Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture – 12
Stereochemistry Determination Using NMR Spectroscopy

Hello, welcome to module 12 of the course on Application of Spectroscopic Methods in Molecular Structure Determination. We are continuing the lecture from module 11, on the use of in our Spectroscopy in Stereochemistry Determination.

In this module, we will consider the Nuclear Overhauser Effect, how it is used for determination stereochemistry of organic compounds. And also, look at some aspects of the Lanthanide Shift Reagent, how they are used for determination of stereochemistry of organic compounds.

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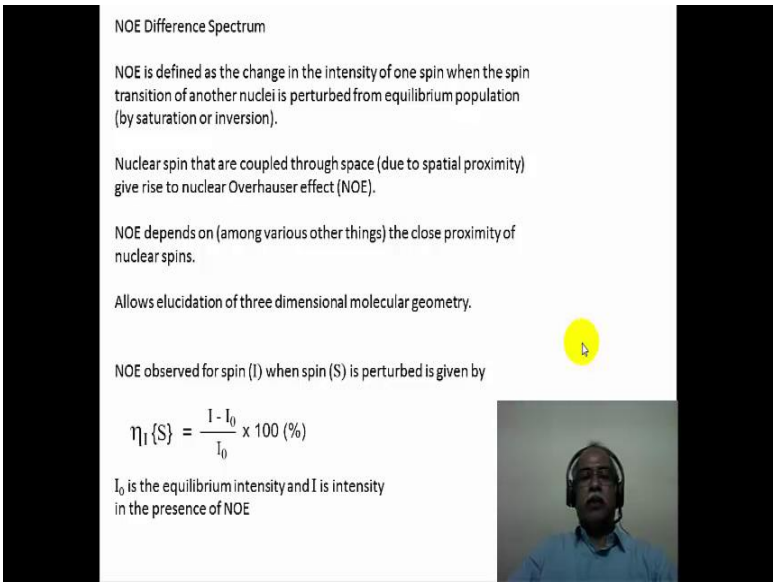

Now, we have already introduced the concept of Nuclear Overhauser Effect in the earlier lectures so, let me just refresh your memory. Nuclear Overhauser Effect is defined as the change in the intensity of 1 spin when another spin transition is perturbed from the

equilibrium population. What is important here, is that the Nuclear Overhauser Effect essentially depends on the close proximity of nuclear spin. In other words, if 2 hydrogens 1 of the hydrogen has to be observed under Nuclear Overhauser Effect conditions it has to be in close proximity to the other hydrogen then, only it is possible to observe the effect of Nuclear Overhauser Effect on this nuclear spins. Therefore, the distance relationship essentially gives important information about the 3-dimensional molecular geometry, so based on the Nuclear Overhauser Effect one can determine the molecular geometries fairly simply.

The Nuclear Overhauser Effect observed for a spin I when spin S is perturbed is given by this expression, this eta I is the Nuclear Overhauser Effect. When the spin I is observed and the spin S is saturated. This would be essentially the difference between the intensity of the signal in the absence and in the presence of Nuclear Overhauser Effect, I is the intensity in the presence of Nuclear Overhauser Effect and I 0 is the original intensity in the absence of Nuclear Overhauser Effect, divided by the intensity in the absence of Nuclear Overhauser Effect times 100 gives the percentage observed Nuclear Overhauser Effect in terms of this expression. Therefore, it is necessary to measure the spectrum under the Nuclear Overhauser Effect condition as well as in the absence of Nuclear Overhauser Effect.

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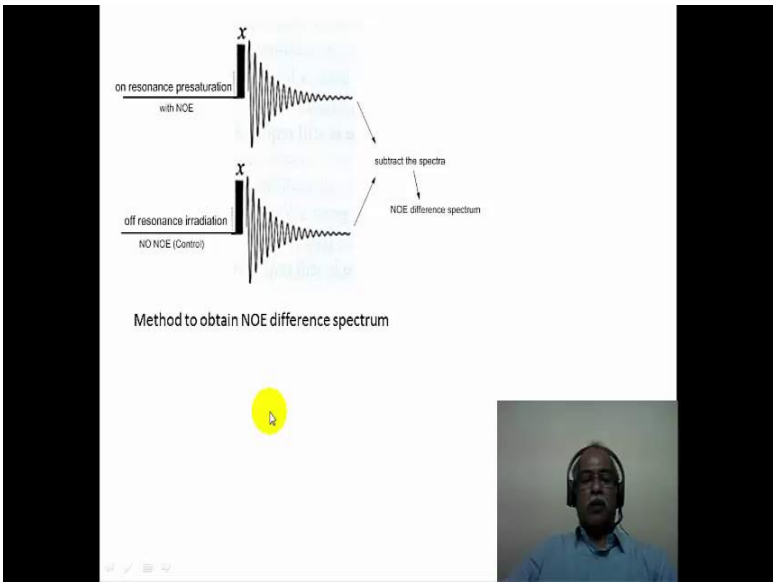

In order to be able to ascertain the Nuclear Overhauser Effect, being felt in the spectrum it is necessary to obtain a difference spectrum. In other words, you record the spectrum under the conditions where Nuclear Overhauser Effect will be present and record the spectrum once again under conditions where Nuclear Overhauser Effect will be absent take the different spectrum that corresponds the NOE difference spectrum. Now, the pulse sequence for the NOE difference spectrum is given here, the top 1 is spectrum accumulated under the conditions where NOE is present, the bottom 1 is under the conditions where no NOE will be present.

