

PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 6

Lecture 28

Week 6: Lecture 28: Cardiac Glycosides

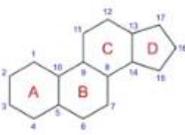
Hello everyone, and welcome to the NPTEL course on Pharmacognosy and Phytochemistry. In the previous session, we learned what glycosides are, the classification of glycosides, and a few drugs containing glycosides, such as aloe and senna. Those were the laxative ones. In this particular session, we will delve into more potent glycoside derivatives, which are the ones that act on our heart. These are what are called cardiac glycosides.

So, welcome to a session on cardiac glycosides. Cardiac glycosides, if you see, are sterol glycosides. By nature, they are steroidal moieties and have a strong ability to act on the human heart, as they are glycosides. We understand that the steroids are attached to sugar molecules, which make them more absorbable and affect their ADME, the pharmacokinetic parameters. The pharmacodynamic parameters are attributed to the steroidal moiety.

Cardiac glycosides can be divided into two categories: cardenolides and bufadienolides. The difference lies in their carbon count. If you see their numbering, the basic steroidal moiety starts from ring A. Steroidal moieties have a four-ring system. A, B, and C are six-membered ring systems, whereas ring D is a five-membered ring system. The numbering starts from ring A and goes in this manner.

Cardiac glycosides

- Cardioactive glycosides are steroidal glycosides, that act on the human heart.
- Cardenolides and bufadienolides.
- **Cardenolides** have a steroid skeleton with **23 C-atoms** and contain γ -lactone (butenolide) ring, e.g. digitoxigenin.
- **Bufadienolides** with **24 C-atoms** contain, on the C-17 carbon atom, a δ -lactone ring (α , β - γ , δ doubly unsaturated), e.g. scillarenin



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So if you can see. It's a typical what we call it as anticlockwise manner. So you have your carbon number 1, 2, 3, 4, 5. Now naturally when you have sterols many of them have a hydroxy group present at the third position. So 1, 2, 3, 4, 5 then 6, 7, 8, 9, 10.

So your ring A and B is a 10 carbon ring system with shared carbons that is carbon number 5 and carbon number 10. Now when you go to ring C, the numbering happens in a slightly different manner as you can see. The numbering happens in this manner. So you will see then after 10 is 11, 12, 13, 14, 15, 16, 17. OK, so now if you see between your ring B and C, the shared carbons are at the carbon 8 and 9 and between C and D, the shared carbons are carbon number 13 and 14 from cardiotoxic activity.

Carbon-12, 14, 16 and 17 are very vital. We will see them as we elaborate more into cardenolides and bufadenolides. But just to give you an out view, cardenolides generally contain what is called as gamma-lactone system whereas bufadenolides contain what is called as a delta-lactone system. So here you will see them very nicely the difference between the two. So this are steroidal moieties.

If you remember, you will have your carbon numbers 1, 2, 3, which I said was hydroxy 4, 5, 6, 7, 8, 9, 10, and then onwards you can recollect 11, 12, 13, 14, 15, 16, and 17. Now here, the sugar is attached in a beta position, and so is the lactone moiety. But in cardenolides, you will see a 5-membered lactone moiety, and in the case of bufadenolides, you will see a 6-membered lactone moiety. One more thing, as you can see, a difference between them is here the number of double bonds is just one, whereas in this case, you have two double bond structures.

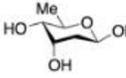
In terms of their occurrence, cardenolides are principally found in plant species, whereas bufadenolides have been found in plants as well as certain species of frogs or toads. So

they are also found in the animal kingdom as well. Cardenolides—you can say examples of cardenolide-containing plants include your digitalis, they include your thivetia, they include your strophanthus, whereas bufadenolide is present in your squill and bufa species of frog. When it comes to the glycone moiety or the sugar moiety, like I said, they have been attached to the 3-hydroxy moiety. Linkages of the steroidal moiety chiefly by a beta linkage.

