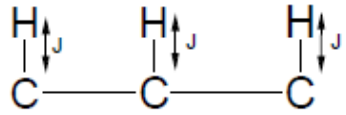
Two Dimensional NMR- Part III

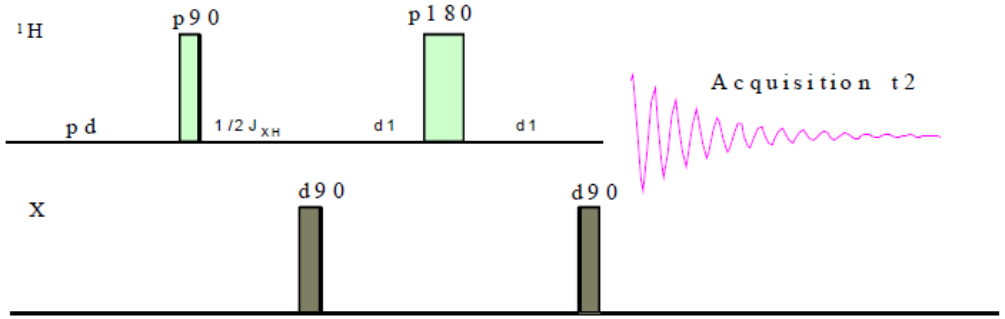
DR. DARSHANA MEHTA

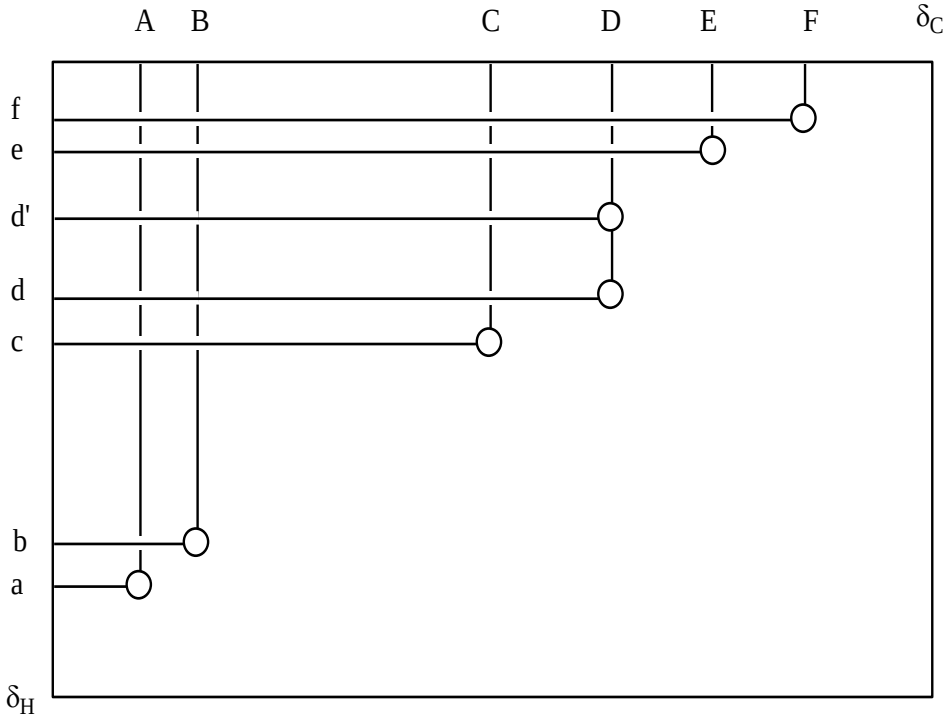
SCHOOL OF STUDIES IN CHEMISTRY AND BIOCHEMISTRY, VIKRAM UNIVERSITY, UJJAIN – 456 010, MADHYA PRADESH, INDIA

HMQC – Heteronuclear Multiple Quantum Coherence



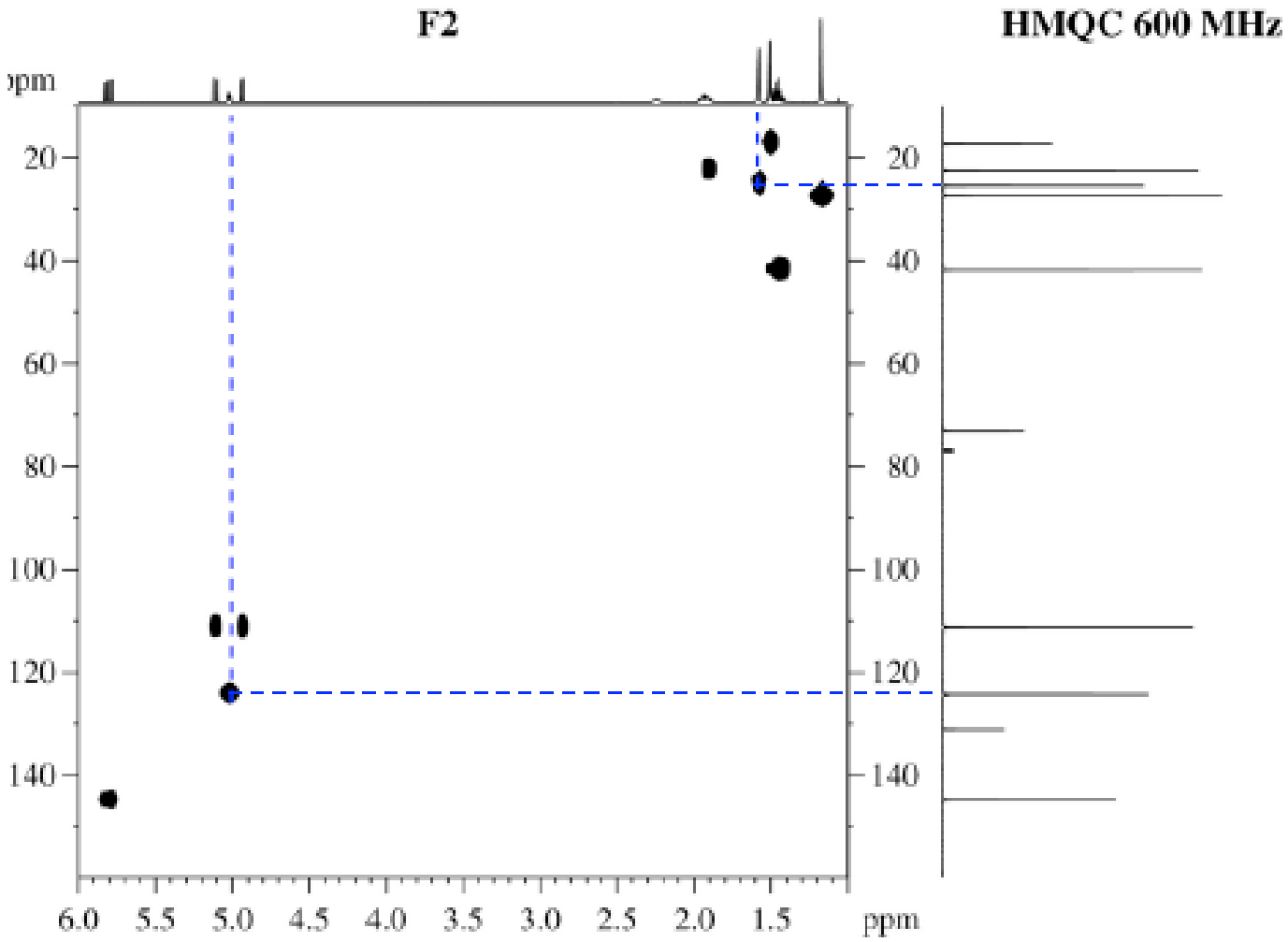
- The experiments show correlation between heteronuclei while detecting protons that are coupled to them.
- The pulse sequence utilizes zero and double quantum coherence between *J*-coupled protons and carbons to label each proton with the frequency of directly bonded carbon.
- For insensitive nuclei such as ^{13}C or ^{15}N, the sensitivity by the factor of $(\lambda_H/\lambda_X)^3$ is theoretically 64 and 1000 times more enhanced than ordinary ^{13}C and ^{15}N one pulse experiments respectively.



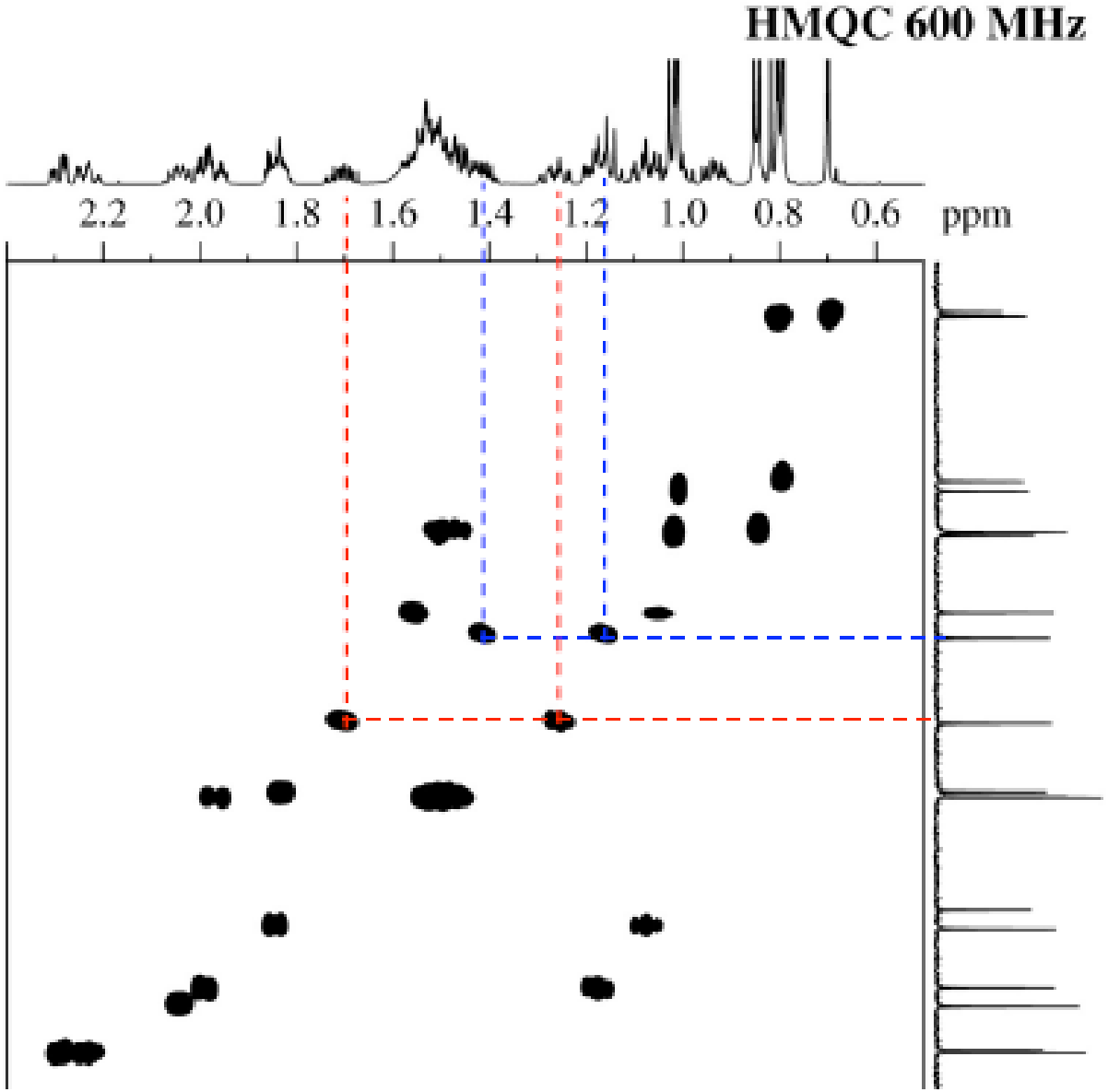


$^1J_{CH}$ = 140 Hz; C-H direct correlations (1 bond)

