PRINSIP DAN STRATEGI ELUSIDASI STRUKTUR 1D NMR
INTRODUCTION

• **Mass Spectrum**: MW (M\(^+\)), Formulae, Fragmentation

• **FT-IR**: Functional group (-OH, -C=O, -COOR, Ar, -C=C-)

• **Spectroscopy NMR**: Chemical Shift information provides a clue about the electronic environment of the nucleus (spin-spin splitting between one nucleus)

• **Identification of species**: genus/species, 

• **Chemical contents**: flavonoid, xanthone, saponin, etc

• **Searching data base** (Constituents), **Chemical shift prediction** (ChemDraw)
Strategy for Structure Elucidation

Pure compound
- MS, NMR
- NMR, IR, UV
- X-RAY

Molecular formula
- NMR, IR

Functional groups
- NMR, IR

Substructures
- NMR

Very secure 3D molecular structure
- Total synthesis

Unsaturation Number (UN)
- Dereplicate by MF

Working 2D structures
- Draw all isomers

List of working 2D structures
- Dereplicate by structure
- NMR, MS, IR, UV

New 2D molecular structure
- NMR
- ORD Molecular modeling

Known molecular structure

Structure Elucidation Strategy using H–H and H–C correlation data

1. Get $^1$H, $^{13}$C NMR spectra; get multiplicities and integrals
2. Get $^{13}$C–$^1$H correlation spectrum (e.g., HMQC)
3. Get one bond $^{13}$C–$^1$H correlations; assign $^1$H resonances to $^{13}$C resonances
4. Get $^1$H–$^1$H correlation data (e.g., COSY)
5. Check assignment of diastereotopic protons using COSY and HMQC
6. Assemble substructures using COSY data
7. Get long range $^{13}$C–$^1$H correlation spectrum (e.g., HMBC)
8. Combine substructures into all possible working structures
9. Check all working structures for consistency with 2D NMR data
10. 2D structure
The NMR strategy and the NMR tools:

- Number of protons, $^1$H chemical shifts, $^1$H-$^1$H couplings --> $^1$H spectrum
- Number of magnetically nonequivalent carbons --> $^{13}$C spectrum
- $^1$H-$^{13}$C direct correlation (one-bond) --> HSCQ, (HETCOR)
- $^1$H coupling network --> COSY
- $^1$H-X (X=$^{13}$C, $^{15}$N, $^{19}$F, $^{31}$P, etc) long-range correlation --> HMBC
- stereochemistry, 3D structure --> NOESY, ROESY
## Introduction to Spectroscopy

Instrumentally aided studies of the interaction between matter (sample being analyzed) and energy (any portion of the electromagnetic spectrum)

<table>
<thead>
<tr>
<th>Method</th>
<th>Abbrev.</th>
<th>Energy used</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet-Visible Spectroscopy</td>
<td>UV-Vis</td>
<td>ultraviolet-visible</td>
<td>nm</td>
</tr>
<tr>
<td>Infrared Spectroscopy</td>
<td>IR</td>
<td>infrared</td>
<td>μm or cm⁻¹</td>
</tr>
<tr>
<td><strong>Nuclear Magnetic Resonance</strong></td>
<td>NMR</td>
<td>radio frequencies</td>
<td>Hz or δ</td>
</tr>
<tr>
<td>Mass Spectroscopy</td>
<td>Mass Spec</td>
<td>electron volts</td>
<td>amu</td>
</tr>
</tbody>
</table>

**Question:** What actually happens to the sample during an analysis? {How do the sample and energy “interact”?}
Matter/Energy Interactions

- What happens when a sample absorbs IR energy?
  - Stretching and bending of bonds (typically covalent bonds)
  - $E_{\text{vibration}}$ increases momentarily

- What happens when a sample absorbs Rf energy (radio frequencies) in an NMR experiment?
  - Nuclei previously aligned in a strong external magnetic field are “flipped” against the field
  - $B_0 =$ external magnetic field

\[
\text{IR} \quad \begin{array}{c}
\text{~3500 cm}^{-1} \\
\text{~100's MHz}
\end{array}
\]
5 mm Varian Probe
(1H-19F)/(31P-15N)PFG
400 MHz NMR Spectrometer

