PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 1

Lecture4

Week 1: Lecture 4: Biosynthesis of Terpenes- Acetate Mevalonate & MEP pathway

Thank you. Hello everyone, and welcome to the fourth session of week one of our course on pharmacognosy and phytochemistry. In this session, we will delve a little deeper into a set of compounds called terpenes. We will understand them based on our previous session, which covered biosynthetic building blocks. We will also try to synthesize a few terpenes from these biosynthetic pathways.

So let's learn what terpenes are and how they are biosynthesized. So if you recall the building block we discussed previously, that is the C5 building block made up of isoprene. The isoprene building blocks follow a rule: whenever you have one isoprene unit, it has a formula of C5H8.

So whenever there are multiples of this, or as isoprenes are added, you just have to multiply. So if my single building block is C5H8, when there are two building blocks, it will become C10H16, that is C5*2=C10 and H8*2=H16. Similarly, depending on that, in nature, you have various terpene compounds. So we will study the C5 building block in detail.

So, the C5 building block is made up of isoprene units and this isoprene unit can be derived from two different biosynthetic precursors. One is mevalonic acid, as you can see here, which comes from the acetate mevalonate pathway, and the second is deoxyxylulose phosphate, which comes from the methyl erythritol phosphate pathway. Now, we are going to learn both of these pathways to derive terpenes.

Now, what are terpenes? Terpenes are C5 derivatives, and based on how many C5 compounds are present, they get their nomenclature. To start with, just take the first example: hemiterpenes. 'Hemi' means half. So, when I say C5, that is the first unit or the basic building block, referred to as hemiterpene. So, if C5 is half, the full one is called monoterpene, and it will be twice.

Thus, C10 derivatives are called monoterpenes. Now, adding 5 to each of them, you will get C15, which is the next building block, called sesquiterpenes. So, you have hemiterpenes, which is C5. You have monoterpenes, which is C10. Then you have C15, which is sesquiterpenes.

Now, surprisingly, in nature, one more phenomenon happens. That is, there are compounds, especially those which are aromatic in nature, that originate from isoprene units. They don't fit in multiples of five. They are called homoterpenes, and generally, they are C5 multiples plus one carbon.

So, you might come across a compound like C11, which is C10 + 1, or you might come across a compound such as C16, which is C15 + 1. They are called homoterpene derivatives. What happens is they are terpene derivatives. They are generally derived as multiples of 5 but undergo some elimination and some

type of reactions to become just one carbon extra to the terpenoid derivatives. So, being still terpenoid in their biosynthesis, they are referred to as homoterpenes. Now, going ahead, if mono is C10, then duplicating it will give you twice the monoterpene which is your diterpene, that is, a C20 derivative. In addition to that, just add 5 to it, and you have what is called sesterterpenes.

And then just add one more 5 to it, and you will have what are called C30 derivatives. Now, C30 derivatives are referred to as triterpenes. C10 is mono, C20 is di, and C30 is tri. 30 is your triterpenes. Now, if you add 10 more to it, you get C40 compounds, which are your tetraterpenes, and tetraterpenes are very big.

In the previous session, we saw an example of carotene. Now, these carotenoid compounds, such as lycopene, are definitely your C40 compounds, which are made up of about eight isoprene units. So, eight times five makes 40, forming tetraterpene compounds. So, all of these terpenes come from these two basic metabolic pathways.

So, let's understand them. Now, moving to the acetate mevalonate pathway—as the name indicates—we derive this pathway from acetyl-CoA, and this acetyl-CoA comes from what is called the Emden-Meyerhof pathway. This is the same pathway that converts glucose into pyruvate, and thereafter, pyruvate is converted into acetyl-CoA. Now, this acetyl-CoA pathway—or acetate mevalonate pathway, or isoprenoid pathway, or HMG-CoA reductase pathway—

are synonymously used and essentially mean the same thing, that is, your acetate mevalonate pathway which involves intermediates such as mevalonic acid. Now, this pathway is used for biosynthesizing compounds such as terpenes and also steroids, especially your hormonal compounds. It is found in the cytoplasm and starts with a simple molecule of acetyl-CoA.

This acetyl-CoA—three such molecules of which add together—forms mevalonic acid, and thereafter you get your isoprenoid units, which are your isopentyl pyrophosphate and dimethylallyl pyrophosphate. So, what happens chemically? Let us try to understand it. So, we start with a simple molecule, that is, your acetyl-CoA. Now, when you see acetyl-CoA, this acetyl-CoA is an acetate that has been added to an enzyme.

