Binary Defense: Cyanogenic Glycosides and Glucosinolates

(toxic products in common food plants)

i) constitutive defenses (always present - many alkaloids and triterpenes)

ii) induced defenses (i.e, nicotine, phytoalexins, protease inhibitors)

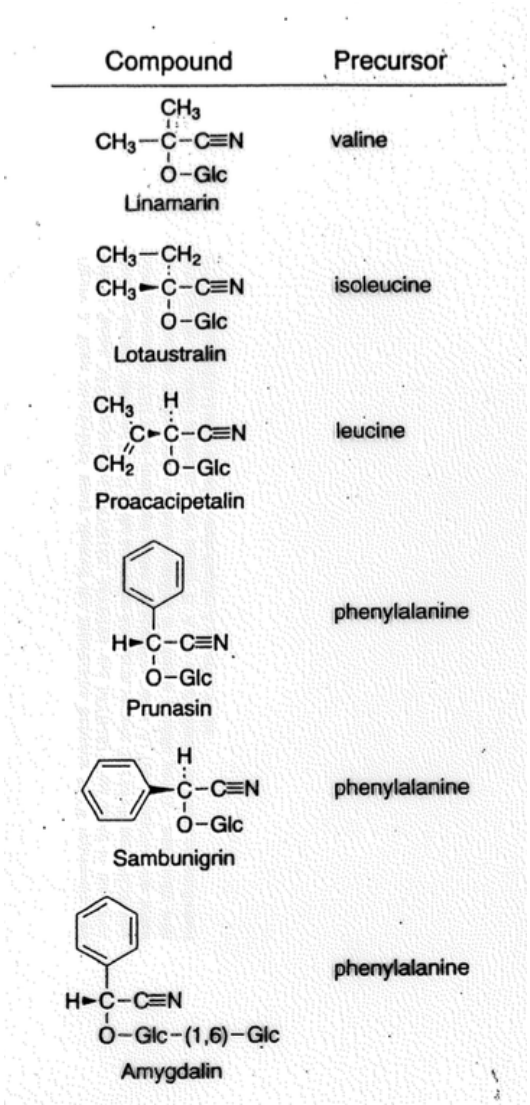
iii) "binary" defense (preformed but not active) - today's lecture

1. Definition: *Glycosides which release HCN when sugaris 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** , *amino acid*-derived **R-group** (variable)
- amino acid derived, often the R-group is still very similar



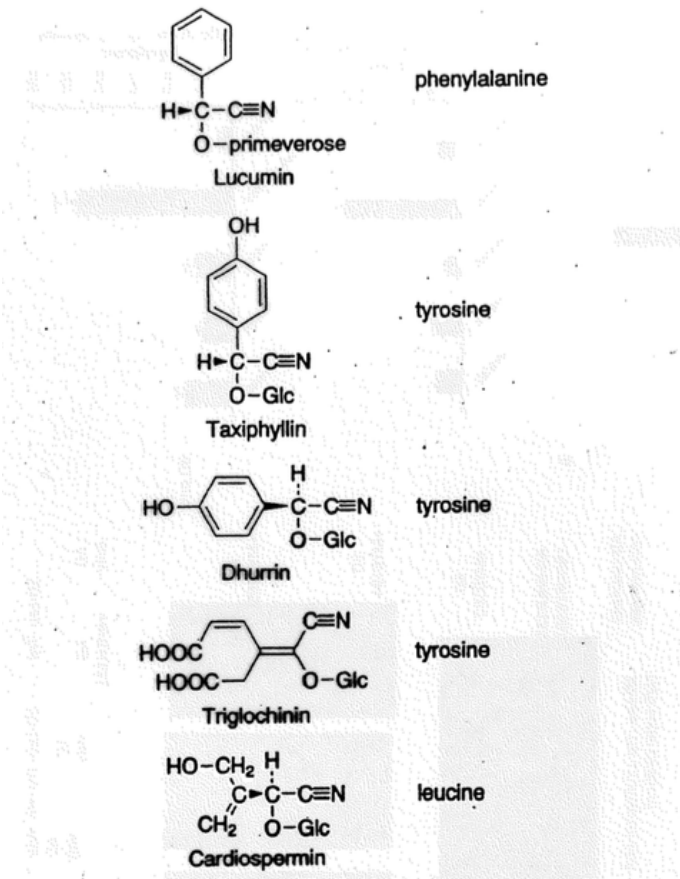


Figure 12.16 Structures of a few common cyanogenic glycosides.

3. Simple Biosynthesis:

- N-hydroxylation/decarboxylation (P450, **aldoxime**)

