

# PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 6

Lecture 29

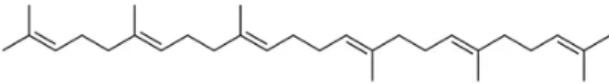
## Week 6: Lecture 29: Triterpene and Diterpene Glycosides

Hello everyone, and welcome to the NPTEL course on pharmacognosy and phytochemistry. This week, we are dealing with a set of compounds called glycosides. In this session, we will learn more about terpenoidal glycosides, especially diterpenes and triterpenes. So, if you remember from our previous chapter on lipids and volatile oils, we discussed a few sets of compounds called terpenes. Those were the ones derived from the isoprenoidal pathway.

Now, these terpenes, depending on the number of isoprene units attached to them, can be categorized as monoterpenes, diterpenes, and triterpenes. So, monoterpenes are the ones we studied in our volatile oils, Comparatively, as the molecular weight increases, we come across diterpenes and triterpenes. Diterpenes are compounds containing 20 carbons, whereas triterpenes are compounds containing 30 carbons. So, let's understand diterpenes and triterpenoidal compounds, as well as their glycosidal derivatives.

So, we'll discuss triterpenes first. Triterpenes are compounds derived from isoprene units, and if you look closely, they are C<sub>30</sub> compounds. So, overall, you will come across six isoprene units joined together. If you try to visualize it, you can see the isoprene units as 1, 2, 3, 4, 5, and just cut it from here; you can see the second isoprene unit. Here, the third isoprene unit continues till here. Now, what happens here is something called tail-to-tail condensation. So, instead of forming a head-to-tail condensation, this tail-to-tail condensation causes almost an inverse symmetry. So, you can just imagine starting from here, you have your next terpene, that is, your isoprene unit.

**Triterpenoids**



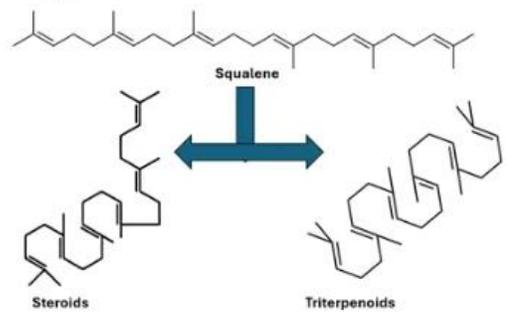
- Triterpenoids are C-30 derivatives
- Biosynthesized from condensation of 6 isoprene moieties

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The next isoprene unit and the last isoprene unit. So you have about 1, 2, 3, 4, 5, 6. Six isoprene units which are joined to each other. Now each of these isoprene units contains five carbons. So 5, 6 are naturally we have C30 compounds which combine to form triterpenoidal compounds.

So If you go to see triterpenoidal compounds, depending upon how they cyclize or how they condense, the biggest linear chain triterpenoidal compound which you see occurring in nature, the aliphatic one, is your squalene. Now this squalene, depending upon the enzymes it encounters, might fold in a way that you will get a four-ring structure. And you can call this A ring, B ring, C ring, D ring, wherein the D ring has five members. This essentially gives rise to a set of molecules which are called steroidal triterpenoids.

