

Two Dimensional Heteronuclear Correlation Spectroscopy

HETCOR

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INTRODUCTION

Correlation Spectroscopy

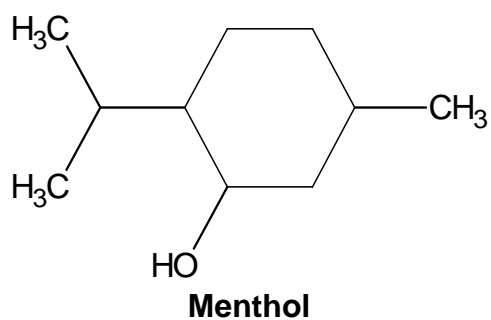
Correlation experiments reveal connections between spin coupled nuclei. Spin, or scalar, coupling between nuclei is established by the appearance of cross peaks (peaks off the diagonal) in the two-dimensional spectrum. Since spin coupled nuclei are usually separated by two or three bonds, the correlation experiment, by revealing this connectivity within the compound, is often enough to establish the chemical structure. Resonances which are coupled through four and five bonds will occasionally show cross peaks also. Although most correlation experiments are designed to emphasize short range coupling, there are experiments designed to emphasize long range correlations as well.

Two-dimensional spectra can be acquired in either of two modes. In the magnitude, or absolute value mode, only the "real" part of the data is collected since the final spectrum is not phased. In the phase sensitive mode, both the "real" and "imaginary" parts of the data are acquired so that the spectrum can be phased. An advantage of a phase sensitive spectrum is that the peaks are much narrower and the resolution greater. A disadvantage of a phase sensitive spectrum is that twice as much data must be recorded in order to extract the phase information. Thus, it takes twice the time to record a spectrum in phase sensitive mode.

HETCOR

The Heteronuclear correlation experiment described here (also called ^{13}C - ^1H COSY) shows connections between carbon atoms and the protons directly bonded to them. The experiment also furnishes information about carbon types. Quaternary carbons, for instance, have no attached protons and consequently, show no correlations. Methine carbons, can show one and only one peak. Methylene carbons can show one or two peaks depending on the chemical shifts of the two protons and methyl groups generally show a single intense peak or a single multiplet. The spectra usually have quite low resolution so the fine structure due to multiplets is minimal. HETCOR spectra are usually acquired in magnitude mode due to the long accumulations required to obtain a reasonable signal to noise ratio.

The HETCOR spectrum of Menthol is shown on a following page. Notice, that the hydroxyl proton shows no correlations. The three methyl (15, 22, 23 ppm), three methylene (24, 34, 45 ppm) and four methine (26, 32, 50, 72 ppm) resonances are all clearly distinguishable. The assignments so far, have all been made without using arguments based on chemical shift!!! The spectrum required about 35 minutes to collect.



References.

- 1) **150** and More Basic NMR Experiments, A practical Course, S. Braun, H.-O. Kalinowski, S. Berger, Wiley-VCH, 1998, pages 375-377.
- 2) Basic One- and Two-Dimensional NMR Spectroscopy, Second, enlarged edition, Horst Friebolin, VCH Publishers, 1993, pages 239-247.
- 3) Modern NMR Spectroscopy, A Guide for Chemists, Jeremy K.M. Saunders and Brian K. Hunter, Oxford University Press, 1987, pages 100-108.

EXPERIMENTAL SETUP

The concentration of the sample should be high enough that you can record a high signal to noise ^{13}C NMR spectrum in 64 - 128 scans. If this is not possible, then the number of scans for the 2-D experiment must be increased. The number required is to the nearest integer multiple of four to: $\text{NS}(2\text{D}) = \text{NS}(1\text{D}) / 16$.