In other words, this will be the control experiment. The weight is done is initially. Let us say, we have 2 hydrogen we want to observe the hydrogen A under the irradiation of hydrogen B then this would correspond to applying a frequency of hydrogen B under this conditions before the pulse of hydrogen A is being set up and this will essentially saturate a hydrogen B and set up the Nuclear Overhauser Effect corresponding to the transfer of spin polarization from hydrogen B to hydrogen A. Then the hydrogen A is excited and the free induction decay is observed.

In the case where the control experiment is done it is not exactly the hydrogen B frequency that is being applied. Off resonance irradiation means that the frequency is not matched it is off set by a few 100 kilo hertz or so in terms of the frequency difference between the actual frequency and the applied frequency, this will not saturate the signal and it will not essentially give you the Nuclear Overhauser Effect either. Under these condition when the spectrum is accumulated this would be a spectrum without the Nuclear Overhauser Effect. So, if you subtract this 2 spectra different spectrum will essentially tell the effect of Nuclear Overhauser Effect and that is how the Nuclear Overhauser Effect difference spectrum is normally recorded.

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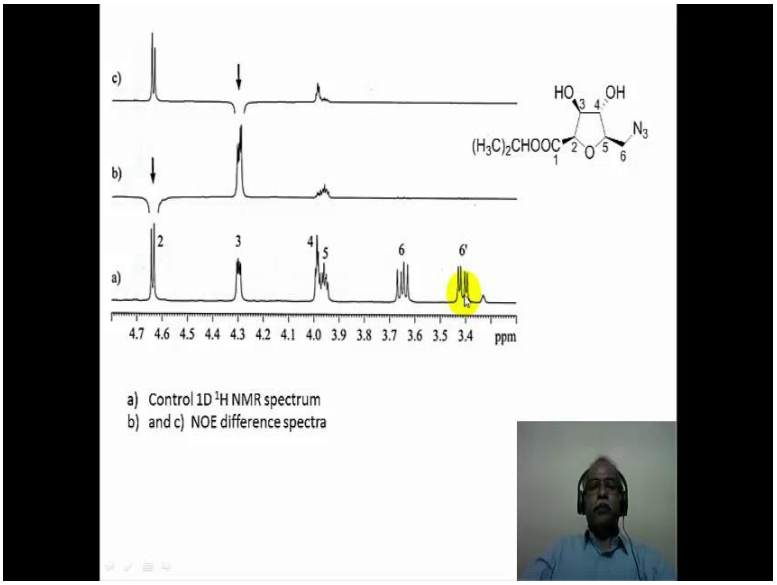

Now, let us consider a simple example of the normal spectrum which is being recorded for this particular sample. This is a assided derivative and in this particular compound the bottom trace is actually the control 1D experiment, in other words this is a normal 1 dimensional NMR spectrum of this particular compound with the peaks assigned to various hydrogen's in this particular molecule.

If we look at the hydrogen number two it comes out the highest delta value because it is corresponding to a hydrogen bearing and on the carbon bearing and oxygen as well as a carbonyl functional group. This is an S R functional group and this is around 4.65 ppm are so. And the second trace is actually a difference NOE spectrum, in other words under the conditions of irradiation of two the molecule is observed and with and without NOE effect it is observed and this spectrum is essentially subtracted from the two with the NOE to get the difference spectrum, so this is a difference spectrum after subtraction of the two spectra accumulated in this manner this for this particular molecule.

So, you do not see these peaks here, because they do not have any changes from the normal spectrum or the control spectrum and the one with the NOE. In other words; these, hydrogens do not undergo any kind of an NOE effect. The only hydrogens that does do show the NOE effect by means of an enhancement in the difference spectrum is

essentially, hydrogen on carbon number three and hydrogen on carbon number five. In other words this hydrogen here is sin with respect to hydrogen this carbon and also the hydrogen in this carbon, so the special close proximity between this hydrogen and this hydrogen makes the NOE to be observed maximally for this particular combination of hydrogen. Namely, hydrogen at the position two and hydrogen at position three. These are cis hydrogen essentially; a large Nuclear Overhauser Effect is what is observed.

Hydrogen 2 and 5 are also cis with respect to each other and you do see a small Nuclear Overhauser Effect, enhancement affect that you seen by means of a positive peaks in this particular case. Since two is saturated you do not see the signal for two and you do not the signal for 4, 6 and 6 prime essentially because they do not have any Nuclear Overhauser Effect as it is seen in the difference spectrum. This essentially tells you that the relative stereochemistry between 2 and 3 must be cis with respect to each other. Similarly, the relative stereochemistry between 2 and 5 also must be cis with respect to each other, the relative stereochemistry between 2 and 4 is actually trans and that is the reason you do not see any Nuclear Overhauser Effect for the proton at carbon number 4 in difference spectrum.

The top trace again is by irradiation of the hydrogen at carbon number three. Carbon number three also has a cis hydrogen with respect to carbon 2 and 5, so one should observe the Nuclear Overhauser Effect corresponding to carbon number two and carbon number 5 when 3 is irradiated. In other words, the hydrogen and carbon number 3 is irradiated, 1 does see the effect on hydrogen on the carbon number 2 and 5 because they (Refer Time: 07:29) with respect to each other. So, this clearly demonstrates the relative stereochemistry of this molecule to be 2 and 3 being cis, 3 and 4 being trans and 4 and 5 being trans, 2 and 5 as a result is also cis with respect to each other. Stereochemistry determination is this. Molecule is essentially done by the difference in the Nuclear Overhauser Effect spectrum.

