Binary Defense: Cyanogenic Glycosides and Glucosinolates
(toxic products in common food plants)

Background: recall plant phytochemical defensive strategies encountered

i) constitutive defenses (always present - many alkaloids and triterpenes)
ii) induced defenses (i.e, nicotine, phytoalexins, protease inhibitors)
iii) "binary" defense (preformed but not active) - today's lecture

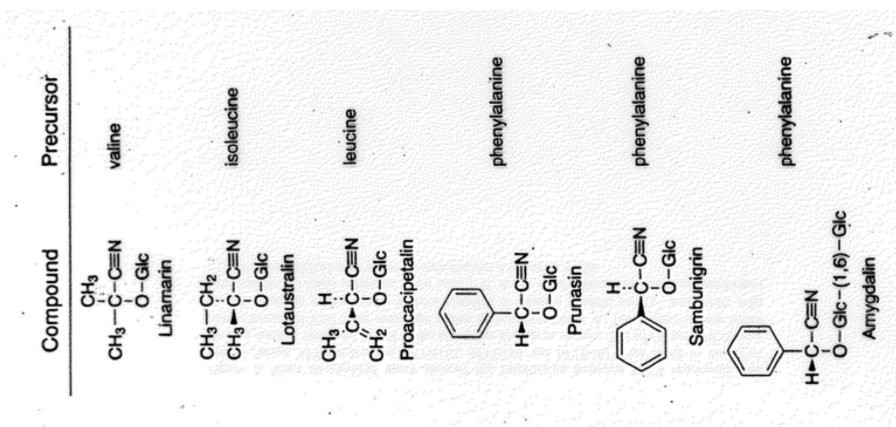
I. Cyanogenic Glycosides

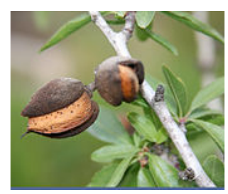
1. Definition: *Glycosides which release HCN when sugar is removed*

- widespread in plants, but in particular in Rosaceae (almonds, peach seed), Fabaceae (Lotus, clover), Graminae (sorghum)
- found in at least 2000 plant species, 60 different structures have been described.
- evolved independently in different taxa.

2. Typical Structure:

- three components: **nitrile**, **glucose** (or other sugar), amino acid-derived **R-group** (variable)
  - amino acid derived, often the R-group is still very similar





3. Simple Biosynthesis:

- derived from one of five amino acids: aromatic (Phe, Tyr) or branched-chain (Leu, Ile, Val)
- steps: - N-hydroxylation/decarboxylation (P450, **aldoxime**) (multifunctional P450)
  - nitrile formation (P450) (multifunctional P450)
  - glucosylation by UDP-dependent glycosyltransferases (*the presence of the glucose stabilizes nitrile*)

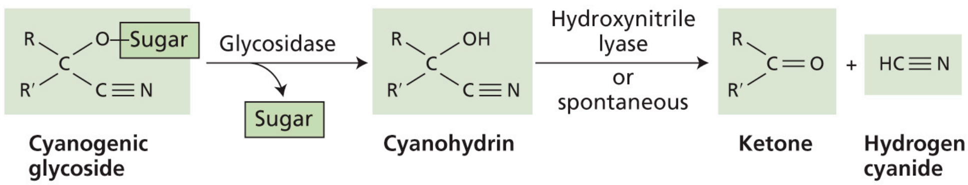
4. Mechanism of cyanogenesis (HCN production):

- *both spontaneous and enzyme-mediated*
- *enzyme kept in separate compartment from the compound itself (vacuolar)*

i) enzyme removes glucose*: ß-glucosidase* (can also happen spontaneously)
ii) 2-hydroxynitrile product is unstable -> *hydroxynitrillyase* (optional) --> HCN is released

Compartmentalization of cyanogenic glycoside and the respective ß-glucosidase is essential! (=binary defense)

(A)



PLANT PHYSIOLOGY AND DEVELOPMENT 6e, Figure 23.16 (Part 1)

6. Defensive Functions and Counteradaptations (Examples)

NB: HCN is a general inhibitor of mitochondrial respiration

i. Clover (*Lotus*) contains **linamarin**

- genetic polymorphisms: low and high HCN genotypes
- higher slug herbivore pressure at lower latitudes favor high HCN genotypes (early season herbivory)

ii. Cassava (*Manioc esculenta*) contains **dhurrin**

- cassava root is a tropical starchy staple (we used it mostly for its starch in tapioca)
- root crop, perennial, good in poor soils, pest-resistant
- bitter and sweet varieties relate to dhurrin content

