# Edited $^{13}C$ Spectra - APT and DEPT

APT and DEPT are techniques for $^1H$-decoupled $^{13}C$ spectra which use the phase (normal or upside-down) or selective deletion (certain peaks missing) of the $^{13}C$ peaks as a way to encode information about the number of protons attached to a carbon (C, CH, $CH_2$ or $CH_3$). These spectra are called "edited" because the peak intensity and phase is modified relative to a normal $^{13}C$ spectrum. There has been much discussion about which experiment to use: APT or DEPT. APT gives all of the information of a normal carbon spectrum with somewhat reduced sensitivity, and it tells you if the number of attached protons is odd ($CH_3$ or CH) or even ($CH_2$ or quaternary). DEPT is much more sensitive than a normal carbon spectrum, and it can unambiguously identify the $CH_3$, $CH_2$ and CH carbon peaks. This requires acquiring and processing three separate spectra, however, and does not detect the quaternary carbons or solvent at all.
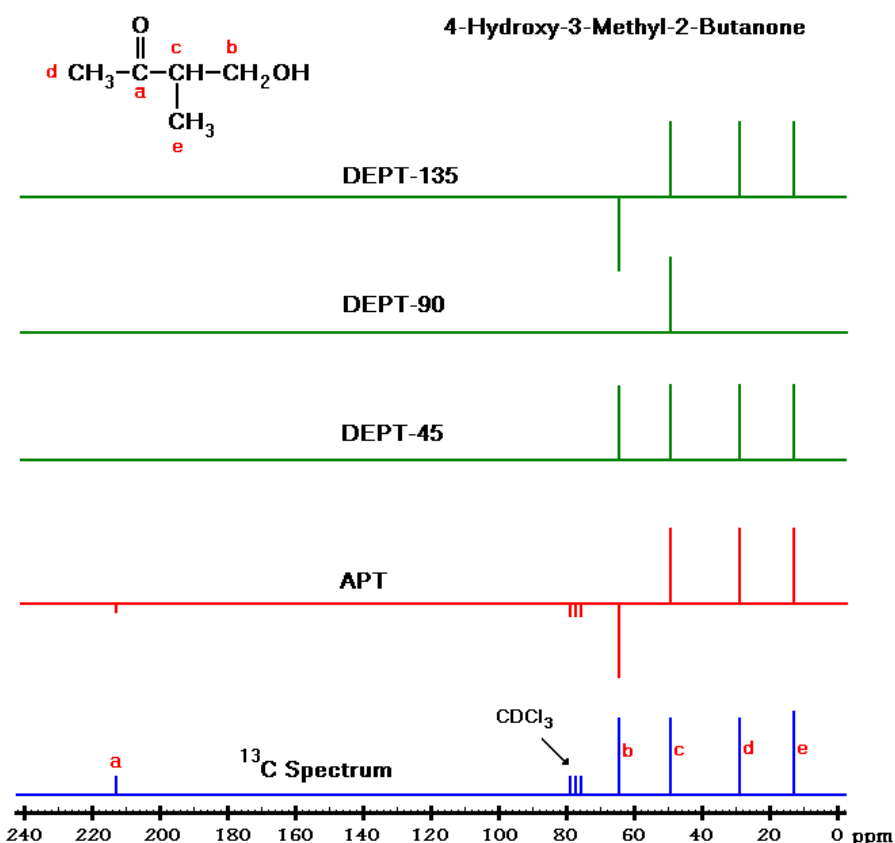


The diagram shows the expected results of a normal $^{13}C$ spectrum, an APT spectrum, and a full DEPT analysis of 4-hydroxy-3-methyl-2-butanone. The APT spectrum shows all carbons including the quaternary C=O and solvent carbons, and sorts the carbons into categories of CH and $CH_3$ ("up" peaks) and quaternary and $CH_2$ ("down" peaks). Note that sometimes APT spectra are presented "upside-down" with CH and $CH_3$ peaks "down" and quaternary and $CH_2$ peaks "up".

