PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 1

Lecture5

Week 1: Lecture 5: Shikimic Acid Pathway

Thank you. Hello everyone, and welcome to session 5 of the NPTEL course on pharmacognosy and phytochemistry. In this session, we are going to learn about a very unique pathway, which is called the shikimic acid pathway. This pathway is unique in the sense that it is seen in plants and certain microorganisms but is absent in humans.

However, it is essential for producing amino acids such as phenylalanine, tyrosine, and tryptophan. These amino acids are often referred to as essential amino acids. The reason is that we lack this particular pathway in our bodies. We have to rely on dietary sources for these amino acids. So, let's learn what the shikimic acid pathway is and what we primarily derive from it,

as well as the secondary metabolites we obtain from it. The shikimic acid pathway is a secondary metabolite pathway that primarily provides us with amino acids such as phenylalanine, tyrosine, and tryptophan via a shikimic acid intermediate. So, what happens is, if you observe a biosynthetic pathway, you have photosynthesis and then you have the Emden-Meyerhof pathway.

Through the Emden-Meerhof pathway, we get what is called phosphoenolpyruvic acid. Now, this phosphoenolpyruvic acid is one of the starting materials for your shikimic acid pathway. The second starting material comes from a shunt pathway called hexose monophosphate. Through this you get a second compound which is a 4 carbon sugar

phosphate that is your erythrose 4-phosphate. So together your phosphoenol pyruvate and erythrose 4-phosphate are used to prepare the amino acids.

Now, this shikmik acid pathway via these amino acids gives us a number of building blocks. In the previous sessions, we have learned about the different building blocks. So, what building blocks do we derive from the shikimic acid pathway? The first building block that we can derive from the shikmik acid pathway is your phenyl propanoids. The second one what we can derive is what you call it as phenyl ethyl amine unit and

The third amino acid that is your tryptophan contributes to what is called the indole C2N building block that is the tryptophan or often referred to as the tryptamine building block. So we get C6C3, we get C6C2N and we get indole C2N as building blocks for our secondary metabolites from these essential amino acids. Now, if you go to see these building blocks, these building blocks are there in numerous organisms.

So, shikmik acid pathway you can see in bacteria, you can see in fungi, you can definitely see it in plants, algae and some protozoans. Now, this helps not only in building amino acids for them, this helps also provide vitamins and some defensive molecules. Now, coming to secondary metabolites, in terms of secondary metabolites,

your shikimic acid pathway gives you compounds such as tannins, Cinnamic acid derivatives, your lignans, lignans, the glowing molecules that is your coumarins, the antioxidant molecules that is your flavonoids and anthocyanins and certain alkaloidal compounds are also derived from your cinnamic acid pathway. Now to start with your shikimic acid pathway happens via two starting materials phosphoenol pyruvic acid

as you can see here that is your phosphoenol pyruvate and the second molecule is a 4 carbon member sugar that is your erythrose 4-phosphate. So we add a C3 and a C4 to get a 7 carbon unit. This 7-carbon unit is called deoxy-d-arabinoheptulocenic acid 7-phosphate. So, what happens?

You can focus here. So, if you see this carboxylic acid, this is the carboxylic acid. The phosphate is removed to get a ketone, and here you can see this carbon. So, here you have this 1, 2, 3. I am just labeling it here: 1, 2, 3.

Now, this joins with the 4. This is your 4. What happens here in the 4 is your aldehyde is now converted into an alcohol. So here you have your 4, 5, 6, and there you have your 7. That is a 7-carbon unit, and that is why you call it a heptulosonic acid derivative.

Now it is a heptulosonic acid 7-phosphate. So the terminal carbon, the 7th carbon, carries a phosphate. Now, this is involved in a condensation reaction, and this condensation reaction is In the presence of an enzyme, again a synthase, that is a dehydroquinate synthase, cyclizes to give you what is called dehydroquinic acid.

You can see here the cyclization has happened here, and during this process, this particular ketone has been converted into an alcohol. So you can see this alcohol moiety that is contributed by this moiety, and you get what is called dehydroquinic acid. Now, from dehydroquinic acid, you can get your quinic acid derivative. Now, dehydroquinic acid is an important intermediate.