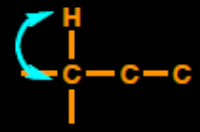
HMQC – Heteronuclear Multiple Quantum Coherence



HMQC – Heteronuclear Multiple Quantum Coherence



Diastereotopic Protons



1 bond H–C coupling

HMQC – Heteronuclear Multiple Quantum Coherence

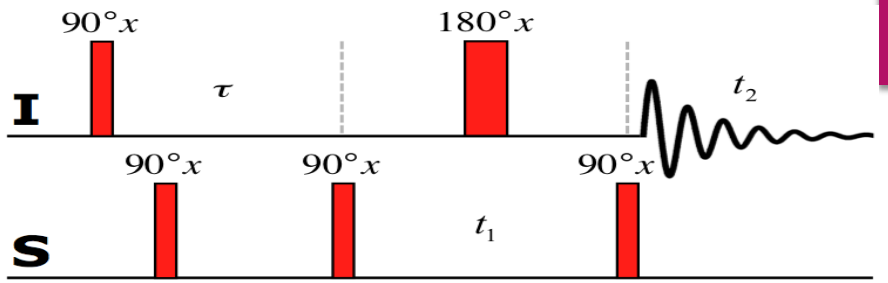
- Reverse Polarization Transfer experiments, also known as indirect detection or heteronuclear multiple-quantum coherence (HMQC) experiments, have become extremely powerful tools for chemists to determine insensitive nuclei such as ^{13}C or ^{15}N.
- This enhancement is achieved by observing the couplings between the X nucleus and the protons from the side of the much more sensitive proton.
- These experiments show correlation between heteronuclei while detecting protons that are coupled to them.
- Actually, the probe for HMQC experiment is designed for the detection of proton signals together with an irradiation coil for the X nucleus broadband decoupling. Because of this, the techniques are often referred to reverse or indirect detection.

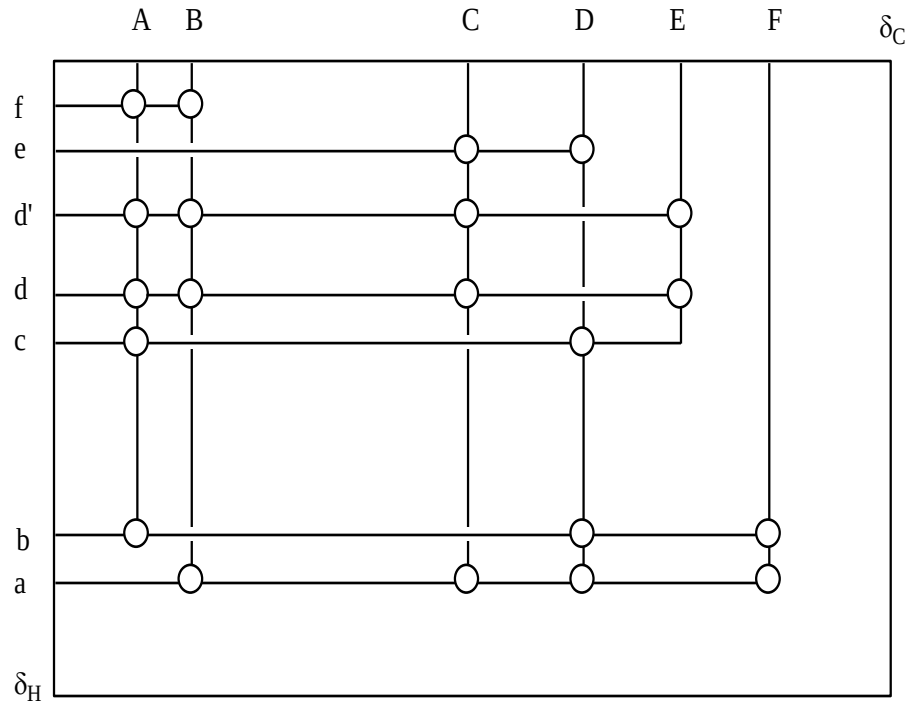
HMQC – Heteronuclear Multiple Quantum Coherence

- The HMQC experiments require the instruments are capable of generating pulse on 1 H and X and possibly decoupling X while observing 1 H.
- All high field NMR instruments should have dual broadband system. The frequencies of both observe and decoupling channels could be set to 1 H to 103Rh or lower.
- Performing an indirect detection experiment simply involves setting the observe channel to observe proton and the decoupling channel to decouple X nucleus.
- It also requires that the decoupler power can fast switch between high power output for the hard pulse and low power output for the broad band decoupling. Some of old instrument do not have this capability, but it still can perform HMQC experiments without decoupling.

HMBC – Heteronuclear Multiple Bond Correlation

- To obtain long-range proton carbon correlations via 2J and 3J, we could use HMQC pulse sequence and set the coupling delay ½ J to about 50-100 ms
- Shows cross peaks for protons and carbons separated by 2 and 3 bonds. The one bond correlations are suppressed.
- One can indirectly obtain the ^{13}C-^{13}C correlation and can correlate the quaternary carbons with nearby protons
- The intensity of the crosspeaks depends on the magnitude of the long range proton-carbon coupling constants (5-20Hz)



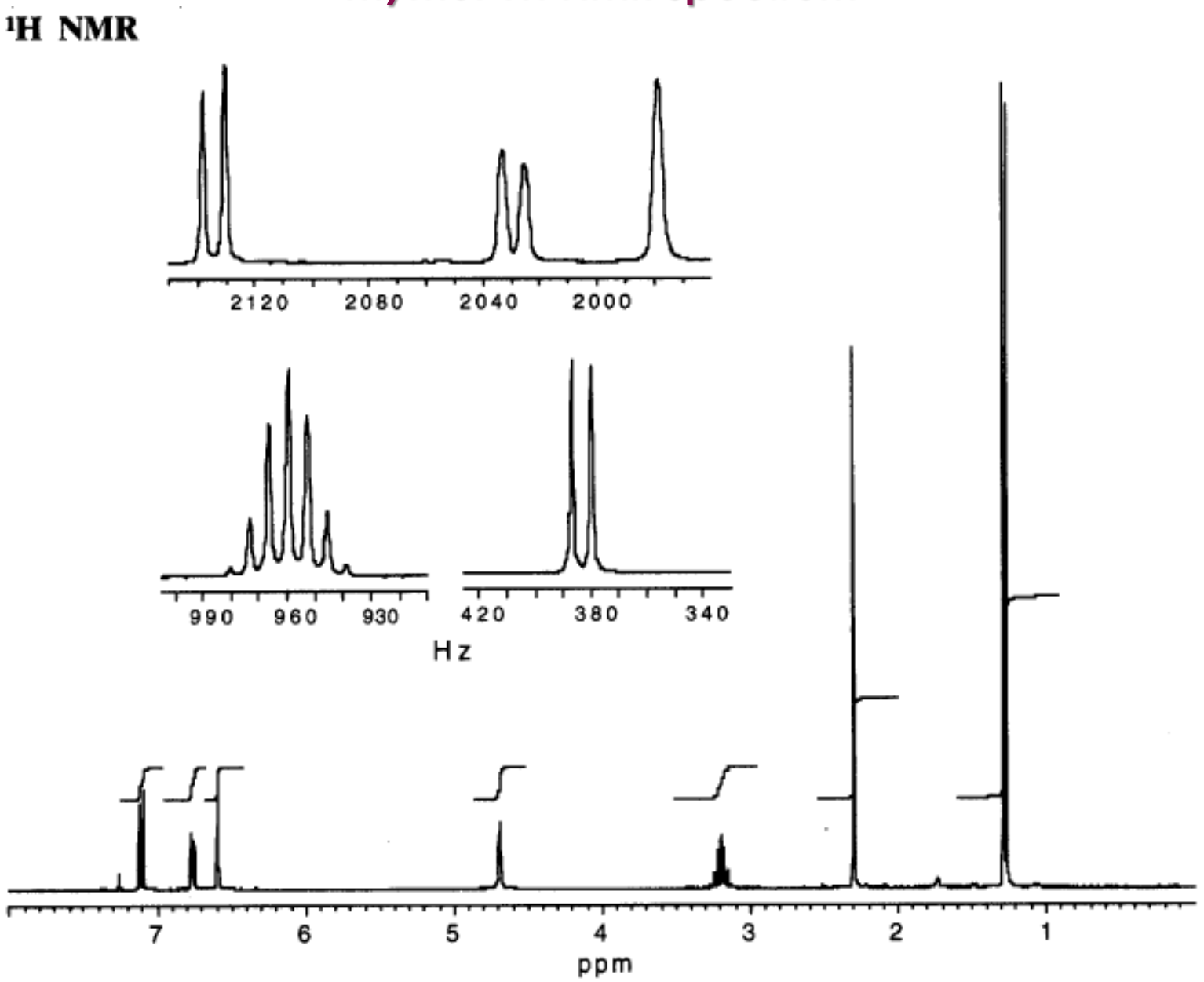


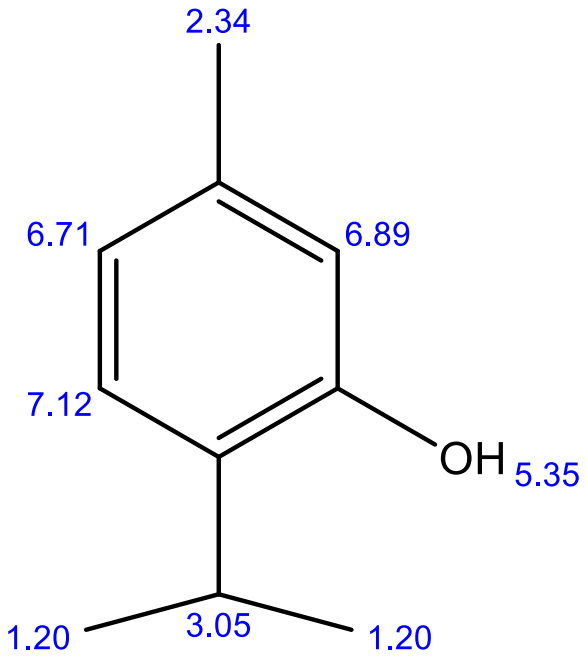
$^{2\text{-}3}J_{CH}$ = 9 Hz; C-H indirect (long range) correlations (2-3 bonds) C-C-H & C-C-C-H

