CHAPTER II

HISTORICAL

Presence of Compounds in Moringa pterygosperma Gaertn.

Chemical constituents isolated from Moringa pterygosperma were reported in NAPRALERT database as benzenoid, steroid, proteid, S-compound, alkaloid, lipid, flavonoid, coumarin, triterpene, vitamin, carotenoid, carbohydrate. List of the compounds found in various part of this plant are shown in Table 1.

Table 1 Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Benzenoids		
4-OH phenylacetic acid,	roasted seed	Villasenor,. *et al.* 1989
4-OH phenylacetonitrile,	roasted seed	Villasenor,. *et al.* 1989
4-OH phenylacetamide,	roasted seed	Villasenor,. *et al.* 1989
4-(α-L-Rhamnosyl-oxy) phenyl	roasted seed	Villasenor,. *et al.* 1989
acetonitrile,	seed	Dayrit, *et al.* 1990
Moringyne	seed	Memon., *et al.* 1985
Vanillin	seed	Saluja, *et al.* 1978
Moringine	root bark	Chopra, *et al.*, 1932.
	bark	Ghosh,*et al.*, 1935.
Proteids		
Alanine	seed	Tandon,. *et al.* 1967
Phenylalanine	seed	Tandon,. *et al.* 1967

Table 1 (continued) Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Hydroxyproline	Seed	Tandon,. *et al.* 1967
Arginine	Seed	Tandon,. *et al.* 1967
Histidine	Seed	Tandon,. *et al.* 1967
Leucine	Seed	Tandon,. *et al.* 1967
Proline	Seed	Tandon,. *et al.* 1967
Serine	Seed	Tandon,. *et al.* 1967
Threonine	Seed	Tandon,. *et al.* 1967
Valine	Seed	Tandon,. *et al.* 1967
Thiocarbamates		
Niazimicin	Leaf	Faizi, *et al.*, 1992
Niaziminin A	Leaf	Faizi, *et al.*, 1992
Niaziminin B	Leaf	Faizi, *et al.*, 1992
Niazinin A	Leaf	Faizi., *et al.*, 1992
Niazinin B	Leaf	Faizi., *et al.*, 1992
Nitrile glycosides		
Niazirin	Leaf	Faizi, *et al.*, 1994
Niazirinin	Leaf	Faizi, *et al.*, 1994
Flavonoids		
Gossypitin	Leaf	Daniel, 1989
Quercetin, 3′-methoxy	Leaf	Daniel, 1989
Quercetagetin	Leaf	Daniel, 1989

Table 1 (continued) Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Quercetin	Flower	Pankadamani, Seshadri, 1952
Kaempferol	Flower	Nair, Sankara, 1962
Quercetin-3-glycoside	Flower	Nair, Sankara, 1962
Rhemnetin	Flower	Nair, Sankara, 1962
Rhamnetin-3-glycoside	Flower	Nair, Sankara, 1962
Rutin	Leaf	Shaft, Ikram, 1982
Triterpene		
Baurenol	Bark	Mohamed, *et al.*, 1965
Coumarin		
4-OH mellein	Stem	Saluja, *et al.*, 1978
Steroid		
β-sitosterol	Stem bark	Kinel, and Gedeon, 1957
Sulfur Compounds		
4-(α-L-Rhamnosyl-oxy)benzyl Glucosinolate,	Seed	Villasenor, *et al.*, 1990
Pterygospermin	Root	Raghunandana,Mariam,. 1949
	Seed	Badgett, 1964
	Bark	Kurup, Narasimha,., 1954
4-(α-L-Rhamnosyl-oxy-benzyl) isothiocyanate	Entire plant	Das, Rao, 1958
Allyl isothiocyanate:	Entire plant	Das, Rao, 1958