**Triterpenoids**

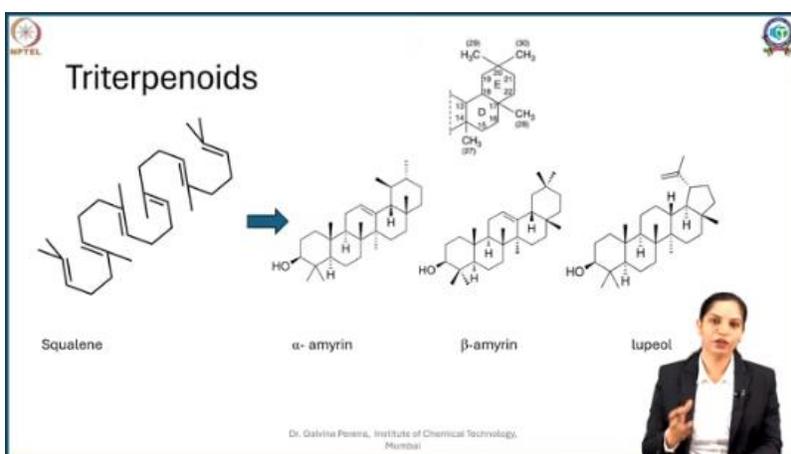


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Now, in this case, what happens is if I just join it here and I'll again get A, B, C, D, but this time D has six carbons and an additional ring which is an E ring. So in this case, your squalene molecule will give rise to compounds which are called pentacyclic triterpenoids.

So your steroidal triterpenoids as well as pentacyclic triterpenoids have their genesis from squalene and are made up of six isoprene units. Now

One good example of a triterpenoidal compound which has a steroidal nucleus is the damarine type of triterpenoids, which we see occurring in ginseng. Another set of triterpenoidal compounds features a five-ring system, known as pentacyclic triterpenoids. Now, there are numerous triterpenoids, and there are more than about a hundred categories structurally. But if we have to broadly classify them, the most common types are alpha-amyrin, beta-amyrin, and lupeol. So, what happens here is, as you can see, alpha-amyrin, beta-amyrin, and lupeol differ somewhat in their E ring. If you go by numbering,



We can have the same numbering: 3, 4, 5, 6, 7, 8, 9, 10. The one which we studied in steroids. So again, this is going to give you 11, 12, 13, 14, 15, 16, 17, 18. So here you are going to have your 18, and then onwards you will see your 19, 20, 21, 22, 23. Now, these substitutions—the methyl substitutions, especially on the A ring—you will see 23, 24.

Then you have your 25. Then you have your 26. Then you have your 27. This methyl and the last two methyl groups of the E ring get the numbers 29 and 30. So, you can see, all in all, we have 30 carbons accounted for.

Now, when the terminal methyl, the last methyl of the E ring, one of the methyl groups occurs at position 19 and the second methyl occurs at the 20th position, we call this set of compounds alpha myrin. But when your 29 and 30, Both of them occur simultaneously on a single carbon, and that is your carbon number 20. We call those compounds beta myrin. Now, a slight change to this is, rather than having a 6-membered ring, if we have a 5-membered ring, what happens here is you can see position 19.

But the 20th carbon—imagine I cut it from here. So the 20th carbon is kind of protruding out. So this is your 19, this is your 20, and again, imagine this to be your 21. So what happens here is again your 29 and 30 are located here. In this case, when you have a 5-membered ring, your 20th carbon is out with the substitutions—that is, the methyl substitutions at 29 and 30.

Such compounds are called lupeol derivatives. Now, you might have other derivatives also, depending upon how the ring cycle is, where the rings open, and also depending upon the nature of substitution, especially with regards to oxygen. Many of the triterpenoidal class are derived from it. So, we start with the first example of a triterpenoidal pentacyclic glycoside compound, and that is your licorice. Now, licorice or liquorice—there are two spellings to it.

The L-I-C-O-R-I-C-E is an American one, whereas L-I-Q-U-O-R-I-C-E is a British one. So, both of the spellings are correct. In the Indian system, we call it cheshtimad or yashtimadu. Now, this contains especially the underground parts. Underground parts include the roots as well as what you call the runners or the stolons.

