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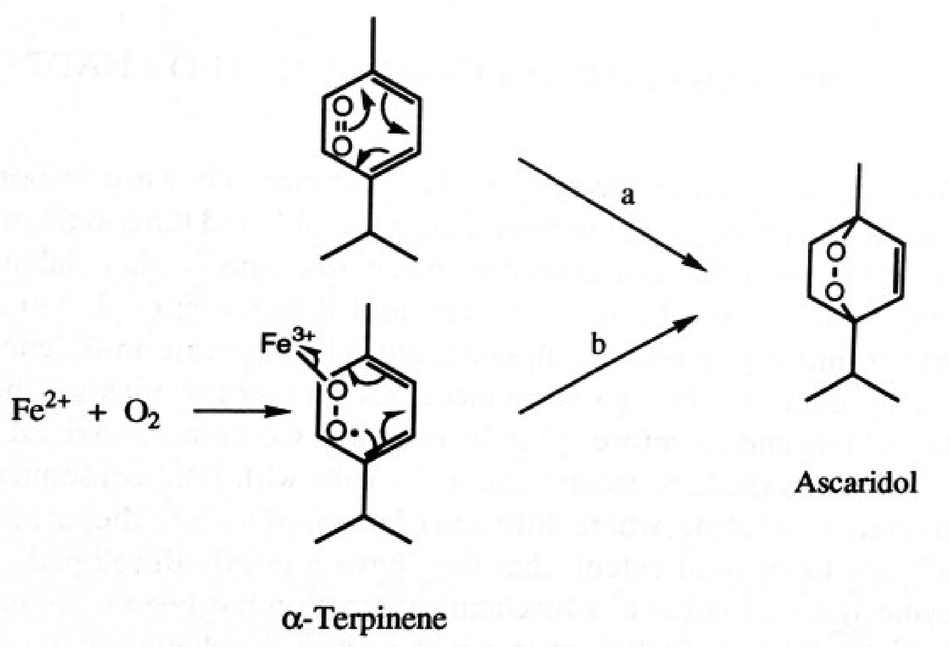
Chapter 4-2
The shikimic acid pathway

Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

Oxidation in nature

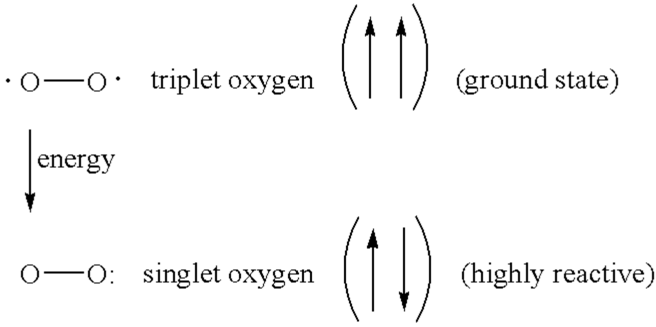
- Singlet oxygen $^1O_2$ is a short lived and very reactive molecule which is thought to participate in oxidations in nature.
- Triplet oxygen $^3O_2$ is nevertheless the most plausible oxidating agent.



Possible pathways to the biosynthesis of ascaridol from α-Terpinene

(a) Using $^1O_2$

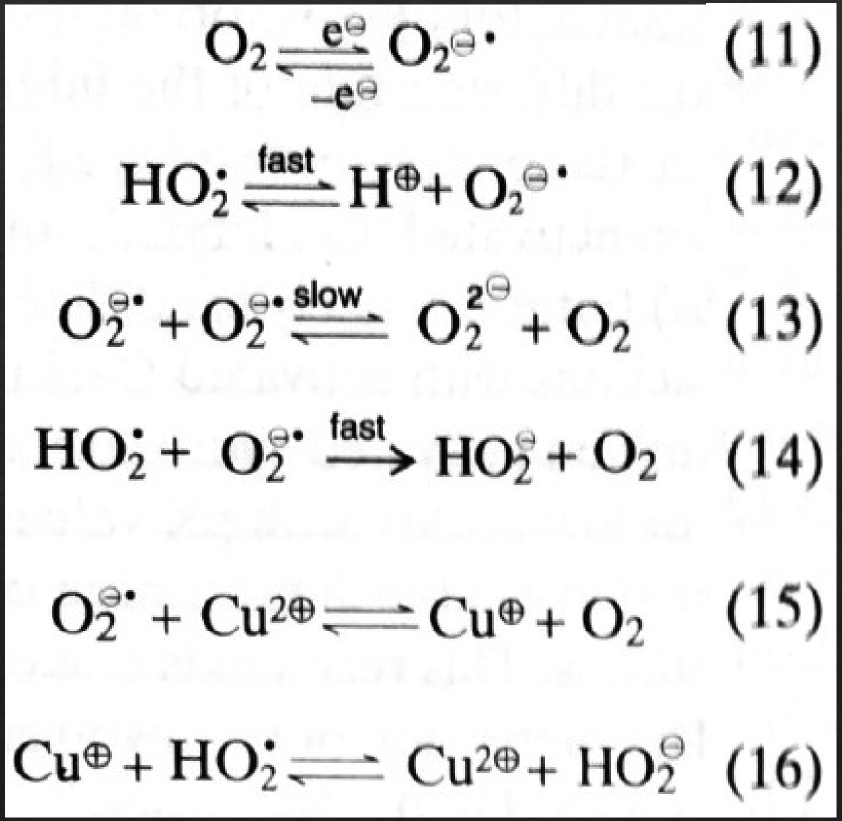
(b) Using $^3O_2$ (iron catalyzed)



Reduction of oxygen to water

(11) Formation of the superoxide radical ion (**not an oxidant**)
(12) Hydrogen peroxide radical formation (at physiological pH, this acidic form is barely present).
(13-16) Impurities and moisture cause a slow formation of peroxide and oxygen (electron transfer). This is accelerated with the protonated specie, and with copper ions.

$O_2^{-\cdot}$: Good nucleophile and mild reducing agent (ex: reaction with alkyl bromide)
$HO_2^{\cdot}$: Strong oxidant, hydrogen abstractor (on unsaturated hydrocarbons, or activated saturated hydrocarbons)



$$O_2 \underset{-e^{\ominus}}{\overset{e^{\ominus}}{\rightleftharpoons}} O_2^{\ominus\cdot} \qquad (11)$$

$$HO_2^{\cdot} \overset{fast}{\rightleftharpoons} H^{\oplus} + O_2^{\ominus\cdot} \qquad (12)$$

$$O_2^{\ominus\cdot} + O_2^{\ominus\cdot} \overset{slow}{\rightleftharpoons} O_2^{2\ominus} + O_2 \qquad (13)$$

$$HO_2^{\cdot} + O_2^{\ominus\cdot} \xrightarrow{fast} HO_2^{\ominus} + O_2 \qquad (14)$$

$$O_2^{\ominus\cdot} + Cu^{2\oplus} \rightleftharpoons Cu^{\oplus} + O_2 \qquad (15)$$

$$Cu^{\oplus} + HO_2^{\cdot} \rightleftharpoons Cu^{2\oplus} + HO_2^{\ominus} \qquad (16)$$

Involvments of $O_2^{-\cdot}$ in biological hydroxylation

- Peroxide is often the first intermediate in aliphatic hydroxylation.
- Example: Biosynthesis of prostaglandins (very important molecules in animals, having diverse hormone-like effects in animals)
  - A radical is formed by hydrogen abstraction, leading to a cyclazation
  - A peroxide is formed, and a peroxidase allow the O-O cleavage.
  - Rearrangements lead to prostacyclin, thromboxane or $PGF_{2\alpha}$