Table 1 (continued) Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Isobutyl isothiocyanate:	Entire plant	Das, Rao, 1958
Benzyl isothiocyanate:	Entire plant	Das, Rao, 1958
	Root	Eilert, *et al.*, 1981
(:4-(α-L-Rhamnosyl-oxy)benzyl isothiocyanate:	Seed root	Eilert, *et al.*, 1981
4-(α-L-Rhamnoside) benzyl isothiocyanate:	Seed	Eilert, *et al.*, 1981
4-[(4′-*O*-acetyl-α-L-Rhamnosyloxy) benzyl isothiocyanate	Leaf	Faizi, *et al.*, 1994
O-methyl,4-[2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl] thiocarbamate)(E)	Leaf	Faizi, *et al.*, 1995
O-methyl,4-[2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl] thiocarbamate)(Z)	Leaf	Faizi, *et al.*, 1995
O-ethyl,4-[2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl] thiocarbamate)(*Z*)	Leaf	Faizi, *et al.*, 1995
Lipid		
Arachidic acid	Seed oil	Sengupta, Gupta,. 1970
Behenic acid	Seed oil	Sengupta, Gupta,. 1970
Pamitic acid	Seed oil	Sengupta, Gupta,. 1970
Myristic acid	Seed oil	Sengupta, Gupta,. 1970
Linoleic acid	Seed oil	Sengupta, Gupta,. 1970
Lignoceric acid	Seed oil	Sengupta, Gupta,. 1970
Oleic acid	Seed oil	Sengupta, Gupta,. 1970
Stearic acid	Seed oil	Sengupta, Gupta,. 1970

Table 1 (continued) Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Palmitic acid	Seed oil	Khan, *et al.*, 1975
Lauric acid	Seed oil	Ferrao,. and Ferrao, 1970
Pentadecanoic acid	Seed oil	Ferrao, and Ferrao., 1970
Octacosanoic acid	Stem	Saluja, *et al.*, 1978
Inorganic compounds		
Potassium	Flower	Rangaswami, Sankarasubramanian, 1946
Calcium	Flower	Rangaswami, Sankarasu-bramanian, 1946
	Fruit	Basu, Ghosh, 1943
Phosphorus	Fruit	Basu, Ghosh, 1943
Sulfur	Root	Kurup, Narasima Rao, 1952
Carbohydrates		
Gum	Flower	Bhattacharya, *et al.*, 1982
Starch	Leaf	Rao, *et al.*, 1979
Carotenoid		
β-carotene	Fruit	Ortaliza, *et al.*, 1969
Vitamins		
Ascorbic acid	Leaf, Fruit	Ramachandran, *et al.*, 1980
Vitamin A	Leaf, Fruit	Ramachandran, *et al.*, 1980
Vitamin B_1	Leaf, Fruit	Ramachandran, *et al.*, 1980
Vitamin B_2	Leaf, Fruit	Ramachandran, *et al.*, 1980
Nicotinic acid	Leaf, Fruit	Ramachandran, *et al.*, 1980
Choline	Leaf	Ramachandran, *et al.*, 1980

Table 1 (continued) Occurrence of chemical compounds in *Moringa pterygosperma* Gaertn.

Compound	Plant part	References
Miscellaneous		
Oxalic acid	leaf, fruit	Ramachandran, *et al.*, 1980
Amylase	Seed	Rao, *et al.*, 1979
Ascorbic acid oxidase	Fruit	Spruyt, 1940

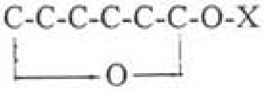
Glycoside

Glycosides are organic compounds in which widely distributed in nature, especially in the plant kingdom. Glycosides play an important role in the life of the plant and are involved in its regulatory, protective, and sanitary functions. Among such a widely variety of compounds one finds many therapeutically active agents. In fact, the group contributes to almost every therapeutic class. Other name of glycosides is Heterosides. (Farnsworth, N.R. 1966)

