PHYTOCHEMISTRY AND ISOLATION OF GLYCOSIDES: AN OVERVIEW

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ABSRACT

A glycoside is an organic compound, usually of plant origin, and comprising a sugar portion linked to a non-sugar moiety in a particular manner. The molecule from which the non-sugar moiety of a glycoside is derived is called the aglycones or genin. The linkage between the sugar and the aglycones is a hemiacetal linkage formed by the reducing group (usually aldehydes or keto group) of the sugar and an alcoholic or phenolic hydroxyl group of the aglycon. Glycosides are important class of secondary metabolite which exhibits numerous important pharmacological actions. This review includes the classification and general methods of isolation of glycosides.

KEYWORDS: Glycosides, genin, secondary metabolites, classification, isolation.

INTRODUCTION

Plant as a source of medicine

Medicinal plants are those plants rich in secondary metabolites and are potential source of drugs. These secondary metabolites include alkaloids, glycosides, coumarins, flavonoids, steroids, tannins, volatile oil etc. A considerable number of definitions have been proposed in the term ‘medicinal plant’. According to WHO, a medicinal plant is any plant which in one or more of its organs or parts contain substances contain substances that can be used for therapeutic purposes or which are precursors of chemo pharmaceutical semi synthesis. Medicinal plants have many characteristics properties. The ingredients of plants produce some synergic effect which damages other pharmacological action. The medicinal plants helps in the treatment of complex cases like cancer diseases which are very effective. For
example vincristine and vinblastine from vinca and curcumin from turmeric. India is one of the few countries were almost all known medicinal plants can be cultivated in some part of the country. The ancient system of medicine make use of most of our native plants. For example Unani, homeopathy, Ayurveda. The uses of medicinal plants are seeds production of high yielding variety of seeds, nutraceuticals herbs or foods having more nutritional value.[1]

**Secondary metabolites in plants**
Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways. Secondary metabolites have shown to possess various biological effects, which provide the scientific base for the use of herbs in the traditional medicine in many ancient communities. They have been described as antibiotic, antifungal and antiviral and therefore are able to protect plants from various pathogens. Besides they constitute important UV absorbing compounds, thus preventing serious damage from the light. Secondary plant metabolites are classified according to their chemical structures into several classes. The classes of secondary plant metabolites include phenolics, alkaloids, saponins, terpenes, lipids, carbohydrates, glycosides.[2]

**Glycosides**
Glycosides are defined as any compound that contains a carbohydrate molecule that is convertible by hydrolytic cleavage into a sugar (glycone) and a nonsugar component (aglycone or genin). Examples include the cardenolides, bufadienolides, amygdalin, anthraquinones, and salicin. Saponins consist of an aglycone with a triterpenoid or steroid backbone linked to a carbohydrate molecule. This confers their ability to form soap-like foams in aqueous solutions.

Glycosides are formed in nature by the interaction of the nucleotide glycosides— for example, uridine diphosphate glucose (UDP-glucose)— with the alcoholic or phenolic group of a second compound. Such glycosides, sometimes called O-glycosides, are the most numerous ones found in nature. Other glycosides do, however, occur in which the linkage is through sulphur (S- glycosides), nitrogen (N-glycosides) or carbon (C-glycosides).