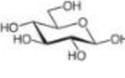



Sugars

- Sugar molecules is connected to the β -OH group of C-3 carbon atom.
- The sugar components of cardioactive glycosides are mainly deoxy-sugars and glucose.
- The deoxy-sugars are 6-deoxy-sugars (e.g. L-rhamnose, D-fucose, D-digitalose, L-tevetose) or 2,6-deoxy-sugars (e.g. D-digitoxose, D-cymarose, L-oleandrose)



D-digitoxose



D-glucose

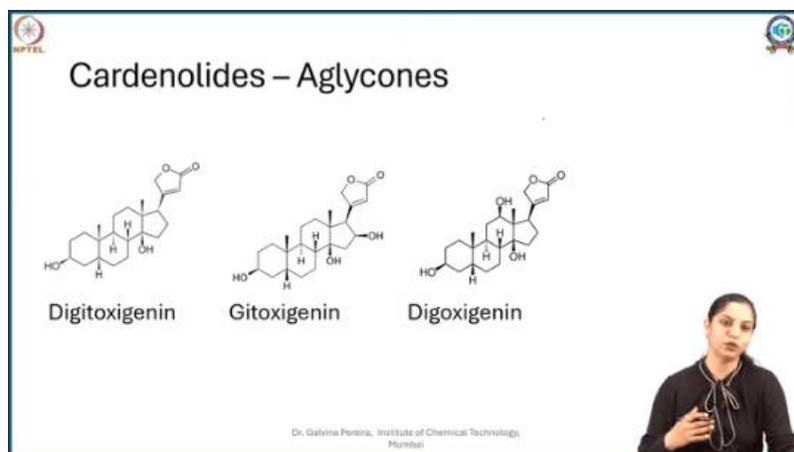


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So carbon number 3, you take a beta linkage and attach a sugar. Now, predominantly these sugars include your deoxy sugars. One good example of a deoxy sugar is digitoxose, which is present in your digitalis. So you can see this particular carbon, OK, it's a deoxycarbon out here, whereas this is your glucose.

Apart from that, some of them are also found to contain rhamnose. So you have your deoxysugars: rhamnose, fucose, digitalose, thivetose, and 2,6-deoxysugars. This is 1,2, but you might also have your 2,6-deoxysugars, such as digitoxose. You can see it clearly. Then, cymarose and oleandrose are some examples of deoxysugars.

Now, glucose, when it is attached to this type of glycoside, is mostly the terminal or outermost sugar. Now, if you see aglycons, I'm just giving you a few examples of aglycons, which are very potent as cardioactive drugs. And if you see in terms of substitution, let's come back here. Here, you remember 1, 2, 3 is a hydroxy, 4, 5, 6, 7, 8, 9, 10.



We are done with this. Then you have 11, 12, 13, 14, 15, 16, 17. I am just repeating it so that you all can visualize it more clearly. So, in case you have a hydroxy at the 14 position. Now, apart from 17, you have your 18, 19 as well.

So, there are a total of 19 carbons in the steroidal moiety. But whenever a hydroxy group is present at the 14th position, we call it digitoxigenin. Whenever the hydroxy group is present at the 14th and 16th carbons, you call it digitoxigenin. And whenever it is present at the 12th and 14th positions, you call it digoxigenin.

Apart from that, you might also come across gitaloxin. Now, in gitaloxin, what happens is you get esterification. So, this hydroxy group is actually converted into an aldehyde. We call it G-tal. That comes from G-toxigenin, the aldehyde of G-toxigenin.

So, G-taloxigenin. So, these are a few common aglycons which occur in cardiac-active glycosides, especially in the Digitalis species. So, let's see what Digitalis is. So, Digitalis is a beautiful Himalayan plant which grows in Europe or mostly temperate climates, depending on the species. Predominantly, two species of Digitalis are used: *Digitalis purpurea* and *Digitalis lanata*.

