One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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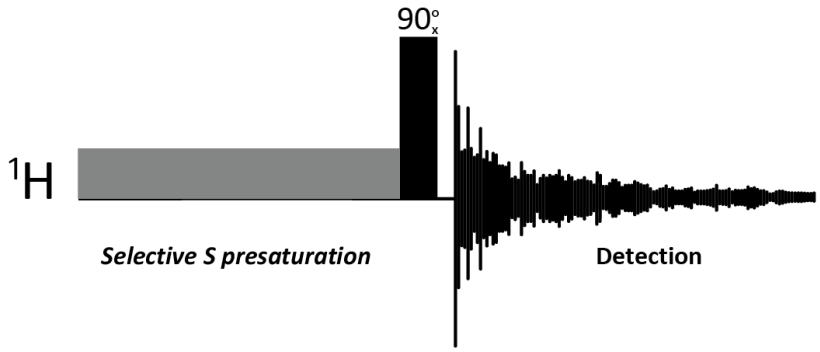
Lecture 57: Steady State NOE

Welcome all of you. Since last couple of classes, we extensively discussed varieties of 2D techniques. Especially in the last class, or in the last one or two classes, we discussed NOESY, its Conceptual Understanding, varieties of NOE experiments, like steady state NOE, ROESY, Heteronuclear Overhauser effect, Transient NOE using 2D, 2D NOESY is the Transient technique. I told you in the previous class, one or two classes before itself, it is a Transient technique. So, the varieties of experiments we discussed. In the previous class, we took large number of examples to analyze and get the conformation of the molecules by the combined use of varieties of two-dimensional techniques like COSY, NOESY, HMBC, HSQC, etcetera. Basically, at the end, NOESY was used to get the conformational information and also to get the information about the substituent in different places in a given molecule, based on the correlation information we have obtained from the NOESY spectrum. Lot of examples we took and in a given molecule, there could be cis-geometry, trans-geometry or both could be present. I took all these examples and we analyzed the spectrum and fairly easily we were able to get the structural information, conformation of all these molecules by using NOE correlations. One has to be very precise, and one has to be very careful. Of course, how you do the NOESY experiment using 2D is very easy. You do not need to worry too much about it. Of course, there are certain complications which I discussed already. We can take care of it. But one problem with the 2D NOESY is it will take lot of time and also depends upon the mixing time and other things. You have to set the mix properly, so that there is enough time for the spins to transfer magnetization between themselves, which are NOE coupled. But, let me say I have a small molecule, not like a big protein. Mind you, I wanted to tell you this NOESY technique I showed simple examples to make you comfortable. See, if I have to analyze a protein spectrum or a big molecule to analyze NOESY spectrum itself will take several hours. Every cross peak we have to identify and then start getting the information about the spatial proximity, structural information and finally, the distance information. So that is a huge task. That is only if you are in that field, if you are working on a particular molecule, you will spend several hours, several days and weeks to get the structure. That is why I took only small simple molecules to make you comfortable. Even then, let us say, a fairly big molecule you are doing, if you are a biologist or a biophysist or a biochemist, you have a fairly big protein, you are trying to work, and then you are getting a very good NOESY spectrum. Let us say which is containing several hundreds of cross peaks. That is fine, you know for such molecule

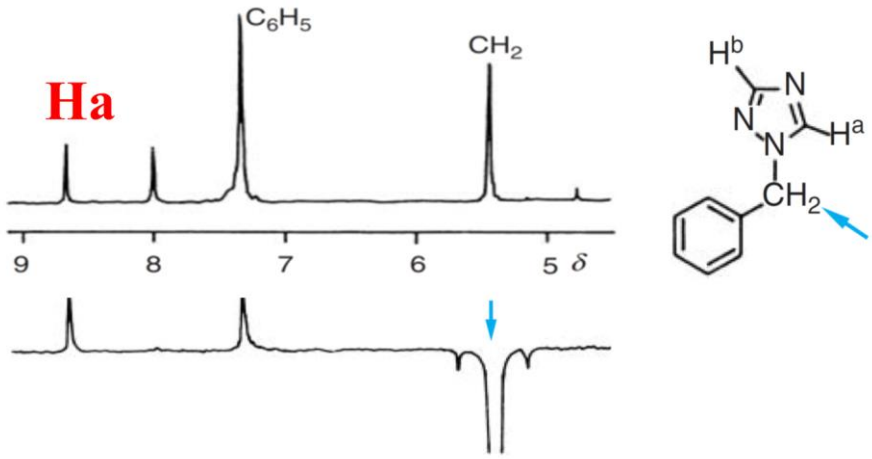
you have to do that. But you have a small molecule let us say, we do not require that much time. You do not have to acquire a 2D data, spend hours or several hours of experimental time. And if it is a simple spectrum like we saw in some of the previous examples of the NOESY, can we do the same 2D experiment in a one dimensional way? That is what I said steady state NOE. Selectively, we can excite one of the protons that is what I told you, and then apply identical RF power far off resonance, far off from the region of interest, and then take that as a reference spectrum. Take the difference between the two. If there is an NOE, you will see the enhancement of the signal intensity. If that is positive or negative NOE, it is a different question. You are going to see that by taking the difference. Of course, if it is a simple molecule like in the previous examples we saw, if you want to find out whether it is cis or trans, and if you have made the assignment by a simple 1D or a COSY experiment, why do you have to spend time to do the NOESY? Can you not do in a 1D way? See many of these 2D experiments like TOCSY, NOESY, HSQC, ROESY, all these things can be done in a one dimensional way. You do not need to go for 2D all the time unless, there is a pressing need for it.