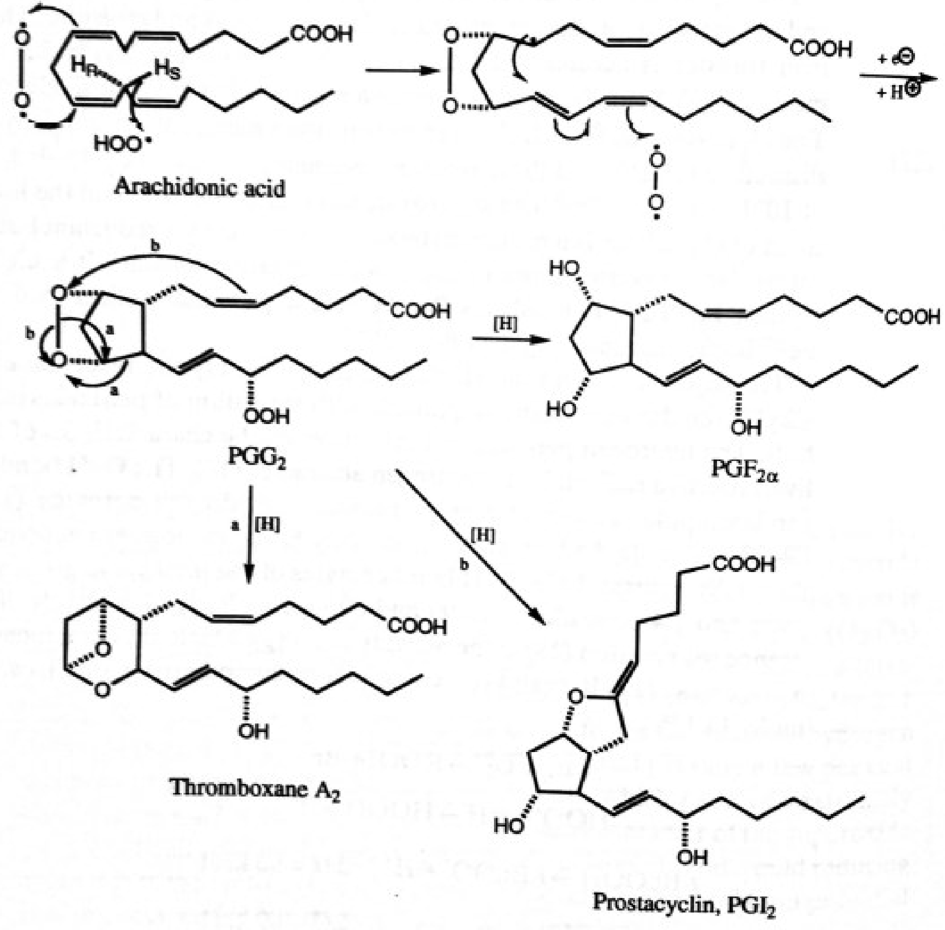
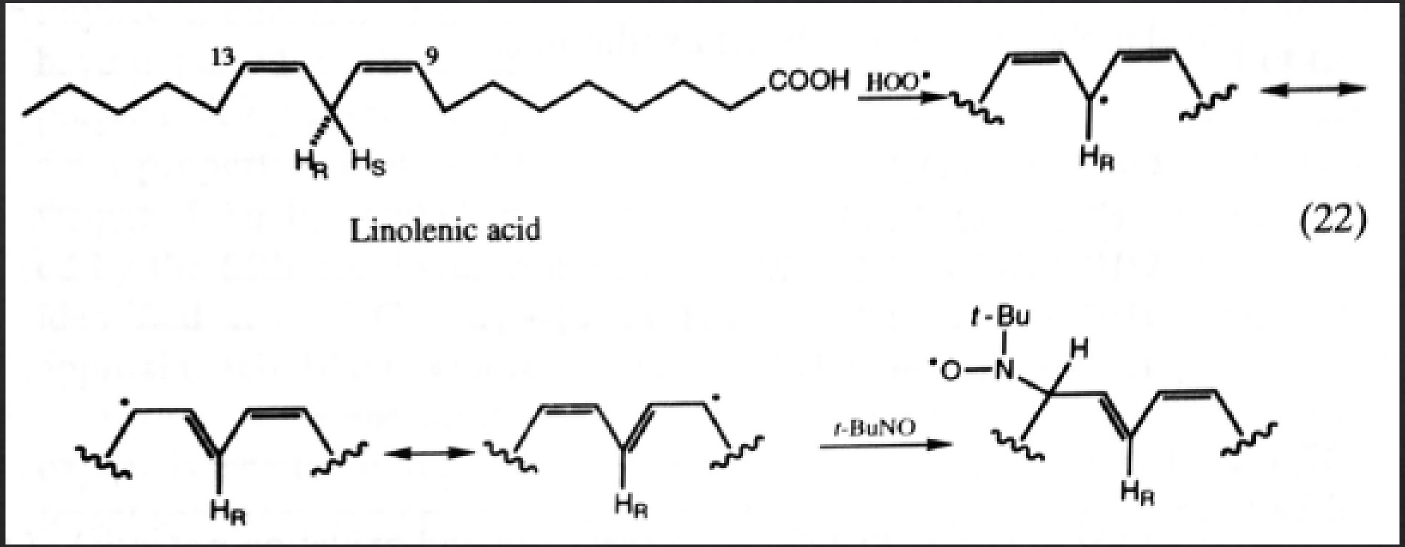


Fig. 5 Biosynthesis of prostaglandins and thromboxanes from arachidonic acid

How can we know that radicals are formed ?