If you see compounds such as your tannins, especially your gallic acid derivatives, if you look at gallic acid, gallic acid has a very simple structure. So there's a good resemblance between dehydroquinic acid and gallic acid. So what you need to do is eliminate this, probably reduce this, and aromatize this. So this is a multi-step process, and eventually, from your quinic acid, you get gallic acid derivatives.

So you have quinic acid or dehydroquinic acid that gives rise to some hydrolyzable tannins. From gallic acid, you can get your ellagic acid and other compounds as well. In some cases, what happens is this hydroxy acid undergoes elimination along with this hydrogen, resulting in a double bond. So this happens in the presence of an enzyme called dehydroquinate dehydratase.

Now when this happens, you get a double bond here, and the compound is your 3-dehydroxy shikimic acid derivative. Now this shikmic acid derivative, especially your 3-dehydro shikmic acid derivative, is then kind of reduced with the help of an enzyme called shikimate dehydrogenase and you get your main compound from which the biosynthesis is labeled.

The compound is your shikmic acid. So this shikmic acid is a kind of marker for plants or for organisms in which this biosynthetic pathway exists. Now, this shikimic acid pathway doesn't stop here. It goes ahead to produce numerous amino acids that we discussed. So let's see them.

So this shikmic acid undergoes what is called a phosphorylation reaction at the third position. So this is the first, second, and we call it the third. Third position. So it undergoes phosphorylation at the third position to give you what is called shikimic acid 3-phosphate in the presence of an enzyme, shikimate kinase.

Kinases are generally the enzymes that are involved in phosphorylation. So now, once you get the shikimic acid phosphate, what happens next is an interesting phenomenon. After phosphorylation, one more molecule is added. So, an additional molecule of phosphoenol pyruvate is added. If you remember, this was your C3.

So, one more C3 gets added. This happens in the presence of the enzyme enoyl pyruvyl shikimate 3-phosphate synthase. So, this gets added, and you get the compound, which is your enoyl pyruvyl shikimate 3-phosphate. You can see that your pyruvic acid gets added here precisely, and these are your three carbons. Now, in order to undergo further processes, what happens is a dephosphorylation reaction.

So, this loses its phosphate, and as it loses its phosphate, you can see the changes in the unsaturation happening. So, this happens in the presence of chorismate synthase, and you get what is called chorismic acid. So, from shikimic acid, your next derivative is chorismic acid. So, from chorismic acid, we get an interesting set of amino acids called tryptophan.

So, this chorismic acid sometimes further undergoes an amination reaction. So, if you see on the phenyl ring, when there is a COH and NH2, we call this compound an anthranilic acid derivative. So, this anthranilic acid derivative is formed here. After the elimination of these two groups.

So, these anthranilates are compounds that come from chorismic acid. And in some cases, amination occurs here also. And this amination gives rise to molecules such as para-

aminobenzoic acid. PABA is a very important metabolite for microorganisms and is also involved in vitamin synthesis. So you get your anthranilic acid, and you get your PABA, especially from chorismic acid.

Now, what happens once you get anthranilic acid? This anthranilic acid will add a sugar, especially your phosphoribosyl pyrophosphate sugar, and later on, through a sugar-opening reaction, forms an amino acid, tryptophan. So it does a sugar-opening, there's a transamination, and it's a multi-step reaction, and eventually, what you get is the amino acid tryptophan.

So from your chorismic acid, we have our first building block, and if you remember, this building block is what gives us our indole C2, that is C1, C2, and N2. After decarboxylation. So you get your tryptophan; from tryptophan, you get numerous alkaloids being there.

Now, another thing that can happen from chorismic acid is sometimes the phenylpyruvic acid which is attached here shifts to move to this position. Now, this happens in the presence of an enzyme called mutase. So chorismic mutase orchestrates this event, and what you get is your compound moving here, so the point of attachment is this. Now,

If you carefully assess, this carbon is what gets attached here. Your oxygen gets converted into a ketone moiety. So you have this carbon, you have this oxygen, which gets converted into a ketone moiety, and you have your carboxylate. So here, if you look carefully, you have your C6, C3 almost in line, but one carbon is extra.

Now, this carbon is eliminated along with water through an enzyme called prefenic acid dehydratase. So, this elimination happens, and you get what is called hydroxyphenyl pyruvic acid. Now, depending on the elimination, this hydroxy group may stay or may get eliminated. So, if the hydroxy group stays, you get what is called hydroxyphenyl pyruvic acid. If the hydroxy group gets eliminated, you just have what is called phenylpyruvic acid.