So, I will show you how we can utilize one dimensional NOESY technique to get the structural information in some of the molecules. We will start with that work today. This is called selective NOE experiment, also called steady state NOE experiment. I discussed this when I was discussing the concept of NOE and varieties of NOE experiments. Steady state NOE experiment is very simple. You apply a pre-saturation RF power, soft pulse, RF pulse for pre-saturating the selected signal of your interest, apply 90 degree pulse, start collecting the signal. That is a very simple one. Of course, I told you, you have to carry out the difference experiment. Selectively saturate the proton by very low RF power for a time t, collect the spectrum. Identical experiment you do, apply same RF power, far off place for the same amount of time, so that the intensity are not getting perturbed because of RF power. Even if there is a perturbation, it should be equal for both of them. Collect the spectrum. That is what I said, and this is a reference spectrum. Now, take the difference between these two. This is what I told you in the steady state experiment. If you do that, difference of that, if there is NOE, you are going to see it. This is all the steady state experiment you can do. Very simple experiment, and of course, lot of things

about selectivity of the particular frequency, and then uniform perturbation of the multiplets, everything, all complications are there. If you do not do properly, what will happen? We discussed. So, in the steady state experiment, selectivity of the frequency for saturation is very important, pre-saturation. Assume that you are all comfortable with that. You know how to do the experiment. Let us look at the applications of some of them by using 1D steady state NOE. I am going to discuss couple of applications today.

One is you can identify E and Z geometry in alkene isomers. Already we saw in the 2D NOESY, finding out cis and trans geometry. We can find out aromatic substitution position. That also we saw. We can make the resonance assignment. That is very important. You can identify while making the assignment, if you get a doubt whether a peak for a particualar proton is this or the other one, which is the resonance frequency for a particular proton, then such times NOE also comes into picture. And then conformational preference if there is any. All these things can be addressed. There are many more applications. I am just trying to show you one or two applications where by doing one dimensional selective NOE, you can always address some of your problems, some of your research interests. You do not require a big 2D NOESY spectrum and everything. Just you can do it by 1D NOESY, the steady state difference NOE experiment. We will start with this one. This is the spectrum of this molecule.

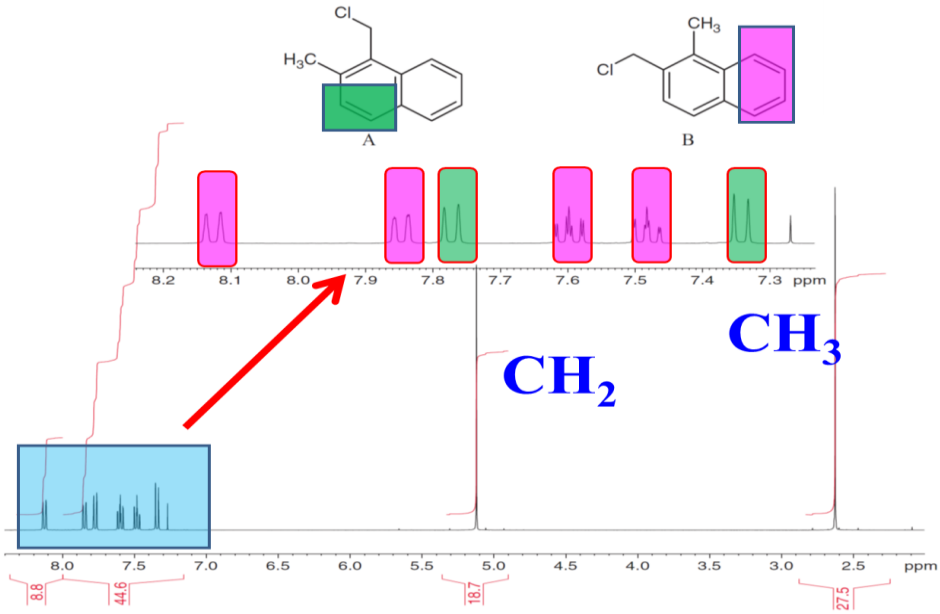


This is a phenyl group. We are not worried about what is the that and everything. This is the phenyl group. Basically, there is a CH2 proton and these two are the protons, two protons which is attached to that ring, 5 membered ring. Now our doubt is which is this proton? There are two here. These two two correspond to HA and HB. One is HA, other is HB. Which is HA, which is HB? I do not know. I could make a mistake, because there is no way I can identify this. C6H5 is far away, I can identify this. CH2 is separately here, I can identify this. Then between these two proton, which is which, this specific assignment of the individual protons could be little bit problematic, although the

