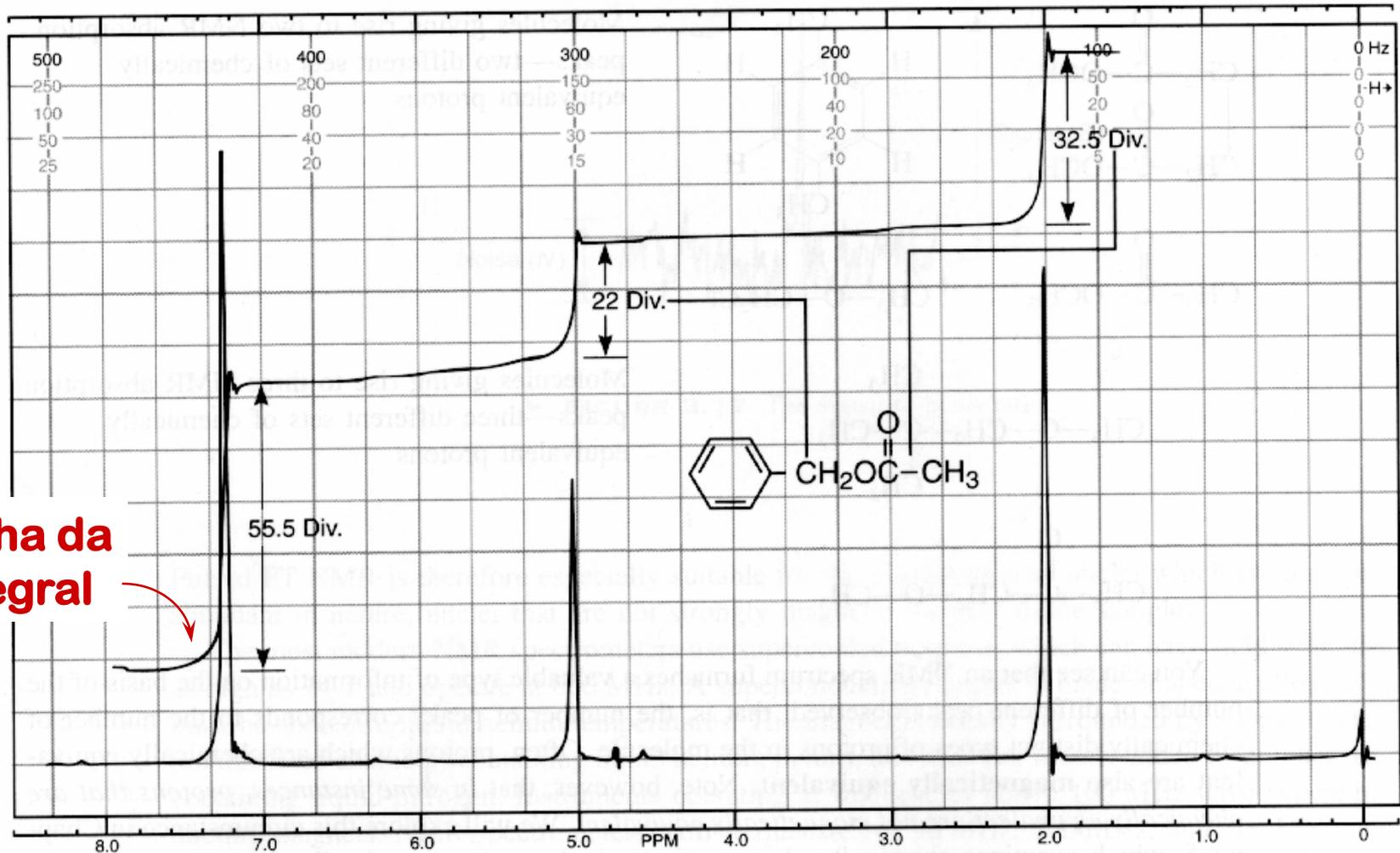


1H

2H

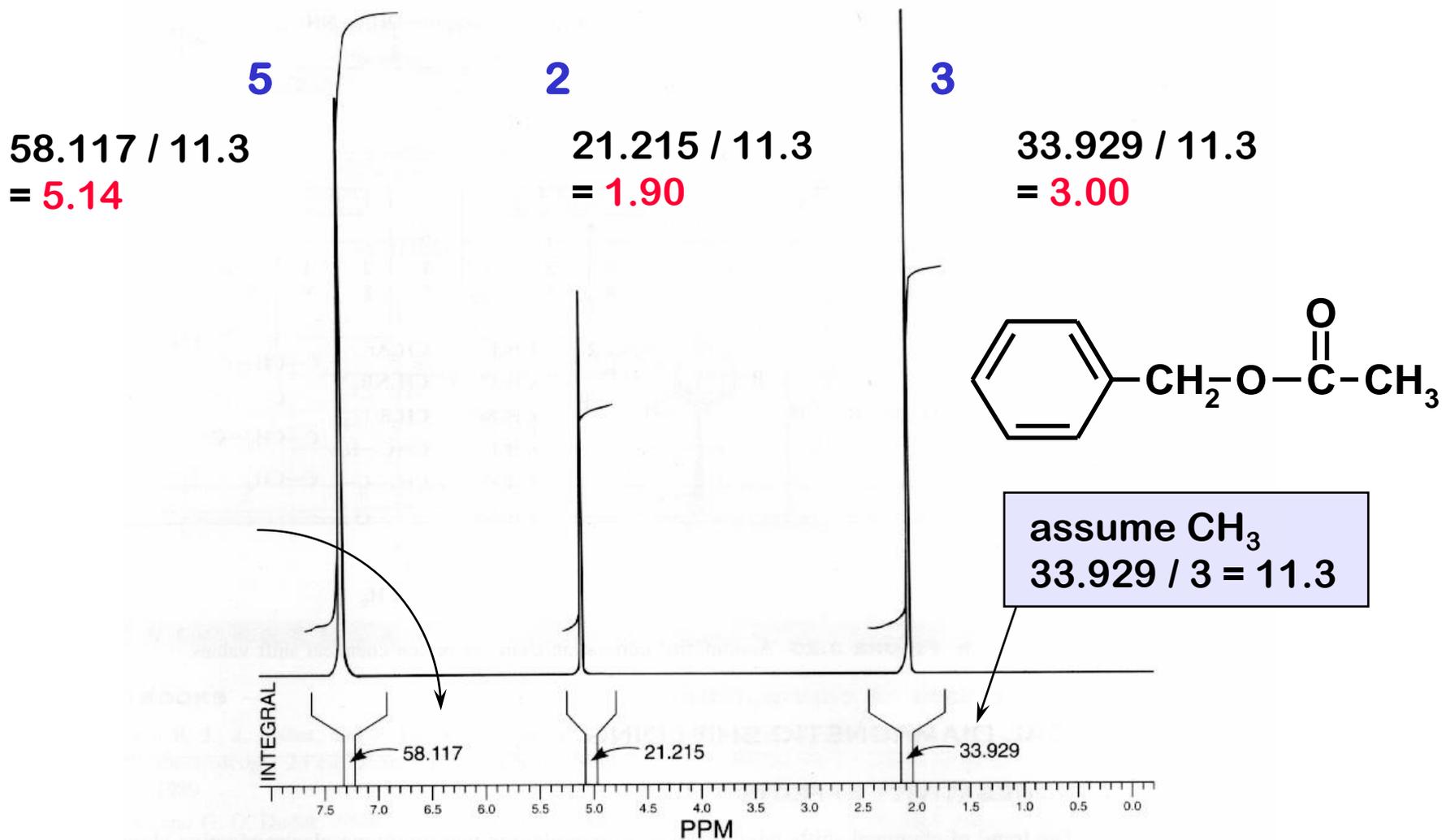
3H

Acetato de benzila



$$55 : 22 : 33 = 5 : 2 : 3$$

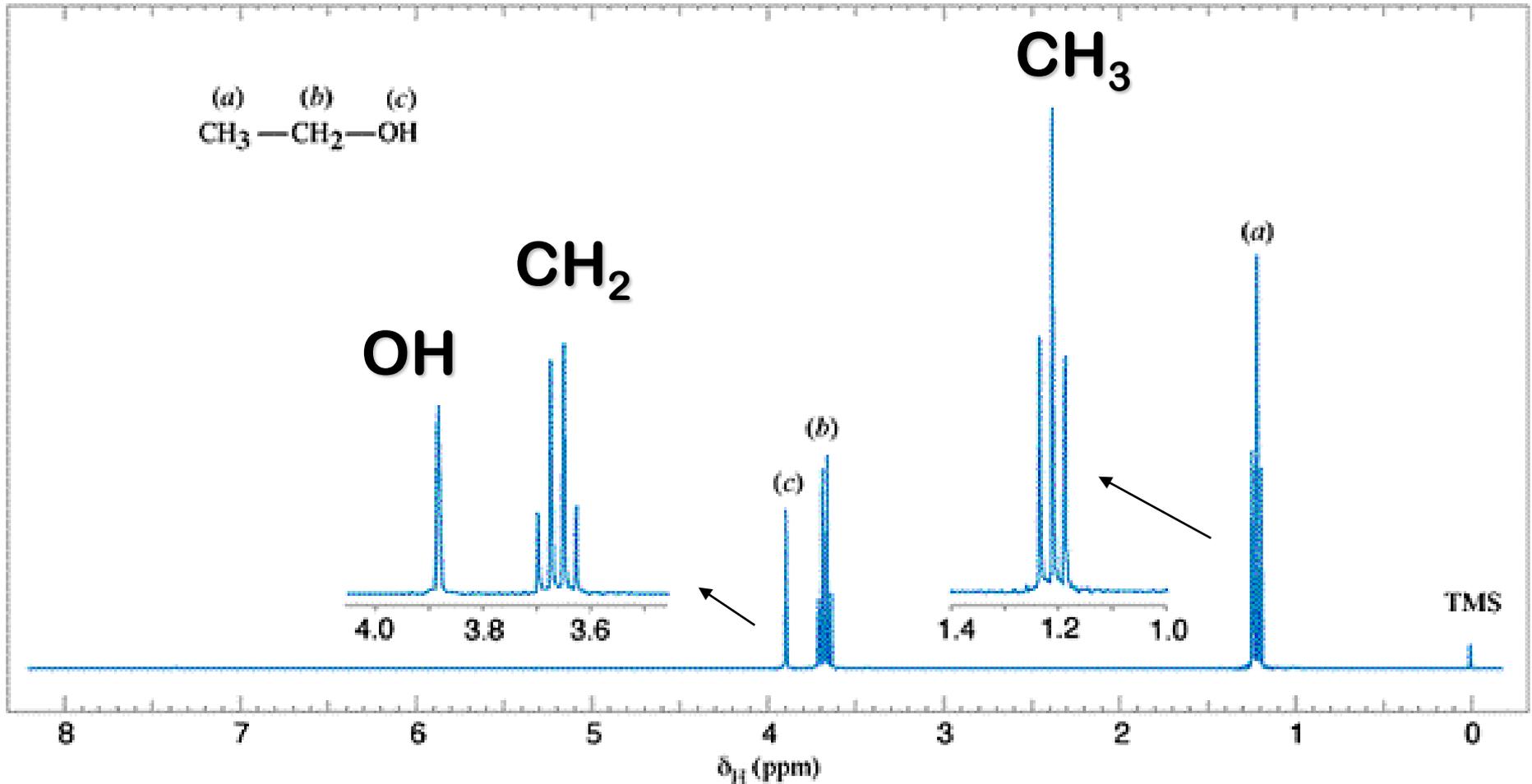
Acetato de benzila



Informações obtidas de um espectro de RMN de ^1H :

- 1) Deslocamentos químicos (δ - ppm);
- 2) Integrações (n^{os} relativo de Hs);
- 3) Constantes de acoplamento (J em Hertz);**

^1H NMR spectrum of ethanol (300 MHz)

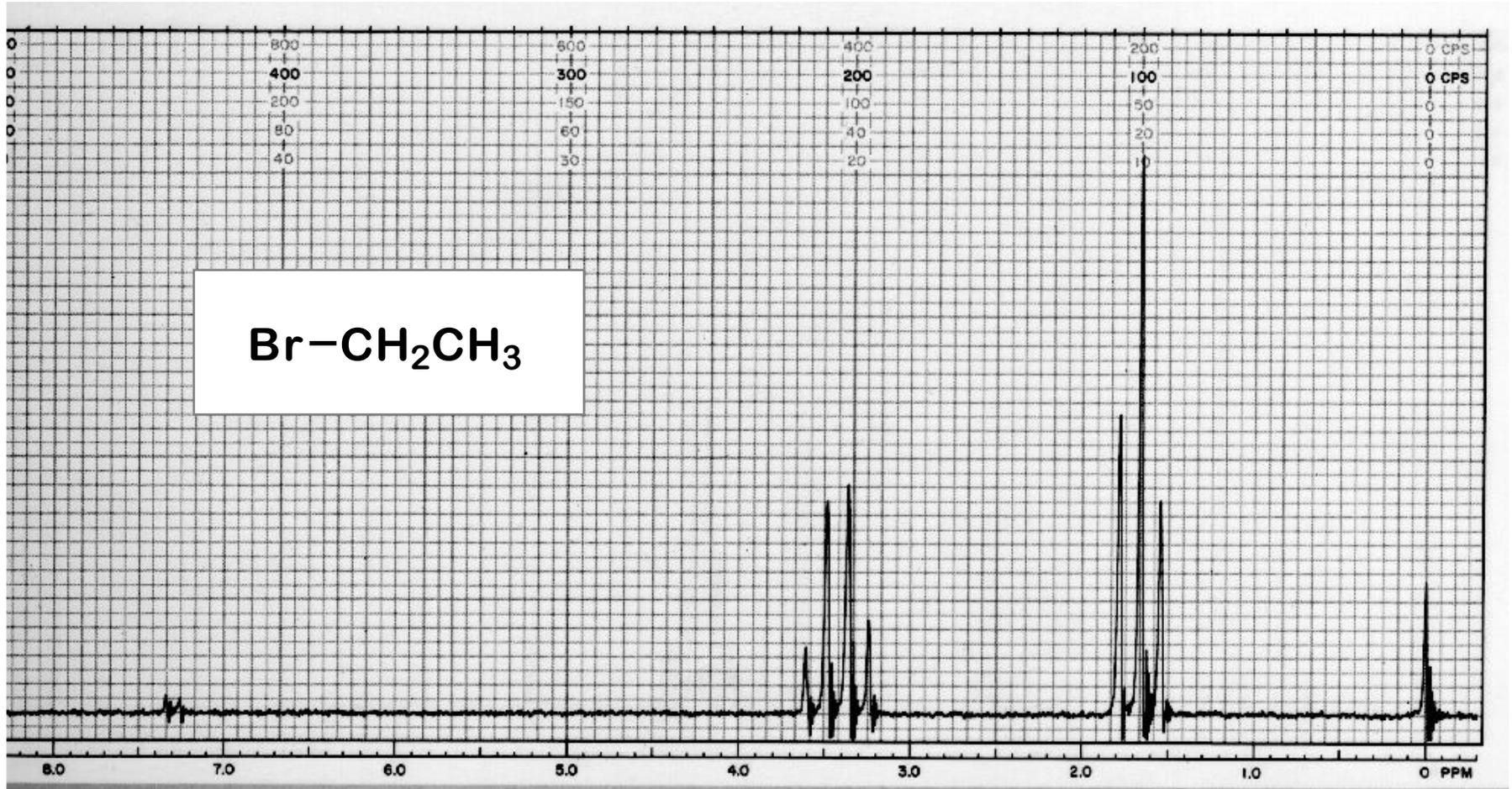


O que são esses
desdobramentos?

