

# DOUBLE RESONANCE EXPERIMENTS

# SPIN DECOUPLING

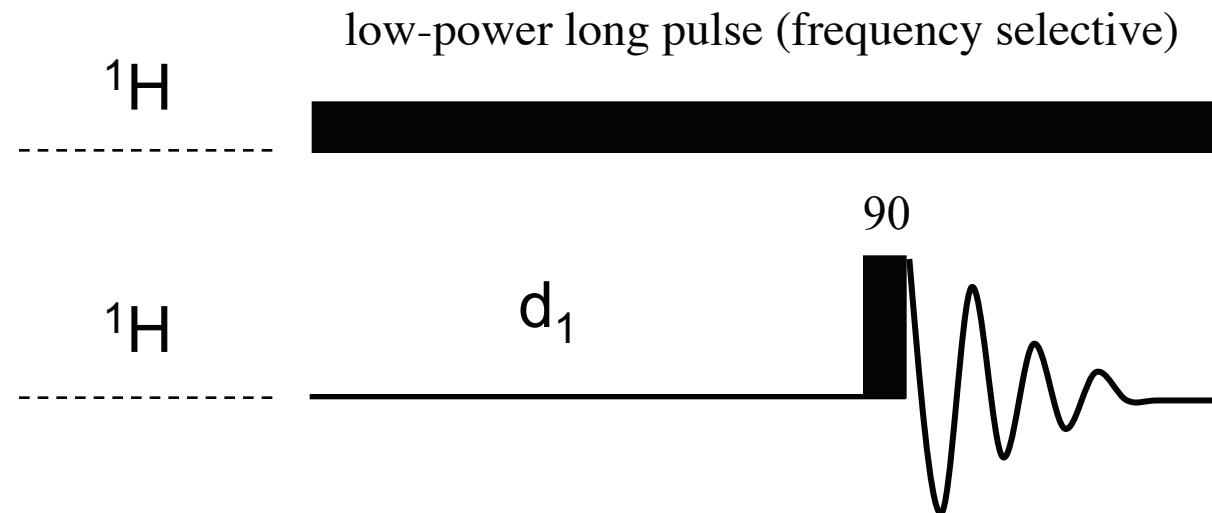
- It is often advantageous to reverse or remove the splitting caused by spin-spin coupling
- This is called *spin decoupling*
- Spin decoupling (or just “decoupling) can be used for several reasons
  - to simplify spectra
  - to assist in identification of coupling between nuclei
  - to improve signal-to-noise
- How do we decouple spins from one another?
- Remember, coupling occurs because the transitions of one spin ( $\alpha$  to  $\beta$  or  $\beta$  to  $\alpha$ ) ensue when the other (coupled) spin is in either the  $\alpha$  or  $\beta$  state
  - thus, to observe the coupling of one spin to another, the lifetime of the coupled spin in a given state must be long enough for the transitions of the other to occur
  - this lifetime ( $\tau_1$ ) must be greater than  $1/J$  ( $J$  is the scalar coupling constant)

$$\tau_1 > 1/J$$

- if this lifetime is significantly shortened, the coupling (splitting of the signal) will not be observed
- Two main types of decoupling: *selective* and *broad-band*

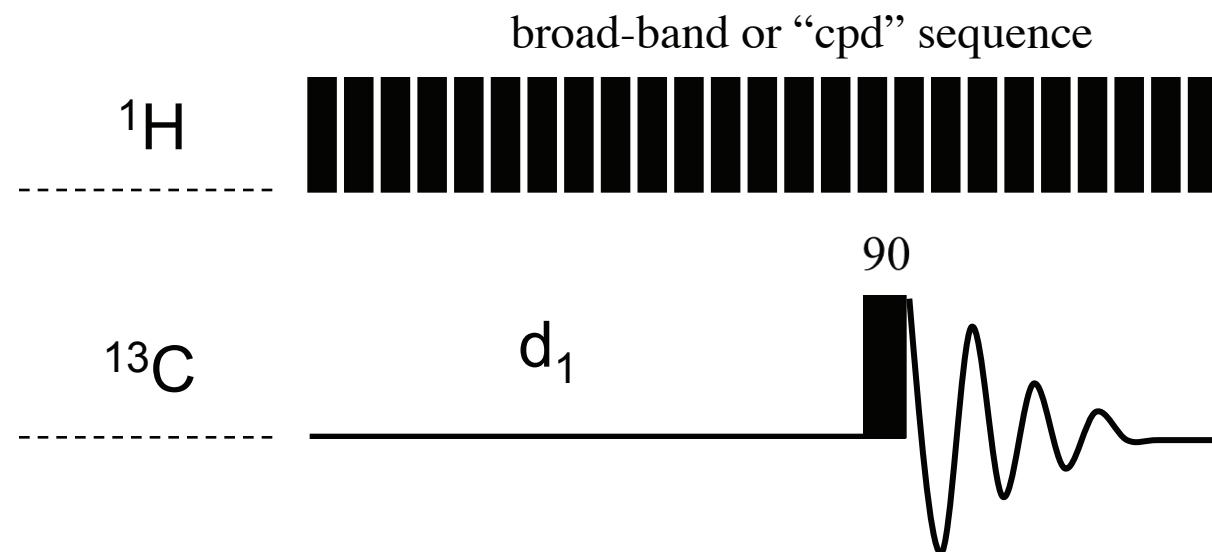
# SELECTIVE DECOUPLING

- selective decoupling
  - selective excitation is accomplished by a very long pulse centered at the Larmor frequency of the signal of interest (remember, the bandwidth is proportional to  $1/\tau_p$ , so a *long* pulse is highly *selective*)
  - the long pulse causes rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions such that the lifetime of any particular spin in any given state is short (i.e.  $\tau_1 < 1/J$ )
  - selective excitation of one signal (spin) in an NMR spectrum leads to multiplet collapse and simplification of the spin-spin splitting patterns of the signals from the nuclei to which the spin is coupled