So, *Digitalis purpurea*, let's talk about it. It's also referred to as purple foxglove for its beautiful purple-colored flowers, which represent or mimic a lady's glove. And It belongs to the family Scrophularaceae. It's commonly found and grown as an ornamental plant in England, Germany, France, North America, and places like India, Japan, Kurdistan, Mexico, Nepal, Spain, and Turkey.

Now, this plant—the leaves of it—are very toxic. So, though it's ornamental, it's often handled with great care. Now, in terms of cultivation, what is done with this plant is the seeds are sown, and you will see in the first outgrowth, it will bear what are called rosette

leaves. Those are not very good or rich in glycosides. Subsequently, it will give you nice-colored,

ovate kind of leaves, and those are the ones preferred for their high cardenolide content. So, it contains somewhere between 0.1 to 0.4 percent of cardenolides in terms of purpurea glycosides. So, purpurea glycosides are nothing but your cardenolides plus sugars. Now, let's break down this molecule one by one. So

To see this, if you remember, whenever you have—now I am being a little quick. So, you have these 10 carbons: 11, 12, 13, 14. So, 14-hydroxy is, if you remember, it was digitoxigenin. Okay. So, if you have your digitoxigenin, digitoxigenin attached to 1, 2, 3 digitoxose sugars.

So, I am just putting it here. Plus 3 into digitoxose plus one molecule of glucose. This is what your purpurea glycoside A is. So, purpurea glycoside A has a glycone digitoxigenin and a glycone moiety consisting of three molecules of digitoxose and a single molecule of glucose. This occurs primarily in plants and is referred to as primary glycosides. Now, what happens is there is also an enzyme present in this plant called digipurpurase.

Chemical Constituents

- The characteristic constituents of the drug are cardenolide glycosides (0.15-0.4%).
- Purpurea glycoside A, purpurea glycoside B and purpurea glycoside C, digitoxin, gitoxin and gitalotoxin.
- steroidal saponins, flavonoids (e.g. apigenin, luteolin), caffeic acid and chlorogenic acid

Purpurea glycoside A

3x *Digitoxigenin*

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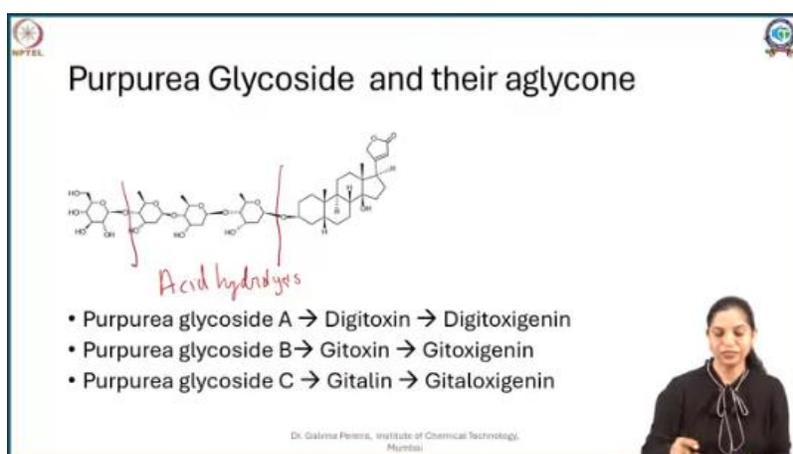
So, whenever the drug is broken, ruptured, or due to some mechanical effects, the enzyme leaves are bruised. In that case, what is going to happen is the enzyme digipurpurase comes in contact with your purpurea glycoside and starts cleaving the sugar. So, in the process, you get this glucose being removed. So now you're left with three molecules of digitoxose sugar plus digitoxigenin, and this together is called digitoxin.

So digitoxin is a glycone plus three digitoxins when you remove a glucose molecule. Now, this is referred to as secondary glycosides. So once the enzyme removes the glucose, you

get this as a secondary glycoside. Now, in a similar way, your purpurea glycoside B will give you gitoxin, and purpurea glycoside C will give you gitaloxin.

