One and Two Dimensional NMR Spectroscopy for Chemist
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Lecture - 39
Analysis of 13C spectra and DEPT

Welcome back. In the last one or two classes extensively we discussed more about carbon 13 NMR and information was given. And we even tried to understand and interpret some of the carbon-13 spectra also and explicitly brought out to you the advantage of broadband decoupling of protons when you are observing the carbon 13 spectra.

There are several types of decoupling where we can get decoupling with nOe, decoupling without nOe, coupling with nOe, coupling without nOe. All those things we can see. It is possible to do all those things. That is what I discussed also; and took one or two examples of how do we make the assignments of carbon-13 spectrum, especially broadband decoupling when there is coupling with other heteronuclei.

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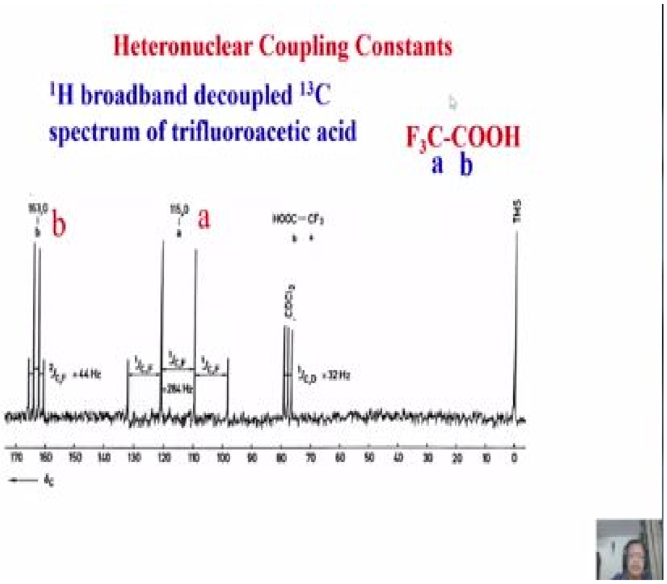

Heteronuclear Coupling Constants

^1H broadband decoupled ^{13}C spectrum of trifluoroacetic acid

$F_3C\text{-}COOH$
a b

We will continue today. So this is what we discussed in last class; that is where we stopped and I explained to you why is this CF3 peak is coming to the downfield and what was the reason for getting this quartet and what was the reason for getting the quartet for this carbon.

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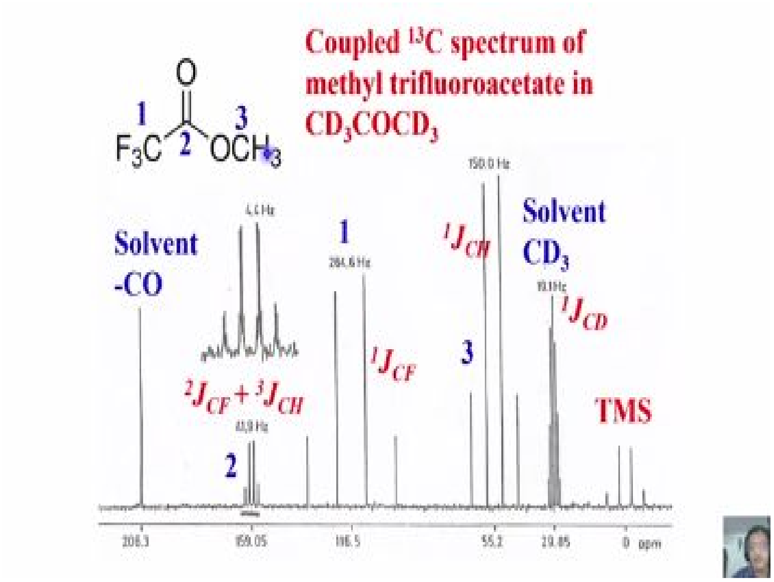

We will go to next this molecule, another molecule. This is a coupled carbon 13 spectrum of methyl trifluoroacetate in CD3COCD3. A very, very interesting thing. Please remember now we are talking about coupled carbon-13 spectrum. What is this one? You have to interpret; what is carbon-13 spectrum? Each and every peak has to be assigned very clearly. How do we go ahead in doing this?

Of course, this possibly we all know; when I took trifluoroacetate CH3 when it becomes CF3. In the previous example, we saw CF3 carbon was coming to the downfield; somewhere around this region. My first guess, this could be CF3 carbon; will start like that. First you write the structure. This is structure of this triflouoroacetate, I guess. Let us make the assignment, with some assumptions we have to start, and then prove it later.

In the previous example, we saw this carbon was coming somewhere around 110 to 120 ppm because it is attached to fluorine atoms, 3 fluorine atoms are there which are directly bonded to carbon. As a consequence, I said, this CF3 carbon is coming very much downfield here. And also we saw in the previous example carbon fluorine 1 bond J coupling was sufficiently large. See this is a well dispersed quartet with large chemical large separation of 4 peaks.

That means, if you measure the any two adjacent peak separation, it gives you J coupling. Look at this the separation, it is quite large. This also tells me because this separation and the quartet pattern; that which is coming also at the down field this must be CF3. this is the first assignment. Next what I have to see. Look for other carbons. What is the next carbon we can see?

