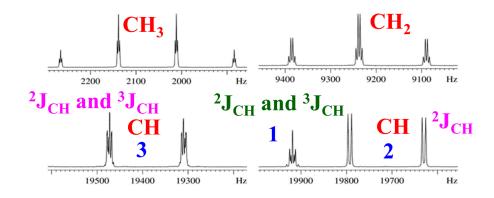
One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis Prof. N. Suryaprakash

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Lecture 25: 13C-NMR-II

Welcome all of you. In the last class, we started discussing about carbon-13 NMR. It is another very important exotic nuclei, very friendly nuclei, because spin half, but of course, it has less abundance, only 1 percent of the two isotropomers of carbon. Carbon 12, as I told is major isotropomer with 99 percent abundance. Carbon 13 is spin half, that is NMR active. Carbon 12 means NMR inactive and this is present only in 99 percent. And also, as gamma is 4 times less than that of the proton, it is very less sensitive. That is what I said. And also, I said if there are N chemically inequivalent carbons present in the molecule there will N peaks. Usually all the carbon 13 spectra are recorded with broadband proton decoupling. That means, we decouple all the protons present, all the protons coupled to carbon. Overall, simultaneous all the carbons coupled to all the protons are broken. As a consequence, you are going to get a single peak for each chemically in equivalent carbon. So, carbon 13 spectrum is fairly simple to interpret. What I said, if this is abundance is only 1 percent, probability of seeing carbon in carbon 13 state is only 1 percent, when there is single carbon. What happens when there are two carbons? I said probability is 1 in 10000, 0.000121, I so told you. What happens if there are n number of carbons? I took the example of 3 and then 6 carbons in the molecules. If there are 6 carbon present, I said the probability of seeing all the carbons in the carbon 13 state is 1 in a quadrillion, 1 in 10 to the power of 12, huge, very, very small. If you have very small abundance of such molecules, practically it is impossible to get the spectrum from such molecules. But in reality, even for a big molecule with large number of carbons, we do get the spectrum. Then the question is how do we get such spectrum? For this, I also explained to you taking an example of ethanol, when there are two carbons present. Each carbon can be individually present in carbon 13 or both carbon 13 and both carbon 12. That is what I explained and both present carbon 12 is NMR inactive, does not give signal. Both carbon 13 present is very difficult to see, probability is very low and there are two isotope farmers, CH3 with carbon 13 per state, CH2 in carbon 13 state will give rise to NMR spectrum. And there is a one bond coupling, I explained to you when there are different carbons attached to different number of protons, carbon attached to CH3 group for example, 3 protons gives a quartet, CH2 carbon gives a triplet, CH carbon is a doublet and quarter a carbon does not split, it gives only a singlet. No coupling, that is what I said. And then in extending that logic, if I go to ethanol spectrum, CH3 will be a quartet and CH2 will be a triplet, but there is also long range coupling,

CH3 carbon can couple to protons of CH2. The CH2 carbon can couple to protons of CH3, in which case spectrum gets complicated. One is going to become a quartets of triplet, other is going to be triplets of quartet. That is a spectrum fully coupled carbon-proton spectrum. What I said we are going to do the decoupling, then all the carbons are decoupled, remove couplings, you will get one single peak for each carbon. But I also said, if you look at the proton-proton coupling, protons are passive spins and they are not getting reflected in the carbon 13 spectrum, this is what we understood. So, with this now we will go further; and I and I told you for a molecule containing N chemically inequivalent protons, the proton spectrum is from a single molecule, whereas the carbon 13 spectrum, if in a given molecule there are N chemically inequivalent carbons, there will be N different isotopomers, in which each isotopomer has one carbon in carbon 13 states. That is one carbon of an isotopomer has one carbon 13, in another isotopomer different carbon will be in different carbon 13 state, like that you have N different isotopomers each having carbon in carbon 13 state. So, the real carbon 13 spectrum is not from a single molecule, it is the superposition of the spectrum of all the different isotopomers that is what we saw for ethyl alcohol that is what I said in this case.