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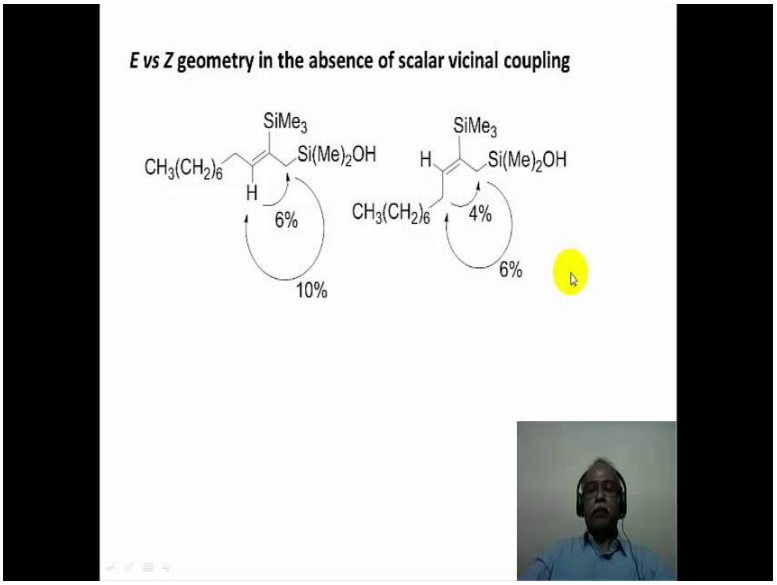

Cis and the trans geometry around a double bond is possible to obtain the stereochemistry information if there is a coupling between the 2 partners, cis and trans coupling is present. However, if there is only 1 olefin hydrogen, how does one observe the stereochemistry of the molecule is the question? Now under these conditions Nuclear Overhauser Effect difference spectroscopy is employed in this particular case, we can look at this hydrogen here this is the only olefin hydrogen present in this molecule so it is should be possible to pick this hydrogen in and irradiate the hydrogen. Under the conditions of irradiation of this hydrogen there is a 6 percent to enhancement of the signal of this particular possession hydrogen, so that indicates their assist with respect to each other.

In other words, there is no enhancement of this particular signal in the NMR spectrum. Similarly, when this particular metaluminous irradiated under the conditions of Nuclear Overhauser Effect difference spectroscopic tells that there is a 10 percent enhancement in the hydrogen signal for this particular hydrogen. So, one can mutually saturate either this hydrogen or this hydrogen and observe the relative Nuclear Overhauser Effects in the difference spectra. If we take the opposite isomer when, this hydrogen is irradiated there is no enhancement here; however, when these two groups which are cis with respect to each other they are mutually irradiated one after the other there is a four percent Nuclear

Overhauser Effect in this direction and 6 percent Nuclear Overhauser Effect in the other direction. This essentially tells that this is the compound where the 2 (Refer Time: 09:24) sorry the hydrogen and the (Refer Time: 09:26) groups are trans with respect to each other. This is a compound where, the hydrogen and this (Refer Time: 09:30) group are cis with respect to each other. In the absence of a vicinal coupling between the 2 partners of a olefinic hydrogen cis and trans it is possible to obtain the Z E stereochemistry of the molecule based on Nuclear Overhauser Effect and the difference spectrum, where the differences in enhancement can be every clearly seen between these two isomers.

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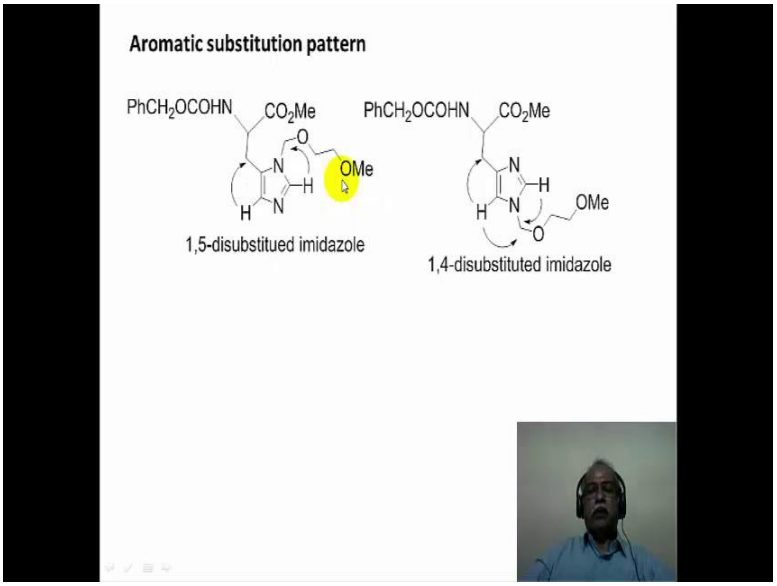

The same is true when, you want to determine the Aromatic substitution. This is a 1, 5 disubstitued imidazole, in other words this is 1, 2, 4, 4, 5 this is a 1, 5 compound and if we look at this particular imidazole this is 1, 2, 3 and 4, so the regioisomers are different in this particular case. In this case the 2 substitute and the nitrogen substitute and the carbon substitute are on the adjacent position. Whereas in this case, the nitrogen substitution and carbon substitutions are not on the adjacent positions. So, this is a 1, 5 disubstitued imidazole and this is 1, 4 disubstitued imidazole. What is interesting about this system is that, when you do irradiation of this particular hydrogen which is easy to pick because this is highest chemical shift value hydrogen in the molecule and these are the 2 aromatic hydrogen's present in this molecules, so you can easily pick out these 2

hydrogen's very easily in the NMR spectrum. And when you irradiate this particular hydrogen there is a nuclear Overhauser enhancement of this CH 2 which is flanked by nitrogen and oxygen, this would be proudly the highest chemical shift value of hydrogen's in the aliphatic region. So, this enhancement can be very clear seen and when this hydrogen is irradiated the enhancement of the signal corresponding to this CH 2 can be easily observed. On the other hand if you do the 2 possession hydrogen irradiation in this molecule that results in the enhancement of this particular signal whereas, when you irradiate the other hydrogen that has a enhancement of both this methylene as well as this methylene because both the methylenes are adjacent possessions with respect to this particular hydrogen. Here, for this hydrogen you see only 1 enhancement whereas, for this molecule this hydrogen irradiation gives enhancement of this CH 2 as well as this CH 2, clearly telling that the 2 substituted positions are with respect to this particular hydrogen in a 1 2 fashion substitution that corresponds to the 1 4 disubstitued imidazole, this corresponds to 1 5 disubstitued imidazole.