400 MHz Avance System

Unix computer

Electronic controls

Superconducting magnet
400 MHz Superconducting Magnet

- Magnetic field strength: 9.4 Tesla (94,000 gauss)

400 MHz is the frequency used for proton detection in this field.

Keep metal (ferromagnetic) objects, pacemakers, and credit cards several feet away!
NMR Sample Position
(prior to release into probe)

Liquid Helium -269°C (4.2 K)

Liquid Nitrogen -196°C (77.4 K)

Superconducting magnets require continuous cooling.
## Spin Number I of Nucleus and Abundances

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Unpaired Protons</th>
<th>Unpaired Neutrons</th>
<th>Net Spin I</th>
<th>(MHz/T)</th>
<th>Nat. Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1\text{H}$</td>
<td>1</td>
<td>0</td>
<td>$\frac{1}{2}$</td>
<td>42.58</td>
<td>99.98</td>
</tr>
<tr>
<td>$^2\text{H}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6.54</td>
<td>0.016</td>
</tr>
<tr>
<td>$^{31}\text{P}$</td>
<td>0</td>
<td>1</td>
<td>$\frac{1}{2}$</td>
<td>17.25</td>
<td>100</td>
</tr>
<tr>
<td>$^{23}\text{Na}$</td>
<td>2</td>
<td>1</td>
<td>$\frac{3}{2}$</td>
<td>11.27</td>
<td></td>
</tr>
<tr>
<td>$^{14}\text{N}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3.08</td>
<td>99.63</td>
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<tr>
<td>$^{13}\text{C}$</td>
<td>0</td>
<td>1</td>
<td>$\frac{1}{2}$</td>
<td>10.71</td>
<td>1.11</td>
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<tr>
<td>$^{19}\text{F}$</td>
<td>0</td>
<td>1</td>
<td>$\frac{1}{2}$</td>
<td>40.08</td>
<td>100</td>
</tr>
</tbody>
</table>
Spin State Energy Differences vs. Magnetic Field Strength

$\Delta E \approx 200 \text{ MHz for } ^1\text{H}$

$\Delta E \approx 400 \text{ MHz for } ^1\text{H}$

randomly oriented nuclei (no magnetic field)

Energy

Magnetic field strength, $B_0$ (Tesla)

High Field NMR
- increased sensitivity
- increased resolution
Dehydrocorticosterone
THE BASIS OF NMR

The case of the spin half nucleus:

A spinning nucleus has more energy when its magnetic field opposes the applied field.

Nuclei are charged and may have spin which makes them magnetic.

Energy gap in field corresponds to radio frequency.

NO FIELD

MAGNETIC FIELD APPLIED

\[ l_z = -\frac{1}{2} \]

\[ l_z = +\frac{1}{2} \]

Relative Energy

Applied Magnetic Field Strength Increases

Recorder

Sample tube

Magnetic field

South pole

North pole

RF receiver

Sample

RF transmitter
Basic 1D-NMR pulse sequence

- Observed nucleus
- Relaxation time $\phi_t$
- Acquisition time $t_i$

$\phi \leq 90^\circ$

FID → Fourier transform → Spectrum

Time → Frequency

FT

$0.5 \rightarrow 1.0 \rightarrow 1.5 \rightarrow 2.0 \rightarrow 2.5$ (sec)

$2.0 \rightarrow 1.5 \rightarrow 1.0 \rightarrow 0.5$ (ppm)
NMR Signals

- The **number** of signals shows how many different kinds of protons are present.
- The **location** of the signals shows how shielded or deshielded the proton is.
- The **intensity** of the signal shows the number of protons of that type.
- Signal **splitting** shows the number of protons on adjacent atoms.
1. Chemical shift - each nonequivalent hydrogen gives a unique signal along the x-axis.

2. Spin-spin coupling - neighboring NMR active nuclei split each other's signal.

3. Integration - peak areas are proportional to the number of equivalent nuclei giving a signal.

$^1$H-NMR Data: 3 components

Two signals split into multiple peaks having a ratio of areas of 2:3.

"quartet"  "triplet"
Tetramethylsilane

- TMS is added to the sample.
- Since silicon is less electronegative than carbon, TMS protons are highly shielded. Signal defined as zero.
- Organic protons absorb downfield (to the left) of the TMS signal.
Chemical shift

Electrons surrounding a nucleus create a magnetic field ("shield") which affects the size of the magnetic field seen at the nucleus.
Penomena deshielded of electron circulation in double bond and Aromatic
Location of Signals

- More electronegative atoms deshield more and give larger shift values.
- Effect decreases with distance.
- Additional electronegative atoms cause increase in chemical shift.

