Application of Spectroscopic Methods in Molecular Structure Determination
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Lecture - 20
Mass Spectrometry

Hello, welcome to the course on Application of Spectroscopic Methods in Molecular Structure Determination. This is module 20 of this particular course, and in this module, we will have a tutorial session on Mass Spectrometry. Now in the earlier module, we post couple of questions and we will see if we can arrive at the structures or the answers for those two questions.

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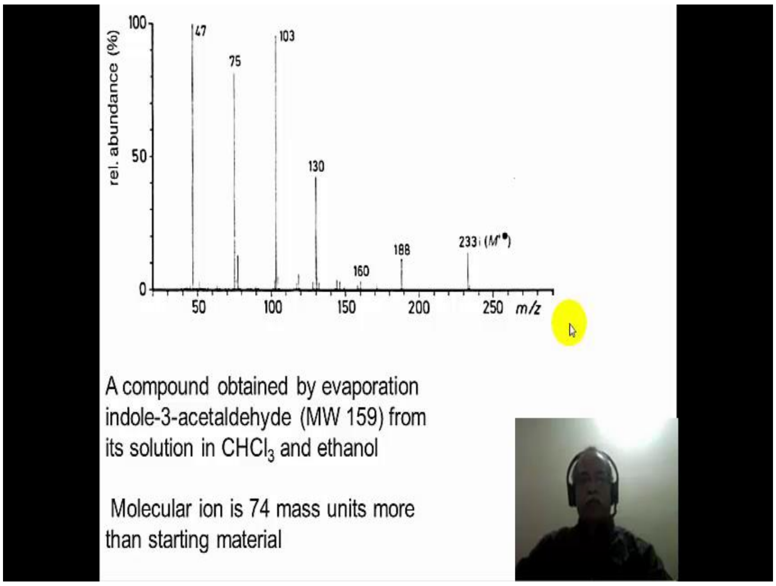

A compound obtained by evaporation indole-3-acetaldehyde (MW 159) from its solution in $CHCl_3$ and ethanol

Molecular ion is 74 mass units more than starting material

The first question is given here; a mass spectrometry is given here. This corresponds to a compound obtained by evaporation of indole-3-accitoldehyde from a solution in chloroform and ethanol alcohol. In other words, when the evaporation of indole-3-accitoldehyde is carried out from a solution of chloroform and ethanol alcohol, a new compound is obtained and this mass spectrum corresponds to that particular compound.

If you observe carefully, the molecular weight of indole-3-accitoldehyde is 159, and that peak is absent in the spectrum, so it cannot because of the indole-3-accitoldehyde. The molecular weight is 333 for this compound as it is mentioned here as m plus ion and this is about 74 mass units more than the starting material. Suppose, if the indole-3-accitoldehyde had reacted with chloroform, then it must contain some chlorinated compound such is not the case because the chlorine isotopes are not see in the molecular ion region. If it had been a reaction with the chloroform, chlorinated product could have been formed and as a result chlorine containing product could have been formed. As a result of that m plus and the m plus two ions would have been seen in the mass spectrum, therefore, the obvious choice is that the indole-3-accitoldehyde has reacted with ethanol alcohol.

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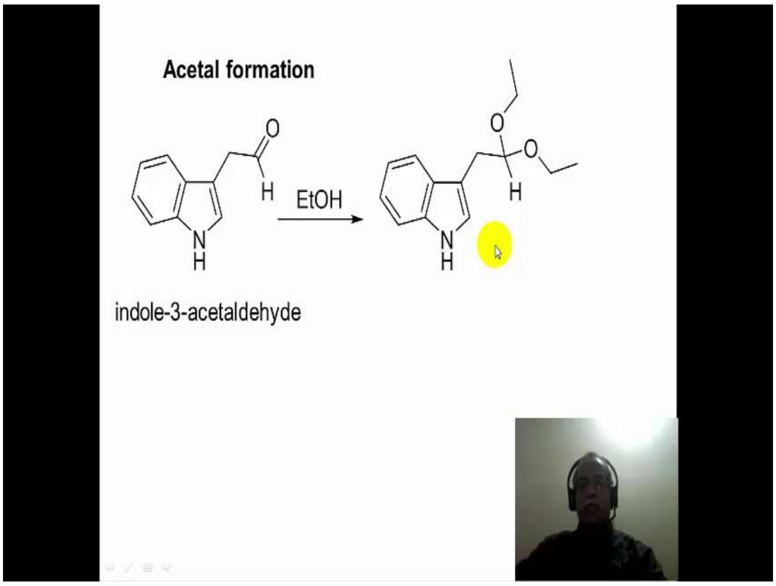

Acetal formation

indole-3-acetaldehyde

All the heads react with alcohol to give accitol as the product. So, the most likely reaction is indole-3-accitoldehyde is reacted with ethanol alcohol to give the diet accitol of indole-3-accitoldehyde. Now the molecular weight of this compound corresponds to 233 matches with the molecular weight of the substance that is shown in the mass spectrum of this compounds. So, let us see the fragmentation pattern of this compound also matches with peaks that are seen in the mass spectrum.