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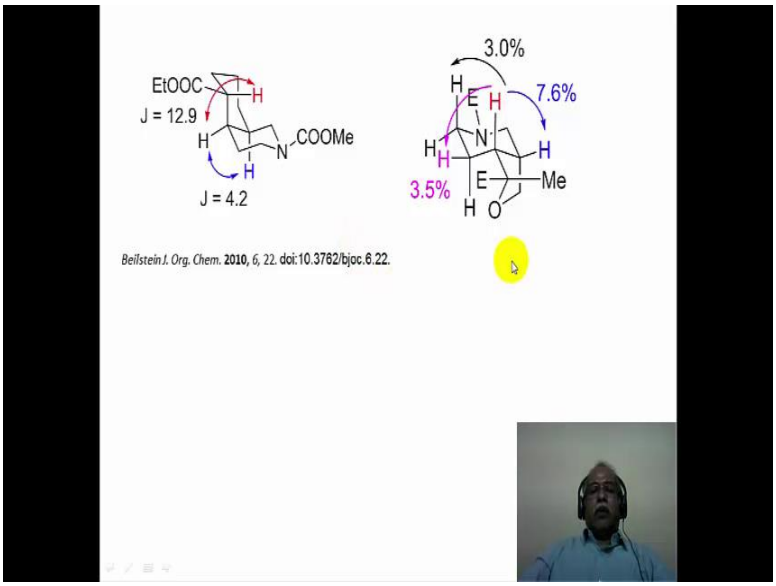

Now, one can use the Nuclear Overhauser Effect in enhancement effect in very effectively when you talk about this kind of fairly complex bicyclic system for example, this is a cis decalin system. In the cis decalin system, this hydrogen and this hydrogen relative stereochemistry is established based on the coupling constant. This is essentially

trans dioxin kind of a coupling so it is a high value in terms of large value of the coupling constant. Whereas, this is a axial equatorial kind of a hydrogen coupling and that corresponds to a volt 4.2 hertz and this corresponds to volt 13 hertz are so. So, based on that this skeleton of this molecule is determined to a cis fused decalin system not decalin system this is A S R decalin system for example, and the relative stereochemistry of these hydrogen is also decided based on the coupling constant information. Where the coupling constant information is difficult to come by it is also possible to use the Nuclear Overhauser Effect.

Let us take this example here, this is again a bicyclic fused, bicyclic molecule and this is cis fusion is what we are talking about in this particular case also. When the red hydrogen is irradiated it in close proximity with this axial hydrogen which is a 1 3 diaxial and this equatorial hydrogen which is 1 2 sorry not, this again an axial type of system this is also a cis hydrogen. So, 1 observes a nuclear Overhauser in enhancement of three different hydrogens when the red hydrogen is irradiated in the molecule, essentially established the relative stereochemistry between these 2 centers and these 2 centers also for this molecule.

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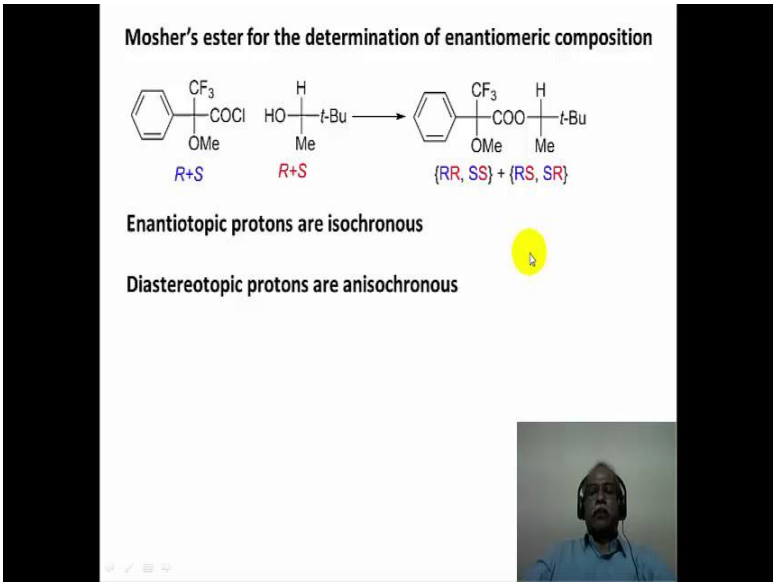

Now, let us see how one can determine for example, ratios of enantiomers because

enantiomers are indistinguishable in the NMR spectroscopy. So, if you want to see different signals for a enantiomers in the NMR spectrum you need a chiral solvent which is too expensive to employ, on the other hand if 1 makes a salt or a ester derivative of enantiomeric mixture in this particular case, this enantiomeric mixture of alcohol if we take and treat it with a chiral acid chloride which is also an enantiomer. These 2 combinations essentially will 4 different isomers, these 2 are enantiomers in nature and these 2 are diastereomeric mixture in nature. So, anisotropic protons are isochronous in other words they have the same chemical shift where as diastereotopic protons are anisochronous, in other words they show a different chemical shift value in the NMR spectrum.