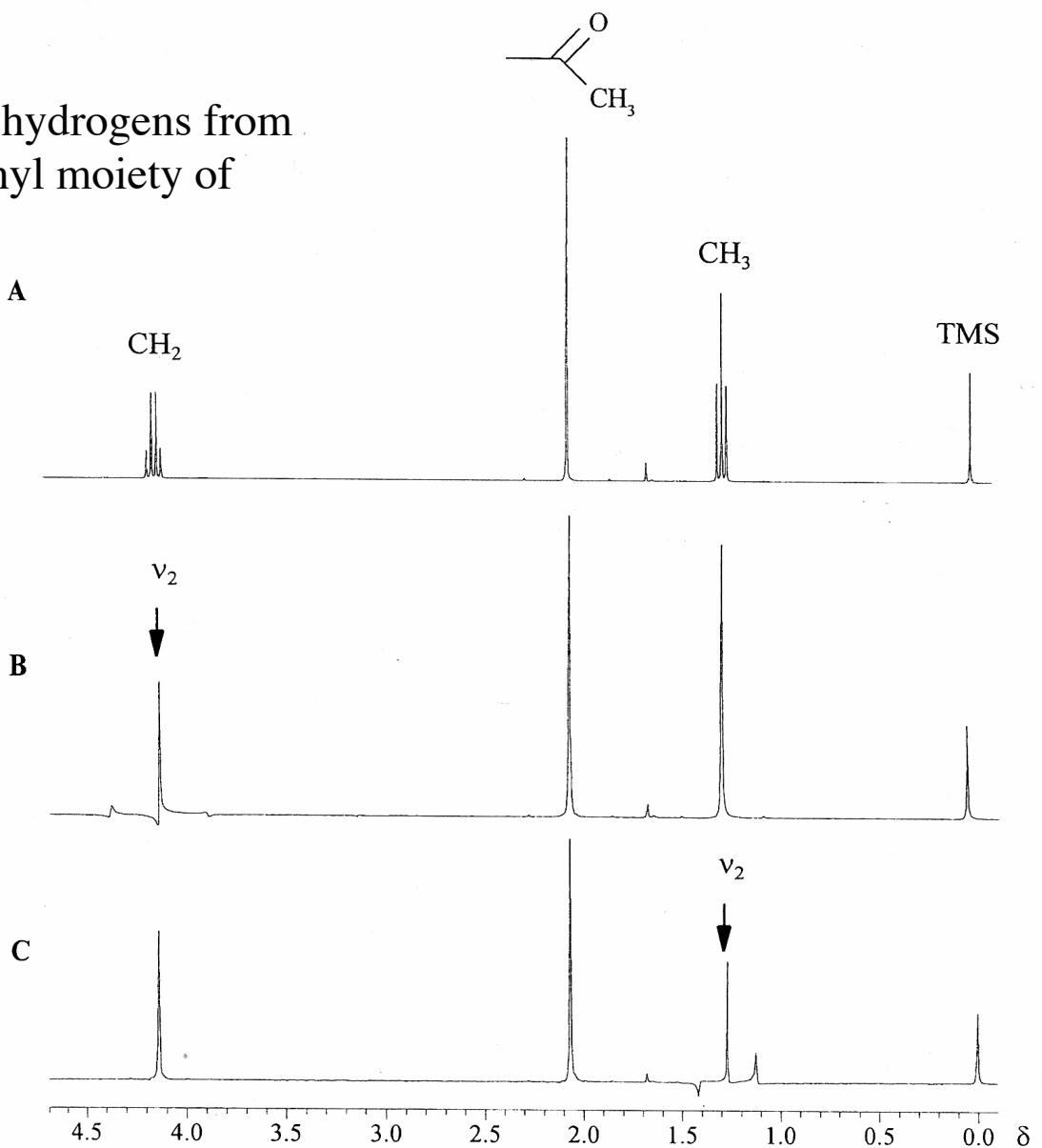
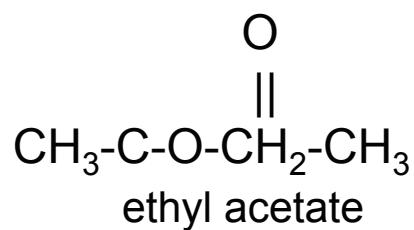
# BROAD-BAND DECOUPLING

- broad-band decoupling (or *composite pulse decoupling*, cpd)
  - excitation of a large frequency range using a train of short pulses (short pulses excite a large bandwidth) applied in rapid succession
  - the rapid succession of pulses causes rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions such that the lifetime of any particular spin in any given state is short (i.e.  $\tau_1 < 1/J$ )
  - broadband excitation of all frequencies corresponding to a particular nucleus type (i.e. all hydrogens) leads to multiplet collapse and simplification of the spin-spin splitting patterns of the signals from coupled nuclei
- often it is useful to remove *all* coupling of a given type
- for instance, it is often useful in  $^{13}\text{C}$  NMR/spectra to remove all coupling from  $^1\text{H}$



# SELECTIVE SPIN DECOUPLING

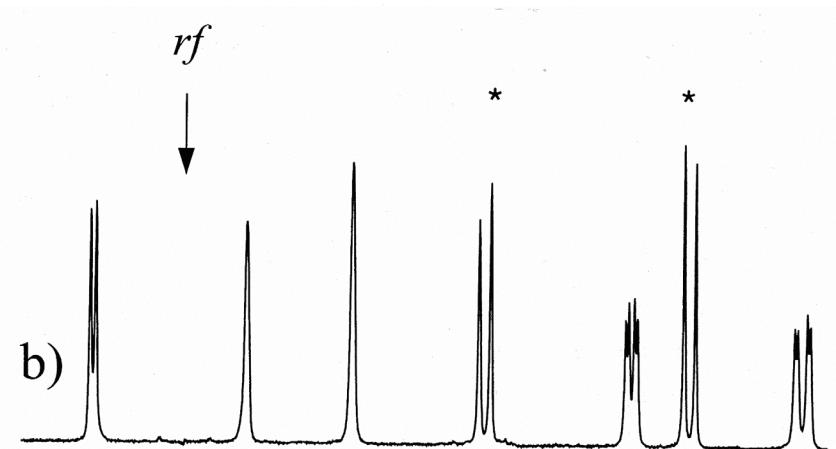
- trivial example
  - selective decoupling of methyl hydrogens from methylene hydrogens in the ethyl moiety of ethyl acetate



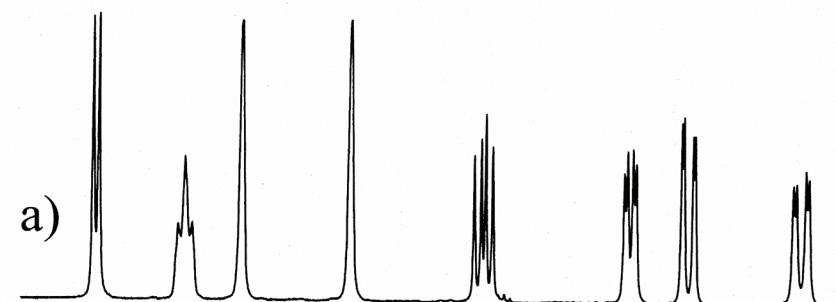
# SELECTIVE SPIN DECOUPLING

- selective decoupling can identify signals from coupled nuclei, thus assisting to establish connectivity/structure
- intensities of signals from coupled nuclei are improved (due to multiplet collapse)
  - integrals are unchanged for signals from coupled nuclei