This one, this is CH3 carbon, you know why? It is attached to three protons and it has to be a quartet, perfect quartet and CH coupling one bond is you know what is that order. We have already discussed in the previous class, what is strength of coupling for sP carbon, sP2 carbon and sP3 carbon. So you have some idea and as a consequence I would say this quartet is corresponding to CH3 protons.

No doubt about it. What is the next one? Why this is a quartet? If you look at this one, this carbon is attached this fluorine; and 2 bond carbon fluorine couplings is not small it is of the order of 30 to 40 hertz or sometimes 50 hertz. Look at it this coupling; it is about 40 hertz. So as a consequence, I would say this is a carbonyl carbon coupled to 3 chemically equivalent fluorines. CF3 group, CF3 fluorines it is coupled.

As a consequence CO carbon is a quartet. That is also fine. But one interesting thing happens, this region is expanded here. If you look at it, this region is expanded; when you expand, it is just not quartets. Each line of the quartet is another quartet. See it is a quartet of quartets. Where is this second quartet coming from? This is because of the long-range coupling of these 3 protons to this carbon. This carbon is experiencing long range coupling with these protons, 3 bond coupling.

Remember in acetonitrile example I told you there is a long range proton carbon coupling; exactly that is what happening here. This carbon is not only experiencing coupling with 3 fluorines, giving a quartet; it is also experiencing coupling with three equivalent protons; giving rise to quartet of quartets that is what it is, then our assignment is correct. No doubt about it. Then what about this one, this is reference TMS.

Since it is not a decoupled spectrum; it is coupled spectrum, TMS gives rise to quartet; because it has 4 CH3 groups and as a consequence, this is TMS. Fine that is also assignable then this is solvent because we have a CD3COCD3, then we have various isotopomers of that CD2COCD3, CDCOCD3; all possible combinations we can think of; and this is going to be a complex multiplicity pattern for solvent.

That is also fine. Then what is this peak? This is the solvent CO. This is solvent CD3 group, this is CO of the solvent. Very simple; right? all the peaks could be assigned now. This is the advantage. This is the coupled spectrum. Just to give an idea. I wanted to say how we can make the assignment with a coupled spectrum also. Suppose if I have to do decoupled spectrum, completely broadband decoupled; what you would have got? a singlet here, a singlet here, singlet here, a singlet here, a singlet here.

Then our confusion could have been why this singlet which is which? Which is coming at the downfield. What is this singlet? Then we do not know whether this is CH3 carbon or CF3 carbon, we do not know. So this is an idea and especially this also tells me idea about long range coupling of this carbonyl carbon with these 3 protons, so these are all advantages. Sometimes to identify peaks; identify particular carbons, we can also make use of coupled spectrum. This all the information we can obtain.

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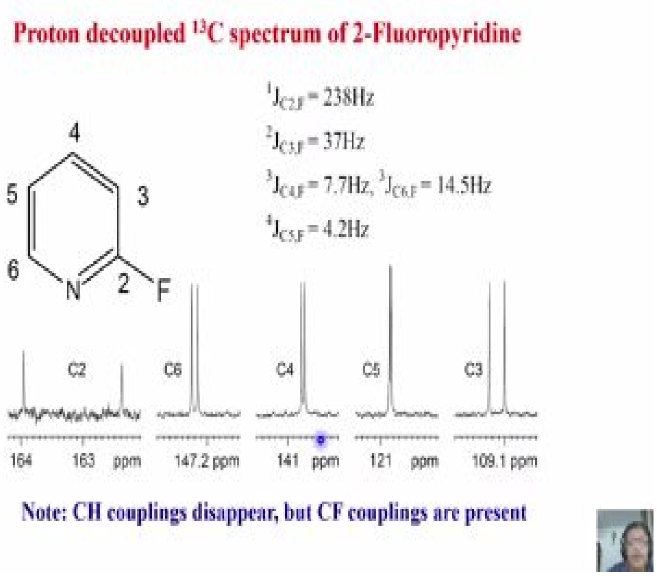

Proton decoupled ^{13}C spectrum of 2-Fluoropyridine

$^{1}J_{C2,F} = 238Hz$

$^{2}J_{C3,F} = 37Hz$

$^{3}J_{C4,F} = 7.7Hz$, $^{3}J_{C6,F} = 14.5Hz$

$^{4}J_{C5,F} = 4.2Hz$

Note: CH couplings disappear, but CF couplings are present

Now take the simple example of a proton decoupled carbon 13 spectrum of 2 fluoropyridine. A simple molecule; there is fluorine here, so what are the things you are observing here. Look at this one. This is a one bond carbon fluorine coupling here. One bond carbon fluorine coupling; this carbon has to be a large doublet because one bond CF coupling is quite large; of the order of about 250 to 300 hertz or even more sometime 350 hertz, you will see.

So this has to be a largest coupling then it is a two bond carbon fluorine; then three bond, then four bond; like that you can see depending upon how many bonds they are separated, the coupling strength between carbon fluorine decreases. Based on the coupling strength itself we can make the assignments here, for each carbon. Look at this one, this is a carbon spectrum. First this is carbon 2. It is proton decoupled. All these protons which are coupled, different carbons are removed. All couplings of these protons attached to these carbons and these carbons, everything is removed. We are experiencing only fluorine carbon couplings here. So we do not have to worry about protons at all. So then our job becomes very simple now. You have to only consider carbon fluorine couplings; and this is the carbon 2, I told you, because of large coupling it is a doublet; this carbon, this is a 1JCF coupling.