Now, what happens here is this acetyl-CoA, which gets added to an enzyme, gets condensed with another molecule of acetyl-CoA in the presence of an enzyme, that is, acetyl-CoA thiolase. Thiolase kind of cleaves the SCOA group, and your acetyl-CoA joins to each other. So, you find this functionality joins here to the methyl-CoA. This is the methyl of the next acetyl-CoA, and you get what is called acetoacetyl-CoA.

Now, this acetoacetyl-CoA undergoes what is called an aldol addition. And one more molecule of acetyl-CoA is involved in the process. Now, this is done in the presence of an enzyme called hydroxymethylglutaryl coenzyme A synthase. Now, HMG-CoA synthase is a very important enzyme, and it is involved in the formation of a molecule which is called beta-hydroxy beta-methyl glutaryl CoA.

So, if you see, this is the typical beta-carbon coenzyme. So, you have your beta-hydroxy beta-methyl glutaryl CoA. Now, you can clearly see what has happened: this particular oxygen of the ketone group has been converted into an OH, and then you add this. This molecule I have kept as red out here.

And you can see carefully how it gets added. So, this is the CH3. This is the C part. So, it gets added, and what we have now is C2 + C2 + C2. That is, we have 6 carbons.

So this is my first acetyl CoA. This is my second acetyl CoA. And this is my third acetyl CoA which is kind of condensed together to form beta hydroxy beta methyl glutaral CoA. Now this undergoes what is called a reduction as well as elimination. The reduction happens here and as a result you can see this getting converted into alcohol.

And then what happens is you can see because the reduction happens, this enzyme is called a reductase. This happens in the presence of a NADPH, a kind of cofactor. Now, this produces one of the key intermediates in your isopreneur pathway and that is mevalonic acid. This is where your acetate mevalonate gets its name from.

Now, in some cases, you might find it represented as an ion that is mevalonate. That's also perfectly fine. I've put it here as mevalonic acid. OK, so what happens here is in your case of mevalonic acid, you might get this mevalonic acid further phosphorylated. And phosphorylation often happens in the presence of an enzyme called kinase.

Now, this kinase phosphorylates it at the fifth position. We initially saw that at the fifth position, the carbon got reduced. Now, what happens is this hydroxy group further gets phosphorylated. Now, this phosphorylation varies from organism to organism. In the majority of cases, you get what is called 5-phosphomevalonic acid.

So just to recollect, you have three molecules of acetyl-CoA condensing to form a 6-carbon derivative which is your hydroxymethylglutaryl-CoA. Now, this hydroxymethylglutaryl-CoA undergoes reduction to form mevalonic acid and thereafter phosphorylation to form phosphomevalonic acid. Now, this phosphomevalonic acid, like I said, depending upon the organism, there are different postulates. One postulate says that it gets phosphorylated one more time at the fifth position to form diphosphomevalonic acid.

One postulate also suggests that instead of at the fifth position, it might phosphorylate at the third position, giving rise to three, five postulates. by phosphomevalonic acid. In some cases, it is suggested that the phosphorylation is eliminated first, the reduction happens and then it gets re-phosphorylated. But going by the most abundant rule,

What we say is the phosphomavulanic acid gets phosphorylated one more time in the presence of an enzyme, 5-phosphomavulonate kinase. And what happens here is you get your Now, this will undergo decarboxylation. So, we are going to lose this. We initially had six carbons.

Now, decarboxylation means one less carbon. So, we are going to have effectively five carbons after decarboxylation, which we precisely want because our isoprene is a C5 unit. Not only that, when this decarboxylation happens, there is also elimination. The elimination of this hydroxy with a hydride from here leads to the formation of a double bond. So, this may undergo elimination, resulting in a double bond and

That's where we get our compound, isopentyl pyrophosphate. Now, this isopentyl pyrophosphate may undergo isomerization to form what is called dimethylallyl pyrophosphate. So, these are our key intermediates: isopentyl pyrophosphate and dimethylallyl pyrophosphate which are involved in the synthesis of all our terpene derivatives.