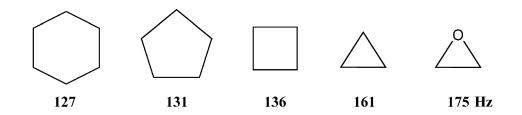


So, now, we are going further, we can see diethylphthalate, a simple molecule. This molecule spectrum is chosen because it is very simple, we have CH3CH2 here. Look at it CH3, this CH3 here is a quartet. As I told you 3 protons coupled to carbon will make this carbon a quartet, but these remote protons are also coupled to this, long range coupling, and this it is going to be quartets of triplets. Similarly, CH2 is going to be a triplet because of 2 protons coupled to carbon and then long range coupling make each of them, each line of the triplet as a quartet, it is going to be triplet of quartets. We also have 3 different protons, there is a symmetry along this axis for this molecule. There is a quaternary carbon and these 2 protons are single proton attached CH carbons. So, as a consequence they are doublets. But of course, they can have a long range couplings. So, this is a very simple spectrum of this molecule, I just wanted to show you. If you look at

the carbon 3 here; for example, this is carbon 3, it has a one bond coupling with proton and there is a long range coupling with different protons, this carbon can have a coupling with this proton. So, that is what we are going to see, the complexity will be there. Similarly, if you come to Carbon 2, Carbon 2 has one bond direct proton carbon coupling, at the same time it has a long range coupling with protons attached to Carbon 3. So, it is going to be complex like this. So, that is how you can interpret. And then since it is going to be a decoupled spectrum, this much of complexity would not be there, you simply look for each peak and one peak for one carbon. So, that is why I said broadband decoupling is an important thing which we do in carbon 13 NMR. Before going to that broadband decoupling, I want to give you the strength of couplings, because that is important.

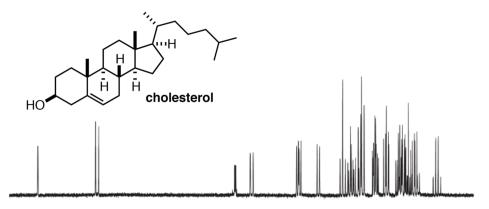
> ¹³C-¹H; ¹J_{CH}= 150 Hz ¹³C-X-¹H; ²J_{CH}= 2 - 10 Hz ¹³C-X-Y-¹H; ³J_{CH}= 2 - 10 Hz

The broadband decoupling requires irradiation with the radio frequency, with another radio frequency at the proton frequency and the strength of this irradiation should be larger than the coupling strength. Then only you can break the coupling. So, for that we should know the strength of the one bond J coupling. One bond J coupling normally is of the order 150 Hz, it can go to 200 or 250 Hz. It is not a dogma. In some molecule it can go up to 200; in some molecule we have seen up to 250 also. Similarly, if you consider the molecule of this type, with two bonds, you know with two bond carbon proton coupling, it can vary to 2 to 10 hertz and three bond can be 2 to 10 Hz again.



And it can so happen sometimes three bond couplins is more than two bond coupling that is also possible, varieties of things can happen. This is just an idea, so that we should know how much RF power we have to apply to break the coupling between carbon and proton. And what are the J values for different type of molecules? if you take benzene group, one bond CH coupling is 127 hertz, for a five member ring 131, four membered ring CH coupling here is 136, three membered ring 161 Hz,. Wee this one, three member with O attached is 175. You see as the size of the ring keeps coming down, the coupling strength keeps on increasing. This is a typical one bond carbon proton coupling in different molecules.