molecule is very simple. Of course, there are ways to do that. You can go into, take the nitrogen 15 HSQC, see the long range correlation, HMBC long range correlation etcetera. That is all, but in a simple way, one dimensional experiment if you want to do to make the site specific assignment of this particular proton, what I will do is a 1D steady state difference NOE experiment. I will irradiate this proton, CH2 proton is here. This is a normal spectrum, and then I take the difference after irradiating this, take the difference. When you take the difference, of course, this will be saturated and there will not be any signal after saturation. So, difference when you take this will be negative. That is what is happening. When you take a difference spectrum, the saturated peak would not be there when you saturate. So, when you take the difference in normal spectrum that will be a negative peak, huge intense negative peak will be there. That is what it is. This tells me I have saturated CH2 protons. This is okay, not a well resolved spectrum. It is a simple slightly broadened and clumsy spectrum, but all this entire group of spectral lines, even if it is resolved, it is attributed to phenyl group. Then what is left? These two, but if you see the difference NOE here, I saturated you know selectively this CH2, and take the difference. Of course, there is some change in the intensity for a phenyl group. That is okay. Some NOE is transferred to one of these protons of the phenyl group. That is expected because it is attached to phenyl group only. But then if you see carefully, this peak there is no change at all. It is not seen at all, but you will see there is a NOE for this proton. This CH2 when you radiate, the closed spatial proximity is this one. So, it can give NOE only to this. Which is that proton? I can specifically assign this proton to HA very easy. So, this is assignment problem. If you need to make the assignment properly, why do you need a 2D NOESY for this simple molecule? See all you need to know is that you have to identify CH2. If you use selectively saturate CH2, If at all there is NOE, it has to be only for this. Then you make the specific assignment. So, assignment problem can be solved. This is one way. So, selective saturation of CH2 clearly establish this is a HA proton.

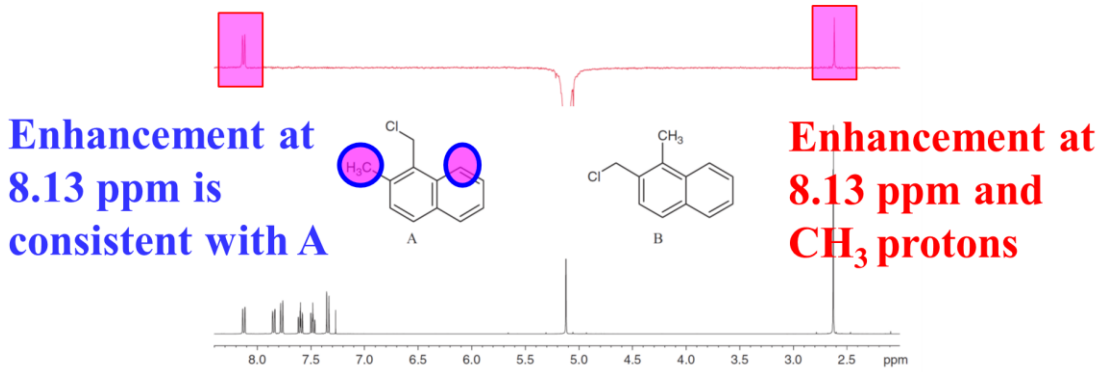
Fine. I will give you a little difficult problem. In a sense, little bit more complex. How do you distinguish two naphthalene structures by steady state NOE? What are these two naphthalene structures? Let us see this. There are two naphthalene structures here. One is see that this group CH2 Cl is here and another is CH3 attached. There are two possible structures we can think of A and B. What is A? In the case of A, this CH2 Cl is here. CH3 is this side. Whereas, in the other case, CH3 is this side, CH2 Cl is here. There are two possible structures for this naphthalene substituted molecule. Our job is to find out which is the correct structure. We do not know. These are the two possibilities and NMR spectrum is taken. Once you take the NMR spectrum, first thing is you have to make the assignment. Assignment is simple. Of course, this entire region is expanded here. If you expand, already I have told you how to assign the phenyl protons. These two are the exclusively two protons which experience only ortho coupling, whether it is this

molecule or this molecule, does not matter. There are only two protons which experience only one ortho coupling and a large doublet will be there. Which is that one? If you look, and of course, this we can assign CH3 proton and this is CH2, which is attached to chlorine. So, little bit down fine. So, assignment can be done. This aromatic proton when you want to assign, I would say these two are for the this phenyl ring. Fair enough, because these are the only two doublets with identical peak separation and this must be from this phenyl group. In this phenyl group, there are four protons. There are different multiplicity patterns you are going to see.