HMBC – Heteronuclear Multiple Bond Correlation

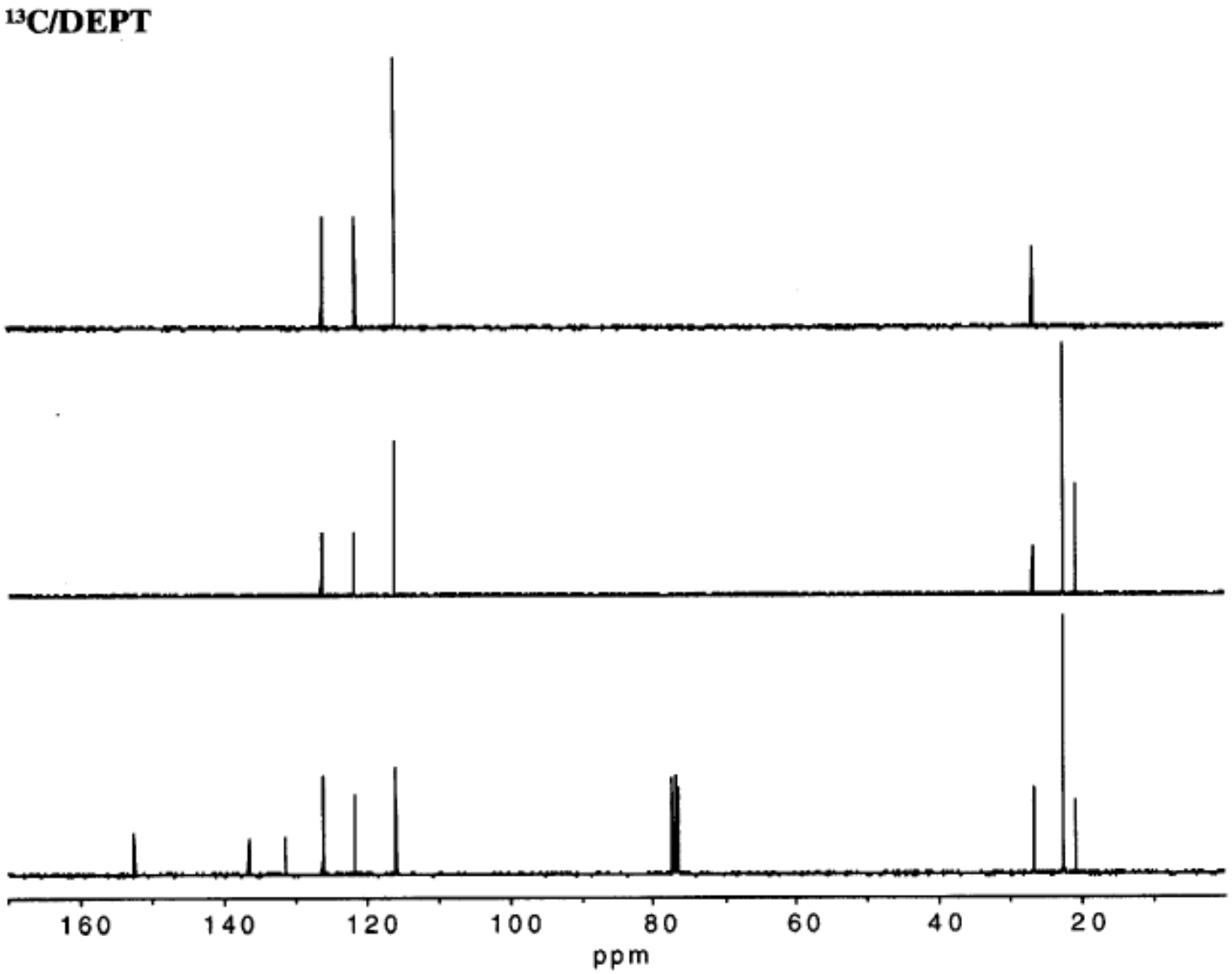
- To obtain long-range proton carbon correlations via ^{2}J and ^{3}J, we could use HMQC pulse sequence and set the coupling delay ½ J to about 50-100 ms, relative to the J= 10 - 5 Hz.
- The disadvantage of HMQC is there are still ^{1}J cross peaks.
- The HMBC (Heteronuclear Multiple Bond Correlation) is a special pulse sequence to acquire the long-range correlations.
- The HMBC experiment does not apply ^{13}C decoupling, so the cross peaks caused by ^{1}J and ^{2}J or ^{3}J can be separated, and also provide their coupling constants.