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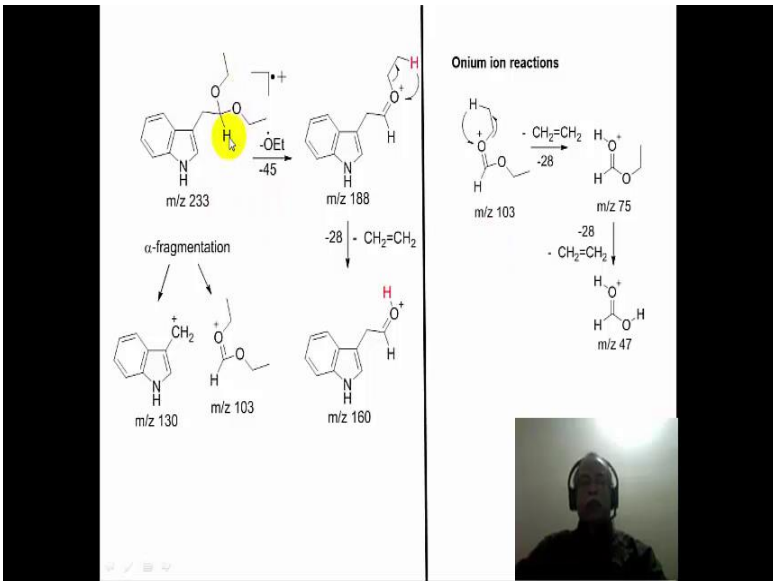
Onium ion reactions

Now, a diethyl accitol of this type can undergo fragmentation of this carbon carbon bond because that will result in a stable carbon ion either this one or a stable carbon ion which is this one. Because this is stabilized, reasoned stabilized by oxygen lone pair participation in the deal of vocalization process. Either of the fragment can form with the corresponding positive charge on it and the molecular weight of the fragment are 130 and 103 respectively for this two fragments. Alternatively, this carbon oxygen bond can be claved that will also give an oxonium ion, which is relatively a stabled species with the loss of 45 mass units corresponding to O E t minus, the loss of O E t for example in this particular case and the oxonium ion has a m by z value of 188.

Whenever an oxonium ion is formed, it is followed by a onium ion reaction. Onium ion reaction is essentially the migration of a hydrogen from the beta position to the onium atom that is this particular case of the oxonium oxygen atom with the concomitant loss of a molecule of etalon in this particular case. So, loss of 28 mass unit will give you a fragment which corresponds to 160. The migration of the red hydrogen on to the oxygen followed by cleavage of the carbon oxygen bound, results in the protonated species of the indole-3-carbon-aldehyde and the loss of 28 mass unit is accounted by the loss from 188 to 160. Now let us see if the mass spectrum of this particular compound has this fragments namely 188, 130, 103 and 160. It does indeed have the peaks corresponding to

188, 160, 130 and 103, 103 seems to be a very intense peak and relatively speaking the other peaks are also not too bad in terms of the intensity except for the 160 peak.

Let us try to identify what is the peak corresponding to 47 and 75 in the fragmentation pattern. This oxonium ion has two athol groups with beta hydrogen, so it can another fragmentation process can take place namely the onium ion reaction can take place by the migration of the beta hydrogen onto the oxygen giving the protonated form of athel format which has the molecular weight of 75. This can further undergo hydrogen transfer from the beta position to the oxygen resulting in the formation of the protonated species of formic acid which is 47 mass units. So, those peaks are also seen 47 mass unit peak is the base peak in this particular spectrum so the structure of this molecule in terms of its molecular weight, as well as the fragmentation pattern have been accounted for by the mass spectrum that is shown in the picture in this particular problem.