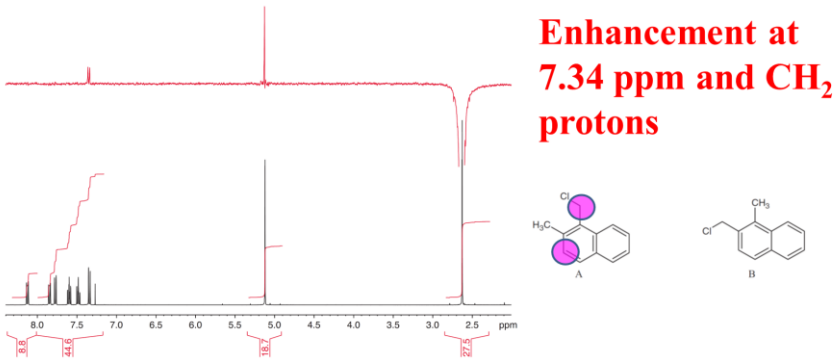


If you see, these two protons will be more like a triplet structure. I told you two ortho couplings and one metacouplings. For this one, if you carefully see these two protons, each one will experience two ortho couplings and one metacoupling, each of them. So, as a consequence, it will be like a triplet of a doublet. So, these two peaks are for these two protons. And of course, remaining two protons are here, one ortho, one meta and para coupling. In principle, each of them should be doublet of a doublet of a doublet, eight line pattern, it does not matter. Well, we are not worried about multiplicity pattern at the moment. We can assign those two very carefully. But I just want to assign these four protons belong to this phenyl group, fair enough assignment. But my problem is whether CH3 is this side or CH3 is this side of the napthalene ring. We will see that assignment. One thing I can tell you this type of assignment, if it is there, what are the possible structures for this? These two protons are here, if I remove that, these two protons, these two substitutions should be ortho to each other. If not, other possibilities should be para to each other. Why? Only then there is a possibility of two protons experiencing the ortho coupling. If it was meta, then we will have a meta coupling, which is much smaller.

Ortho coupling is quite larger. And there is only one ortho coupling for both of them. As a consequence, the first conclusion is these two substituents are ortho to each other or para to each other. One of the possibilities we can think of, it must be either ortho or para to each other. Of course, para can be there, but we can rule it out by doing some 1D difference NOE experiment. What we will do is, you selectively saturate CH2 proton.

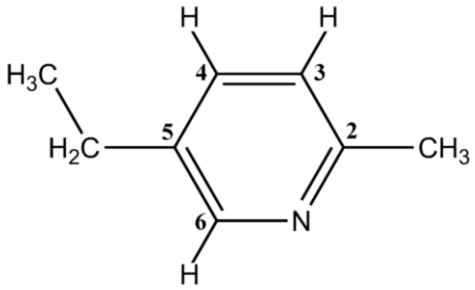


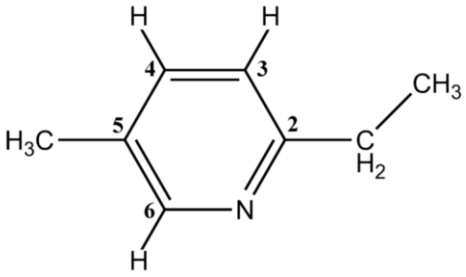
This is the molecular structure, these two, this is CH3 and you see this proton is enhanced. What is that proton? If you carefully see, this proton has been assigned, these two are for the one phenyl ring and these two for other phenyl ring. This when you irradiate CH2, enhancement is seen at 8.13 ppm and also for CH3 protons. When it can happen? This can happen only if you carefully see this. Let us look at this. This can happen if I am irradiating this CH2, you cannot get NOE for this. You can get NOE for CH3, that is correct, that possibility is there. But for one of the phenyl protons when there is NOE, it must be this, it is to the proton of the other ring, other phenyl ring, not where CH3 is attached. So, I would say this is the possible structure for this. This says enhancement to another proton is consistent with the structure. It cannot be this. If it is this, let us say this can give to this, one for this proton and not for other proton. See, assignment we have already done. So, it is not possible. This assignment is clearly known. So, obviously, if this is the structure only, you can get enhancement at this proton and enhancement for CH3. That is one conclusion. But we can further confirm it by doing selective irradiation of CH3 proton. CH3 is here, selectively I will saturate that.



Now, when I do that, there is enhancement for CH2 and also there is enhancement for one of the protons at 7.34 ppm. This 7.34 ppm proton, we know it is from this phenyl group, from the assignment we already made. Understand? So, I saturate CH3, I am getting enhancement at CH2 and also at the phenyl proton. Then this cannot be the structure, because if I saturated this one, CH3, I may get enhancement for this, NOE for this, but not for this. That is not possible. It can give for this like we saw in the previous case. So, it cannot give for this. Obviously, this must be the structure because if you saturate this CH3, you get enhancement here and enhancement for CH2. Look at the structure here. We have saturated CH3, we are getting enhancement at CH2 and enhancement of one of the protons of this phenyl ring. So, that must be the structure. So, this irradiation of both CH2 and CH3 confirms and this must be the correct structure of the molecule. You understand? Now, we could get the correct structure of the molecule. This is the way we could identify the substitution in the naphthalene rings.