**TABLE 13-2 Chemical Shifts of the Chloromethanes**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Shift</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-CH-H</td>
<td>80.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8 ppm</td>
</tr>
<tr>
<td>H-CH-Cl</td>
<td>83.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3 ppm</td>
</tr>
<tr>
<td>H-CH-Cl</td>
<td>85.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9 ppm</td>
</tr>
<tr>
<td>H-CH-Cl</td>
<td>87.2</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Each chlorine atom added changes the chemical shift of the remaining methyl protons by about 2 to 3 ppm. These changes are nearly additive.*

=>
## Typical Values

<table>
<thead>
<tr>
<th>Type of Proton</th>
<th>Approximate $\delta$</th>
<th>Type of Proton</th>
<th>Approximate $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkane ($\text{--CH}_3$)</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alkane ($\text{--CH}_2$--)</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alkane ($\text{--CH}$--)</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{O}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C--CH}_3$</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C=CH}$</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{R--CH}_2$--X</td>
<td>3–4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>($X = \text{halogen, O}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C=C--H}$</td>
<td>5–6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C=C}$</td>
<td></td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>$\text{Ph--H}$</td>
<td></td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td>$\text{Ph--CH}_3$</td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>$\text{R--CHO}$</td>
<td></td>
<td></td>
<td>9–10</td>
</tr>
<tr>
<td>$\text{R--COOH}$</td>
<td></td>
<td></td>
<td>10–12</td>
</tr>
<tr>
<td>$\text{R--OH}$</td>
<td></td>
<td></td>
<td>variable, about 2–5</td>
</tr>
<tr>
<td>$\text{Ar--OH}$</td>
<td></td>
<td></td>
<td>variable, about 4–7</td>
</tr>
<tr>
<td>$\text{R--NH}_2$</td>
<td></td>
<td></td>
<td>variable, about 1.5–4</td>
</tr>
</tbody>
</table>

*Note:* These values are approximate, as all chemical shifts are affected by neighboring substituents. The numbers given here assume that alkyl groups are the only other substituents present. A more complete table of chemical shifts appears in Appendix 1.
O-H and N-H Signals

- Chemical shift depends on concentration.
- Hydrogen bonding in concentrated solutions deshield the protons, so signal is around $\delta$ 3.5 for N-H and $\delta$ 4.5 for O-H.
- Proton exchanges between the molecules broaden the peak.
The Chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard.

$$\delta = (\nu - \nu_{\text{ref}}) \times 10^6 / \nu_{\text{ref}}$$

Chemical Shifts value for $^1$H-NMR Spectrum

- Electronegative substituents are “deshielding” and shift NMR signals of nearby hydrogens to higher δ values.
- Ring currents associated with π-systems are “deshielding” and shift NMR signals of nearby H’s to higher δ values.
- H’s attached to O or N have highly variable chemical shifts which are concentration, solvent, and temperature sensitive.

Source: Professor P. R. Young, University of Illinois at Chicago
Interpretation of $^1$H-NMR Data

**Multiplisitas H-H:**
the splitting pattern expected (if all coupling constants are equal) varies as described by Pascal's triangle:

Spin-spin coupling (splitting) - neighboring NMR active nuclei split each other.

- nonequivalent nuclei three bonds (or less) apart mutually interact with each other causing their NMR signals to be split into multiple peaks.

$n = \# \text{ of neighbors} \quad n + 1 = \# \text{ of peaks in signal}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>$n + 1$</th>
<th>pattern</th>
<th>peak ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>singlet (s)</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>doublet (d)</td>
<td>1 : 1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>triplet (t)</td>
<td>1 : 2 : 1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>quartet (q)</td>
<td>1 : 3 : 3 : 1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>pentet (p)</td>
<td>1 : 4 : 6 : 4 : 1</td>
</tr>
<tr>
<td>many</td>
<td>many + 1</td>
<td>multiplet (m)</td>
<td></td>
</tr>
</tbody>
</table>