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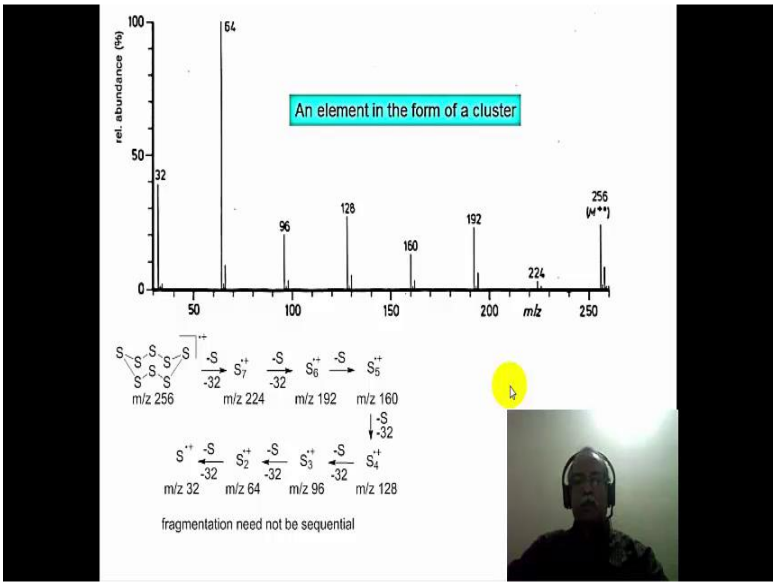

An element in the form of a cluster

fragmentation need not be sequential

Let us move on to the next problem, the next problem deals with an element which is in the form of a cluster, in other words if you look at the mass spectrum carefully there are many peaks, but if you consider the adjacent peaks, each one of the adjacent peak corresponds to a mass unit difference of 32. So, 32 plus 32 is 64, 64 plus 32 is 96 plus 34 is 128 plus 34 is 160 and so on all the way up to 256. So, 8 times 32 is 256 and an element with an atomic weight of 32 would be sulfur, so we are talking about that sulfur acid cluster. Sulfur acid is supposed to have a ground structure like, this with the molecular weight of 256 with the loss of sulfur atoms one after the other, it will result in the formation of the mono atomic sulfur cater and radical and at m by z 32.

All the intermediate stages mass corresponds to the various peak that are seen in this particular spectrum, the fragmentation actually need not be sequential as it is found in this particular mechanistic scheme, two bonds can break at any point of time in this molecule resulting in the s 3, s 4 or s 5 s 6 fragments from the s 8 fragment directly. As a result of that the following fragmented ions can be formed either sequentially or non-sequentially in a particular manner to account for the various peaks that are seen in this particular spectrum.

Mass spectrometry is a very powerful tool to identify substitution pattern, particularly if the substitution is because of a higher isotope of that element.

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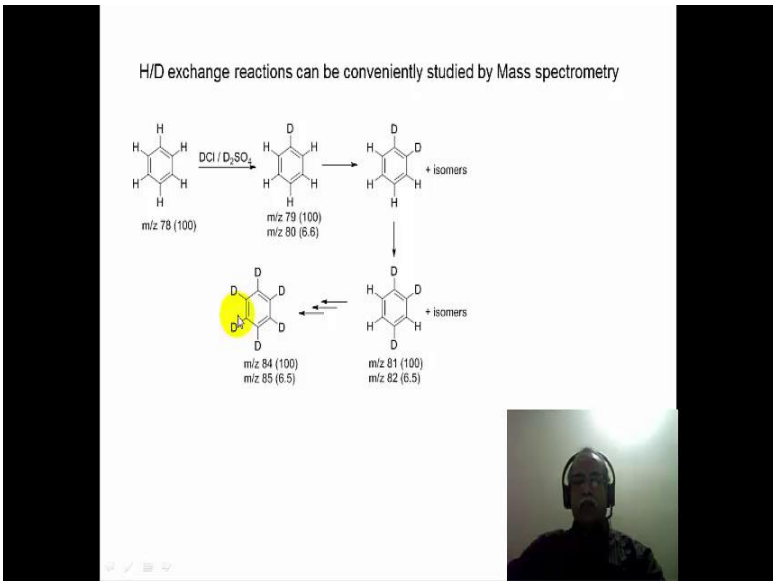

H/D exchange reactions can be conveniently studied by Mass spectrometry

Let us consider this reaction, here the deuterium is substituting the hydrogen sin benzene all the way up to benzene defaces the molecule. The most convenient way to follow this particular reaction is using mass spectrometry because with the incorporation of every deuterium, the mass of the resulting molecule changes from the parent molecule. So, benzene has a molecular weight of 78 so if you look at the benzene mass spectrum, it will have a base peak at m by z value 78. Upon substitution of a deuterium by heating it with d c l and d 2 s o 4, both of them are diluted with d 2 o for example, the hydrogens can be substituted sequential by deuterium so the mono deuterium of compound has a molecular weight of 79. Once the mono deputation is complete, the spectrum would correspond to 79 m by z value of the base peak of the molecular ion.