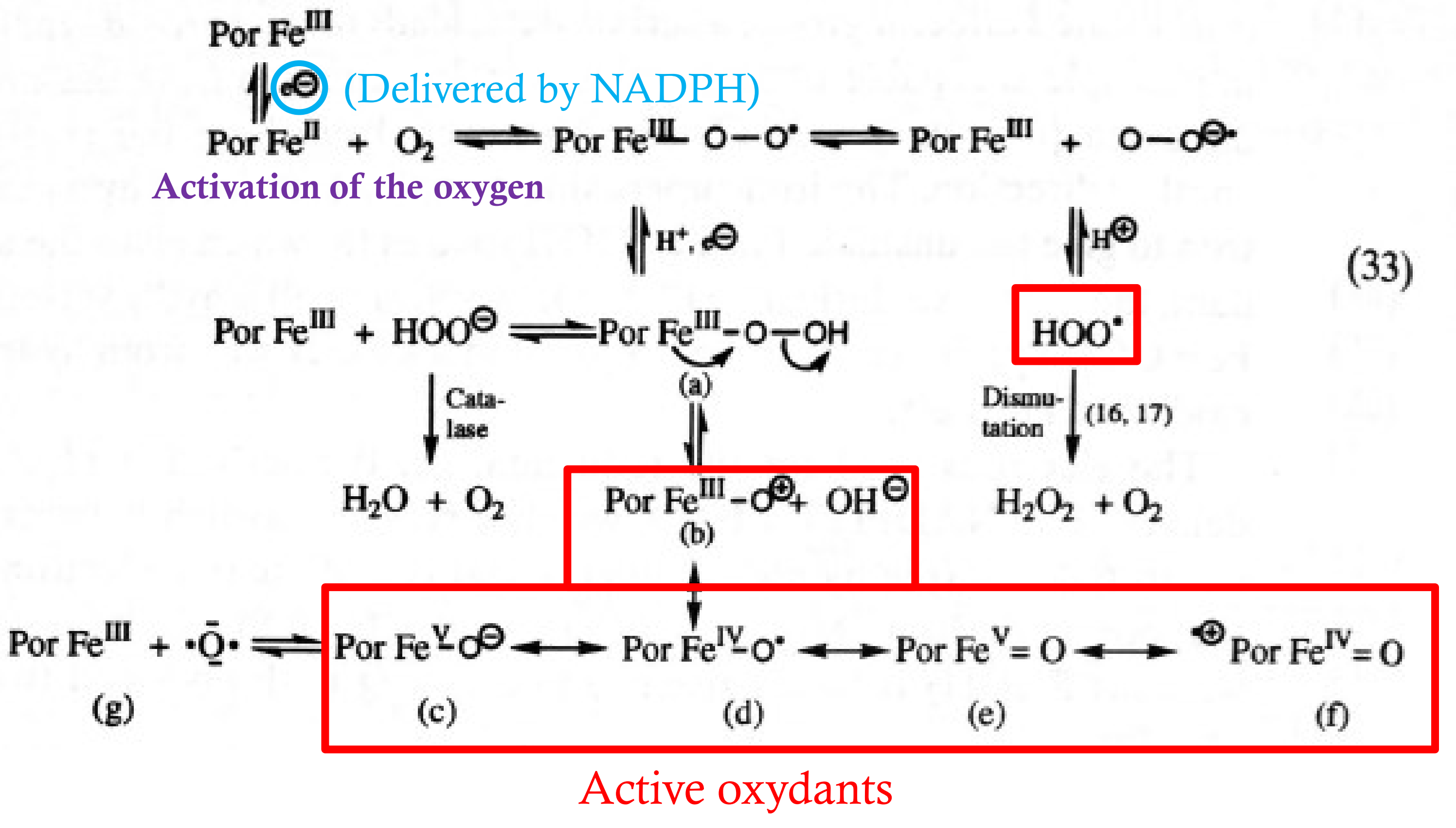


2-methyl-2-nitrosopropane is added as scavenger to a mixture of linolenic acid, oxygen and lipoxygenase.

It competes with oxygen for the radical.

Hydroxylation of non-activated aliphatics

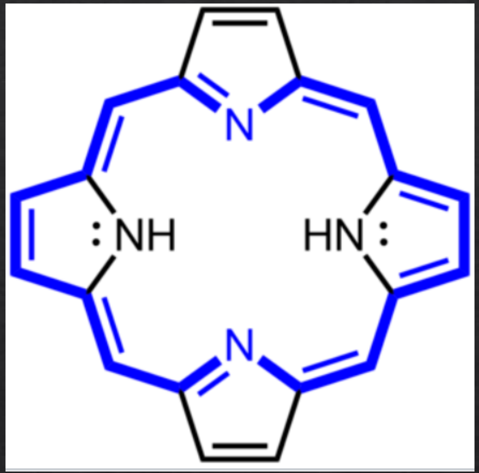
- Iron-porphyrin complexes are playing a major role.



(Delivered by NADPH)

Activation of the oxygen

Active oxydants



Exemple of porphyrin

$$\mathrm{PorFe^{IV}-O^\bullet + H_2O_2 \rightarrow PorFe^{IV}OH + HOO^\bullet}$$

PorFe^{IV} can break non-activated C-H bonds

Oxidation of aromatics

- Oxidation of aromatics happens during their detoxifying mechanism.
- The epoxide is just an intermediate, but can still be present in significant quantities, especially in some polycyclic aromatics.
- Epoxides of polycyclic aromatics display carcinogenic properties…

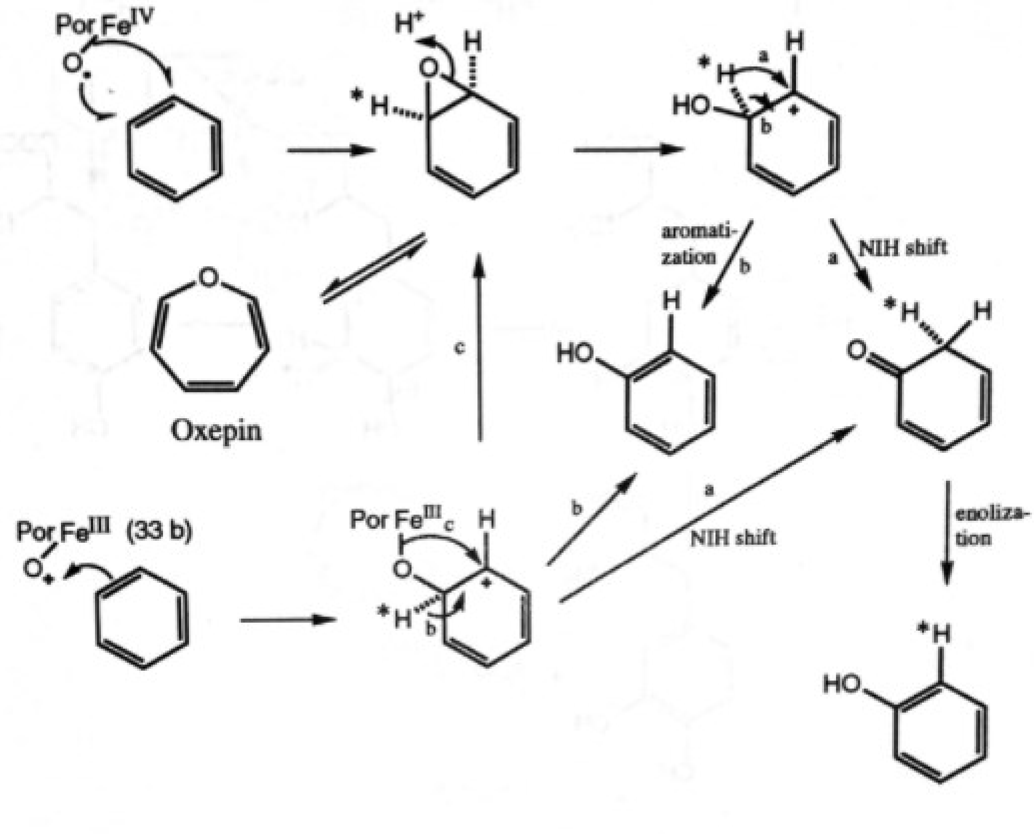
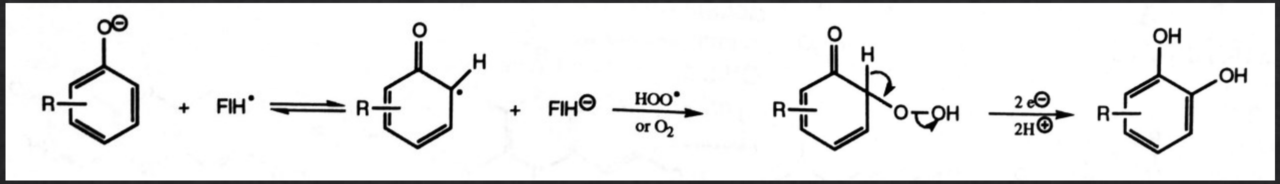


Fig. 8 Possible mechanistic pathways for P450 mediated aromatic hydroxylations

Flavin dependent oxygenases

- Flavins act as converters between 2 e^- oxidants (NAD^+), and 1 e^- oxidants ($PorFe^{3+}$)
- It is oxidized to its quinoid form by oxygen.
- The blue radical is also an active species in hydroxylation of phenols and indoles