selective decoupling

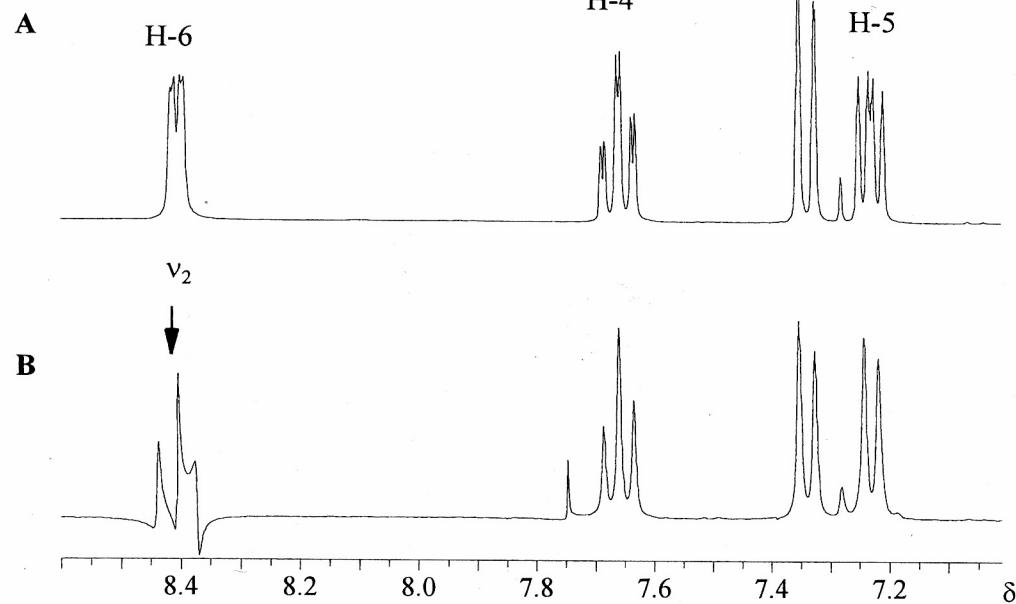
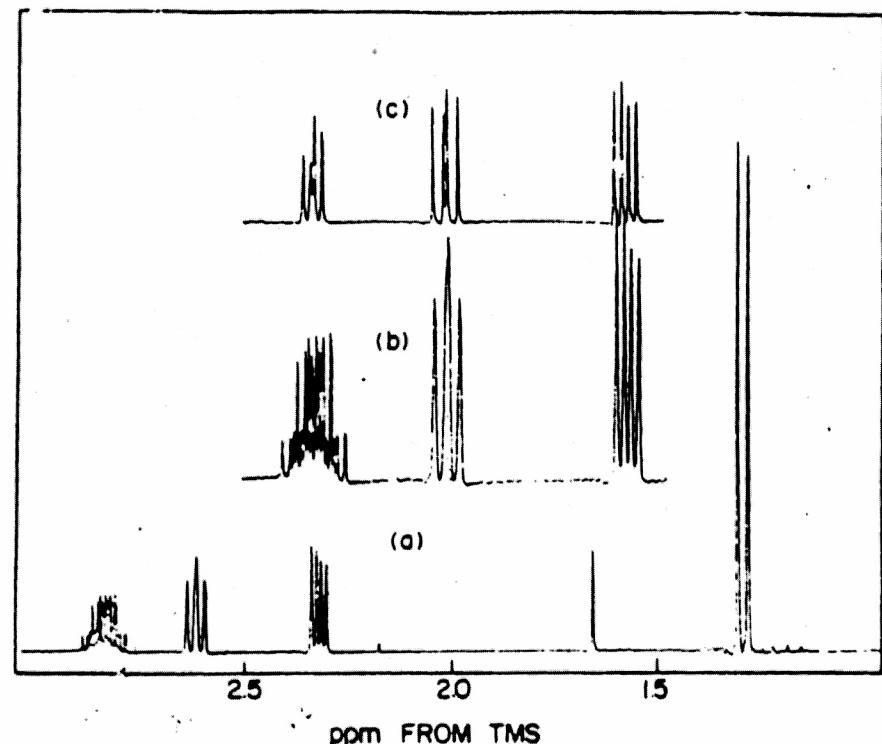
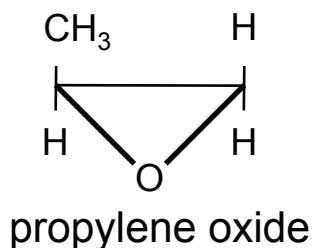


no decoupling

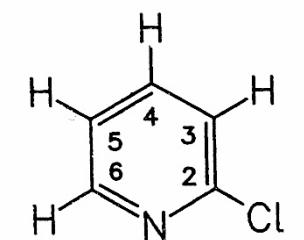


## SELECTIVE SPIN DECOUPLING

- (right) selective decoupling of the methyl hydrogens (1.3 ppm) of propylene oxide confirms identity of signal from methine H (2.8 ppm)



- (left) selective decoupling of H-6 of 2-chloropyridine leads to assignment of signals from H-3, H-4 and H-5



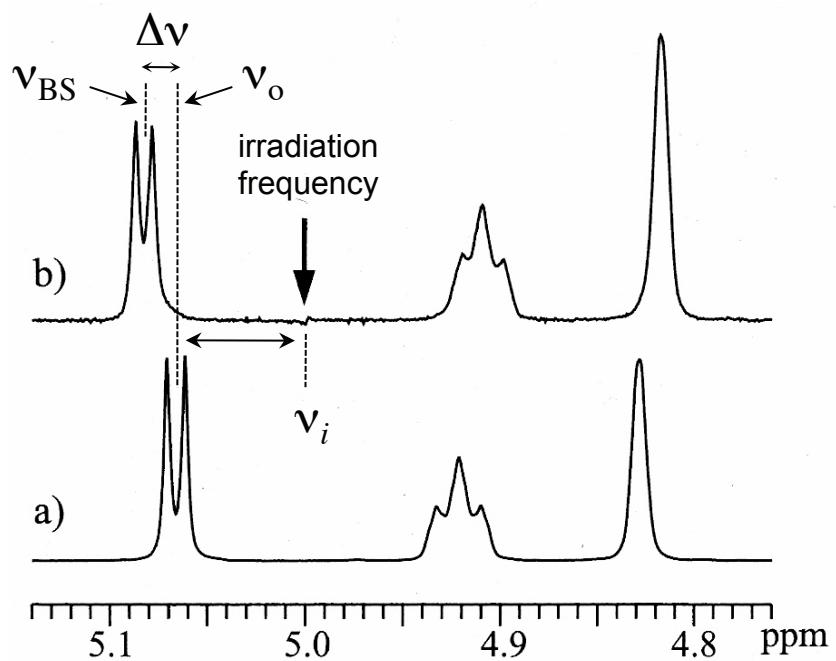
## BLOCH-SIEGERT EFFECT

- In selective homonuclear decoupling experiments, if the decoupling field is applied during the acquisition of the FID, signals in the spectrum ('b', below) can move relative to their positions in the control spectrum ('a', below) where no decoupling field is applied
- This is demonstrated in the spectra shown (below), where the arrow indicates the frequency at which the decoupling field was applied for spectrum 'b'
- This effect is known as the Bloch-Siegert effect. The shifts in positions of signals are known as Bloch-Siegert shifts
- The effective field experienced by a nucleus is altered by the presence of the decoupling field, thus causing the frequency changes
- It is not observed if the decoupling field is turned off prior to acquisition
- If the decoupling field is not too strong (if  $B_2 \ll (\nu_o - \nu_i)$ ), then the Bloch-Siegert shifts can be easily calculated:

$$\Delta\nu = \nu_{BS} - \nu_o = \frac{B_2^2}{2(\Delta B)} = \frac{B_2^2}{2(\nu_o - \nu_i)}$$

$B_2$  is the decoupling field strength in Hz

- In many homonuclear (selective) decoupling experiments, the requirement for selectivity keeps  $B_2$  small, but if  $B_2$  is large and is not turned off during acquisition, Bloch-Siegert shifts can be very large