· nitrile formation (P450) (multifunctional P450)

glucosylation by UDP-dependent glycosyltransferases

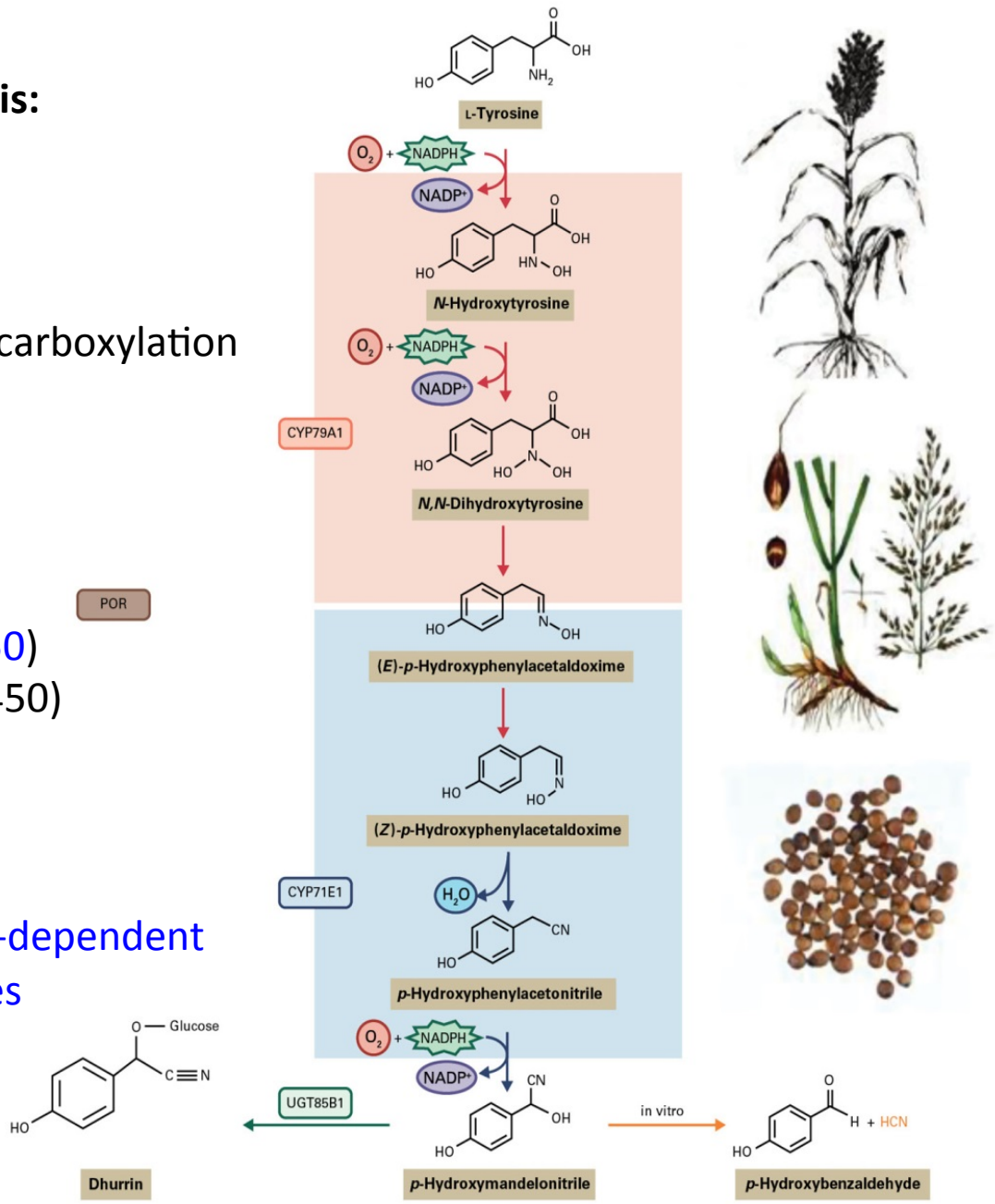


FIGURE 24.20 *Biosynthesis and degradation of the tyrosine-derived cyanogenic glycoside dhurrin in* Sorghum bicolor. *The enzymes catalyzing the different steps are shown. Each cytochrome P450 enzyme catalyzes more than one step in the pathway. These steps are indicated by red*

Cyanogenic glycoside enzymes from a metabolon

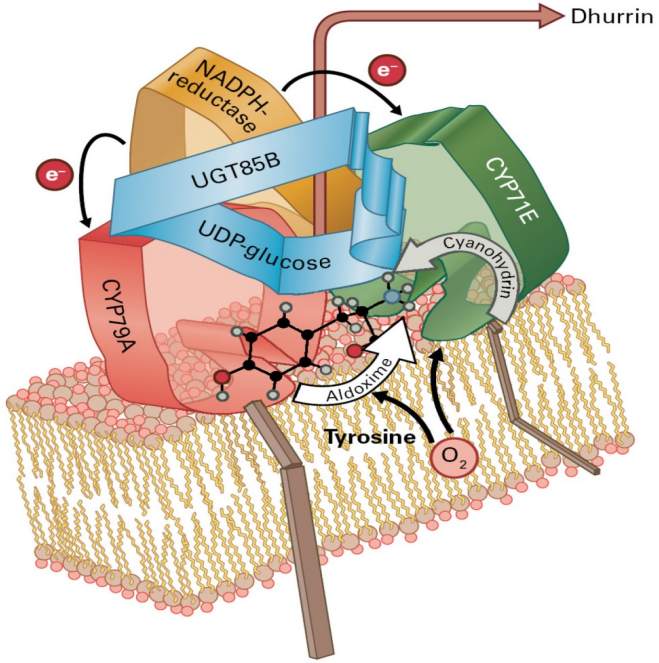


FIGURE 24.22 *Metabolon formation in cyanogenic glycoside synthesis. The two cytochrome P450 enzymes (CYP79A1 and CYP71E1) and UDP-glucosyltransferase (UGT85B1) interact to channel intermediates in the biosynthetic pathway directly into formation of dhurrin.*

Biochemistry & Molecular Biology of Plants, Second Edition. Edited by Bob B. Buchanan, Wilhelm Gruissem, and Russell L. Jones.