Going to the second pathway. That is your methylerythritol phosphate pathway. Now, this occurs in some bacteria, some eukaryotic parasites, as well as plastids in plants, you know, which produce chlorophyll. So, your chlorophyll biosynthesis or most of your terpenes, which are involved in your electron transport reactions,

mostly are prepared in plants by the methylerythritol pathway. Basically, your methylerythritol phosphate pathway. Now, the intermediates which are required as starting materials for this are your pyruvate and glyceraldehyde 3-phosphate. Your pyruvate and glyceraldehyde 3-phosphate are also products of your Emden-Meyerhof pathway,

which is nothing but your glucose pathway. So here, in this particular pathway, your glucose gets converted into your pyruvic acid, and glyceraldehyde 3-phosphate is one of the intermediates during the process. Now, both of these molecules condense to give you a key intermediate.

which we call it as deoxyxylulose 5-phosphate and this deoxyxylulose 5-phosphate then undergoes rearrangement to form an isoprenoidal group. This isoprenoid then polymerizes to give compounds such as chlorophyll, carotenoids, tocopherol, prenalquinones and so on. So let's understand this pathway.

So a molecule of pyruvate condenses with glyceraldehyde 3-phosphate. Now what happens in this process is you can see your pyruvate being condensing here and our CO2 gets eliminated. So when CO2 gets eliminated this joins here. Now you will see one more process being happening and that is this aldehyde gets converted into an alcohol.

So you will see pyruvate condenses with glyceraldehyde 3-phosphate undergoes a decarboxylation in presence of an enzyme that is 1-deoxyxylulose 5-phosphate synthase that is it is involved in the synthesis. Now once this synthase is involved the second step what happens is it undergoes reduction as well as isomerization. So reduction of what? So if you see here there is a ketone.

This ketone gets converted into alcohol. So you have a reductase and you also have an isomerase. Now, what happens or where does the isomerization occur? You have a methyl here. This methyl shifts to this carbon.

So you will see the methyl is shifted to this carbon and the ketone which was there on this carbon has now been converted into alcohol. And that is done in the presence of an enzyme, which is 1-deoxyxylulose 5-phosphate reductoisomerase because reduction is also happening, as well as isomerization. Now, this gives rise to the compound methylerythritol 4-phosphate.

Now, this methylerythritol 4-phosphate adds a cytidine triphosphate. Now, in the presence of cytidine triphosphate, you will see an adduct being formed, and this is the cytidine adduct. The compound name is 4-diphosphocytidyl-2-C-methyl-D-erythritol. Now, this compound further undergoes one more phosphorylation in the presence of an enzyme kinase. More specifically, 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate kinase.

Now, this kinase phosphorylates here. And as a result, you have three phosphate groups in this. So, the resultant compound that is formed is 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate. Now, this molecule carrying three phosphates undergoes a process of cyclization. But for that cyclization to happen, an elimination is necessary.

So in the process, we lose what is called cytidyl monophosphate. So here, your cytidyl monophosphate is lost or eliminated, and we are left with the compound with just two phosphate groups. Now, these two phosphate groups cling to each other to cyclize. So you get what is called a cyclodiphosphate adduct.

So, in the presence of the enzyme 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, you get a cycloadduct, which is 2C-methyl-D-erythritol 2,4-cyclodiphosphate. Now, what happens here is another interesting phenomenon, and that is a kind of conversion with the removal of a water molecule. So here, you will see that carefully, this is being eliminated.

Along with this moiety. So it's a kind of water elimination simultaneously. And this gives rise to a double bond out here. So I have my CH2OH. I have my CH3.

And a double bond. And now you can visualize your isoprene. So this happens in the presence of an enzyme synthase. That is 1-hydroxy-2-methyl-butanyl-4-diphosphate synthase. So as a result, you get this compound, which is 1-hydroxy-2-methyl-butynyldiphosphate.

which is very close to isoprene but just has an extra 1-hydroxy group. Now, this hydroxy group is removed by reduction, and the enzyme involved in this process is 1-hydroxy-2-methyl-butynyldiphosphate reductase. Once the reduction happens, you get the same isomers that came in from your acetate mevalonate pathway. That is your isopentyl diphosphate and dimethylallyl pyrophosphate.

So you get dimethylallyl pyrophosphate, which kind of isomerizes and can be converted into isopentyl diphosphate. Now, since the isomers are common from here on, we take the pathway as a common step. So we call it a terpene biosynthesis. So two such building blocks. You can clearly see this.