Chemically, they are the acetal derivatives of carbohydrate. The hemiacetal hydroxyls in their cyclic pyranose or furanose structures, can be substituted with a variety of alkyl or aromatic residues by a hydroxyl group of the nonsugar component and the secondary hydroxyl is condensed within the sugar molecule itself to form an oxide ring. The ether linkages formed between these hemiacetal hydroxyls and the various kind of residues are known as glycosidic linkages. More simply, glycosides may be considered as sugar ethers. These linkages usually connects the anomeric carbon of oligosaccharides containing one or more kinds of monosaccharides constituents (glycone) with an alcohol or phenolic hydroxyl of a second nonsugar molecule (aglycone) or a genin. This type of linkage give rise to the so-called O-glycosides, the most common type of glycoside found in plants. If the anomeric carbon of the glycone is attached to an aglycone though sulfur, the S-glycosides are formed. A third group are the N-glycosides which involve attachment of the glycone to an amino group of an aglycone. Finally the C-heterosides involve a carbon to carbon linkage of glycone and aglycone. The formation of an O-glycoside may be represented :

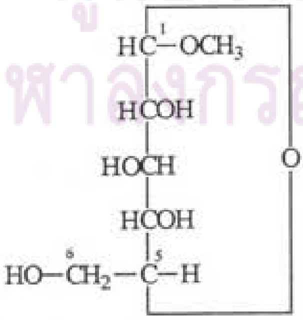
$R\text{-}OH + HOX \leftrightarrow R.OX + H_2O$ eg.

sugar aglycone glycoside

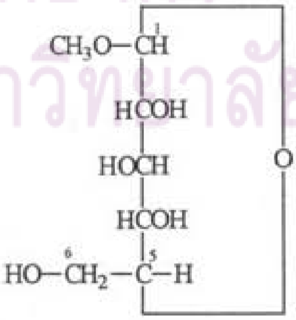

$C\text{-}C\text{-}C\text{-}C\text{-}C\text{-}C\text{-}O\text{-}X$

As regards the carbonhydrate constituents in glycosides, hexose and pentose are generally known. A special examples, deoxy-sugars and O-methyl sugars have been identified as the carbohydrate constituents of cardiac glycosides, and D-apiose, a branched carbohydrate is an uncommon carbohydrate constituents of oligosaccharide. But several glycosides are known where the sugar moiety is not a true sugar, but a derivative such as uronic acid (Evans, 1989)

Structure, since sugars exist in isomeric α- and β-forms, both types are theoretically possible. Two series of stereoisomeric glycosides are known, the α- and β-glycosides, depending on the stereoconfiguration of the glycosidic linkage. Practically all natural glycosides, however, are of the β-type, although the α-linkage is found in nature in some carbohydrates such as sucrose, glycogen and starch. Taking the methyl-D-glucosides as a simple exam, they are represented by



α-Methyl-D-glucoside



β-methyl-D-glucoside

The glycosidic linkage is formed by dehydration involving a hydroxyl gr. of the aglycone (methanol here) and the hydroxyl group on the hemiacetal carbon of the sugar, thus forming an acetal type of structure if the OR (in the above case, OCH_3) group is in the same steric sense as the $-CH_2OH$ group on C-5 (for D-family sugars), the glycoside configuration is designated β; if in the opposite steric sense, it is designated as α (Gennaro,1990)

Localization and Distribution of Glycosides

Glycosides are distrubuted widely in the plant kingdom. They not only occur in Angiospermae, certain families of which (Apocynaceae, Rhamnaceae) are particulary rich sources, but also in lower plants as shown by the complex N-glycosides in *Streptomyces* species. Glycosides of animal origin are relative rare. Since the discovery of salicin (salicoside) in the bark of the willow in 1830, many hundreds of glycosides have been isolated. And now they are found in all parts of the plant roots, bark, leaves, flowers (Anthocyanins), fruits and seeds.

They are generally present in the vacuole, and are sometimes localized in particular cells. They often show large variation in concentration during the growth of the plant (hydrolysis during germination, disappearance after flowering, accumulation in leaves or bark, and occasionally seeds). Their concentration often diminishes or they disappear completely, leaving only aglycones, due to enzyme action during drying of plant tissue; these phenomena greatly complicate the study of their distribution in plants(Swain, 1963:Gennaro, 1990).