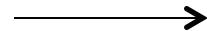


# BROAD-BAND DECOUPLING

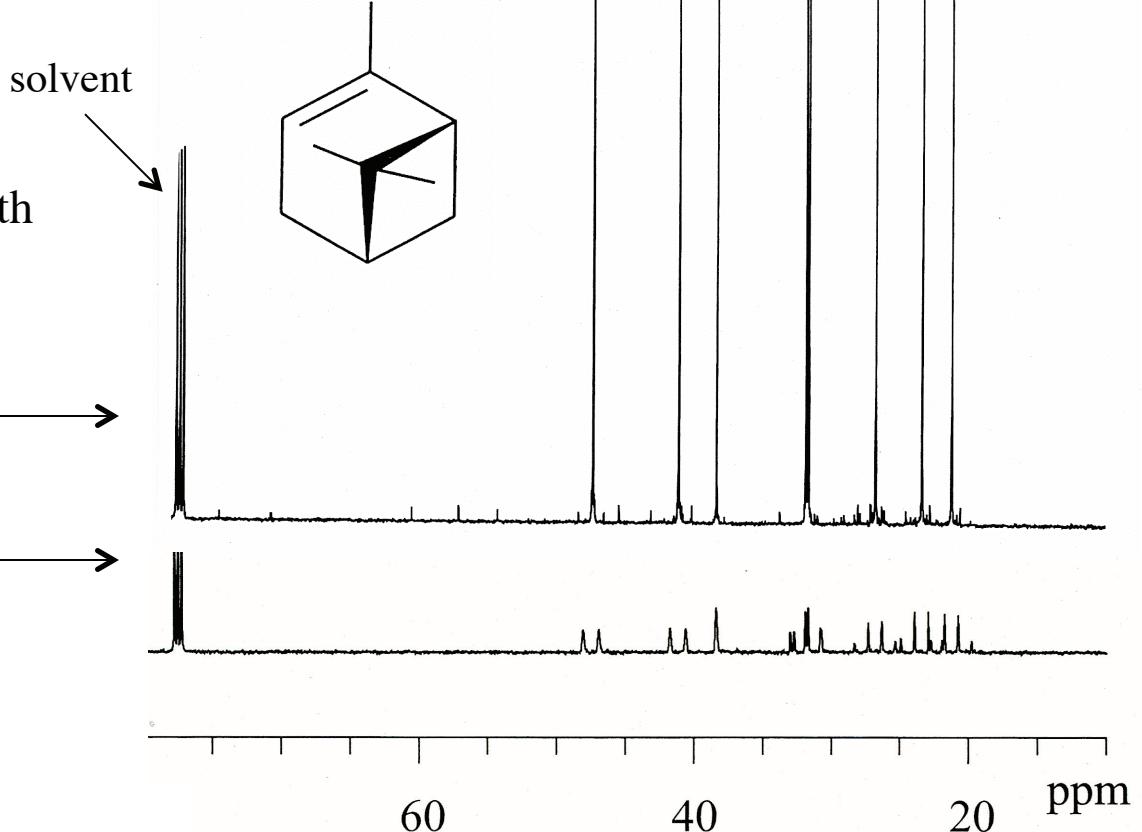
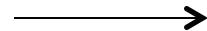
- Substantial spectral simplification (multiplets collapse to singlets)
- Substantial signal-to-noise gains
- Signal-to-noise gains arise from two sources:
  - collapse of the multiplet into a singlet
  - nuclear Overhauser effect (NOE)
- The signal-to-noise enhancement by the NOE is not uniform, so quantitative information is lost

- example: acquisition of  $^{13}\text{C}$  spectrum of alpha pinene with broad-band  $^1\text{H}$  decoupling

- broad-band  
 $^1\text{H}$  decoupling

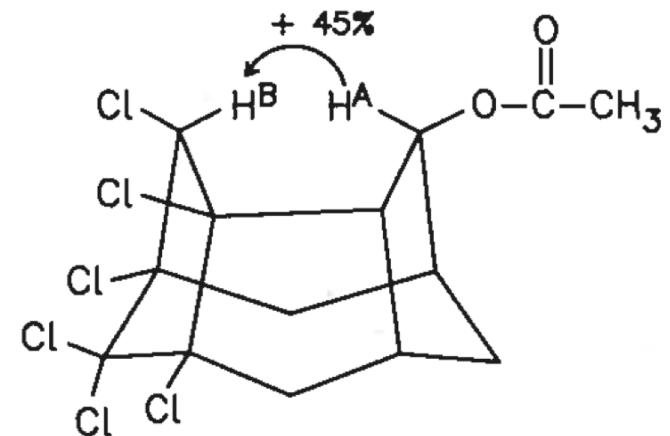


- no decoupling



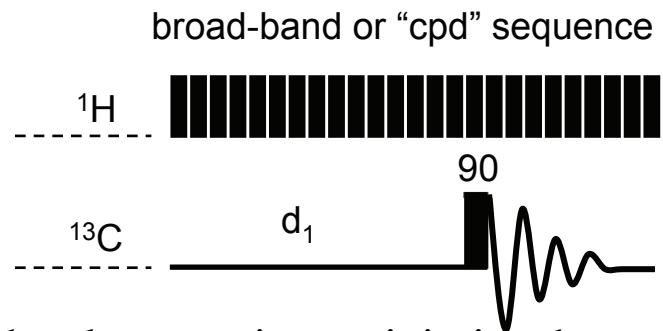
# BROAD-BAND DECOUPLING: INTRODUCTION TO THE NUCLEAR OVERHAUSER EFFECT

- The nuclear Overhauser effect (NOE) is the change in intensity of the NMR signal from a nucleus when the spin populations of a nearby nucleus are changed (for instance, by an electromagnetic pulse)
- This is a *through space* effect (independent of the presence of a chemical bond)
- The effect is *distance dependent* (upper internuclear distance limit of 5-6 Å); shorter distance, larger effect
- The effect is *time dependent*: it does not develop instantaneously
- The effect is closely tied to relaxation (**much more later in semester**)
- For broadband decoupling of <sup>1</sup>H from <sup>13</sup>C:
  - the effect (signal-to-noise-enhancement) is proportional to number of <sup>1</sup>H nuclei attached to a given <sup>13</sup>C nucleus
  - effect is proportional to density of <sup>1</sup>H nuclei (attached or not attached) surrounding a given <sup>13</sup>C nucleus
  - <sup>13</sup>C signal may be increased up to 200% by nuclear Overhauser effect
  - quantitative information lost because relative integral values are dependent on the signal-to-noise enhancement by the NOE which is not uniform for all <sup>13</sup>C nuclei



# BROAD-BAND DECOUPLING

- The broadband decoupling or “cpd” sequence can be as simple as a series of 90 or 180 degree pulses, or quite sophisticated, employing multiple pulse angles, multiple pulse phases, and “shaped” pulses (non-rectangular pulse profiles)
- A goal of cpd (*composite pulse decoupling*) sequence development is to minimize the effects of pulse imperfections, maximize the exitation bandwidth and minimize pulse power (decoupling pulse strength, or  $B_2$  field strength) to avoid problems such as sample heating
- These sequences have “interesting” names, such as MLEV-16 (“Malcolm Levitt”), WALTZ-16 (“1-2-3, 1-2-3”), GARP (“Globally Optimized Alternating Phase Rectangular Pulse”), DIPSI-2, SEDUCE, FLOPSY-8, etc.
- Examples: MLEV-16, WALTZ-16 and GARP



**MLEV - 16:** RR $\overline{R}$ R $\overline{R}$ RRR $\overline{R}$  RRRR, where  $R = 90_x \ 180_y \ 90_x$  and  $\overline{R} = 90_{-x} \ 180_{-y} \ 90_{-x}$