$3J_{HH} = \text{vicinal coupling (Hz)}$
Common Splitting Patterns

Singlet (s)  doublet (d)  triplet (t)  quartet (q)  pentet (p)
1:1        1:2:1       1:3:3:1    1:4:6:4:1

Ethyl $\text{CH}_3\text{CH}_2-$

Ethyl signature: quartet / triplet with integration ratio of 2 / 3

Isopropyl $\text{CH}_3\text{CHCH}_3$

septet / doublet & 1 / 6 ratio
Exercise

\[ C_4H_6O_3 \]

\[ C_6H_8O_4 \]

\[ C_6H_{10}O_4 \]

\[ \text{CH}_3\text{COCCCH}_3 \]
\[ \text{CH}_3\text{OCOCOCH}_3 \]
\[ \text{CH}_3\text{OCCH=CHCOCH}_3 \]
\[ \text{CH}_3\text{OCCH}_2\text{CH}_2\text{COCH}_3 \]
\[ \text{CH}_3\text{OCOCOCCH}_3 \]

\[ \delta, \text{ ppm} \]

\[ \delta, \text{ ppm} \]

\[ \delta, \text{ ppm} \]
Enantiotopic Distinctions

- If H’s are in environments that are mirror images of each other, they are **enantiotopic**
- Replacement of each H with X produces a set of enantiomers
- The H’s have the same NMR signal (in the absence of chiral materials)

The two hydrogens on C2 (and the two hydrogens on C3) are **enantiotopic** and have the same NMR absorption.

The two possible replacement products are enantiomers.
Diastereotopic Distinctions

- In a chiral molecule, paired hydrogens can have different environments and different shifts.
- Replacement of a pro-$R$ hydrogen with $X$ gives a different diastereomer than replacement of the pro-$S$ hydrogen.
- **Diastereotopic hydrogens** are distinct chemically and spectroscopically.

The two hydrogens on C3 are diastereotopic and have different NMR absorptions. The two possible replacement products are diastereomers.
Equivalent H’s

- Two H’s that are in identical environments (homotopic) have the same NMR signal
- Test by replacing each with X
  - if they give the identical result, they are equivalent

The 6 CH₃ hydrogens are homotopic and have the same NMR absorption.

Only one possible replacement product
**Coupling Constants**

- **Coupling constant (J):** the distance between peaks in an NMR multiplet, expressed in hertz
  - J is a quantitative measure of the magnetic interaction of nuclei whose spins are coupled.

```
<table>
<thead>
<tr>
<th>Coupling</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HaHb</td>
<td>6-8 Hz</td>
</tr>
<tr>
<td>Hb</td>
<td>8-14 Hz</td>
</tr>
<tr>
<td>Ha</td>
<td>0-5 Hz</td>
</tr>
</tbody>
</table>
```

```
<table>
<thead>
<tr>
<th>Coupling</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=H</td>
<td>11-18 Hz</td>
</tr>
<tr>
<td>Hb</td>
<td>5-10 Hz</td>
</tr>
<tr>
<td>C=H</td>
<td>0-5 Hz</td>
</tr>
</tbody>
</table>
```
Coupling Constants ($J$)

- 7.5 Hz (Ortho)
- 1.5 Hz (metha)
- 0.8 Hz (para)

- Multiplicities?
- t(ortho)
- d(meta)
- d(tfet)
Sucrose in D$_2$O
$^1$H Spectrum
Varian Unity-300

HOD

d, J = 3.7

d, J = 8.8

t, J = 8.5

9.5

t
10.0, 3.8

9.3

dd

CH$_3$OH

Figure 1.15
\[ \text{H}_1' \quad J = 3.6 \text{ Hz (}\alpha\text{)} \]

\[ \text{H}_1 \quad J = 3.6 \text{ Hz (}\alpha\text{)} \]

\[ \text{1}^\text{H} \text{ Spectrum} \]