Now, the sub reaction can proceed further and in duration of during the course of the reaction if the aliquot amount of the samples can be analyzed by a g c mass (Refer Time: 08:01), it will clearly reveal whether it has gone to mono substitution, di-substitution or tri substitution or complete substitution to benzene d 6, when it goes all the way to benzene d 6, the m by z value corresponds to 84, 6 mass units more than the molecular weight of benzene itself for example. So, there is mass spectrometry is a very convenient way to follow such reaction, where the isotope substitution reactions are carried out. This is further illustrated in the next example.

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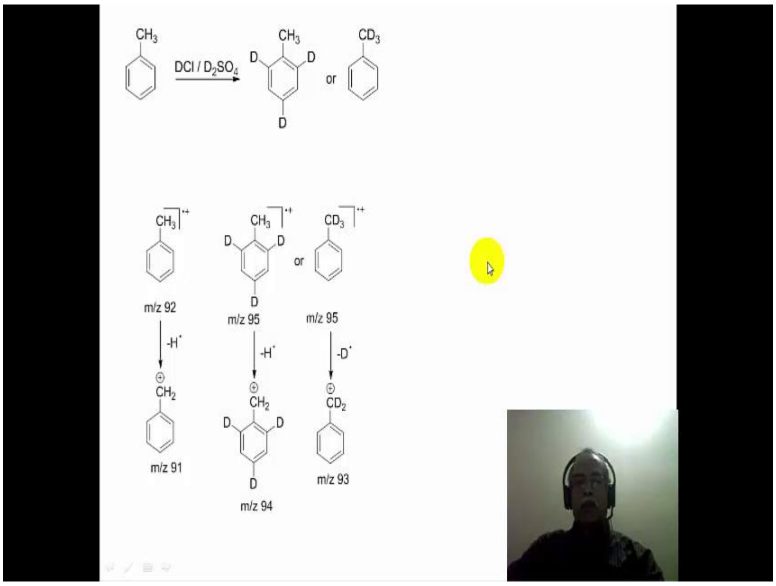

Suppose, if you take toluene and do the reaction and stop the reaction at the tri-substituted stage, one can have either this product or this product. This is a product where the aromatic ring protons have been substituted by deuterium at specific positions of ortho and para position with relative to the methyl or the three hydrogens of the methyl, themselves are substituted by deuterium to give a c d 3 compound. How do we identify whether the particular reaction has given substitution on the aromatic ring? or substitution of the side chain in this particular reaction.

Now, if you look at the molecular weight of this compound and this compound which contains 3 deuterium; this is 3 mass units more than toluene. But they cannot be identified, they cannot be distinguished purely based on their m by z value because both of them has 95 m by z value because the molecular weights are same. However, if you look at the fragmentation pattern, the most prominent fragmentation pattern of toluene is loss of hydrogen from the side chain to give the benzene canton which is mass spectrum m by z value 91, which rearranges to the trapezium canton eventually. If such a reaction where to be fassil with toluene, it should also be fassil with the deuterated toluene, this a tri deuterated toluene, this is also a tri deuterated toluene. This particular case this can lose only a hydrogen atom to give the tri substituted benzyl cation with a by a z value of 94. Whereas this particular molecule with the c d 3 would lose a deuterium to given 1 mass unit lower, in other words this is the benzyl cation with a, c d 2 unit present in the system.

So, one can easily now distinguish based on the mass peak of the benzyl cation, if it is toluene and reacted substrate if it is there it will give 91, if it is a nuclear substituted derivative; in other words, the aromatic ring substituted derivative it will give a base speak around 94 in the mass spectrum or if it gives a spectrum at 93 m by z value, then it would indicate that c d 3 is actually the compound that is formed. In reality when you do the reaction, it is actually the ring hydrogens are substituted by deuterium to give the (Refer Time: 10:43) toluene because the identification of m by z value 94 would correspond to only this particular peak and this could be 93 and this would be 91. So, mass spectrum is a very valuable tool, even when you do substitution reaction particularly when the substitution is a higher isotope of a given element or example.

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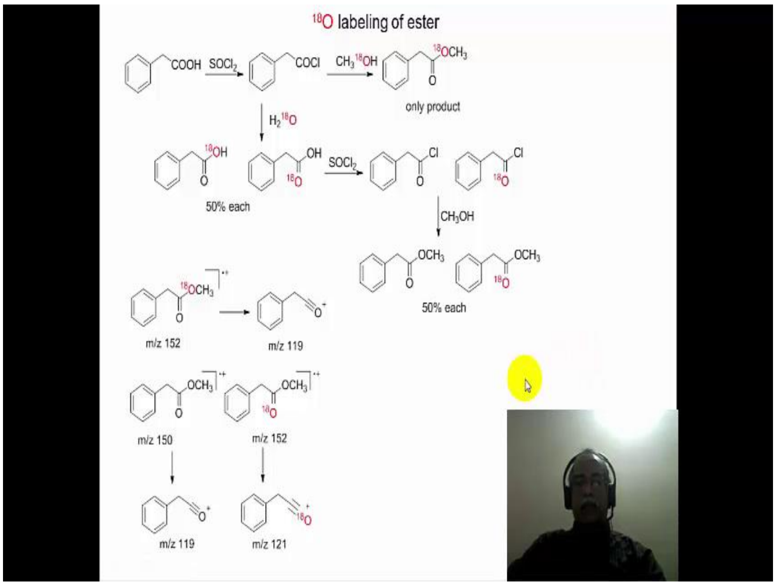