Which is the next largest? This is next largest separation is this is, 2JCF, then I would safely say this is carbon 3. What is the next one? this is again, one, two, three, bond coupling; this is C6, C4, 123 bond coupling, of course this is quite larger than this. There could be some anomaly to identify which carbon, whether this is carbon 6 or carbon 4? OK, there is a way to identify that later. But now what is carbon 5, carbon 5 is this. This is 1, 2, 3, 4 bonds away, this is carbon 5; So all the carbon based on the strengths of the coupling we can make the assignments. Very easy in this case, this is what it is. In the proton decoupled carbon 13 spectrum of this based on the coupling strength of heteronuclei; between carbon and fluorine; all carbons can be assigned. We do not need to worry about the proton couplings at all, in this example.

All these things are how to make the assignment of different carbons attached to different protons. But, generally, there are only 4 different types, not generally always, there are four different types of carbons present in the molecules. CH3 carbon, CH2 carbon, CH carbon and carbon not attached to any proton at all. But in a crowded spectrum with so many peaks, even after decoupling, I showed you one example of a complex spectrum even after decoupling, even if you get one peak for each of the carbons present in the molecule, there can be many carbons, many peaks. Then the question of identification of the carbons, whether it is CH2 carbon, whether CH3 carbon or a CH carbon, still remains, to make the assigned. Unlike in the previous example here, where we have to go by the coupling, we get a fully coupled spectrum we can make the assignment, but we get a complicated situation. So we have to think of an option, a possibility where we must get a decoupled spectrum. That is one peak for each carbon, at the same time I must assign whether is a CH3 carbon, a CH carbon, a CH2 carbon or a quaternary carbon. Based on the number of protons attached to carbon, can I do the editing? This is possible, this is a common experiment which, most of the time, the chemists make use of it for identification of carbons based on the protons attached to it. Individual carbons can be assigned. You can identify the carbon. I can say it is a CH3 carbon, I can say **it is** CH2 carbon, without going into or looking into the coupling pattern.

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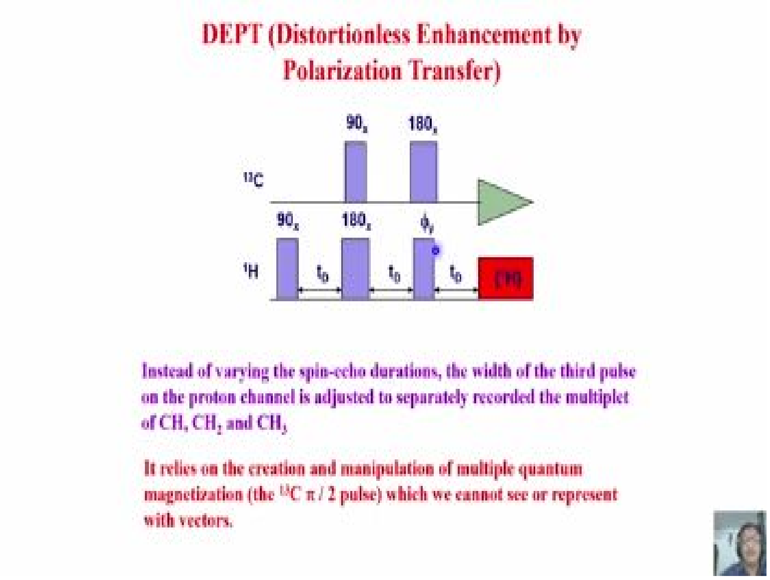

DEPT (Distortionless Enhancement by Polarization Transfer)

Instead of varying the spin-echo durations, the width of the third pulse on the proton channel is adjusted to separately recorded the multiplet of CH, CH_2 and CH_3

It relies on the creation and manipulation of multiple quantum magnetization (the ^{13}C π / 2 pulse) which we cannot see or represent with vectors.

That is an experiment people use, called DEPT. DEPT means distortionless enhancement by polarization transfer. Remember this is very commonly used experiment, and this is the pulse sequence. I will explain about polarization transfer in detail in one or two classes. Next or subsequent one or two classes I will explain about what is a polarization transfer, how we do that. The advantage of this is, in this case we can transfer the magnetization of protons to carbon, you understand?

Because proton is rich, in the sense it is a large magnetization. More magnetization is there. There is a way we can take the magnetization the proton and give it a carbon. Then what will happen it is like robbing the rich and giving the poor. Now the carbon which are less sensitive become more sensitive. Because it has more magnetization, the detection becomes much better, you can enhance the signal to noise ratio. There is a way to do that, this is a polarization transfer technique; the detailed discussion will follow.

But at the moment you understand this is a distortional enhancement polarization transfer, you can understand all the pulse sequences by vector diagram, sometimes it is not possible, then you can use product operators. But we are not going to do all those things. I do not want to frighten you with all those things. But some examples, I will give you especially INEPT, APT, where I will give you vector diagram explanations, in the later classes.