Let us assume for the time being that we are taking only the R isomer of this particular acid chloride which is called a Mosher's ester chloride. Then we will have R of the blue, red of the other one. So, red R and R combination will be there and R and S combination also will be there, in other words the blue R and the red S combination will be there. Those are diastereoisomers and they can be readily distinguished by NMR spectroscopy.

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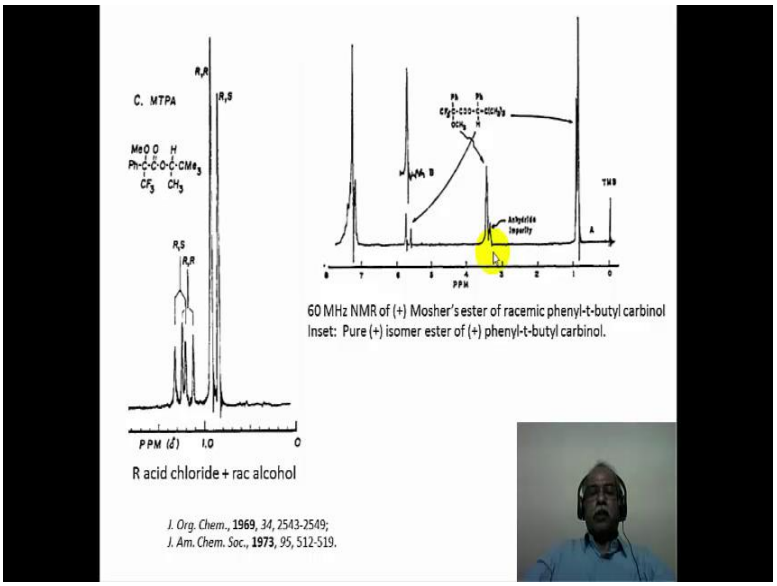

This is what is illustrated here, the R acid chloride namely the acid chloride is taken only R isomer and a racemic mixture of this particular alcohol is taken and the ester is formed.

The resulting ester gives you nicely 2 different signals for this particular hydrogen here, this hydrogen which is a tertiary hydrogen here which is adjacent to the in other words let us sorry, let us take this methyl group. This is a methyl region is what we are looking at, these 2 peaks are corresponding to the tertiary butyl functional group in this molecule and this corresponds to the CH 3 functional group in this molecule for example. If you take the alcohol alone this will simply give a doublet for this hydrogen this methyl group because it is split by this hydrogen into a doublet and this also will give only 1 kind of a tertiary butyl group corresponding to a mixture of an (Refer Time: 15:52).

On the other hand when it is reacted with an optically active acid chloride and this ester is formed, this is a diastereomeric ester. You have the R acid chloride and the racemic alcohol, so you will have R,R combination and R,S combination and that corresponds to 2 diastereomers being present. The 2 diastereomers are easily distinguished is identified this means CH 3 which was originally doublet because of the hydrogen. Now 2 doublets are seen, corresponding to the R,S isomer the other 1 corresponding R,R isomer. So, if 1 integrates the space 1 can easily identify how much of R,R and how much of R,S is present in this molecule, which in term can tell you about the (Refer Time: 16:36) access or a (Refer Time: 16:37) ratio of the 2 molecules. Since we have taken a racemic alcohol the ratio is nearly 1 2 1 in this particular case.

Similarly, the tertiary butyl group is also taken as the R,R and R,S isomer each 1 gives a singlet for the tertiary butyl functional group. This integration also 1 can tell that we are dealing with racemic alcohol of compounds in this particular case. Now this is a 60 megahertz NMR spectrum of the plus isomer of the mosher ester of racemic phenyl tertiary butyl carbinol, in other words this is the compound essentially and this is essential the difference is that you have a methyl group here whereas, this is a phenyl group here that is a difference. The pure isomer plus isomer of the ester and the pure plus isomer of the alcohol is shown in the inset here let me just see for example, this is a recemic mixture of alcohol with a acid chloride and this is one of the pure anisochromers of the alcohol, we can see here one of the diastereomer corresponds to the alcohol plus alcohol which is over here and the other diastereomer which is not present in the pure plus alcohol is missing in this particular case.

So, the enantiomerically pure alcohol gives only 1 peak, the enantiomerically not pure in other words the racemic mixture of alcohol gives 2 peaks corresponding to this particular signal there is no coupling partner to this hydrogen so it is a diastereomeric mixture of asters is what is formed then it is treated with Mosher's acid chloride. So, the example here is essentially illustrates the frank that the Mosher's ester can be formed using this acid based acid alcohol acid chloride alcohol reaction when it does so, if we take a pure Mosher's acid chloride and a racemic mixture it will give a diastereomeric mixture of esters which can be easily distinguished, enantiomeric cannot be distinguished but diastereomeric can be distinguished easily by NMR. That is a basic principle behind the technique that is being used. And the 1 advantage of Mosher's salt are the Mosher's esters that the diastereomeric signals are fairly well separated as you can see, so the integration of the signals and hence the ratio determination will become easy. Not only that 1 can use also flurine NMR to look at the CF 3 group in this compounds, that is also useful to distinguish the or get the enantiomeric access ratio of this compounds in the molecule.