Nomenclature

The term 'glycoside' is a very general one which comprises all the many and varied combinations of sugars and aglycones. More precise terms are available to describes particular classes. Some of these terms refer to the sugar part of the molecule, others to the aglycone, while others indicate some well-defined physical or pharmacological property. Thus, a glucoside is a glycoside having glucose as its sole sugar component; a pentoside yields a sugar such as arabinose; rhamnosides yield the methyl-pentose rhamnose; and rhamnoglucosides yield both rhamnose and glucose.

Total classification on the basis of the aglycones, while feasible, is intricate because of the large variety of aglycones; however, with certain classes of glycosides, e.g., phenol, anthraquinone and sterol glycosides. The older system of naming glycosides using the termination '-in', e.g. senegin, salicin, aloin, strophanthin, is too well established to be easily changed. Those suffixes which is often used, should be reserved for nitrogen-containing compounds, and to avoid confusion with alkaloids, it is recommended that the suffix '-oside' be used instead, e.g. sennoside, the rest of the name being related to the botanical origin of the substance in question (e.g. aucaboside (aucubin) from *Aucuba japonica*, franguloside (frangulin) from *Rhamnus frangula*) and so on. However, compounds whose names have been well established, such as digitalin ouabain, strophanthin, should retain such names. When the same glucoside has been isolated from several plants, it should be denoted by the earliest given name; for example, strophanthin g would better be called ouabain (Swain,1963).

Biosynthesis of Glycosides

Consideration of glycoside biosynthesis necessarily consists of 2 parts. The general reactions couple a sugar residue to an aglycone. Presumably this transfer reaction is similar in all biologic systems. This contrasts with the pathways for biosynthesis of the various types of aglycones, which tend to be diverse and must be considered individually.

Available evidence indicates that the principal pathway of glycoside formation involves the transfer of a uridyl group from uridine triphosphate to a sugar

1-phosphate. Enzymes catalyzing this reaction are refered to as uridylyl transferase (1) and have been isolated from animal, plant, and microbial sources. Phosphates of pentoses, hexoses, or various sugar derivatives may participate. The subsequent reaction, imediated by glycosyl transferase (2), involves the transfer of the sugar from uridine diphophate to a suitable acceptor (aglycone), thus forming the glycoside.

$$\text{UTP} + \text{Sugar-1-P} \overset{(1)}{\leftrightarrow} \text{UDP-Sugar} + \text{PPi}$$

$$\text{UDP - Sugar} + \text{Acceptor} \overset{(2)}{\leftrightarrow} \underset{\text{(Glycoside)}}{\text{Acceptor - Sugar}} + \text{UDP}$$

Once such glycoside is formed, other enzymes may transfer another sugar unit to the monosaccharide moiety, converting it to a disaccharide. Enzymes occur in various glycoside-containing plants that are capable of producting tri- and tetrasaccharide moieties of the glycosides by analogous reactions.

To illustrate the biosynthesis of an aglycone moiety and the stereospecificity that can be involved in the glycosyl transferase reaction, the formation of cyanogenic glycosides is an interesting case to note the process of glycoside formation is shown in Figure 1 for prunasin, a cyanogenic glucoside. The amino acid phenylalanine, which arises from the shikimate pathway, is the starting precursor. An aldoxime, a nitrile, and a cyanohydrin are involved as intermediates in the pathway. The presence of a chiral center in mandelonitrile provides the opportunity for 2 β-glucosides to occur. In wild cherry, Prunus serotina, prunasin (D-mandelonitrile glucoside) is formed. The isomeric sambunigrin (L-mandelonitrile glucoside) is formed in Sambucus nigra. Apparently, these compounds do not occur in the same species, further confirming the stereospecificity of the glycosyl transferases that catalyze their formation.