Companion website: www.wiley.com/go/buchanan/biochem

WILEY Blackwell

4. Mechanism of cyanogenesis (HCN production):

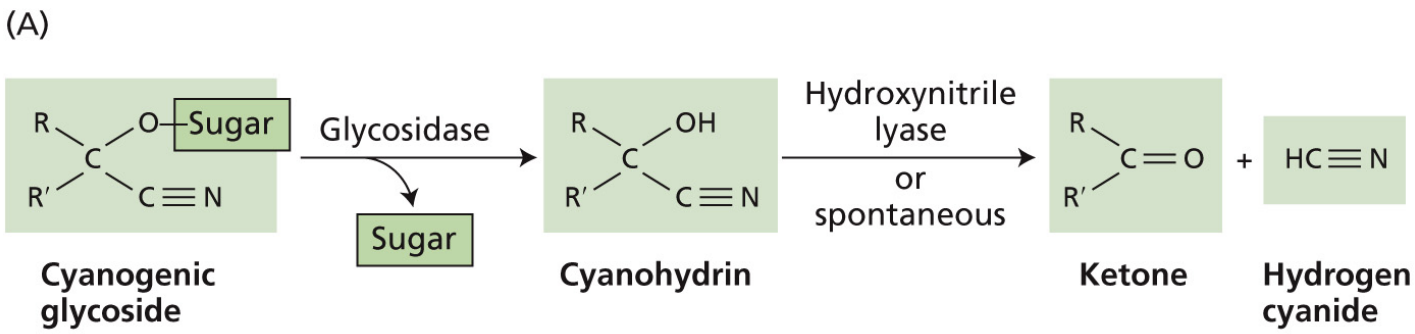
- *both spontaneous and enzyme-mediated*
- *enzyme kept in **separate compartment** from the compound itself (vacuolar)*

i) enzyme removes glucose*: ß-glucosidase* (can also happen spontaneously)

ii) 2-hydroxynitrile product is unstable -> *hydroxynitrillyase* (optional) --> HCN is released

<u>Compartmentalization</u> of cyanogenic glycoside and the respective ß-glucosidase is essential

Enzyme-catalyzed hydrolysis of cyanogenic glycosides to release hydrogen cyanide (HCN)



***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 (milder winters) favor high HCN genotypes (early season herbivory)

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

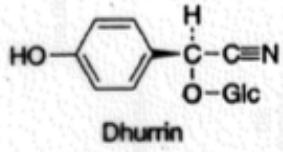
ii. Cassava (*Manioc esculenta*) contains **dhurrin**

- cassava root is a tropical starchy staple (also, 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)

Cassava plant and preparation



C

D

BOX 24.4 continued

Qualities Targetted by BioCassava Plus Project

Genetic Engineering of Cassava for:

1. reduced cyanogenesis
2. enhanced pro-vitamin A (see Golden Rice)
3. increased Fe++ and Zn++ bioavailability
4. improved protein content
5. gemini virus resistance (30-50% loss)
6. improved shelf life and storage

Effect of antisense inhibition of linamarim biosynthesis P450s in roots and leaves in cassave

leaf promoter

root promoter

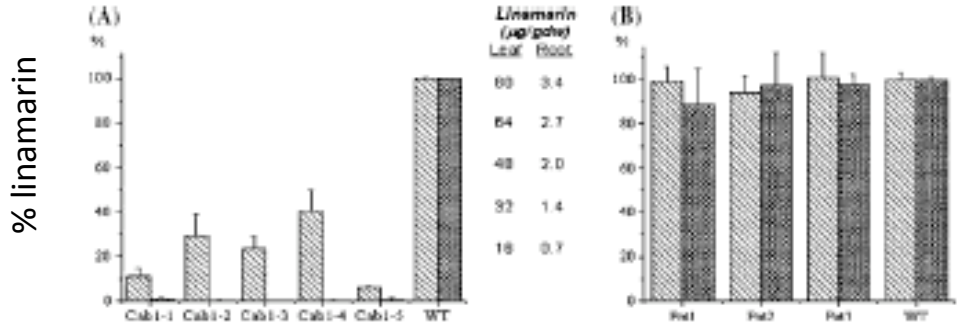


Figure 5. Linamarin content of roots (▨) and leaves (▩) of transgenic cassava plants in which the expression of the *CYP79D1/D2* genes was selectively inhibited in leaves (A) or roots (B).

Siritunga and Sayre, Plant Mol Biol 2004

Linamarin utilization and metabolism by cassava

666

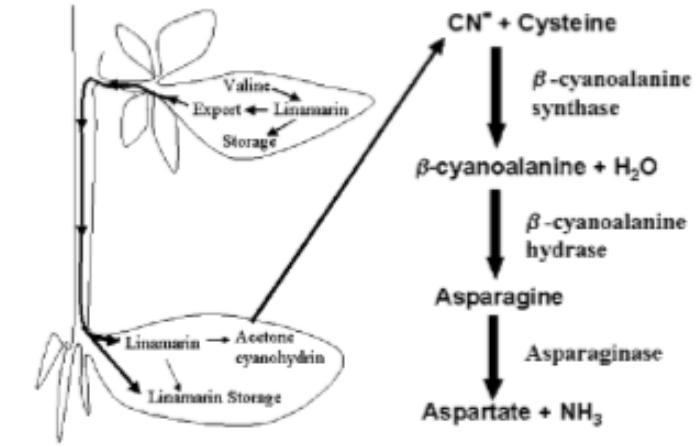


Figure 6. Proposed pathway for the transport of linamarin to roots and its metabolism to produce asparagine.

