

PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 7

Lecture35

Week 7: Lecture 35: Coumarin containing drugs (Psorelea, Ammi majus, Aflatoxin)

Thank you. Hello everyone, and welcome to the Week 7 Session 5 of the NPTEL course in pharmacognosy and phytochemistry. In this session, we will be dealing with a very interesting set of compounds, and those are coumarins. Now, imagine a set of compounds that have the ability to fluoresce. Fluoresce means glow, and you might have seen something like radium or a

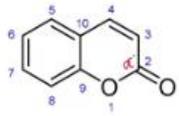
substance that you see in your glow sticks at a discotheque. Such compounds have the ability to fluoresce. So, this set of compounds that we are going to study now in this session are compounds responsible for fluorescence. They were earlier also used to indicate the authenticity of currency notes because they used to give a typical color fluorescence when those currency notes were placed under UV light, and these compounds are called coumarins. So, what are coumarins?

Let's delve into it. Coumarins are a set of compounds that are defined as benzo-alpha-pyrone. So here, you have your alpha-pyrone. They are often found or formed as a part of cyclization. So, coming back to our phenylpropanoids.

Now imagine something like cinnamic acid. So here we are. With the cinnamic acid. But in this case, I am drawing cinnamic acid like this. OH.

NPTEL

What are Coumarins?



- Coumarins are formed by the cyclization phenylpropanoid,
- They are derivatives of benzo- α -pyrone
- They are derived from **Shikimic acid pathway**
- They are often hydroxylated to possess phenolic groups
- Coumarin possesses a sweet, hay-like scent

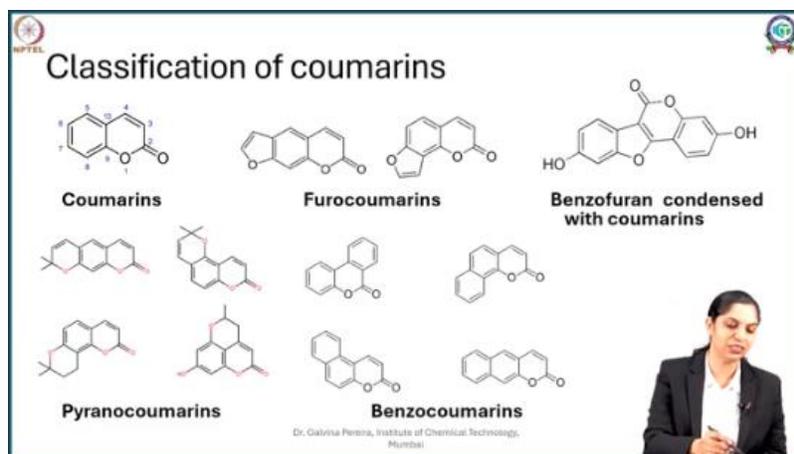
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Now when we have something like a hydroxyl out here, and it undergoes a water loss, what you get in the process is what we call coumarins. So they are nothing but the same phenylpropanoid derivatives, which have this time undergone cyclization. Previously, we saw condensation with flavonoids, then we saw dimerization with lignans, and now we are seeing cyclization leading to a set of beautifully fluorescent compounds called coumarins. So since they are phenylpropanoids, they have been derived from the shikimic acid pathway, mostly via phenylalanine or tyrosine, via the formation of a coumarin co-intermediate.

Now, coumarins occur in plants as such. So you can see this beautiful molecule; it is present in a few of those plants, and when it is there, it gives you a very sweet, hay-like scent. So maybe if you are into perfumes, you might have heard of a perfume ingredient called tonka bean. So tonka bean contains a good quantity of coumarin, which gives it a sweet, hay-like scent. But due to some carcinogenicity issues or other concerns now,

Which are also to do with the hepatotoxicity. The government mostly across worlds have kind of regulated the substances. But you will still find this in some of the perfumes. I mean it is still illegally added in some of the perfumes for its beautiful scent. Which is difficult to replicate with any other ingredients.