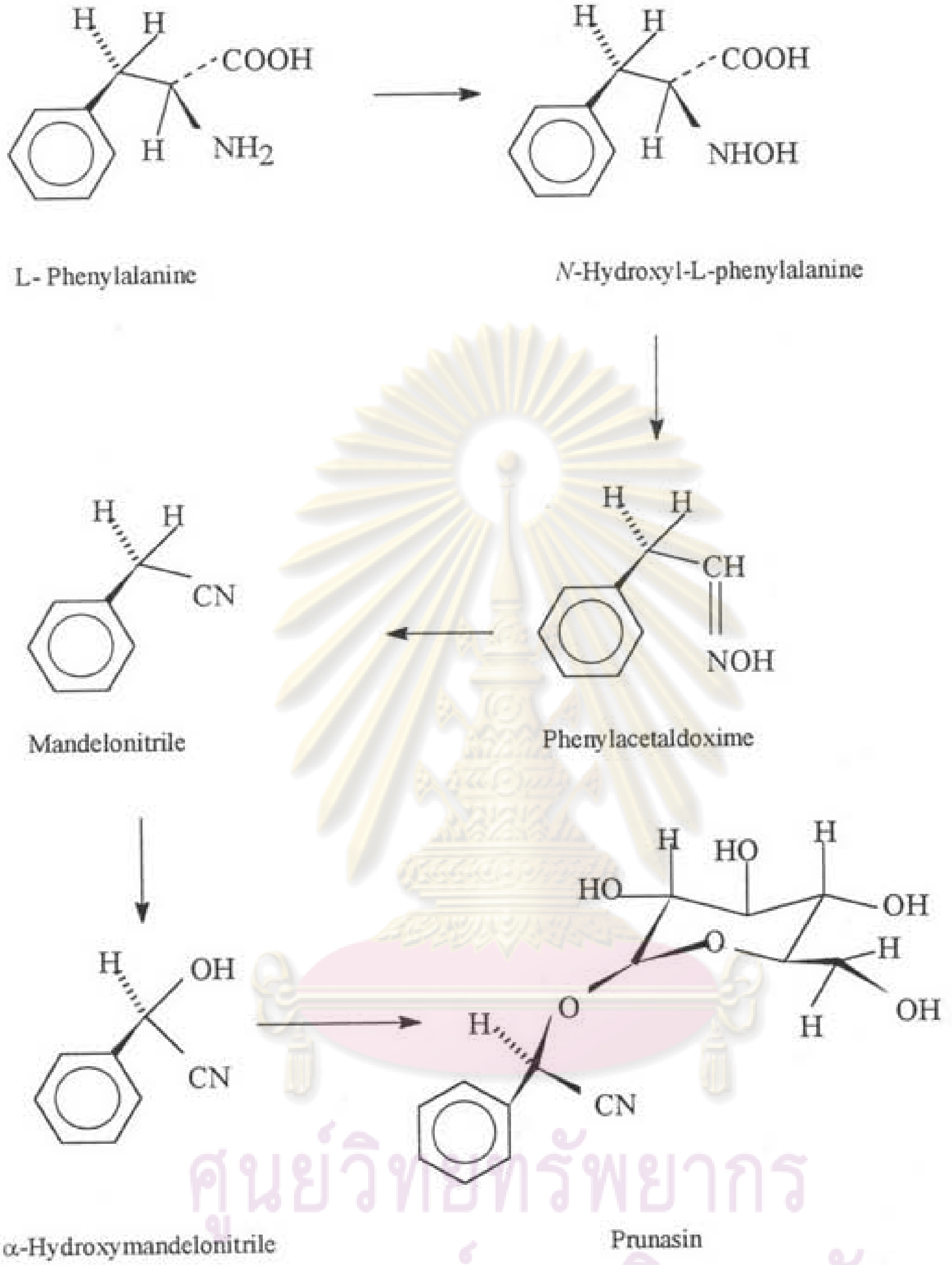


Figure 1 The biosynthetic pathway for the cyanogenic glycoside, prunasin.