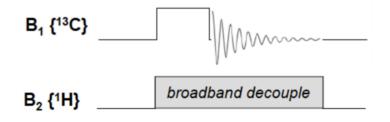
And in a linear chain molecule like aliphatic group, this one bond coupling carbon proton and one bond carbon proton to this one for CH3 group is of the order of 125. In a molecule like the CH3OH, it is 142. If I have a molecule like this one bond coupling between carbon proton is 155. Wherever I have highlighted, it is that carbon coupled to its attached proton. For example, in this molecule this CH2 coupling is160 to 170 hertz. Come to this phenyl carbons, see this is a strange example. In the phenyl group, the carbon attached and there is another triple bond CH, the coupling can be 250 Hz, quite large. Take for example, CHC13 coupling is close to 210 Hz. So, this is how we know the values of couplings for different carbons; the strength of the couplings of different carbons with one bond coupling to its directly attached proton. So, a real complex spectrum we will see. Look at the spectrum, this is a very very complex spectrum of a cholesterol.



How many carbons present in that? You can count yourself 1, 2, 3, 4, so many carbons are present. Now, we know how you got the spectrum. I already explained to you, this is superposition spectra from, several isotopomers, each time one carbon is in carbon 13 state. That is the spectrum we got. So, complex with all proton couplings you see. You can easily identify here the patterns. For example, here is a quartet. For example, this is a doublet like this. How do you simplify this analysis? There are so many multiplicities present here, they are overlapped. How do you identify triplet, quartet, etcetera, multiplicity? What we do is, we simply do the decoupling. When we do the decoupling, this complexity of the spectrum gets enormously simplified.

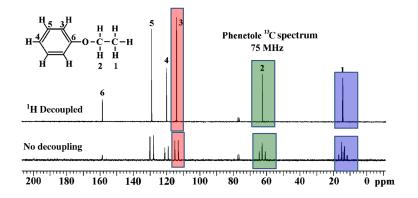
Now, the question is what is decoupling? I vaguely discussed this thing earlier and I just

wanted to tell you this has been discussed extensively in one of the previous courses. Just for your idea, the broadband decoupling of carbon 13, how it is done is, you see we are going to apply a radio frequency pulse on carbon, start collecting the signal. And then, we are going to apply another RF pulse at the proton channel, at the proton frequency. This is called a broadband with a continuous wave decoupling. Continuously, we keep on applying the RF pulse with a high power.



Power is such that it shall be larger than, at least two times larger, than the carbon proton coupling. In which case, what is going to happen is, ou will break the coupling between carbon and proton. See what happened? In reality, this is diagrammatically like this. At the proton frequency, you keep on applying RF power to saturate proton. What do you mean by saturation? We already discussed in the very first or second class. Saturation means the population between two energy states are equal. You keep on applying RF power in such a way, the spins in the low energy state goes to high energy state and spins in the higher energy state will come to low energy state. And this rapidly happens when you continuously pump the RF Then, what will happen? This carbon will not see this proton in alpha state or beta state, neither of the states because of the rapid transition that is going on between two states, or in other words, it is saturated. You will not see any population at all in either alpha state or beta state. Then, it will see the average, neither in alpha nor in beta states, it is somewhere here, in which it appears as if it is not coupled at all. You have broken the coupling. This is what is called decoupling and we are going to collect the signal while applying the RF pulse continuously. You collect the signal on carbon 13. This is what we do. So, that means, when you are applying another RF power at the proton channel and you collect the signal, you will break the coupling between all protons coupled to all the carbons. So, irradiation, this is what I said, causes proton to get saturated and the spins undergo rapid transitions among all the possible spin states. So, carbon nuclei senses only the average spin state, not either alpha state nor beta state. There are n number of distinct spin states. It does not see all of them. It will see only the average. So, in effect, all the spin interaction between carbon and proton are averaged to 0. This is because of rapid transition spins among the spin states. This is what is called broadband decoupling. Broadband because broad range of frequencies of all carbons to all protons will be broken. This is called broadband decoupling.