^{18}O labeling of ester

This is further illustrated in the oxygen labeling of this particular ester, this is phenol acetic acid. Phenol acetic acid can be converted into the corresponding acid chloride, when the acid chloride gets hydrolyzed by water o 18, there are two acids that are formed because of the rapid proton exchange that can take place between these two oxygens. The oxygen that is originally present in the molecule is o 16 and the oxygen that enters from the water molecule is o 18 so one can have either the carbonyl oxygen as o 18 or the o h oxygen has o 18. In fact, we will get a 1 is to 1 mixer of these two compounds when this reaction is carried out.

On the other hand, if methanol o 18 is used for (Refer Time: 11:47) the acid chloride then one gets specifically the o c h 3 where the o oxygen is 18 labeled oxygen that would be the only product that is formed here. Now the mixer of these two acids can be converted into the acid chloride which will give this two mixer of acid chloride with o 18 label and without o 18 label subsequently when it reacts with menthol alcohol for example, it will form two esters one ester divide of any oxygen 18 isotope, another ester where the carbonate oxygen is labeled with an oxygen 18 isotope. So, one can easily distinguish the formation of the differential labeled. In this particular case the labeling is on the carbonate oxygen, whereas in this particular case the labeling is on the o c h 3 of the ester.

How do we differentiate this labeling? By simply looking at the fragmentation pattern. The most prominent fragmentation of this particular molecule could correspond to the loss of methoxy radical, to give a oxonium ion or the aisle cation of this type with a m y z value of 119 with the loss of the oxygen 18 isotope of the molecule during the fragmentation process. On the other hand, if mixture of these two esters are present in the system, in other words if the reaction has proceeded in this manner and the labeling has left in the carbonyl oxygen compound, then this fragmentation pattern will have both 119 as well as 121. In this reaction, 119 will be the soul fragmentation process, whereas in this case since this is the mixer of two compounds both 119 and 121 will be formed, indicating the oxygen of the carbonyl is the one that is labeled in this particular system whereas the oxygen of the o c h 3 is what is labeled in this particular reaction. So, this illustrate essentially the utilization of mass spectrometry for the identification of isotopically labeled substrate very effectively, because the masses are different and mass spectrometry works on the basis of the masses of the molecular.

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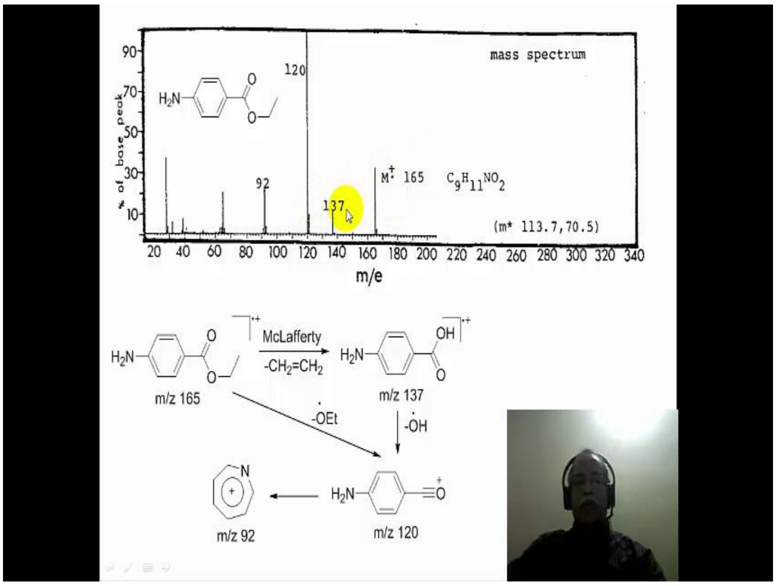

Now, let us try to analyze this particular spectrum, the spectrum corresponds to para amino ethyl benzoate. Para amino ethyl benzoate is a pain killer and this mass spectrum corresponds to the electron impact and a session mass spectrum of this particular compound. The molecular formula is given c 9, h 11, n 2 and the structure of the compound is also given here. If you look at the mass spectrum, the fragmentation corresponds to the loss of 45 to give a mass at 120 and loss of 28 corresponding to a mass at 137. The ester of this type, in other words the ethyl ester always gives a always gives a peak with the loss of 28 mass unit because of the Mclafferty rearrangement.