So coumarins if you go to classify basis their structure the coumarins can be native coumarins as you can see here. So this native coumarins is benzo α pyrone as we discussed and the ketone group is located at α . Now in some cases this groups may also possess hydroxyl moieties. So in this case, they are called as hydroxycoumarins. So you may have coumarins and you may have hydroxycoumarins.



So whenever it's hydroxy, you can trace back instead of phenylalanine, the hydroxy might have come from your tyrosine residues. In some cases, what happens here is you get some adducts. So some other functionalities add to this and you have what is called as furanocoumarins. So in furanocoumarins this is a classic structure of soralin and this is angelicin. So you have your furanocoumarins wherein a furan ring is attached to a coumarin.

Now in some cases instead of furan you can even have a benzofuran attached. So this is like a benzo furan attached or condensed with coumarins. In some cases rather than furan you might have a pyran group. So this is an example of pyran. Now depending upon where exactly you can see four different types of pyrano coumarins are possible.

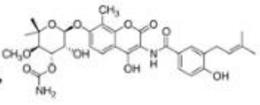
And last case, instead of pyran, you might have a benzene ring attached to it, just a simple benzene. And in this case, you will get your benzocoumarins. So you can have native coumarins, sometimes substituted as hydroxycoumarins. If furan is added, you will have furanocoumarins. Again, you might have subclasses in that depending upon where exactly the furan is added.

You might have benzofuran condensed coumarins, you might have pyran condensed coumarins or you might have benzene condensed coumarins which are your benzo coumarins. So depending upon what attaches to this coumarins you have the classification of coumarins. We go across occurrence. Now, coumarins is one class of compounds you will see which occurs not only in plants but also in fungus and certain bacteria. Like we discussed when we talk about plants, a good ingredient is tonka beans which has tonka.




Occurrence

- **Plants:** Tonka beans, Sweet clover, Lavender, Cinnamon, Angelica, Psoralea, horse chestnut and Citrus fruits.
- **Fungus:** Endophytic fungus, *Aspergillus* species (*Aspergillus flavus* and *Aspergillus parasiticus*)
- **Bacteria:** Streptomyces bacteria (novobiocin and coumermycin)



novobiocin



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Up to about 1% of coumarins. Then you have sweet clovers, lavender, cinnamon, angelica, soralia, and horse chestnut. That is your *Aesculus hippocastanum*. And citrus fruits, even your bale, which is there. Egil, marmalade, that also contains coumarin.

It is also present in certain endophytic fungi. Endophytic fungi are the fungi that thrive inside the plant. It's present in some endophytic fungi. It is present in *Aspergillus* species. So, what happens is, you must have seen, especially during the monsoon season,

within your onion peels or within your garlic, you might encounter a black powdery fungus, which, you know, kind of dusts off spores very easily. Those are the *Aspergillus*. Now, two species of those *Aspergillus*, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, are known to biosynthesize coumarins. So they contain or they prepare coumarin derivatives. We'll discuss them shortly.

Now apart from that your bacteria in certain species of streptomyces they produce coumarin derivatives. So a very famous example of coumarin derivatives produced by your bacteria are antibiotic compounds. So if you see your antibiotic compound like novobiosin. So antibiotics are slightly bulkier or bigger molecules but if you carefully concentrate You can see a little hydroxycoumarin derivative occurring in here.

So this is where your coumarin is located. And you can see it is substituted by other functionalities and made big by the bacteria so that it can kill other bacteria or other group of microorganisms. Another one is your coumarin. So these two are a very lead molecules or good antibiotic examples of drugs containing coumarins. Now let's see few examples.