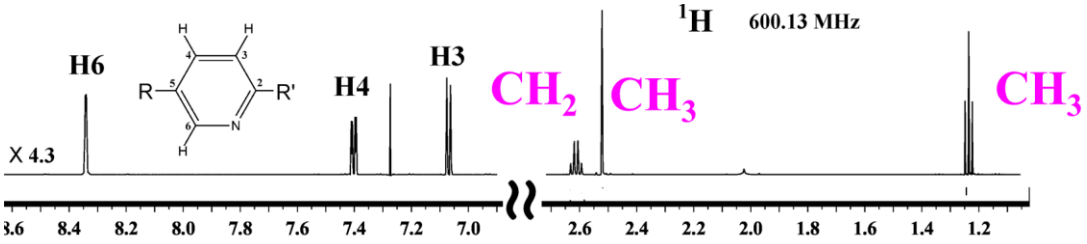
We can do another thing, the regiochemistry of two alkyl substituents of the pyridine ring, I will see. This is the pyridine ring. Now, there are two possible structures given here. One is CH2CH3 his ere and CH3 is here. This is the hydrogen. So, this is CH3 and this is CH2 CH3. Alternately, this is CH2 CH3 and CH3 is here. Understand? CH3 could be between these two protons or CH2 CH3 could be between these two protons. So, this is called 2-methyl-5 -ethyl pyridine or this is 2-ethyl-5-methyl pyridine. See, methyl ethyl pyridine or ethyl methyl pyridine. These are the two possible structures.





2-Methyl-5-Ethylpyridine **2-Ethyl-5-Methylpyridine**

How do you identify which is which? We will go for this. This is the NMR spectrum, and of course, there are only three protons. CH2 , CH3 is here. We know that. CH3 here, CH2 here. Of course, which is methyl CH3, you can identify. One CH3 is here. That is other one is CH2 CH3.

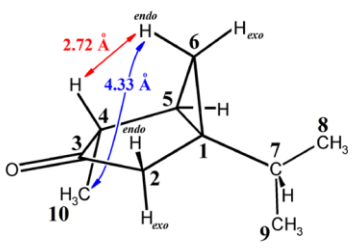


R = Ethyl or methyl? **R' = Methyl or Ethyl?**

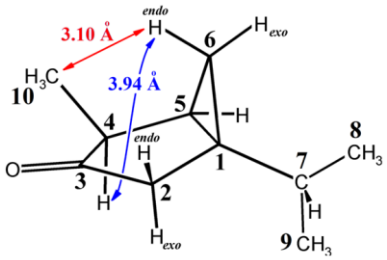
It has to be a quartet and a triplet. This is from CH2 CH3 group. Other CH3 is here. Fine. this is more like a singlet. So, this has to be, CH3 has to be a singlet. There is no way it can get coupling to anything and long range coupling. So, it is CH3, it is a singlet we can make the assignment. This CH3 singlet, and CH2 and CH3 for the methyl and ethyl group are assigned. Then, what is left over? phenyl group. In the phenyl group, there are three protons. This is very easy to assign, because consider this proton. This has only ortho coupling and para coupling. If it is not resolved, it is only a simply a doublet. What is that possibility? You can look at it. There is a doublet here. This is possibly experience one ortho coupling and para coupling is not resolved. Whereas, if you look at this proton H4, this experiences ortho coupling with this, and a meta coupling with this, it is going to be doublet of a doublet. So, it is H4. What is left over? This one, and this one is H6, and has to be a doublet, because it is coupled only to this. So, based on this, assignment of all the four phenyl protons have been made with knowledge of multiplicity pattern. Now, the question still remains is whether the ethyl group is here or here. R and R prime is methyl or ethyl. If R is ethyl, R prime has to be methyl. If R prime is methyl, other one has to be ethyl. So, we have to find out what is what? What we will do is, we will selectively do the inversion of one of the CH2 or selective saturation. We will do one of the protons. Which one? We are going to do the CH2. The CH2 CH3 is there. Selectively, we have to invert the CH2 protons, I mean CH2 protons of the CH2CH3 group. That is done selectively inverted and difference NOE is taken. So, this is our normal spectrum and this is difference spectrum, after selective radiation. Where are we seeing the peaks? See, there is enhancement for H6, enhancement for H4 and there is enhancement for CH3 with some distortion because of the multiplicity. As I told you, if you do not excite the group properly, there is going to be a problem of antiphase. This is what I told you. In the multiplicity, uniform perturbation should be there. Otherwise, this anti phase character can nullify the intensity. But anyway, there is some distortion, but not so much. Let us consider the intensity enhancement. If you saturate CH2 proton, you are going to see the enhancement of H6 and H4 and also CH3. What is the possibility? The conclusion is ethyl group is located at the position 5 of the phenyl ring. This is ethyl group, not methyl