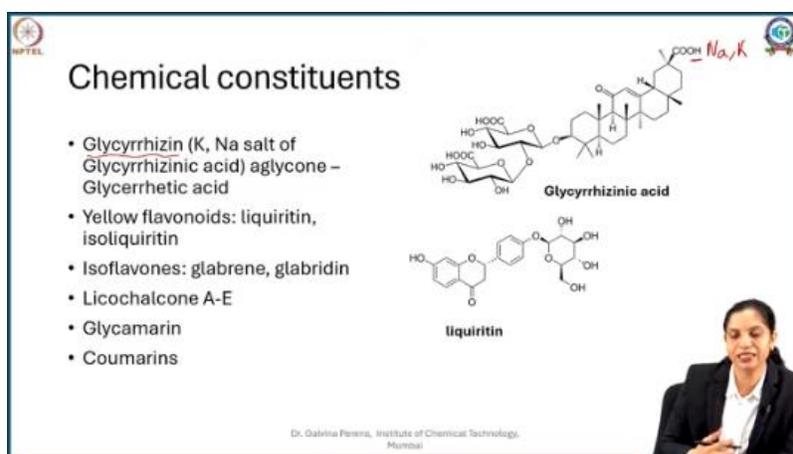
Both of them are cut and collected. So you might have the entire one, or you might have it cut. Now, in some cases, the outer peel—we call it cork—has been removed due to processing. In some cases, it is there. So you might have it peeled, unpeeled, fresh, dried, cut, or uncut.

So there are numerous species of licorice which exist. The most common ones in the market are *Glycyrrhiza glabra*, *Glycyrrhiza inflata*, or *Glycyrrhiza uralensis*. So these are the common ones and belong to the family Fabaceae. Now, most commonly, liquorice is cultivated and occurs in Russia, Iran. If you go to Middle Eastern countries, you will find a lot of these liquorice candies. It is also cultivated in Spain, Afghanistan, and India.

Now, the main ones, the main licorice of commerce are the Spanish and the Russian ones. So if you see Spanish, they are a little shorter or smaller with a length of about 14 to 20 centimeters and a diameter of about just five millimeters or 20 millimeters, like two centimeters max, with a reddish-brown cork. Whereas if you see the Russian variety, it is much thicker and The diameter may be as big as 5 centimeters as opposed to 2 centimeters, and the length is about 30 centimeters or so, about a foot long, and has a slightly purplish-colored cork. But if you break this, you might see internally a slight yellowish color pigment is present, and that's attributed to the compounds present in it.

So licorice contains triterpenoidal glycosides, especially pentacyclic triterpenoidal glycosides belonging to the ursane category. Here you have what is called a pentacyclic triterpenoidal compound called glycyrrhizinic acid. Now, in the plant, it occurs in salt form. So you might have a sodium compound. Potassium salt out here, and the sodium-potassium salt of glycyrrhizinic acid is called glycyrrhizin, so the most common

saponin, what we can say, is glycyrrhizin. Now, why am I calling it a saponin? So saponins are the ones which show surfactant-like properties. So if you observe this molecule carefully, you see a little polar entity out here made up of sugars, that is a disaccharide, and if you see here, here is a more nonpolar entity. So when you have a nonpolar entity with a polar head, they act like surfactants.



And if you put them in water, they tend to produce foam. So glycerin or glycerinic acid is basically saponin in nature and responsible for the foam produced when licorice is soaked in water. Now, if you cut out the sugars. You get the aglycon part, and the aglycon part is named as glyceritic acid. So glyceritic acid is the aglycon part of glycyrrhizinic acid.

Now, apart from that, we were just discussing licorice. When you cut it open, you see a slight yellow coloration, and that yellow coloration is attributed to flavonoids, which are slightly yellow in color, such as licoritin. Iso-licoritin, which is a kind of chalcone, isoflavones, glabrin, glabridin, licochalcones A2E. You also have compounds such as glycomerins and some coumarins present. So these are the ones which make up the chief composition of licorice. Now, you can check licorice by doing numerous chemical tests.