This is alpha, the oxygen is alpha, this is beta and this is gamma carbon. The gamma hydrogen which is the metal hydrogen can be transferred on to the oxygen with the loss of ethylene unit which is a 25, 28 mass unit loss corresponding to a fragment at 137, which would be para aminobenzoic acid in this particular case. Para aminobenzoic acid these molecule can also lose a ethoxy radical to directly give a loss of 45 mass units to give the 120.

The fragmentation pattern can be seen here from the molecular ion, a Mclafferty rearrangement gives you 137 fragment, which is this particular fragment namely para aminobenzoic acid, from the para aminobenzoic acid one can lose the o h dot to give the aisle cation which is it at 128 or directly from the molecular ion it can lose a ethoxy radical to give the same aisle cation in this particular case, this looses a molecular of carbon monoxide with a loss of 28 mass unit from 120, resulting in the formation of a fragment at 92, which probably corresponds to this particular structure similar to the tropylium ion structure for example, except it is substituted with one nitrogen because of the nitrogen present in the para position.

So, the fragmentation pattern essentially is consistent with the structure or the mass spectrum is consistent with the fragmentation pattern that we have seen and the important message is that ethel estates always give a Mclafferty rearrangement with the loss of 28 mass unit from the parent molecular ion to give a fragment ion which is 28 mass units less than the molecular ion peak

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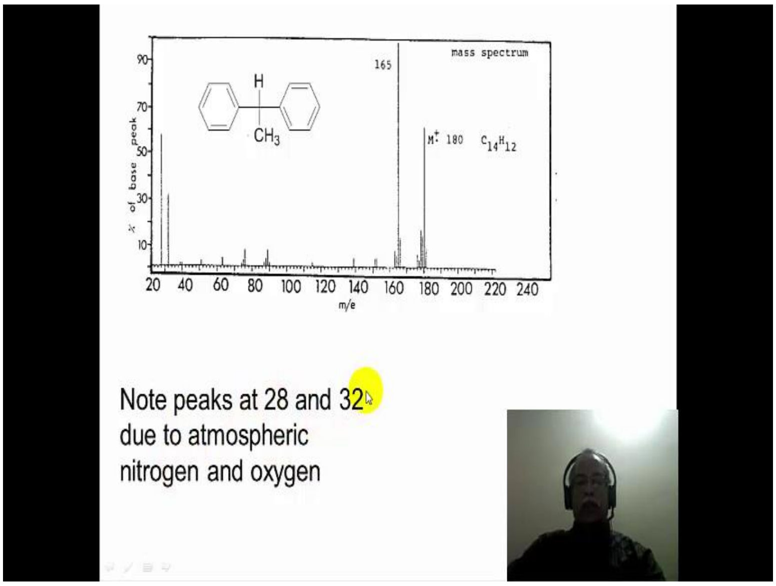

Note peaks at 28 and 32 due to atmospheric nitrogen and oxygen

It is an interesting mass spectrum this is a hydrocarbon molecule with the molecular weight of 180. So, one actually sees the molecular ion peak fairly intern peak is seen in this particular case and the second fragment that is seen which is a peak is 165 corresponding to 15 mass unit loss in this particular case. The 15 mass unit can only be a cleavage of this carbon carbon bond with the loss of the methyl group, resulting in diphenyl methyl cation which is a resistant stabilized stable cation. You do not see any for the fragmentation because of the relatively high stability of the diphenylmethyl cation, which is recorded as the base peak in this particular case. Interestingly the mass plectrum also shows some impurity peaks, essentially coming from the atmospheric nitrogen and oxygen there is a peak if you observe carefully at 28 m by z value and 32 m by z value, most likely these peaks are coming from the contamination from the atmospheric nitrogen and oxygen corresponding to 28 molecular weight and 32 molecular weight respectively for these two elements.