Anomeric Region

ppm

1.000

1.087

HOD
## Chemical Shifts Value of Solvents

<table>
<thead>
<tr>
<th>Solvent (deuterated)</th>
<th>$^1$H NMR Chemical Shift</th>
<th>$^{13}$C NMR Chemical Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>11.65 (1), 2.04 (5)</td>
<td>179.0 (1), 20.0 (7)</td>
</tr>
<tr>
<td>Acetone</td>
<td>2.05 (5)</td>
<td>206.7 (1), 29.9 (7)</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.94 (5)</td>
<td>118.7 (1), 13.9 (7)</td>
</tr>
<tr>
<td>Benzene</td>
<td>7.16 (1)</td>
<td>128.4 (3)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>7.26 (1)</td>
<td>77.2 (3)</td>
</tr>
<tr>
<td>DMSO</td>
<td>2.50 (5)</td>
<td>39.5 (7)</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.87 (1), 3.31 (5)</td>
<td>49.1 (7)</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>5.32 (3)</td>
<td>54.00 (5)</td>
</tr>
<tr>
<td>Pyridine</td>
<td>8.74 (1), 7.58 (1), 7.22 (1)</td>
<td>150.3 (1), 135.9 (3), 123.9 (5)</td>
</tr>
<tr>
<td>Water (D$_2$O)</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>

Multiplicities: 1 (singlet), 2 (doublet), 3 (triplet), et al
**Chemical Shift of $\text{H}_2\text{O}$ (or HOD)**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>$\delta_{\text{H}_2\text{O}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>2.8</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>2.1</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.4</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.6</td>
</tr>
<tr>
<td>Dimethyl Sulfoxide</td>
<td>3.3</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.8</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>1.5</td>
</tr>
<tr>
<td>Pyridine</td>
<td>4.9</td>
</tr>
<tr>
<td>Water (D$_2$O)</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Integration only gives information on the relative number of different hydrogens, not the absolute number.

$^1$H spectrum of Taxol
**Garcinia nervosa**: dulxanthone G

**1H NMR Spectral Data**

- **B**: 6.74 (d, J = 10 Hz)
- **A**: 5.58 (d, J = 10 Hz)
- **D**: 1.52 (s, 6H)
- **R1**: 6.38 (s)
- **R1**: 13.36 (s)
- **2 x -CH₃**: 4.087
- **4 x OCH₃**: 3.863
- **J = 10 Hz**
Gambar IV. 2. Spektrum $^1$H-NMR senyawa porxanthone A (1) dalam CDCl$_3$
Oleanolic acid

Catechin

b-Amyrin

Stigmasterol

Quercetin : \( R = H \)
Querctrin : \( R = \text{Glc} \)
<table>
<thead>
<tr>
<th>PPM</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.275</td>
<td></td>
</tr>
<tr>
<td>2.393</td>
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</tr>
<tr>
<td>2.270</td>
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<td>1.972</td>
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</tr>
<tr>
<td>1.773</td>
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</tr>
<tr>
<td>1.748</td>
<td></td>
</tr>
<tr>
<td>1.565</td>
<td></td>
</tr>
<tr>
<td>1.557</td>
<td></td>
</tr>
<tr>
<td>1.551</td>
<td></td>
</tr>
<tr>
<td>1.508</td>
<td></td>
</tr>
<tr>
<td>1.488</td>
<td></td>
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<tr>
<td>1.397</td>
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<td>1.352</td>
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<td>1.295</td>
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<td>1.186</td>
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<td>1.055</td>
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<td>1.011</td>
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<td>1.008</td>
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<td>0.961</td>
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<tr>
<td>0.893</td>
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<tr>
<td>0.879</td>
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<td>0.876</td>
<td></td>
</tr>
<tr>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

**Fridelin**

**Stigmasterol**
Differences in $^{13}$C Technique

- Resonance frequency is $\sim$ one-fourth, 15.1 MHz instead of 60 MHz.
- Peak areas are not proportional to number of carbons.
- Carbon atoms with more hydrogens absorb more strongly.
Spin-Spin Splitting

- It is unlikely that a $^{13}$C would be adjacent to another $^{13}$C, so splitting by carbon is negligible.
- $^{13}$C will magnetically couple with attached protons and adjacent protons.
- These complex splitting patterns are difficult to interpret.
Proton Spin Decoupling

- To simplify the spectrum, protons are continuously irradiated with “noise,” so they are rapidly flipping.
- The carbon nuclei see an average of all the possible proton spin states.
- Thus, each different kind of carbon gives a single, unsplit peak.
Off-Resonance Decoupling

- $^{13}$C nuclei are split only by the protons attached directly to them.
- The $N + 1$ rule applies: a carbon with $N$ number of protons gives a signal with $N + 1$ peaks.