This is a dimethyl allyl pyrophosphate and this is attached to your isopentyl pyrophosphate. In the process, it will undergo phosphate elimination and dilution. Since these two groups are being added up this C5 plus C5 is C10 and C10 is your geranyl diphosphate. So C5 plus C5 adds together in presence of an enzyme geranyl diphosphate synthase to give you geranyl diphosphate. Now to this you can have one more isoprene unit being added.

So when one more isoprene unit is being added, again it will undergo phosphoryl elimination and then you get what is called as a farnesyl diphosphate. Again, it is easy to visualize. This is dimethylallyl pyrophosphate. This is isopentyl pyrophosphate.

This is isopentyl pyrophosphate. So you have three C units. So this is C10. This is C15. Now, here you can add one more to this, and what will happen?

Your C15 plus 5, you will get your C20. So, again you have your dimethylallyl pyrophosphate, isopentyl pyrophosphate, isopentyl pyrophosphate, isopentyl pyrophosphate to give you geranyl geranyl diphosphate. Now, in some cases, this monomer may dimerize to give you edox. So, say for example, your geranyl-geranyl diphosphate can be a dimer of C10. Whereas your farnesyl, that is C15, may dimerize in the following manner.

So, I just remove this phosphate, and I take this carbon and attach it to this carbon. Now, generally what happens here is, we saw previously what is called head-to-tail condensation. That is, we say 1, 2, 3 is the head and 4, 5 is the tail, which is attached to the next head. 1, 2, 3, 4, 5. Now, which is attached to the next head, which is 1, 2, 3, 4, 5.

But what happens here if I am just talking about the head 1, 2, 3, 4, 5? Now, this is a 5, 5 attachment, so this is a tail-to-tail condensation, and like we saw here, the molecule shows a different kind of symmetry. So, in the presence of the enzyme squalene synthase, this molecule will join and fold. Now, if you see, the foldings are very proper, and then it will undergo the formation of

what is called squalene epoxidase and thereafter give you—I am just... making—I'm not adding any carbon; I'm just joining the dots, and you can clearly visualize that if I just join these double bonds, I break these double bonds and join this molecule or cyclize this molecule, I will get steroids. So, squalene is one of the key intermediates involved in steroidal synthesis. It is also involved in the synthesis of triterpenoidal compounds, so from here, we will get our steroids.

We will get our triterpenoids. And what we require in the process is C5 *6. So, you have C15, which is C3. So, we have C15, which is C5 *3. So, two of such molecules.

So we call it C5 *6, which is your C30. Now, moving further, what might also happen is these big bulky molecules—you remember C20 plus C20. So, C20 and C20 may join to each other to form a big bulky C40 group. So, this is done in the presence of an enzyme called phytoene synthase. Now, this phytoene synthase, when it joins them together, will just form a plain linkage.

You need unsaturation. You will see naturally when I talk about tetraterpenes; I am talking about carotene-like compounds. And then, when I talk about carotene, these are colored compounds. The color intensity depends on double bonds. So, in nature, you'll find most of the carotenes with double bonds.

So, this synthase undergoes a few more unsaturations in the presence of the enzyme phytoene desaturase to become what is called carotene. which is present in your carrots. Now, this carotene will undergo further unsaturation to form lycopene, which is an intense red pigment present in your tomatoes and then you will see cyclization. Now this cyclization happens in the presence of an enzyme called lycopene cyclase and it gives rise to both alpha and beta carotene. For convenience, I have just put beta carotene here, but you can visualize it. It's very simple.

You can just see it is the same thing. So I'm just starting it from here. 1, 2, 3. 4, 5. I cut it here again 1, 2, 3, 4, 5.

I can cut it here 1, 2, 3, 4, 5 and I can go till here. So you can see the same kind of 4 isoprenes are there on each side which are joined to each other here by a tail to tail linkage is an important distinguishing characteristic of your tetra terpenoidal compounds. Now going to a few examples of compounds.

So when I talk about hemiterpenes, hemiterpenes are C5H8 compounds. Now when I say terpenes, I mean hydrocarbons. But in some places, you will see the word terpenoids. Terpenoids are when your terpenes are oxygenated. Carotenes are when they are hydrocarbons.