Classification

The classification of glycosides is a difficult matter. If the classification is based on the sugar group, a number of rare sugar are involved, the structures of which are not too well known; it the aglycone group is used as a basis of classification, one encounters groups from probably all classes of plant constituents. A therapeutic classification, although excellent from a pharmaceutic viewpoint, omits many glycosides of pharmacognostic interest (Tyler,Brady and Robbers, 1981).

Although glycosides form a nature group in that they all contain a sugar unit, the aglycones are of such varied nature and complexity that glycosides vary very much in their physical and chemical properties and in their pharmacological action. This chapter deals with explanation according to that aglycone fragment with which the often occur in this study.

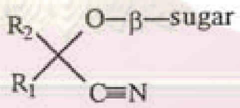
CYANOGENIC GLYCOSIDE

1. Introduction

Many plants yield hydrocyanic acid (HCN) or prussic acid on treatment with enzymes or with acid at elevated temperatures, thus they termed cyanogenesis, and sometimes designated as cyanogenetic glycoside or cyanophore glycoside. However, the cyanogenesis observed when cyanophoric plant tissues are crushed or their cellular structure otherwise disrupted is the result of the action of enzymes in the plant acting on the cyanogenetic glycosides (Bell and Charlwood,1980)

2. General structure

The cyanogenic substances in plants are usually carbohydrate derivatives, specifically, β-glycosides of 2-hydroxynitriles (cyanohydrins). They have the general formula and as such are glycosidic derivatives of 2- hydroxynitriles.



The structural variations are of three types involving,

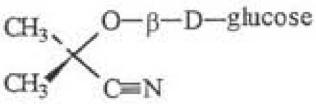
1. The nature of the sugar moiety

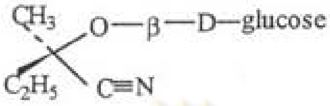
The sugar moiety in all but three of the known cyanogenic glycosides is the monosaccharide, D-glucose. This sugar is linked to the carbinol carbon by an *O*-β-glucosyl bond. The three exceptions are amygdalin(I), vincianin(II), and lucumin(III) which have disaccharides as their carbohydrate moiety.

2. The nature of the groups R_1 and R_2

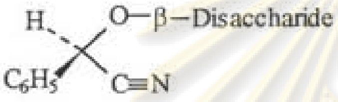
R_1 is either an aliphatic or aromatic group in a majority of the cyanogenic glycosides; the exceptions are triglochinin(IV) and its methyl ester(V) In the latter, R_1

and R_2 are the elements of the cyclopentene ring. R_2 in a majority of the cyanogens is H; the exceptions, besides triglochinin(IV), methyl triglochinin(V) are linamarin(VI) and lotaustralin(VII). In these two compounds R_2 is -CH_3.





Linamarin(VI) (R)-Lotaustralin(VII) Triglochinin(IV)



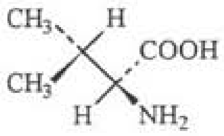
Disaccharide =Gentiobiose; (R)-Amygdalin(I) methyl Triglochinin(V)
= Vicianose; (R)-Vicianin(II)
= Primaverose; (R)-Lucumin(III)

3. The chirality of the carbinol carbon atom.

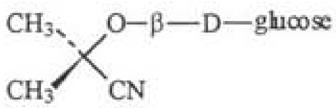
Since R_1 is usually not the same as R_2 in these compounds, the carbinol carbon atom is chiral. This introduces the possiblity of epimeric pairs of glycosides which would differ only in configuration at that carbon. Several such epimeric pairs do occur in nature, usually not in the same plant or even in related families.