$=>$
Interpreting $^{13}\text{C}$ NMR

- The number of different signals indicates the number of different kinds of carbon.
- The location (chemical shift) indicates the type of functional group.
- The peak area indicates the numbers of carbons (if integrated).
- The splitting pattern of off-resonance decoupled spectrum indicates the number of protons attached to the carbon. =>
Two $^{13}$C NMR Spectra

$^{13}$C NMR

\[ \text{O} \]
\[ \text{CH}_3 - \overset{\text{C}}{\text{C}} - \text{CH}_2 - \text{CH}_3 \]

2-butanone

\[ \delta (\text{ppm}) \]

TMS

=⇒

Chapter 13
Hydrogen and Carbon Chemical Shifts

- COOH
- δ11-δ12

O
-C=H

X

C=H

X = O, hal

O
-C=H

10 9 8 7 6 5 4 3 2 1 0

200 150 100 50 0

aromatic C

C≡C

C—Cl, C—Br

C=O

C=C

C=O

alkanes
Uses of $^{13}$C NMR Spectroscopy

- 1-Methylcyclohexene has five $sp^3$ resonances ($\delta$ 20-50) and two $sp^2$ resonances $\delta$ 100-150 (see Figure 13-11)

- Signal to noise ratio much better with multiple scans!
Combined $^{13}\text{C}$ and $^1\text{H}$ Spectra
Chemical Shift for $^{13}$C-NMR Spectrum
$^{13}\text{C}$ spectrum of Taxol

Experimental time: 14h
CH Multiplicities (DEPT dan APT)

DEPT (Distortionless Enhancement by Polarization Transfer)

APT (Attached Proton Test).

DEPT spectra shown in the figure below are, from top to bottom:

- DEPT-135: CH and CH3 peaks up, CH2 peaks inverted
- DEPT-90: CH peaks only
- DEPT-45: all protonated carbons
- Normal 13C spectrum
2. The COSY 90 pulse sequence (COSY stand for: CORrelated Spectroscopy).
3. The COSY 45 pulse sequence.
4. The long range coupling; COSY.
5. The relayed COSY or one homonuclear step relayed COSY.
6. COSY experiment with a double quantum filter: (DQF COSY).
7. The NOESY sequence (NOESY stand for: Nuclear Overhauser Enhancement Spectroscopy).
8. Phase sensitive COSY and NOESY.
9. TOCSY and ROESY.
1. The XHCORR sequence.

2. The COLOC sequence (COrelation via LOng range Coupling).

3. Th
HSQC - Heteronuclear Single Quantum Coherence

(correlates X-$^1$H chemical shifts via one-bond $^1$J(XH) couplings)
Gradient HSQC spectrum of Taxol

nt=1 per increment
256 complex points in F1
exp. time=15 min
Gradient HSQC spectrum of Taxol (expansion #2)
### Results from the HSQC experiment:

<table>
<thead>
<tr>
<th>Proton-Carbon Pairs</th>
<th>Quaternary Carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.06 (2) 130.21 (d*)</td>
<td>203.61 (s)</td>
</tr>
<tr>
<td>7.67 (2) 127.04 (d*)</td>
<td>172.72 (s)</td>
</tr>
<tr>
<td>7.54 (1) 133.71 (d)</td>
<td>171.22 (s)</td>
</tr>
<tr>
<td>7.44 (2) 128.70 (d*)</td>
<td>170.35 (s)</td>
</tr>
<tr>
<td>7.42 (1) 131.97 (d)</td>
<td>167.04 (s)</td>
</tr>
<tr>
<td>7.41 (2) 127.04 (d*)</td>
<td>167.01 (s)</td>
</tr>
<tr>
<td>7.35 (2) 129.03 (d*)</td>
<td>141.95 (s)</td>
</tr>
<tr>
<td>7.33 (2) 128.72 (d*)</td>
<td>137.99 (s)</td>
</tr>
<tr>
<td>7.28 (1) 128.37 (d)</td>
<td>133.65 (s)</td>
</tr>
<tr>
<td>6.91 (1)</td>
<td>133.23 (s)</td>
</tr>
<tr>
<td>6.21 (1) 75.57 (d)</td>
<td>129.18 (s)</td>
</tr>
<tr>
<td>6.16 (1) 72.41 (d)</td>
<td>81.19 (s)</td>
</tr>
<tr>
<td>5.72 (1) 55.04 (d)</td>
<td>79.05 (s)</td>
</tr>
<tr>
<td>5.61 (1) 74.98 (d)</td>
<td>58.65 (s)</td>
</tr>
<tr>
<td>4.87 (1) 84.41 (d)</td>
<td>43.19 (s)</td>
</tr>
</tbody>
</table>