proposed synthesis of asn and asp from HCN

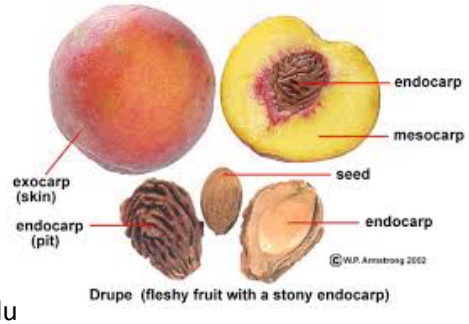
Linamarin is a transport form of N, and N source for growing roots

Siritunga and Sayre, Plant Mol Biol 2004

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

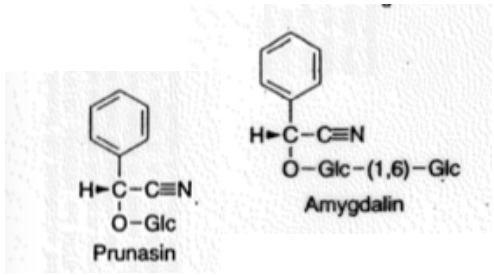
- bitter almonds are toxic but used for flavor (benzaldehyde)
- 'sweet' almonds have mutation and less amygdalin
- cyanogenic prune or peach stones: ~300 mg HCN /100 g
- apple seeds: ~60mg/100g (2 cups = lethal dose??)

Wikipedia



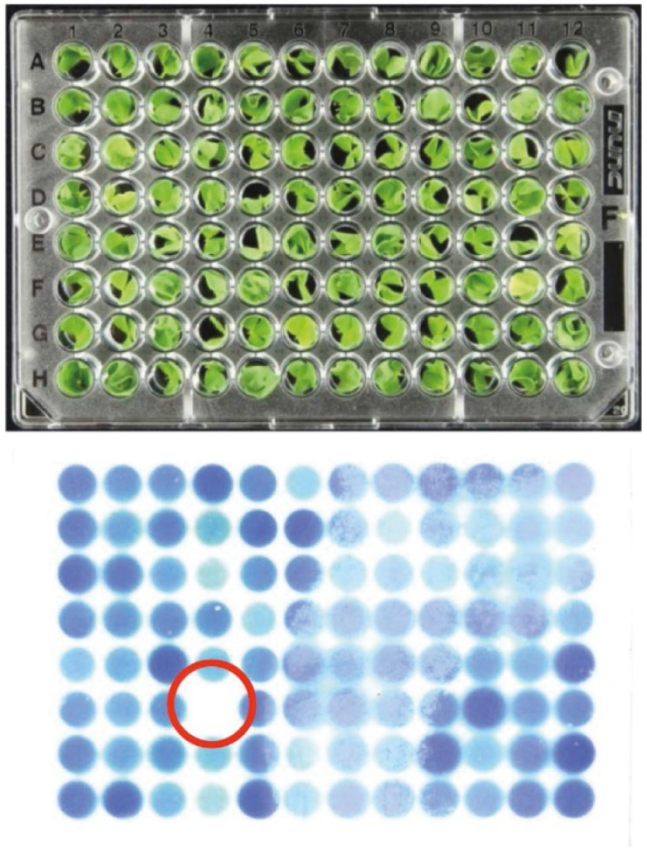
Drupe (fleshy fruit with a stony endocarp)

http://waynesword.palomar.edu



High throughput screen for clover linamarin (HCN) mutants

A



B

BOX 24.3

Biochemistry & Molecular Biology of Plants, Second Edition. Edited by Bob B. Buchanan, Wilhelm Gruissem, and Russell L. Jones.