The first one is you can check if it contains the flavone derivatives, especially the chalcone derivatives. Now, when you take this plant or a section of it and then add about 80% sulfuric acid, you will see that initially this part of the plant, which is exposed to 80%

sulfuric acid, produces an orange-yellow coloration, and this happens because your flavones get converted into chalcones therein, and that gives you a nice coloration. yellow-orange coloration. The second one is because it contains flavonoids. You know, more than 20-30 flavonoids have been reported in this plant, and some papers cite more than 50 flavonoid derivatives being reported. So if you take this, and this is what the Shinoda test is all about. So if you take the alcoholic extract of this plant—that is, you take the plant powder and boil it in alcohol—and to that, if you add your magnesium turnings in the presence of concentrated hydrochloric acid.

Reduction of flavonoids take place and they get converted into anthocyanins. This anthocyanins are colored compounds and gives you color ranging from orange to pink depending upon the nature of flavonoids. So this is your Shinoda test and as you can see here this is the extract because of the yellow flavonoids present it's already showing yellow but when you treat it with magnesium turnings and concentrated hydrochloric acid you will see that the color intensifies changes to a little orange pink hue. And that indicates some anthocyanins have been formed during the process. And that's a Shenoda positive.

Whereas if you go to see the next test tube, that is what we call it as the saponin test. We discussed earlier your glycerin is a saponin. So if you just soak your licorice or boil your licorice and then gently shake it, you will see that it produces a foam. Now this foam despite keeping it for a period of 5 minutes also doesn't subside and that's what we call it as stable foam and this foam is stabilized by surfactants. What are surfactants here?

The surfactants here are your glycyrrhizine. OK, so because of the glycyrrhizin, you get a saponin test or foam test positive. Another test what you can do for saponins is a hemolysis test. So if you take a blood sample, this is not possible much to do in a chemical lab. But if you have access to blood samples, if you take blood samples and if you drop saponin test,

extracts or extracts containing saponin there that produces hemolysis that is your RBCs split open and that hemolysis test is also a test for saponins. Applications, licorice is being used or gestimus has been used in Ayurveda. It is known as a rasayanam or a rejuvenator. Licorice is used in candies for the reason the glycerin it has the saponin it has has a very sweet taste or a sweet flavor almost up to you know 100 to 150 times as that of your sugar that is your sucrose. So you get a derivative in market called as ammonium glycerin which is nothing but an ammonium salt of glycerin and that is also approved as a sweetener.

It has anti-inflammatory activities. It has demulcent activities. And as a result, you will see that in Ayurveda, even in your cough syrups, small amount of jeshtimad or licorice has been added. So it acts as a mild expectorant, as an anti-inflammatory agent. It helps you in dealing with the peptic ulcers.

Now, one more important thing about the licorice is it has what is called as mineralocorticoid or adrenocortical activity. Now what is this is in near your kidneys there are adrenal glands which secrete something called as cortisol. So in patients suffering from Addison's disease those are not produced and as a result the especially the sodium and the doesn't occur properly.

Now, in such cases, when you consume licorice beyond a particular dose, it acts as a mineralocorticoid, helping in water and sodium retention and, as a result, providing relief to the patients. It is also reported to have an antibacterial effect. It helps in dealing with chronic inflammation, and this property is attributed to the flavonoid derivatives, especially for arthritis, skin inflammation, and rheumatoid arthritis. Now, one more interesting finding is that in cosmetics nowadays, a derivative—a flavonoid present in licorice—is glabridin. It is studied to inhibit tyrosinase, the enzyme involved in melanin synthesis.

And since it stops melanin synthesis, there is a whitening effect observed in the skin. So, glabridin is used in skin-brightening creams. Now, licorice, because of its adrenocortical activities, needs to be taken with caution. It is known to alleviate. So, if you consume licorice for a longer duration of time, it can lead to what is called elevated blood pressure and hypokalemia.

So, these two things need to be considered with caution if you're consuming licorice on a long-term daily basis. Now, we move on to the next drug, and the next drug is a part of—if you've seen the prescription or the label of your vitamin supplement or many other immune modulators—you might come across this drug called ginseng. So, we'll study ginseng—what it is. So, ginseng is actually the root of a plant belonging to the genus *Panax*. Now

This is a huge genus, but many subspecies of it show very good immunomodulatory or adaptogenic effects. These include *Panax ginseng*, also referred to as Korean ginseng. *Panax notoginseng*, which is Chinese or South China ginseng. *Panax quinquefolius*, also referred to as American ginseng. They belong to the family Araliaceae, and their roots resemble human-like curves.