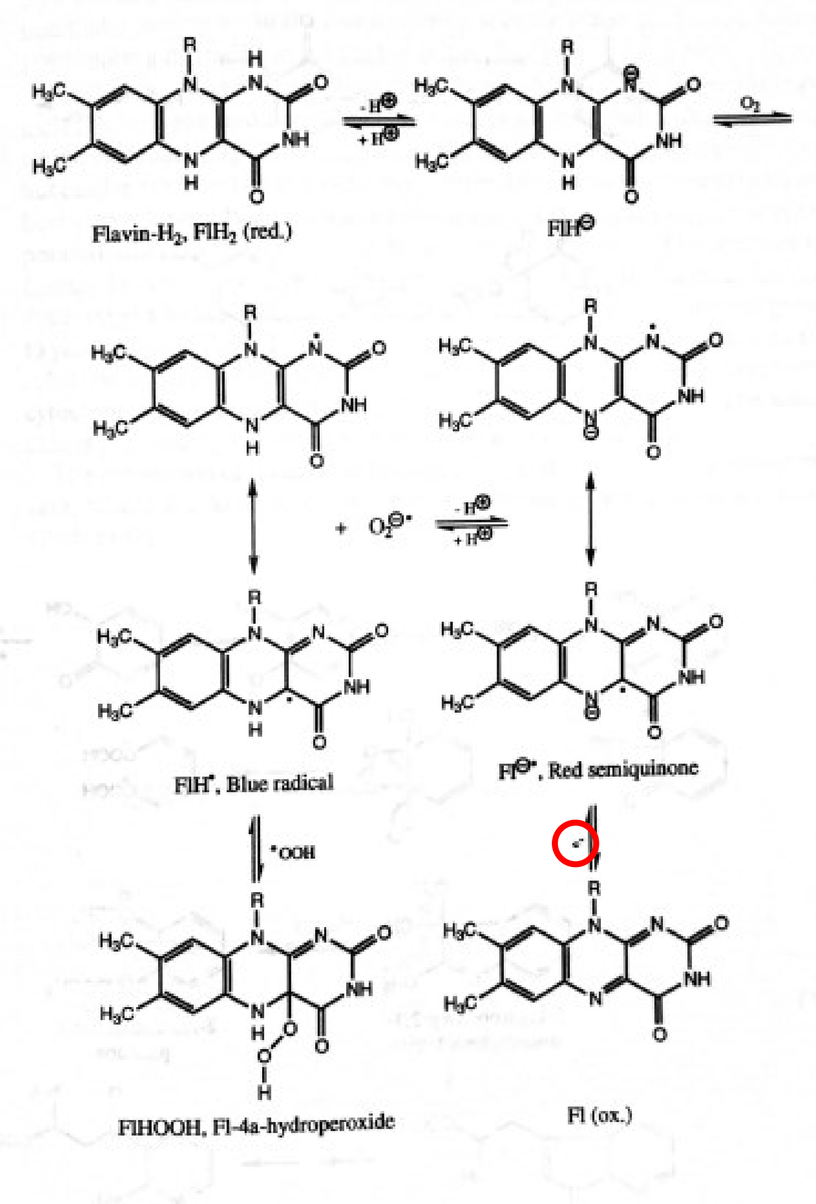


Fig. 11 Stepwise one-electron transfers; oxidation of flavin-H_2 by oxygen

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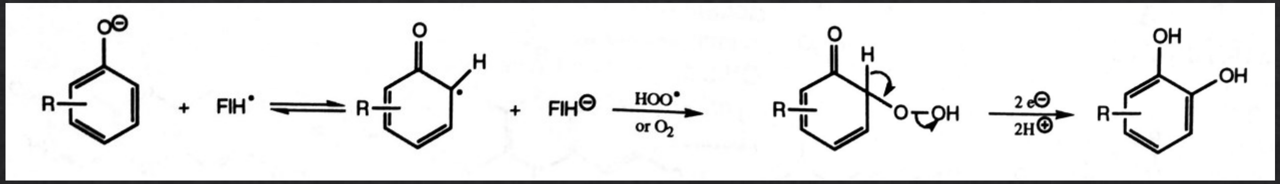
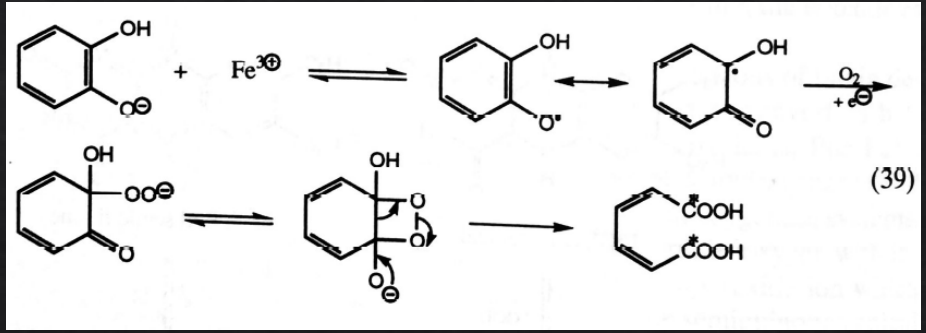


- This is part of the degradation of aromatics (phenol → catechol → ring opening):



(39)

