

PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 6

Lecture30

Week 6: Lecture 30: Cyanogenetic, Isothiocyanate and Phenolic Glycosides

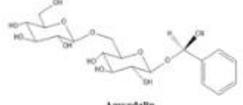
Thank you. Hello everyone, and welcome back to the session on glycosides. So far, we have learned about different types of glycosides, anthraquinone glycosides, and various compounds. Now, we are moving on to a set of very unique compounds: cyanogenetic, isothiocyanate, and phenolic glycosides. To start with the first category, that is cyanogenetic glycosides,

These are glycosides containing a cyano group, which is cyanogenetic or cyanophoric. Basically, these groups have what is called a cyano linkage. Have you heard of this gas or liquid called hydrogen cyanide? Hydrogen cyanide is a very poisonous gas that acts as a contact poison. For humans and animals, if you carefully observe the effects of hydrogen cyanide, in humans, it inhibits the ability to carry oxygen smoothly when this gas is inhaled.

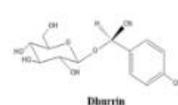
In animals, it proves to be really lethal, often interfering with the electron transport chain. So, cyanogenetic glycosides or cyanophoric glycosides are Compounds, or you can say, a plant's way of hiding this powerful biological weapon called hydrogen cyanide. This biological weapon, hydrogen cyanide, is hidden in a way that it is kept concealed. It is in a form conjugated with sugars.

Cyanogenetic/ Cyanophoric glycosides $H-C\equiv N$

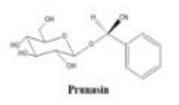
- Cyanogenic glycosides (CNGs) are characterized as α -hydroxynitriles (cyanohydrins) that are stabilized by glycosylation.
- They are present in plants and biosynthesized from amino acids



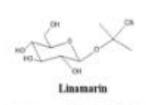
Amygdalin



Dhurrin



Prunasin



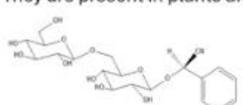
Linamarin

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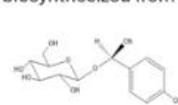


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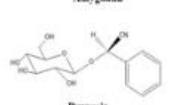
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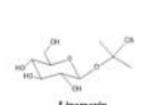
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Now plants try to keep it. Now, if the plant tries to save the hydrogen cyanide in itself, it will be toxic to the electron transport chain or to the plant itself. So, as a result, what happens is the plant wants to kill the animal consuming it, but it doesn't want to harm itself. So what it does is it converts this hydrocyanic acid into a set of compounds by adding sugars to it. Cyanogenic glycosides are characterized as cyanohydrins or also referred to as α -hydroxynitrile derivatives, which are stabilized in plants by glycosylation, wherein a sugar or disaccharide has been added to it.

So you will see numerous such examples, such as amygdalin, durin, linamarin, and prunacin. Now, the common feature of these is, suppose you focus on this particular carbon, you will see bearing linamarin—all three of them have a hydrogen except here, where there is a CH_3 . Then you have cyano linkages. Then you have glucosidyl linkages.

And in this fourth case, you might have a phenyl ring. Now, this phenyl ring in linamarin is replaced by a methyl moiety. So, linamarin is slightly different, but you can generalize

the other three. So now, what is going to happen is whenever an animal consumes this plant, the plant wants to release its toxicity.

So if you remember, plants have an enzyme called glucosidases. So these glucosidases will gradually break down the linkages. I'm sorry, from here, and as a result, convert it into a hydrolyzed product. Now, this hydrolyzed product will later form a ketone and release HCN. This HCN, which is released, will then prove lethal to the animals.

Now, let's see this. So take an example where you have your linomerin. The simplest one. I am just drawing CH₃ for your convenience. Now, the first thing that will happen is a glucosidase will enter.

The slide features a title 'Cyanogenetic/ Cyanophoric glycosides' on the left. To the right is a chemical structure of a cyanoglucoside, which is a glucose molecule with a cyanide group (-C≡N) attached to the anomeric carbon (C1). Handwritten red annotations show 'CH₃' and 'C#N' near the cyanide group. Below the title is a list of two bullet points:

- Cyanogenesis--they release toxic hydrogen cyanide as defense against generalist herbivores.
- Cyanogenic glycoside + β-glycosidase → Cyanogenic aglycone + Glucose + Hydroxynitrile lyase → HCN + Aglycone + Glucose

At the bottom of the slide, there is a footer: 'Pharmacognosy © Institute of Chemical Technology' and a small number '3'.

And this glucosidase kind of acts here. It releases your sugar, and what you get in the process are your derivatives. Now, the derivatives you will get in the process are first, a hydroxy—a little unstable, but yes—a hydroxy, what you call it, a hydroxynitrile derivative. And then this hydroxynitrile derivative will slowly convert into an aldehyde. Now, as a result, what you get here is a ketone, and plus, you will get HCN.