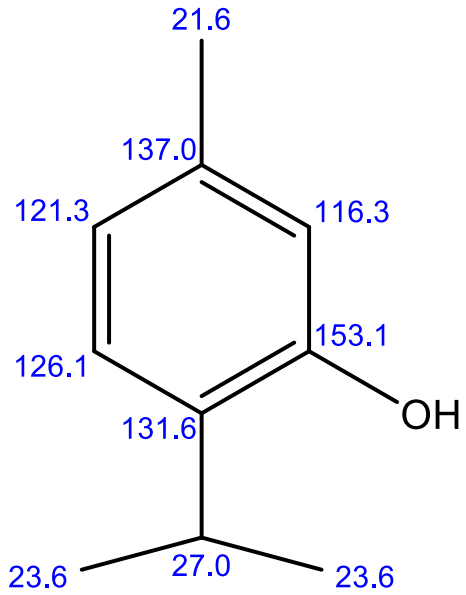
Thymol 1H NMR Spectrum



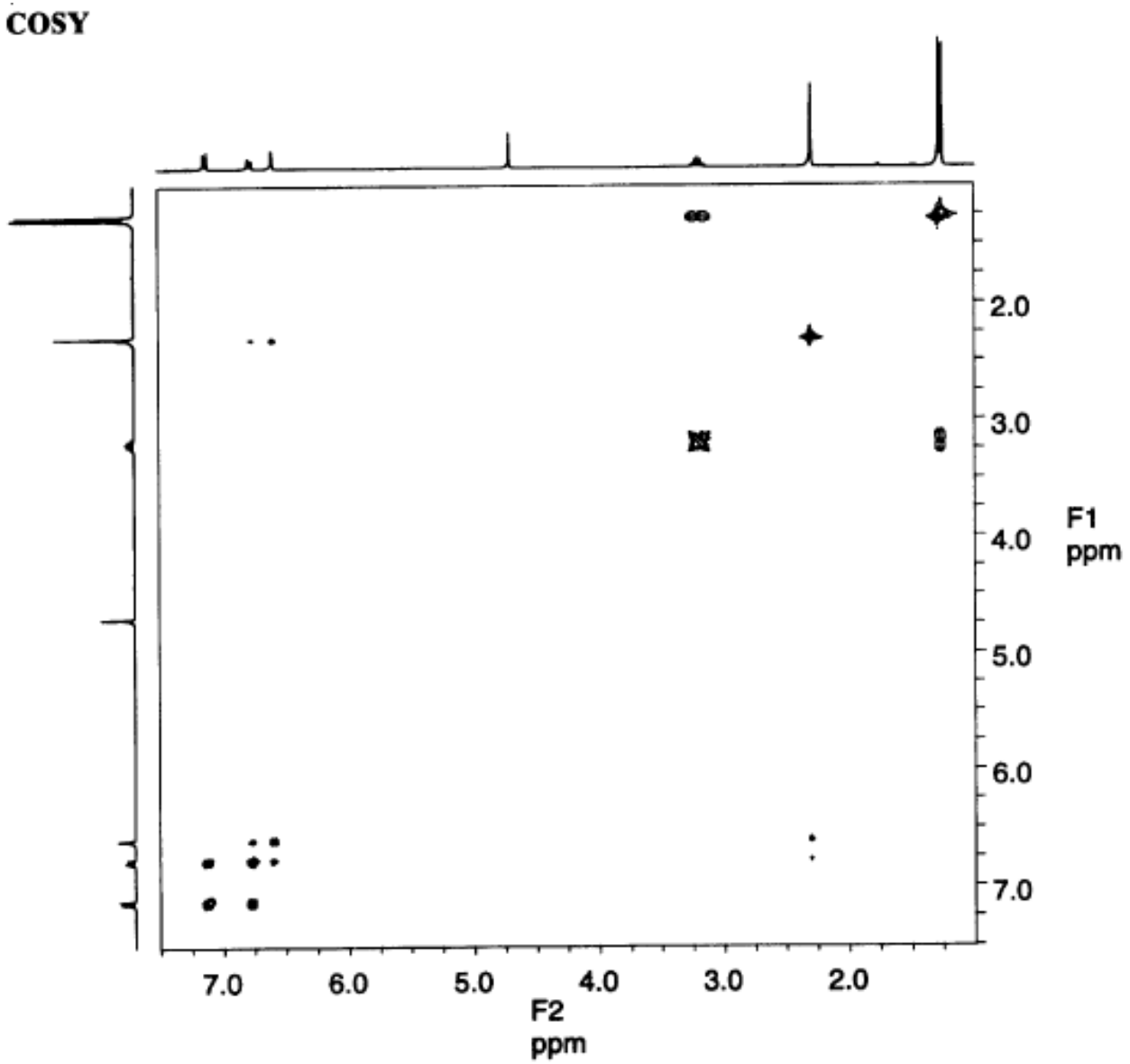


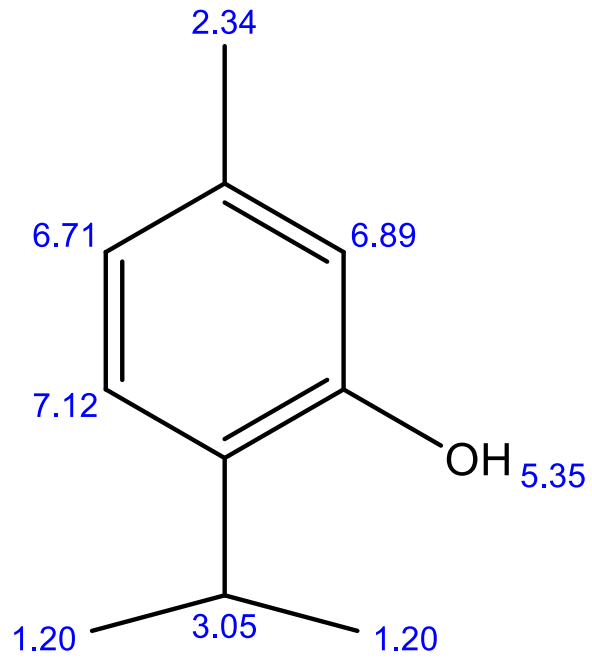
Thymol DEPT Spectrum



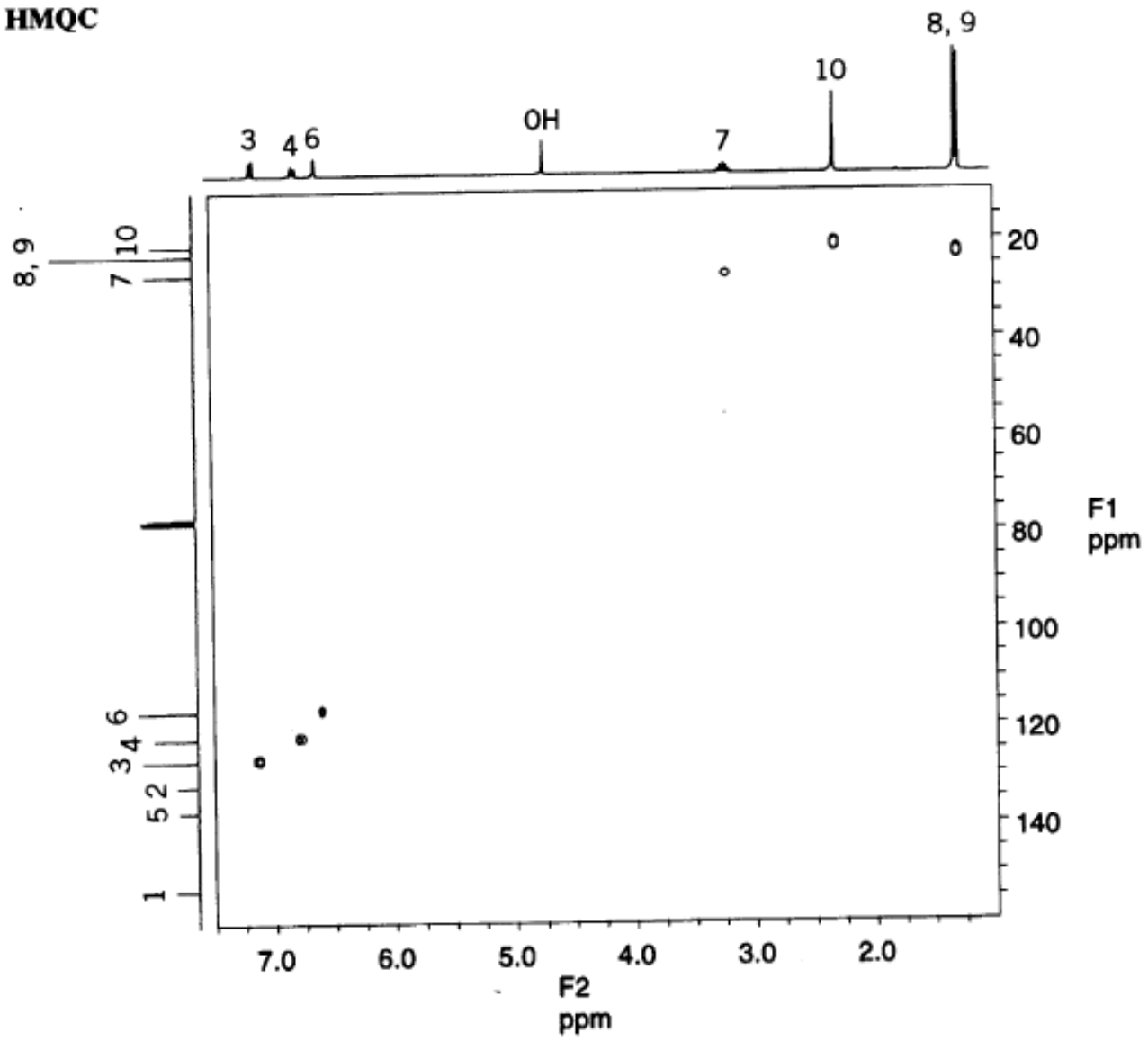


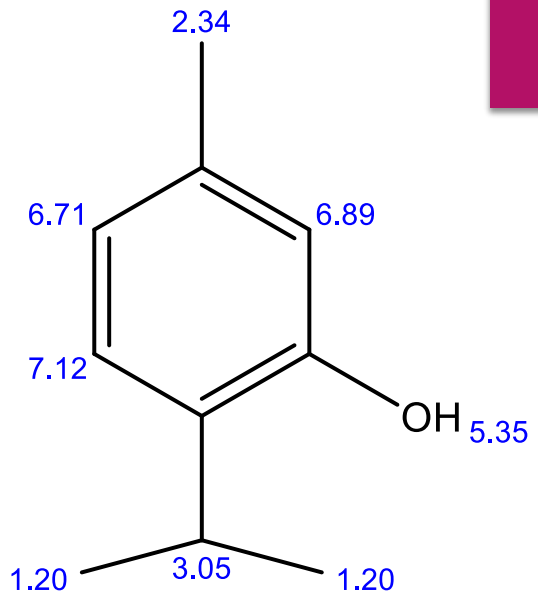
Thymol COSY Spectrum

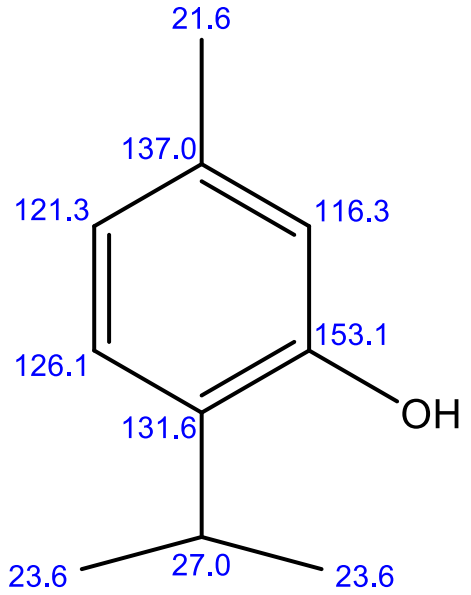




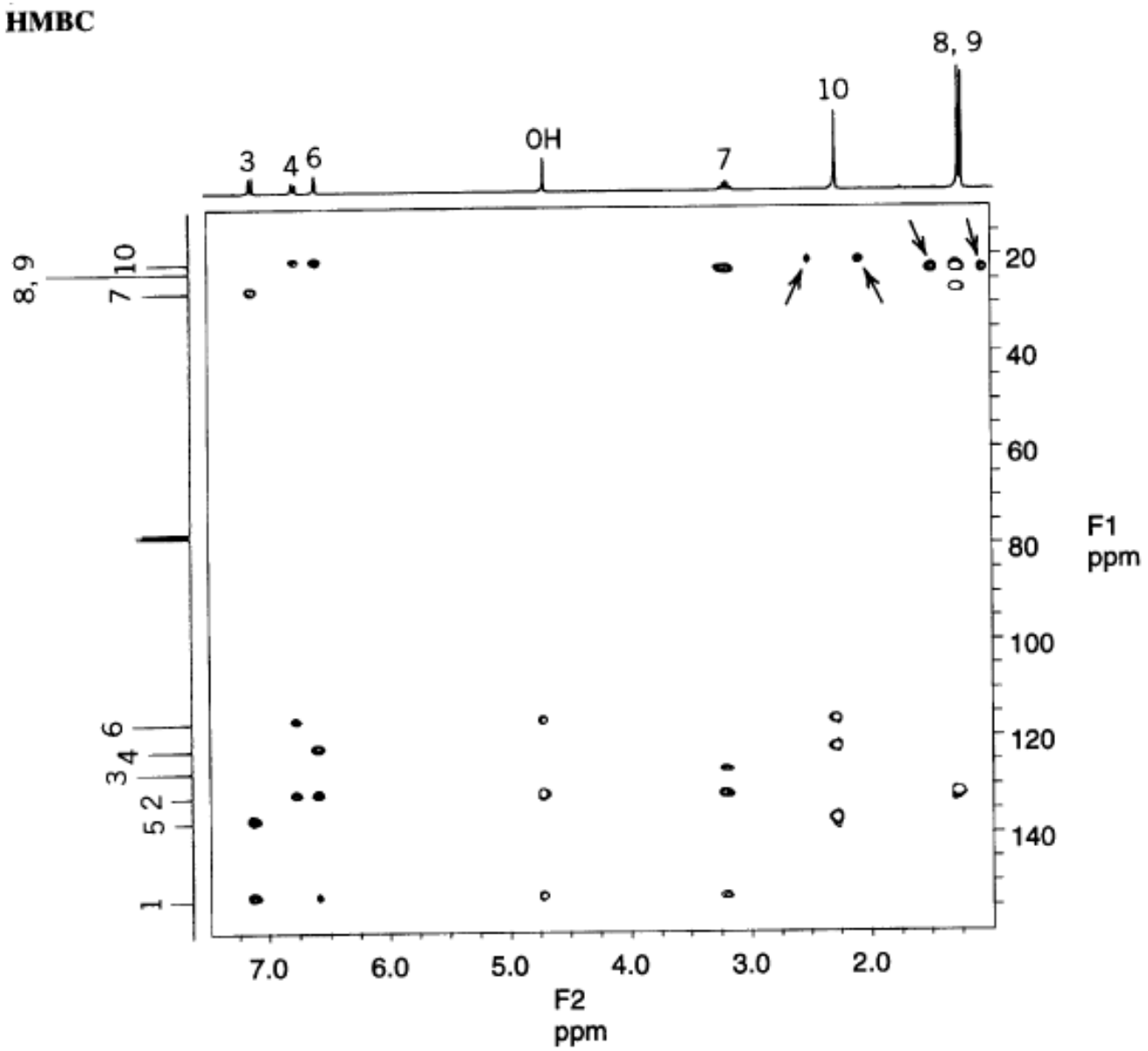
Thymol HMQC Spectrum

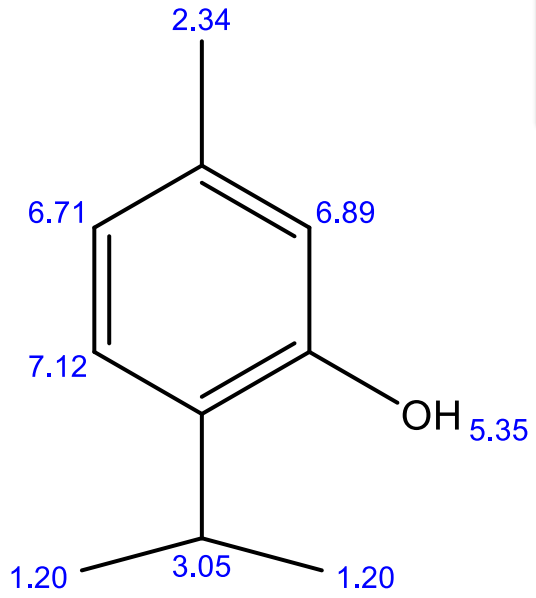


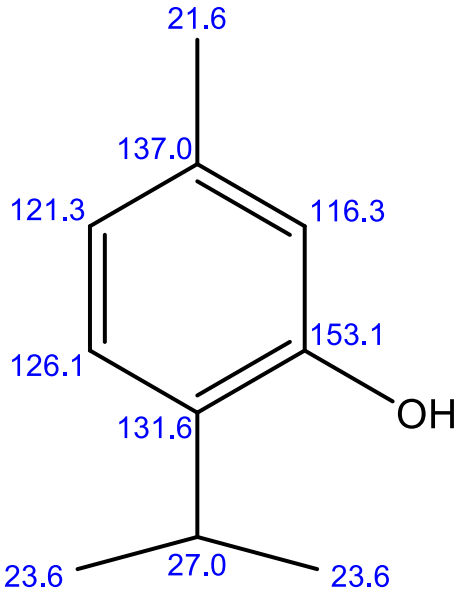


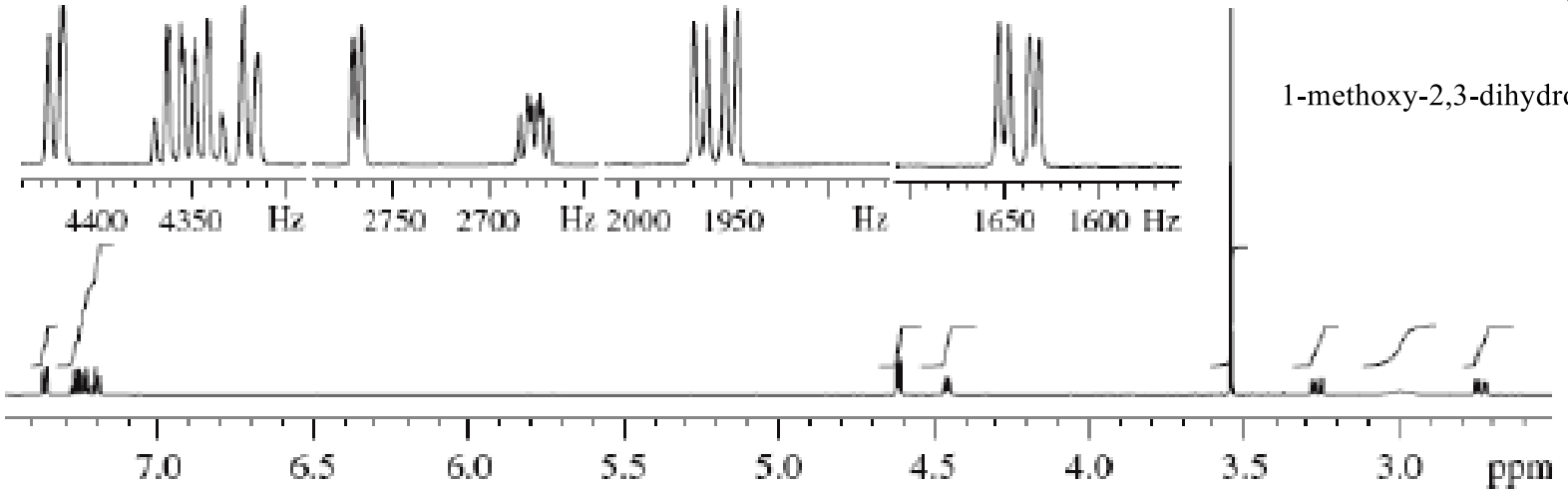


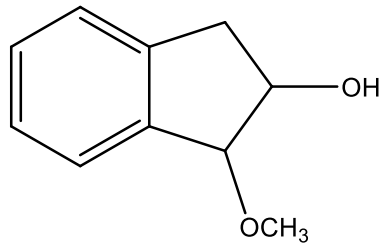
Thymol HMBC Spectrum



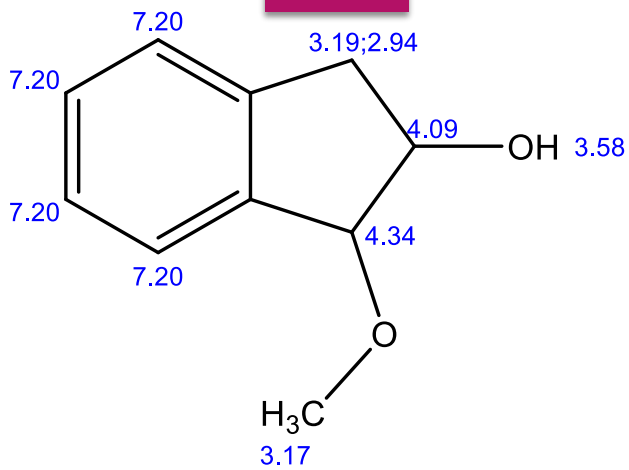




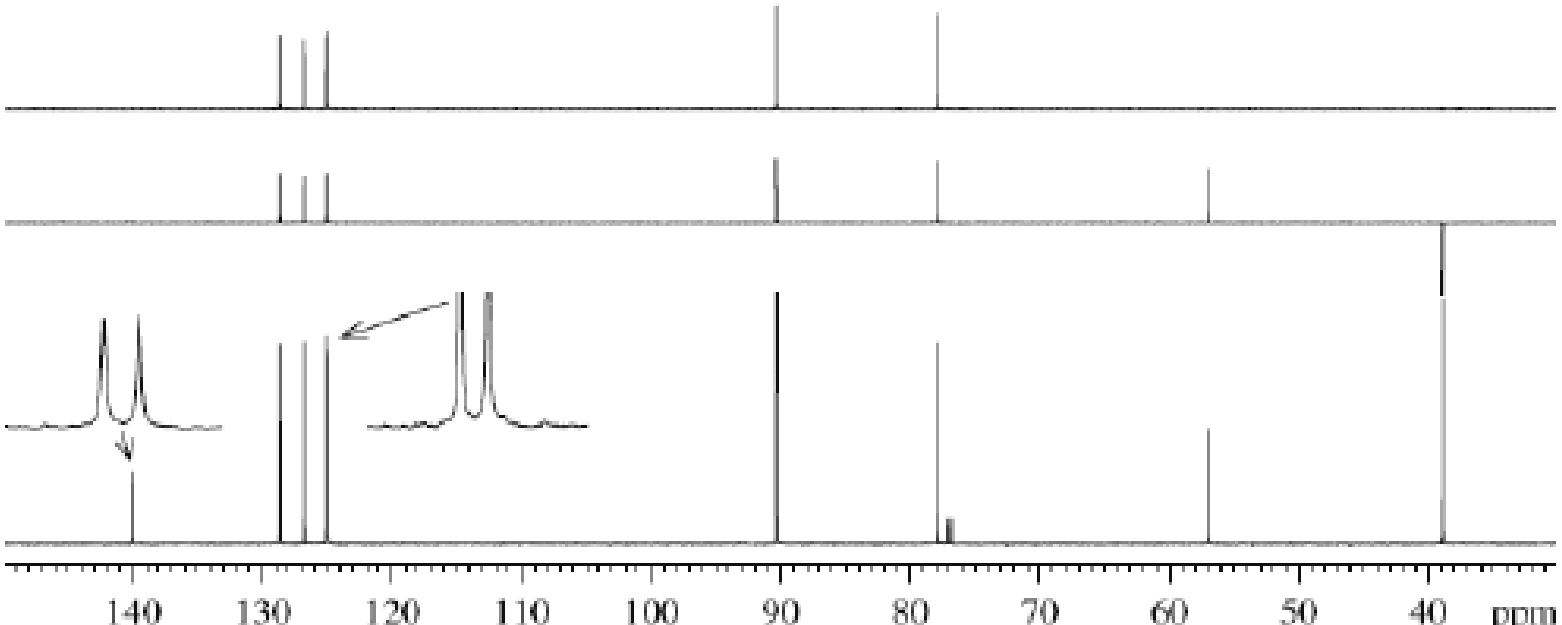


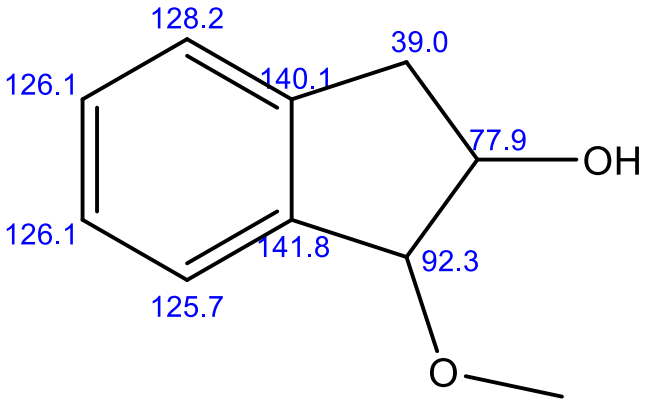


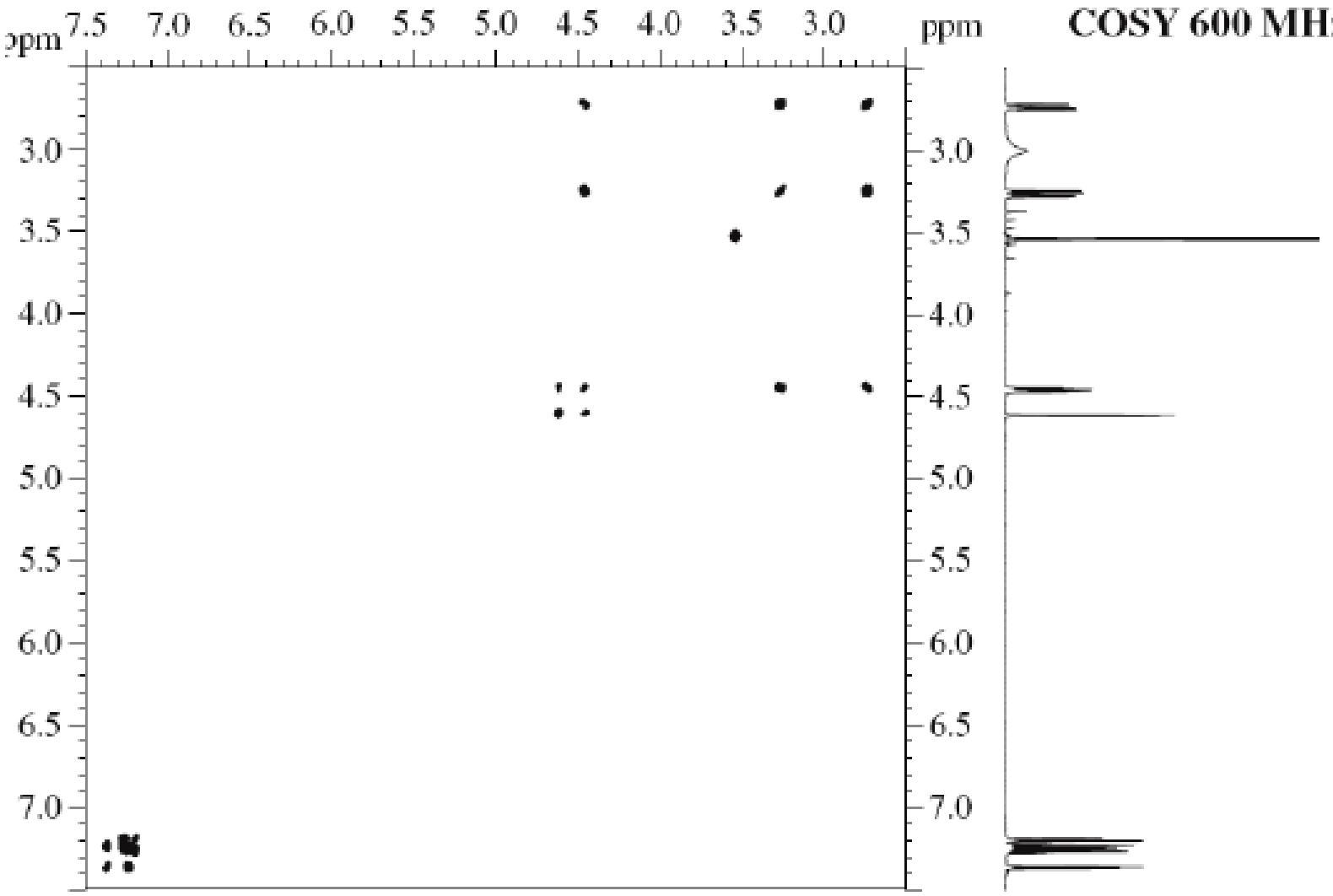
1-methoxy-2,3-dihydro-1*H*-inden-2-ol



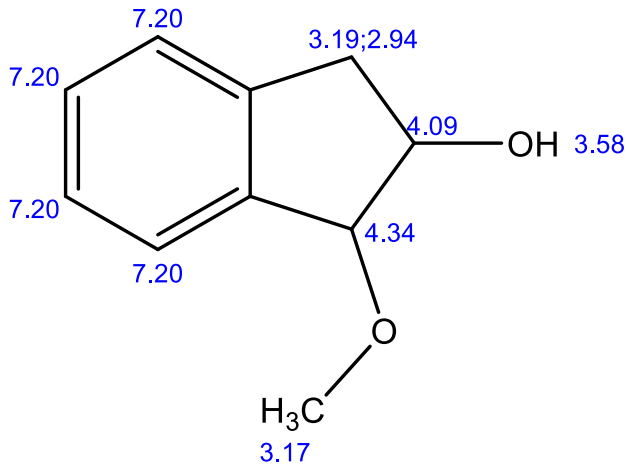
^{3}C/DEPT NMR 150.9 MHz



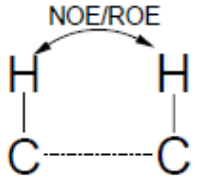



ppm 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 ppm
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

COSY 600 MHz


7.20
3.19;2.94
7.20
4.09
OH 3.58
7.20
4.34
7.20
O
H3C
3.17

Nuclear Overhauser (NOESY) Spectrometry Proximity Through Space



- NOESY for very large molecules, ROESY for mid-size molecules
- These spectra are used to locate protons that are close together in space
- Can be a 1D or 2D NOESY technique
- nOe is a through space effect
- It has nothing to do with connectivity in the molecule
- A proton that is close in space to the irradiated proton is affected by the NOE whether or not it is coupled to the irradiated proton; if it is coupled, it remains at least partially coupled because the irradiation is week in comparison with that used for a decoupling experiment.