Companion website: www.wiley.com/go/buchanan/biochem

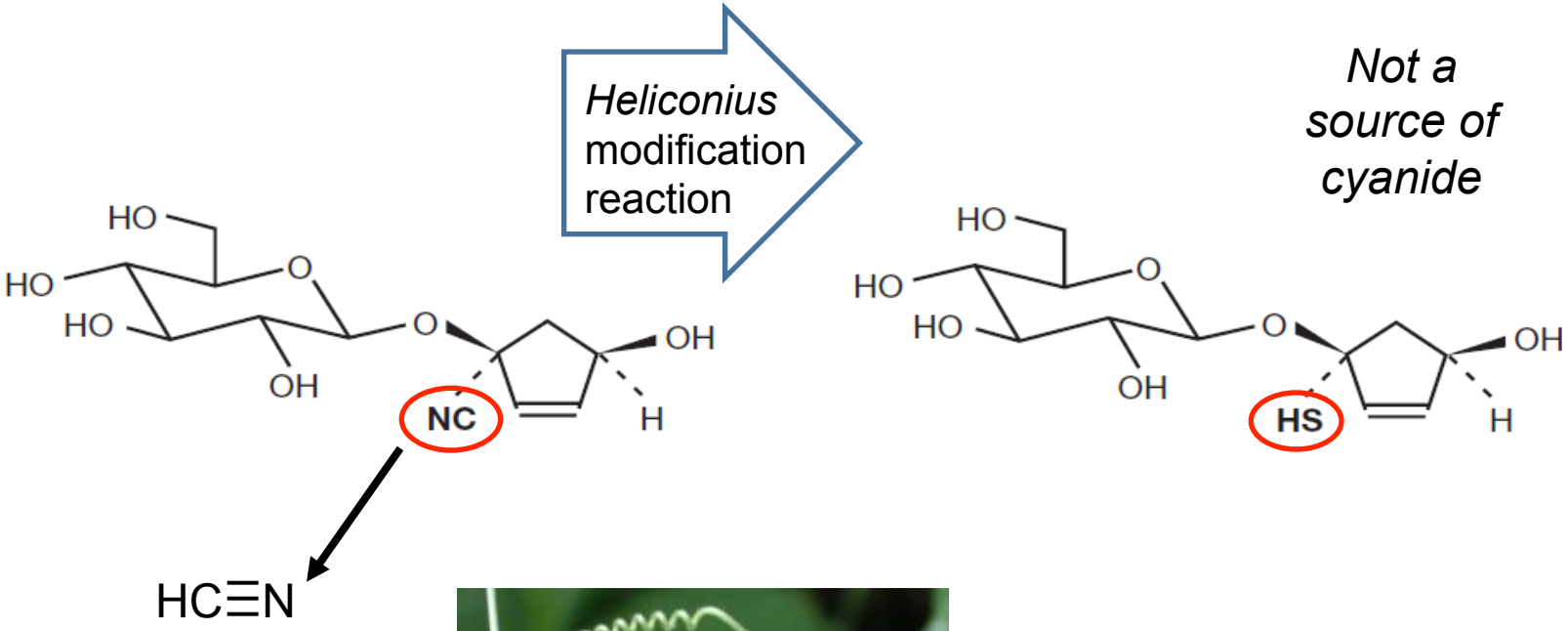
WILEY Blackwell

Case study: *Heliconius* butterflies and passion flowers

South American *Heliconius* butterflies were collected and studied as early as the 17th century, and provided the basis for ideas about mimicry

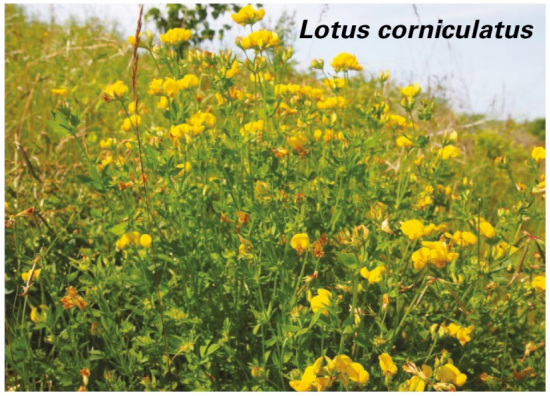
Maria Sybilla Merian 1705; William M. Ciesla, Patricia M. Ciesla, Forest Health Management International, Bugwood.org