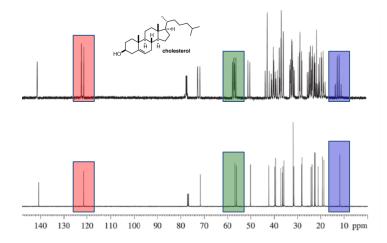
Now, the question, I already told you, how much of the RF decoupling power you have to apply? It should be at least twice larger than the largest CH coupling. The CH coupling in the molecule is, let us say, 250 Hz. At least the RF power should be larger than double of that, about 500 Hz, just to be sure. Even more than one CH is enough, but to make sure that there are no other effects, we have to make it double of that, or slightly larger. In such a case, all the carbons coupled to all protons are broken. Where do you put this RF? Where do you send the second RF to saturate protons? At the center of the proton spectrum, remember carbon is coupled to proton. We have a proton spectrum. Go to the proton spectrum here. Go to the center of this proton spectrum. At that place, you apply the RF power. When you apply the second RF, decoupling RF, then this thing happens. So, this decoupler offset has to be set to the center of the proton spectrum, and its power should be at least twice larger than the CH coupling. The broadband spectrum, as I told you, gives us a single and a distinct peak for each chemically inequivalent carbon. Very simple. Now, take the example of this molecule. How simplified, how the coupled and decoupled spectrum appears?



It is a molecule, phenetole recorded 75 megahertz. So, the proton frequency is 300 MHz. This is the spectrum without any decoupling. We have CH3 group, CH2 group and phenyl group. CH3, as I told you, when it is coupled, it will be a quartet. Three protons are coupled to this carbon. CH2, both these protons are coupled to this carbon, a triplet, and each of the CHs, there is symmetry here and they are equivalent, this thing and these are equivalent. So, there are only three chemically inequivalent carbons. Each of them is a doublet because it is attached to a single proton. Very nice. At the same time, there is also ipsocarbon, which is not attached to any proton. That is called a quaternary carbon. This is the total spectrum. Here, it is easy, simple molecule. I can interpret.

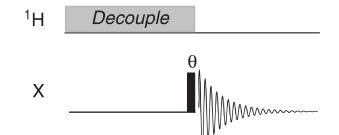
What happens to a big molecule? It is difficult. So, to understand we do the decoupling, proton decoupling for this carbon 13 spectrum. Now, what happens, you see, this quartet coupling disappears. And all the peaks merge into a single peak. So, you will get a single peak at the center of this multiplicity that corresponds to chemical shift of carbon CH3. Similarly, this triplet, all the lines will collapse and appear at the center of this. That is the

chemical shift of carbon CH2. Similarly, chemical shift of the different carbons here and this is a quarternary carbon. And so, you see, there are six carbons here. In this molecule, there are six peaks. That is the difference between coupled and decoupled spectrum.

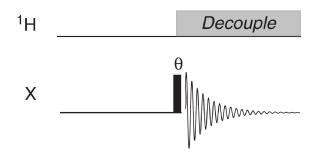


If you want to go further, here you can see the changes what you see here. More important thing I showed you in the cholesterol molecule. This is where you see. This was the quartet. This is coupled and this is broad band decoupled. Look at this is a quartet. This is a doublet. I can understand this also doublet. What do you get out of this? It is a clumsy thing. You do not know, but there are so many carbons present here. So, what we do is, we do the broadband decoupling and then we identify the number of carbons present in that very easily here, which is not possible from this. This is a carbon 13 broadband decoupling we do. But then, just to give you an idea.

How do you do the broadband decoupling? I already told you. You apply the RF power at the proton frequency and saturate the protons while detecting the carbon that is decoupling, a hand waving argument. The broadband decoupling can be done in different ways. What are those different ways? I can acquire the carbon 13 spectrum in different ways. Before going further, here I want to tell you one thing. I am using the term called NOE, meaning nuclear overhauser effect. I have not discussed this. That is to be extensively discussed. After this carbon 13, may be I will introduce NOE. Also, I am going to introduce what is called DEPT. Polarization transfer is needed. That also I have not explained, but that is used in this class in understanding carbon 13 NMR. Both polarization transfer and NOE will be discussed subsequently in the remaining classes. But please remember, NOE is enhancement of the signal because of decoupling of one. Then, what will happen? The neighboring carbon or neighboring proton will have a enhanced signal intensity. This is because of Overhauser effect. I will discuss this later.