NOESY (Nuclear Overhauser Effect SpectroscopY)

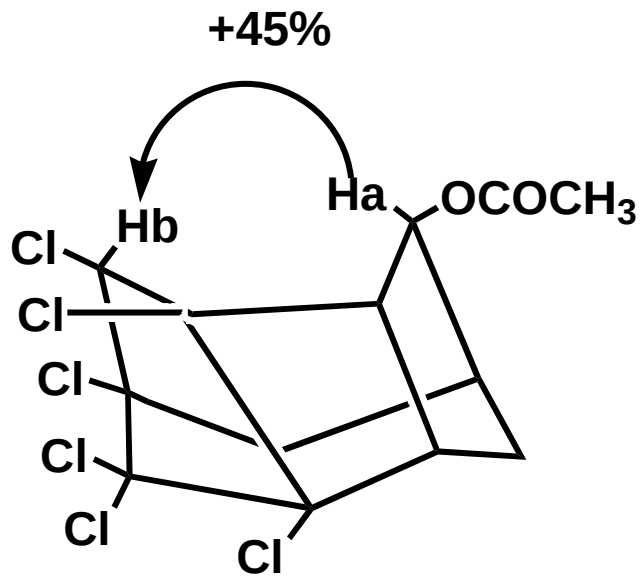
- This technique is developed to observe NOE in the two dimensional spectroscopy.
- The NOEs between all spatially close protons can he observed as cross peaks simultaneously.
- It is very important and useful technique in molecular structure elucidation and molecular conformation determination.
- The experiment NOESY yields a pure adsorption correlation map on the basis of incoherent magnetization transfer processes such as dipolar coupling and chemical exchange.
- The incoherent magnetization transfer happens during the mixing time that should be properly set so that the maximum NOE could be observed.

NOESY (Nuclear Overhauser Effect SpectroscopY)

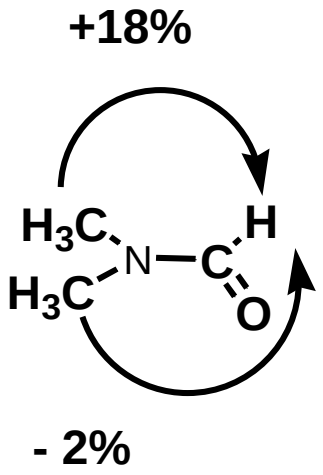
- The NOESY is a homonuclear, shift correlated experiment, in which cross peaks result from dipolar interactions between spins.
- Dipolar couplings result from through-space interactions and only depend on the distance of the spins, but not on the number of intervening bonds.
- They are observable for nuclei separated by up to 4-5 Å.
- Because the sign of the NOE depends on the molecular reorientation time τc of the molecule, peaks in the NOESY may be positive (large molecules) or negative (small molecules). The reorientation time is largely influenced by the viscosity of the solvent.

NOESY (Nuclear Overhauser Effect SpectroscopY)

Which methyl signal belongs to which group?

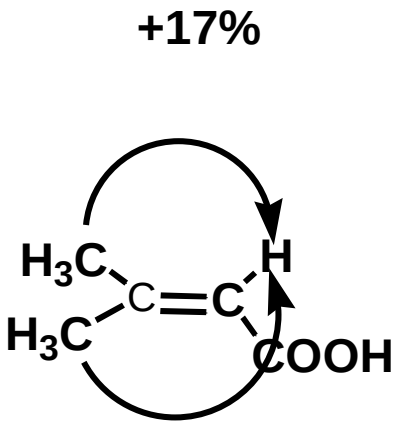


semiclathrate



- 2%

Dimethylformamide



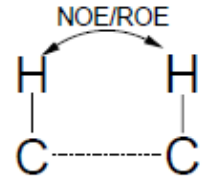
- 4%

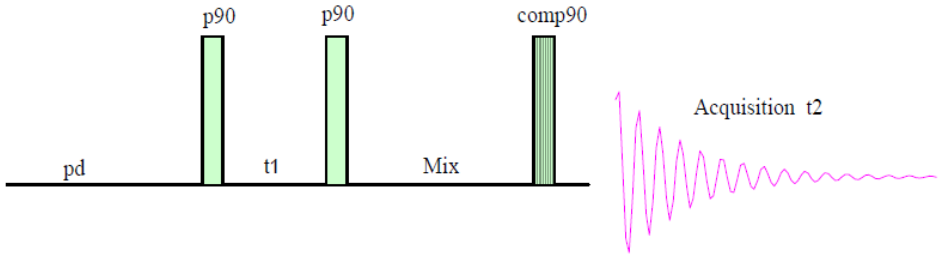
3-methylcrotonic acid

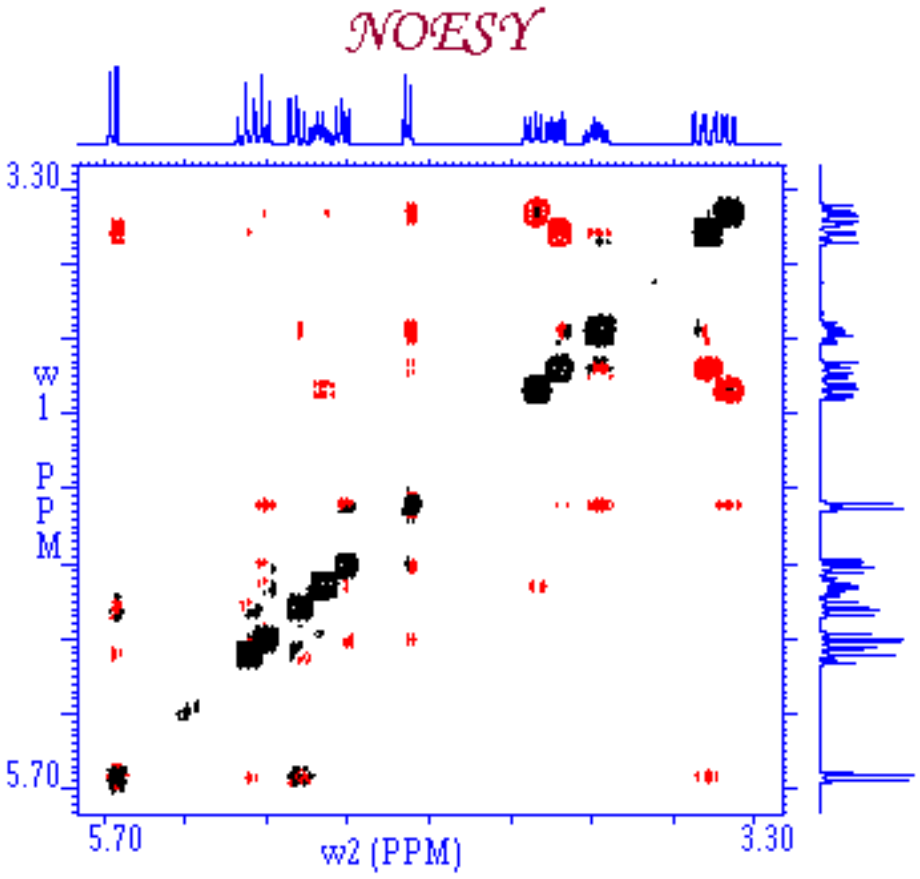
Two Me groups are non-equivalent owing to hindered rotation about the C-N bond. Both Me signals are therefore found At δ 2.79 and δ 2.94, together with a singlet at δ 8.0 for the formyl proton. If one now saturates the Me signal at δ 2.94, the intensity of the formyl proton signal increases by 18%. When instead the other methyl signal is saturated, a decrease of 2% is observed.

NOESY (Nuclear Overhauser Effect SpectroscopY)

- Cross-peaks appear between protons that are within 5 Å of each other.
- Diagonal peaks are correlated by through-space dipole-dipole interaction (NOE)
- NOE is a relaxation factor that builds-up during the "mixing-time" (t_m)
- Short τc gives cross peaks with the opposite sign as the diagonal peaks
- The effect relies on molecular size. The NOE effect ~ 0 at 1000 Da. It works well for small molecules (t_{mix} ~ 800 ms) and macromolecules (t_{mix} ~ 100 ms).
- True NOE and ROE peaks are negative. In NOESY can get COSY peaks showing (positive).