Now, why this is important is that the next reaction is a transamination reaction. During the transamination reaction, this ketone moiety gets aminated. So, if this hydroxyl group

is intact, you get the amino acid tyrosine. But if this hydroxyl group is missing, that is, from phenylpyruvic acid, you will get phenylalanine. which is nothing but tyrosine missing a para-hydroxy group.

So, from this particular pathway, that is from chorismate, you could get tryptophan, you could get phenylalanine, as well as tyrosine. So, all these amino acids are involved in the preparation of numerous primary as well as secondary metabolites. So, let's see what these are. So, if you see phenylalanine, phenylalanine is something

which helps contribute along with tyrosine in the preparation of plant pigments such as flavonoids, isoflavones, anthocyanins, and proanthocyanidins which later get converted into condensed tannins Not only that, it helps in the preparation of many defense compounds This includes cutin, suberin, lignin, lignans, as well as cinnamic acid derivatives

Now, talking about tyrosine, tyrosine is also one of the important essential amino acids The reason being it is involved in the biosynthesis of numerous pigments as well as neurotransmitters Neurotransmitters are vital for the conduction of neuronal signals So it is involved in the synthesis of L-dopamine Dopamine, epinephrine, norepinephrine, phenylethylamine, as well as tyramine

In terms of pigments, it is involved in the formation of melanin-like pigments which are eumelanin and pheomelanin, also thought to be melanin precursors Now coming to tryptophan, tryptophan is involved in the synthesis of vitamins and numerous cofactors such as niacin, NAD, and NADP It is involved in plant hormones such as auxins, indole, acetic acid derivatives, tryptamine, and

Also present or also involved in the synthesis of alkaloids. The amino acid tryptophan is also involved in the preparation of neurotransmitters. such as serotonin, melatonin, and tryptamine. Now, coming to secondary metabolites, let's see how they are processed further. Now, these amino acids are your primary metabolites.

They need to be prepared in order to undergo or divert from their primary metabolism and enter secondary metabolism. So this happens in an enzyme called phenylalanine

ammonia lyase, if it is phenylalanine, or tyrosine ammonia lyase if it is tyrosine. So lyase is an enzyme which cuts off. So we need our amino acids to be converted into something like phenylpropanoid derivatives,

wherein if we don't want amino acids, in that case, the ammonia lyase will take care of it. Now that the amino groups have been reduced, you get what is called transcinnamic acid or in this case, hydroxy transcinnamic acid derivatives, and later on they will get converted into coumaric acid derivatives. So, hydroxy transcinnamic acid derivatives are called coumaric acid derivatives. Now, you can derive that directly from tyrosine via tyrosine ammonia lyase or via phenylalanine.

where it first gets converted into cinnamic acid and later on gets hydroxylated in the presence of an enzyme called transcinnamic acid hydroxylase to become coumaric acid. Now, this coumaric acid, once it is ready, gets added to an enzyme called coenzyme A. This happens in the presence of an enzyme that is 4-coumaryl-CoA ligase. Ligase is something with joints or sticks.

So once this ligase acts, you have your paracoumaryl coenzyme A. Now, this paracoumaryl coenzyme A is an important precursor. Now, if you see that in terms of the nucleus, you have your C6, which is your phenyl ring. You have your 1, 2, 3. C3.

So, all your C6, C3 derivatives can be derived from this cumural CoA via different mechanisms or different enzymatic pathways. The important compounds that we get from this are your lignans, which are nothing but C6, C3 into lignins, which are C6C3 into N. What happens here is your lignins are nothing but polymers of C6C3. So what you can do in this case is take your polymers C6C3 and just multiply them N times to get your lignins.

You can get compounds such as coumarins, which show fluorescence, and you can get cinnamic acid derivatives. These show very good defensive action because of their acidic nature. So, let's see the cinnamic acid derivatives. So, once you have your coumarin CoA, it gets oxidized to coumaric acid. So, this is your coumaric acid.

This coumaric acid further undergoes hydroxylation in the presence of an enzyme called coumarate 3-hydroxylase. Now, this coumarate 3-hydroxylase will add a hydroxyl group here and give you a compound called caffeic acid. Now, this caffeic acid will undergo methylation. Remember your building blocks. This can be done with the help of an enzyme called methyltransferase.