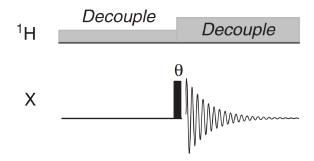


So, now I can have a coupled spectrum with NOE, meaning I am going to apply radio frequency pulse and collect the signal on carbon 13. X is carbon 13 for me. But what I am doing? Remember, they are different channels. Omn the proton channel, till we acquire signal, before this we are applying decoupling power, and then I am collecting the signal. Here, RF power is not applied. That means, here I am not decoupling. I am going to get a coupling here. But why did I apply decoupling power before? That is a phenomenon. When I do this, applying decoupling power, prior to that, we get enhancement of the signal intensity due to overhauser effect. This is an experiment if you do, you will get a coupled spectrum, but with enhanced intensity. This is called coupled spectrum with NOE.



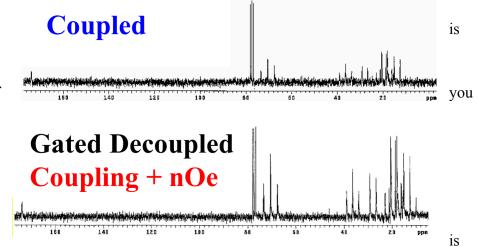
On the other hand, I can do one more thing. I can start acquiring the signal and do not do NOE at all. This is a spectrum, where because I am applying decoupling power while acquiring the signal, I am breaking the coupling between carbon and protons.

But I am not doing NOE. There is no NOE here. This is what is called inverse gated decoupling experiment. This is called inverse gated decoupling experiment, where I am decoupling the carbon with protons, but no NOE. There is no enhancement in the signal intensity. This is an experiment, but NOE develops and affects the longitudinal magnetization. So, as a consequence, you do not get NOE. So, since the acquisition is already present here, there is not going to be any buildup of the signal. That is why this is called inverse gated, no NOE and we are going to decouple.



I can think of other situation. I can apply continuous RF power, may be of different strength. This is different strength. This is different strength. Can be of similar strength? No problem. This is called high power decoupling. I have NOE. At the same time, I am also doing decoupling. This is an experiment. The decoupling is on continuously throughout the experiment. Even before applying the pulse, after applying, pulse while collecting the data, continuously RF power is on at the proton channel or at the proton frequency. This is an experiment called high power decoupling. Here, both decoupling is going on and NOE is going on. Simultaneously, both are going on. Then, what you are going to get here? You are going to get a decoupled spectrum; completely removed carbon proton coupling plus signal intensity will be enhanced because of NOE. This is a decoupled

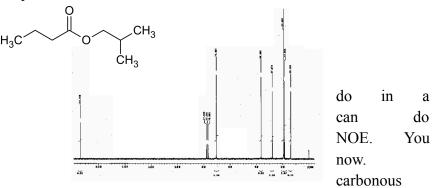
spectrum with NOE and this the routine carbon 13 NMR all of will be doing. Remember. when you record а carbon 13 NMR spectrum, this



what you are doing without your knowledge. Your operator will be recording the spectra for you.

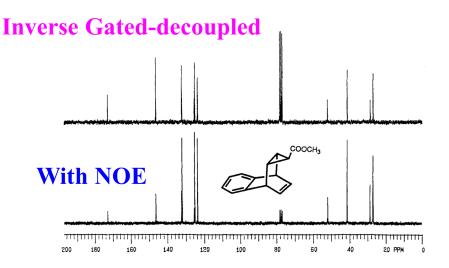
Look at the example, how do you compare this thing? This is a carbon 13 NMR spectrum of this molecule, very complex. It is coupled. Coupled means each of these carbons has

multiplicity patterns because of coupled protons, quartet, triplet, doublet, all those things, etcetera. All are overlapped here. You see, look at the spectrum. It is very clumsy. Lot of noise is there, very noisy here. On the other hand, we do this gated decoupling. What is gated decoupling? I told you. We are doing the coupling. At the same time, we are doing NOE before applying RF pulse. That is called inverse gated decoupling. When you do that, you see, still you get a coupled spectrum. But at the same time, you will see this is still a triplet. You see here, this is still a triplet. We are not decoupling. They are coupled, but NOE is there. Because of NOE, you can see the intensity here, enormous rise in the intensity here. Look at the signal here. Look at the signal here. Coupling with NOE is there, enhanced spectrum plus NOE.