- processing roots by grating and soaking followed by drying or heating detoxifies (removes HCN)
- humans have **rhodanese** enzyme to detoxify HCN (induced)

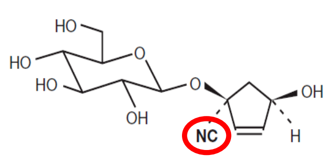
(NB: lethal dose of dhurrin: 0.5-3.5 mg/kg body wt)

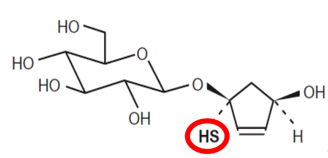
iii. Almond, apple, peach (Rosaceae) seeds contain **amygdalin & prunasin**)

- bitter almonds are toxic but used for flavor (benzaldehyde)
- 'sweet' almonds have mutation and less amygdalin
- cyanogenic prune or peach stones: ~300 mg HCN /100 g
- apple seeds: ~60mg/100g (2 cups = lethal dose??)

iv. Insect Counteradaptations

- sequestration and storage linamarin from Lotus (clover) by burnet moth larvae. *These insects have additionally independently evolved their own linamarin synthesis pathway (convergent evolution).*
- Heliconius larvae detoxify cyanogenic glycosides (see below)





7. Additional functions in N transport

- Attempts to reduce linamarin in transgenic cassava led to the realization that these compounds are transported, and used by the plant as transport forms.

- in this case they function as primary as well as secondary metabolites.

II. Glucosinolates (Thioglucosides, or Mustard Oils) *(= sulfur containing glycosides)*

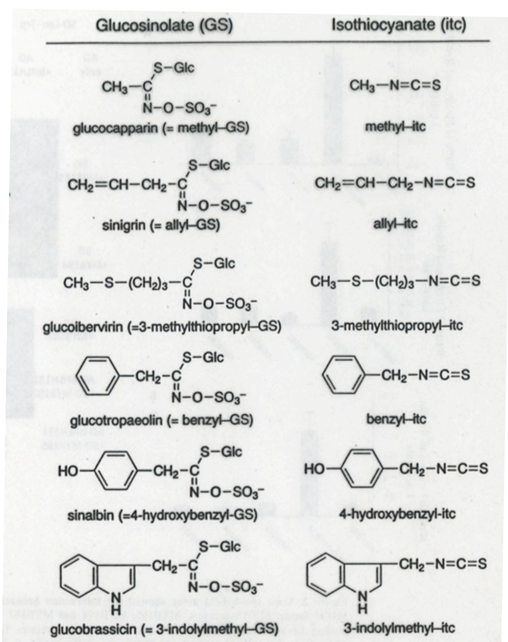
1. Introduction

- amino acid derived (aromatic, aliphatic)
- limited distribution (Brassicaceae: *Brassica, Arabidopsis, etc)*
- they are very important in the human diet (flavor, health)
- interact with enzyme = binary defense) → lead to **isothiocyanate** and related compounds

2. Structure

Three components: thioglucoside, hydroximinosulfate ester,
& variable R-group (amino acid-derived)

- based on Phe & Tyr (aromatic), Trp (indole),
  Leu, Ile, Val (aliphatic)
- 120 structures known (evolved from cyanogenic glycosides?)



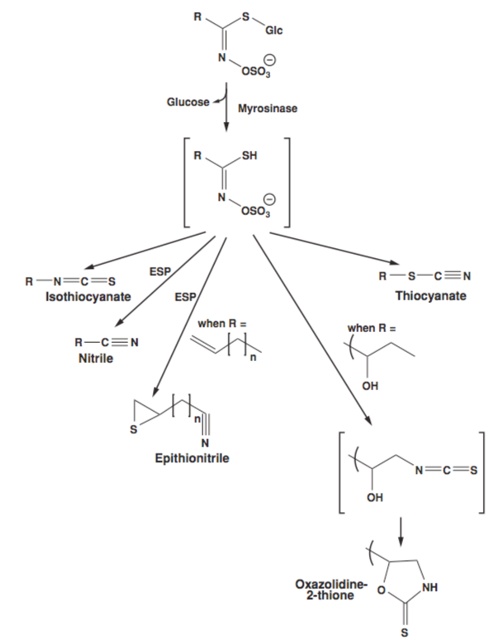


Figure 5
Outline of glucosinolate hydrolysis. Brackets indicate unstable intermediates. Abbreviations: ESP,