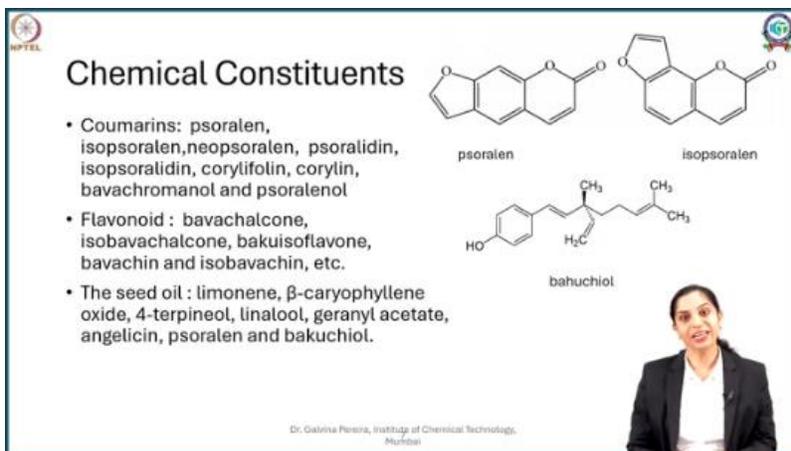
For examples I have picked soralia, aminages which are the plants and for fungus I have picked up aflatoxins. So let's see soralia first. Now soralia derives its name from a A Greek word which means basically to treat leprosy. So, you know, the itch which is responsible

for leprosy after your, you know, lepromatous, that is your mycobacterium leprae attacks it, causes a lot of itching.

And that itch, which was associated with it, was known to be cured by soralia oil. So, even in the Indian system of medicine, we call it kushtanashni. So, it kind of destroys the kushtarog, or what you call leprosy. It's also called bakuchi or bavachi oil. So, these are the seeds, or these are the kind of fruits.

Both of them are used, mostly the fruits or dried ripe fruits of kushtarog. *Suralia caurifolia*, which belongs to the family Leguminosae, are taken. Now, this plant is cultivated and grows abundantly in Asia, especially in India, China, Pakistan, Nepal, and Vietnam. So, the seeds are taken, and from those seeds, the oil is obtained. The good part of this is that coumarins are compounds which are very lipophilic in nature, and as a result, even in oil, you will find those coumarins present.

Now, these coumarins, once they enter the oil, attribute their medicinal properties to the oil and hence show beneficial biological effects. So, what are these compounds present in bakuchi oil? The main compounds present in bakuchi oil basically belong to the few ranocoumarin groups. So, in that, you have soralin. And you have isosoralin.



Soralin and isosoralin differ in their point of attachment. The molecular weight of both of them remains the same. Apart from that, you also have neosoralin, soralidin, isosoralidin, corifolin, corilin, babachrominol, and soralinol. So, these are a few compounds which are kind of thought to be furanocoumarin derivatives and other derivatives that also contribute to the properties of soralia, such as the antibacterial or anti-inflammatory activity of soralia.

especially the soralia oil. Apart from that, there is another group of compounds called flavones, which are present in the fruit as well as to some extent in the seed. These

flavonoid derivatives include bavachalcone, which belongs to the chalcone group, isobavachalcone, bavaisoflavone, bavachin, and isobavachin. Now, this is not all; it contains a lot of other flavonoids, but since we are focusing right now on coumarins, I have restricted it here. Now, the seed oil, apart from coumarins, also contains terpenes, and these terpenes include limonene, caryophyllene, 4-terpineol, linalool, geranyl acetate, which give it a good aroma.

Angelicin, which is again a coumarin, soralin, and bakuchiol. Now, angelicin and soralin, which are present in the oil, are coumarin derivatives. But bakuchiol, if you look closely, is a terpenoid derivative. More recently, there has been a good market for this compound.

The reason being, if you see cosmetics which are in the anti-aging domain, they use what is called vitamin A derivatives or retinoid derivatives. Now, the problem with retinoid derivatives is they cause a lot of redness. In people using them, so a little milder version of that is required, and a little milder version has been offered by bakuchiol. So it does essentially the same function—that is, it stimulates collagen generation, it gives you, you know, anti-wrinkle effect, and this too at a very lower dosage. So bakuchiol, you will find it in numerous cosmetics, comes from the very same oil. That is your soralia oil. So applications now, because these are coumarin compounds, they will have a lot of effect when it comes to killing bacteria.