But this one you cannot do give vector diagram explanation for this, because this is a different thing see it relies on manipulation of the multiple quantum magnetization, so it is very difficult to represent this by vectors. So that is why, I am explaining this here itself before polarization transfer experiment. There we can be invoke vector diagram to understand the behavior of magnetization, how it is happening so that we can understand everything.

So, since it is little difficult, not easy, although we can do upto certain level, but we cannot do all the time. As a consequence I do not go by vector diagram method, but simply I tell you this is the pulse sequence we use, it is like echo-pulse; this is called spin-echo sequence and this is what we use for polarization transfer. Most important thing what you should know is this angle.

Normally we use the 90 degree pulse, 180 degree pulses in all the experiments. In this sequence this last pulse on the proton is not a 90 degree pulse. It is defined by flip angle φ. Φ is an angle to which the magnetization is tilted. Let us say the magnetization is along Z axis. You can take the magnetization to the middle of the first quadrant, this is called 45 degree, you can bring it to X axis, this is called 90 degree.

You can take further down 135 degree or 180 degree like that, you can play with magnetization depending upon the pulses. This is the pulse whose flip angle can be varied. It is in our hands. What we do is; for the identification of protons attached to different carbons, we do only 3 types of experiments. In each experiment only φ is varied, all other things remains constant.

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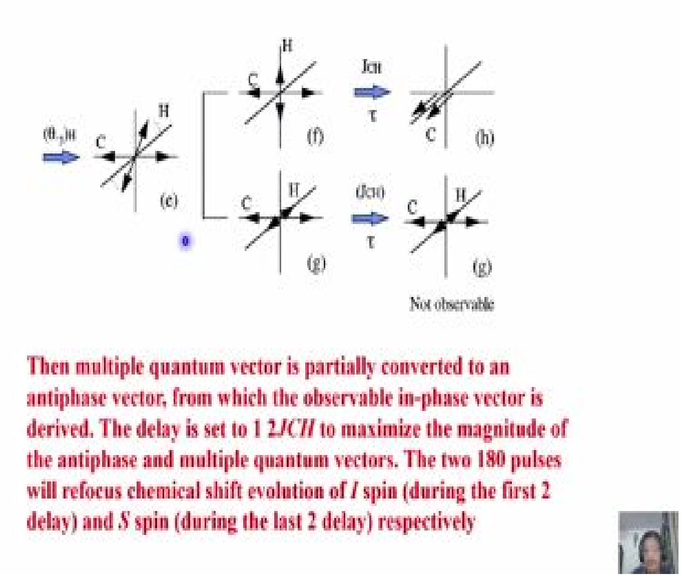

Then multiple quantum vector is partially converted to an antiphase vector, from which the observable in-phase vector is derived. The delay is set to 1 2*JCH* to maximize the magnitude of the antiphase and multiple quantum vectors. The two 180 pulses will refocus chemical shift evolution of *I* spin (during the first 2 delay) and *S* spin (during the last 2 delay) respectively

Of course, to some extent it is given first time, I will not go to that. I will come to this thing and discuss later because when I come to expkain the INEPT polarization transfer everything; this will be explained.

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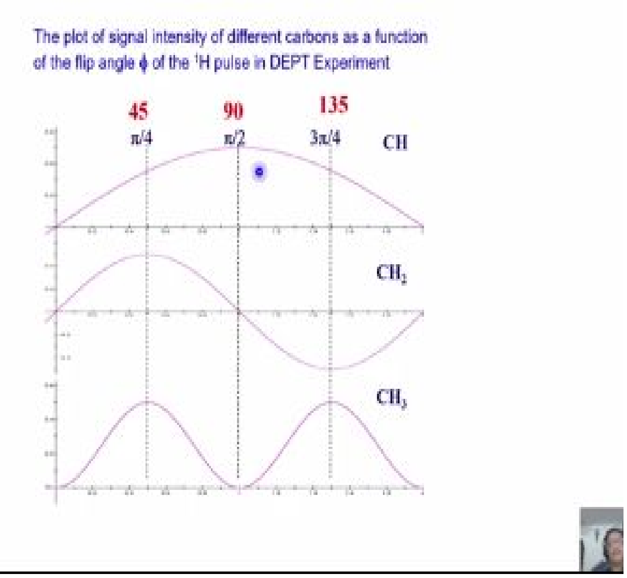
The plot of signal intensity of different carbons as a function of the flip angle ϕ of the ^{1}H pulse in DEPT Experiment

But remember one thing; because in carbon 13 that I want to complete I am telling you; keep this in mind, what is happening to the magnetization at different points, here, here, here, here. I will explain in the next one or two classes. So now what happens as a function of phi angle is what we can see. We can plot the intensity of different peaks, different carbons. I vary this angle, starting from 1 degree to 5 degree, 10 degrees in steps of 5 degree, 10 degree, keep on varying. I have a molecule which contains CH3 carbon, CH2 carbon and CH carbon all the three. That is what, let us say I assume, I have it.

Now what I will do is, I will record the spectrum, proton decoupled, so I get three different peaks for CH3 carbon, CH carbon and CH2 carbon. Measure the intensity of each of these peaks, by varying the angle φ, I varied the angle phi, 5 degrees I will take one spectrum, measure the intensity of each of the carbon. Vary angle to 10 degrees or 15 degrees always in equal steps, I can do, 5 degrees, 10 degrees like that. Every time I keep measuring the intensity of all the three peaks, individually CH, CH2 and CH3 intensities I measure.