Only the DEPT-90 experiment can distinguish between $CH_3$ carbons and CH carbons. Taken together, the three DEPT experiments can unambiguously assign all "protonated" (i.e., non-quaternary) carbons to one of the three categories CH, $CH_2$ and $CH_3$. Note that the quaternary C=O and solvent carbons are missing from all DEPT spectra.

A comparison performed on a test sample of 43 mg cholesterol (MW 386.7) in $CDCl_3$ on the Varian Unity-300 will give you some idea of the relative time requirements for these experiments. The times required for more dilute samples were estimated from the 43 mg data.

Note that a $^{13}$C **and** a full DEPT analysis takes less acquisition time than a single APT spectrum with the same signal-to-noise ratio.

| mg | mM | S/N | $^{13}$C | APT | DEPT-135 | Full DEPT |
|----|-----|-----|----------|----------|----------|-----------|
| 43 | 148 | 16 | 4.1 min | 10.3 min | 1.2 min | 4.3 min |
| 10 | 34.5 | 16 | 1.3 hr | 3.2 hr | 22 min | 1.3 hr |
| 5 | 17.2 | 16 | 5.1 hr | 12.7 hr | 1.5 hr | 5.3 hr |
| 1 | 3.4 | 16 | 5.3 days | 13.2 days | 37 hr | 5.5 days |

These numbers can be used as a rough guide for $^{13}$C-detected experiments if you compare the concentration (in mM) of your sample rather than the weight (in mg). If you can live with an S/N of 4, these experiment times can be cut in half (S/N is proportional to the square root of the number of transients). Clearly, for a 5 mg sample of molecular weight similar to cholesterol, the DEPT-35 is the most practical experiment. For a 1 mg sample size an inverse 2D $^{13}$C-$^{1}$H correlation experiment is probably the only feasible way to get $^{13}$C chemical shifts.

**APT (Attached Proton Test) spectrum.** If you only want to do one $^{13}$C spectrum, and quaternary peaks are important, an APT is the experiment of choice. Insert the sample, lock and shim, set up for a routine $^{13}$C spectrum, and acquire a short $^{13}$C spectrum and phase-correct it. Even if you can only see solvent peaks, this will give you good starting parameters for the APT spectrum. Enter **apt**. This runs a macro (short computer program) which sets up the parameters for the APT experiment and displays some information about the experiment and its parameters.

| | |
|---|---|
| **d1=1** | relaxation delay - longer to see quaternary carbons better |
| **d2=0.0071** | set d2 delay to 1/J where J is the one-bond CH coupling (~140 Hz) |
| **p1** | set to the 180$^{o}$ $^{13}$C pulse at power **tpwr** (twice the 90$^{o}$ pulse, see blackboard) |
| **pw** | normal $^{13}$C pulse for simple 1D spectrum |
| **d3=0.001** | short delay |
| **dm='yny'** | decoupler off during the first d2 delay |
| **nt** | set to acquire enough scans for a good $^{13}$C spectrum |

Enter **go** to start acquisition. Save and transform the FID, **without using aph**. The deuterated solvent peak should be upside-down; if you have to phase-correct, do it carefully and only phase one peak in the spectrum. The quaternary and $CH_2$ carbons peaks should be "down", while the CH and $CH_3$ carbon peaks will be "up". Plot the spectrum with **vp** (vertical position) set at mid-screen (**vp=75**) to allow for the negative peaks.

**DEPT (Distortionless Enhancement by Polarization Transfer).** The DEPT spectrum is much more sensitive than a normal $^{13}$C spectrum because the observed carbon signal originates from an attached proton and is transferred to the carbon ("polarization transfer"). The down-side to this is that carbons without attached protons (quaternary carbons) cannot be observed. A full DEPT analysis involves acquiring three spectra, varying the parameter **mult**:

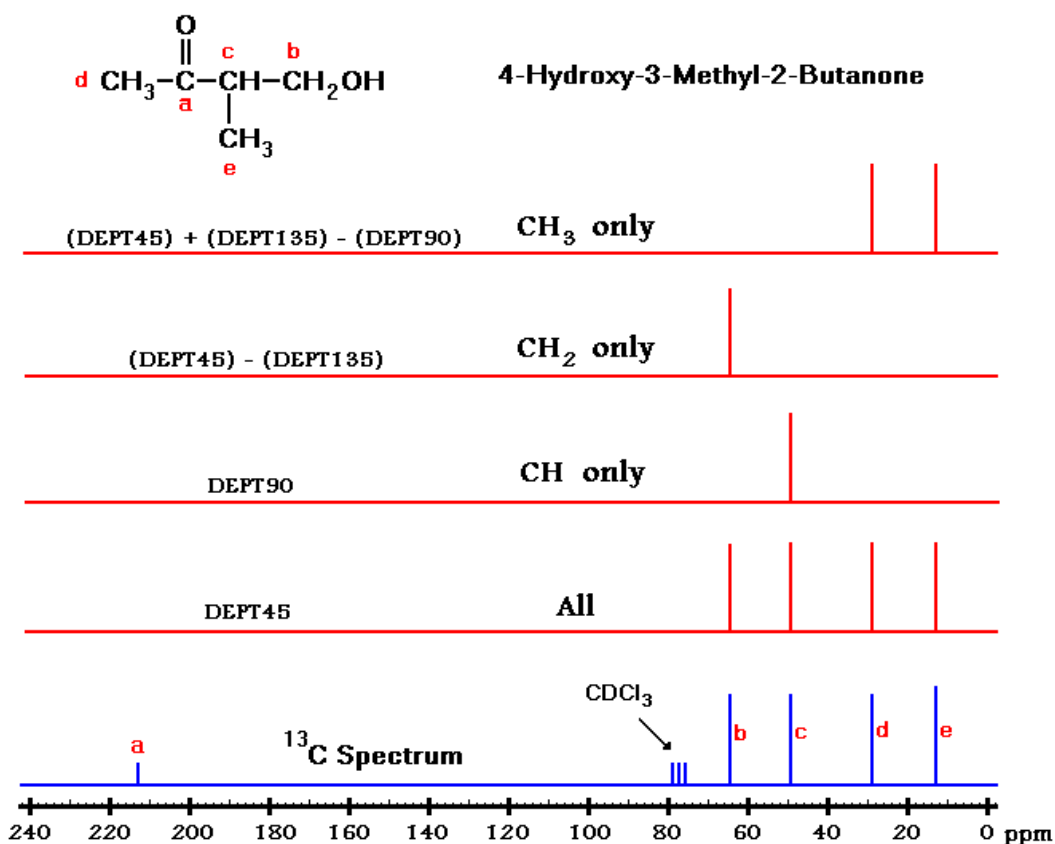| | | |
|---|---|---|
| **mult**=0.5 | "DEPT-45" | All carbons with attached protons are observed "right-side up" |
| **mult**=1.0 | "DEPT-90" | Only CH carbons are observed |
| **mult**=1.5 | "DEPT-135" | CH and $CH_3$ carbon peaks "up", $CH_2$ carbon peaks "down" |

With the information from these three spectra, each carbon peak can be assigned to one of the three categories: $CH_3$, $CH_2$ and CH.  If you have a very dilute sample, you may want to run a DEPT-135 (**mult**=1.5) only.  To run a full DEPT analysis, the parameter **mult** is arrayed with four values: 0.5, 1.0, 1.0, 1.5.  The DEPT-90 spectrum is repeated because it is less sensitive.