because if CH2 is irradiated, only these two are getting enhancement, H4 and H6. It is possible only if ethyl group is here. If the ethyl group is here, these two cannot get the enhancement. You understand? So, that rules out methyl here. So, R has to be ethyl group containing CH2CH3. That is very easily you can understand. So, ethyl group is situated at the position 5 of the pyridine ring. Then obviously, other one has to be methyl. To confirm further, you can do other experiments also. We saw enhancement at H4, H6. Now selectively irradiate H6. Obviously, you must see the enhancement at CH2. CH2, you see that and also CH3 also, very easy. So, you could see that. So, if you selectively irradiate H6, you got enhancement for CH2. Similarly, you do for H4, you are seeing enhancement for CH2, CH3 and of course, some disturbance is there, that neighboring peak is also getting hit. When you hit H4, this also get enhanced. That is OK. That is not our interest, but you understand H4 gives correlation to this, and this, and also with the CH3. That clearly confirms this CH2 CH3 is next to each other. It is not on this side of the pyridine ring. Further, what you can do is, you can also do irradiation of the H3 proton here. If you do H3 proton irradiation, where do you expect the enhancement? It has to be only this and this, nothing else. None of these should get the enhancement, exactly. See this, you are irradiating this, selective saturation of H3 gives you enhancement for H4 proton and also for this CH3 proton, and bit of disturbance for other things. That is not important. More important is H4 and CH3. These are enhanced because of hitting CH3 proton. What does it tell you? This tells me CH3 has to be on the other side, this side next to nitrogen. So, this must be methyl. So, different NOE experiments, selective NOE confirms which is the isomer which you are looking at, whether the R group is methyl or ethyl, we can easily identify.

We can do one more, take a little complicated molecule. In principle, for this you require 2D NOESY, but I can tell you sometimes even in such big molecules, even simple one-dimensional difference NOE can also help. And there we are considering the molecule thujone. Thujone has two isomers, alpha and beta. What is that alpha and beta? This alpha, this is beta.