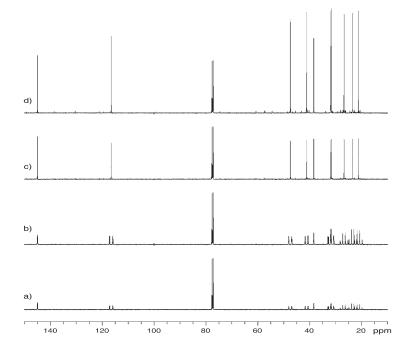
Same thing you can different way. You decoupling with see, look at this one Completely all the

couplings are broken. You get enhanced intensity. Look at the signal intensity here. Look at the signal intensity here. Signal to noise ratio is very poor here. Look at this one, huge signal and very little noise here. This is a real CW spectrum with decoupling and of course, there is NOE there.



And this is with inverse gated decoupling for another molecule. Just to compare, no NOE and this is decoupled with NOE. You see the effect? You see the effect here. Look at this

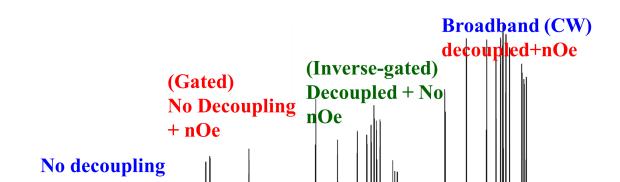
peak. Look at this peak. Look at this peak. Enormous gain in the intensity. This is because of NOE. So, this is the way how we can enhance the signal intensity of the carbon by doing decoupling with NOE.



will tell comparison you. This is a spectrum without any decoupling. Can you see this one? So, signal to noise ratio is so poor, signals are very weak in intensity, no decoupling because there is lot of multiplicity. Each peak split into multiplicity, doublet, triplet, quartet like that. As a consequence, signal to noise ratio is very poor. Next one, gated decoupling. That is coupled spectrum with NOE. Same thing with signal intensity more. See the comparison. Already you have got better signal to noise ratio. Go further. This is a inverse gated decoupling. Decoupling without NOE. I am decoupling here, but no NOE. And the last experiment, decoupling with NOE. Fantastic. There are four possibilities. No decoupling, coupled with NOE, decoupling without NOE and decoupling with NOE. And decoupling with NOE is a real spectrum; compare this and this. All multiplicities are broken here and we are going to get a singlet for each carbon plus look at the enhancement in the intensity. Huge intensity is there. So, this is what is important. Signal intensity goes up because of NOE when you record the spectrum of carbon with decoupling with NOE. What you have to do for that? You have to put a decoupler power on throughout, before acquiring the signal, before you apply the pulse, why after applying the pulse, even while

And

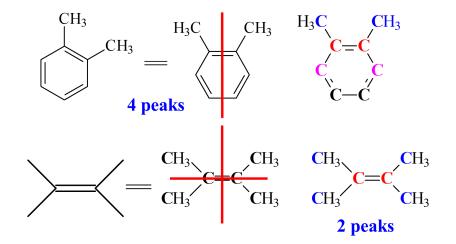
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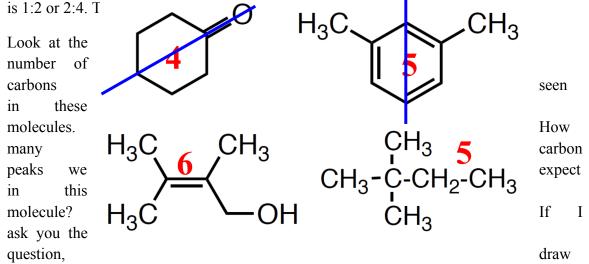
acquiring the signal, continuously decoupler power is on in which case you are going to get this one. And this is a simple comparison for a sucrose molecule.