Apart from that, your plant also contains what are called steroidal saponins, flavonoids such as apigenin, luteolin, and some cinnamic acid derivatives such as your caffeic and chlorogenic acid. So you can see here clearly: purpurea glycoside A, remove the glucose, and you get your digitoxin. Remove this. Now, how do you remove this? This is generally done by what is called acid hydrolysis.

Acid hydrolysis will give you aglycone. But the problem is, if you get your aglycone, The aglycone is going to be inactive because it doesn't have the required ADME effects. Now, care has to be taken during the harvesting stage. It is said that if you keep the leaves—so these leaves are generally harvested when the plant is in bloom or at least three-fourths of the inflorescence is open.



So that time you collect the leaves having high cardenolide content. Now these leaves are to be dried immediately. The reason is if you don't dry it immediately, the digi-purpurase remains active. And if the digipurpureas remain active, like I said, it starts cleaving the sugars, being the glucose being first. So enzymatic hydrolysis predominantly takes care of this.

And to this point, it is okay. But if further it deteriorates and it removes your digitoxose moiety as well, in that case, you get a genin. And the genin has a lot of issues in terms of its bioavailability and absorptivity. So It doesn't show the desired pharmacological effect.

And as a result, the sleeves are not thought to be of good quality. So ideally, the moisture content during the drying stage is brought to below 5% and then stored to prevent it from enzymatic hydrolysis. Similarly, your purpurea glycoside B will give you gitoxin, which

in presence of acid will get hydrolyzed to gitoxigenin. purpurea glycoside C will give you gitalin. You remember the aldehyde substitution and this will give rise to on acid hydrolysis gitaloxygenin.

So, There are numerous tests. I have stated a few, but let's discuss them. So one of the tests which can be used for detection is the budget test. The budget test takes place.

You can do it on a leaf. So you just take a very thick section of a leaf and to that leaf, you can add sodium picrate directly. When you add sodium picrate directly, it gives you a yellow to orange color. Alternatively, if you don't have sodium picrate, what you can do is initially add a drop of picric acid and then to that, you add an alkali like sodium hydroxide. Eventually, your picric acid will form a sodium picrate salt, and you will get the same result.

So it is said that the picric acid gives you a strong coloration, whereas the presence of a base, like sodium hydroxide or sodium salt or picric acid in this case, intensifies the color to an orange shade. The next test is the legal test. So in this case, what you can do is take the pure cardiac glycoside. So you can dissolve or extract the drug in pyridine.

Now, this is treated a little bit with sodium nitroprusside solution and made alkaline. In a similar manner, but this time you will get a pink to red color. Now, you can also do what is called a test for digitoxose sugar. That is a test for deoxysugar. In this case, you can take the isolated compound or the plant extract.

You can do a hydroalcoholic extraction, filter it, and then you add a good amount of glacial acetic acid. Now, to that, just add a drop of ferric chloride solution and from the sides gradually add concentrated sulfuric acid. Now, we know that concentrated sulfuric acid, owing to its density, will form a layer at the lower side of the test tube. So, gently observe the color change.

Initially, the interface between your ferric chloride-glacial acetic acid layer and your sulfuric acid layer will be reddish-brown in color, and slowly the ferric chloride and glacial acetic acid layer acquires a bluish coloration. So, if this happens, it's a good chance that it contains a deoxysugar. Apart from that, you also have Kedde's test. Kedde's test also helps you distinguish between cardenolides and bufadienolides.

So what you do with that is you extract your plant or you dissolve your compound and treat it with small quantity of Cades reagent, which is actually a mixture. It is you have to take equal quantities of 2% solution of 3,5-dinitrobenzoic acid in methanol and about 7.5%

aqueous KOH. So when you take this in an equal ratio, you should get a bluish violet coloration. which gradually takes time to fade like one to two hours. If no color change is seen, there's a high chance that it's a bufadenolide and not a cardenolide.