Record a ^1H spectrum.

| | |
|------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| NEW (or EDC) | Create a new data set for your sample. |
| NAME name | Data set name. |
| EXPNO 1 | Experiment number (must be 1). |
| PROCNO 1 | Process data number (must be 1). |
| [SAVE] | Save the data set. |
| RPAR +proton all | Read in the standard proton parameters. |
| NS, etc. | Adjust NS and other parameters as necessary. |
| RGA, ZG | Acquire some data. |
| FT, APK, ref | Fourier transform, phase and reference the spectrum. |
| Zoom | Zoom in on the region of interest. The expanded region need not contain a reference peak. |
| [sw-sfo1] | Set the sweep width and spectrometer frequency to cover the zoomed region. Reduce TD if the acquisition time (AQ) is unnecessarily large. |
| RGA, ZG | Acquire a spectrum of the zoomed region. |
| FT, etc. | Fourier transform, phase etc. |

Record a ^{13}C spectrum.

| | |
|--------------|----------------------------------------|
| NEW (or EDC) | Create a new data set for your sample. |
| NAME name | Data set name. |
| EXPNO 13 | Experiment number (must be 13). |
| PROCNO 1 | Process data number (must be 1). |
| [SAVE] | Save the data set. |

If you have already collected a ^{13}C spectrum, skip the next section, set up a spectrum of a zoomed region, and acquire that.

| | |
|------------------|----------------------------------------------|
| RPAR +proton all | Read in the standard proton parameters. |
| NS, etc. | Adjust NS and other parameters as necessary. |
| RGA, ZG | Acquire some data. |

| | |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| FT, APK, ref | Fourier transform, phase and reference the spectrum. |
| Zoom | Zoom in on the region of interest. The expanded region need not contain a reference peak. |
| [sw-sfo1] | Set the sweep width and spectrometer frequency to cover the zoomed region. Reduce TD if the acquisition time (AQ) is unnecessarily large. |
| RGA, ZG FT, etc. | Acquire a spectrum of the zoomed region. Fourier transform, phase etc. |

Set up the ^{13}C - ^1H correlation experiment.

The following AU program sets up all of the parameters for hetcor.

XAU su_hetcor Set up magnitude mode hetcor. You must be attached to experiment 13 to execute this.

[OK] Answer "Delete `meta.ext` files ?" with [OK].

The AU program expects or sets the following file parameters. Use EDC to display the results.

| File parameter | | |
|----------------|---------------|------------------------------------------------------------------------------------------------------------------------|
| EXPNO | 1 13 14 | Experiment number 1, for 1-D proton. Experiment number 13, for 1-D carbon. Experiment number 14, for 2-D hetcor. |

The AU program sets the following acquisition parameters. Use EDA to display the results.

| Acquisition Parameters | | |
|------------------------|-------|---------------------------------------------------|
| Time domain 2 (F2) | | |
| PULPROG | hxco | PULse PROGram for hetcor. |
| TD | 1024 | Time Domain points. |
| NS | 8 | Number of Scans (integer multiple of 4). |
| DS | 16 | number of Dummy Scans for 1st row. |
| D1 | 2 sec | relaxation Delay. |
| CNST2 | 145 | One bond C-H coupling constant, J_{CH} . |

| Acquisition Parameters | | |
|------------------------|--------|----------------------------------------------------------|
| CNST11 | 3 | Used to calculate the refocusing delay, D3. |
| Time domain 1 (F1) | | |
| TD | 256 | Time Domain points. |
| ND0 | 2 | Number of D0 periods per cycle. |
| IN0 | 1 / SW | Increment in t1 (calculated). |
| SW | | SW of ¹ H spectrum collected in experiment 1. |
| SFO1 | | ¹ H frequency used in experiment 1. |

ASED

Check that acquisition parameters are set correctly. A brief description of other parameters, not described above, is given in the pulse program at the end of this document.
Save the acquisition parameters.

[SAVE]

EXPT

Calculates the approximate length of time to do the experiment. This may help you to decide if you want to collect more or fewer slices or scans or points etc. The Menthol spectrum required about 35 minutes.

DATA ACQUISITION

[Spin on/off]

TURN THE SPINNER OFF (press the Spin On/Off button on the BOSS keyboard).

ZG

Start the acquisition.