This resemblance is why they are believed to be beneficial for the body. If you're feeling tired, it helps you adapt to stress, making it a stress-relieving agent. So, what are the types of ginseng? In the market, numerous types are available. In China, you can find what is called fresh ginseng. Fresh ginseng is generally harvested from plants less than four years old and eaten fresh. It is simply cut and eaten. That is your white ginseng. It may also be peeled, dried, and eaten, resulting in white ginseng.

In some cases, it is heated or steamed. During heating or steaming, some extraneous compounds deteriorate, and some sugars caramelize, giving it a reddish outer coating. This is sold as red ginseng. Sometimes, it is also baked or puffed. You can also find puffed red ginseng in the market, which can be consumed like a snack. There is also sun ginseng, which is steamed white ginseng processed at much higher temperatures and pressures.

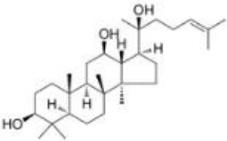
The last one is kaffir. Sort out Korean black ginseng. Now, what is done is this is the same red ginseng, but it has been steamed and cooled multiple times. So, some of them say about nine times it is steamed, cooled, dried, and this process instead of red, it now achieves an extraneous black coloration, and that is sold in the market as black ginseng. So, if you see the chemical composition,

The chemical composition of ginseng is more of a damarine type of compounds, what we studied, that is a steroidal triterpenoids. Now, in steroidal triterpenoids, this is your 10, and this is your 19. So, if you see with respect to 20, this 20, you get 20 R and 20 S isomers. So, it is said that your 20 R isomers of ginseng are called ginseng oocytes. Especially the glycosides of it are much, much more potent.

**Chemical composition**

- Ginsenosides (Saponins 20(R) and 20(S) isomers)- ginsenoside, chikusetsusaponin, panxoside etc.
- Dammarane-type saponins are generally classified into
  - Protopanaxadiol (PPD) type- saccharide(s) to C3 and/or C20
  - Protopanaxatriol (PPT) type- saccharide to C3, C6, and/or C20.
- Ginsenosides Rb1, Rb2, Re, and Rg1 are most common
- Gintonin.
- Polysaccharides (glycans) known as panaxans .

**(20S)-Protopanaxadiol**



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Now, this aglycone part is called protopanaxodial or protopanaxotriol. Now, protopanaxodial will have especially a diol at C3 and or at C20, whereas if you see your

protopanaxotriol, it will have an additional hydroxy here. This is your C6 position. So, you will have C3, you will definitely have your C20, but in addition, there will also be a hydroxy at your C6 position in addition to your protopanaxodial.

So you have protopanaxodial, protopanaxotriol, and to all these hydroxides, you will see your sugars being attached. Now, the sugars, when they are attached, will make the molecule more and more polar. So there is an interesting finding. Now, in naming the ginsenosides, what they did is, if you remember in your chromatography, there is a technique called TLC. So in TLC, you have something called a stationary phase and a mobile phase.

So assuming your normal TLC, your stationary phase is generally polar, and your mobile phase is generally nonpolar. So what happens here is when I spot my compounds in TLC, because my mobile phase is nonpolar, all my polar compounds stay back. And all my nonpolar compounds run with the mobile phase. So interestingly, because ginsenosides were many, the nomenclature came wherein R was your retention factor. So RA was the most polar one.

We even have R0, to be frank. And then you go on like RB, RC, RD, RE, and so on till RG. And then, depending upon the slight fractions, you had RA1, 2, RA3, RG1, and 2. So RA1 is the most polar, So having up to five sugars attached to it, whereas as you go up more and more nonpolar, the sugars decrease.