The wide range of toxicity among the cardiac glycosides resides in the complex interaction of the steroid nucleus, sugar moiety, and the lactone ring with ATPase. The steroid nucleus is key to the cardiac glycoside interacting with ATPase. Plant cardio toxin sterols differ from
mammalian sterols in the ring orientation. The difference is mostly in the orientation of the A ring to the B ring. In mammalian steroids, the A/B ring juncture is in a trans configuration. However, plant cardiac glycosides have an A/B ring juncture that is cis, and the two hydrogen are on the same side of the rings.\textsuperscript{17} The change of the A/B junction does not necessarily imply a decrease of activity of the steroid, although it does for the corresponding glycosides, indicating that the main influence of A/B junction arises from its ability to place the sugar into a suitable position. Although the fundamental pharmacologic activity of these plant toxins resides in the steroid nucleus, the sugar residues play an integral role in their activity. The sugar residue increases the water solubility of the steroid nucleus, making it more available for translocation into the myocardium. The lipophilic steroid nucleus is important in the compound's onset and duration of action. The presence of an acetyl group on the sugar moiety also affects the lipophilic character and the kinetics of the entire glycoside. In general, cardiac glycosides with more lipophilic character are absorbed faster and exhibit longer duration of actions as a result of slower urinary excretion rate. Lipophilicity is markedly influenced by the number of sugar residues and the number of hydroxyl groups on the aglycone part of the glycoside. As the steroidal rings are substituted with polar hydroxyl substitutes, the onset of action becomes faster and the duration decreases.\textsuperscript{18} Cardiac glycosides with monosaccharide sugar residues appear to have great potency, suggesting that only the first sugar molecule is involved in receptor binding.\textsuperscript{[3]}

**Classification of glycosides**

The glycosides can be classified based on the glycone, type of glycosidal linkage and by the aglycone.\textsuperscript{[3,4]}

**Classification On the Basis of Glycone**

If the glycone group of a glycoside is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide, etc.

![Figure 1: Glucuronide.](image-url)
1. Classification On the Basis of Glycosides

1. O-glycosides: Sugar molecule is combined with phenol or –OH group of aglycon, for example, Amygd-aline, Indesine, Arbutin, Salicin, cardiac glycosides, anthraxquinone glycosides like sennosides etc.

![Rhein-8-Glycoside](image)

**Figure 2: Rhein-8-Glycoside.**

2. N-glycosides: Sugar molecule is combined with N of the –NH (amino group) of agycon. For example, nucleosides which are the essential components of DNA, RNA, cofactors etc.

![Adenosine](image)

**Figure 3: Adenosine.**

3. S-glycosides: Sugar molecule is combined with the S or SH (thiol group) of aglycon, for example, Sinigrin.
4. **C-glycosides**: Sugar molecule is directly attached with C—atom of aglycon, for example, Anthraquinone glycosides like Aloin, Barbaloin, Cascaroside and Flavan glycosides, etc.

2. **On the Basis of Aglycone**

The various classes according to aglycone moiety are given below:

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Class of glycoside</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anthraquinone glycoside</td>
<td>Aloe, rhubarb, senna</td>
</tr>
<tr>
<td>2.</td>
<td>Cardiac glycosides or sterols</td>
<td>Digitalis, squill, thevetia</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin glycosides</td>
<td>Liquorice, dioscorea, ginseng</td>
</tr>
<tr>
<td>4.</td>
<td>Thiocynate and isothiocynate glycosides</td>
<td>Black mustard</td>
</tr>
<tr>
<td>5.</td>
<td>Flavone glycoside</td>
<td>Ginko, liquorice</td>
</tr>
<tr>
<td>6.</td>
<td>Cyanogenetic and cyanophoric glycosides</td>
<td>Bitter almond, wild cherry</td>
</tr>
<tr>
<td>7.</td>
<td>Aldehyde glycoside</td>
<td>vanillin</td>
</tr>
<tr>
<td>8.</td>
<td>Phenol glycoside</td>
<td>Bearberry</td>
</tr>
<tr>
<td>9.</td>
<td>Steroidal glycoside</td>
<td>Solanum</td>
</tr>
<tr>
<td>10.</td>
<td>Bitter or miscellaneous glycoside</td>
<td>Gentian, picrorrhiza, chirata</td>
</tr>
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</table>
General method of isolation of glycosides