Some *Heliconius* butterflies can detoxify a cyanogenic glucoside

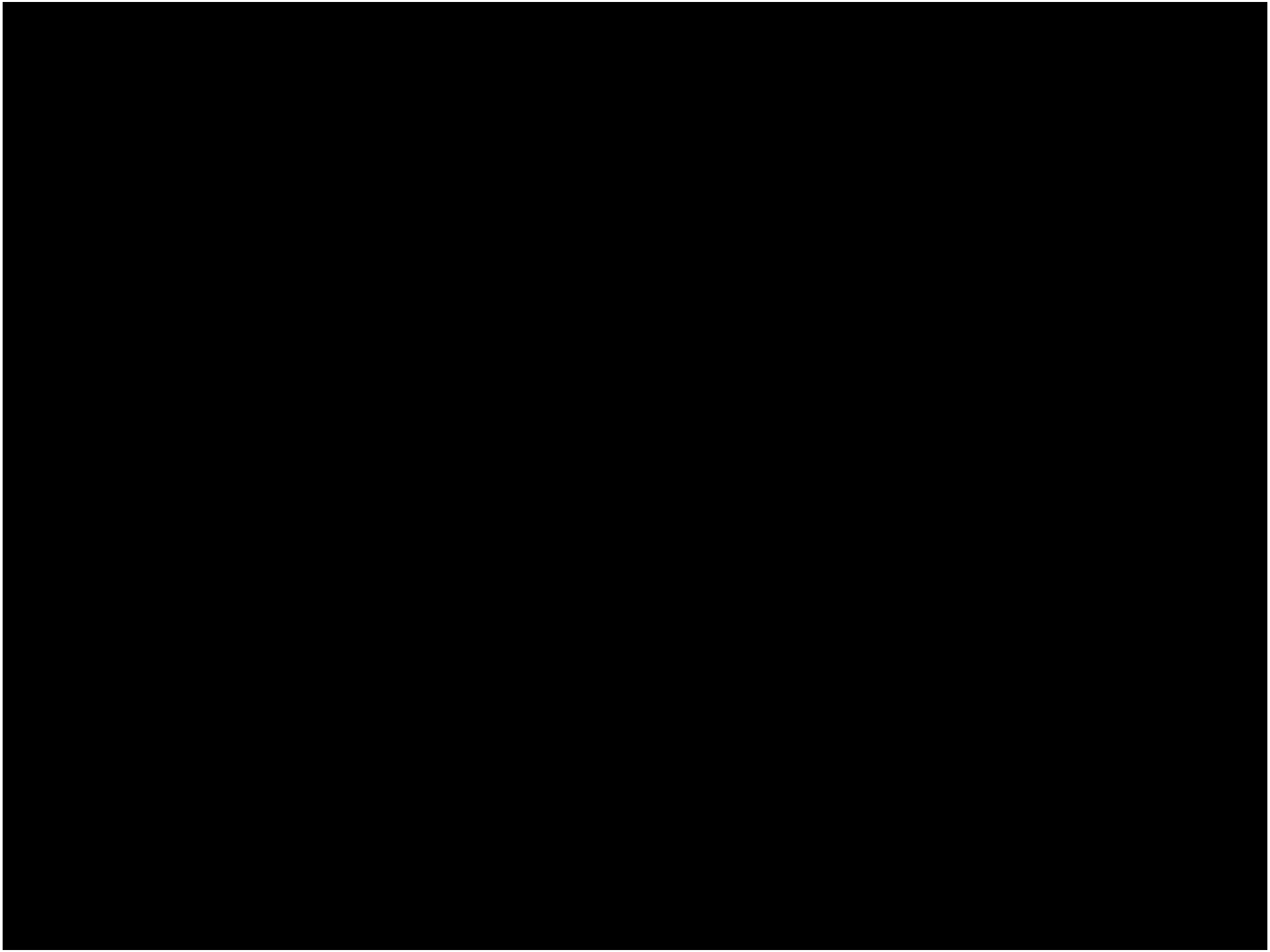


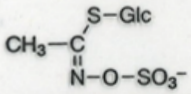
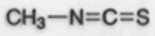
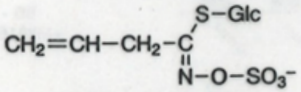
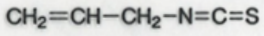
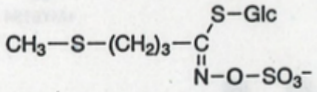
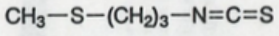
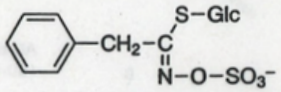
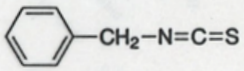
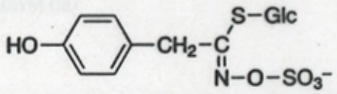
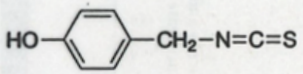
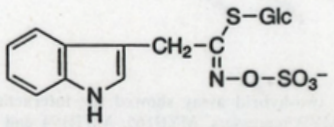
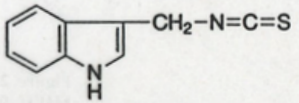
The larvae of more than 60 species of *Heliconius* butterflies are specialized feeders of *Passiflora* and are tolerant of their various secondary metabolites

Some insects can tolerate, and even produce their own cyanogenic glycosides
*(Male burnet moth transfers **nuptial gift of cyanogenic glycoside**s)*



BOX 24.5



Glucosinolate (GS)	Isothiocyanate (itc)
 glucocapparin (= methyl–GS)	 methyl–itc
 sinigrin (= allyl–GS)	 allyl–itc
 glucoibervirin (=3-methylthiopropyl–GS)	 3-methylthiopropyl–itc
 glucotropaeolin (= benzyl–GS)	 benzyl–itc
 sinalbin (=4-hydroxybenzyl-GS)	 4-hydroxybenzyl-itc
 glucobrassicin (= 3-indolylmethyl–GS)	 3-indolylmethyl–itc

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

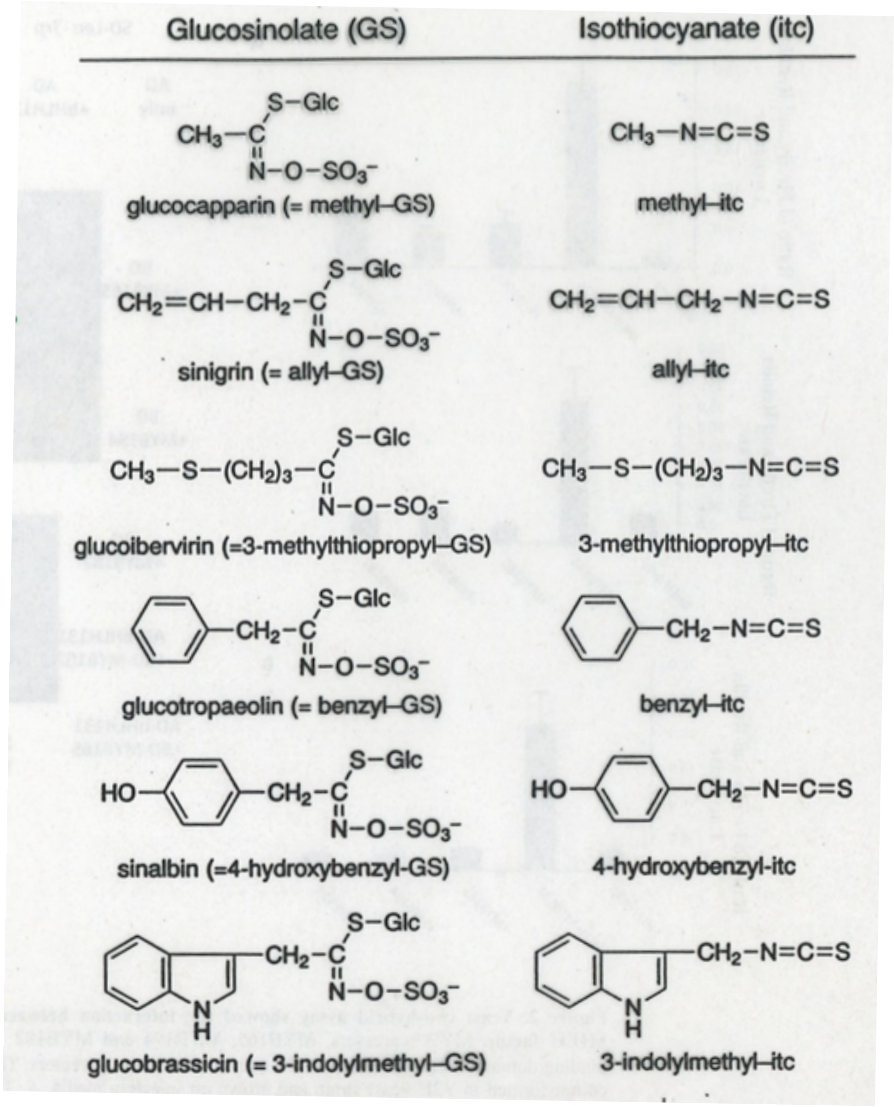
Glucosinolates (Thioglucosides, or Mustard Oils) - Brassicaceae