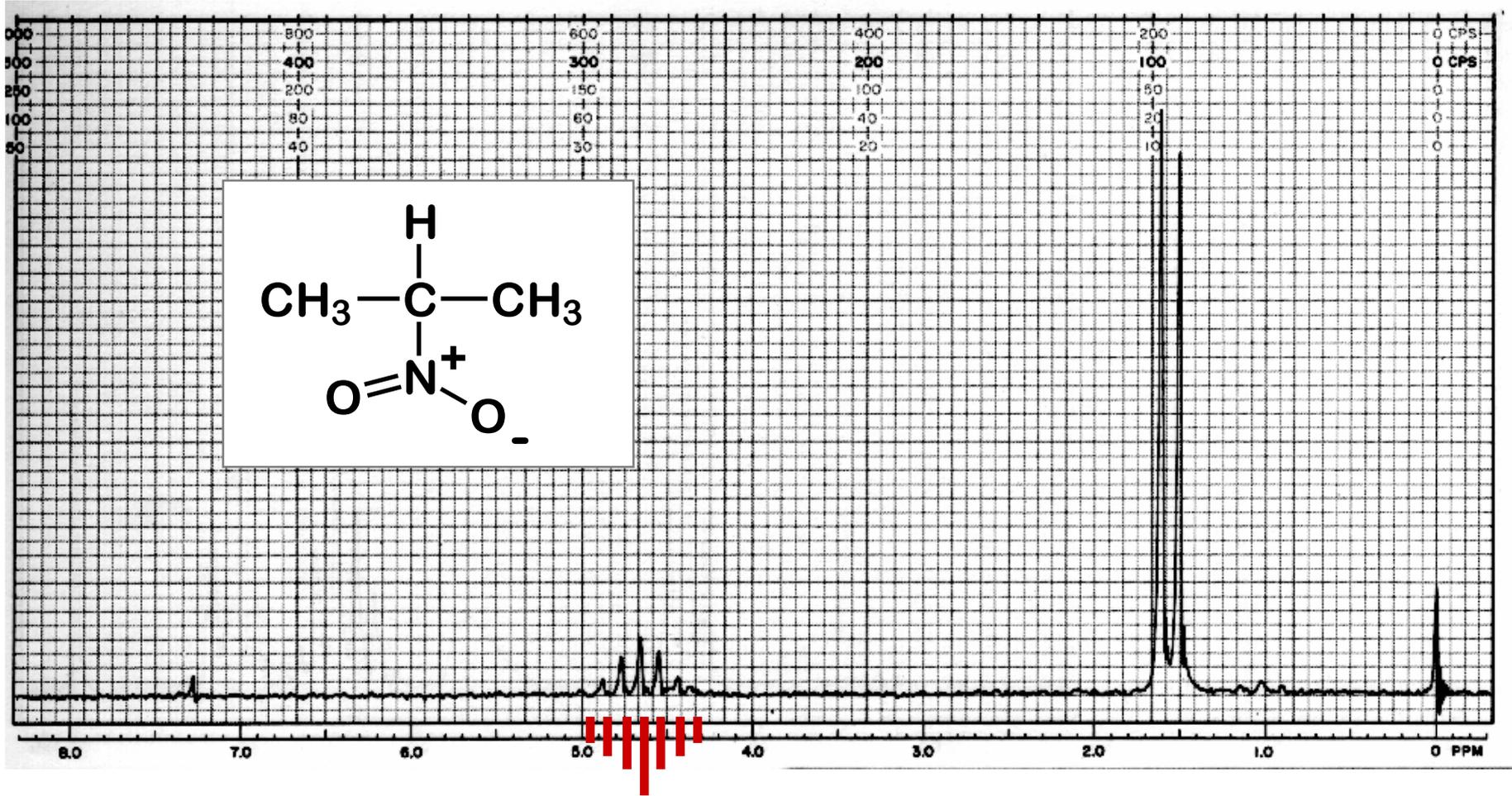
Aproximação (rudimentar) para se definir a multiplicidade em um acoplamento

$$\text{multiplicidade} = \text{número de Hs vizinhos} + 1$$

Bromoetano

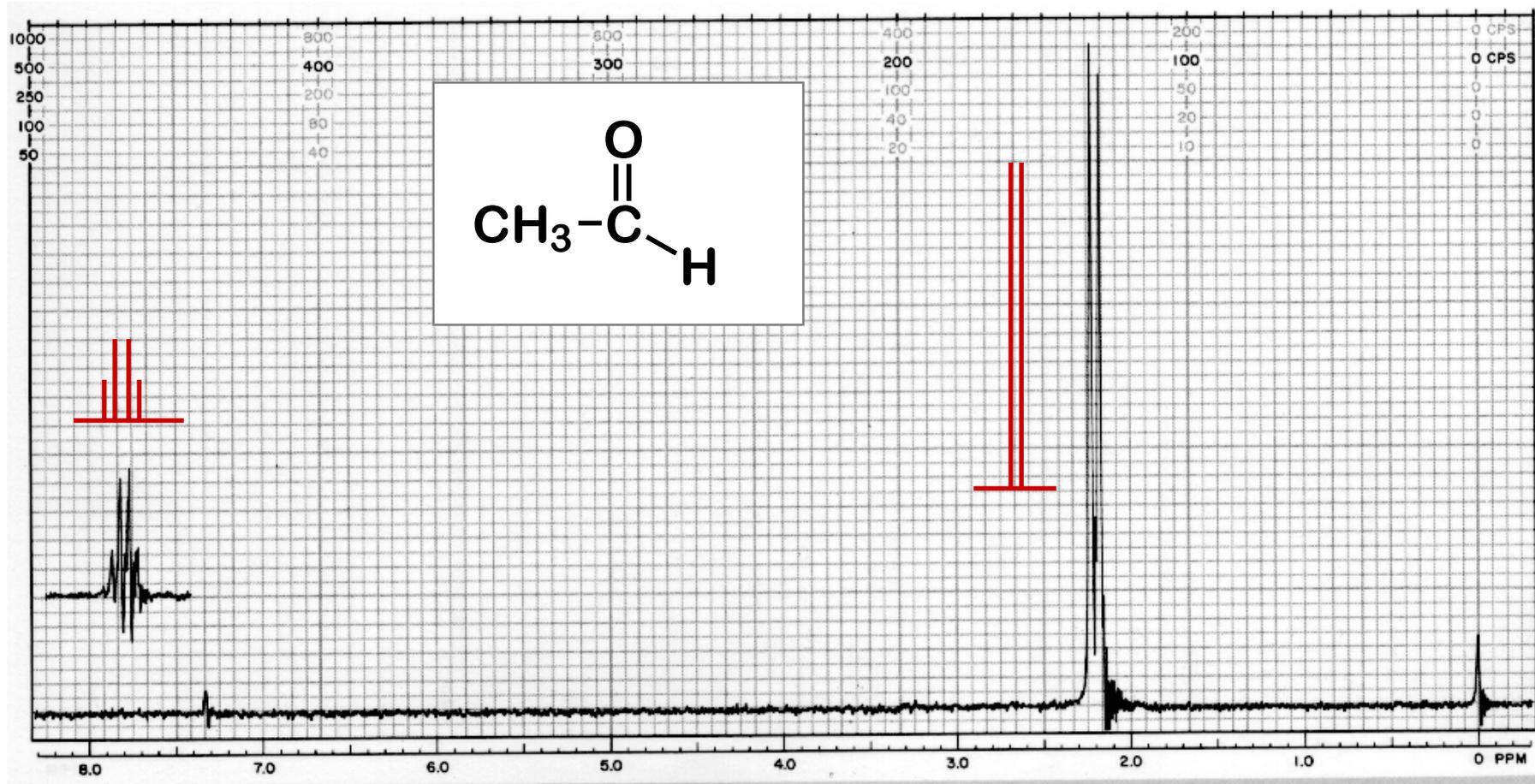


2-Nitropropano



1:6:15:20:16:6:1 in higher multiplets the outer peaks are often nearly lost in the baseline

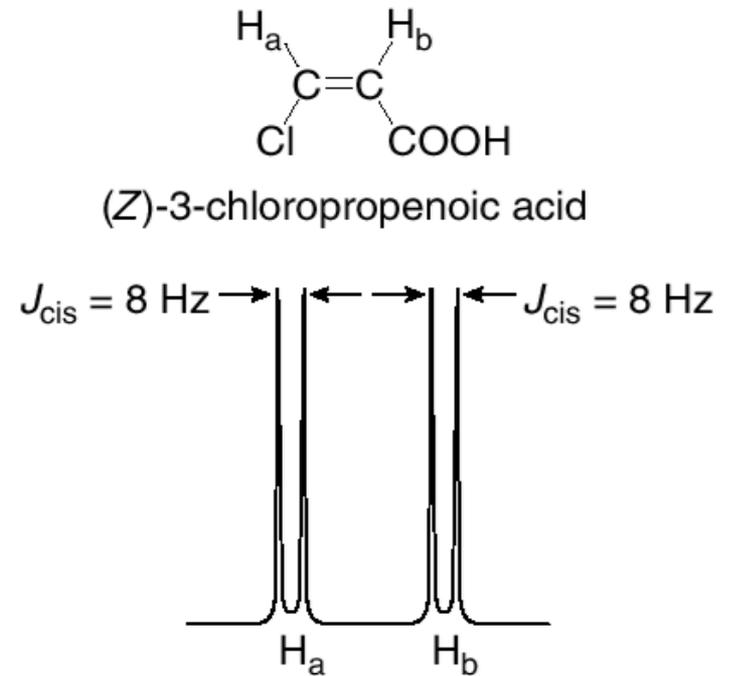
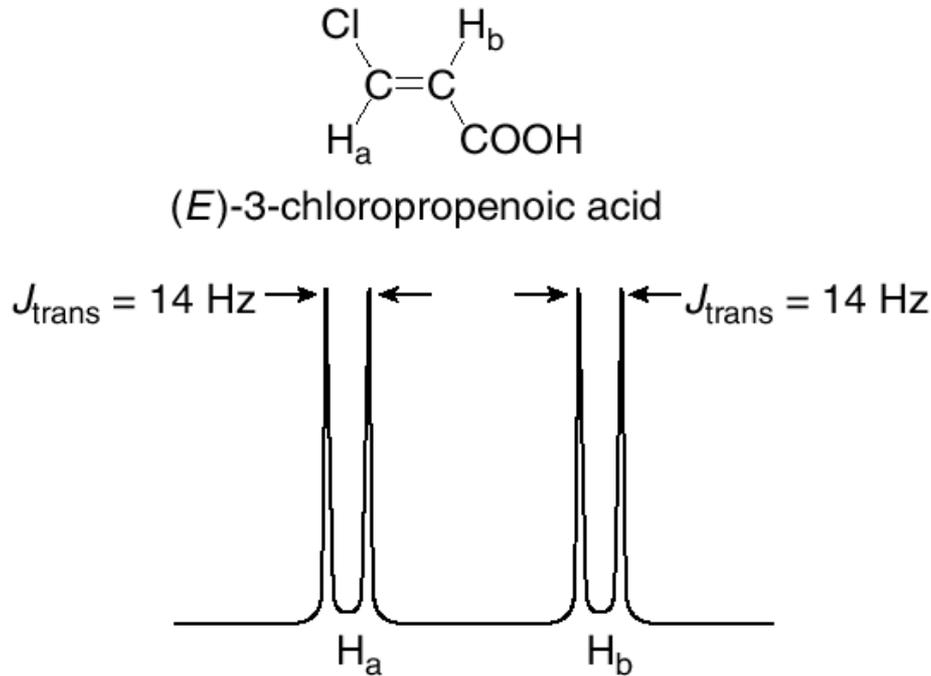
Acetaldeído



offset = 2.0 ppm

Acoplamento mais simples para Ha diferente de Hb

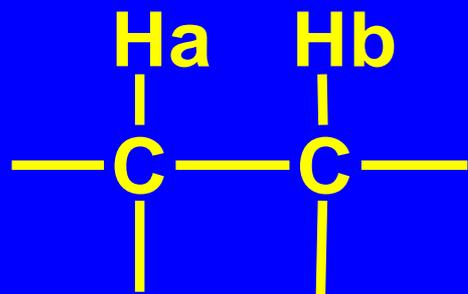
Acoplamento spin-spin (coupling constant)



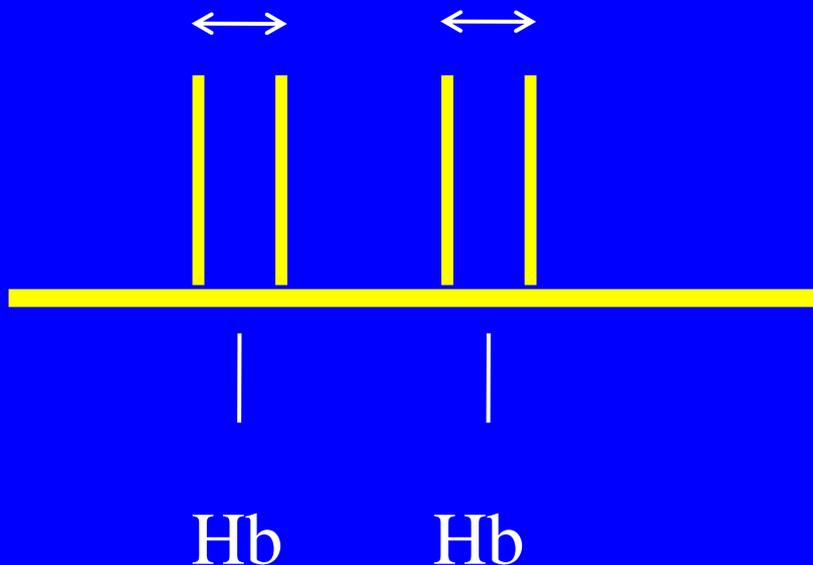
Os dois isômeros podem ser distinguidos com base nos valores de J (constantes de acoplamentos): $J_{trans} > J_{cis}$

Acoplamento mais simples para diferentes Hs (Ha possui ambiente eletrônico diferente de Hb)

Mesmo valor de constante
de acoplamento, pode identificar os
hidrogênios que estão acoplados entre si.



$$J_{ab} = J_{ba}$$



Dois sinais distintos
(dois deslocamentos químicos)

Excitação de um núcleo hipotético HA (com ligeiro excesso no Estado Fundamental)

$$\nu_{HA} = B_0 + B_{HB}$$

direção do
campo
magnético

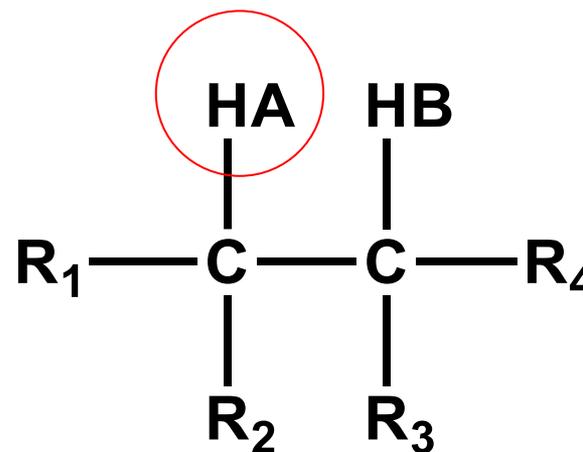
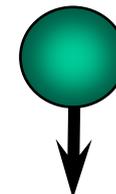
B_0



HA

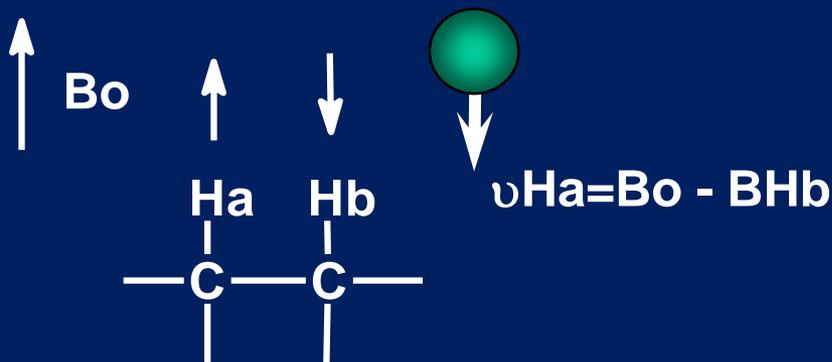
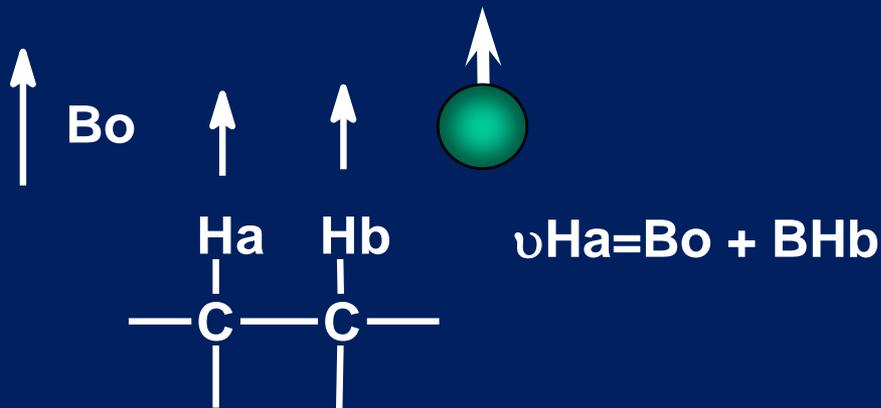
BHB: campo
gerado pelo
núcleo HB

HB ~1:1

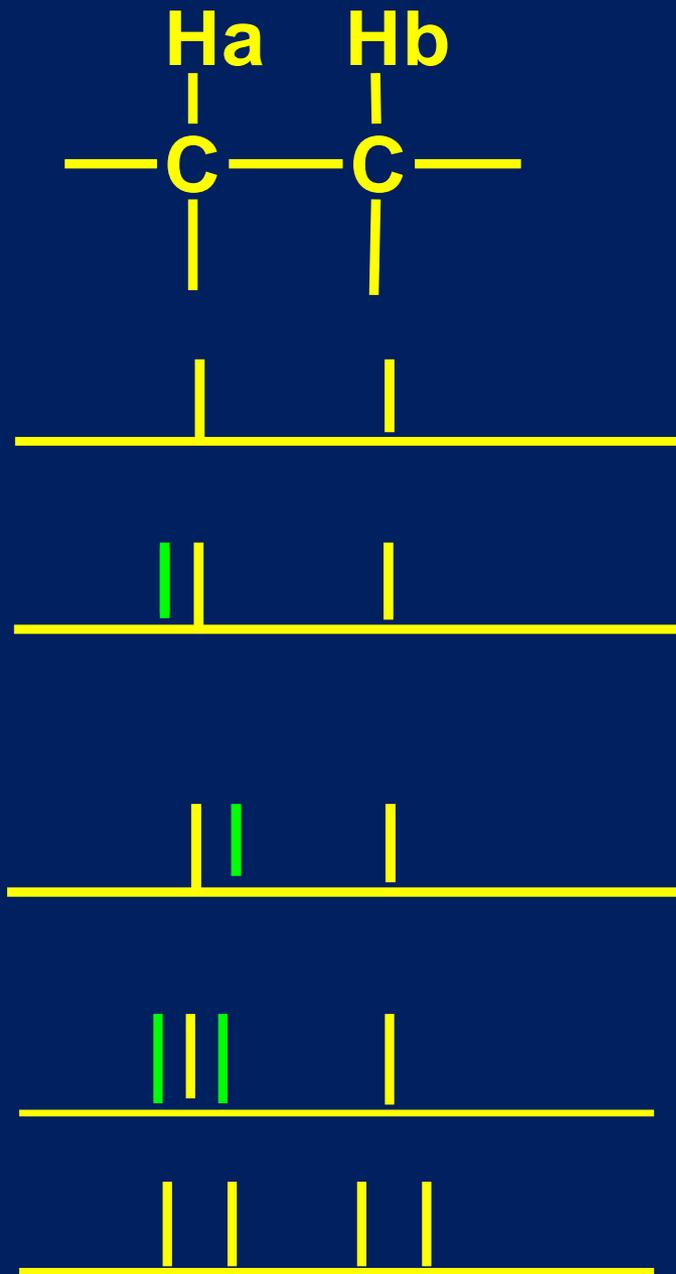


Acoplamento mais simples:

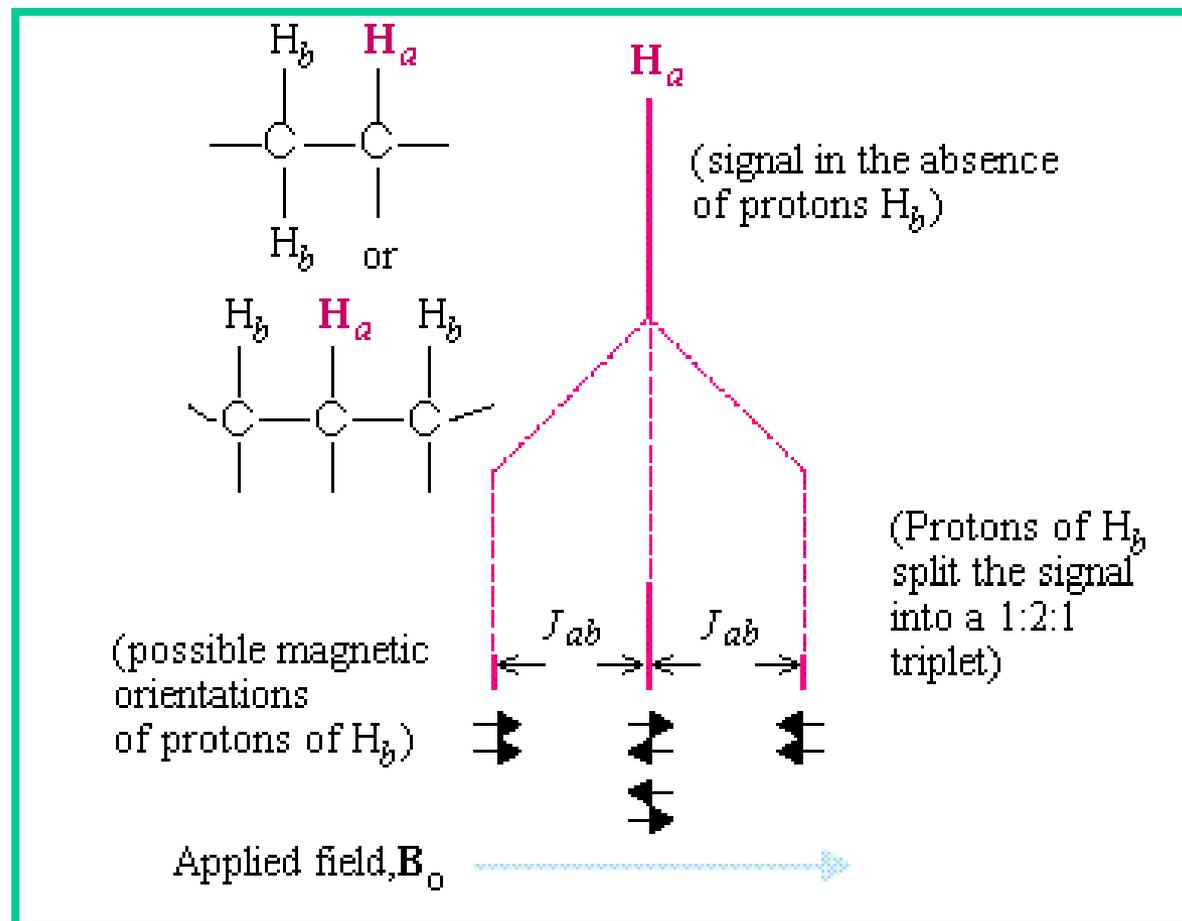
Observando-se Ha:



$$J_{ab} = J_{ba}$$



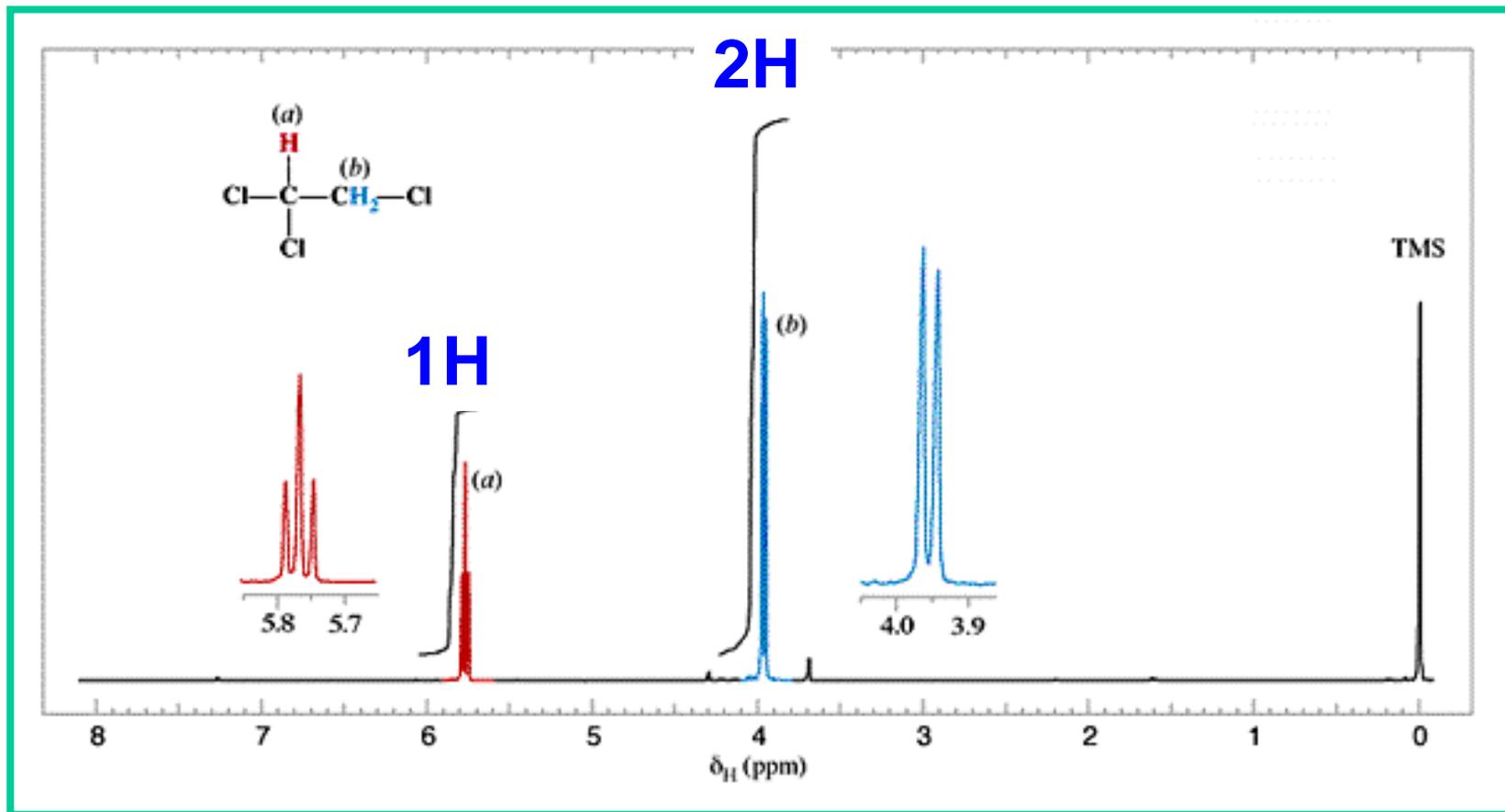
Ha acopla com dois Hb e é desdobrado como um tripeto de intensidades 1:2:1



E qual a multiplicidade de Hb?

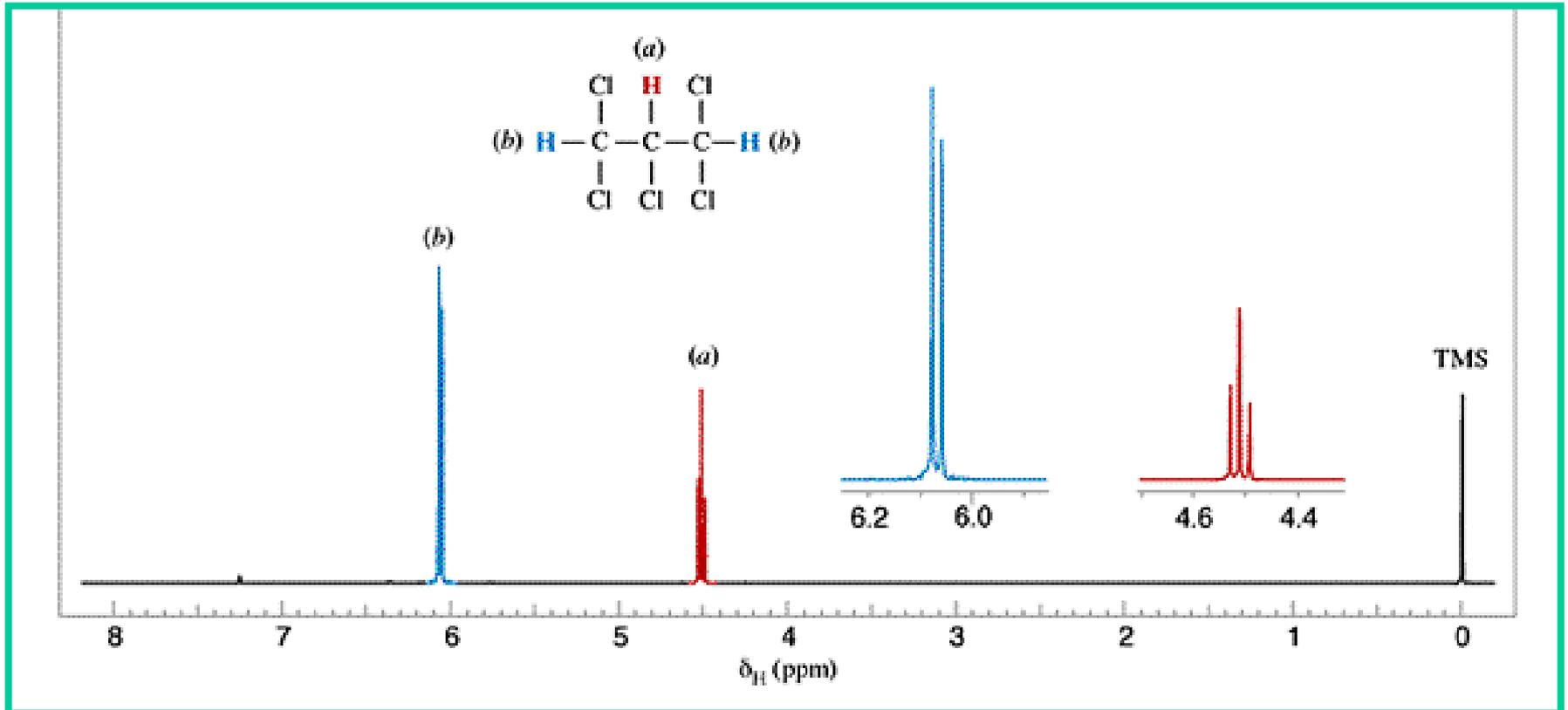
1 Ha acopla com 2 Hb

Espectro de RMN de ^1H (300 MHz)
do 1,1,2-tricloroetileno

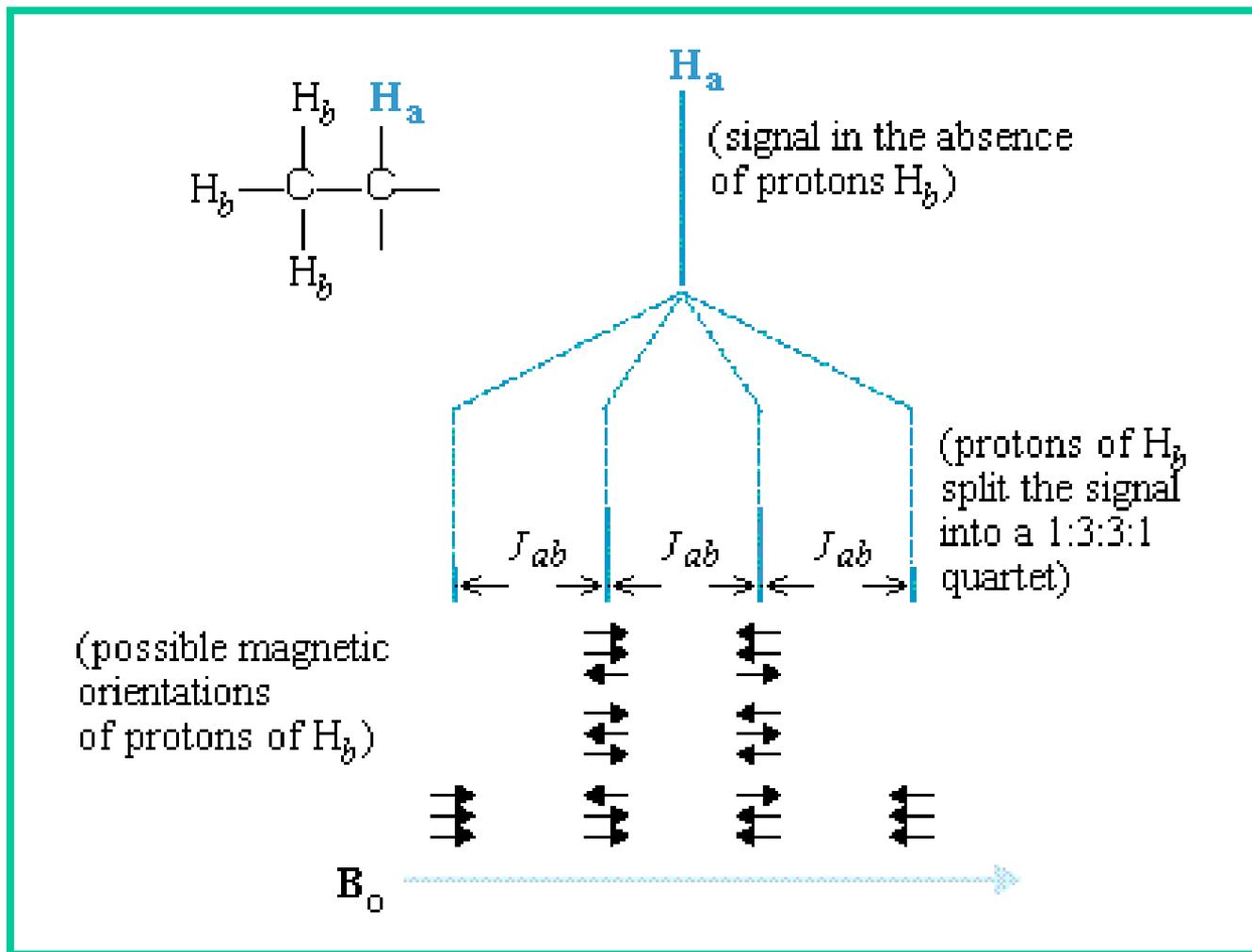


1 Ha acopla com 2 Hb iguais (ainda que em dois carbonos)

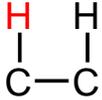
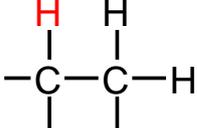
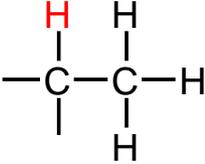
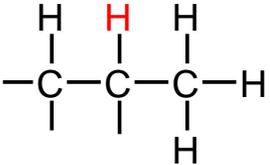
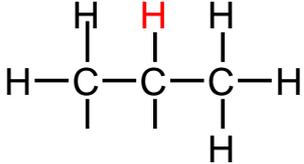
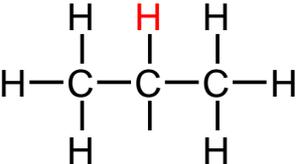
Espectro de RMN de ^1H (300 MHz)
do 1,1,2,3,3-pentacloropropano



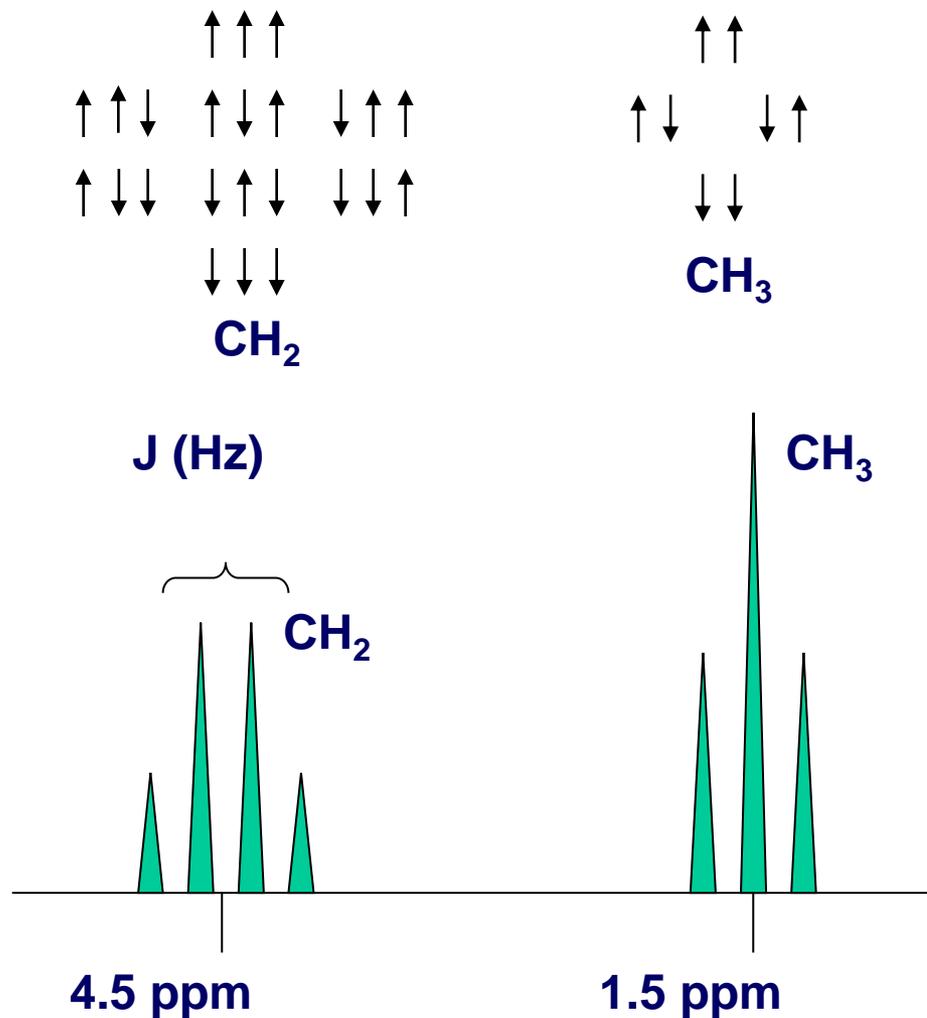
Como se forma um quadrupeto?



