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Review Article

Plant cyanogenic glycosides: an overview

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ABSTRACT

The cyanogenic glycosides have consisted of α-hydroxy nitrile aglycone and a sugar moiety that producing hydrogen cyanide (HCN) during the hydrolysis process. This HCN-producing property underlines the toxicity of the plants commonly associated with these compounds in humans and animals. However, new evidence shows that the toxicity of these compounds is lower than those theoretically caused by the equivalent released HCN. In order to provide a deeper understanding of cyanogenic glycosides, an overview of these compounds is given in this article. It covers the chemistry, distribution, biosynthesis, toxicity, determination, as well as extraction and isolation of the compounds.

1. INTRODUCTION

Cyanogenic glycosides are glycosides of α-hydroxy nitriles composed of aglycone and sugar parts. The most commonly occurred sugar moiety is D-glucose and is followed by gentiobiose. The aglycone part of cyanogenic glycosides can be aliphatic or aromatic substituents. Triglochinin, sutherlandin, linamarin, and lotaustralin are the examples of the first-mentioned group, while dhurrin, prunasin, sambunigrin, and amygdalin are the representatives of the later. Most of the cyanogenic glycosides, such as dhurrin, prunasin, linamarin, and lotaustralin have a ß-glycosidic linkage between the aglycone and D-glucose, but some other compounds, including amygdalin and linustatin, possess the second sugar moiety attached by ß-1,6 linkages. The structures of some representative of cyanogenic glycosides are shown in Figure 1 (Vetter, 2017).

The glycosidic bond in cyanogenic glycosides can be hydrolyzed by a two-step enzymatic process utilizing β-glucosidases and hydroxy nitrile lyases. The hydrolyzing enzyme of a given cyanogenic glycoside is synthesized by the same plant but is stored in a different location. Cyanogenic glycosides are stored in the vacuoles and mainly in leaf tissues, while that of β-glucosidase enzymes are in the apoplastic space, and commonly attached to cell walls, in the cytoplasm, vesicles, or chloroplast. Besides, the hydroxy nitrile enzymes are accumulated in the cytoplasm. Once plant tissue is disrupted, the cyanogenic glycosides and enzymes are brought in contact, and cyanogenic glycosides are degraded into cyanohydrins, hydrogen cyanide (HCN), and ketones (Vetter, 2017).

For example, when bitter almond (*Prunus dulcis* (Mill.) D.A.Webb, Rosaceae) kernels are crushed, amygdalin will come into contact with amygdalin hydrolase and release one of its D-glucose moiety. The resulted prunasin molecule will further react with prunasin hydrolase and release the remained D-glucose as well as produce mandelonitrile. Mandelonitrile is a benzaldehyde cyanohydrin than eventually will be degraded into benzaldehyde and HCN with the presence of mandelonitrile lyase (Dewick, 2009). Cyanogenic glycosides can also be hydrolyzed by enzymes produced by microorganisms residing inside the rumen of mammals when they eat

the compound-producing plants. A study reported that amygdalin, prunasin, and linamarin were hydrolyzed at different rates by various pure cultures of rumen bacteria (Majak & Cheng, 1987).

2. OCCURRENCE AND DISTRIBUTION OF CYANOGENIC GLYCOSIDES

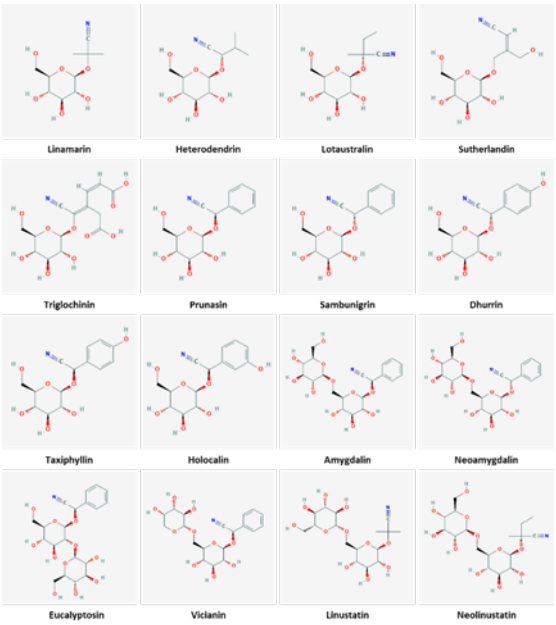
Cyanogenic glycosides commonly occur in families of Euphorbiaceae, Leguminosae, Linaceae, Myrtaceae, Poaceae, and Rosaceae (**Table 1**). The genera that are often connected with the presence of these compounds are including *Sambucus*, *Triglochin*, *Linum*, *Eucalyptus*, *Bambusa*, *Sorghum*, *Prunus*, and *Malus* (Panter, 2018; Vetter, 2017). Some cyanogenic glycosides-containing plants are used as traditional medicine. For example, bitter almonds and peach (*Prunus* spp., Rosaceae) contain amygdalin, prunasin, and sambunigrin. Both plants are traditionally used as antitussive in Chinese folk medicine for the small quantities of HCN generated from their kernels (Bone & Mills, 2013).