So they have bactericidal effect. They have cytotoxic effect. Antioxidant effect comes from your flavonoids, bakuchiol. Anti-inflammatory, antifungal, as well as immunomodulatory activity. Now this, like I said, it's a coumarin derivative, so this coumarin is because of its ability to fluoresce, because of its ability to kill, is used in psoriasis as well as in leprosy now. In some cases, when you want to kill a bacteria or kill a microorganism, one good effect was observed with soralia oil is when you apply it to the skin.

Now, what happens is the soralin derivatives, especially the furanocoumarin derivatives, stay on your skin there. Now, once they stay on the skin, you can expose your skin to sunlight or you can specifically expose your skin to UVA light. Now, UVA light is a little bit of what you call it as a longer wavelength light. UVA light is something which cannot cause that much damage or redness as compared to your UVB or UVC. UVB, UVC are kind of more intense when it comes to skin penetration.

UVA is something that has lower energy and a higher wavelength. So that is beneficial to us. So specifically, if you don't want to cause redness or itching or less harmful effects of UV, more specifically, a UVA lamp combined with psoralen is used. Now when you apply

it to the skin, it will start producing fluorescence. That is, it acts as what is called a photosensitizer.

So they will absorb, start emitting fluorescence, and emission happens in all directions. So they will emit, they will kind of trigger the cells, and this happens. This emission of fluorescence can kill the bacteria and fungus in the vicinity. So in the early stage of mycosis, wherein the fungus has slightly infiltrated the skin but not deeply, in that case, you can use UVA because for deeper penetration you will need UVB or UVC. Similarly, you will see 8-methoxypsoralen is also used, and 5-methoxypsoralen is also used, but

It is said that if you take a slightly advanced or higher dose of this, especially when consumed internally, it can cause vomiting, headaches, or in some cases, nausea. So apart from that, this effect, like I said, the fluorescence effect, is also used for the treatment of vitiligo. So in patients suffering from vitiligo, you know, they have lighter or white patches. So compared to your skin, those patches appear much lighter. So in order to make it, or to match that color with the skin, you want to increase melanin secretion in those white patches.

So how is it done? You apply your furanocoumarins there or you apply your bhavachi oil there. Now, this compounds because the fluorescence will stimulate it. melanin so whenever your skin is exposed to sunlight you have melanin secretion to protect the skin so same way now imagine your photosensitizer is right there on your skin so it will cause more enhanced melanin secretion so in that white patches Because the melanin secretion increases, you will see that the color darkens of those white patches, nearly matching that to the skin.

Now, this doesn't happen in a single sitting. This happens in a multiple sitting. So, you know, like every week you have to take a certain mg of your soraline dose with certain joules or, you know, like 5 to 15 joules energy of your body. UVA light so that combination you do it for a four to eight weeks time is generally recommended for you know clearing or matching the skin tone with the vitiligo patches so your vitiligo patches now will turn dark enough to match the remainder of the skin so that is how it is used to treat the vitiligo. Another drug which is also used very similarly is amemages.

Now this is not a leguminosa family, this is actually an ambelifera family. Now what is an ambelifera? You see in your kitchen there are fruits such as your fennel, jeera, that is your cumin. You will see your, you know, ajwai. You will see your caraway.

So all of them, you know, are very tiny. Those are fruits, and those have ridges if you observe very carefully. So this is also Umbelliferae. That means your inflorescence, as I've shown here, is a typical umbel shape or umbrella-shaped inflorescence. So the fruits of these plants are taken.

It's also called lace flower for that beautiful delicate thing, or it's also called a bishop's wheel. Now, this grows throughout, especially in the Middle East, Egypt, where it's cultivated in India, in West Asia, as well as in some parts of West Africa. Also, this plant or this crop is taken. Now, once the fruits of it mature, you can harvest it by threshing, and you will get Ammi majus fruit. Now, Ammi majus fruits also contain furanocoumarins.

Now, they are more specifically xanthotoxin and methoxsalen. So you will see your xanthotoxin here. So it is like, if you put this as one, put this as two, three, four, you can then put it as five, 6, 7, 8. So this is like your 8-methoxsalen, whereas your bergapten is something like your 5-methoxsalen.