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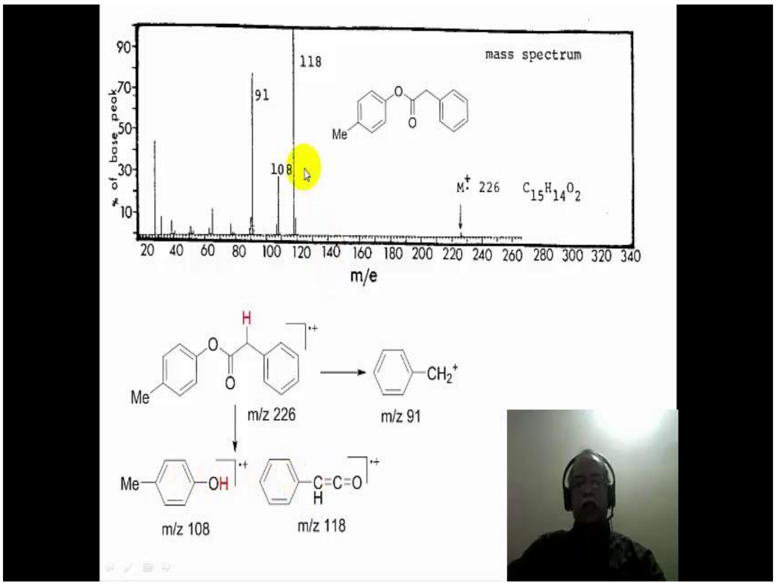

Now, let us consider the mass spectrum of this particular ester, this is the phenylacetic acid, phenolate ester is what is formed here. The molecular weight is 226 what is important to observe in this particular molecules fragmentation is there are two fragments formed which is roughly half of the 226, in other words 108 and 118 are the two fragments that are formed. If you add 108 and 118, it corresponds to 226, in other words the molecule fragments and the charge resides on both the fragments corresponding to 118 and 108 in this particular case.

Let us see the fragmentation process, the fragmentation process is essentially migration of this beta hydrogen, this is alpha carbon with respect to the oxygen it is alpha and beta hydrogen migration to the oxygen resulting in the formation of para methyl phenyl in otherwise para cresol at molecular weight of 180 m by z 108 this particular case and simultaneously it also gives the phenyl (Refer Time: 18:25) as the product with the molecular weight m by z value of 118.

In other words, this particular fragments such that the two fragments, either of them can have a cationic charge in the resulting structure for example. If you add up these two numbers that correspondence to 226, a simple fragmentation of the aisle carbon bond can result in the benzylic cation, which is rearranges to a tropylium cation with a molecular weight of 91 and the 91 peak is also shown in this particular spectrum. Again you speak at 28 probably corresponding to carbon oxide that may be coming from this particular fragment for example, resulting in the formation of the c o is seen in the mass spectrum as a peak around 28. So, the fragmentation essentially corresponds to a hydrogen migration with the concomitant breaking of the carbonyl carbon bond in this particular bond, in this particular molecule for example, resulting in the formation of these two fragments which account for the overall fragmentation of this particular molecule.

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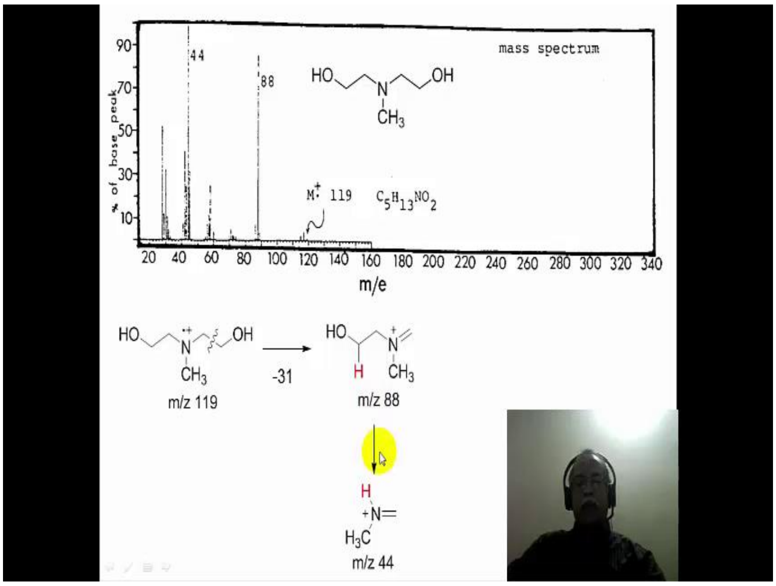

Here is an interesting aliphatic compound, this aliphatic compound undergoes fragmentation quiet extensively because the molecular ion peak is a very week peak in this particular case. The molecular weight is 119, it is an odd molecular weight because it is a nitrogen containing molecule. The difference between 88 and 119 is 31 mass unit so one has to account for 31 mass unit, if you break this particular bond and look at the resulting fragments c h 2, o h for example, loss of c h 2, o h will be a fragment corresponding to loss of 31 mass unit. In other words, this breaking of this particular bond essentially from the cation radical results in the formation of (Refer Time: 20:17) cation that (Refer Time: 20:19) cation has a molecular weight of 88 and the structure correspond to the (Refer Time: 20:24) cation is this particular (Refer Time: 20:26) cation is quite possible that the (Refer Time: 20:28) cation actually exist in cyclic form because this is 1, 2, 3, 4, 5 form of five membered ring for example and as a result of that this could be a stabled ion to give a peak which is fairly intense peak in terms of the relative abundance.