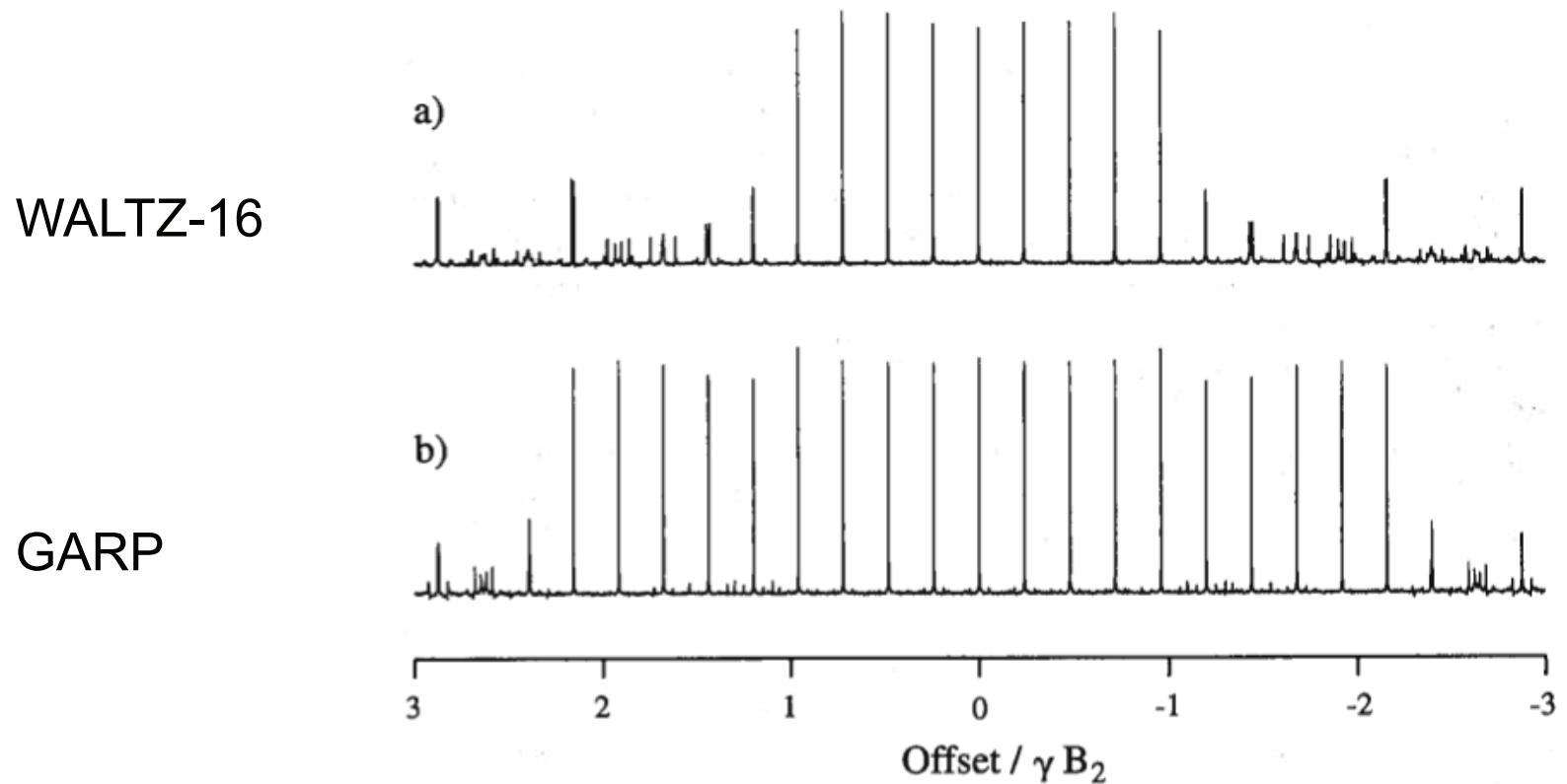
**WALTZ - 16:** Q $\overline{Q}$ QQ, where  $Q = 270_{-x} \ 360_x \ 180_{-x} \ 270_x \ 90_{-x} \ 180_x \ 360_{-x} \ 180_x \ 270_{-x}$

**GARP:** RRR $\overline{R}$  $\overline{R}$

where  $R = 30.5 \ \overline{55.2} \ 257.8 \ \overline{268.3} \ 69.3 \ \overline{62.2} \ 85.0 \ \overline{91.8} \ 134.5 \ \overline{256.1} \ 166.4 \ \overline{45.9} \ 25.5$   
 $\overline{72.7} \ 119.5 \ \overline{138.2} \ 258.4 \ \overline{64.9} \ 70.9 \ \overline{77.2} \ 98.2 \ \overline{133.6} \ 255.9 \ \overline{65.6} \ 53.4$

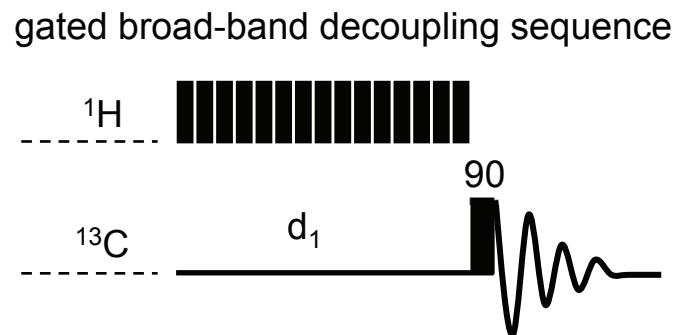
## BROAD-BAND DECOUPLING

- “Decoupling offset profiles” indicate the effective decoupling bandwidth ( $\gamma B_2$ )
- Example: note the bandwidth of GARP is nearly twice that of WALTZ-16



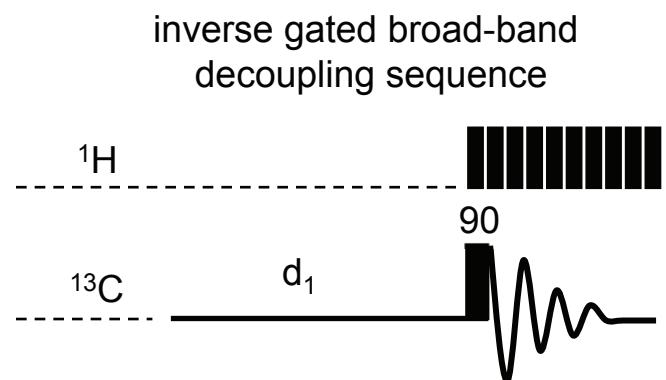
# BROAD-BAND DECOUPLING: GATED BROAD-BAND DECOUPLING

- The gated broad-band decoupling experiment is designed to:
  - improve signal-to-noise via the NOE (or nOe)
  - retain coupling information (retain signal multiplets)
- The NOE develops during the time that decoupling is performed (during the  $d_1$  delay time)
- $^1\text{H}$ - $^{13}\text{C}$  coupling returns *instantaneously* when decoupling is ceased (just before acquisition), so coupling information ( $^{13}\text{C}$  signal splitting due to attached hydrogens) is present in the spectra
- The signal enhancement from the NOE begins to be lost after decoupling ceases, but the loss depends on  $T_1$  relaxation times, which are generally long for  $^{13}\text{C}$  in small molecules, so losses are minimal
- *Signal-to-noise enhancement by the NOE is not uniform for all  $^{13}\text{C}$  nuclei, so quantitative information is lost*
- So, the gated broad-band decoupling sequence is useful when both high signal-to-noise (from the NOE) and coupling information (signal splitting) are desired or required