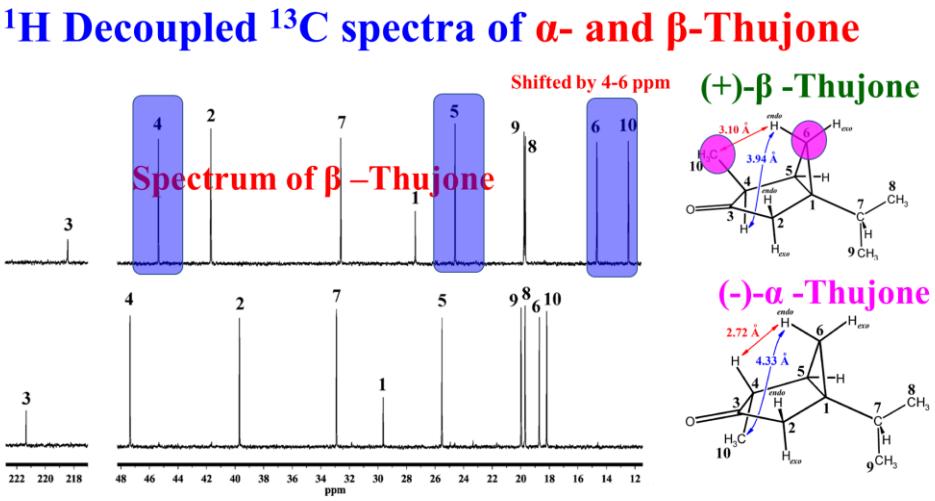


(-)-α -Thujone



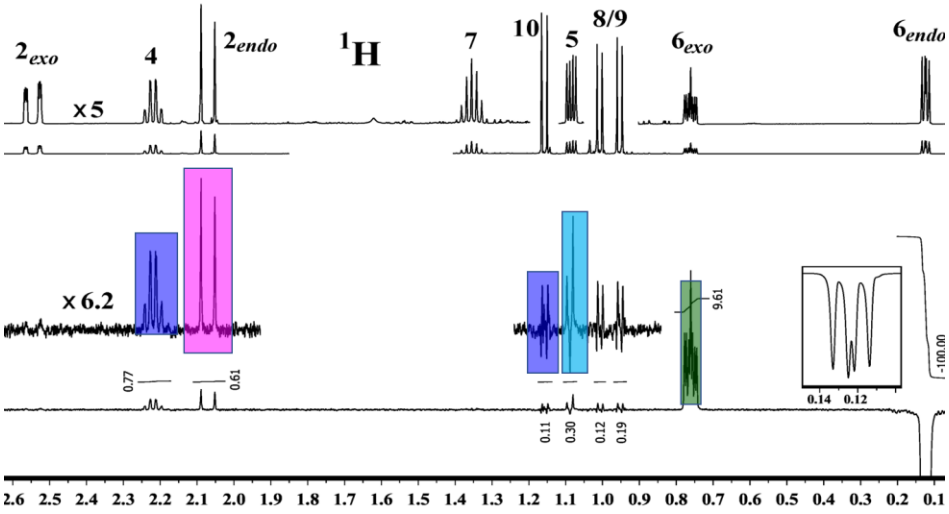
(+)-β -Thujone

In the case of alpha, what will happen is C6 is a gamma carbon. These have both Gauche and anti relationship. That means, once proton, H4 proton is here, in the other case H4 proton is opposite. CH3 is here. See, CH3 and H4 proton position gets interchanged for alpha and beta thujones. In the alpha thujone, H4 is close to H6 endo, say H6 Ndou, whereas in beta thujone, CH3 is close to endo, H6endo. These are the only two conformational changes you can notice. Then it is easy, if you identify what is H6, simply do the selective irradiation of that, do the NOE, selective NOE. Then if you find out whether there is enhancement, whether it is on the CH3 proton or H4 proton, then you know whether it is alpha thujone or beta thujone. Easily we can do that and this is alpha thujone. In this case, we have C6, C10 are on the opposite side, C6 is here and C10 is here, opposite, CH3. They are on the opposite side of the five membered ring. Whereas in the beta thujone, of course C6 and C10 here, they are on the same side. See, they are on the same side of the five membered ring. In the previous case, they are opposite to each other. C6 is this side, C10 is below, opposite side. Now, of course, we can also do 13C NMR. Of course, mind you, carbon-13 NMR also gives a lot of information about the isomers, stereoisomers. Although I did not explicitly tell you, the carbon1-13 chemical shifts can also be used to get the stereoisomer information.



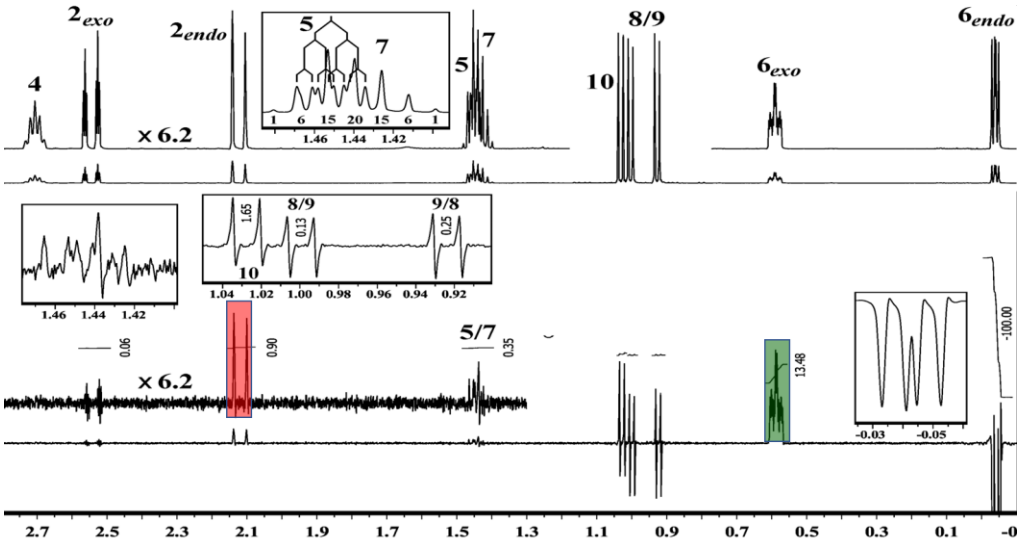
For example, in the alpha thujone and beta thujone if you consider, see this protons 4 and these protons 6 and 10, especially in beta thujone are shifted quite a bit by 4 to 6 ppm. Similarly, this proton 4 and 5 are also shifted. This is because of steric hindrance there, you know, steric clash is there between C6 and C10. And that will push these carbons a little far away. Similarly, for C4 and C5, it pushes to high field. Both these carbons compared to alpha thujone, they are moved high field because of steric clash. Of course, if you go to see the carbon -13 NMR, very, very carefully, there is lot of things to discuss. Then, you can also get the information about isomers by looking at the chemical shifts information. Chemical shift also tells you based on how much it has moved high field or low field, some idea we can get, we need some expertise on that, you can also get

information. Assuming that we are not interested, we do not know that much. We want to observe only 1D selective NOE and see that. In the NOE, here what we can do, we can selectively invert the 6 endo here, 6 endo not exo, 6 endo peak in thujone. You do not know which is which, does not matter. We will take that, then both could be 6 endo. When you invert, take a look at the NOE at positions for H4 and H10. That is what I told you. If there is enhancement for H10, then it is beta. If there is enhancement at H4, it is alpha. Very easily you can do selective NOE. You do not need to go for 2D NOE for this. So, alpha thujone should give a very strong peak for H4 and weak NOE for methyl H10 for this very weak. Converse is the case for beta thujone. So, beta will give strong NOE for this one, and weak NOE for this one. You see the distance matters. Distance is less than 5 angstroms here. This is 3.1 Å, this is nearly 4 Å. That makes a difference. We will see that how this experiment is done. This is selective NOE on one of the diastereomers.