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?)



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)

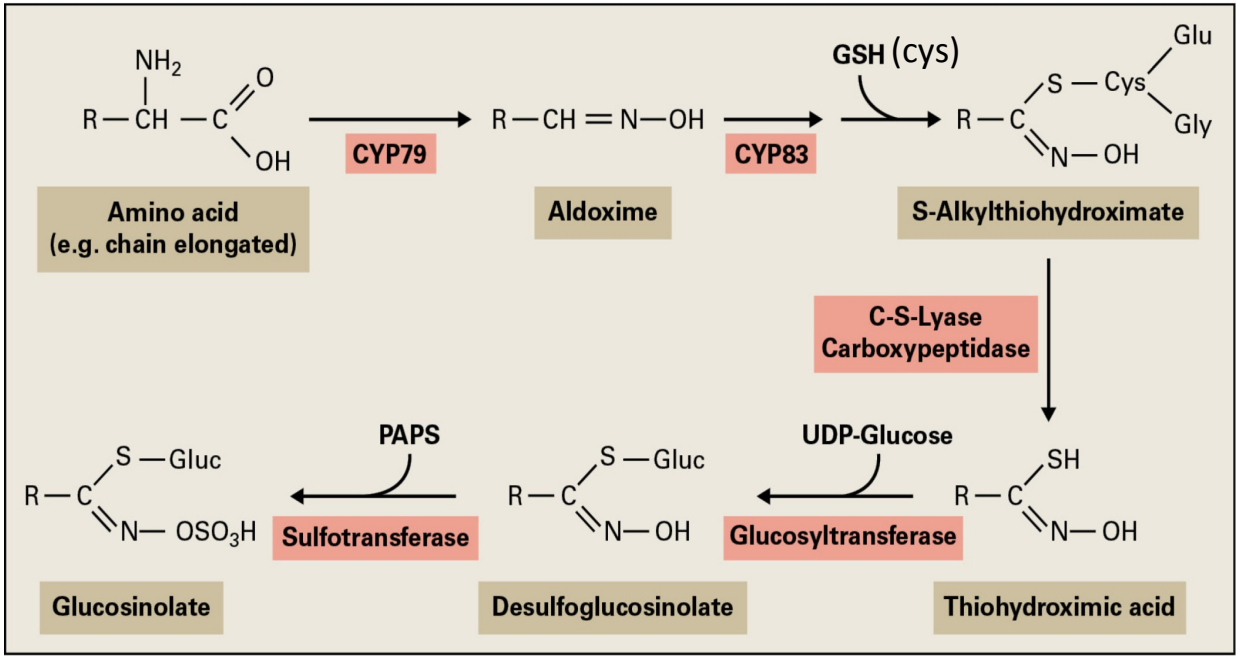
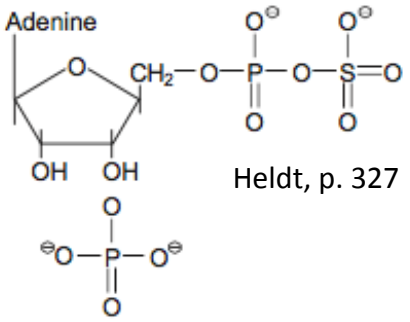


FIGURE 24.25 *Biosynthesis of glucosinolates. Cleavage of the glutathione (GSH) conjugate may be achieved by multiple routes, including action of a carboxypeptidase or the C-S lyase SUPERROOT 1 to produce the thiohydroximic acid.*



PAPS
(3-Phospho-AMP-sulfate)

Heldt, p. 327

Glucosinolate Products and their modification

epithiospecifier protein (ESP): directs rearrangements to **epithionitrile**

ESM1 (epithiospecific modifier) promotes epithionitrile by inhibiting nitrile formation)