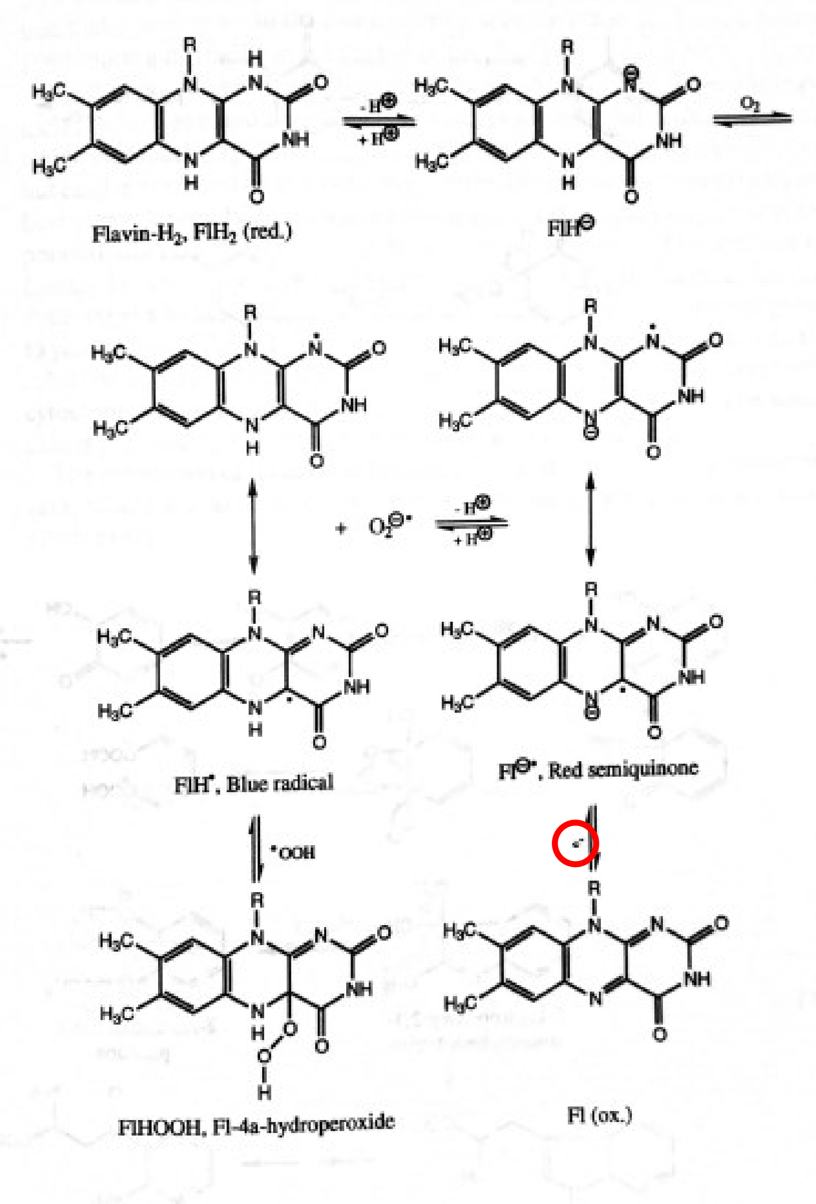
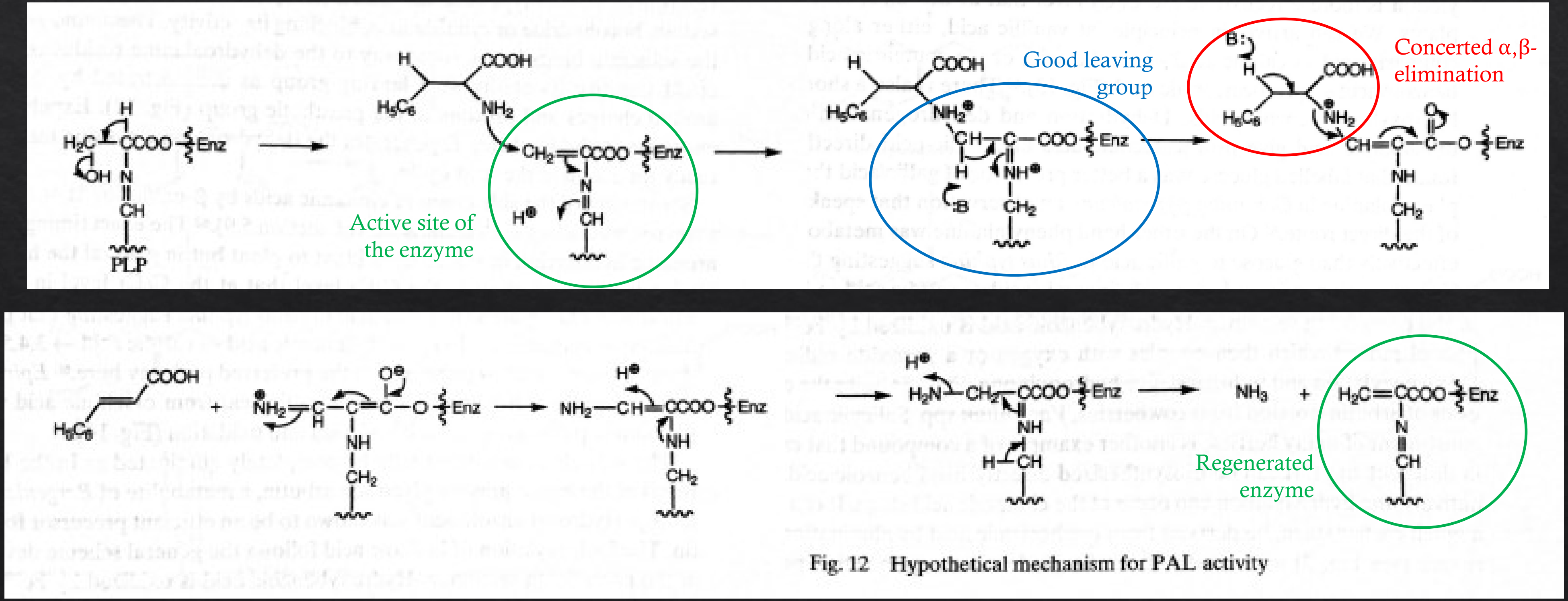


Fig. 11 Stepwise one-electron transfers; oxidation of flavin-H₂ by oxygen

Cinnamic and benzoic acid

- Cinnamic acids are widespread in nature and are formed from phenylalanine by enzymatic elimination of ammonia followed by aromatic hydroxylation and methylation.
- Enzyme: PAL (phenylalanine ammonia lyase)



Active site of the enzyme

Good leaving group

Concerted α,β-elimination

Regenerated enzyme

Fig. 12 Hypothetical mechanism for PAL activity

Biosynthetic network of cinnamic and benzoic acids

- The starting material is phenylalanine.
- Different paths are available for the synthesis of, for instance, vanillic acid:
  - Passing by coumaric acid
  - Or by benzoic acid