On other hand, further fragmentation by a hydrogen migration to the emin followed by loss of vinyl alcohol, result in the formation of a loss of 44 mass unit which corresponds to the vinyl alcohol resulting in the formation of this particular aminium ion at 44 mass unit. So, the 44 mass unit this is exactly half of the molecular weight of a 88 giving 44 with the loss of vinyl alcohol as a fragment in this particular case. So, essentially all the major peaks in the compound fragmentation process has been accounted by the simple fragmentation process in this particular molecule.

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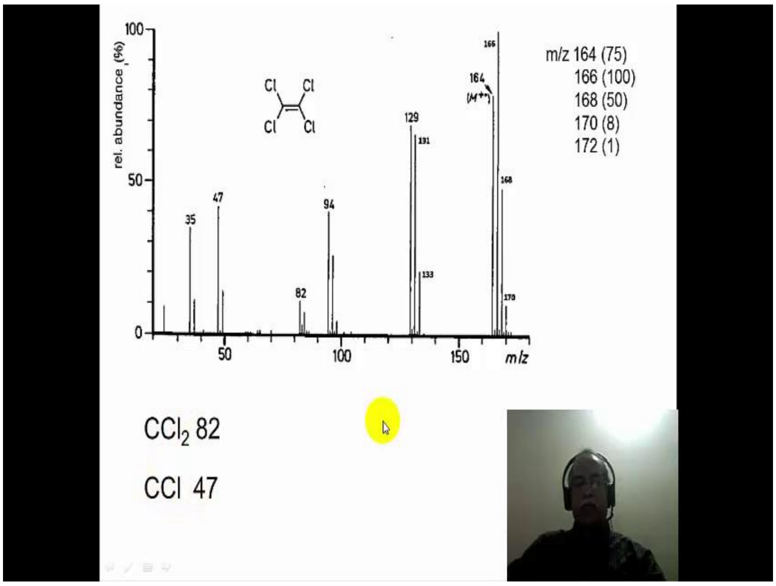

m/z 164 (75)
166 (100)
168 (50)
170 (8)
172 (1)

CCl_2 82

CCl 47

Finally let us consider an interesting example of a polyhalogenated compound, this is tetrachloroethylene is the molecule. Tetrachloroethylene has four chlorine atoms therefore, it must have the following molecular ion cluster peak is what is to be seen. All the 34, all the chlorine atom being 35, the molecular weight will be 164, if one of the chlorine atom is 37 and the rest are 35, then it will be 166, then two of them are 37 for example, would give 168, three of them has 37 would give 170, all four of them are 37 would give 171. Indeed, one sees the cluster of peaks in the molecular ion region for this particular compound, this is a positive ion mass spectrum is what is shown here in this particular spectrum and the molecular region one can see 164, 168 sorry 164, 166, 168, 170, the 172 peak is probably too small to be seen that will let you to the base peak which is 100. This is only 1 percent whereas the 166 peak is the 100 base peak in this particular spectrum.

One can also see the relative intensity to be essentially following the trend that is shown here this is calculated based on the expected line intensities of this based on the natural abundance of the 37 and the 35 isotopes of chlorine in nature for example and 164 is about 75 percent and the base peak is 166 as predicted by the calculated values of the relative abundance of this peaks. What you see subsequently is the loss of one chlorine mass unit corresponding to this cluster of peaks another chlorine mass unit corresponding to this cluster of peak and finally, another chlorine mass unit last corresponding to a chlorine cation radical itself is seen here 35 and 37 are seen very clearly.

The peaks at 47 and 82; essential correspondence to dichloro (Refer Time: 23:22) cation radical which is 82 mass units or c c l fragment correspondence to 47 mass unit. So, essentially the entire range of peaks that are seen in the mass spectrum of this compound can be clear accounted for by the fragmentation, by the sequential loss of chlorines from this molecule, as well as the fragmentation corresponding to the breaking of the carbon carbon bond resulting in the formation of c c l 2 and c c l as fragments which accounts for all the masses in this spectrum.

Now, what we have seen in this module is sort of a tutorial session, trying to solve some problems based on the mass spectrum. Most important thing is that we have demonstrated how a isotopes substitution reaction can be conveniently studied by mass spectrum, we have three examples of isotopes substitution first two examples of deuterium substitution on toluene and benzene and the second example is oxygen 18 substitution in an certification reaction.

Thank you very much for your attention.