I do not know whether it is alpha or beta. Some molecule has been chosen. Whether alpha or beta, I do not know. This is a normal one-dimensional spectrum and selective inversion is done for 6 endo. Assuming that assignment has been done by using COSY or using your knowledge of multiplicity pattern, everything which can be done. You simply by using COSY, you can make the assignment. Especially exo and endo, one important thing is in the sugar molecules like this, what you should remember is exo will be always down filled and endo will be in the high field. The exo-exo coupling, J coupling between the exo is much larger here. You can see, splitting is much larger than this one. But remember, exo will be little down filled compared to endo. Not always true, but most of the time you can identify that. So, now 6 endo is here selectively inverted and this is the difference spectrum. See, what is the difference? There is enhancement for proton 4, large enhancement. Where is proton H10? Very small, that is the distorted one. And another enhancement is proton H5, and I have H6exo. Of course, when you hit H6 endo, H6exo has to have enhancement because they are just they are geminal protons. So,

obviously, if hit one, other one has a strong enhancement. That is not important thing. But what is important is H4 has a very strong NOE. This is what it is, very strong NOE. H10 also has noe, a bit of distorted one. So, the largest NOE is for H6exo because of geminal distance and NOE for H2endo is also there and H4endo, H4proton is large because the distance is very small. A strong NOE for H4 peak and a weak for H10 confirms this is alpha tujone. That is what I told you. So, I did not know the molecule. I did not know whether it is alpha or beta isomer, but I knew by the structure if I hit 6endo, H4 if it is intense, that is if NOE is stronger there, that must be alpha. Otherwise, if H10 is stronger, that is beta. OK. We will confirm that. The fourth NOE peak is something H5 that all not important for us. Now, from this NOE experiment, you can conclude H4 is on the endo phase of the 5 membered ring close to H6endo and H10 methyl group on the exo phase, far from the exo endo. Obviously, this sample is alpha Tujone, and not beta Tujone, because of the NOE. H4 is on the endo phase of the 5 membered ring, that is very close to H6 endo and the H10 is on other side, exo side. Because of that, we can say it is a alpha isomer.



Now, we will take other stereoisomer. Same thing, I do not know which one is which. Now, what we will do is the selective irradiation of H6 endo again and take the difference spectrum. What is that you are seeing? You see the enhancement for H2 endo. And of course, always exo is known becausethey are in they have a geminal relationship. Another important thing is, you will see enhancement for proton 10. Of course, there is anti phase relationship because of some issue here, does not matter, but you can see enhancement is more for H10 and little bit for 8, 9 and major is there for H2 endo. So, look at this largest NOE for this H6 exo is 0.76%, a strong NOE for H2 endo is also revealed. And then a strong NOE combined with the subtraction artifact is observed for H10. Subtraction artifact is 2 multipllets are positive, 2 multiplates are negative intensity.

It could be subtraction artifact or it could be because of improper excitation of multiplets, we do not know, but there is an artifact does not matter. But still it gives a largest NOE for that. So, what is the conclusion? H4 is on the exo phase of the 5 membered ring, H4 is far away from endo and the H10, H methyl is on the endo phase, close to H6endo. That shows the sample is beta thujone and not alpha thujone. Very easily you could distinguish two isomers, alpha and beta isomers of thujones, by just doing selective NOE experiment. So, this is what I wanted to tell you about this thing. Now, the time is getting up, what I am going to do is I will stop now. I will continue with one or two more examples of where do you apply the selective NOE, 1D selective NOE, so that you can get the conformational information. I took simple molecule and then slowly we went to a little bit bigger molecule. In this class what we understood is by selective irradiation of one of the protons, you could identify the close spatial proximity of other protons and get conformation, whether it is cis-trans, and E- Z geometric isomers, we can identify.

There are lots of experiments we can do. Only thing requirement is the frequency selective excitation has to be proper, otherwise you will be getting distortions. There is a leakage of the frequency if you selectively excite neighboring protons, then it could be confusing. We also have found out we can identify isomers and regiospecificity depending upon the substitution in the phenyl group, in which place the substitution is present. And in regiospecificity on substitution we understood whether the methyl and ethyl group are on which side of the phenyl ring, also we understood. All these because simple one dimensional difference NOE experiment. The conclusion is you do not need to go for a 2D NOESY, a big experiment and spend lot of instrument time and more time. Instead of that in your molecule just by looking at it, if you know which to irradiate simply do the one dimensional difference NOE you will get the structural information. So, with this I am going to stop here. In the next class we will continue with one or maybe another example where you can get different type of information and then we will go to a different type of one dimensional experiment. Thank you very much.