It is possible to classify the cyanogenic glycosides on the basis of their origin. This will be the basis for the classification used in this review (Bell and Charlwood, 1980)

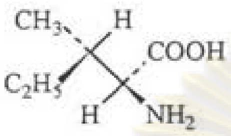
I. Glycosides derived from Valine and Isoleucine



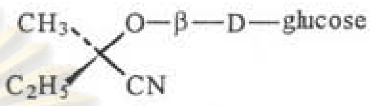
Valine



Linamarin(VIII)



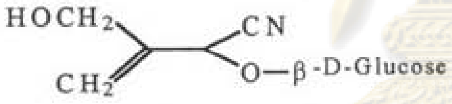
(3S)-Isoleucine



(R)-Lotaustralin(IX)

Linamarin(VIII) and (R)-Lotaustralin(IX) always occur in the same plant although not necessarily in the same ratio i.e in the ratio 19:1 in *Phaseolus lunatus* while in the reversed ratio of 1:19 was observed in *Lotus tenuis.*

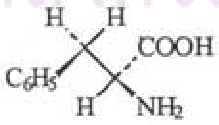
II. Glycosides derived from Leucine e.g.



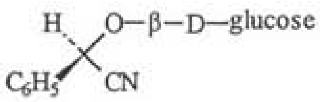
Cardiospermin(X)

Cardiospermin(X) was isolated from *Cardiospermum hirsutum,* a Sapindaceous species ,known to contain one or more cyanolipids of closely related structures.

III. Glycosides derived from Phenylalanine



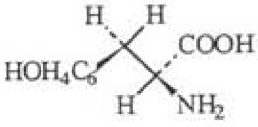
Phenylalanine



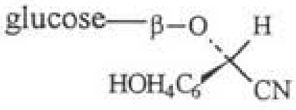
(R)-Prunasin(XI)

(R)-Prunasin(XI) was isolated from leaves of the cherry laurel, *Prunus lauracerasus.*

IV. Glycosides derived from Tyrosine



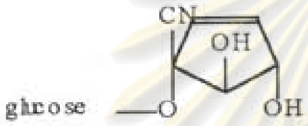
Tyrosine



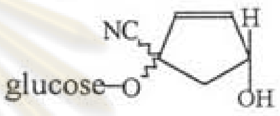
(S)-Dhurrin(XII)

(S)-Dhurrin (XII) which occurs in *Nandina domestica, Thalictrum aquilegifolium* and *Goodia lotifolia.*

V. Glycosides with Cyclopentene rings e.g.



Gynocardin(XIII)



epi-Tetraphyllin B(XIV)

Gynocardin(XIII) is the only one for which the structural assignment is complete. An East African species has also yielded evidence for the existence of *epi*-Tetraphyllin B(XIV).

3. Occurrance

No general rule can be established with regard to the distribution of these compounds in various plant tissues. This compounds are widely distributed, having been detected in more than 50 natural orders of plants, including ferns, basidiomycetes, and phycomycetes(Farnsworth,1996).

Although the number of cyanogenic plant species is large, the number of known cyanogenic compounds is not, numbering only 32 if one includes the cyanolipids and pseudocyanogenic glycosides. Families noted for their production of cyanogenic glycosides are the Rosaceae (150 species), Leguminosae (125), Gramineae (100), Araceae (50), Compositae (50), Euphorbiaceae (50), and Passifloraceae (30) (Bell and Charlwood, 1980)..

4. Biosynthesis

The sequence indicated in this figure 2.