3. Biosynthesis

- amino acid: decarboxylation/N-hydroxylation
- add **S** from cysteine
- glucosylation (UDP-glucose)
- add **SO_4^{-2}** from PAPS
  (PAPS = phosphoadenosine phosphosulfate = key sulfur donor)

4. Hydrolysis products and biological effects

- hydrolysis by the enzyme **myrosinase** (= **thioglucosidase**) removes glucose.
- the aglycone part of the molecule is unstable → leads to spontaneous rearrangements
- **isothiocyanate** is main product, but smaller amounts of other products are formed. These include **thiocyanate**, **nitriles**, **epithionitriles**
- the products that are ultimately produced depend on the R-groups, and the presence of additional proteins:

i) **epithiospecifier protein** (ESP) directs rearrangements to epithionitrile
ii) works with **ESM1** (**epithiospecific modifier protein**)

5. Biological Effects of glucosinolates, and breakdown products.
- products are lipophilic -> they penetrate membranes, irritate nerves, impact ion channels ... 'wasabe'
- isothiocyanates attack -SH, $-NH_3$ groups in biomolecules
- compounds act as general antifeedants and toxins
- good examples of specialist insects that have learned to adapt.

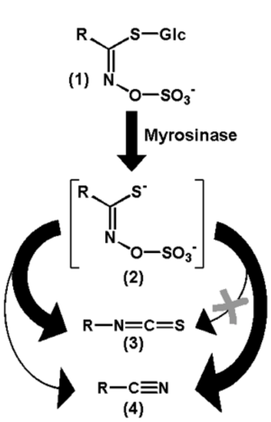
i) Isothiocyanates are toxic to generalist pests
- toxic effects of sinigrin to swallowtail larvae shown via clever "celery infiltration" experiment: 0.1% sinigrin =100% mortality

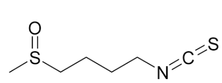
ii) Some well-studied specialists are adapted to glucosinolates
- cabbage butterfly can only feed and reproduce on *Brassicas*
  sinigrin = feeding stimulant for larvae, oviposition stimulant for adults
- flea beetles: feeding stimulant is a mixture of chemicals including glucosinolates

iii) How specialist insects detoxify glucosinolates
- cleave sulfate: prevents **myrosinase** action (diamondback moth)
- redirect ITC products via **Nitrile Specifier Protein** (**NSP**)
  = *novel insect protein*, in cabbage butterfly
- also: examples of sequestration (w. insect-derived myrosinase)

iii) The importance of glucosinolates in the human diet
- *Brassica oleracea* (cabbage, cauliflower...) & *B. rapa* (turnip, bok choy) are common vegetables
- *B. nigra*, *Sinapis alba* species, (black and white mustards) are condiments (mustard taste due to isothiocyanate)
- breeding led to low glucosinolate content in seed for improved canola oil (also low erucic acid & low sinapine)
- anticancer effects ascribed to **sulforaphanes** (high in brussel sprouts)

Sulfurophanes: *specialized products of some rearranged glucosinolates linked to anti-cancer effects via their ability to induce phase II enzymes (conjugation of xenobiotics, cell cycle control)*





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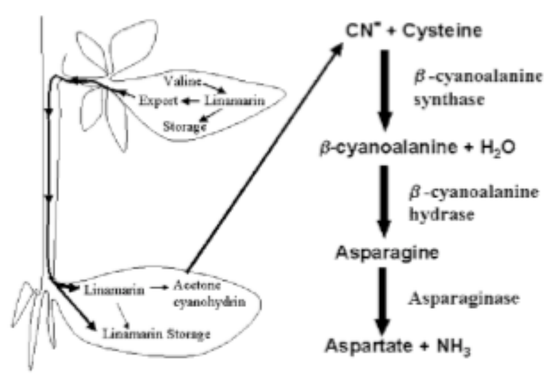

Figure 6. Proposed pathway for the transport of linamarin to roots and its metabolism to produce asparagine.