This HCN gas, or the HCN which is released, is going to prove lethal to the animal consuming it. So, it's a way of the plant's defense system. Now, interestingly, the Cyanogenic glycosides, as well as the enzymes, are spaced—that is, in plants, they are kept away from each other in slightly different cell organelles. But at the time of consumption, when the animal is chewing, these organelles are ruptured, so glycosidase comes in contact with the cyanogenic glycoside. It has no option but to lyse the sugar, and as a result, hydrocyanic acid is released. Once hydrocyanic acid is released, it causes all the toxic effects.

Now, one good example of a hydrocyanic acid-releasing compound, or one such example of a cyanogenic glycoside, is bitter almond. Now, we have tasted sweet almonds. So, it's a very close plant. With a slightly different variety, it's *Amygdala amara*, or it consists of dried seeds which appear almost similar to almonds but taste a little bitter. This bitterness is attributed to the compounds present in it, so this is also *Prunus amygdalus*. When you are talking about the sweet almonds that we consume, it is the variety *Dulcis*.

But when you are consuming bitter almond, it is *Prunus amygdalus*. It is the variety *Amara*, which is different. Both of them belong to the Rosaceae family and bear beautiful flowers. Now, these bitter almonds have been cultivated throughout You will see them in slightly temperate regions, wherever there's a colder climate.

Iran, Asia, you'll find it in Sicily, Portugal, Morocco and even in South France. Now, this cannot be consumed as they are bitter. They are not clearly edible because they are toxic over a period of time. If you eat more of this, this hydrocyanic acid initially causes sedation and may prove lethal in humans also. But what you can prepare out of that are two things that your oil and flavor.

Now, chemically, if you see your almond, it contains or chemically, if you see a bitter almond, it contains a sign for a glycoside called as amygdalin. OK, now this amygdalin has two sugars attached to it, a phenyl ring and a hydrogen like we saw generically. Now what is going to happen in your amygdalin is it has enzyme called as emulsin. Now this emulsin contains amygdalase, prunase and hydroxynitrillase. So let's understand them.

So first thing amygdalase will cleave up here. So this is removed and So now your amygdalin once one sugar is removed it get converted to what is called as prunacin. This minus one sugar is prunacin. Now this prunacin is acted by the second enzyme called as prunase and prunase will cut it out here.

So your second sugar is also gone. You will be left with a hydrolyzed product of prunacin. Now, this compound. will undergo lysis again in the presence of hydroxynitrilease. Now, this hydroxynitrilease, when this happens, will convert this hydroxy into an aldehyde.

If it is a CH₃ out here, you can call it a ketone, but since it's a hydrogen out here, it will convert into an aldehyde, and so I'm just redrawing the whole thing here. I will get what is called this is my carbene. This is my aldehyde, and this is my H. So I get benzaldehyde plus hydrocyanic acid. So your amygdalin gets converted by amygdalase into prunacin.

This prunasin is then acted upon by the enzyme prunase, which converts it into a hydroxynitrilase derivative.

Chemical constituents

- Fixed oil (40-50 percent),
- Protein, emulsion, and volatile oil
- bitter glycoside amygdalin (1-3 percent)
- The cyanogenetic glycoside is obtained from the cake of bitter almonds or seeds after the expression of oil.
- Emulsin: amygdalase, prunase, hydroxynitrilase etc.
- Bitter almond oil contains benzaldehyde and hydrocyanic acid.

amagdaline Prunasin

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That is your benzaldehyde derivative. But this is further converted by your enzyme hydroxynitrile into benzaldehyde and hydrocyanic acid. So your beta almond oil, which is obtained by expressing or squeezing out, is rich in benzaldehyde and hydrocyanic acid. On average, your almond contains about 1 to 3% of amygdalin, and that's the reason it's very bitter in nature. This compound is not present in your sweet almonds.

So you can check whether you're consuming sweet or bitter almond by doing some tests which are particularly specific to amygdalin because of its ability to release hydrocyanic acid or HCN. Now, one such test is the ferrousferrocyanide test. What you have to do is extract a gram of the drug and crush it. The moment you crush it, the enzymes as well as your amygdalin is going to come in contact, and the process of lysis will begin.