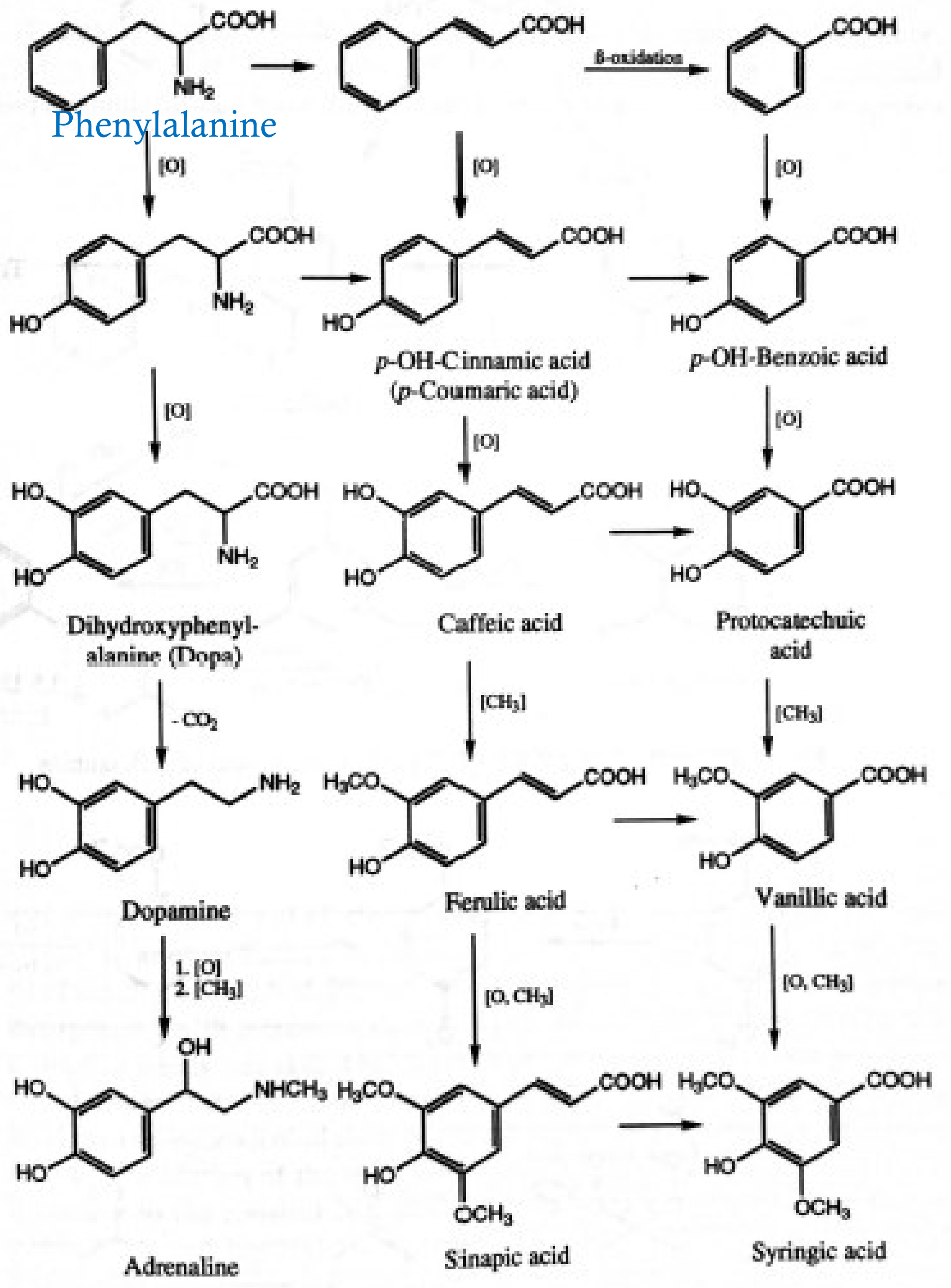


Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

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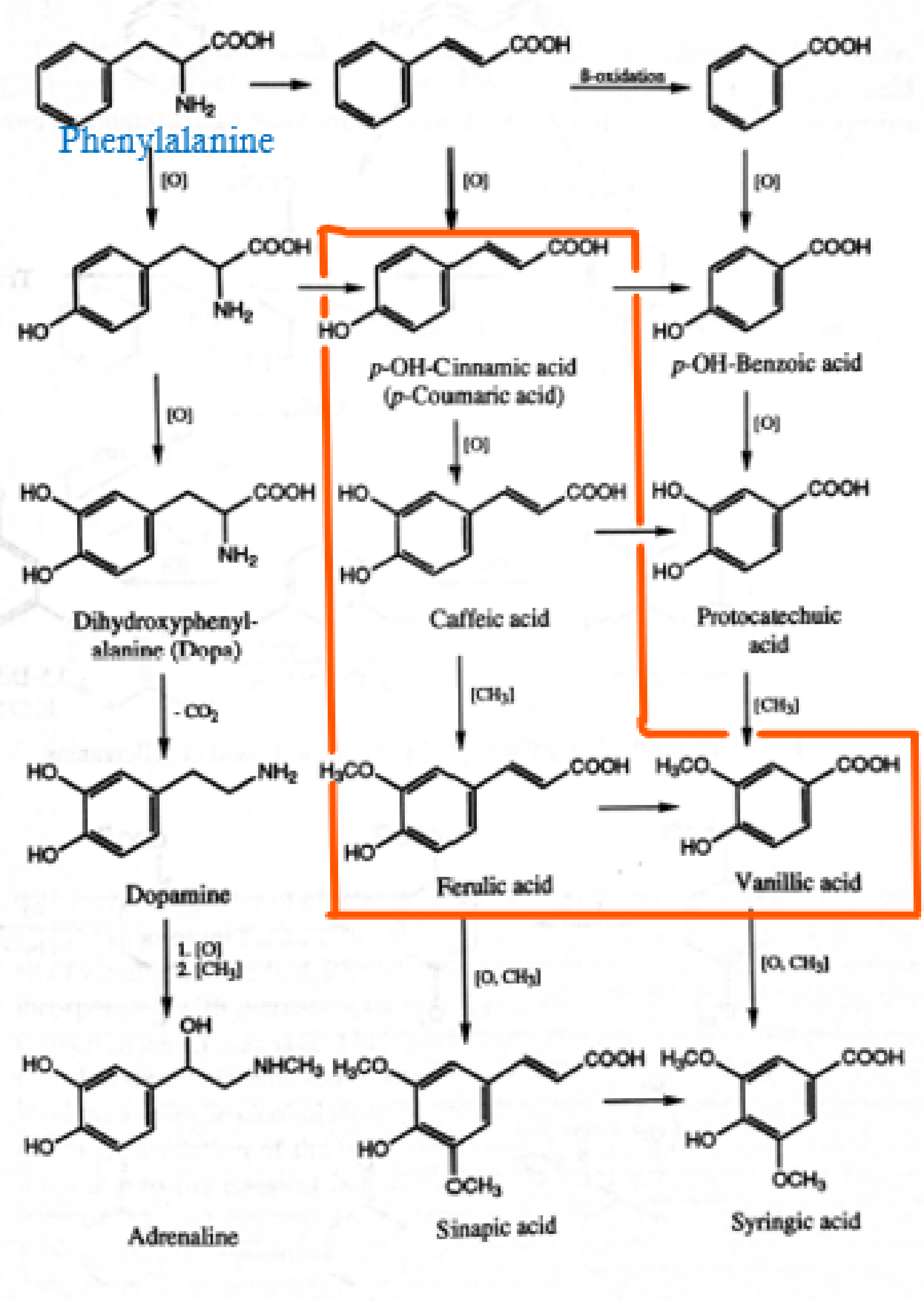

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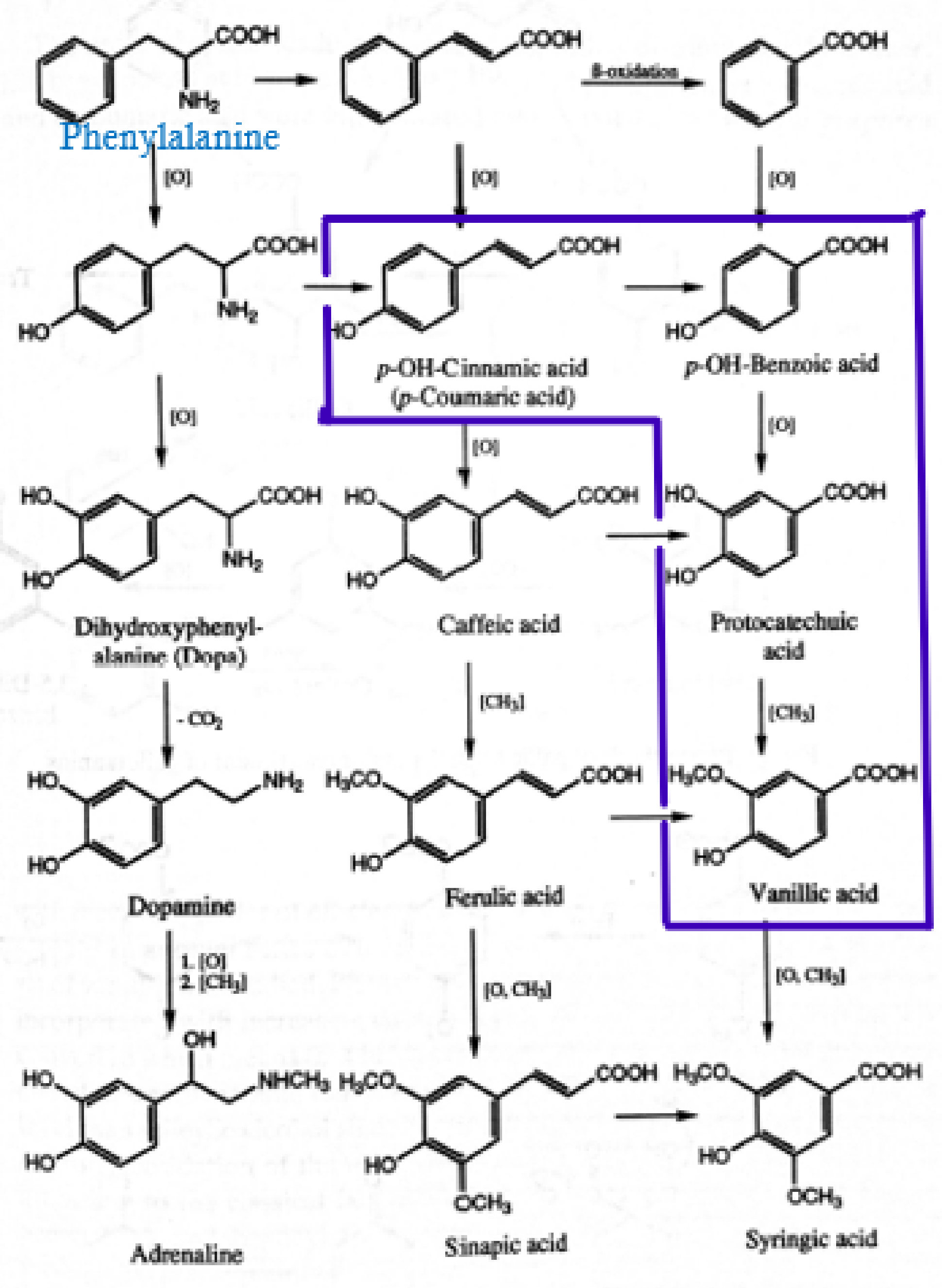


Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

Biosynthetic network of cinnamic and benzoic acids

- Dehydration and dehydrogenation of 3-dehydroshikimic acid leads directly to gallic acid.
- Glucose is a better precursor of gallic acid than phenylalanine in *Geranium pyrenaicum*, but not in *Rhus typhina*

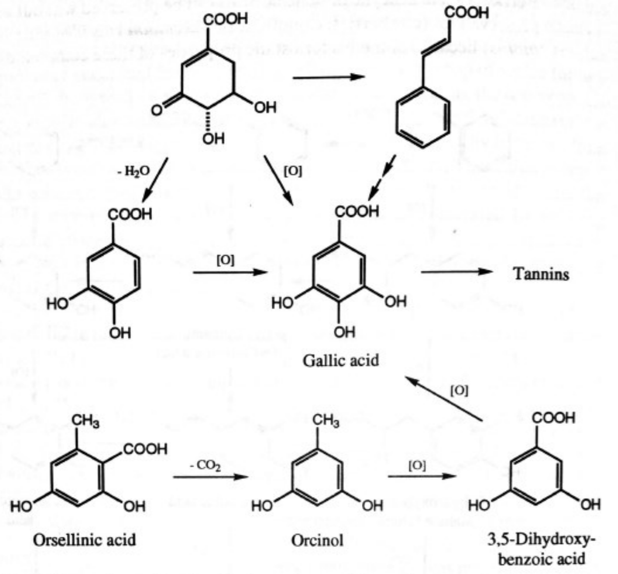


Fig. 14 Biosynthesis of gallic acid, the major constituent of gallotannins

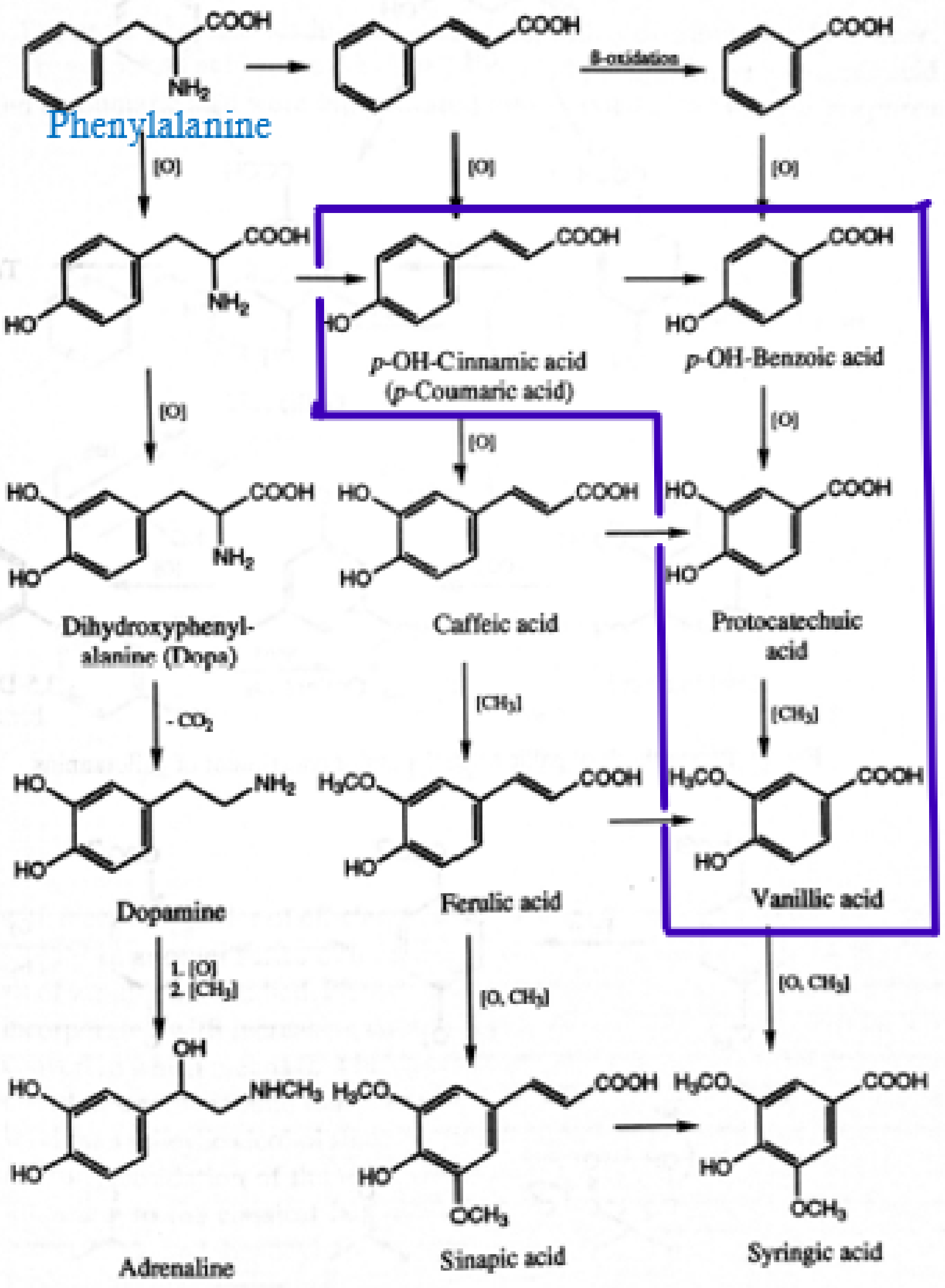


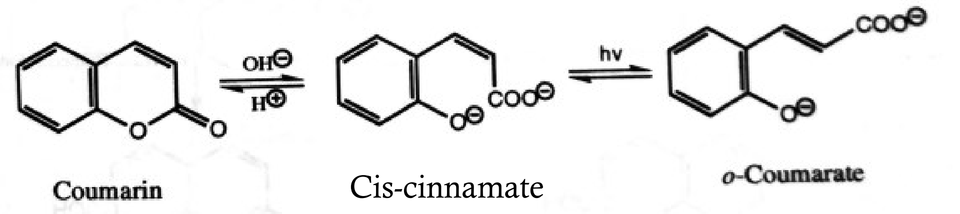
Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