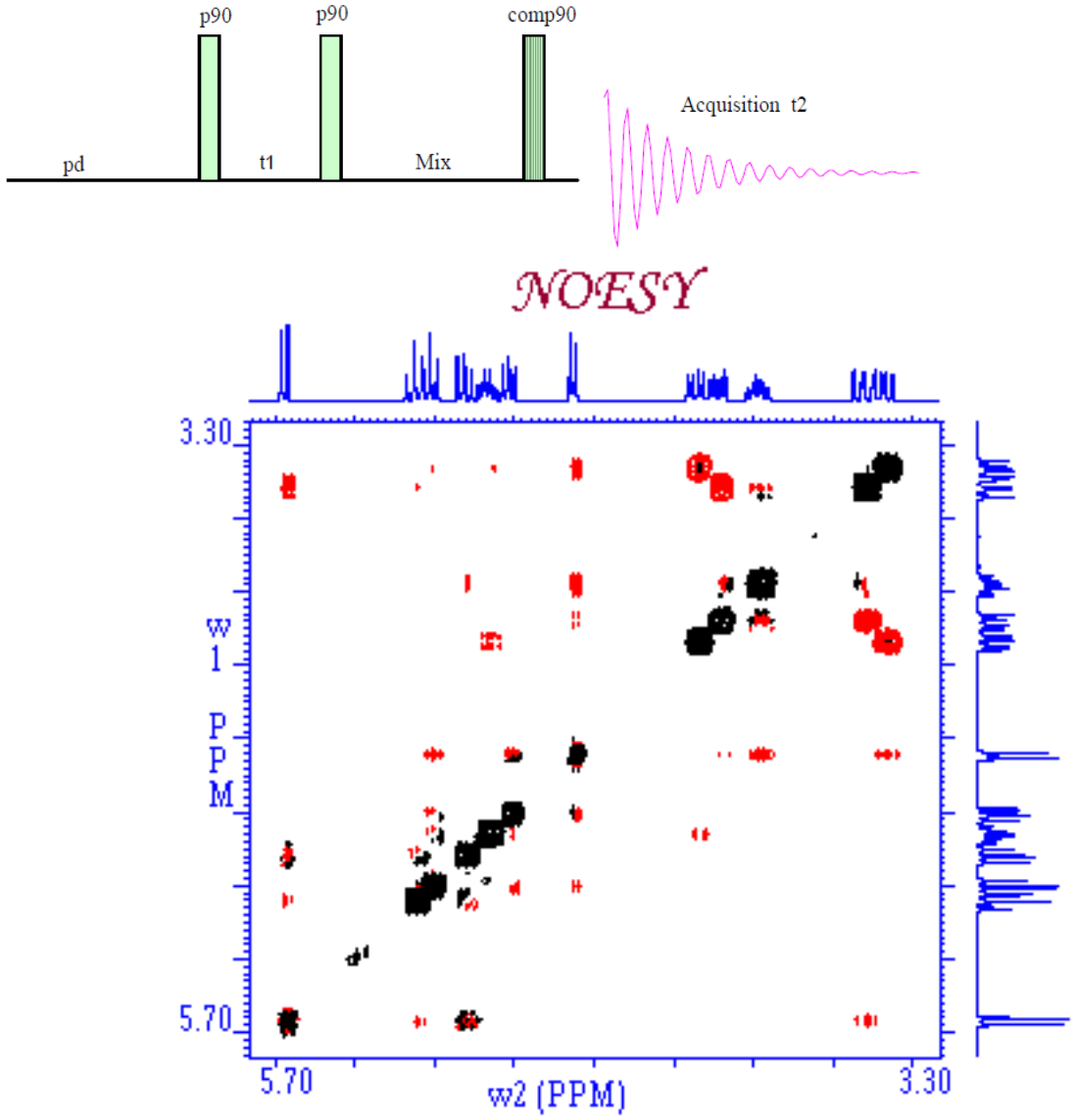






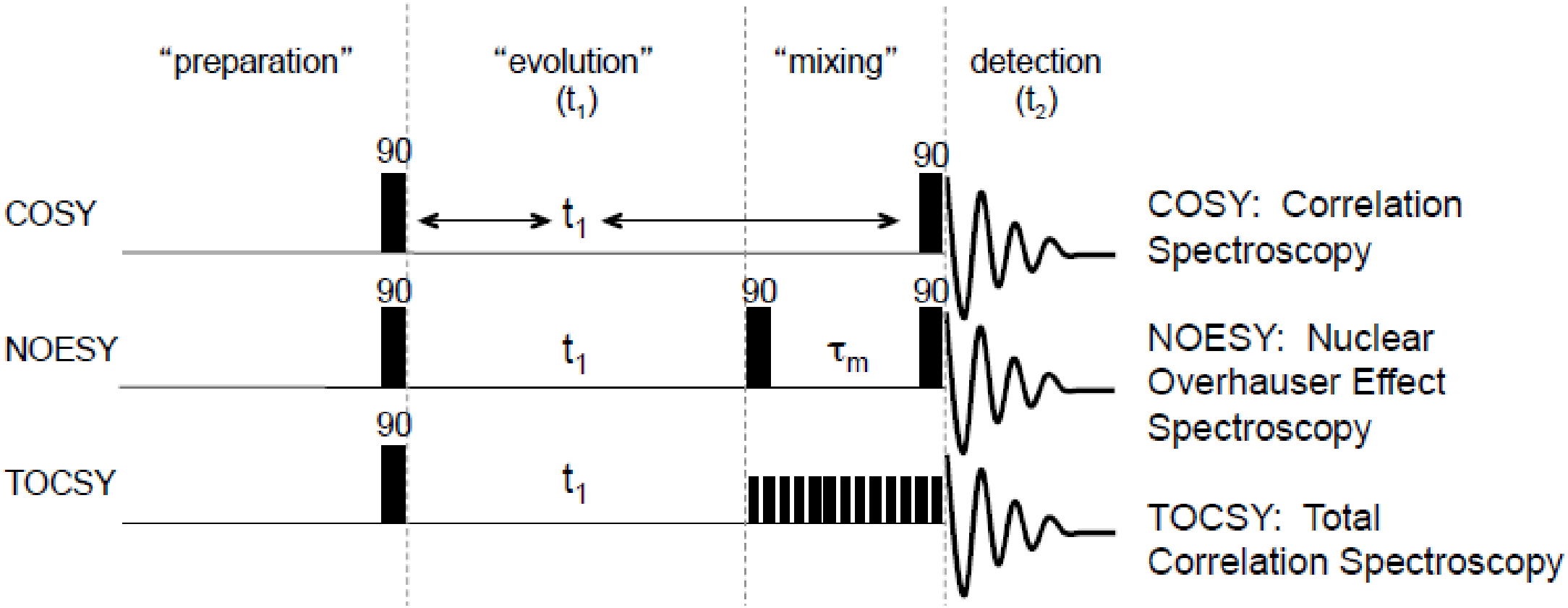
NOESY (Nuclear Overhauser Effect SpectroscopY)

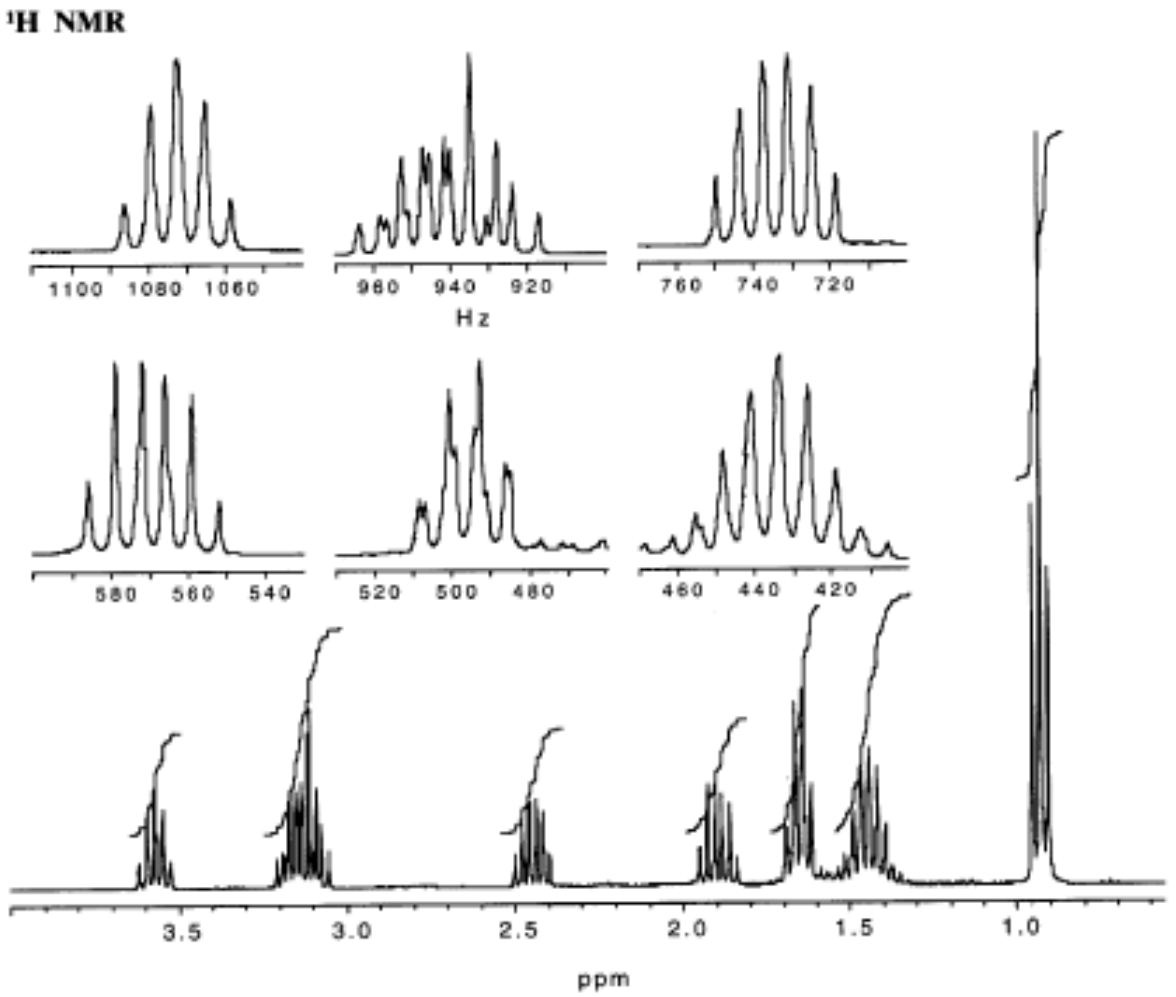
- The pulse sequence consists of three 90 degree pulses.
- The first pulse creates transverse magnetization, the magnetization is then frequency labeled during the evolution time.
- The second pulse flips the labeled components to -z axis. These components are mixed through cross relaxation processes during the mixing time.
- The last composite 90 degree pulse rotates the magnetization to transverse plane again for observation.

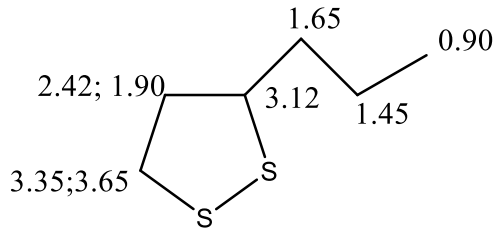


NOESY (Nuclear Overhauser Effect SpectroscopY)

- This technique is developed to observe NOE in the two dimensional spectroscopy.
- The NOEs between all spatially close protons can he observed as cross peaks simultaneously.
- It is very important and useful technique in molecular structure elucidation and molecular conformation determination.
- The experiment NOESY yields a pure adsorption correlation map on the basis of incoherent magnetization t
- Transfer processes such as dipolar coupling and chemical exchange. The incoherent magnetization transfer happens during the mixing time that should be properly set so that the maximum NOE could be observed.