You also have other tests such as your Raymond's test. Now, where do you apply this cardioglycosides? Cardiac glycosides are known to act on a specific system called a sodium potassium ATPase pump. They inhibit the sodium potassium ATPase pump. And as a result, what will happen is you will see an increase in intracellular amount of sodium as well as calcium.

And this calcium in the cardiomyocytes or the heart cells, as you call it, will increase the contractility. This contractility is referred to as a good inotropic effect. It also exhibits a good thing on a good effect on a tone. It's called as tonotropic effect. So good inotropic, it maintains the muscle tone and this contractility helps us in a way that since it's a better contraction, more cardiac emptying will happen.

Now, imagine a case of congestive cardiac failure where your heart is unable to pump. This gives you more energy to pump, more vigor. And as a result, the heart performs better. Hence, this class of compounds is also referred to as cardiotonic agents. Apart from that, they also show mild diuretic activity, which is also said to be a result of their cardiotonic effects.

Now we move on to another plant which also contains cardenolides, and that is *Digitalis lanata*. Unlike purpurea, which had purple-colored flowers, as you can visibly see, this one has a little brown to yellow color in its inflorescence. So it is obtained from the dried leaves of *Digitalis lanata*, which belongs to the Scrophulariaceae family, and it's again found almost similarly in Europe, England, California, and India. But you can see there is a notable difference between the leaves and the flowers of this plant. There is also a significant difference between the chemical constituents.

In this case, apart from not finding purpurea glycosides, you find compounds called lanatosides. Now, what is the difference between purpurea glycosides and lanatosides? So if you see this carefully, this is a lanatoside C. Now, the glycone here is digoxigenin. Digoxigenin is absent in purpurea.

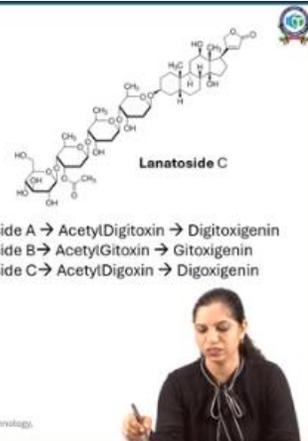
Chemical constituents

- The characteristic constituents of the drug are cardenolide glycosides (1%).
- Lanatoside A, lanatoside B, lanatoside C, lanatoside D, lanatoside E, digitoxin, digoxin, acetyldigoxin.
- Their aglycones are: digitoxigenin, gitoxigenin, digoxigenin, dignatigenin and gitaloxigenin.
- Other constituents are steroidal saponins, flavonoids (e.g. apigenin, luteolin), caffeic acid and *p*-coumaric acid and anthraquinone-derivatives.

Lanatoside C

Lanatoside A → AcetylDigitoxin → Digitoxigenin
 Lanatoside B → AcetylGitoxin → Gitoxigenin
 Lanatoside C → AcetylDigoxin → Digoxigenin

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So this glycone doesn't happen or doesn't occur in your *Digitalis purpurea*. Now apart from that you have your three sugars which are digitoxos. And one sugar which is a glucose. But at the third position, so this is like one, second and third. At the third position of third digitoxor sugar, you will see an acetyl substitution.

So that's the major difference between your purpurea glycoside and lanetocside. So in lanetocyte, the third position of the third digitoxose sugar is acetylated. The aglycons remain more or less same, but there is an additional aglycon which is found in laneta and not in purpurea and that is your digoxygenin. So you will have primary glycosides, lanetocyte A, B, C and so on. You will have your secondary glycosides.

Secondary glycosides in this case will give you acetyl because this is not gone but this later on this acetyl can also be hydrolyzed. So after the acetyl is hydrolyzed you will get your digitoxin, gitoxin and digoxin. Yes hydrolysis of this acetyl group is also possible. Now then In the end, when complete hydrolysis is done, you get the corresponding genin.

So, you will get digitoxygenin, gitoxygenin, digoxygenin, and so on. Apart from that, it has other moieties, like your dignatigenin, which comes from your lanotocyte C and gitaloxygenin. So, Apart from cardiac glycosides, you have steroidal saponin derivatives, flavonoids, apigenin, and luteolin. Again, you have cinnamic acid derivatives and some anthraquinones, which are also present in this plant.