Now, you can also facilitate the process by adding alkalis. In this case, alcoholic KOH is also facilitating the hydrolysis. Now, once the hydrolysis is done, the drug is kind of boiled, and complete hydrolysis is taken care of. You add an aqueous solution, a mixture of almost equal quantities of ferrous sulfate (2.5%) with ferric chloride, and then heat it again for 10 minutes. You take this solution and transfer it to another test tube which contains about 20% hydrochloric acid. You will see a nice Prussian blue color appearing, and that's due to the presence of hydrocyanic acid. Hydrocyanic acid is also known to precipitate mercury from your mercuric nitrate solution, so when you take your

any almond, especially the bitter almond, crush it, heat it slightly, warm it, but don't extensively heat it, otherwise your hydrocyanic acid will vanish. So if you're heating it, ensure that the vessel is covered, or just very gently heat it, and then treat it with your

aqueous mercury nitrate solution. You will see that after some time, tiny black-colored particles or dots appear at the bottom of the test tube. That is nothing but your precipitated mercury, which has been precipitated due to hydrocyanic acid. You can also do what's called a Grignard's reaction test. Grignard's reaction test can be observed again in most of the cyanogenetic glycosides, which have the ability to release HCN.

So what you can do here is take your filter paper and treat it with sodium picrate. Now, if you don't have sodium picrate available, don't worry. First, dip it in picric acid solution, followed by dipping it in sodium carbonate. The picric acid will form a sodium salt, and that's your sodium picrate. Now, allow this sodium picrate filter paper to dry for some time.

Once it is dry, in a conical flask, take your drug—maybe about 5 grams—crush it in a mixer grinder with a little water so that the enzyme and the cyanogenic glycosides come into contact. As the reaction starts, HCN will be released. Now, as it is being released, hang this filter paper, which you have soaked in sodium picrate solution. Now, this sodium picrate gets converted into sodium isopurpurate. Sodium isopurpurate is brick red in color, but this reaction happens very slowly.

So, you will observe that after an hour, the filter paper you have put inside the conical flask, or kept hanging inside the conical flask, gradually turns to a brick red color. This is due to the Grignard reagent. Another one is, you know, like with your guaiacum or guac resin.

So. You take your guai gum or guai resin and dissolve the resin in alcohol. We want filter paper, you know, just slightly coated with the guai resin. So take this guai resin ethanolic solution, dip your filter paper two or three times so that it coats nicely. A thin coat of guai resin forms over it.

Now, dip this filter paper in copper sulfate. It will acquire a slight bluish coloration due to the copper sulfate, and then this paper you keep in contact with a freshly crushed drug containing your cyanogenetic. You will see a distinct deep stain on the paper, attributed again to the presence of hydrocyanic acid. So, these are a few tests you can perform to check if the almond samples you have are bitter almonds or to check if any drug contains cyanogenetic glycosides.

Bitter almonds, as I said, are not safe for consumption. If you consume them accidentally, you will recognize them by their bitter taste. But still, in smaller doses, people earlier used to give bitter almonds as a sedative. See, it's slightly toxic but causes sedation at low doses. The oil...

Still containing mild doses, it is used as a demulcent. Then the refined oil, which is now free of benzaldehyde and hydrocyanic acid, is a good, cheaper variant for sweet almond oil and can be used in skin creams and moisturizers as an emollient. Now this This preparation, or these bitter almonds, have also been used to prepare or purify amygdalin or even prepare bitter almond water for the release of hydrocyanic acid. Now we move on to the next set of compounds, which are commonly seen in Indian tradition.

The drug is mustard, and the category is what are called isothiocyanate glycosides. You may have seen people, such as bodybuilders, apply a little mustard oil on themselves. So sarso or mustard—you must have seen a lot of this. Cultivation of mustard occurs across northern India, especially in Punjab, Haryana, the northern plains, and also in the Kashmir region. It consists of dried, mature seeds of *Brassica nigra* and *Brassica juncea*.

These are members of the Cruciferae family. This is the black mustard. If you're talking about white mustard, it is *Brassica alba*. But white mustard is not as pungent or spicy as black mustard. As a result, traditionally, especially in Indian cuisine, you will find black mustard.

Apart from that, black mustard is also used for preparing sarson oil or black mustard oil. Geographically, it grows in the northern parts of India, China, Canada, and it's cultivated in North America as well. If you see this plant, it has nice beautiful yellow flowers, and then it bears these pods upon maturing. These pods, if you open them, will split, and within these pods will be your black-colored seeds.

These plants take about 90 days to mature. Once matured, they are dried properly. They are threshed. During threshing, the pods open up and release the seeds. They are then winnowed, and the seeds are collected.

If you cut open or break the seeds, you will see internally they are yellowish in color, and externally the seed coat or the testa is black in color. For *Brassica alba*, the seed coat is white in color. Now, black mustard contains compounds, especially those called glucosinolates. What are glucosinolates? You can see here there is a sugar, and glucosinolates are the category of what we call S-glycosides.

So here, the sugar linkage is attached to the aglycone by the sulfur. So, glucosinolates are S-glycoside type compounds. Now, one glucosinolate derivative present in black mustard is sinigrin. Now, this sinigrin also contains nitrogen apart from sulfur, and there is an enzyme called myrosinase. Myrosinase will specifically act on this.