However, cyanogenic glycosides-containing plants are more popular for their toxicity. Many reports are mentioning that these compounds are responsible for multiple disease conditions both in animals and people. *Prunus* spp. (wild cherry, black cherry, chokecherry; Rosaceae), *Sambucus spp.* (elderberry, Adoxaceae), *Linum* spp. (flax, Linaceae), *Sorghum* spp. (sorghum, Poaceae), *Triglochin* spp. (arrow grass, Juncaginaceae), *Manihot* spp. (cassava, Euphorbiaceae), and *Bambusa* spp. (bamboo, Poaceae) are examples of plants known for causing poisoning in animals and humans (**Table 1**).

3. BIOSYNTHESIS OF CYANOGENIC GLYCOSIDES

Amino acids are the precursors in the cyanogenic glycosides biosynthesis. Phenylalanine is the precursor of prunasin, sambunigrin, and amygdalin, while dhurrin and taxiphyllin are derived from tyrosine. Valine, isoleucine, and leucine provide backbones for linamarin, lotaustralin, and heterodendrin, respectively. The biosynthetic reaction of these compounds is divided into three main steps. The first one, the amino acid precursor molecules, are converted to aldoxime intermediates catalyzed by an enzyme from cytochrome P450 family. In the next step, aldoxime molecules are converted into cyanohydrins by a second member of the cytochrome P450 enzyme family. Lastly, the cyanohydrins are glycosylated by an UDP-glucosyltransferase to form cyanogenic glycosides (Dewick, 2009; Vetter, 2017).

For example, the precursor for dhurrin is tyrosine, which is subsequently N-hydroxylated by and CYP79A1 to form N.N dihydroxy-L-tyrosine. Through decarboxylation–elimination with the same enzyme, it is further converted into E-aldoxime, which is later undergoing a non-enzymatic trans-cis isomerization into Z-aldoxime. The second enzyme, CYP71E1, dehydrates the oxime to form 4-hydroxyphenyl acetonitrile. The same enzyme proceeds a further reaction by oxidizing it and produce (S)-4-Hydroxymandelonitrile. At the final step, dhurrin (Figure 1) is formed when D-glucose is incorporated into the molecule by utilizing 4-hydroxymandelonitrile-O-glucosyltransferase (Dewick, 2009). However, the details of all enzymes and reactions involved in the



Figures 1. The prominent cyanogenic glycosides (PubChem, 2019).

Table 1. The plants are containing cyanogenic glycosides and their respective released HCN .

Scientific Name	Common Name	Family	Cyanogenic Glycoside Type	HCN released (mg/100 g Plant)	References
Sambucus spp.	Elder berry	Adoxaceae	Sambunigrin, prunasin, holocalin	-	(Dellagreca, Fiorentino, Monaco, Previtera & Simonet, 2000)
Manihot esculenta Crantz	Cassava	Euphorbiaceae	Linamarin, lotaustralin	50-400	(Tivana et al., 2014)
Phaseolus lunatus L.	Lima beans	Leguminosae	Linamarin, lotaustralin	10-400	(Shlichta, Glauser & Benrey, 2014)
Vicia spp.	Common vetch	Leguminosae	Vicianin, linamarin	-	(Ahn, Saino, Mizutani, Shimizu & Sakata, 2007)
Trifolium repens L.	White clover	Leguminosae	Linamarin, lotaustralin	-	(Olsen, Sutherland & Small, 2007)
Triglochin spp.	Arrowgrass	Juncaginaceae	Triglochinin	77	(Eyjólfsson, 1970)
Linum spp.	Flax	Linaceae	Linamarin, linustatin, neolinustatin	53	(Waszkowiak et al., 2015)
Eucalyptus spp.	Eucalyptus	Myrtaceae	Prunasin, sambunigrin, eucalyptosin	-	(Gleadow et al., 2008)
Bambusa spp.	Bamboo	Poaceae	Taxiphyllin	300-800	(Choudhury, Sahu & Sharma, 2012)
Sorghum spp.	Sorghum	Poaceae	Dhurrin	10-240	(Aikman, Bergman, Ebinger & Seigler, 1996)
Prunus spp.	Peaches, apricot, and almonds	Rosaceae	Amygdalin, prunasin, sambunigrin	125-160	(Campa et al., 2000)
Malus spp.	Apple	Rosaceae	Amygdalin, prunasin, neoamygdalin, and sambunigrin	50-90	(Bolarinwa, Orfila & Morgan, 2015)