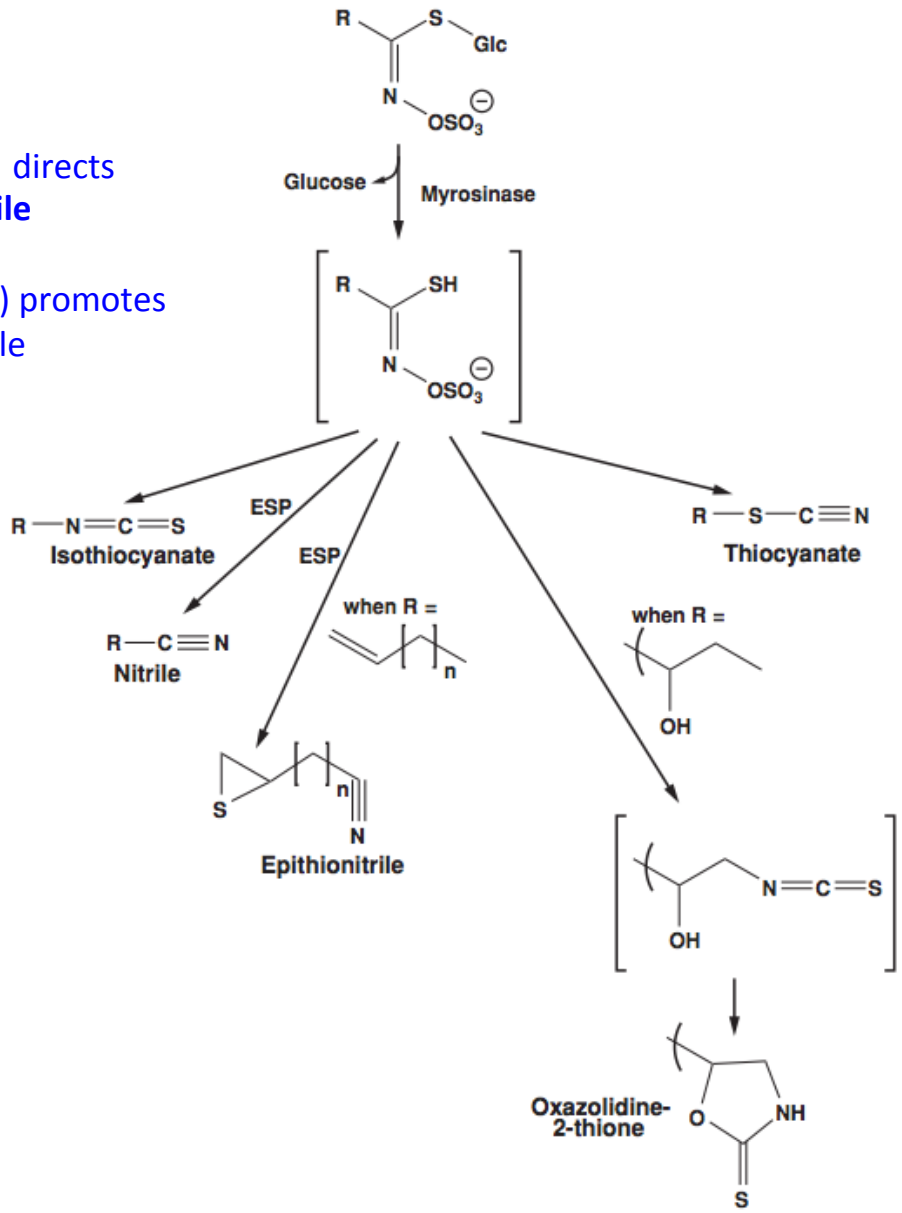


Figure 5

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

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

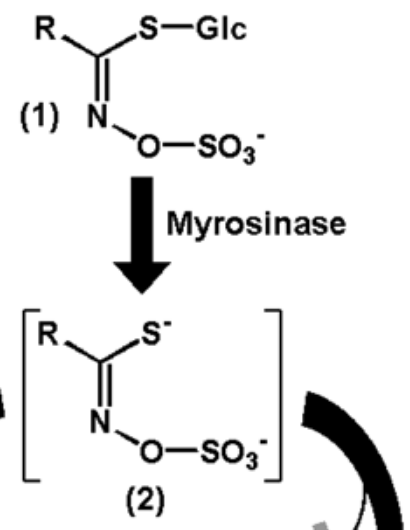
Cabbage butterfly larvae: sequester glucosinolates, and redirect degradation into less-toxic products using the nitrile-specifying protein

The larvae sequesters the glucosinolates as protection against predators

Adult form

Wittstock, U., Agerbirk, N., Stauber, E.J., Olsen, C.E., Hippler, M., Mitchell-Olds, T., Gershenzon, J., and Vogel, H. (2004). Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. USA 101: 4859-4864; David Cappaert, Michigan State University, Bugwood.org

Cabbage butterfly larvae: sequester glucosinolates, and redirect degradation into less-toxic products using the nitrile-specifying protein



Standard product isothiocyanate: more toxic

Nitrile: less toxic

NSP: nitrile-specifying protein

The larvae sequesters the glucosinolates as protection against predators

Adult form

Wittstock, U., Agerbirk, N., Stauber, E.J., Olsen, C.E., Hippler, M., Mitchell-Olds, T., Gershenzon, J., and Vogel, H. (2004). Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. USA 101: 4859-4864; David Cappaert, Michigan State University, Bugwood.org

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)

iii) The importance of glucosinolates in the human diet

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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)*