Triangulo de Pascal para diferentes multiplicidades

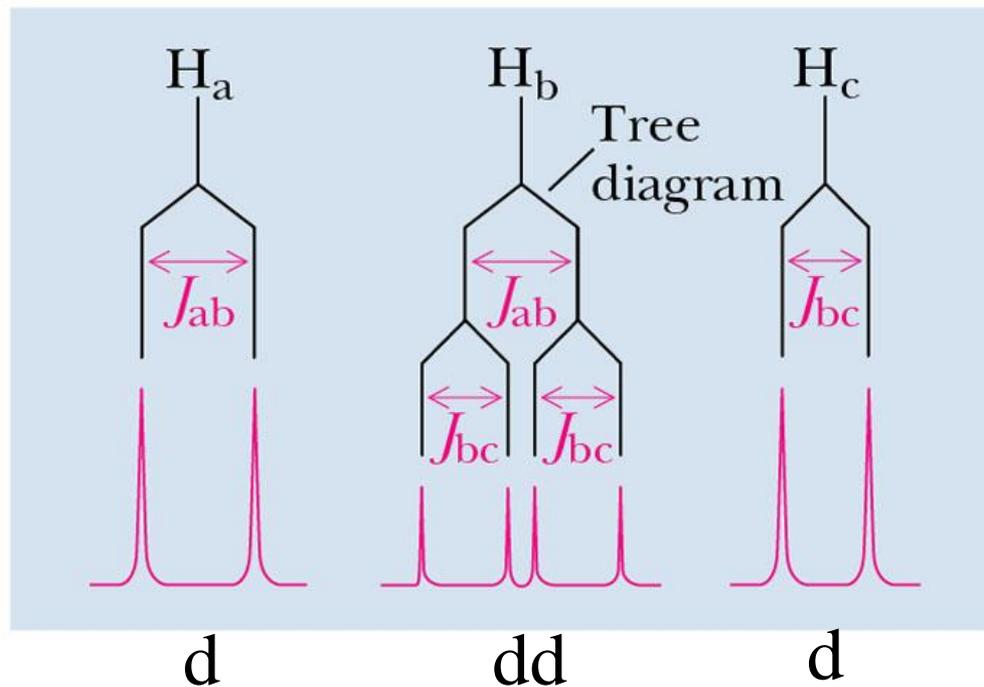
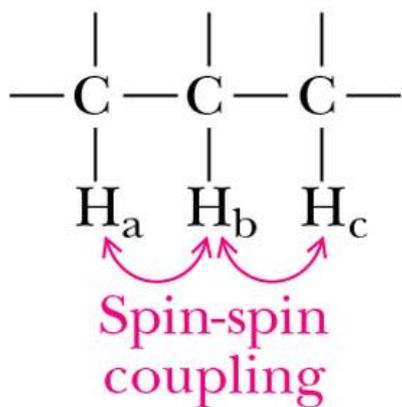
no. of neighbors	relative intensities	pattern	example
0	1	singlet (s)	
1	1 1	doublet (d)	
2	1 2 1	triplet (t)	
3	1 3 3 1	quartet (q)	
4	1 4 6 4 1	pentet	
5	1 5 10 10 5 1	sextet	
6	1 6 15 20 15 6 1	septet	

Como reconhecer uma etoxila (OCH_2CH_3)?



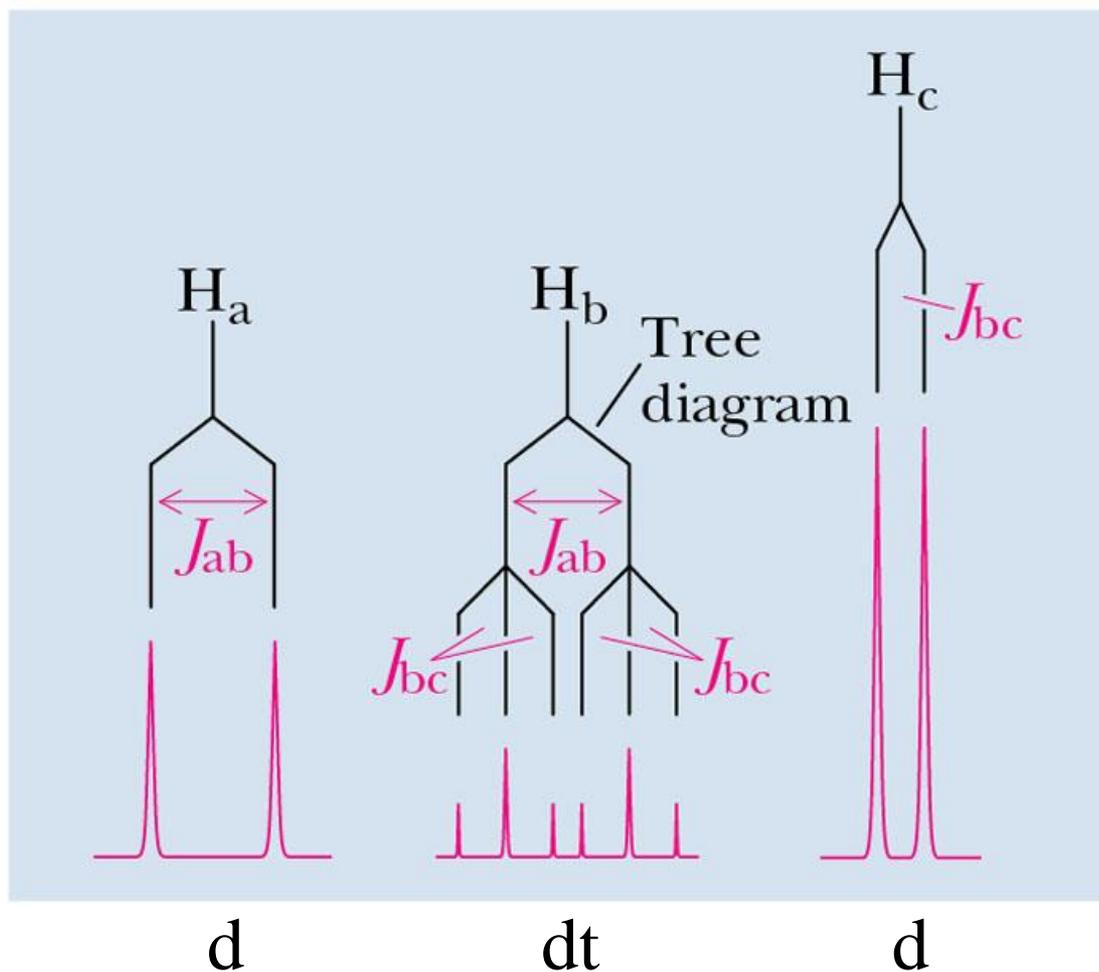
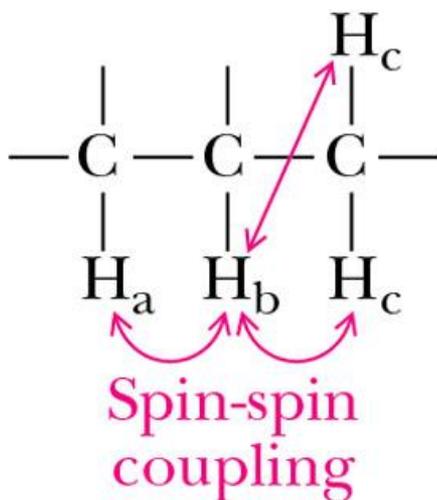
Padrões de acoplamentos com hidrogênios não equivalentes

Um diagrama de árvore mostra que, quando H_b acopla simultaneamente com dois hidrogênios diferentes H_a e H_c, forma-se um duplo dubleto (dd; J_{ab} diferente de J_{bc})



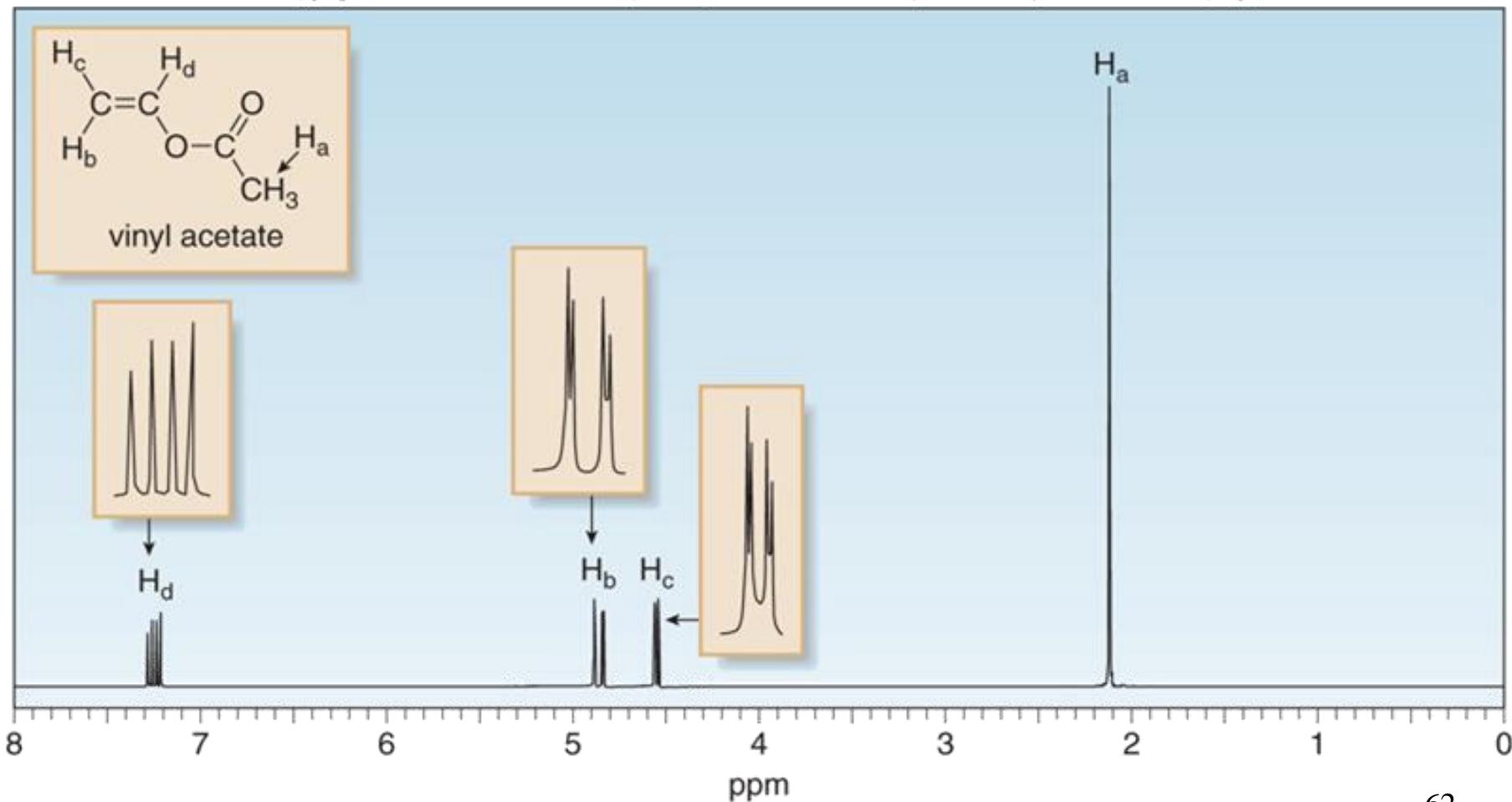
Padrões de acoplamentos com hidrogênios não equivalentes

Se H_c possui 2Hs, o padrão de acoplamento é de um duplo triplete para H_b

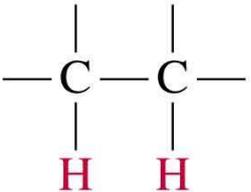
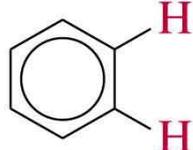
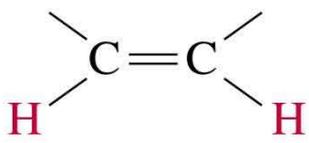
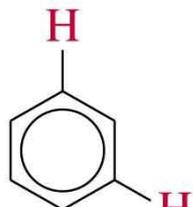
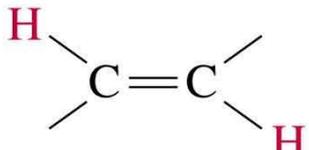
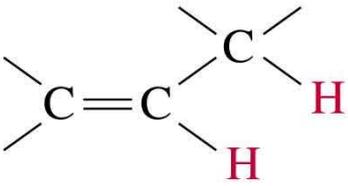
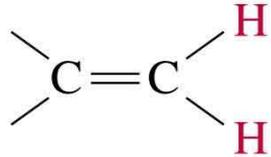
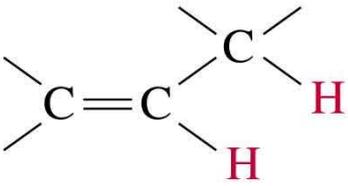


^1H NMR—Spin-Spin Splitting: Non-equivalent coupling

H_a , H_b , H_c and H_d are not equivalent to each other



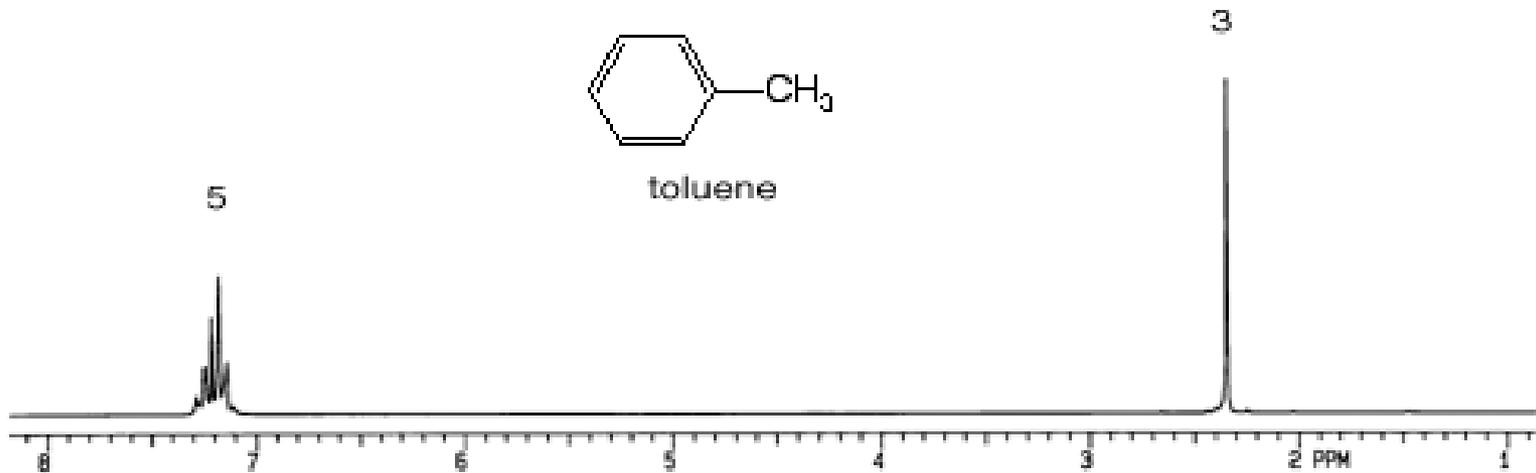
Alguns valores típicos de constantes de acoplamento

		<u>Approx. J</u>			<u>Approx. J</u>
	(free rotation)	7 Hz ^a			8 Hz
	(cis)	10 Hz			2 Hz
	(trans)	15 Hz			6 Hz
	(geminal)	2 Hz			6 Hz
				(ortho)	
				(meta)	
				(allylic)	

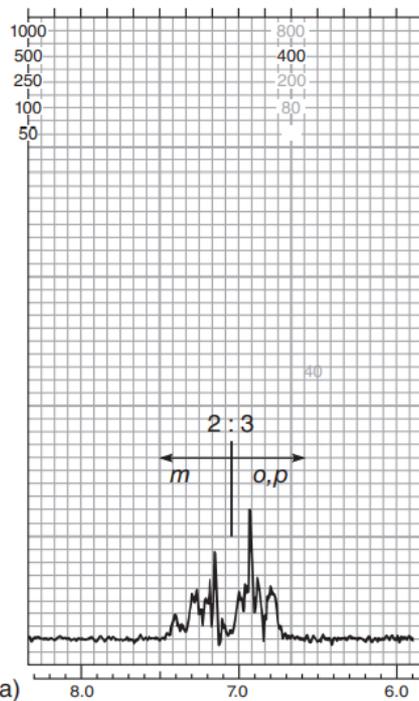
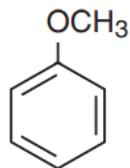
^aThe value of 7 Hz in an alkyl group is averaged for rapid rotation about the carbon-carbon bond. If rotation is hindered by a ring or bulky groups, other splitting constants may be observed.