Chemical constituents

- Furanocoumarins: xanthotoxin (methoxsalen, 8-methoxypsoralen (1.15%), imperatorin (0.75%) and bergapten (5-methoxypsoralen, up to 1.88%),
- Small quantities of Visnadin, marmesin, isoimperatorin, heraclein and isopimpinellin

COC1=CC=C2C(=C1)OC(=O)C=C2
Bergapten

COC1=CC=C2C(=C1)OC(=O)C=C2
Xanthotoxin

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So you remember previously we discussed, so same your PUVA therapy for vitiligo, you can consume these compounds or these compounds can be applied to obtain the same effect. Apart from that, it also contains your imperatorin, and small quantities of another furanocoumarin derivatives or other classes of derivatives. So this include your viscidin, isomarmicin, isoimperatorin, Heraklenin and isopimpelinin.

So these are few compounds which are also there which are also kind of coumarins and all of them put together will also enter in your amimages oil. In some cases the extracts of this fruits are also being taken where as far as therapeutic applications are concerned. This medicine have been used in you know Unani as well as Indian system of medicine. So it's

used in treatment of especially psoriasis. In a very similar manner, vitiligo, you also have application of this in your tinea vesicular.

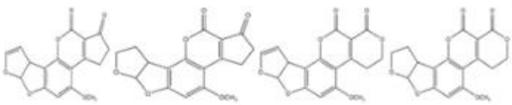
And because this is a coumarin derivative, you can correlate with this. It produces fluorescence. It produces light. And as a result, it is responsible for, you know, sider, that is killing sider. of bacteria and you will see lot of sider effects this includes your bactericidal fungicidal insecticidal larvicidal nematocidal ovicidal viricidal as well as it can kill other herbs or plants that means it's also a herbicidal now apart from that you know especially when it comes to vasculature

It is seen that Ammi visnaga also induces a relaxant effect on vascular smooth muscles. Now, when you are taking coumarins, you need to be a little cautious because if you know, a drug anticoagulant called warfarin. That's also a coumarin, as well as your vitamin K, which is also a coumarin. So they might exhibit some interactions with that, and that may lead to toxicity. Not only that, consuming excessive amounts of these compounds has also been associated with some phototoxic reactions.

So you need to be careful while handling coumarins. But when handled well, they produce a very, very good fluorescence. In fact, many of the chemical tests for psoralia include taking your extract, treating it with a little alkali, and just observing it under UV. You will see a nice yellow-colored fluorescence. There are a few tests, or you know, you can easily detect coumarins by just putting them under a UV lamp because coumarins glow.

And especially, the addition of bases helps in the detection of coumarins. Now we move on to a little about lethal coumarins, and those lethal coumarins are your aflatoxins. These are not drugs; basically, these are poisons. Now, why we are discussing this is because they have significant importance when it comes to the herbal drug industry. And these compounds are aflatoxins.

Aflatoxins

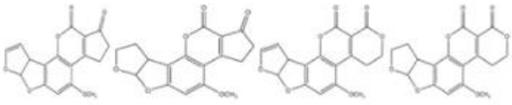


AFB₁ AFB₂ AFG₁ AFG₂

- Aflatoxins are a family of mycotoxins, meaning they are toxins produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus*.
- **Source:** Peanuts, Corn, almonds, pecans, walnuts, Spices, Cocoa beans and certain vegetable oils
- Difuranocoumarins:
- AFB₁, AFB₂, AFG₁, AFG₂

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Aflatoxins



AFB₁ AFB₂ AFG₁ AFG₂

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Aflatoxins are mycotoxins. That means they have the ability to kill us. They have the ability to kill animals as well. They are produced by fungi, which are your *Aspergillus* species. You remember the black powdery substance in your onions, in your pistachio shells, or in your groundnut pods.

That powdery substance is the *Aspergillus* fungus. Particularly if you have *Aspergillus flavus* or *Aspergillus parasiticus*, They give rise to these mycotoxin compounds. So there are numerous sources. Naturally, aflatoxins do not occur in these fruits or cereals.