# BROAD-BAND DECOUPLING: INVERSE (“QUANTITATIVE”) GATED BROAD-BAND DECOUPLING

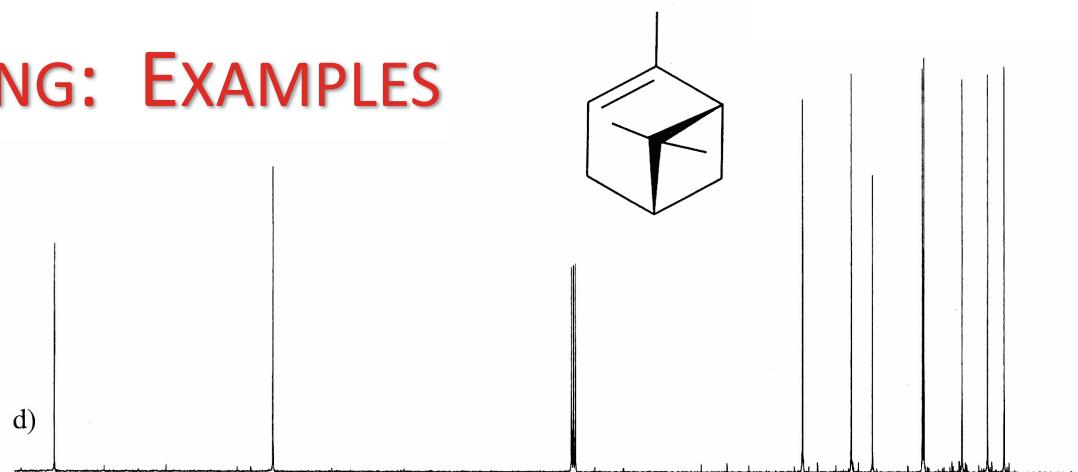
- The inverse gated broad-band decoupling experiment is designed to:
  - *minimize* signal-to-noise enhancement by the NOE
  - remove signal splitting (thereby improving signal-to-noise due to multiplet collapse)
- The NOE does *not* develop during  $d_1$  because broad-band decoupling is *not* applied during this time
- $^1\text{H}$ - $^{13}\text{C}$  decoupling commences when acquisition begins), so there is *no* signal splitting from  $^1\text{H}$ - $^{13}\text{C}$  coupling
- Acquisition times are kept short to minimize development of signal-to-noise enhancement from the NOE that can develop when decoupling is on (i.e. during acquisition)
- Because signal-to-noise enhancement by the NOE is minimized, quantitative information is available from integrals of the signals
- So, overall, coupling information (signal splitting) is lost, signal-to-noise is improved due to multiplet collapse, and integration can give reliable quantitative information



# BROAD-BAND DECOUPLING: EXAMPLES

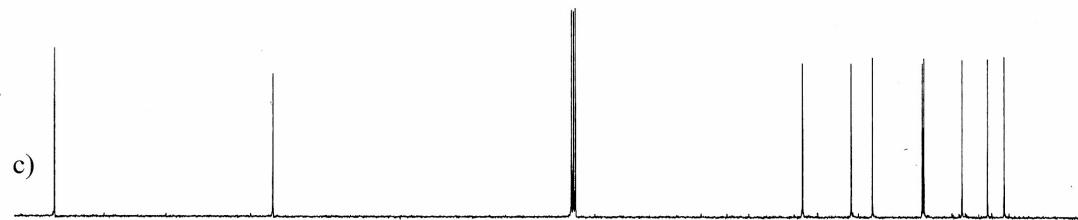
*broad-band decoupling*

-decoupled spectrum, with NOE



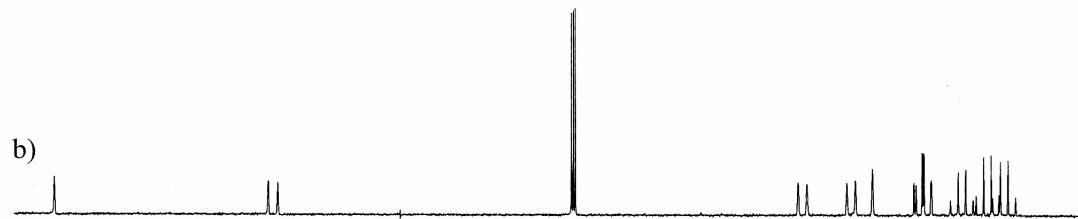
*inverse gated broad-band decoupling*

-decoupled spectrum, no NOE



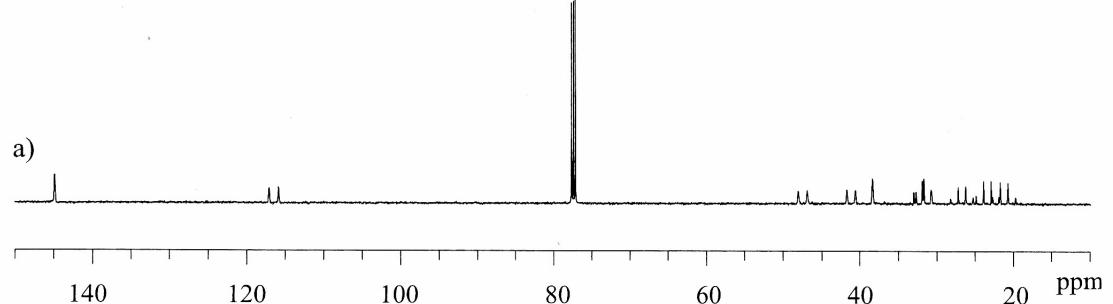
*gated broad-band decoupling*

-coupled spectrum, with NOE



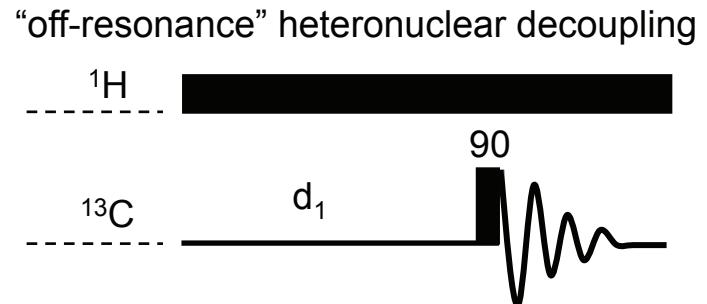
*no decoupling*

-coupled spectrum, no NOE



## OTHER DECOUPLING: OFF-RESONANCE DECOUPLING

- $^{13}\text{C}$  spectra are routinely subjected to  $^1\text{H}$  broad-band decoupling in order to improve signal-to-noise via the NOE and resolve overlapped signals
- This often allows unambiguous identification of individual signals and their chemical shifts at the expense of loss of coupling information (multiplet structure) useful for identification of -CH, -CH<sub>2</sub>, and -CH<sub>3</sub> groups
- There is a compromise method, “off-resonance”  $^1\text{H}$  decoupling of  $^{13}\text{C}$  spectra. The utility of this method stems from the following attributes:
  - it permits some signal to-noise enhancement via the NOE
  - it permits some coupling information to remain in order to assist in identification of CH, -CH<sub>2</sub>, and -CH<sub>3</sub> groups
  - it also reduces heteronuclear ( $^1\text{H}$ - $^{13}\text{C}$ ) coupling constants so as to reduce overlap of multiplets and thus help resolve individual signals
- In this off-resonance decoupling method, a low power (but not *too* low)  $^1\text{H}$  “pulse” is applied at some frequency in the  $^1\text{H}$  spectral window where there are no signals (hence, “off” resonance)
- Often this is at one edge of the spectrum
- In the  $^{13}\text{C}$  spectrum, some NOE enhancement is observed,  $^1\text{H}$ - $^{13}\text{C}$  couplings are reduced, multiplet structures are (normally) preserved but spectral overlap (multiplet overlap) is reduced