RMN de ^1H

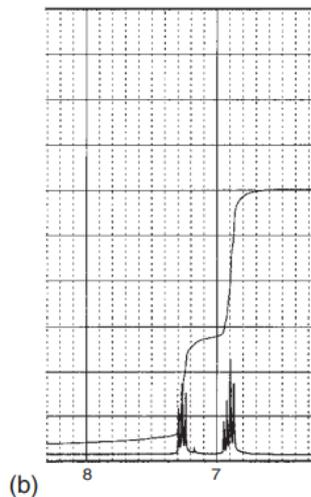
Padrão de substituição em anéis benzênicos



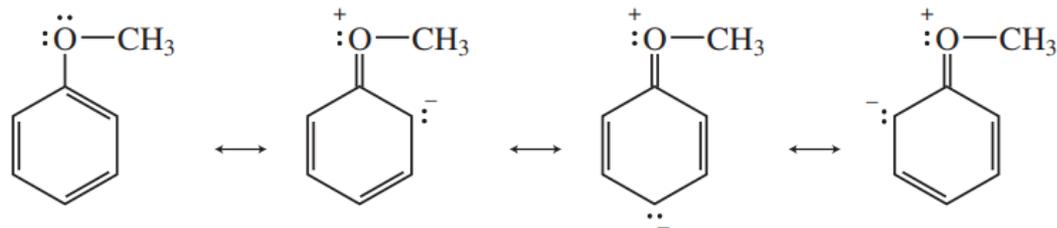
Efeito da metoxila sobre os hidrogênios do anisol no espectro de RMN de ^1H



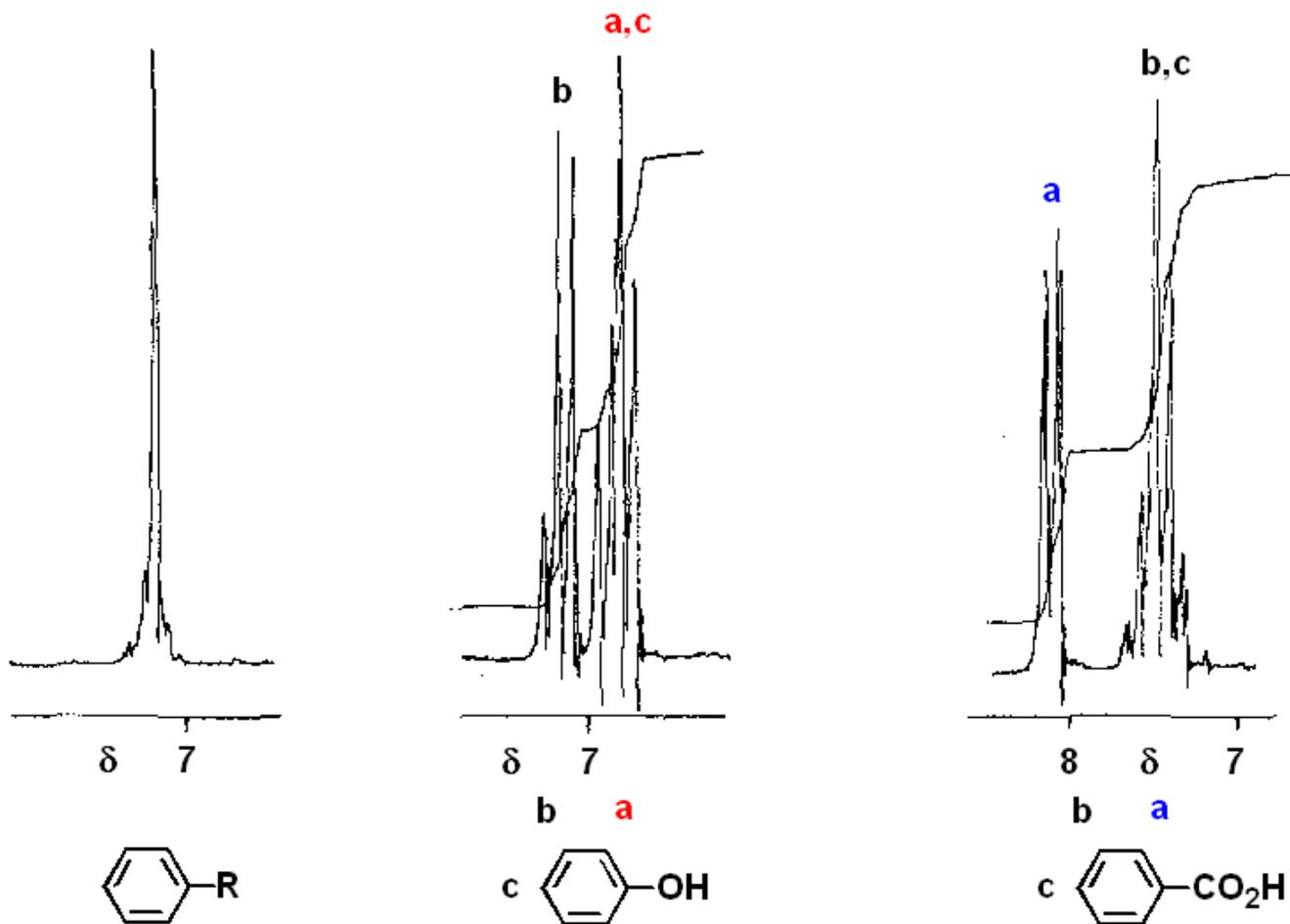
(a) 60 MHz



(b) 300 MHz



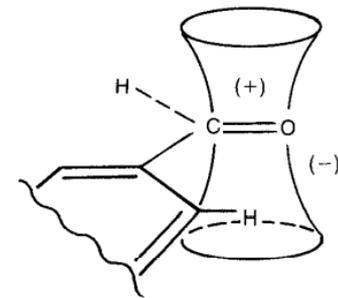
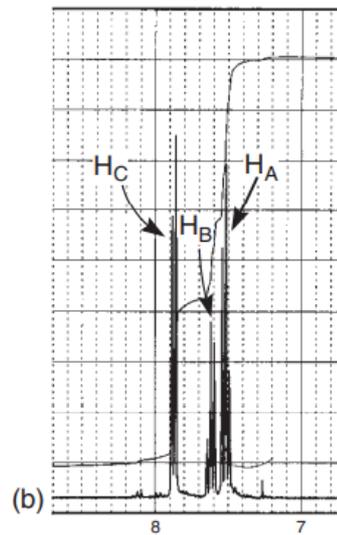
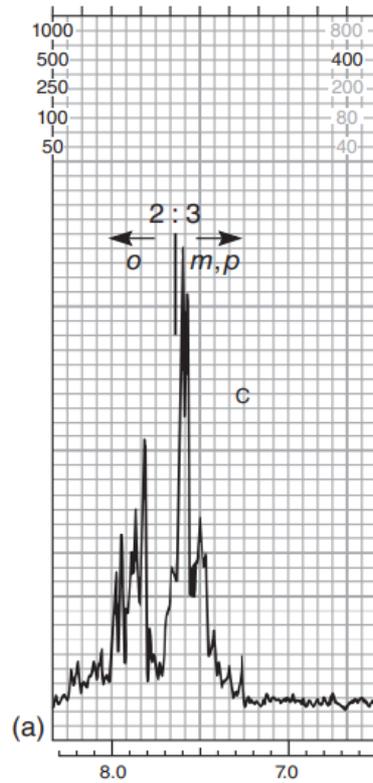
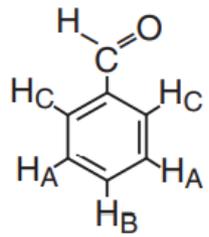
Aneis benzênicos monossustituídos



Proteção dos hidrogênios
orto e *para* do fenol
por ressonância

Desproteção dos
hidrogênios *orto* do
Ácido benzoico
por anisotropia

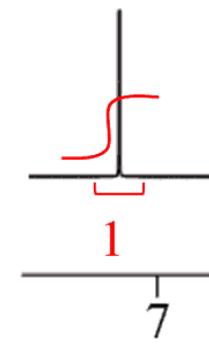
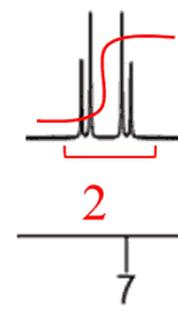
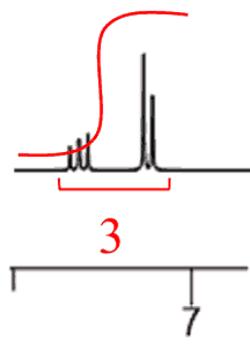
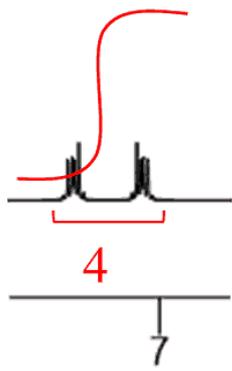
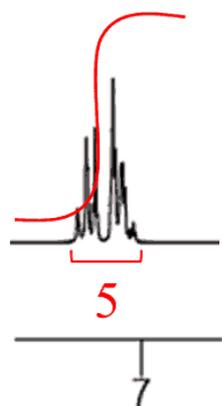
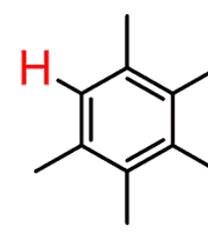
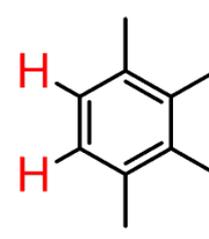
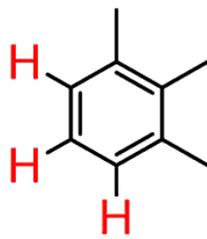
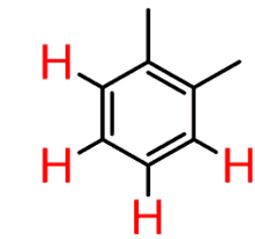
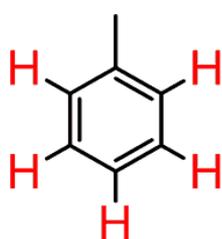
Aneis benzênicos monossustituídos



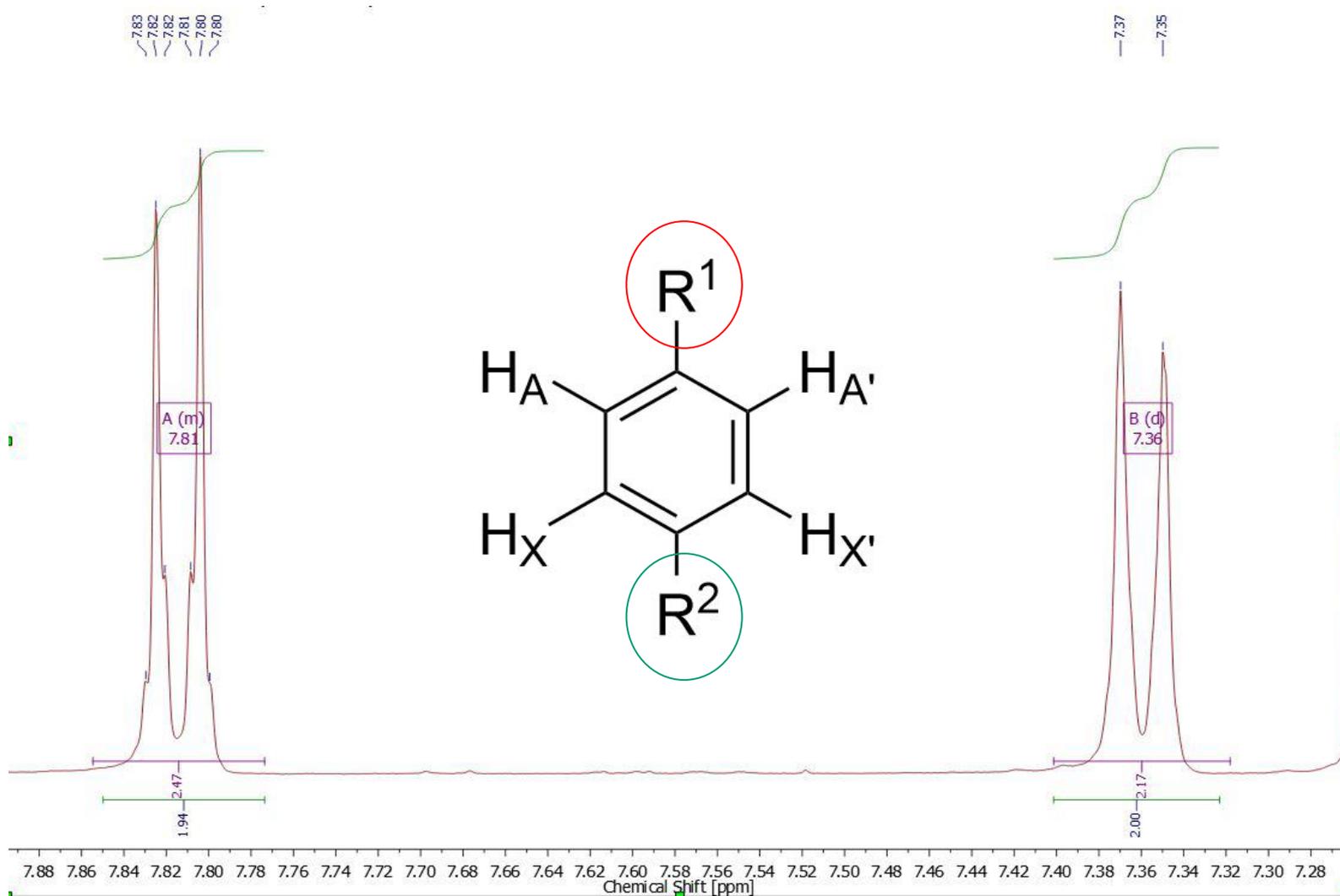
Desproteção
anisotrópica dos
hidrogênios *orto* do
benzaldeído

RMN de ^1H

Padrão de substituição em anéis benzênicos



Aneis benzênicos *p*-dissubstituidos

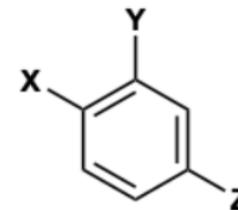
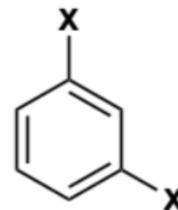
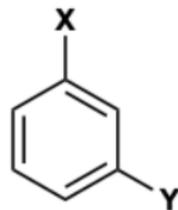
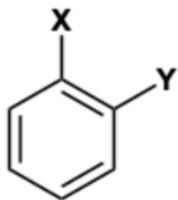
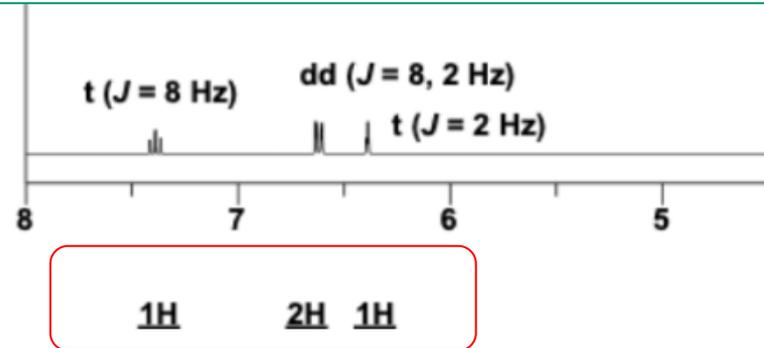
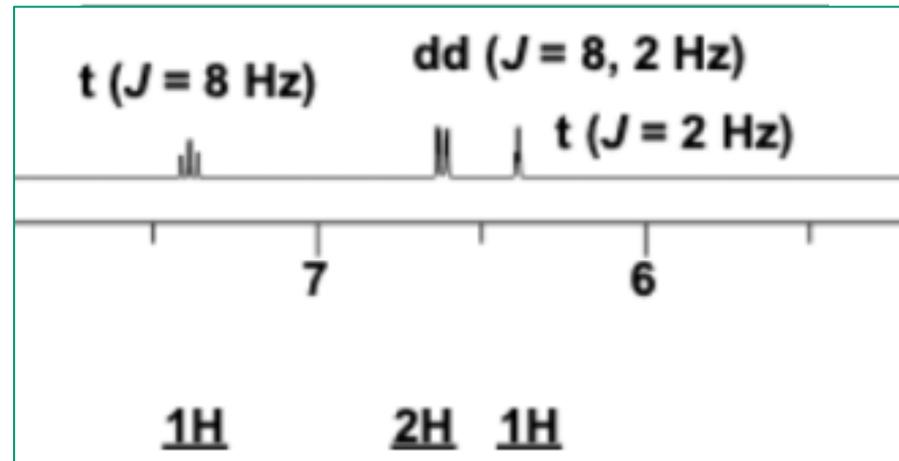


Qual das estruturas é a mais compatível com o espectro apresentado?