biosynthesis of cyanogenic glycosides in most plants are not clearly understood (Sun et al., 2018)

4. TOXICITY OF CYANOGENIC GLYCOSIDES

Cyanogenic glycosides are considered as part of the components of the defense system of their respective producing plants. HCN released during their decomposition is toxic for humans and grazing animals. Hence, the pharmacology aspect of cyanogenic glycosides is usually associated with their toxicity. Once cyanogenic glycosides are ingested, the free HCN is readily absorbed through the gut and lungs. The cyanide ion has a strong affinity to bind with the trivalent iron component of the cytochrome oxidase molecule and further prevents cellular respiration. A characteristic of cyanide poisoning in animals is the cherry red venous blood. This occurs when oxygen saturated hemoglobin cannot release O2 at the tissue level because the cytochrome oxidase is inhibited. In most animal species, the acute lethal dose of HCN ranges 2-2.5 mg/kg body weight, with an exception on pandas (Panter, 2018).

Oral intake of cyanogenic glycosides does not always result in a toxic event, especially if it is in the short-term. HCN is slowly released upon hydrolysis of the compounds in the digestive tract or by hepatic enzymes, which is readily detoxified by the body. Ingestion of oral amygdalin to humans at 500 mg three times a day produced no toxic effects and only moderately raised blood cyanide levels. The β-glucosidase, the compounds-hydrolyzing enzyme, is inactivated by the acidity of the gastric, and hence the released HCN from the compounds is decreased as they pass through to the ileum. The absorbed HCN is further distributed to the somatic cells, where it is transformed into thiocyanate, a harmless metabolite, by the rhodanese enzymes (Bone & Mills, 2013; Schulz, Hansel, Blumenthal & Tyler, 2004).

A crossover study involving 12 healthy adults confirmed that the bioavailability of HCN in whole blood after consumption of cyanogenic glycosides-containing foods was varied. Foods from plant materials that necessarily fast and completely destructed and have a highly specific β-glucosidase, including bitter apricot kernels and fresh cassava roots, might maximally release HCN and hence result in the high bioavailability. For

food with isolated cyanogenic glycosides, for example, persipan paste prepared from debittered apricot kernels, the inactivated or missing specific β-glucosidase leads to a much lower bioavailability (Abraham, Buhrke & Lampen, 2016). In general, numerous studies demonstrated that the toxicity of cyanogenic glycosides is less than that suggested by their theoretical HCN equivalent (Cressey & Reeve, 2019).

However, chronic intoxication may take place when cyanogenic glycosides are ingested for a long time, especially if the compound-containing plants are part of daily food. The accumulation of rhodanide and cyanocobalamine that are produced during the metabolism of the compounds may be related to neurotoxic syndromes. The tropical ataxic neuropathy in Nigeria and konzo, an endemic upper motor neuron disease in Africa, are considered as the result of chronic intoxication of cyanogenic glycosides (Bone & Mills, 2013).

5. DETERMINATION OF CYANOGENIC GLYCOSIDES

There are two methods for qualification and determination of cyanogenic glycosides, and they are direct and indirect ones. The direct method uses cyanogenic glycosides as the target molecules, while the latter addresses the released HCN after hydrolysis.