Now, applications are very similar. So, it has a positive inotropic and tonotropic effect. It is a cardiotonic, so it acts by inhibiting your sodium-potassium ATPase pump. But the presence of digoxin has some differences. So, you will see in the market that you have digitoxin.

and digoxin. So, digitoxin and digoxin are both marketed, and they have slightly different applications. Compared to digitoxin, digoxin has a rapid onset—as fast as 15 to 30 minutes—and it peaks quickly, reaching optimum concentration within three to six hours. So, whenever a rapid onset is desired, in that case, your digoxin is preferred over digitoxin. Digitoxin takes a little more time. Now, one thing about this is that your digitoxin is cleared mostly through hepatic clearance, whereas your digoxin is cleared exclusively by the kidneys. So, people who have renal failure or kidney failure—in that case, you have to be very careful while handling digoxin, as it may not get eliminated quickly because of kidney malfunction.

Again, our difference lies between the doses. If you see your digoxin, digoxin is effective at a very low dose. You can say like 0.5 to 2 nanogram per ml dose. of body fluids whereas if you say digitoxin it has a little wider nice margin that is 10 to 25 nanogram per ml so even if you see toxicity toxicity of digoxin occurs very fast and hence this given very carefully under the supervision of a practitioner or a basically under hospitalization whereas a digitoxin In some cases, it's prescribed even as oral tiny tablets for long-term dosage whenever required because it has good sustainability and a very wide margin of safety as compared to your digoxin.

Now, if you go to see your bufadenolides, a good example of a bufadenolide is squill. Squill is onion like sliced bulbs, which is obtained from *Arginaria* species, *Arginaria maritima*. And if you go to see Indian squill, it's *Arginaria indica*. So from that what they do is the outer sheathing scales have been removed. This is onion leg so belongs to the family Liliaceae.

It is cultivated in Mediterranean countries and West Asia. The white squill which is the medicinal squill is cultivated in Spain, Portugal, Sardinia, Malta, Cyprus, Greece. Whereas red squill if you go to see is more native to Algeria and Morocco. Now, red squill is considered to be a little toxic and not preferred pharmaceutically. In terms of chemical composition, as you can see, it's a bupherdenolide.

It has a six-membered lactone with two double bonds. In terms of sugars, as you can see here, this is one molecule of glucose and one molecule of rhamnose. which is attached to the 3-hydroxy moiety of the sugar. Now, apart from that, it also contains an enzyme, like how digitalis had digiperpurase. This one has skeletonase, which causes hydrolysis.

Now, once it causes hydrolysis, the glucose is lost, and what you get is what is called proskelarin. And then You have another acid hydrolysis. So you get your genin being formed. So it has a sclerinin.

So it has a glycone, sclerinin, glucoskelerinin, and especially proskelerinin. The red squill, which is the toxic variant, contains the principal glycoside, which is scleroside and glucoskeleroside. They are a glycone part, which is referred to as scleroside. Now, this is the one which, you know, is sometimes also referred to, or certain species of red squirrel have also been used to poison rats. So it was very famous as a rat poison initially, but it is no longer used for it.

Apart from that, it also has other flavonoids and some derivatives called xanthoskeliide. Applications, if you see sclerin or proscleridin A, especially from white squill, which is the pharmaceutical one that gets easily absorbed from the GI tract and hence is given as an oral dose as a therapeutic in a number of cardiovascular conditions such as iota insufficiency, angina pectoris, and edema, which is also associated with nephritis. A counter effect of all of this is that when given in small doses, it can relieve a cough and is used as an expectorant. But if you administer it in large doses instead of small ones, there is a good chance that it will cause vomesis. So this is with your cardinolides and bufadinolides, the chiefly cardiac-acting drugs.

And here, if you wish to read more about this. There are a few references, and thank you everyone for your patient listening. Thank you.