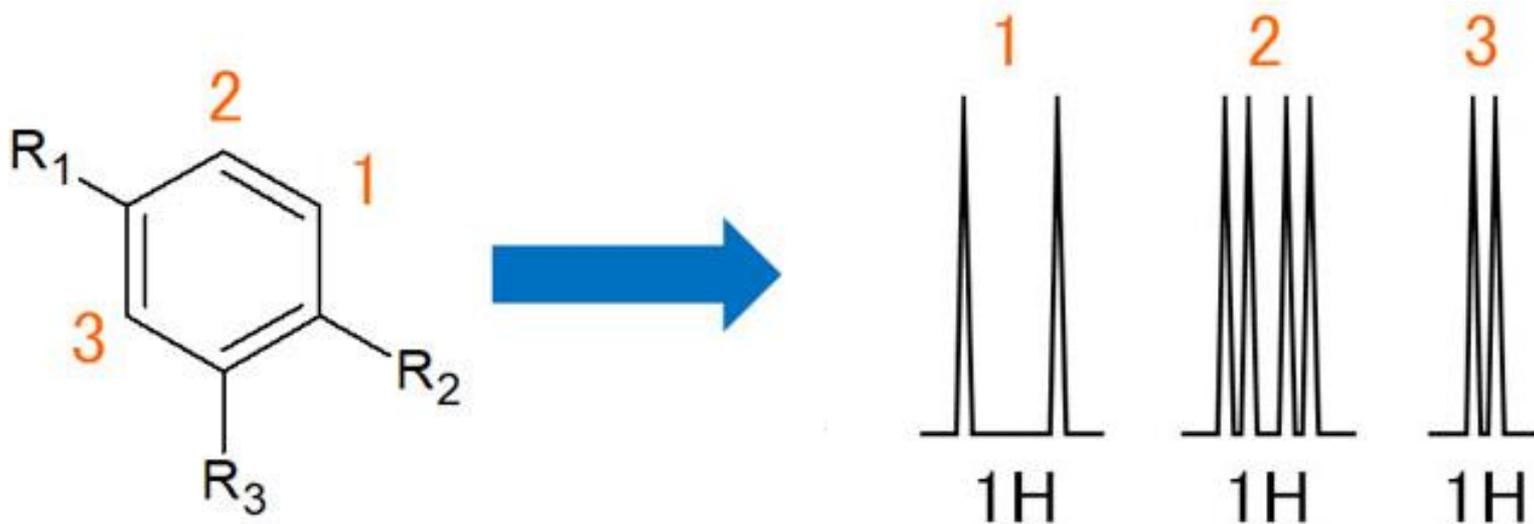
7,40 (t, 8,0 Hz)

6,62 (dd, 8,0, 2,1 Hz)

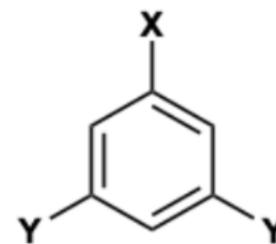
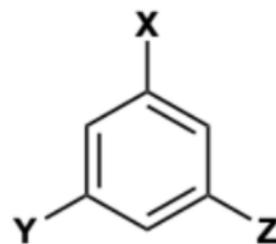
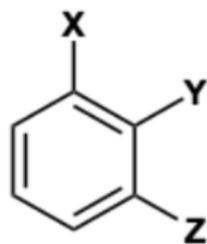
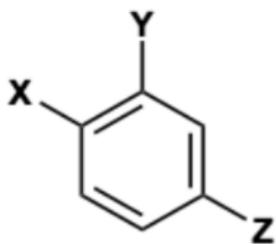
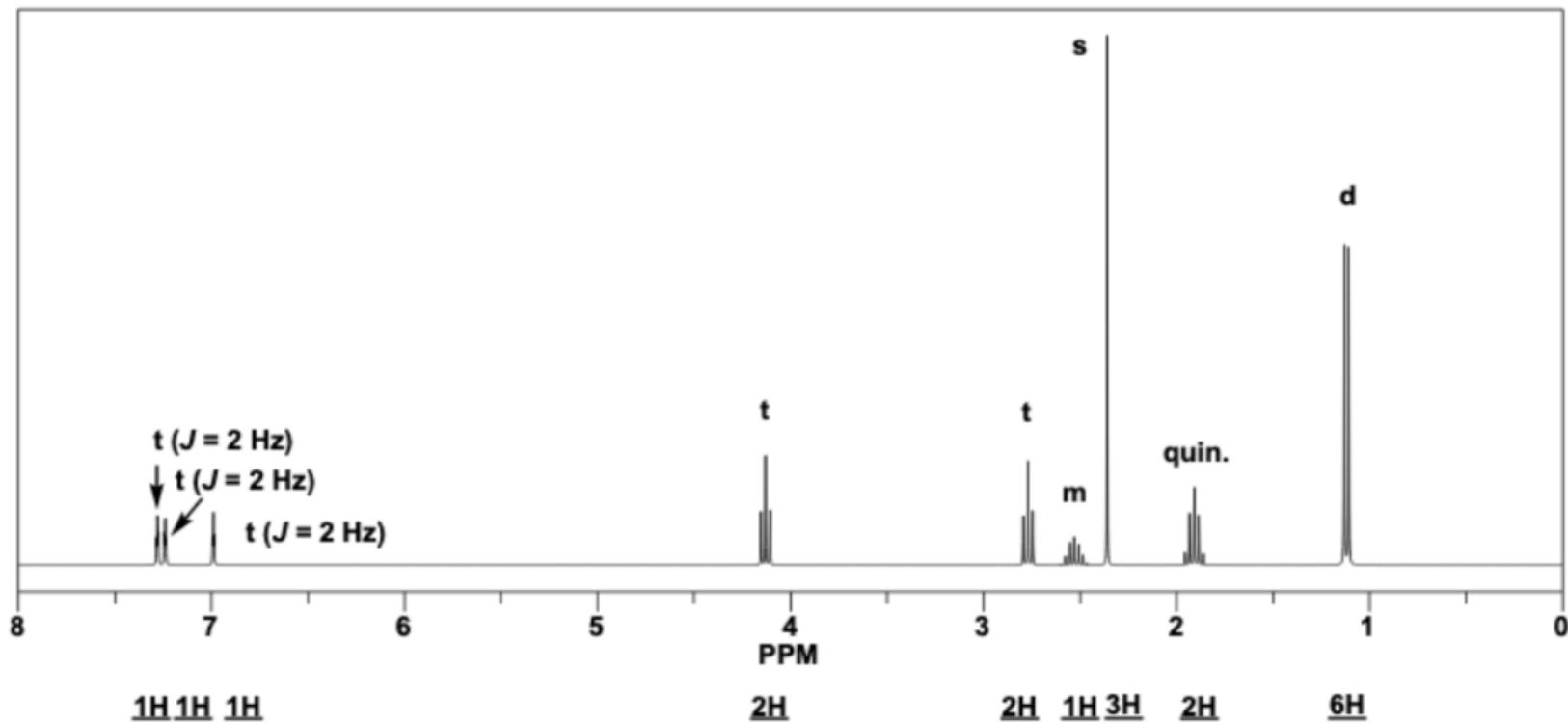
6,40 (t, 2,1 Hz)

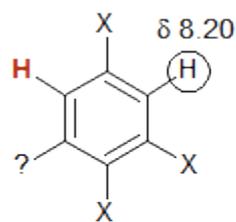


Aneis benzênicos trissubstituídos

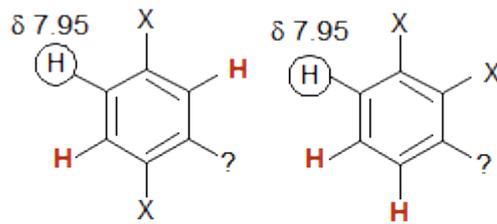


Aneis benzênicos trissubstituidos

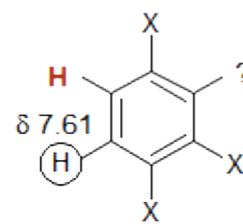


J⁴**A**

J ca 2 Hz
one meta H only

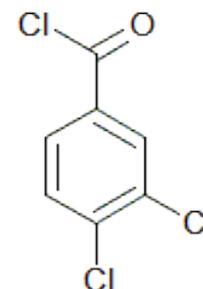
J⁵**B**

J ca 2 and 8 Hz
one ortho and one meta H

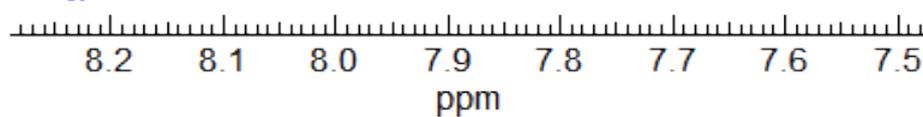
C**J³****D**

$J = 8$ Hz
one ortho H

R-19M C₇H₃Cl₃O
270 MHz ¹H NMR Spectrum (CDCl₃)



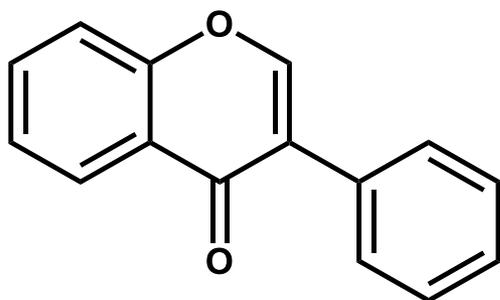
40 30 20 10 0 Hz



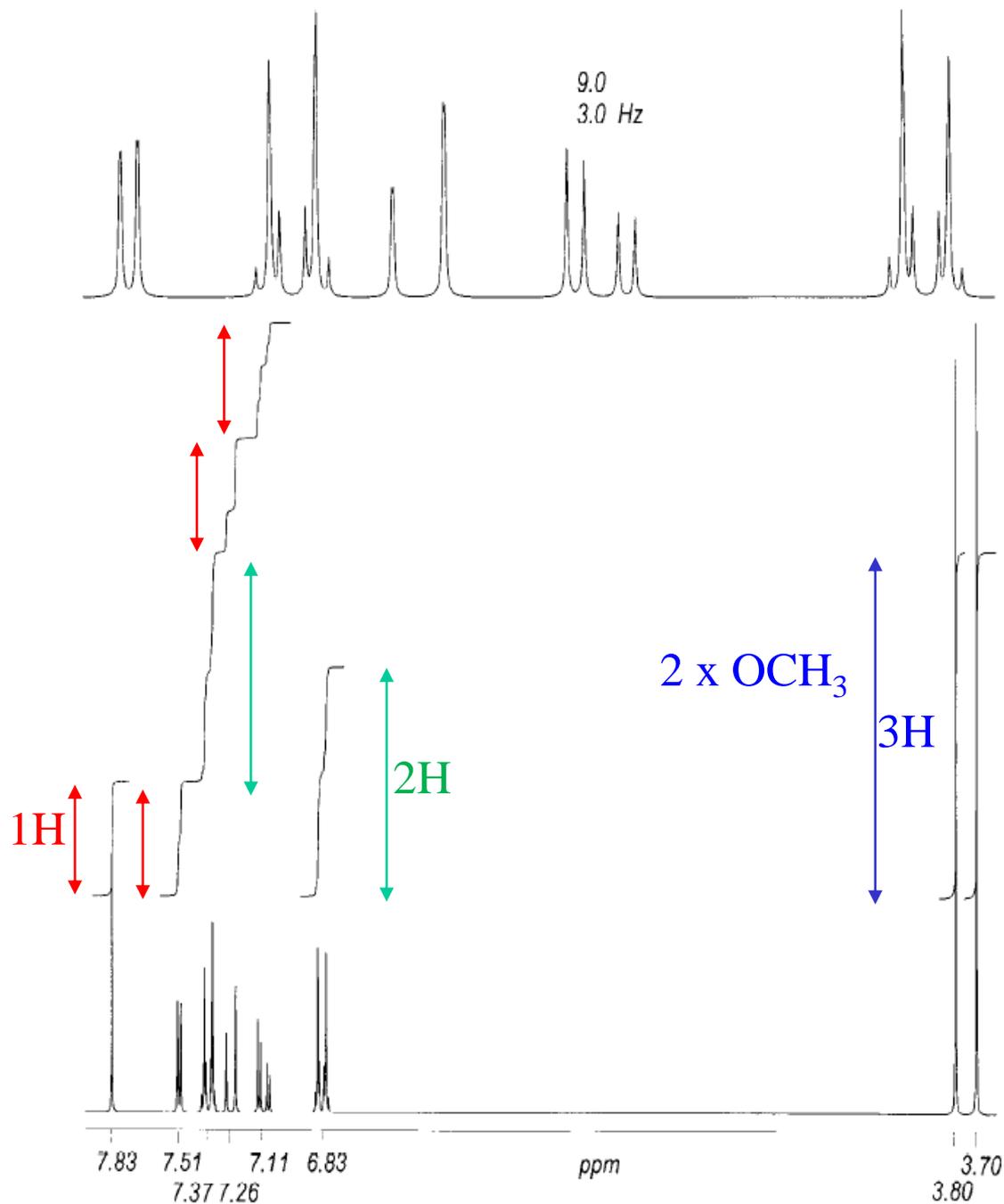
9 8 7 6 5 4 3 2 1 0

ppm

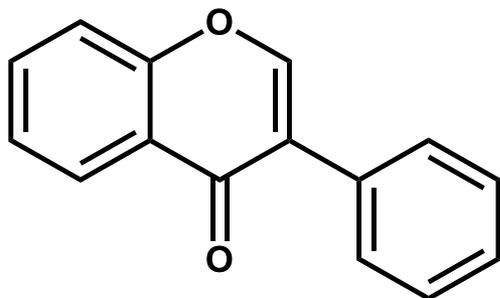
Qual a estrutura da
isoflavona?



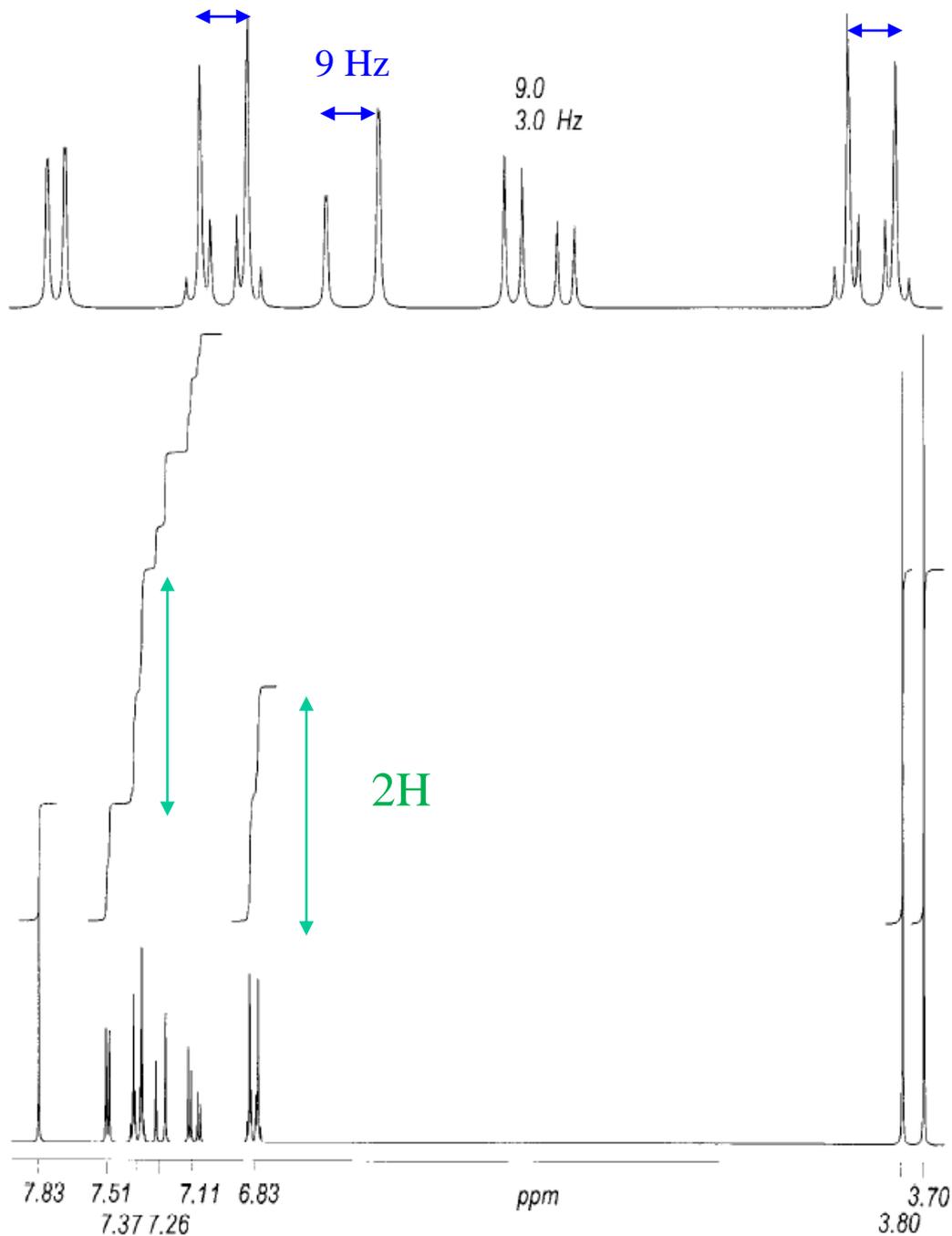
Espectro obtido em $CDCl_3$, 25 °C, 200 MHz.
Os valores de constantes de acoplamento são
dados e Hertz.