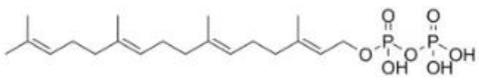
So when you come up to RG, you might just be left with one sugar attached to it. Apart from that, you have your genotonins and your polysaccharides, called panaxans. Now, where do you use it? Like I said, ginseng is used as a tonic and stimulant. It is an adaptogen; it helps you deal with stress and the noxious effects of many factors—physical, chemical, and biological—and it improves memory and intellectual skills.

It is also used in alcohol intoxication therapy because it's good for the liver. It's said to be liver-protective. And, as I said, our derivatives are more potent. So, 20 R-Panax ginsenosides have been used or studied extensively for cancer and therapies and chemoprevention.

All in all, ginseng is an excellent immunomodulatory, neuroprotective, cardioprotective, and anti-inflammatory agent. Moving on to the next set of compounds, which are diterpenes. These contain C20, meaning they contain five-carbon units. Here, you have

your four isoprene units. These four isoprene units join head-to-tail to form what are called diterpenes.

**Diterpenoids**



- Triterpenoids are C-20 derivatives
- Biosynthesized from condensation of 4 isoprene moieties

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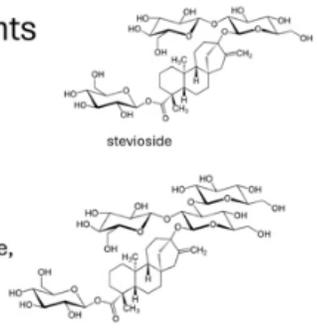


They might again, depending upon the enzyme, cyclize and give rise to various compounds. A good example of diterpene glycoside is a sugar derivative called a sugar substitute, known as stevia. Now, stevia is obtained from the leaves of a plant which belongs to the Asteraceae family, and that's your *Stevia rebaudiana*. Now, there are numerous species in that, but *Stevia rebaudiana* is thought to be the sweetest variety among them. So, stevia cultivation happens in South America, Paraguay, Brazil, and Japan.

Also, it started in India with, you know, our own stevioside-producing factories now in India. Now, the sweetness of stevia is due to different stevia glycosides. The aglycone, that is, this inner part, is referred to as steviol. And to this steviol, depending upon the number and nature of sugars attached, you have your stevioside, which you can clearly see has three sugars. You can see your rebaudioside.

**Chemical constituents**

- Stevia's sweetness is due to 30 different steviol diterpene glycosides, especially stevioside, rebaudiosides (from A to F and U,T,R,S), steviolmonoside, rubusoside, dulcoside A, and steviolbioside



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The steviol is the same; the sugars change, which has four sugars. And numerous stevioside derivatives have still been discovered from the plant. Apart from that, other derivatives include your steviomonoside and rebaudioside. Dulcoside and steviolbiosides. So these are also compounds which are diterpenoids and contribute to the sweetness of the plant, but the majority in terms of their content and sweetness are your stevioside and your rebaudiosides.

Now applications of steviocide is if you see countries like Japan, they are trying to replace their sugar completely by steviocides as a non-calorific alternative. So it is safe to give for people suffering from diabetes, high blood pressure and obesity where you really want to curtail the saccharide or the sugar intake. In terms of their sweetness, they can almost replace, if you see stevioside, it can replace like almost 150 to 300 times sugar. Or if you see rebaudiosides, they are still more sweeter and then can replace 250 to 400 times sugar. Now from this set of molecules, if you specifically pick up rebaudiocide M, that has been commercialized by companies such as Pure Circle and Coca-Cola.

Now rebaudiocide when studied in certain animal models are also reported to help increase the insulin production. So that's also a little advantage of stevia. So with this, we end our today's session on terpenoidal, especially the triterpenoidal and triterpenoidal glycosides. Here are a few references for your reading. And thank you everyone for your patient listening.

Thank you.