High-Performance Liquid Chromatography (HPLC) has been the mainstay for the direct determination of cyanogenic glycosides. A column with porous graphitic carbon and also C-18 ones are commonly used, altogether with various mobile phase systems: methanol/water (9:1), water/methanol (80:20), or gradient of water and acetonitrile. Ultrahigh-Pressure Liquid Chromatography (UPHLC) and Gas-Liquid Chromatography (GLC) analysis were also successfully developed for the determination of these compounds (Vetter, 2017). Another direct determination of cyanogenic glycosides utilized 1H nuclear magnetic resonance (NMR), which was found to be a very suitable tool to perform analysis of cyanogenic glycosides in flax seeds (Roulard et al., 2017). Micellar Capillary Electrophoresis (MEKC) was also developed for the separation of amygdalin, prunasin, neoamygdalin, and sambunigrin from seeds of peach and apple. Excellent and rapid separation of those four cyanogenic glycosides, as well as the detection of prunasin and amygdalin in peach seeds, could be obtained with this method (Campa et al., 2000).

The most common indirect determination of cyanogenic glycosides is the Guignard sodium picrate test. It detects HCN released from fresh plant material when the glycosides are hydrolyzed. Toluene is added to the samples for damaging the cuticle and permitting the passage of HCN to the outside of the cells. The HCN then reacts with sodium picrate absorbed by the paper and turns its color from yellow to red (Müller-Schwarze, 2009). Chemosensors are gradually developed for the identification of HCN released from hydrolyzed cyanogenic glycoside. The hydrolytically released HCN from fresh cassava reacts with the aquacyanocobyrinic acid (ACCA, a derivative of vitamin B12), producing dicyanocobyrinic acid (DCCA). The presence of this reaction is marked by a color change from orange to violet. This reaction takes place within seconds and does not interfere with different anions or other biological molecules of the plant sample (Tivana, Da Cruz Francisco, Zelder, Bergenståhl & Dejmek, 2014).

6. EXTRACTION AND ISOLATION OF CYANOGENYC GLYCOSIDES

Since of cyanogenic glycosides are considered as anti-nutrients, in the food science area, extraction of plants associated with these compounds are mainly for optimizing the composition of their beneficial compounds and minimizing cyanogenic glycosides in the obtained extract. Aside from the content of phenolic compounds and their antioxidant activity, the utilization of water or 60 % ethanol for the extraction of flaxseed significantly affected the content of cyanogenic glucosides in their extract. These compounds were much lower in aqueous extract than in ethanolic extract (Waszkowiak, Gliszczynska-Swiglo, Barthet & Skrety, 2015).

However, in phytochemistry, a natural approach is used to isolate cyanogenic glycosides. In general, they are isolated from plant materials by grinding, with subsequent or concomitant extraction with solvents such as ethanol, methanol, water, or mixtures thereof. The mixture is heated to boiling for several minutes to deactivate enzymes. The slurry is filtered and centrifuged to remove particulate material. The extraction can be performed at -80oC to avoid problems of enzymatic and possible thermal, followed by filtration at room temperature. The solvent is then removed under vacuum and the remained extract is re-dissolved in water. Any precipitate which may occur at this stage should be removed by filtration. The aqueous solution should be extracted with light petroleum or CHCl3 to remove lipids. The extract can be treated with lead acetate, followed by hydrogen sulfide to remove undesirable acidic components. Phenolic compounds can be removed by chromatography on the

polyvinylpyrrolidone column, while many acidic, essential, and ionic impurities can be eliminated with mixed bed ion exchange resins or a combination of acidic and basic ion exchange resins. The isolation can be performed with continuous liquid-liquid extraction using ethyl acetate, paper chromatography using various solvent systems, or column chromatography on cellulose, silica gel, kieselgel, or florist stationary phase (Seigler, 1975).

7. CONCLUSIONS

Cyanogenic glycosides are considered as an anti-nutrient for their capacity releasing the poisonous HCN during hydrolysis. These compounds are mainly occurred in dicots, particularly in Euphorbiaceae, Leguminosae, Linaceae, Myrtaceae, Poaceae, and Rosaceae; originated from amino acid precursor and biosynthesized through a three-step enzymatic process. The toxicity of cyanogenic glycosides in mammals is considered lower than their theoretical HCN equivalent since they are readily metabolized, and hence severe disease usually only shown after ingestion in high doses and/or in a long time. The presence of these compounds in crude drugs and foods can be qualitatively determined directly using various modern techniques or indirectly by evaluating the released HCN. The use of subsequent liquid-liquid extractions and chromatographic methods are useful for conducting the extraction and isolation of these compounds.

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