Four beautiful spectra here. See this is spectrum no decoupling, no NOE. Inverse get a decoupled, no NOE. This is get a decoupled NOE. This is broadband decoupled NOE. Look here. What a gain in the intensity you are going to get. And this is the experiment all of you would be doing every day. This is what I wanted to tell you about decoupling. I hope you got the feeling about decoupling.

We have got individual peaks for different carbons. But how intensities are same. Here look at this example. You go here, somewhere here. This also okay. This is not bad. Here for example, if you look at this one, you will see that different carbons have different intensities. What did I say? Each peak corresponds to one carbon because it is decoupled. If that is the situation, when each carbon correspond to one individual carbon, why there should be difference in the intensity? all peaks carbon should be of identical intensity. There should not be any change in intensity. But actually in reality, intensity of the carbon spectrum all are not of same intensity. They are different relaxation times. At the same time, molecular symmetry also ensures that some carbons have double the intensity or some have less intensity. So intensity of the carbon peaks depend upon symmetry of the molecule and how individual carbons are relaxing.



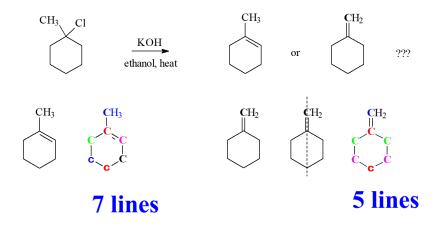
Look at these molecules. It is a simple molecule. So, we have a symmetry along this axis. Three molecules are there. I am putting this into this format. Now, symmetry if you conside,r how many chemically inequivalent carbons are there? You put this one carbon, these two are equivalent, these two are equivalent, these two are equivalent and these two are equivalent. So, how many carbon peaks you get? Four different carbons and each of them double double intensity. So, but they overlap, you get four peaks. That gives four peaks. And what about this one? There is a perfect symmetry along this axis and this axis. Now, all the CH3 carbons are equivalent and both these carbons are equivalent. So, how many peaks you get? Two peaks. But what is the intensity? This is 1:2 intensity. These two overlap and give one peak. These four will overlap and one peak. The intensity ratio



the symmetry axis here. How many different chemical in equivalent carbons are there? Four, because this and this are same, this and this are same, this carbon and this carbon are different.

You get four peaks. What about this one? There is a symmetry axis here. This carbon, this carbon is same, this and this are same, this and this are same, this and this are different. So, how many carbons you get? One, two, three, four, five. See, five carbons are there. What about this one? There are six carbons. All are chemically in equivalent. One, two,

three, four, five, six carbons. Similarly, this one, this molecule has five carbons. This is how it goes. You can even predict the reaction product just by looking at the carbon that is spectrum.

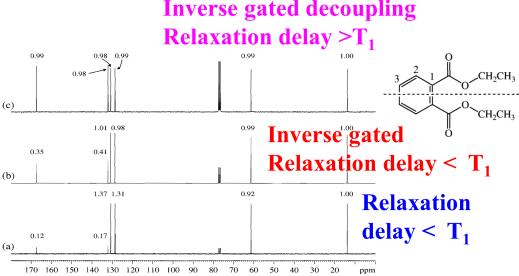


Take for example, a molecule like this. Somebody was doing a chemistry reaction of this molecule with KOH, was confused whether you get this peak or this peak. I mean this molecule or this molecule. How do you know which is the molecule? You have the reaction and this is the product. Which is the product? Simply what you do is take a carbon that is spectrum of this molecule. If this is your product, for example, this one, how many peaks you expect? If this is the product, you get seven lines. All carbons are inequivalent. See, there is the double bond here. There is no double bond. This, three, one, two, three, four, five, six, all are different and this one, seven carbons. Whereas, here, there is a symmetry axis. So, this is same, this is same, this, this, then this. There are five carbons. So, what you do is when you do this reaction, take the product. Take the carbon 13 NMR spectrum and if you get seven lines, then this is the product. If you get five lines, this is the product. You do not have to worry about interpretation. Just count the number of lines. Then you know what is the product of this reaction. So, you can predict the reaction based on the symmetry. Based on the number of peaks you get, which depends upon the symmetry of the molecule. So, that is what I was telling you. The number of peaks that you are going to get in the carbon 13 and its intensity depends upon relaxation and the symmetry of the molecule.