And then I am going to make a plot like this. Very interesting observations, you make here. What is the interesting observation you are seeing here. When φ is 45 degree. When the flip angle of the last pulse, on the proton channel is 45 degrees, you see CH3 carbon, this is the intensity of the peak along this axis, this is the angle; this is intensity. Intensity is along the Y axis; the flip angle is along X axis. So now you see at 45 degree; CH3 carbon, CH2 carbon and CH carbon; all of them have positive intensity. So if I do an experiment, take the spectrum, with φ of 45 degree, I will see all the carbons; CH3, CH2, CH, all are positive intenity. Fine, Now I will make the experiment with 90 degree phi angle. Very interesting thing happens, you see that. The intensity of CH2 carbon becomes 0; Intensity of CH3 carbon becomes 0; whereas CH carbon is positive, well that is one way to identify.

I take a molecule with different carbons. I do a DEPT experiment keeping the flip angle of the pulse as 90 degree. Then what did you do you will remove all carbons which are CH2, CH3 types, that will not come at all, you will get only CH carbons. CH only spectrum you get, very interesting right? So, you are editing a spectrum, when you do a DEPT 90 experiment all the peaks you observe are only CH carbons, very simple. Then the question comes how do you distinguish CH2 and CH3 carbons?

What are they? I may have CH2 and CH3, how do I identify them? What we do is, there are two questions to answer. First you filtered out only CH carbons, fine. Next we have to assign CH2 and CH3 carbons; that is the second point, and the third is between these two I have to distinguish what is what? What I do is, I will do another experiment with last flip angle pulse as 135 degrees. Remember I do 3 experiments; one is 45, one is 90 degree, other is 135. In each case you look at intensity of the signal; CH is positive, CH3 is positive and CH2 is negative.

When you do 135 experiment, DEPT 135, it is called DEPT 45, DEPT 90 and DEPT 135 experiment. When you do DEPT 135, simply look at the peak at negative intensity. That all correspond to carbons attached to 2 protons. There are CH2 carbons; so you identified. A DEPT 90 you identified only CHs, DEPT 135, find out all the peaks with negative intensity, negative peaks, then you know there are CH2s.

So do this experiment and this experiment; then you can identify the left over peaks. They are CH3 peaks, because in DEPT 45 all are positive; and in the DEPT 45 all are positive, in DEPT 135, this is positive, this is negative; and this is positive and DEPT 90 only this is observed, so then you do this experiment first, identify all CH carbons, do this experiment later, then identify all these carbons, then take the difference between this and this then you know, which are CH3 carbons.

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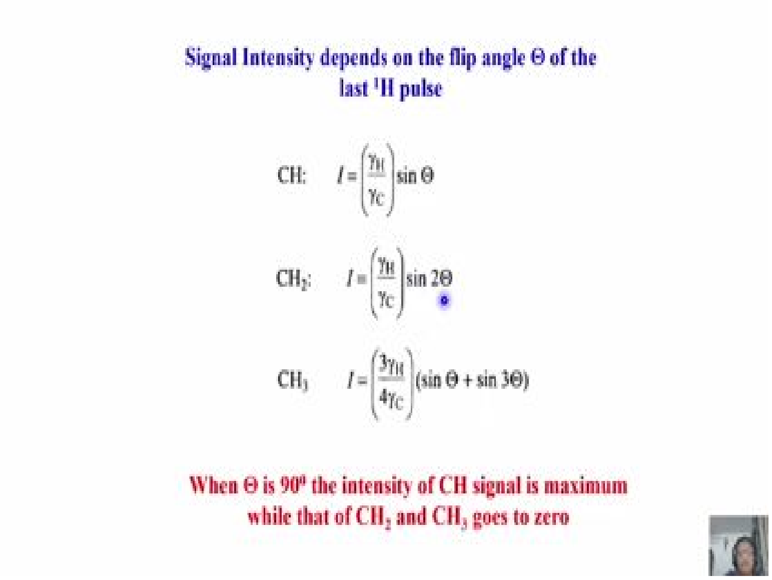

Signal Intensity depends on the flip angle Θ of the last ^1H pulse

CH: $I = \left(\frac{\gamma_H}{\gamma_C}\right) \sin \Theta$

CH_2: $I = \left(\frac{\gamma_H}{\gamma_C}\right) \sin 2\Theta$

CH_3 $I = \left(\frac{3\gamma_H}{4\gamma_C}\right) (\sin \Theta + \sin 3\Theta)$

When Θ is 90^0 the intensity of CH signal is maximum while that of CH_2 and CH_3 goes to zero

Very easy, so this is called spectral editing. And how the intensity of the signal varies, you can derive. The pulse sequence you can understand, what is happening mathematically and if you go into theory how as a flip angle phi varies, how intensity variation works. Based on this only that graphs have been drawn. You can look at it when phi is equal to 90 degree. What is happening here? Sin 90, what is sin 90? 1, So this is 1, maximum signal intensity you will get. What happens for CH2, CH3 when it is sin 90. Look at it, then these 2 signals will go to 0. As a consequence, very clearly you say when it is 90 degree pulse we see only this. So based on this theoretical understanding how the intensity of the different peaks, or different carbons attached to different protons vary, as a function of angle φ, we know how we can edit the carbon-13 spectrum.