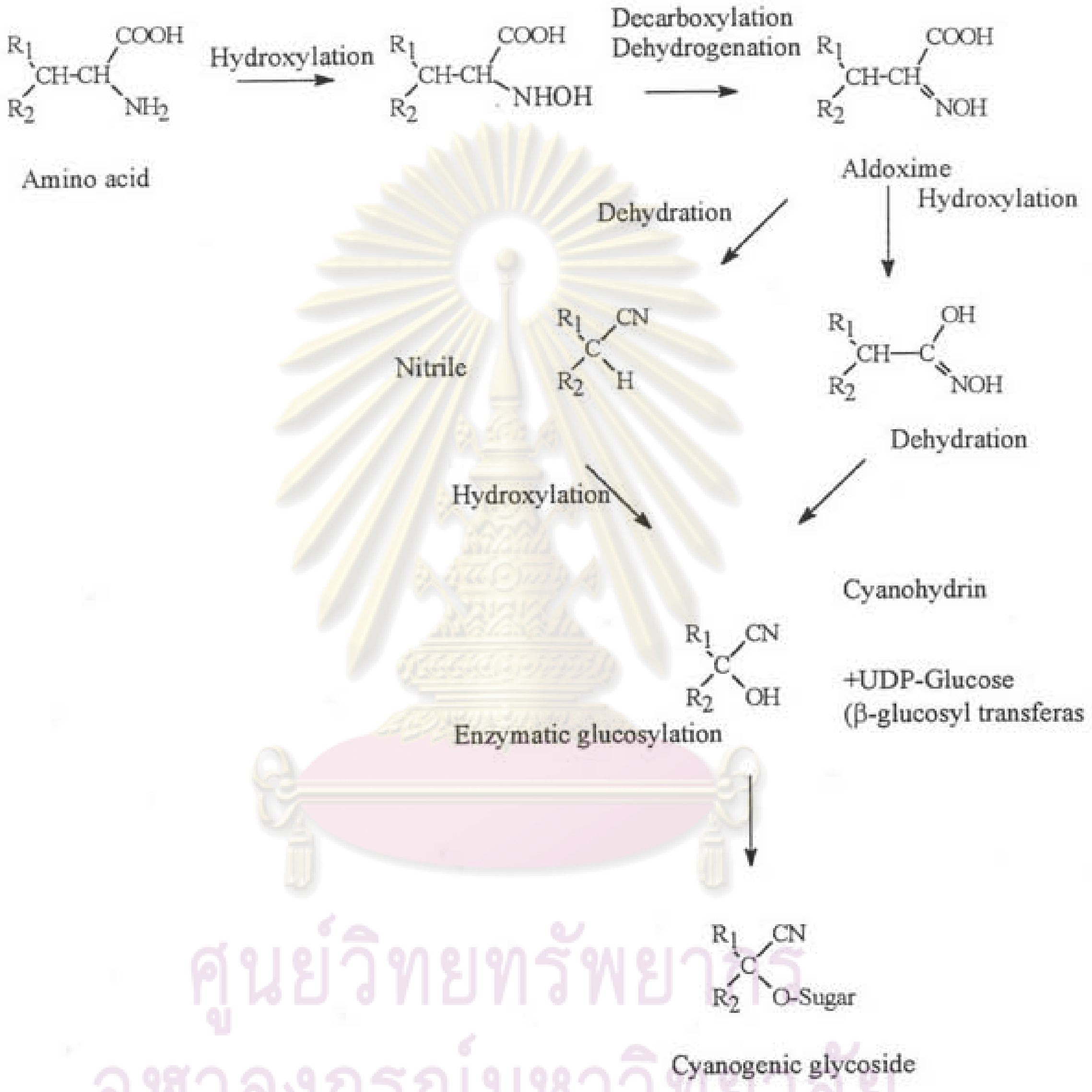


Figure 2 The biosynthetic pathway for the cyanogenic glycoside (Bell and Charlwood,1980)

5. Physiological Role of Cyanogenic glycosides

The function of cyanogenic glycosides is frequently said to be that assigned to secondary plant compounds as a group generally, that is, the role of repelling or inhibiting herbivores, pathogens, or competitors, with subsequent selection favoring the better-protected individuals.

The cyanogenic glycosides have received special attention because of their widespread distribution in plants and some evidence indicating its role in the primary metabolism of the plants. It was suggested that the cyanogens might be primary or essential metabolic compounds which had been selected in some plants (i.e., 'cyanogenic species') because of their defensive role.

There appears to be little direct relationship between the presence of this glycoside and the position of the plant in the system of classification. However, at the species level one may use the presence of cyanogenetic glycosides for differentiation (Swain, 1963; Bell and Charlwood, 1980).