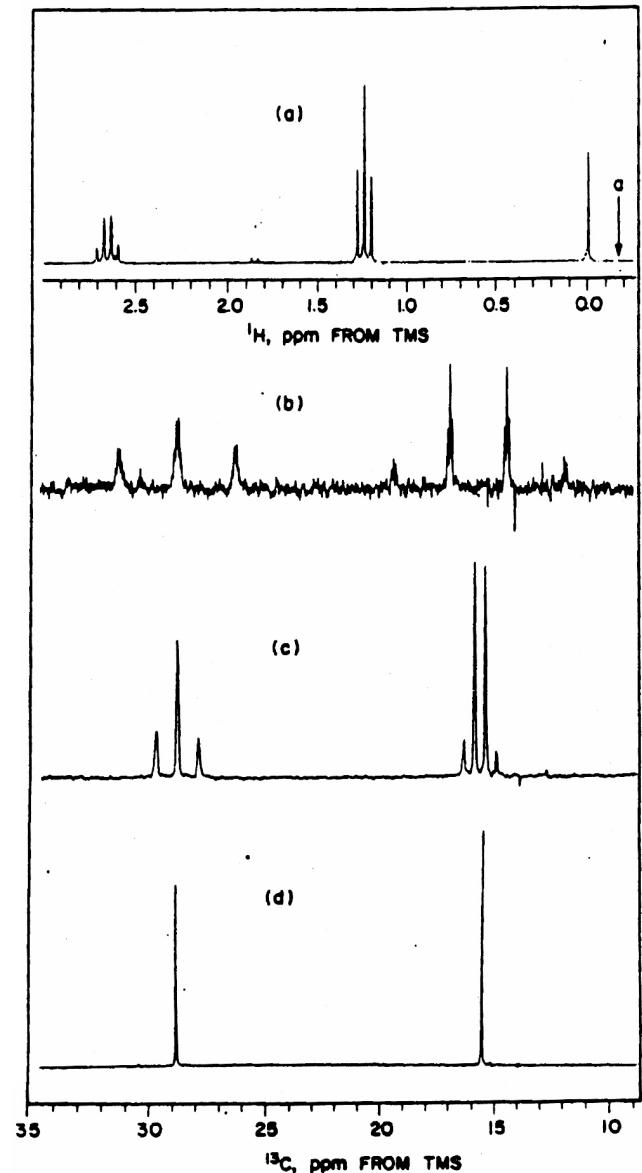


## OTHER DECOUPLING: OFF-RESONANCE DECOUPLING

- $^1\text{H}$  ('a'), and  $^{13}\text{C}$  ('b', 'c', and 'd') spectra of the ethyl group of ethylbenzene are shown
- As indicated in the  $^1\text{H}$  spectrum 'a', a low power pulse is applied at approximately -0.2 ppm (indicated in the spectrum) during the entire experiment
- In 'b' is shown the normal (no decoupling)  $^{13}\text{C}$  spectrum (note the large  $^1\text{H}$ - $^{13}\text{C}$  couplings in the triplet and quartet)
- In 'c' is shown the off-resonance decoupled spectrum
  - note the decreased  $^1\text{H}$ - $^{13}\text{C}$  coupling constants, and how this could be important for spectra where multiplets are overlapped
  - note the improvement in signal-to-noise from the NOE
- In 'd' is shown the broad-band decoupled  $^{13}\text{C}$  spectrum
- The magnitude of the observed  $^1\text{H}$ - $^{13}\text{C}$  coupling constant ( $J_r$ , r for "reduced") in an off-resonance decoupled spectrum is related to the actual coupling constant ( $J$ ), the decoupler field strength ( $B_2$ , in Hz) and the frequency offset between the decoupler field and the  $^1\text{H}$  signal of interest ( $\Delta\nu$ , Hz):

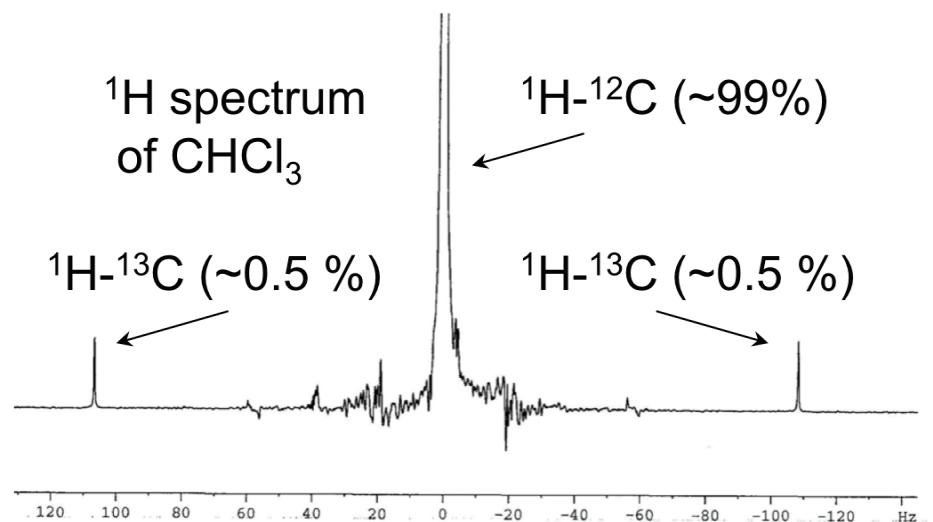
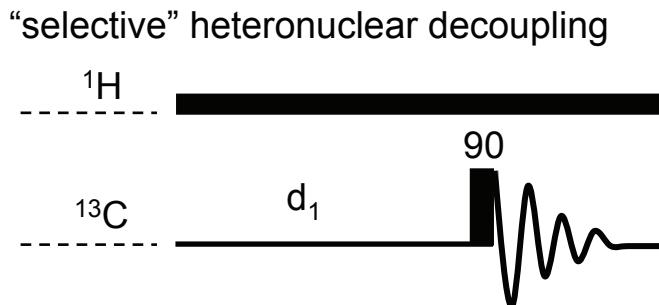
$$B_2 = \frac{\Delta\nu\sqrt{J^2 - J_r^2}}{J_r} \approx \frac{J\Delta\nu}{J_r}$$

the approximation holds only  
when  $B_2 \gg \Delta\nu$



## OTHER DECOUPLING: SELECTIVE HETERONUCLEAR DECOUPLING

- Decoupling  $^1\text{H}$  from  $^{13}\text{C}$  can also be performed selectively
- The frequency of a selected signal in the  $^1\text{H}$  spectrum is saturated, and the  $^{13}\text{C}$  signal of the  $^{13}\text{C}$  bound to that hydrogen collapses to a singlet
- This is very useful for correlating the signals in the  $^1\text{H}$  and  $^{13}\text{C}$  spectra (deciding which hydrogens are bound to which  $^{13}\text{C}$  nuclei)
- This is done in a manner very similar to “off-resonance” decoupling, except the decoupling pulse frequency is “on” some resonance (signal), and the power level generally is lower
- It is important that the signal(s) in the  $^1\text{H}$  spectrum corresponding to  $^1\text{H}$  bound to  $^{13}\text{C}$  are those that are saturated with the low power  $^1\text{H}$  pulse (not the  $^1\text{H}$ - $^{12}\text{C}$  signal/peak)
- The signal corresponding to  $^1\text{H}$ - $^{13}\text{C}$  is split by  $^1J_{^1\text{H}-^{13}\text{C}}$  coupling constant, and the peaks lie on either side of the  $^1\text{H}$ - $^{12}\text{C}$  peak
- These peaks are sometimes called the “satellite” peaks or “ $^{13}\text{C}$ -satellite” peaks