Now, this methyltransferase can vary depending on the substrate. When it is caffeic acid, it will be caffeic acid O-methyltransferase. So, COMT is caffeic acid O-methyltransferase. Now, once this methylation happens, you get a compound called ferulic acid. And then you have hydroxyferulic acid.

Now, this is again done by ferulic acid hydroxylase. Now, what has happened here is this is your position 1, 2, 3. This is 4 and 5, and that's the reason it is ferulic acid 5 or ferulate 5 hydroxylase. Now, once you do that, you can even methylate it. Now, in this case, you can have your hydroxyferulic acid O-methyltransferase and

you will have your cinnamic acid present. So, cinnamic acid, ferulic acid, caffeic acid, and coumaric acid are all cinnamic acid derivatives which play a very vital role in defense because of their acidic nature. Now, what happens in plants is these acids can also get converted into alcohols. So, you have your coumaric alcohol, wherein this will get converted into CH2OH.

For all of them, you will have your ferulic alcohol. You will have your coniferyl alcohol. So, these compounds will also get converted or reduced to their corresponding alcohols in plants and these alcohols can give rise to a new set of metabolites. So, cinnamic acid derivatives are produced from your coumaroyl-CoA.

Then, the next set of compounds, like I said, because they are acids and they can be reduced. Here, you have three compounds which we take from them: coumaril alcohol, coniferyl alcohol, and sinipil alcohol. Your coniferyl alcohol came from your ferulic acid. So, if you see this carefully, your ferulic alcohol or your coniferyl alcohol is nothing but the same COOH being converted into CH2OH.

So, cumeric alcohol, coniferous alcohol, and cinnamic alcohol undergo condensation. And this condensation, if it is a dimerization, the compounds thus formed are called lignans. So, lignans are the compounds formed by dimerization of lignans. Most of what are called cinnamic acid derivatives are later reduced to their alcohols. So, these alcohols you can see very clearly here.

This is a dimer of coniferous alcohol. So, what happens is your double bond, which was there, is now involved in the formation of this bond, and two molecules of coniferous alcohol give you a lignan derivative called secoisolarisiresinol. Similarly, sometimes what can happen is these compounds or these substituents may cyclize within themselves.

and give rise to compounds such as your matairesinol. And sometimes a rearrangement plus addition helps, but you can still see that the phenylpropanoid nucleus is maintained. So this is your phenyl, and you can see 1, 2, 3. This is your phenyl, and here you can see 1, 2, 3.

So they are still phenylpropanoids, and this little adduct comes because you can see here methoxy that has been involved in a kind of cyclization, and this is the compound sesamin, which is present in your sesame seeds. Lignans are very good for health and are thought to be phytoestrogenic in their action. The next set of compounds are lignans.

So if you take these alcohols, they are also referred to as monolignols. Dimers will give you lignans, but the polymers will give you lignins. Lignans are involved in the strengthening of stems, and what happens is once you have your phenylpropanoid compounds especially in the form of alcohols, the monolignols are transported into the cell wall. And once they are transported into the cell wall, they are polymerized in themselves through radical coupling.

Here you have unsaturation. So, it is very easy to initiate the coupling. And there are enzymes such as laccases and peroxidases, which further convert them into free radicals. And help them in the polymerization reaction. As a result, you have a big

kind of C6, C3, or monolignol addition products, and these are called lignins. So, as you can see, as discussed from the previous session, you have a lot of your C6, C3 being

added to give you a huge polymer, and that polymer is your lignin. The next set of compounds derived from your phenylpropanoids are your coumarins. Now, coumarins are cyclic compounds.

They involve lactonization. So, what happens here is from phenylalanine and tyrosine, you get a compound called coumarin CoA, which we have discussed. Now, this coumarin CoA further undergoes hydroxylation. And this hydroxylation is done in the presence of an enzyme called hydroxylase. And further, it undergoes lactonization.

That is, it will undergo cyclization. Now, when it undergoes cyclization from here. You will see this will join, and you will get a coumarin, that is, a lactone-like structure. And this is catalyzed by an enzyme called coumarin synthase. So, if you can carefully see here, this is your tyrosine.