Because of allyl isothiocyanate and erucic acid, it acts as a counter-irritant and rubefacient. Besides that, the oil extracted from black mustard is used for cooking—that's for culinary purposes. We now move on to the next set of glycosides, which are phenolic glycosides. And in this case, we have a plant that is a beautiful orchid family member, and that's your vanilla. Now, vanilla is a very exquisite and expensive spice or flavorant, which is used in the food industry and is obtained from dried fruits—or what we call the dried beans—of *Vanilla planifolia*, sometimes referred to as *Vanilla fragrans*, which is an Orchidaceae family member.

So, being an orchid, it prefers a slightly moist, tropical, warm climate. It is not something that can bear frost. It's not something that can bear arid conditions. It prefers a slightly damp, moist, and warm climate.

So, it's cultivated mostly on islands. If you look at Madagascar, Seychelles, Mauritius, Sri Lanka, the Indonesian islands, and the Réunion islands. Also, it's cultivated in Mexico and, in some cases, in the South Indian Plateau region where it matches the climatic conditions of Indonesia and Sri Lanka. So, some plantations have been actively established in India as well.

But the majority of the supply of vanilla comes from island nations. So, this vanilla is actually from an orchid plant. So cuttings are taken and grown, but you have to be very careful while sowing them in the soil. Mostly, it is allowed to rise vertically for a meter or two and then it is allowed to bend or turn on the plants. Now, this plant, once it matures in about three to four years, starts bearing flowers, and these flowers last for only a day.

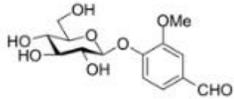
So now, the important part to produce a fruit is pollination. The natural pollinator of vanilla is a stingless bee, which is now thought to be extinct and is not present in many countries. So, as a result, people manually pollinate. So vanilla pollination is something that is mandatorily 100% done manually, as you will see. And for that, you have to take the style and stigma and manually touch them.

So within those 24 hours, during the morning when the flower is most active and receptive, they say that is the time you attach the style to the stigma, allowing a few pollens to fall in. But this is not done for all the flowers because if you do it for the whole inflorescence, it will lead to many fruit formations, resulting in smaller and lower-quality fruit. So selectively, a few flowers are fertilized and allowed to develop into fruit, as you can see here. The beans are generated. Now, these beans, if you see or take a whiff or consume, will not have the flavor or aroma of vanilla.

The reason being, in this particular pod, your vanilla is there as a glucoside. So you will have your vanillin glucoside. And this doesn't have the aroma. So you need to use the beta-glycosidase. Now, this beta-glycosidase will act, especially once the harvesting is done.

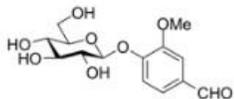
Chemical Composition

- The characteristic colour and odour of the commercial drug are only developed as a result of enzyme action during the curing.
- Vanillin a phenolic aldehyde is a key flavor compound, the primary taste and aroma of vanilla
- High-quality beans will yield a significant amount of vanillin (around 2-2.5%)
- Other compounds include vanillic acid, vanillyl alcohol, p-hydroxybenzaldehyde, p-hydroxybenzoic acid and p-hydroxybenzylalcohol.



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So what is done is there is processing of vanilla beans. So once they are cut, they're kind of killed by putting them in warm water, about 60 to 65 degrees Celsius, by boiling. Heating it and then removing it after three minutes—so that is what is called killing. Then they are allowed to sweat, so they are kept in a closed box and let the moisture evaporate or come out of those beans. So during the sweating and after that, this is followed by drying. This is called curing, so curing of vanilla beans is done. And just to show you, So these are your raw vanilla beans. During the curing process, you see that they develop color.

They develop an aroma profile because the sugar is hydrolyzed, and you get what is called your vanillin. Now, this vanillin is the main aroma principle, and if you see good or nice cultivated varieties, they have from 2 to 2.5% of vanillin. Apart from vanillin, suppose if you change this to a COOH, you get what is called vanillic acid. Suppose you change this

to a CH_2OH , you get what is called vanillin alcohol. And if, suppose, you remove the methoxy, you get what is called hydroxybenzoide.

Then you will get your hydroxybenzoic acid. And you will have your parahydroxybenzyl alcohol. So these compounds are present in your cure. The process of curing may last somewhere between three to six months altogether. And by the end of curing, you'll get a product of this type, which is soft, pliable, and ideally should not break open.

So during the application, the pod is opened, and you will see numerous seeds inside. The seeds are scraped with a knife, which contains tiny crystals of vanillin as well as the seeds, and that is what is used in the food industry. In terms of applications, you will see that vanilla has been used as a flavoring agent. It is used in perfumes. You might find numerous vanilla perfumes, and also in pharmaceutical applications as a sweet therapeutic agent and as a sweetening agent.

So here are a few more references for additional reading. And thank you, everyone, for your time.