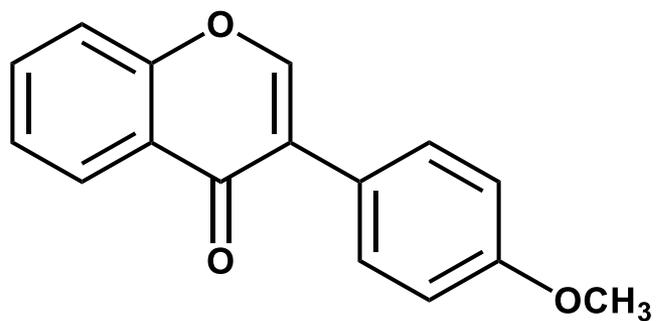
Qual a estrutura da isoflavona?



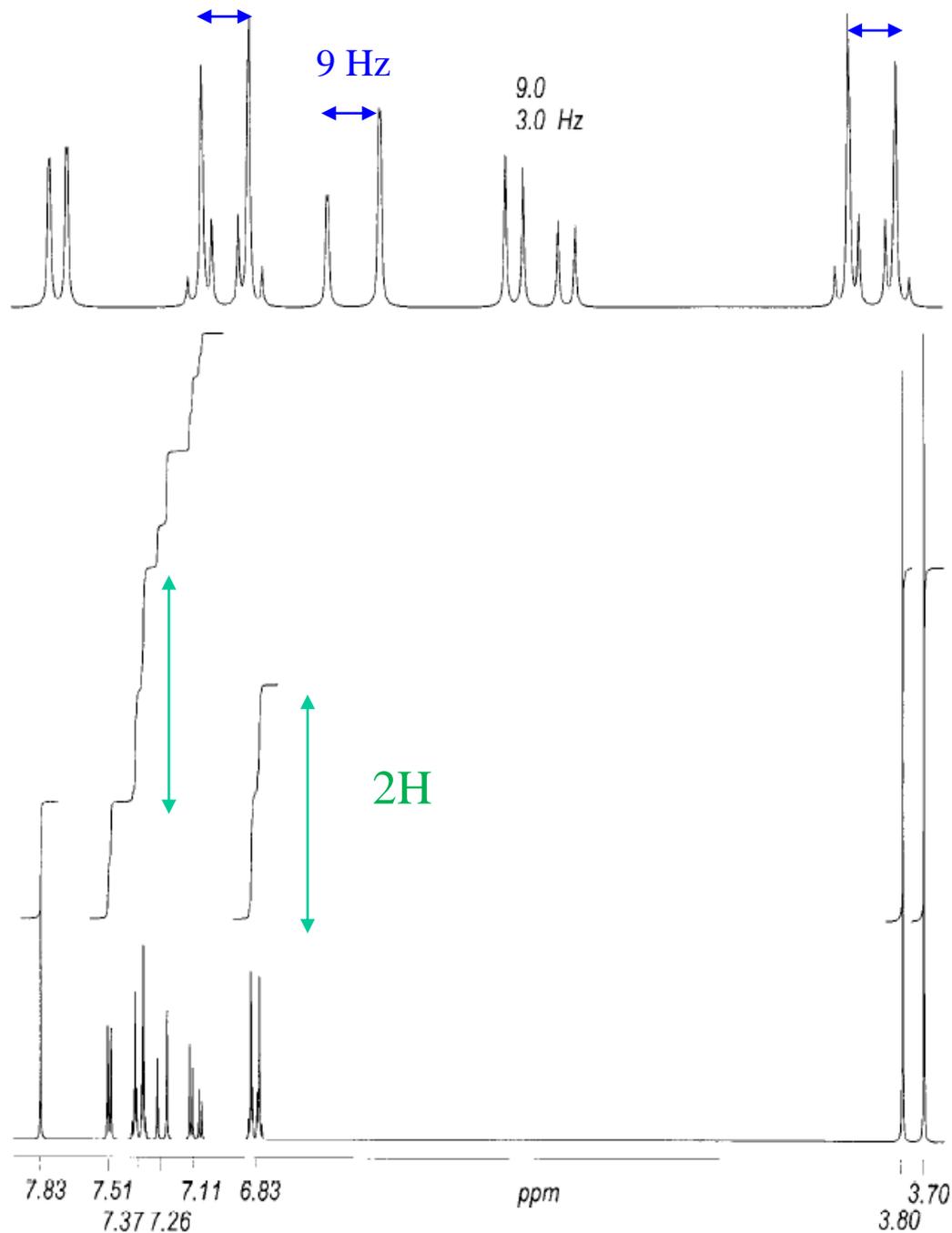
Espectro obtido em $CDCl_3$, 25 °C, 200 MHz.
Os valores de constantes de acoplamento são dados e Hertz.



Qual a estrutura da isoflavona?

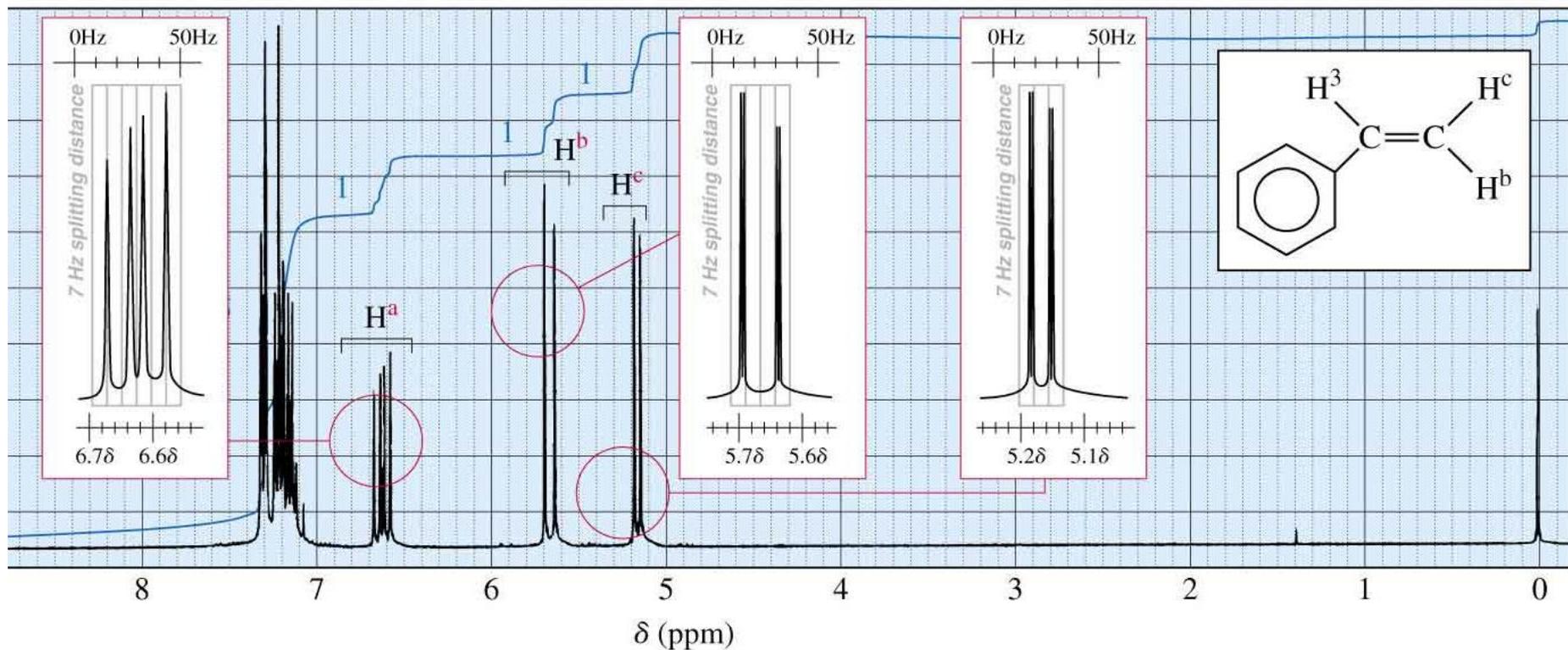


Espectro obtido em CDCl₃, 25 °C, 200 MHz.
Os valores de constantes de acoplamento são dados e Hertz.

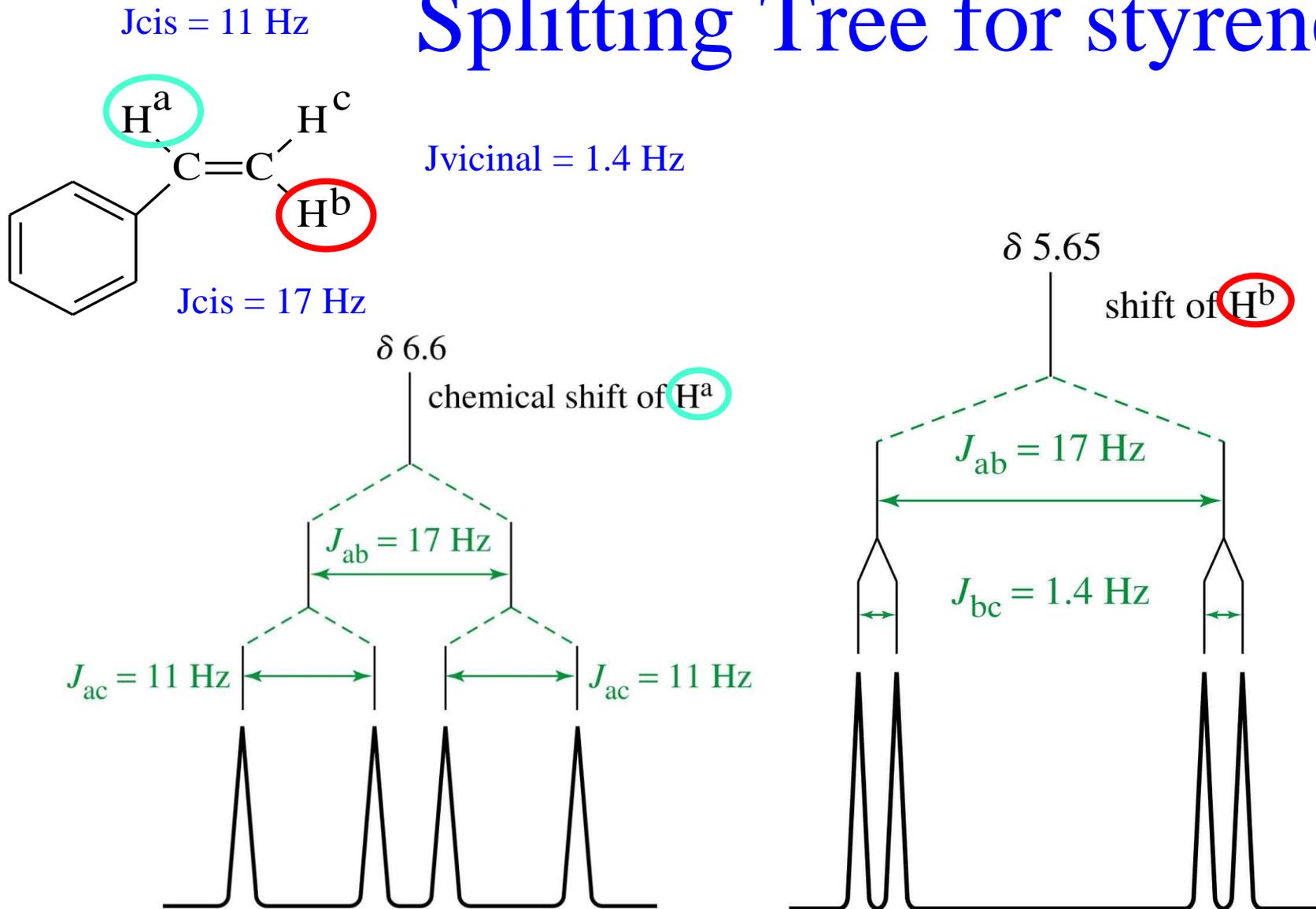


Como seria o padrão de
desdobramento no espectro de
RMN para os hidrogênios
para o estireno?

Espectro de RMN para o estireno

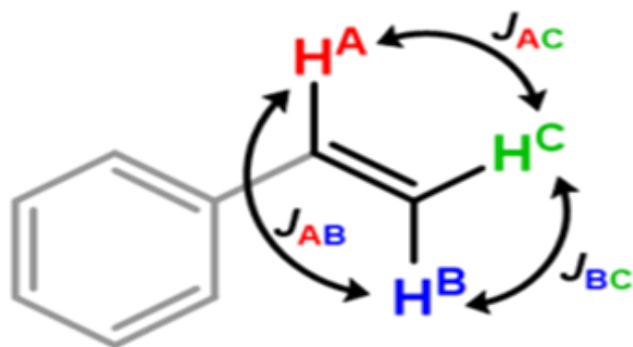


Splitting Tree for styrene



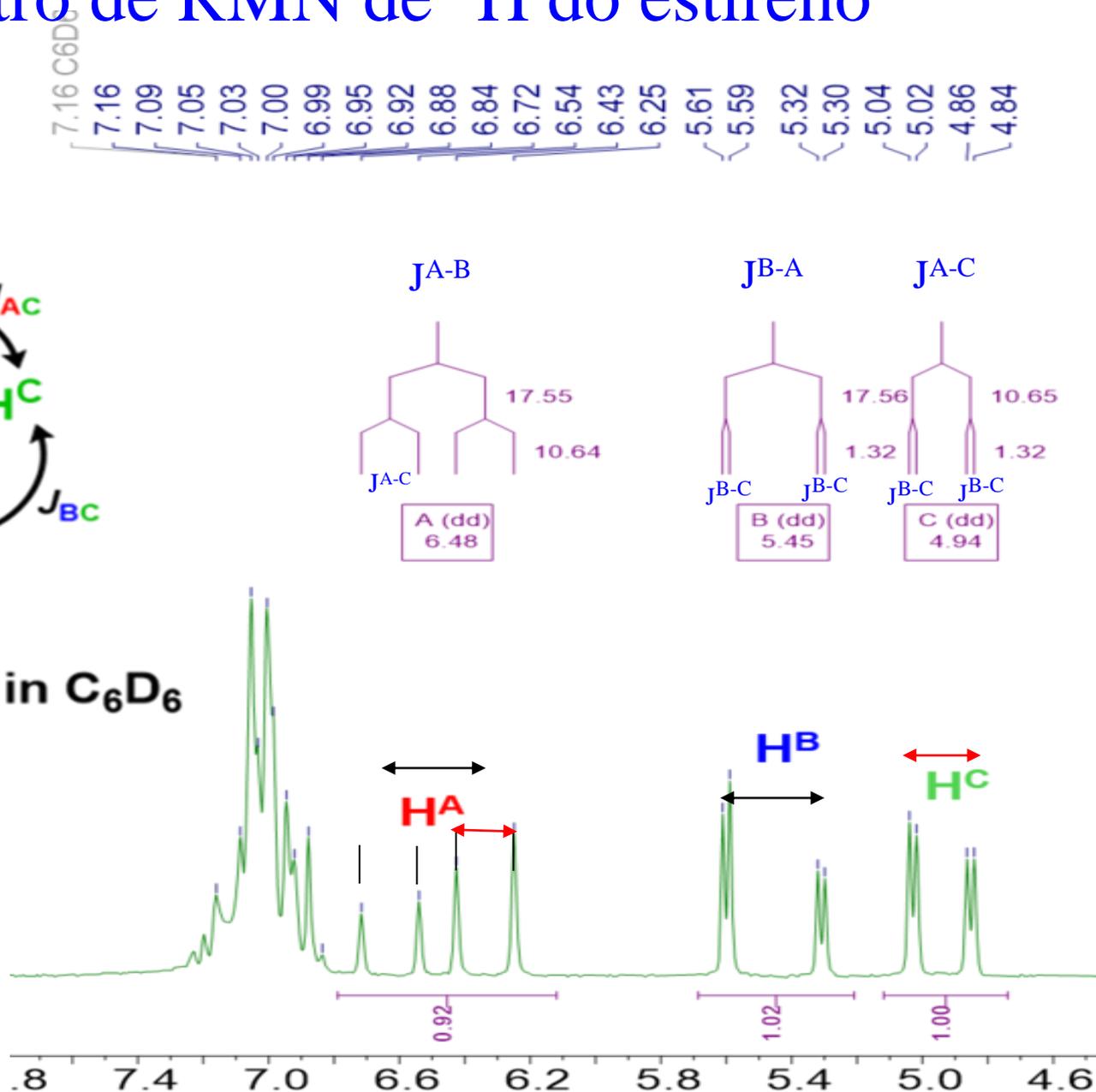
Como seria o sinal para H^c ?