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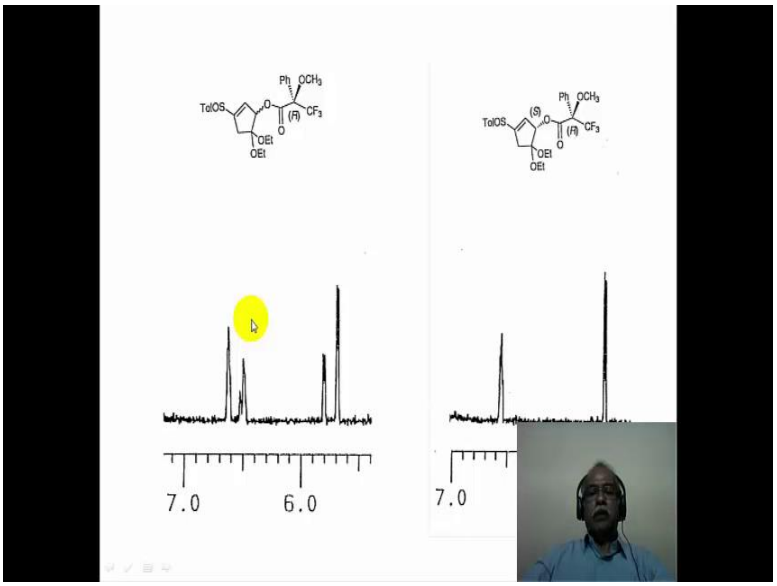

This is another example of a racemic mixture is taken and a pure S isomer is taken with a R isomer of the Mosher ester in other words R isomer of Mosher acid chloride is reacted with racemic alcohol here and a pure S alcohol in this particular case. R and S denotes the obsolete stereochemistry please recall and otherwise refer to the (Refer Time: 19:26)

book as to determination of the obsolete configuration of this centers in this molecules.

In this particular case the olefin hydrogen appears in this region. In the racemic mixture of course there will be 2 isomers corresponding to the R,R and the R,S, here R and S configurations are there here only R configurations is there. So, R,R combination and the R,S combinations diastereoisomers they will give 2 different peaks in the NMR for this particular hydrogen. Similarly, this hydrogen which is bearing the aster functional group that also comes as to isomers, so integration of this peak essentially tell you how much of the R alcohol and how much of S alcohol is being present in the system as for as the enantiomeric enrichment is concerned or enantiomeric access is concerned.

In the case of the pure R,S isomer this only 1 enantiomer that we are talking about, so it gives it only peak. This peak which is coming around 6.5 are so and 6.6 are so, this peak here essentially corresponds to this peak here and this peak here which is 5.57 and this is around 5.757 essentially corresponds to the S isomer. So, one can assign this tall peaks to the S isomer of the alcohol and this small peaks to the R isomer of the corresponding alcohol.

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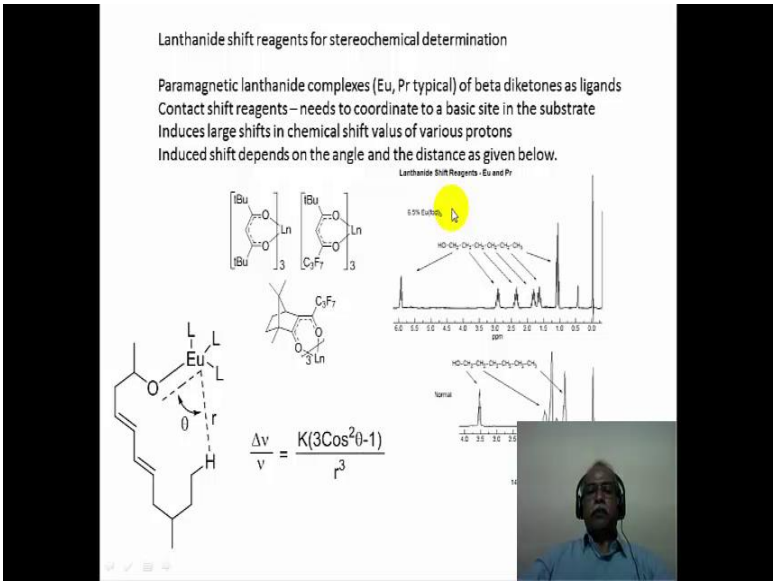

Now, we have also seen the effect of Lanthanide shift reagents on chemical structures

when it coordinates to certain louis basic sides in the organic molecule. This is familiar to you already this has been introduced. This is a methanol equation it depends on the angle of the coordination site to the europium to the hydrogen under observation divided by R cubes, since there is a distance relationship, since it is varying with respect to the power 3 of the distance it rapidly falls off as the distance moves further and further away from the europium complexation center.

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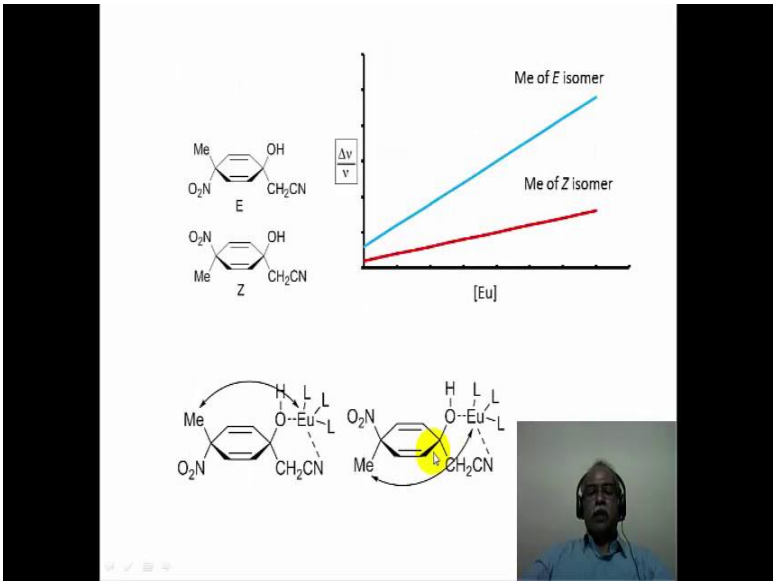

This is nicely illustrated in the determination of stereochemistry of these 2 isomers. If we look at this molecule there is a chiral center here and there is another chiral center here, but these 2 isomers are diastereoisomers, the nitro and OH are trans here, the nitro and oh are cis with respect to each other, so this is called the Z isomer and this is the E isomer of the compound. When the europium shift reagent is added it is going to complex to the most basic site which is this particular oxygen here and with respect to this oxygen if we look at in the europium complexed here, the methenyl group is cis with respect to that particular europium complexation site.