Set up for a standard $^{13}C$ spectrum and enter **dept** to run the DEPT macro, which sets the parameters for a full DEPT experiment with **mult** arrayed, and check the following parameters:

| | |
|---|---|
| **pw** | $90^o$ transmitter $^{13}C$ pulse width at **tpwr=60** (see blackboard) |
| **tpwr=60** | transmitter power |
| **pp** | $90^o$ decoupler $^1H$ pulse width at power **pplvl=63** (start with 15 μs) |
| **pplvl=63** | power level for decoupler during high-power pulses |
| **j=140** | average one-bond $J_{CH}$ coupling |
| **d1=1** | relaxation delay |
| **il='y'** | interleave acquisition |
| **bs=64** | block size for interleaved acqusition |
| **nt** | set to fill available time, or set **nt**=10000 and stop by aborting acquisition |

If your sample is fairly concentrated (40 mg or more), you can calibrate the value of **pp** on your sample.  Otherwise use the value on the blackboard or calibrate on a standard sample such as menthol in $CDCl_3$.  To calibrate **pp**, set **mult=1.0** to run a single DEPT-90 experiment.  If some $CH_2$ peaks are "down", the **pp** value is too large.  If the $CH_2$ peaks are "up" the value is too small.  The correct **pp** value should just null the $CH_2$ carbon peaks.

To perform a full DEPT analysis, enter **mult=0.5,1.0,1.0,1.5**.  Enter **da** to display the array and check the values.  By setting **il='y'** with **bs=64**, each FID in the array (each value of mult) gets only 64 transients before moving to the next one.  In this way you can check the results after the first cycle, which is only a small fraction of the full experiment time.  Enter **go** to start the acquisition.  Each time the FID number indicated in the acquisition status window changes from 4 to 1, you have completed one cycle of all four **mult** values and you can enter **wft** and **dssh** to transform all four spectra and display them side-by-side.  Phase-correct the first spectrum by entering **ds(1)** and **fd** (full display) to treat it as a single spectrum.  Then use **aph** or phase manually.  Repeat the command **dssh** to show all four spectra side-to-side.  You should see only positive peaks (CH carbons) for the second and third spectra, and some positive and some negative ($CH_2$) peaks for the fourth spectrum.  When acquisition is complete, save the data (**svf**), transform the spectra (**wft**), and display the first spectrum and phase correct (see above).  Because you will not get any solvent peak (deuterated solvents have no attached protons), you will have to set the reference using a previously determined chemical shift value (from a normal $^{13}C$ spectrum) for one of the peaks.  To plot the "raw" DEPT data, display a vertically stacked plot (**vsadj  vs=vs/4  ho=0  vo=vs  dss**), edit the text file (**textvi**) and plot the stacked spectra (**pl('all')  pltext  pscale  page**).  Label the spectra DEPT-45, DEPT-90, DEPT-90, and DEPT-135 from bottom to top.

**4-Hydroxy-3-Methyl-2-Butanone**

(DEPT45) + (DEPT135) - (DEPT90)    CH$_3$ only

(DEPT45) - (DEPT135)    CH$_2$ only

DEPT90    CH only

DEPT45    All

CDCl$_3$

$^{13}$C Spectrum

240  220  200  180  160  140  120  100  80  60  40  20  0 ppm

You can generate four pure subspectra ( all protonated carbons, CH only, CH$_2$ only and CH$_3$ only) by forming linear combinations of these spectra. This is diagrammed above for 4-hydroxy-3-methyl-2-butanone (the actual linear combinations used are more complicated). Display the first spectrum (**ds(1)**) and set the threshold for peak picking somewhere above the noise level and well below the tops of the peaks. Enter **printer='DeskJet'** and **printon dg da adept printoff** to calculate the pure subspectra and print a list of parameters, **mult** array and a peak list with each peak labelled as D, T, or Q for doublet (CH), triplet (CH$_2$) and quartet (CH$_3$). The **adept** macro does the proper additions and subtractions to replace the array of four spectra with an array of four "pure" subspectra. Once you have run the **adept** macro, you can't run it a second time or it will try to make the linear combinations of your linear combinations, leading to a mess. If this happens, enter **wft** again to regenerate the original "raw" spectra. To plot the pure subspectra, display a vertically stacked plot (**vsadj vs=vs/4 ho=0 vo=vs dss**), edit the text file (**textvi**) and plot the stacked spectra (**pl('all') pltext pscale page**). Label the spectra ALL, CH, CH$_2$ and CH$_3$ from bottom to top.