DATA PROCESSING

The AU program sets the following processing parameters. Use EDP to check or modify the values.

| Processing Parameters | | |
|-------------------------------------|------|---------------------------------------------------|
| Time/frequency domain 2 (F2) | | |
| SI | 1024 | the Size in F2 (zero fill rows). |
| WDW | EM | Exponential multiplication. |
| LB | 3 | 3 - 5 Hz. |
| PH_mod | NO | No phase correction. |
| PKNL | TRUE | Required with digital filter. |
| BC_mod | QUAD | Background correct quadrature data. |
| SF | | Spectrum reference frequency for ¹³ C. |
| Time/frequency domain 1 (F1) | | |
| SI | 512 | the Size in F1 (zero fill columns). |
| WDW | SINE | Sine multiplication. |
| SSB | 2 | 90° shifted sine bell. |
| PH_mod | MC | Magnitude calculation. |
| BC_mod | NO | No background correction. |
| MC2 | QF | Forward quadrature FFT. |
| SF | | Spectrum reference frequency for ¹ H. |

XFB

Background correct, window, zero fill, Fourier transform, phase and reference. The whole kaboodle in one command. It is OK to execute this command on a partial data set, during acquisition.

2-D CONTOUR DISPLAY

X-Y Expansion the spectrum.

| | |
|-----------|--------------------------------------------|
| [Limits] | Set the limits of the plot region. |
| [PlotReg] | Forces XWinNMR to display the plot region. |

Setting the intensity scale.

| | |
|---------------|--------------------------------------------------------------------------------------------------|
| [DefPlot] | Sets the intensity scale for plotting to be the same as the currently displayed intensity scale. |
| [contours] | Displays the equi-intensity contours of the data. |
| [intensities] | Displays ranges of intensity as a color map. |

PLOTTING

The AU program sets the following plotting parameters. Use EDG to check or modify the values. Once EDG has started, press either the [EDPROJ2] or [EDPROJ1] buttons.

| Plotting Parameters | | |
|------------------------------------------|----------|----------------------------------------------------|
| Projection for Frequency domain 2 | | |
| PF2DU | z | Disk Unit of the data set. |
| PF2USER | username | Your user name. |
| PF2NAME | name | Name of the data set. |
| PF2EXP | 1 | Experiment number of the ¹ H spectrum. |
| PF2PROC | 1 | Process number of the ¹ H spectrum. |
| Projection for Frequency domain 1 | | |
| PF1DU | z | Disk Unit of the data set. |
| PF1USER | username | Your user name. |
| PF1NAME | name | Name of the data set. |
| PF1EXP | 13 | Experiment number of the ¹³ C spectrum. |
| PF1PROC | 1 | Process number of the ¹³ C spectrum. |
| Additional plotting parameters | | |

Pulse Program for HETCOR

```
;hxco
;avance-version
;2D heteronuclear shift correlation
;A. Bax & G.A. Morris, J. Magn. Reson. 42, 501 (1981)
```

```
#include <Avance.incl>
```

```
"p2=p1*2"
"d0=3u"
"d2=1s/(cnst2*2)"
"d3=1s/(cnst2*cnst11)"
"d11=30m"
"d12=20u"
```

```
1 ze
2 d11
3 d1 do:f2
  d12 p12:f2
  (p3 ph1):f2
  d0
  p2 ph3
  d0
  d2
  (p3 ph2):f2 (p1 ph4)
  d3 p112:f2
  go=2 ph31 cpd2:f2
  d11 wr #0 if #0 id0 zd
  lo to 3 times tdl
  d2 do:f2
exit
```

```
ph1=0
ph2=0 2 1 3
ph3=0 0 0 0 2 2 2 2
ph4=0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
      2 2 2 2 2 2 2 2 3 3 3 3 3 3 3
ph31=0 2 1 3 0 2 1 3 1 3 2 0 1 3 2 0
        2 0 3 1 2 0 3 1 3 1 0 2 3 1 0 2
```

```
;p11 : f1 channel - power level for pulse (default)
;p12 : f2 channel - power level for pulse (default)
;p112: f2 channel - power level for CPD/BB decoupling
;p1  : f1 channel - 90 degree high power pulse
;p2  : f1 channel - 180 degree high power pulse
;p3  : f2 channel - 90 degree high power pulse
;d0  : incremented delay (2D) [3 usec]
;d1  : relaxation delay; 1-5 * T1
;d2  : 1/(2J(XH))
;d3  : 1/(3J(XH)) for all multiplicities
;d11 : delay for disk I/O [30 msec]
;d12 : delay for power switching [20 usec]
;cnst2: = J(XH)
;cnst11: = 3 for all multiplicities
;in0: 1/(2 * SW(H)) = DW(H)
;nd0: 2
;NS: 4 * n
;DS: 16
;td1: number of experiments
;MC2: QF
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
```