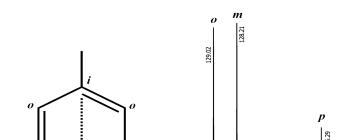
How do you know it depends upon relaxation? This is an example now. Look at this molecule, which we already analyzed. This is a spectrum taken in a conventional way, where signal keeps on acquiring. We acquire signal one after another several number of times to enhance the signal intensity, but the relaxation delay is kept smaller than T1,

smaller than longest T1, whatever the carbon which has longest T1. For example, if the T1 of the carbon is 5 seconds. You set the relaxation delay you give between the pulses, different pulses to acquire a signal. If you keep it less than that maybe 2 second or 3 second, acquire the signal. This is what you are going to get. See for example, this carbon, it is this carbon. They are not of equal intensity, but they are individual carbons. Why you are not getting individual carbons? See, when there is an overlap, I understand. When there is no overlap, why you are not getting individual peaks of equal intensity? So, then that is a problem. So, that is one aspect. It is a possibility because we are given relaxation delay less than that. On the other hand, we can do inverse gated decoupling with same to enhance the signal intensity. Identical experiment with the inverse gated that means there is NOE, but still the relaxation delay is less than T1, but that has not helped you. That has not made all the peaks are equal. Intensity of all the peaks are still not equal. There is a problem. So, on the other hand, do the last experiment. Give the delay larger than T1, not just larger. It should be 5 times T1, 5 times the longest T1, of the longest carbon which has the longest T1. You have to consider give that much delay. You understand, for example, different carbons have different relaxation times. One may have 1 second, 2 second, 3 second, 4 second. I have not discussed relaxation in this course, but I have discussed previously, but when there is an opportunity comes, I will talk about relaxation soon may be after couple of classes, but different carbons may have different relaxation time. Consider the carbon which has the longest relaxation. Give 5 times that as the acquisition dealy and record the spectrum with the same NOE. Look at what is happenir





Example of the symmetry and relaxation both define in the non-protonated carbons here.



You see, ipsocarbons non-protonated. These are all protonated. They have different intensities. Ipsocarbons are not attached to carbon, not attached to proton. This carbon has a very weak intensity because such carbons relax very slowly. This intensity ratio is 1 is to 2, it is understandable. Whereas this one is not understandable. That is because non-protonated carbons always relax slowly. So, this is how we can get the understand about the peak intensity, depending upon the symmetry and the relaxation of the molecules. So, what I am going to do is we will understand more about the interpretation of the carbon 13 spectra and little bit more details in the next class. Right now, in this class, what I discussed is varieties of decoupling techniques available in carbon 13 to enhance the signal intensity. Finally, what you are going to do is the Broadband decouping with NOE. That is the C-W decoupling. Continuously, you apply RF power both before applying the RF pulse and after applying the pulse, while collecting the signal. The decoupling power should be applied at the center of the proton frequency. When you do that, you get a spectrum fully decoupled and the intensity differs because of the symmetry of the molecule. It depends on the relaxation time of different carbons. Do an experiment with gated decoupling or C-W decoupling, whatever it is, with a relaxation time delay given five times the longest T1 of a carbon in a molecule. Then, you get all peaks are of equal intensity. Usually, quaternary carbons which are non-protonated have less intensity. So, intensity depends upon the symmetry of the molecule and intensity depends upon the relaxation times of the carbons. So, this is what we generally discussed about it. I also discussed what is the decoupling, what do you mean by decoupling, how much power you require for decoupling, broadband decoupling where all carbons are decoupled with all protons. So, with this idea, we will stop here. We will come back and continue in the next class where we discuss much more about carbon NMR. Thank you very much.