Whereas, here this would be trans in other words the distance between europium, oxygen and the methenyl will be much larger in this Z isomer compared to the case of the E isomer. So, the E Z isomer because the distance is much larger should show us smaller

effect in terms of the induced chemical shift value to the europium concentration, as you keep increasing the europium concentration more and more complex should be formed and the chemical shift of value of this methyls to methyls should keep increasing as you increase the europium concentration. But to what extend it increases is decided by the slope of this particular curve, the 1 with the larger slope if you look at for example, the methyl group which is cis with respect to the, in the other words the E isomer where the OH and the NO 2 are different, but the methyl is cis to the OH the E Z and E R determined between the NO 2 and the OH group.

But if we look at the methyl and the OH relationship in the E isomer that is cis with respect to E isomer, so the E isomer should give the strongest effect in terms of the induced shift as increase the concentration of the europium. Whereas, the E Z isomer where the methyl and OH are trans with respect to each other gives a smaller slope or slower smaller slope with respect to the E isomer. So, when you have both the isomers if we determine the relative slope of these 2 curves that are seen here, the one with the largest slope obviously belongs to the one where the europium and the methyl coordination, the europium coordination at the methyl are cis with respect to each other which is the E isomer in this particular case.

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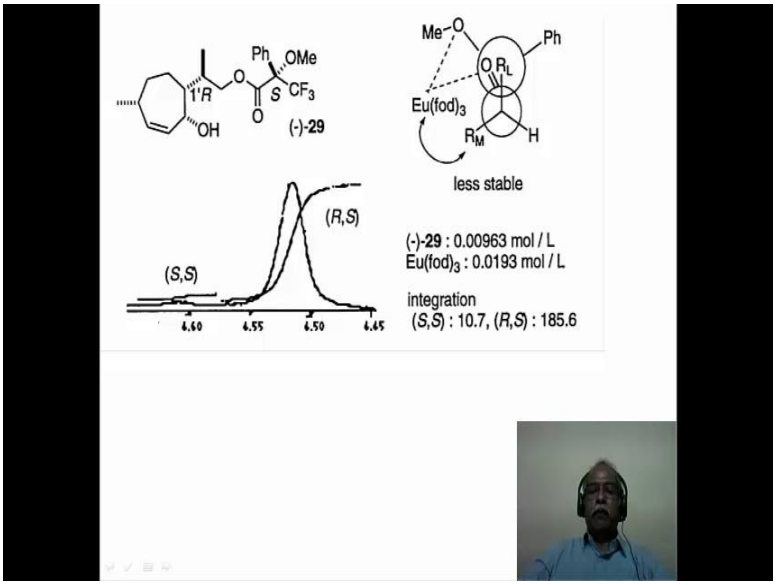

One can also use the chiral shift reagent along with the Mosher's (Refer Time: 23:45) differentiate between the diastereoisomers, in other words the diastereoisomers mixture of ester is formed in addition to that europium is also ordered to increase the shift where the resolution is not so good you can also add the europium. Europium will essentially complex to one of these basic sites here and induced a large shift between the diastereoisomers. So, the S, S isomer is the minor isomer here whereas, the R,S isomer is the major isomer in this particular mixture.

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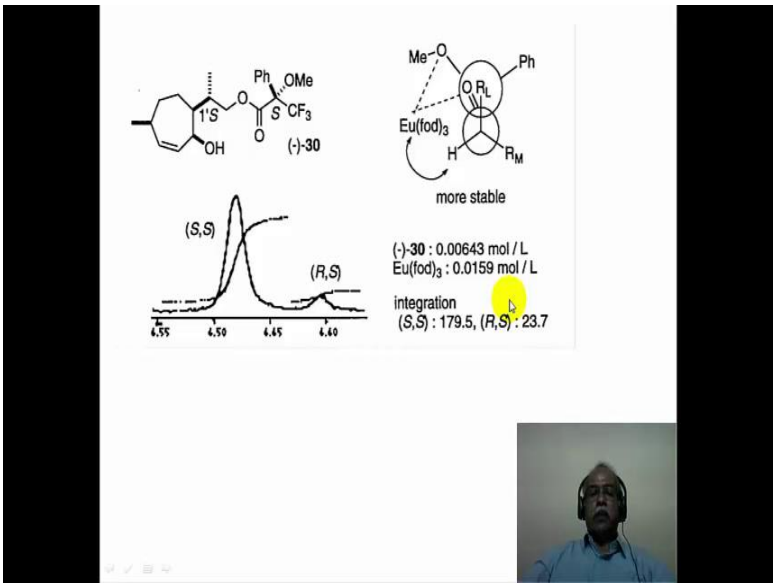

Whereas, if you take the opposite mixture namely the S, S being smaller here whereas, the S,S being higher here, the integration ratios tells us that this is at the ratio of 179 is to 23 whereas, this is in the ratio of 185 is to 10 as per as the 2 diastereoisomers are concerned. Here, a combination of Morsher's ester along with the europium Eu fod 3 is used to distinguish and separate the 2 diastereoisomers signals in the NMR spectrum.