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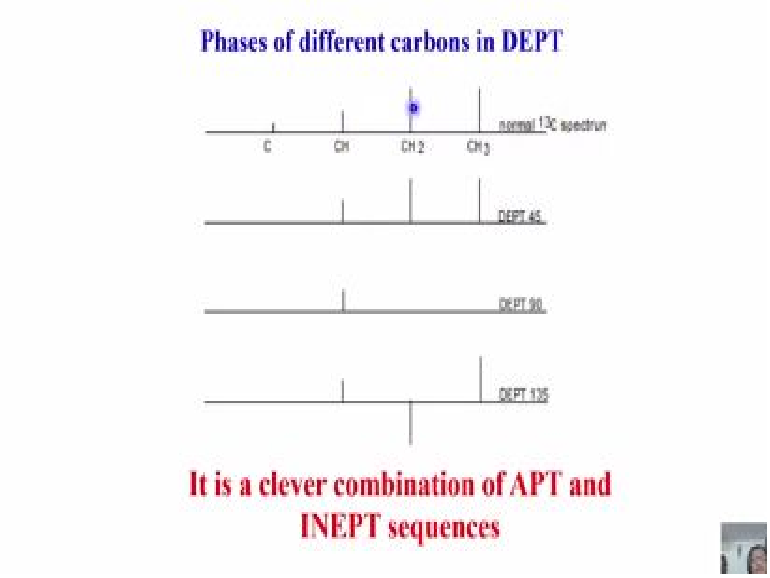

Phases of different carbons in DEPT

It is a clever combination of APT and INEPT sequences

This is a example how we can see. I will explain what is APT and INEPT later. I have not discussed, it is called Attached Proton Test and Insensitive Nuclei Enhancement by Polarization Transfer. First, I have to explain to you polarization transfer, and these are all the methods to identify carbons also. We will come to that later. Since DEPT is most commonly used experiment, I am discussing this now.

This is a stick plot, not a real spectrum. When you do a real carbon 13 experiment, you see all carbons CH3, CH2, CH, quaternary. I do DEPT 45. I do not see quaternary why? Because thye are not attached to protons, so there is no question of detecting these. So do one thing, do a DEPT45 experiment. Find out this difference between this and this. You know, all the quaternary carbons, that is one way, then the identification of quarterly carbons is done.

Next DEPT 90 you do you get only CHs that means you already identified these, because of this and you already identified these because of this DEPT 90. Now, we will take DEPT 135. In the DEPT 135, CH is positive all carbons attached to odd protons are positive. Carbons attached to even protons are negative, so CH2 is negative; so now you identified this also. In these 3 experiments you identify this, this and this. Then find out the sum and difference of the others then you can identify this also. very easy.

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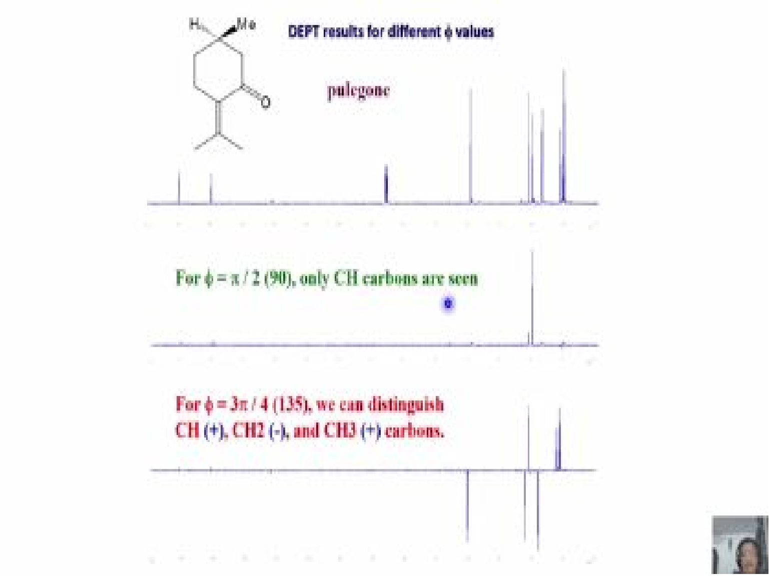

DEPT results for different ϕ values

pulegone

For $\phi = \pi / 2$ (90), only CH carbons are seen

For $\phi = 3\pi / 4$ (135), we can distinguish CH (+), CH2 (-), and CH3 (+) carbons.

So is it could cleverly combine varieties of all these three experiments, and you can do this spectral editing, identify all the particular carbons which are attached to protons. It is a very easy experiment please remember that.

Simple example is that one molecule called Pulegone this has got CH, CH2, CH3 carbons, look at this. This is a normal spectrum. This is DEPT 90, you see only the CH carbons here. All the things are efficiently suppressed; all other carbons the except CH2 carbon are efficiently suppressed here, nothing is here. DEPT 135 degree. What is happening here? CH2s are negative CH and CH3s are positive. So that means there are three carbons here with two protons, these are all CH2 carbons. You see this one, this one and somewhere here this one, these three carbons are CH2 carbons.