|        | 4.72 (1) | 73.20 (d) | 4.33 (1) | 72.18 (d) | 4.23 (1); 4.13 (1) | 76.52 (t) | 3.73 (1) | 45.64 (d) | 2.47 (1); 1.81 (1) | 35.63 (t) | 2.38 (1) | 22.63 (q) | 2.29 (1); 2.22 (1) | 35.72 (t) | 2.17 (3) | 20.84 (q) | 1.73 (1) | 1.72 (3) | 14.83 (q) | 1.62 (3) | 9.57 (q) | 1.17 (3) | 26.88 (q) | 1.08 (3) | 21.83 (q) |

### Conclusions:

- **a**, cross-peaks identify C-H pairs
- **b**, no cross-peak at a $^1$H chemical shift: exchangable proton
- **c**, no cross-peak at a $^{13}$C chemical shift: quaternary carbon
- **We have:**
  - 15 quaternary
  - $17 + 6^* \text{CH}$
  - 3 CH$_2$
  - 6 CH$_3$ carbons
COSY - CORrelation Spectroscopy

(correlates scalarly coupled protons)
DQF-COSY of Taxol
(expansion #2)

Known from TOCSY:
6.91, 5.72, 4.72 3.48

Known from $^{15}$N-HSQC: 6.91 is NH

Known from $^{13}$C-HSQC: 3.48 is OH
HMBC - Heteronuclear Multiple Bond Coherence
(correlates $^{13}$C-$^1$H chemical shifts via long-range $^n$J(X,H) (n=2,3,4?) couplings)
TERIMA KASIH
Magnet - Normally superconducting. Some electromagnets and permanent magnets (EM-360, EM-390) still around.

- **Frequency generator** - Creates the alternating current (at $\omega_0$) that induces $B_1$. Continuous wave or pulsed.

- **Detector** - Subtracts the base frequency (a constant frequency very close to $\omega_0$) to the output frequency. It is lower frequency and much easier to deal with.

- **Recorder** - XY plotter, oscilloscope, computer, etc., etc.
What is Spin?

Atoms are made up of neutrons, protons, and electrons. They are characterized by:

- an atomic number, $Z$, equal to the number of protons (or electrons)
- a mass number equal to the number of neutrons
- a spin quantum number which is a property of interactions between the protons and neutrons
- Spin is a type of *angular momentum* and follows the applicable quantum mechanics
- Angular momentum is a vector.

- Isotopes with even mass number have zero or integer spin
- Isotopes with odd mass number have half-integer spin (e.g. $^{13}$C, $^{1}$H, $^{31}$P, $^{19}$F, $^{15}$N)
- Even # of protons + even # of neutrons $\rightarrow$ no spin (e.g. $^{12}$C and $^{18}$O)
- Odd # of protons and odd # of neutrons $\rightarrow$ spin = integer $> 0$ (e.g. $^{14}$N)
Interpretation of $^1$H-NMR Data

**Chemical shifts** - nonequivalent H’s give unique signals along the x-axis of the spectrum. The small, discrete *energy differences* between signals are measured in $\delta$ or ppm units.

• Radio frequency energies are needed to “flip” nuclei from an aligned ($\alpha$, lower energy state) to an opposed ($\beta$, higher energy state) orientation in an external magnetic field. The Rf energy required is *influenced by the degree of electron shielding* of the nucleus.

• These data provide direct evidence of the number and kinds of hydrogens in a molecule and indirect evidence of how carbon, nitrogen, oxygen, and other atoms are connected.

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<table>
<thead>
<tr>
<th>10</th>
<th>9</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>$\delta$</th>
</tr>
</thead>
</table>

increasing deshielding
increasing shielding

TMS

reference signal
tetramethylsilane
Si(CH$_3$)$_4$ = 0.00 $\delta$