# OTHER DECOUPLING: SELECTIVE HETERONUCLEAR DECOUPLING

- In 'A' is shown the  $^1\text{H}$  spectrum of crotonic acid, and the  $^{13}\text{C}$  spectrum is shown in 'B'
- If the  $^{13}\text{C}$  satellites in 'A' corresponding to the methyl group are saturated, the upfield quartet in 'B' collapses to a singlet

*off-resonance decoupling  
( $^1\text{H}$  decoupling frequency  
 $\nu_2$  at 0 ppm as in figure)*

*off-resonance decoupling  
( $^1\text{H}$  decoupling frequency  
 $\nu_2$  at 7.5 ppm as in figure)*

*selective heteronuclear  
decoupling of  $^1\text{H}$  from  
 $^{13}\text{C}$  in methyl group*

$^{13}\text{C}$  spectrum (no decoupling)

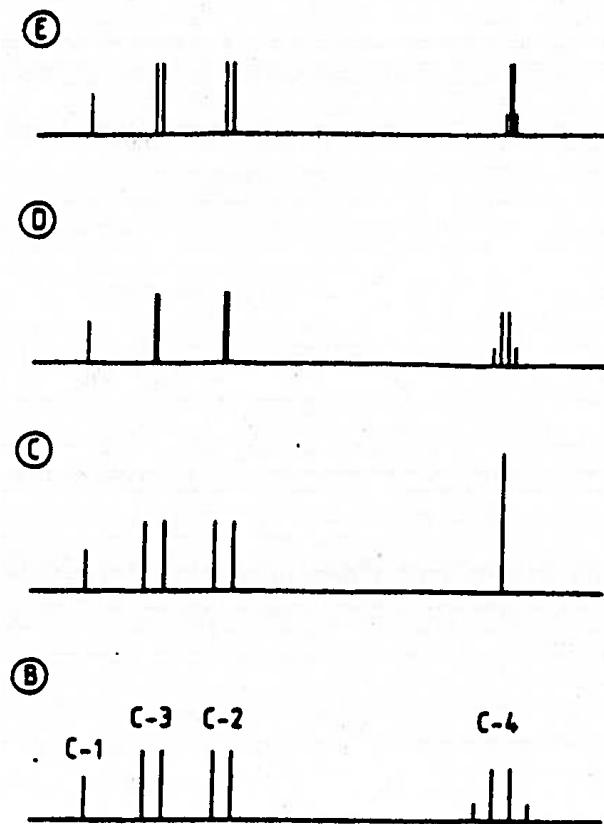
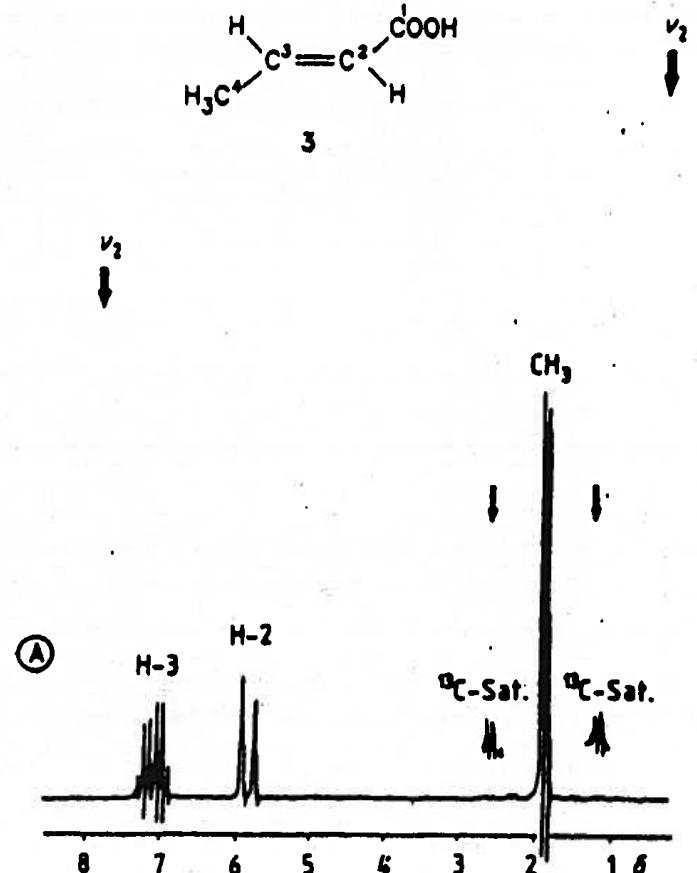
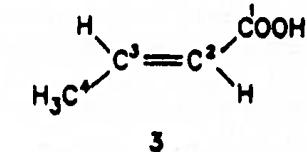


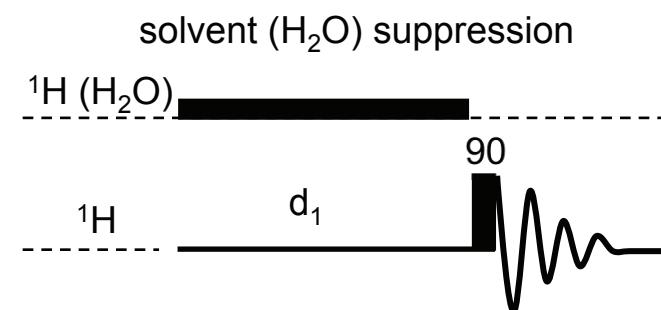
Figure 5-7.

A: 90 MHz  $^1\text{H}$  NMR spectrum of crotonic acid (3).  
 B to E:  $^{13}\text{C}$  NMR stick spectra of 3; B: without  $\text{C}, \text{H}$  decoupling;  
 C: selective  $^1\text{H}$  decoupling of the methyl protons;  
 D:  $^1\text{H}$  off-resonance decoupling, with decoupling frequency  $\nu_2$  at the left-hand end of the proton spectrum



## OTHER DECOUPLING: SOLVENT ( $H_2O$ ) SUPPRESSION

- $^1H$  NMR in protonated solvents is problematic
- The concentration of  $H_2O$  in  $H_2O$  is  $\sim 55\text{ M}$  ( $^1H$  concentration  $\sim 110\text{ M}$ )
- Signals from other molecules are obscured by the large  $H_2O$  signal
- One way to attenuate the  $H_2O$  signal is by “decoupling”, or saturating the resonance (called solvent suppression by “saturation” or “presaturation”)
- A long, selective, low power pulse is used to saturate selectively the  $H_2O$  frequency, which greatly attenuates the signal in the spectrum



$^1H$  NMR spectrum of a 1 mM solution of a protein molecule

←no water suppression

with water suppression→