You understand; of course, DEPT 90 when you do, you know that. Of course, you observe this and in this case you also see this, you do not see that at all. So you also identify quaternary carbons here, that is not seen. In this one you observed CH carbons. In this you observed CH2 carbons now. You can identify using all the knowledge you gained by doing editing, addition or subtraction and seeing which peak is coming in which spectrum, the left over peak is CH3. You can easily identify CH3 carbon.

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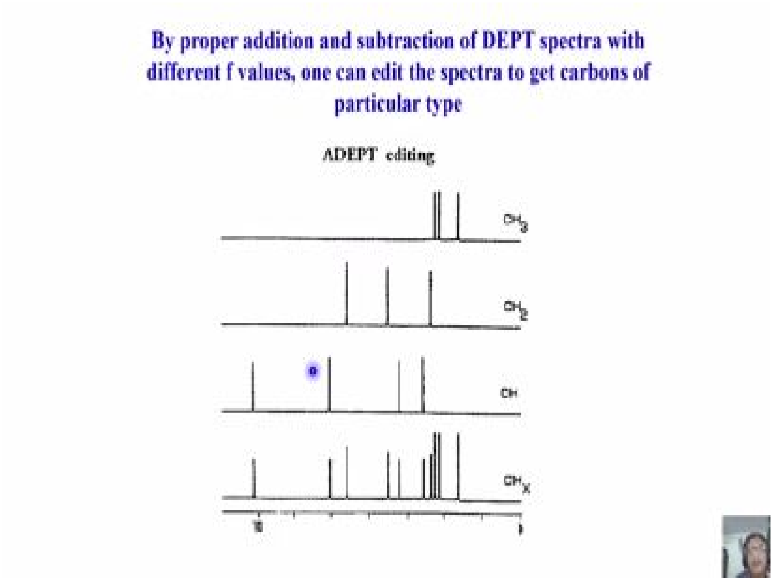

By proper addition and subtraction of DEPT spectra with different f values, one can edit the spectra to get carbons of particular type

ADEPT editing

So this is a simple example, this is called editing. Proper addition and subtraction of the DEPT spectra with different values. Of course, you have to scale the intensities you know all intensities are same that is called a intensity scaling factor. You can scale it and then make a subtraction. When you do that, it is called ADEPT editing. You get CH3 only per spectrum; you get CH2 only spectrum, you get CH only spectrum and you get completely all other things; and of course you can get quaternary, also separately.

Very easy now. I can get the spectrum corresponding to different carbons individually, I can detect by making addition and subtraction, making combinations of all these three different experiments. I can filter out the peaks corresponding to each carbon attached to different protons. This is a DEPT experiment extremely useful to identify the carbons, assign the carbons, at the same time doing decoupling. There is no coupling information. What is advantage? you get signal to noise ratio more. Secondly, this is an experiment done by using what is called as polarization transfer; the added advantage is you bring the polarization of protons to carbon, enhance the signal intensity. That is another advantage; so this experiment has many advantages.

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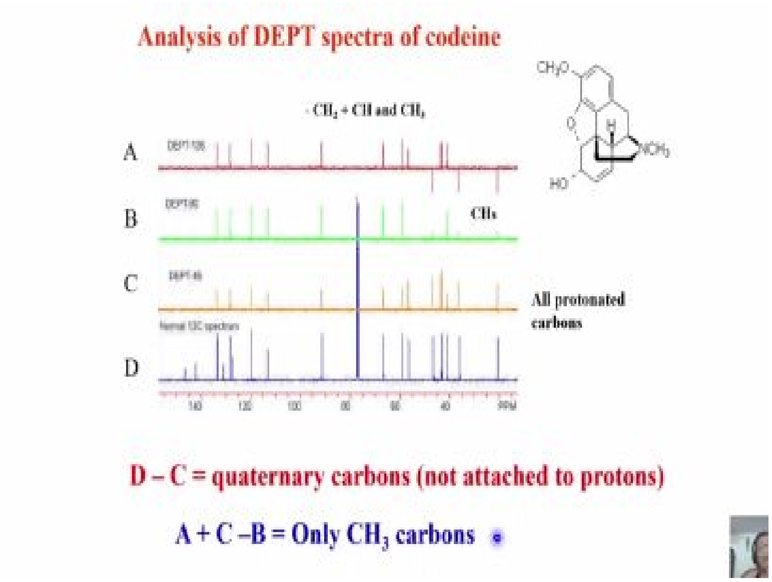

Analysis of DEPT spectra of codeine

D – C = quaternary carbons (not attached to protons)

A + C –B = Only CH_3 carbons

So this is commonly done and utilized by all the chemists. This is a bigger molecule like codeine which is taken from the literature. Various combinations you can do if A, B, C, D. The D is a normal spectrum and in this case, C is all protonated carbons will come at 45. Scale it and then take the difference between these two, you will get only quaternary. Similarly you take A+B, A is a DEPT,135, B is DEPT 90, C is DEPT 45 and add this and subtract of this, you get one CH3 carbons.

Like that you can play with it, by adding and subtracting by scaling the intensity by some ratio. You can get filter the spectrum, corresponding to individual carbons attached to particular number of protons.