The general method of extraction is the Stas-otto-process. The dried plant material is rendered into a moderately coarse powder. The powder is then extracted in a Soxhlet apparatus by continuous hot percolation with aqueous alcohol. The non-glycosidal impurities which get extracted along with glycosides are removed by precipitating them with lead acetate solution. The excess of lead acetate is then removed by passing hydrogen sulphide gas through the extract. Lead gets precipitated as lead sulphide, which is filtered out, the extracted solutes are collected. The glycosides are purified from the crude extract using fractional solubility, fractional crystallization and chromatographic techniques. The isolated purified compound is characterized by UV, visible, IR, mass, NMR spectra and elemental analysis.[5,6]

Isolation of Cardiac glycosides

The drug is pulverized and extracted with 50% ethanol at low temperature, followed by the addition of lead acetate solution to remove the impurities, the precipitates are removed by centrifugation, the cardiac glycoside present in the supernatant are extracted with chloroform, the chloroform extract is evaporated under vacuum and the residue (cardiac glycoside) left behind is further purified by chromatography.[7,8]

Isolation of Flavones and Flavonols

The plant material containing flavones or flavonols is extracted with boiling water and the tannins are removed as lead salts by means of lead acetate the filtrate is diluted with water, acidified with HCl and boiled for some hours when the sugar free flavones or flavonols are precipitated.

They are extracted with alcohol and may be purified by fractional crystallization of their acetates or by re-crystallization from some organic solvents like benzene, carbon disulphide,
alcohol, etc.

**Isolation of Anthraquinone glycosides**

A method was developed for extracting anthraquinone aglycones and their corresponding glycosides from plant materials and for separating them in pure form. The method consists of rendering the glycosides and aglycones insoluble in chloroform and then removing, stepwise, the interfering extractable substances. The method makes use of the fact that the aglycones are chloroform soluble whereas the glycosides are not. The method was applied with good results to the isolation of the aglycones and glycosides of cascara and senna.⁹

![Figure 7: Anthraquinone glycosides.](image)

**Isolation of Saponin glycosides**

A new furostanol pentaoligoside and spirostanol tetraoligoside were isolated for the first time from yam tubers (Dioscorea pseudojaponica Yamamoto) from Taiwan, together with four known yam saponins, methyl protodioscin, methyl protogracillin, dioscin, and gracillin. The structural identification was performed using LC-MS and 1H and 13C NMR. The methanol extract of yam tubers was fractionated by XAD-2 column chromatography using a methanol/water gradient elution system to yield furostanol and spirostanol glycoside fractions. Preparative high-performance liquid chromatography, employing a C18 column and a mobile phase of methanol/water, was used to separate each furostanol glycoside, whereas a mobile phase of methanol/water was used to resolve the individual spirostanol glycosides. The conversions from steroid saponins to diosgenin after acid hydrolysis were around 68 and 90% for furostanol and spirostanol glycosides, respectively.¹⁰
Phenolic compounds constitute a major class of plant secondary metabolites that are widely distributed in the plant kingdom and show a large structural diversity. These compounds occur as aglycones or glycosides, as monomers or constituting highly polymerized structures, or as free or matrix-bound compounds. Furthermore, they are not uniformly distributed in the plant and their stability varies significantly. This greatly complicates their extraction and isolation processes, which means that a single standardized procedure cannot be recommended for all phenolics and/or plant materials; procedures have to be optimized depending on the nature of the sample and the target analytes, and also on the object of the study. Main techniques for sample preparation, and extraction and isolation of phenolic compounds include classical solvent extraction procedures to more modern approaches, such as the use of molecularly imprinted polymers or counter-current chromatography.\cite{11,12}

CONCLUSION

A glycoside is an organic compound, usually of plant origin, and comprising a sugar portion linked to a non-sugar moiety in a particular manner. The molecule from which the non-sugar moiety of a glycoside is derived is called the aglycones or genin. The linkage between the sugar and the aglycones is a hemiacetal linkage formed by the reducing group (usually aldehydes or keto group) of the sugar and an alcoholic or phenolic hydroxyl group of the aglycon. Numerous methods for isolation of glycosides are present. Glycosides are important class of secondary metabolite which exhibits numerous important pharmacological actions.

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