So, you can convert your tyrosine into coumarin CoA. Then, it is hydroxy coumarin CoA. And then, when you cyclize it from your coumarin CoA, you will get your umbelliferone. In some cases, your coumarins can be substituted. So, instead of a

Having your coumaric acid, if you have seen previously in terms of monomers, you had your coniferyl alcohol. Suppose coniferyl alcohol is the compound to be cyclized. In that case, what will happen is you have a hydroxy, you have your methoxy, and then involve this in cyclization. So, this is what you get from coumaric alcohol: scopoletin. So, cyclization of different types of phenylpropanoid alcohols gives you different coumarin derivatives.

Not only that, these coumarin derivatives may combine with other small metabolites to form furanocoumarins, pyranocoumarins, and so on. The next set of compounds, which are obtained from phenylpropanoid derivatives, are slightly different and in this case, it is not C6C3 but C6C2N. Now, C6C2N is referred to as the phenyl ethylamine unit.

This happens when decarboxylation is carried out. So, rather than losing the amine, as in the case of phenylalanine ammonia lyase, we removed the amine. In this case, we will not remove the amine but will remove the carboxylic acid. So, the decarboxylation reaction converts them into compounds such as phenyl. This is 1, 2.

So, we call it phenyl ethylamine derivatives. And phenyl ethylamine derivatives are present in numerous alkaloids. To give you a few examples, as we have previously discussed, I'm keeping it the same for your reference. So, mescaline is a good example of phenyl ethylamine. And so is the analgesic compound, which is present in opium.

That is your morphine. So from morphine also, you'll have your 1. You will have your 2 and you will have your amine that is the next group. Not only that if you see your amino acid tryptophan that contributes to your indole C2N. Now this indole C2N we visualized last time this is your indole moiety.

And this is your 1, this is your second and this is the N. So this is your indole C2N. Now what happens in the plant is your indole C2N condenses with different different set of compounds. These compounds can be something like your cyclogannin. in case of reserpine. So, a series of enzymatic transformations follows.

So, you can see a secologanin, you can see a tannin that is something like a trimethoxygalic acid derivative and later on all of them condense together to give you a reserpine. Now, reserpine is a hypotensive agent which is found in your Rawolfia species. So, the next set of compounds which we obtain are not clearly or pure phenylpropanoid derivatives but they are adducts.

So half of these compounds have been taken from your shikimic acid pathway and half of these compounds have been derived from other pathways. And these compounds involve your condensed tannins and flavonoids. So what happens in this case is your coumaryl CoA condenses with 3 units of malonyl. So from your previous building blocks, if you recollect, malonyl is a reactive form of acetyl CoA.

So when acetyl-CoA adds up, you get a C2 building block. So when you have your 3-malonyl-CoA, you have 3 into C2, which is nothing but your C6. So you get what is called a phenyl ring. But if you remember, acetyl-CoA has an aldehyde group. So you have an alternate route to it.

So this is nothing but this part of it is contributed by your coumaryl CoA and this what happens is to this, you will have coumaryl CoA, your first that is from here. You will

have your second and you will have your third acetyl group being added in the form of malonyl-CoA adducts. This condenses together initially in the form of a chalcone synthase to give you chalcones, which is an open-ring structure, but later on gets converted into a closed-ring structure in the presence of an enzyme.

which is called chalcone flavone isomerase. Now, this flavone isomerase can further convert these flavonoids into different sets of flavone derivatives. either by adding hydroxyl groups or by adding unsaturation. So, a series of enzymatic processes follows, and you will get numerous flavonoid compounds. Now, in some cases, a hydroxyl group can also be introduced by a simple enzyme hydroxylase.

That is your flavanone 3-hydroxylase. In that case, you will get your 3-hydroxy-substituted flavonoid derivatives. 3-hydroxy-substituted flavonoid derivatives are very potent antioxidant agents. And if you introduce unsaturation, you get compounds such as quercetin, or compounds like which are involved in the formation of flavonoid glycosides.

So, extremely polar compounds can be obtained when you hydroxylate your flavone, especially at the third position. So, from your shikimic acid pathway, you get numerous secondary metabolites and some of these are not pure, but some of these can be adducts. Here are a few references if you wish to delve more into these biosynthetic pathways. And thank you, everyone.

See you next week. Thank you.