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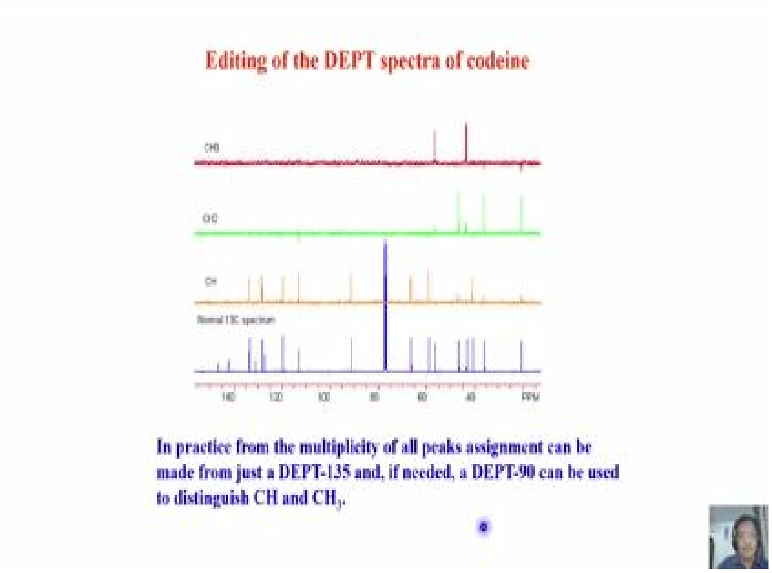

Editing of the DEPT spectra of codeine

In practice from the multiplicity of all peaks assignment can be made from just a DEPT-135 and, if needed, a DEPT-90 can be used to distinguish CH and CH_3.

This is what is edited spectrum of codeine is a CH3 only carbon, CH2 only carbon, CH only carbon, and the normal spectrum.

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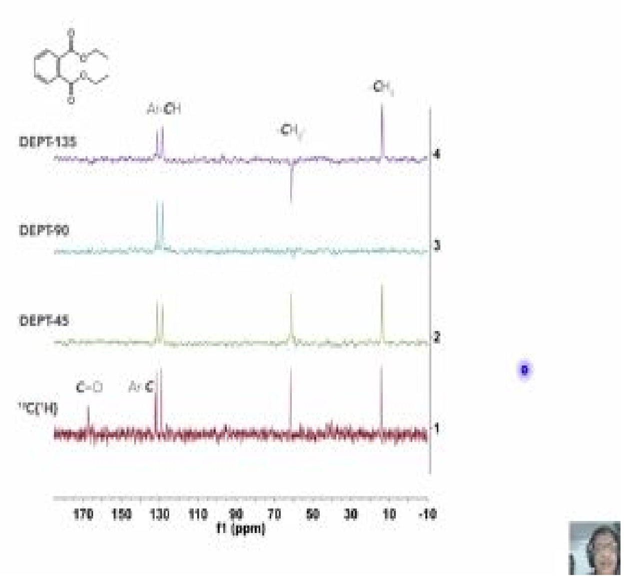

There is no point in the giving more examples; Today what I discussed is carbon 13 NMR, most important we should understand, we can get fully decoupled carbon-13 spectrum with various possibilities of decoupling, I explained already; normally what you do is decoupling with NOE, you get a single peak for each carbon; in which case the assignment is challenging task. You have to assign which carbon corresponds to which group, that is CH3 carbon, CH carbon or CO carbon, etc. For that one way is, you have to take the coupled spectrum and make assignment. I gave an example, but then coupled spectrum complicates the issue. So it is like asking too much. We want to decouple, and that the same time we want to make the assignment easy. So this is what we want to have the cake and want to eat it too.

That is the thing now we have to do. So alternative for that is a polarization transfer experiment called DEPT experiment. Expansion for it is this distortionless enhancement by polarization transfer. If that experiment is done what is going to happen is, the pulse sequence is simple, there is a spin-echo sequence and also polarization transfer both are present there. If you understand that, there is a last pulse on the proton channel. It is the detection pulse with the flip angle φ, you can vary the flip angle φ. Normally the experiments have been done and the function of various angle phi and measuring the intensity of different things of carbon. It is observed when phi is equal to 45 degree or all are positive intensity. All the three types of carbon. In the normal spectrum everything will come. When phi is equal to 90 you get only CH carbon. When phi is equal to 135, all carbon attached to odd number of protons, that is, CH carbon and CH3 carbons are positive in intensity, CH2 carbon is negative in intensity. Understand. So of course you may ask me question, why it is negative what to do with that? This is only phasing, when we do the practical consideration, I tell you this phasing. You can always make it positive, the phase correction we can always do. Now with proper combination, with the scaling factor, proper addition and subtraction of all these can give you CH only spectrum, CH3 only spectrum, CH2 only spectrum and quaternary only spectrum. This way if you have a very complex molecule, you can do decouple spectrum; and also identify the carbons based on the protons attached and make the assignment.

So this is an important experiment DEPT. Please remember, you need to DEPT 45, DEPT 90 and DEPT 135 for identification. So with this I think I will stop the carbon 13 NMR. This was I discussed extensively for more than one or two classes, because this is extensively utilized and you require more information about these things. Please go back and read a lot more things, as I have been telling you NMR is an ocean of knowledge.

What I am giving you only the tip of an iceberg, please go back read more about carbon 13, more about the type of experiment we need to do for identification of the carbons. So in the next class I will go to heteronuclear interactions; and analysis of a couple of spectra of different nuclei.