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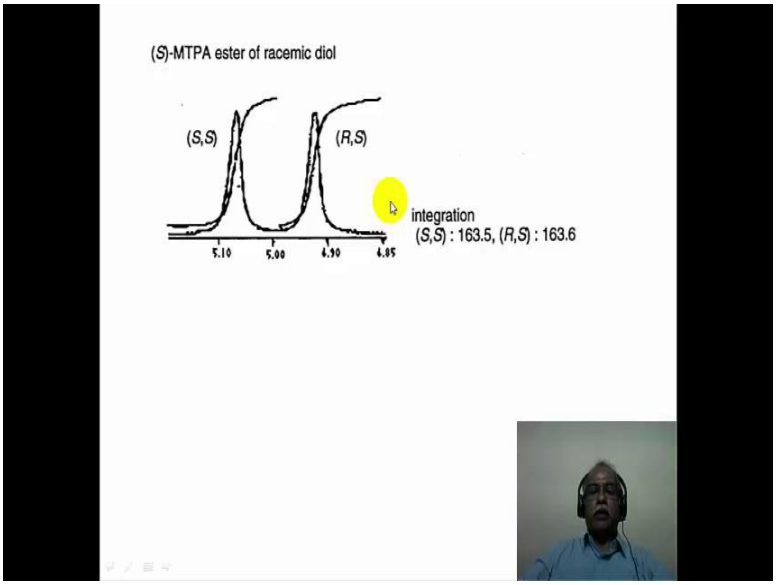

On the other hand, if we take a racemic mixture, we get 1 is to 1 signal intensity ratio for the racemic mixture along with the europium Eu fod 3 being to the added to the mixture to for the separation of the signal.

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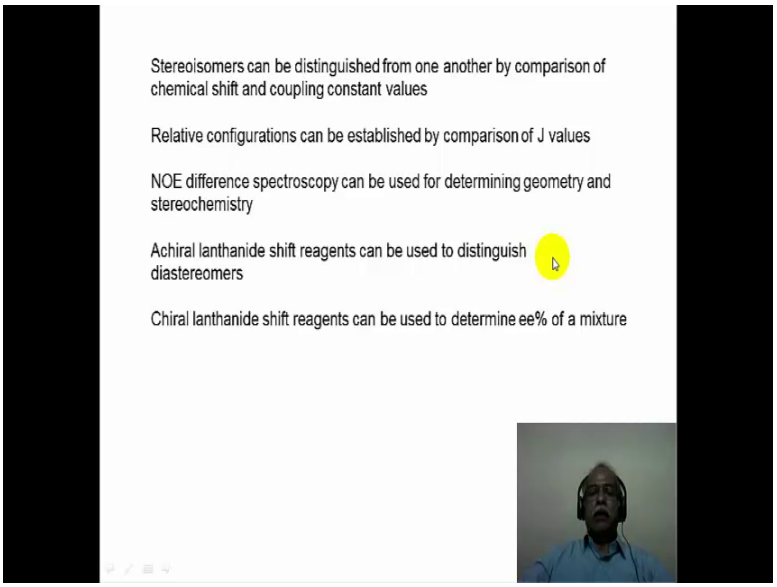

So, what we have seen in this particular module which is a brief module, is that

stereoisomers can be distinguished by one another, stereochemistry can be distinguished from one another by comparison of chemical shift as well as coupling constant value. This was done in the previous module. Relative configurations can be established comparison of the J value this is also we saw in the module 11. NOE difference spectroscopic can be used for determining molecular geometry and stereochemistry, this we saw in this particular module. Achiral lanthanide shift reagents can be used to distinguish diastereoisomers and chiral lanthanide shift reagents can be used to determine the anisochromeric access of a mixture and of course, the Mosher's salt method, Mosher's ester method can be used for distinguishing anisochromeric access of compounds in a racemic mixture or in a anisochromeric access compound it is possible to distinguish the NMR spectroscopy by forming the diastereoisomeric mixture of esters using a Mosher acid chloride.

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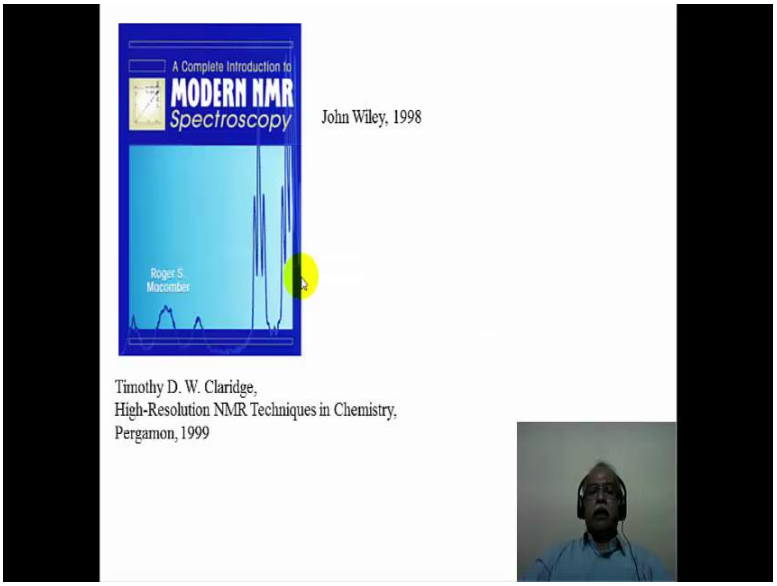

Thank you very much for your attention, once again I would like to recommend these books for the stereochemistry aspects or the NOE affect and so on can be learnt from this nicely written books.

Thank you for attention once again.