But when infested by *Aspergillus* and when the conditions are moist and damp, like in the rainy season, where the moisture is more conducive to fungal growth, they start producing these toxins, and the food becomes contaminated. If you don't wash it properly and then consume it, the problem will go into your stomach, and you will ingest all the aflatoxins. These ingestions are also related to food poisoning, especially. A lot of food poisoning is associated with weddings, where bulk quantities of food are stored, especially raw

materials like onions and other items, which are later used without washing. The chances of food poisoning, especially due to aflatoxins, increase in these cases.

Now if you see your aspergillus, aspergillus flavus gives you aflatoxins B1 and B2 that is AFB1 and AFB2 whereas if you see aspergillus parasiticus it gives you all four that is aflatoxin B1, B2, G1 and G2. Now if you see these compounds they are basically difuranocoumarin. Why they are called as difuranocoumarins is you can carefully see that there is a good amount of You can see that there are bifurans in all four. So these are bifurans attached to coumarin.

So this you can see a coumarin here. So I'll just quickly draw. So this is the difuran moiety. This is the coumarin moiety. And attached to it is in this case, that is when you're talking about aflatoxin B1, B2, it is cyclopentanone.

So aflatoxin B1, B2 are difuranocoumarin. cyclopentanone derivatives whereas when it comes to aflatoxin G1, G2 it is difuro, cumero but this time a pyrone kind of derivative which is more specifically a lactone. So AFB1, B2, G1, G2 are the ones which are very toxic not only that in some cases what happens is animals consume this fodder which is kind of contaminated with fungus especially cows and And this aflatoxins are metabolized by them. Now, when they get metabolized, they are converted into metabolites such as aflatoxin M1, M2, aflatoxin H and so on.

Not only are the plain aflatoxins toxic, but these aflatoxin metabolites are also toxic to us. So if a cow is having a mycotoxin and we consume the milk of that cow, there's a good chance that we are exposed to aflatoxin, but to aflatoxin metabolites in this case. Now, they are very poisonous to an extent; even 6 milligrams per kg is lethal for us. So it is lethal for us. It is lethal for animals as well.

But you can easily detect why, because we said coumarins fluoresce. So if you take this, You can easily see the fluorescence in the extracts. They are soluble in chloroform ether. So you just have to take your drugs and see it.

Now, the problem, like I said in the herbal drug industry, is when you deal with extracts. So imagine you have a whole plant contaminated with aflatoxins. And you extract it in a solvent like chloroform. Say, for example, all the aflatoxins will dissolve. And suppose if you have 20 kilograms of material.

So when you concentrate it, this aflatoxins will also concentrate. And when you prepare a medicine out of it, they are going to enter your patients. So this is a very important. you

know crucial metabolite that the industries check does not enter in their product now what effect does it cause so if it is an acute poisoning like just one time poisoning but i consume something which is contaminated i'll get nausea vomiting a little abdominal pain typical signs of food poisoning and in some cases depending upon the dose my liver will also start to damage now they localize in liver they metabolize in liver and as a result they affect the liver more severely but chronic exposure imagine I am having a slight dose of aflatoxin but I am having it on the daily basis in that case because of continual exposure or chronic exposure there are good chances of hepatic cancers especially hepatocellular carcinoma and if it is given to children it cause growth retardation

This is called immune suppression and eventually cirrhosis. So I need to be very careful and most of this are associated, you know, like with peanut butters. Peanut butters initially, you know, when they are not prepared in a good GMP established facility because peanuts have that little black aspergillus in them. They are good sources of aflatoxin to children. So peanut butters are

are contaminated with aflatoxins have led to growth retardation in children. Now, not only does it affect humans, it also affects animals. So when my cow or when my poultry animals are consuming this, you will see that there's a decreased growth rate in them. There's a lowered milk production or lowered egg production in chickens. There's a carcinogenicity and also similar kind of immunosuppression.

So these molecules have to be handled and treated with care. And you know, a simple fluorescence test is something that we can do to check for these molecules. So here are a few references if you wish to know more about this set of interesting molecules. And thank you, everyone, for your patient listening. © transcript Emily Beynon