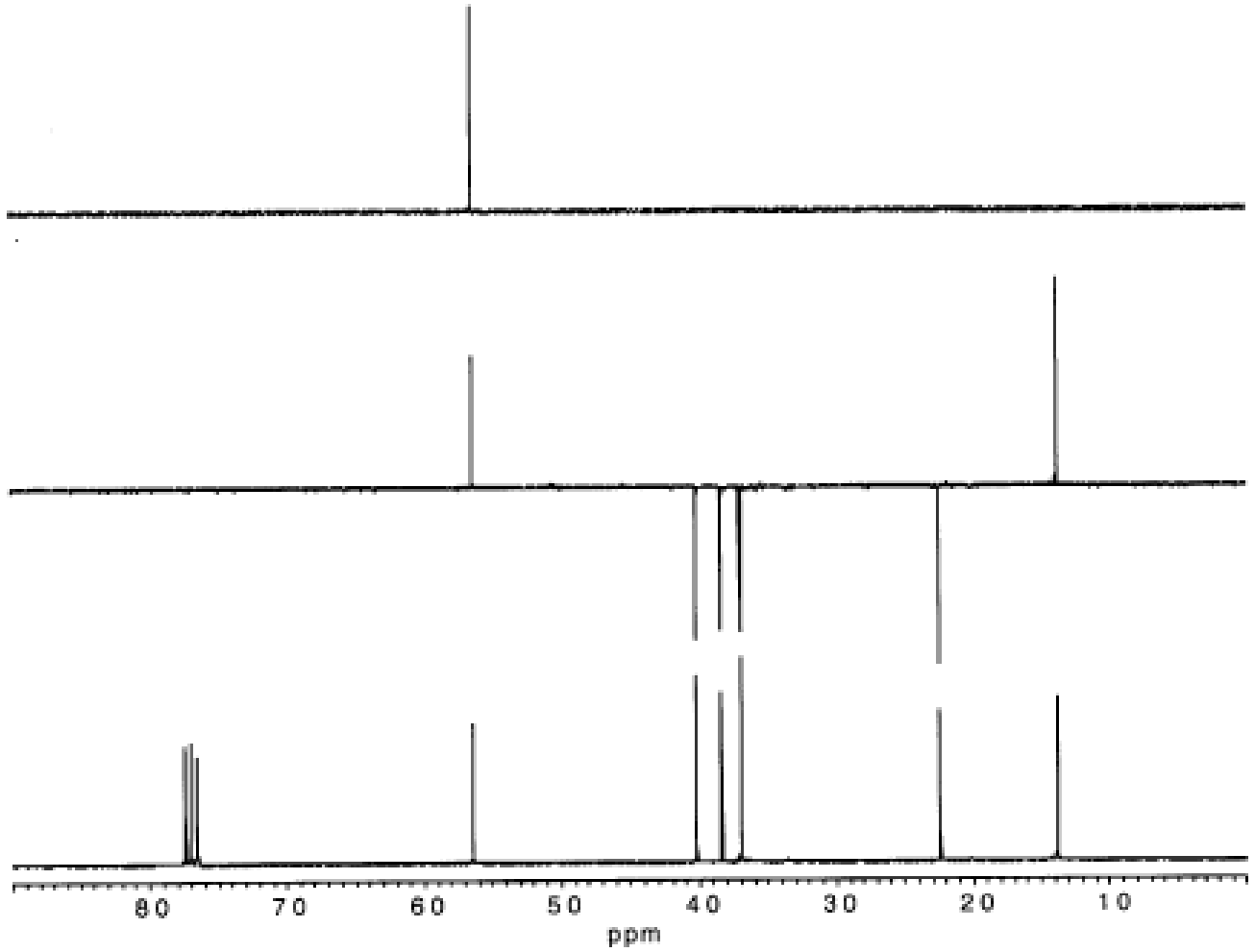

"preparation"
"evolution"
(t_1)
"mixing"
detection
(t_2)
COSY
90
t_1
90
COSY: Correlation Spectroscopy
NOESY
90
t_1
90
τ_m
90
NOESY: Nuclear Overhauser Effect Spectroscopy
TOCSY
90
t_1
TOCSY: Total Correlation Spectroscopy

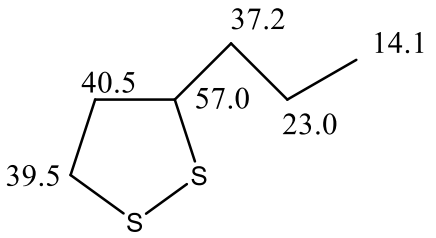




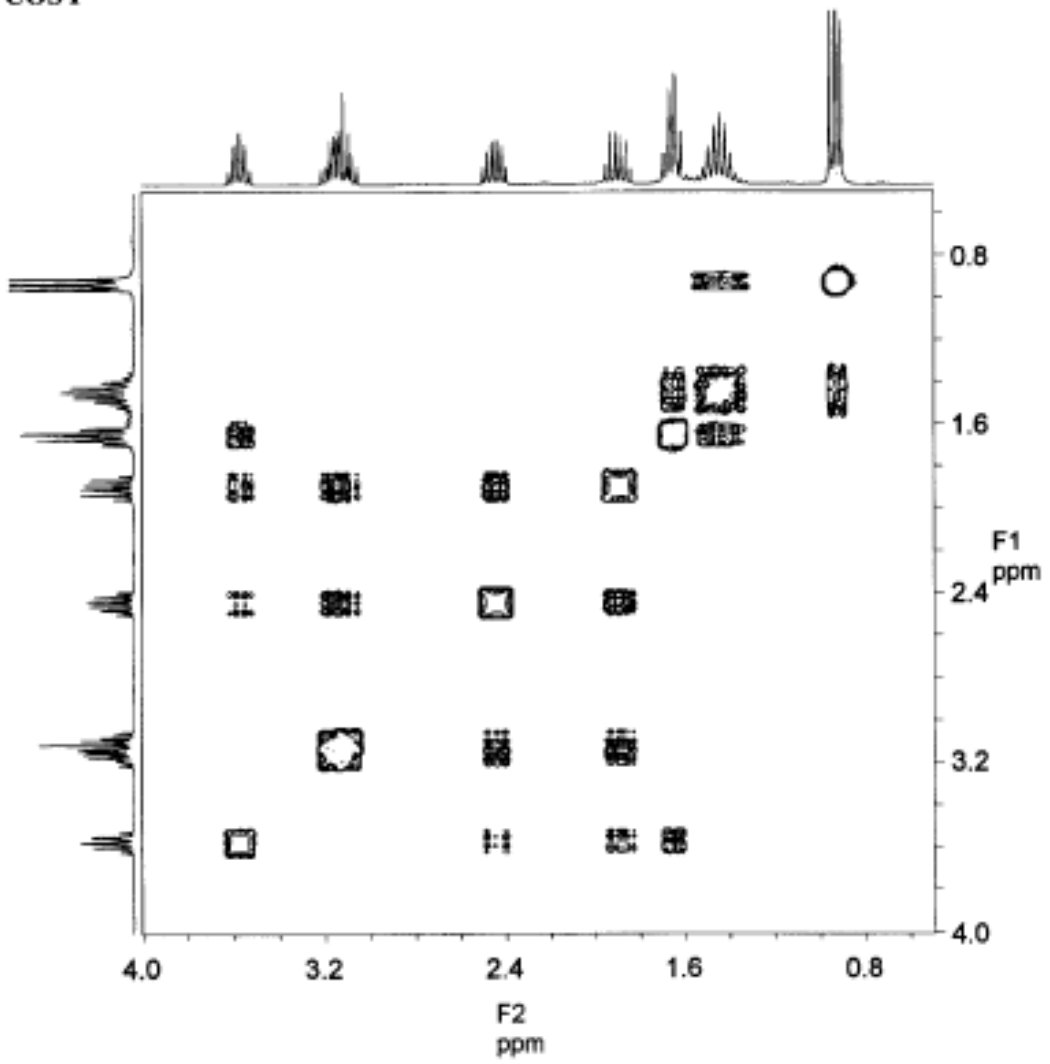
3-propyl-1,2-dithiolane

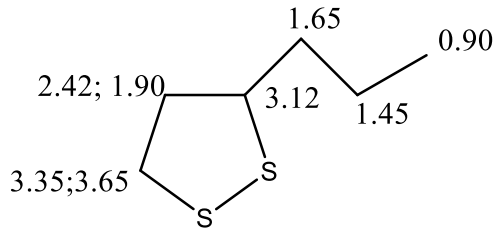
^{13}C/DEPT





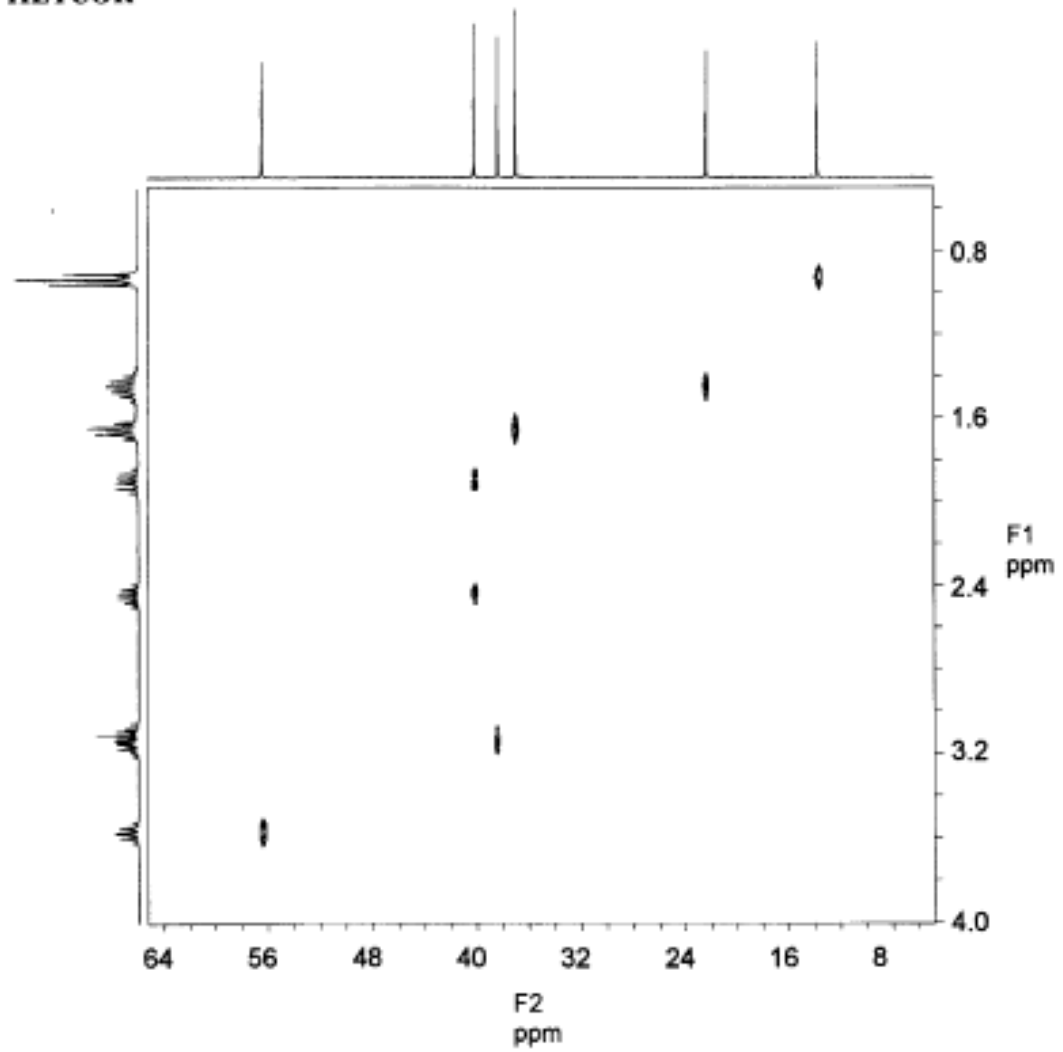
COSY

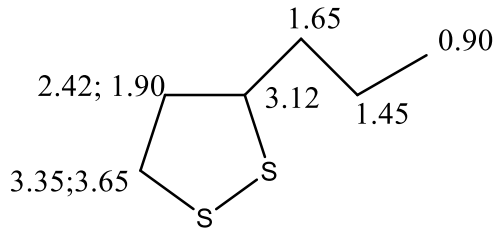
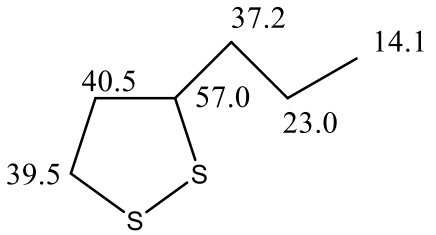




3-propyl-1,2-dithiolane

HETCOR





3-propyl-1,2-dithiolane