Espectro de RMN de ^1H do estireno

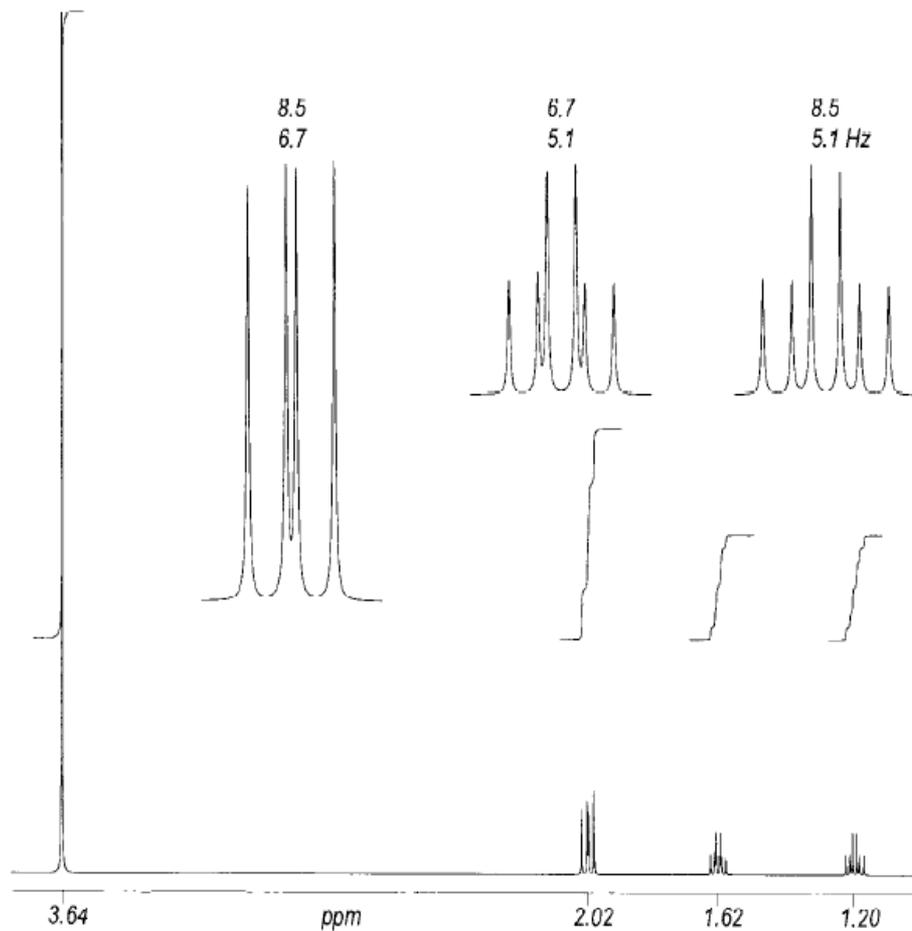


Styrene

^1H (60 MHz) NMR in C_6D_6

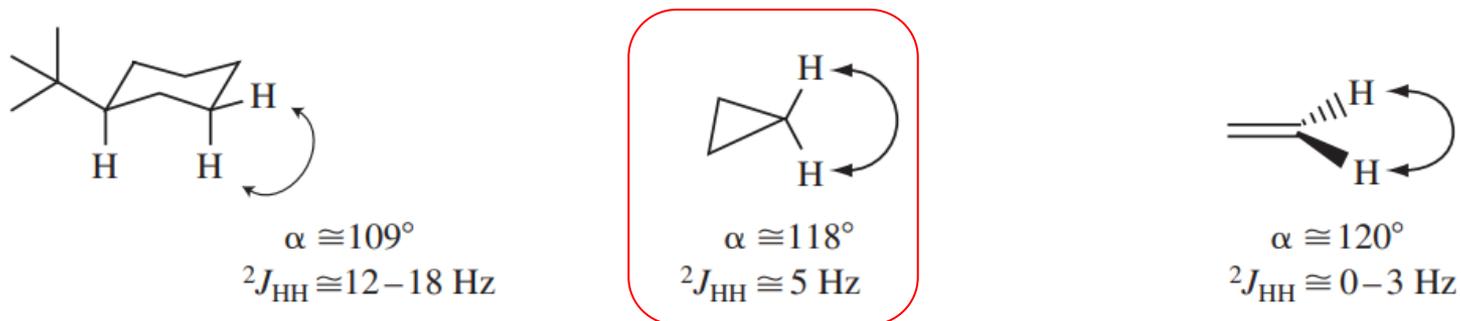


Espectro de RMN de ^1H do dimetilciclopropano dicarboxilato de dimetila

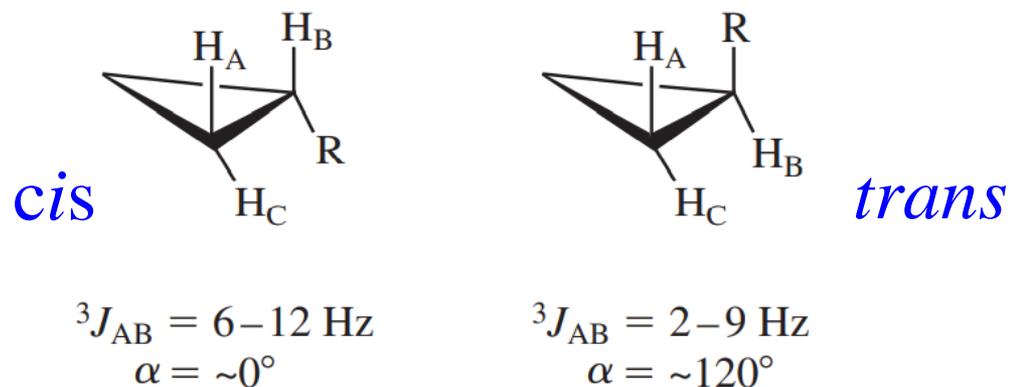


Qual a configuração?
Cis ou trans.

O acoplamento geminal 2J é inversamente proporcional ao ângulo entre os hidrogênios

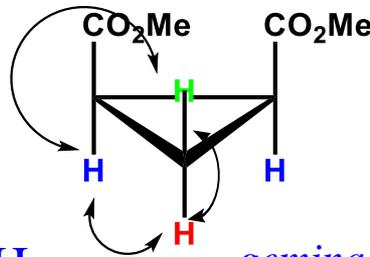


Acoplamento vicinal 3J em ciclopropanos



Ciclopropano dicarboxilato de dimetila

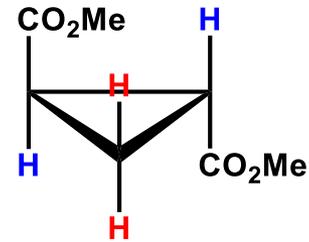
trans : 4 – 8 Hz



cis : 6 – 12 Hz

geminal: 5 – 6 Hz

cis



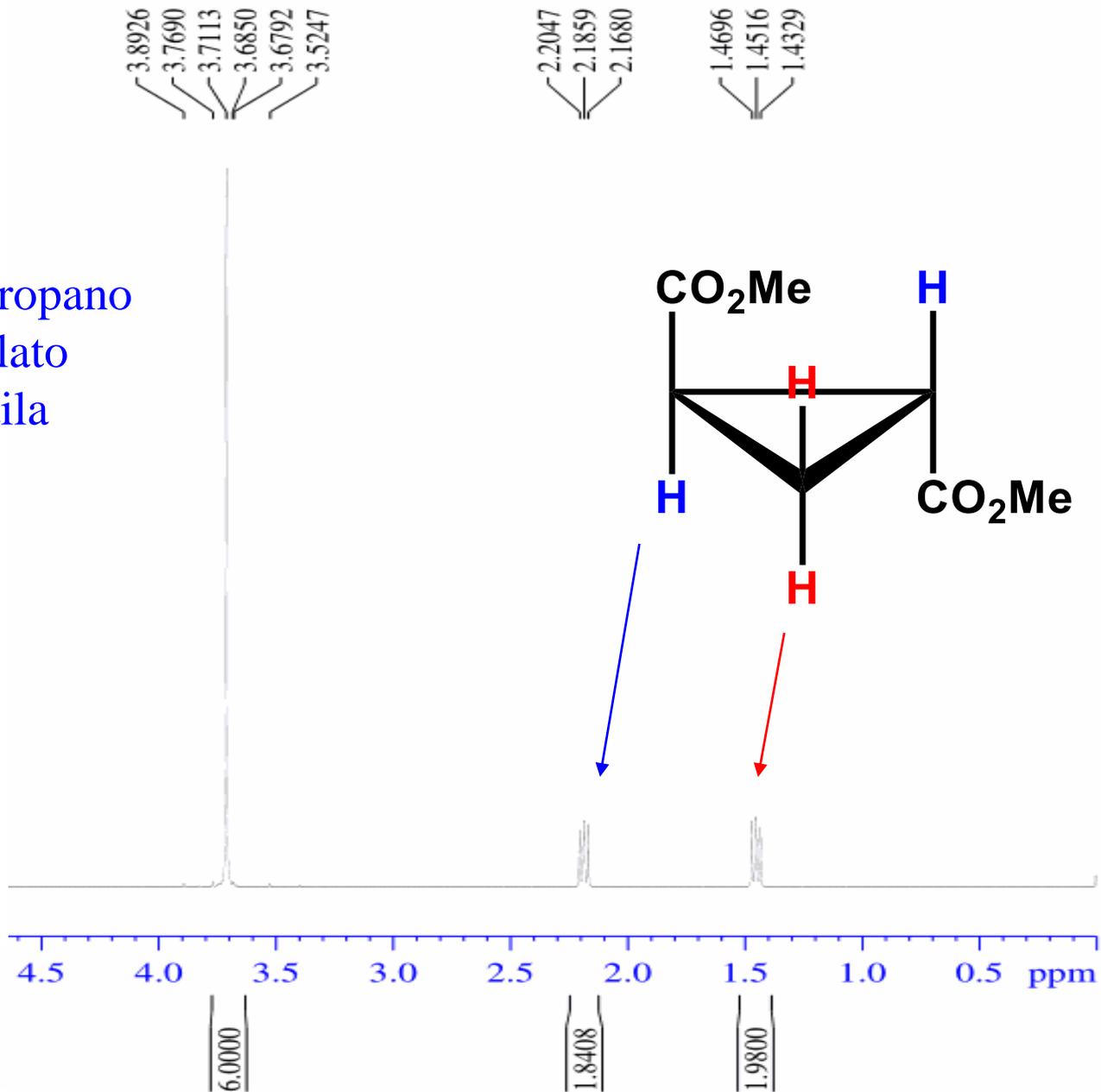
trans

Quantos hidrogênios diferentes temos em cada caso?

Três hidrogênios diferentes
(2H - 1H - 1H)

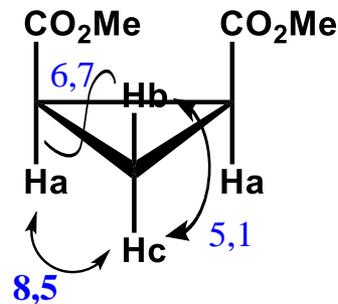
Somente dois hidrogênios
diferentes (2H e 2H)

Trans-ciclopropano
dicarboxilato
de dimetila



cas 826-35-7
CDCl₃ 400 MHz

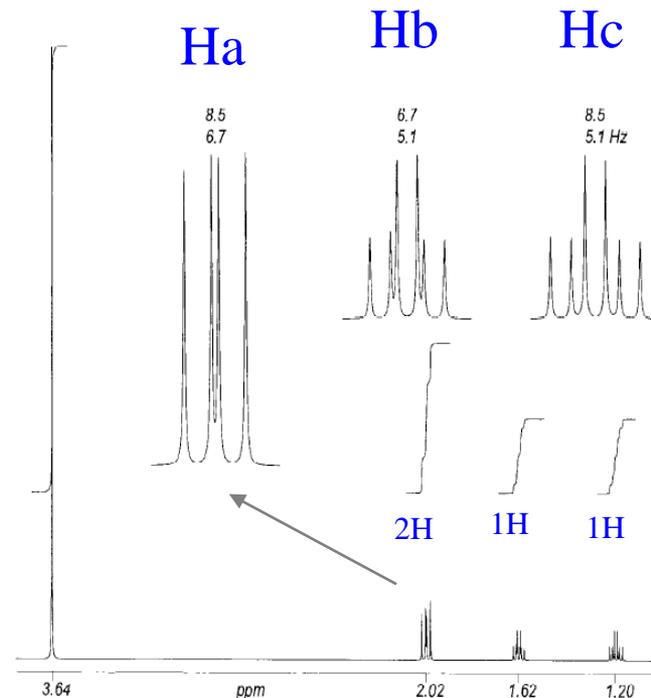
Cis-ciclopropano dicarboxilato de dimetila



Ha (2H): dd 8,5 e 6,7

Hb (1H): td 6,7 e 5,1 Hz

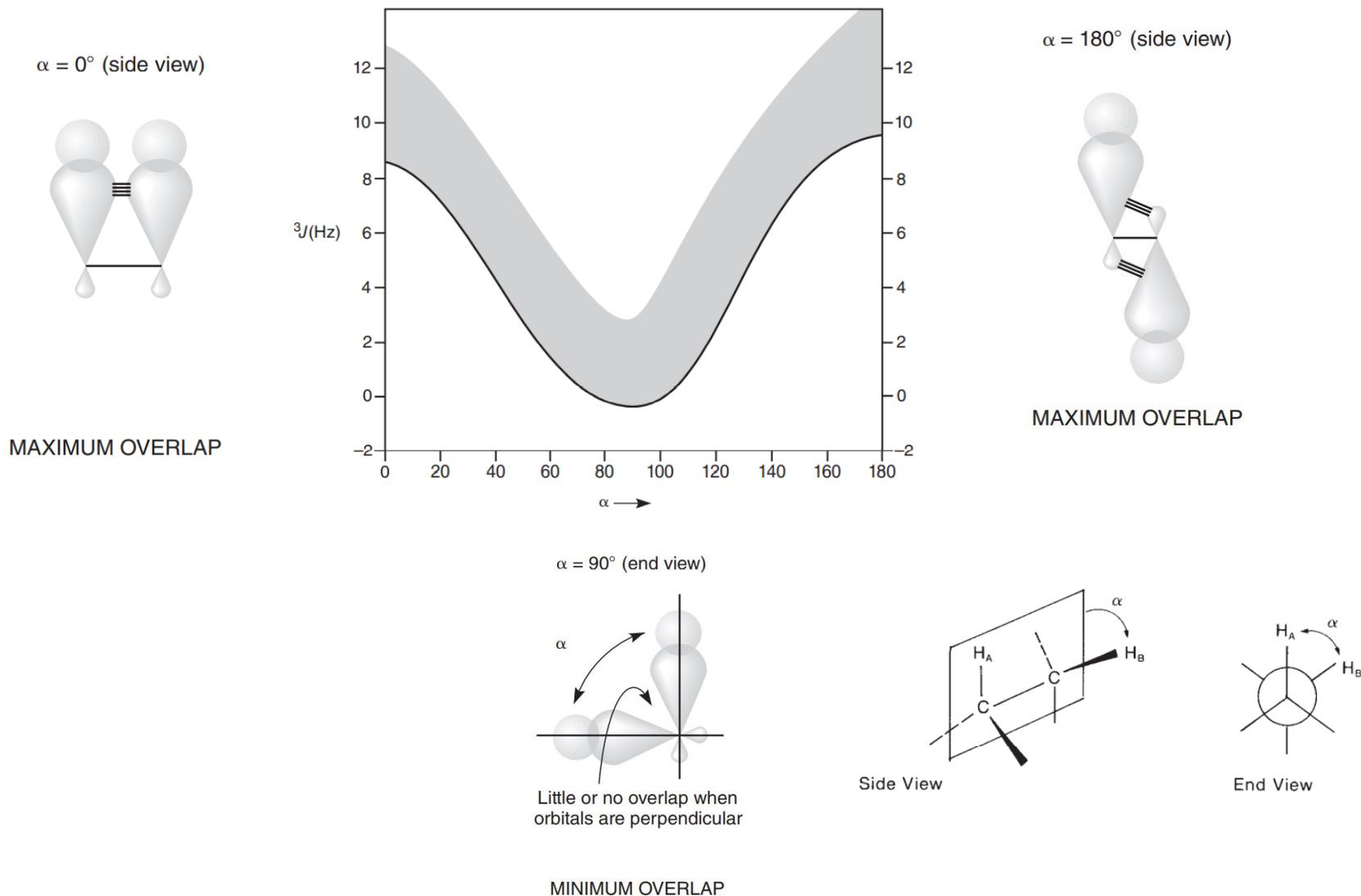
Hc (1H): td 8,5 e 5,1 Hz



Os Hb e Hc são triplo dubletos (td), pois acoplam com dois Ha formando um triplo (6,7 e 8,5 Hz, respectivamente). Esses são desdobrados pelo acoplamento geminal (5,1 Hz).

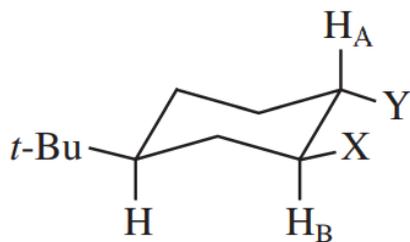
O acoplamento maior é especificado primeiro por isso é um triplo dubleto. Caso contrário, no caso do J do dubleto ser maior, seria um duplo triplo.

Correlação de Karplus—variação da constante De acoplamento 3J com o ângulo diedro α



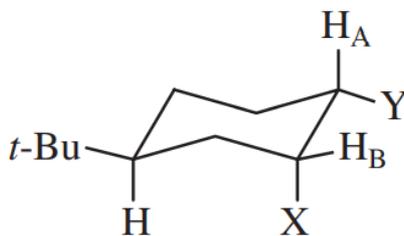
Acoplamentos vicinais 3J em ciclohexanos

a,a



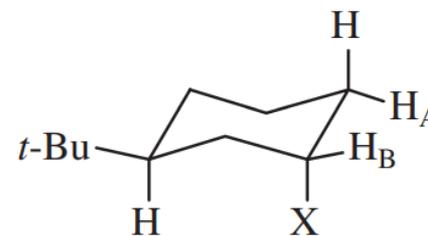
$$J_{AB} = 10-14 \text{ Hz}$$
$$\alpha = 180^\circ$$

a,e



$$J_{AB} = 2-6 \text{ Hz}$$
$$\alpha = 60^\circ$$

e,e



$$J_{AB} = 2-5 \text{ Hz}$$
$$\alpha = 60^\circ$$