Carotenoids are when they are oxygenated. So whenever they are oxygenated, you replace the word terpenes with terpenoids. An example of a hemiterpene is pranol. In this case, it is isoprene, but when it is pranol, you call it a terpenoid. Now, one more example of isoprene is shown here.

Isovaleric acid has the same functionality. So this is my 5 here. Now we move to monoterpenes. Monoterpenes are C10. Now if you examine monoterpenes as C10, they might be cyclized.

So, I am just giving you one example. So, 1, 2, 3. I got here 4 and 5. Now, if I cut it here, I can see the second one. 1, 2, 3.

4, 5, so this is basically two isoprenes joined in this manner. Similarly, you can do this for terpene oil. As well, it's very similar. Want to try the next one? The next one is your sesquiterpenes. Sesquiterpenes have three isoprenes, so by their nature, they are C15H24 compounds. An example of that is your humulene and farnesol, so you can see here. Okay. Precisely, three molecules of your IPP join to each other to give you humulene.

Now, when you see monoterpenes or sesquiterpenes, they are low molecular weight. They are aromatic. They have a nice scent. So, if I am talking about lemon or limonene, it gives you a strong smell of oranges. When I say terpineol, it is your turpentine smell.

So these monoterpenes and sesquiterpenes, as well as your hemiterpenes, are volatile compounds by nature. But as the molecular weight increases, their volatility decreases. Next, you have diterpenes. Diterpenes are like your C20 compounds. For example, if you look at something like retinol, which is a vitamin A derivative.

Again, we can visualize them. This is 1, 2, 3 for us. 4, 5. I cut it here to 3 again. 4, 5. I cut it here. So you can see this is 1, this is 2, this is 3, and this is 4. So you have 5 into 4, which is your C20.

Similarly, if you see your phytol, phytol is also a C20 derivative. Similarly, for your squalene, which is represented in a cyclized manner, but you can represent it in a linear manner. Here, I have marked the isoprene so you can see clearly. Joined to each other by tail-to-tail condensation. Now you have your sesquiterpenes.

Example is your geranyl farnesol that is C25 compounds. Then you have your triterpenes, squalene we have done. Tetra terpenes, lycopene is an example if you recollect which is there in your tomatoes which is there in your watermelon. Then you have your beta carotene which is there in your carrots. Then you have your polyterpenes.

Your rubber latex is an example of a polyterpene which is actually a polymer. Now, in addition to that, you also have a little different or additive compound called meroterpenes. Now, meroterpenes are terpenes in which the terpenes have been attached to slightly different molecules. They are not pure terpenes. So they are terpenes plus something else.

So take, for example, bakuchiol, which is nowadays there in your cosmetic creams, which is replacing your retinol. So you can see a typical head out here. Okay, that's the one. And then you can see the second one. 1, 2, 3, 4, 5.

But you can see this molecule is not a terpene. So it is attached to something else. Similarly, if you see this molecule, which is present in cannabis, that is your tetrahydrocannabinol. Okay, you can clearly see a terpenoid group out here. We just represented it previously under the monoterpene.

So you can see 1, 2, 3, head. 4, 5. And then the second terpene: 1, 2, 3, 4, 5. But from this side, it's no longer a monoterpene derivative or no longer has isoprene units. So you can say it is an adduct.

In addition to that, polyterpenes occur in nature also as some of the monoterpene meroterpene derivatives. This includes your ubiquinone. So you can see in ubiquinone, which is involved in your electron transport chain. The terpenoid 1, 2, 3, 4, 5 is there, and you will see that numerous such molecules are repeating monomers, which are present, and this is attached to a benzoquinone nucleus, whereas when you see tocopherols or

which are called vitamin E derivatives, which are your natural antioxidants. Again, in that, you will see a good symmetry of isoprene units. So now you can easily draw 1, 2, 3, 4, 5. You will see that this unit is no longer a terpene moiety. So that tail or hydrophobic part of tocopherol is contributed by your isoprene units.

So these are a few examples of terpenoidal derivatives. They are biosynthesized. What we learned is the acetate mevalonate pathway and also your methyl erythritol phosphate pathway is Your methyl erythritol phosphate pathway is mostly used for the production of plant pigments. Whereas when you see your terpenes and aromatic compounds,

It is generally the acetate mevalonate pathway which is used. So here are a few references if you wish to learn more about these biosynthetic pathways. And thank you, everyone, for your patient listening. Thank you.