Cinnamic and benzoic acid derivatives

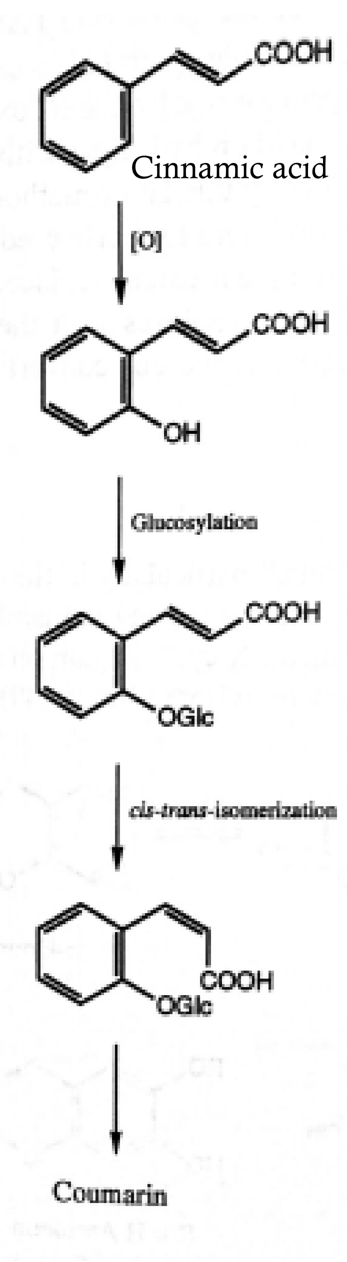
- In Vanilla planifolia, phenylalanine, cinnamic acid, and ferulic acid were better substrate to vanillin than vanillic acid.
- In higher plants, free benzoic acids are poorly reduced.

Coumarins

- Coumarins are lactones which open on treatment with base and cyclize again on acidification. Irradiation causes cis-trans isomerization of the cis-cinnamate.

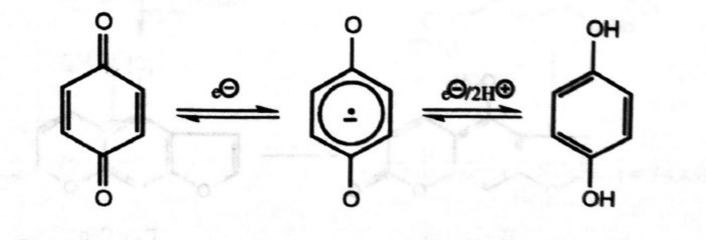


- Coumarins derive from shikimic acid via cinnamic acids.



Quinones

- Quinones include some pigments, antibiotics, coenzymes, and vitamin K.
- They can serve as one-electron transfer agents.



- Quinone biosynthesis are very diverse, and two structurally resembling quinones can have very different origins.

Plumbago europaea

From the polyketide pathway

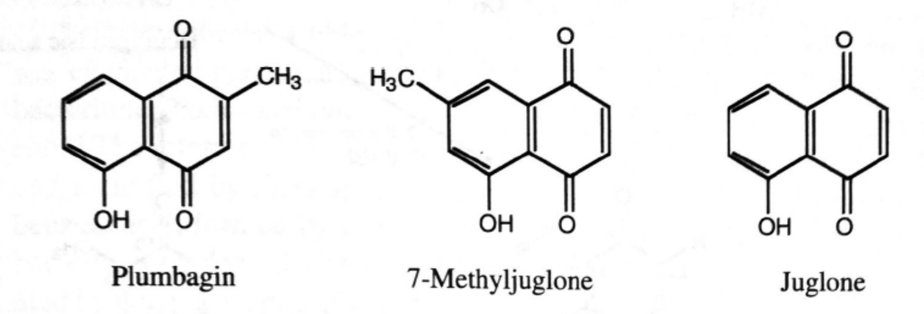


Fig. 20 Compounds of similar structure but of different biogenesis

From skikimic acid

Juglans regia

Quinones

- In bacterias, p-Hydroxybenzoic acid is obtained by elimination of pyruvic acid from chorismic acid
- In plants and mammals, it is obtained by degradation of phenylalanine.

- Vitamin E is structurally related to plastoquinones

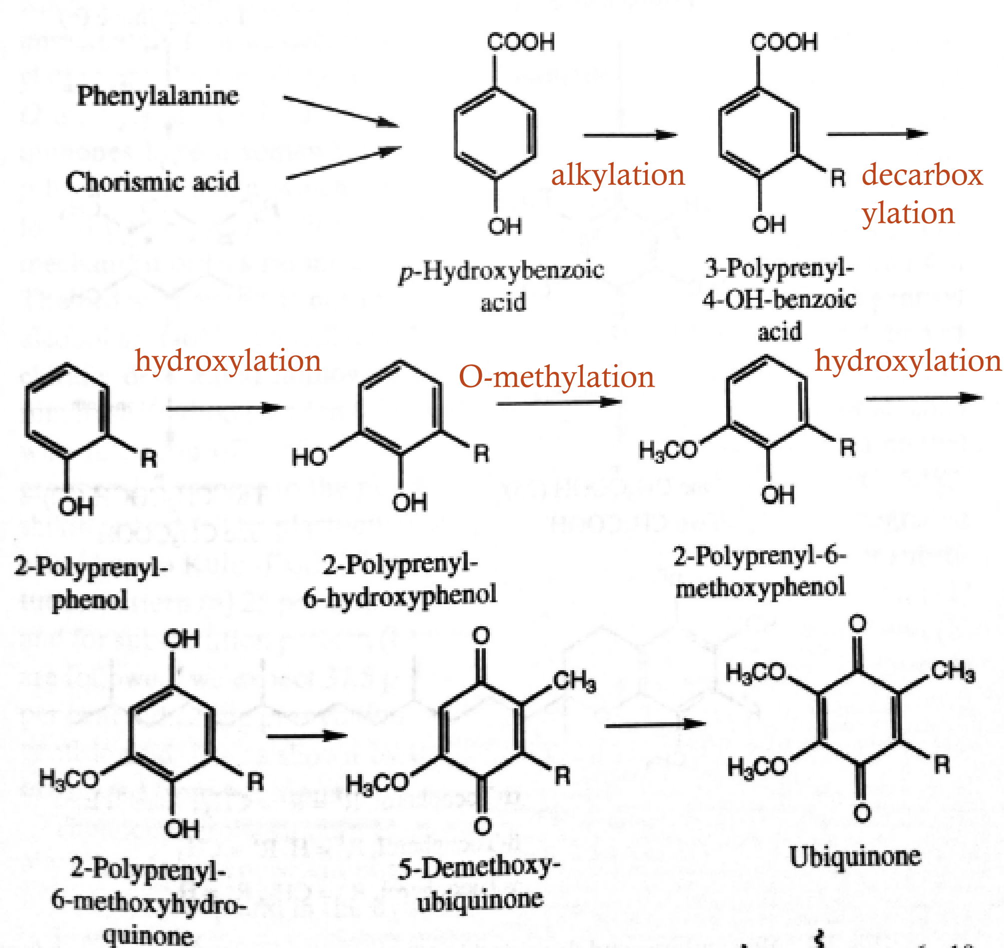
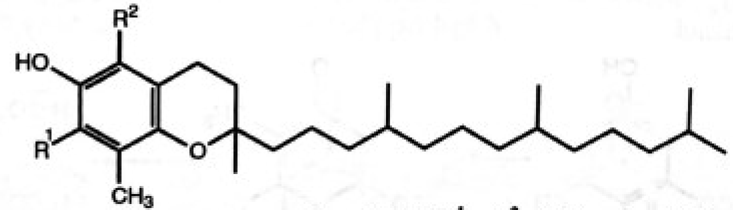
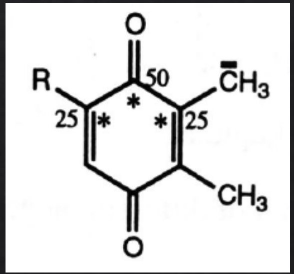


Fig. 21 Biosynthesis of ubiquinones. R = polyprenyl chain, , n = 6 - 10



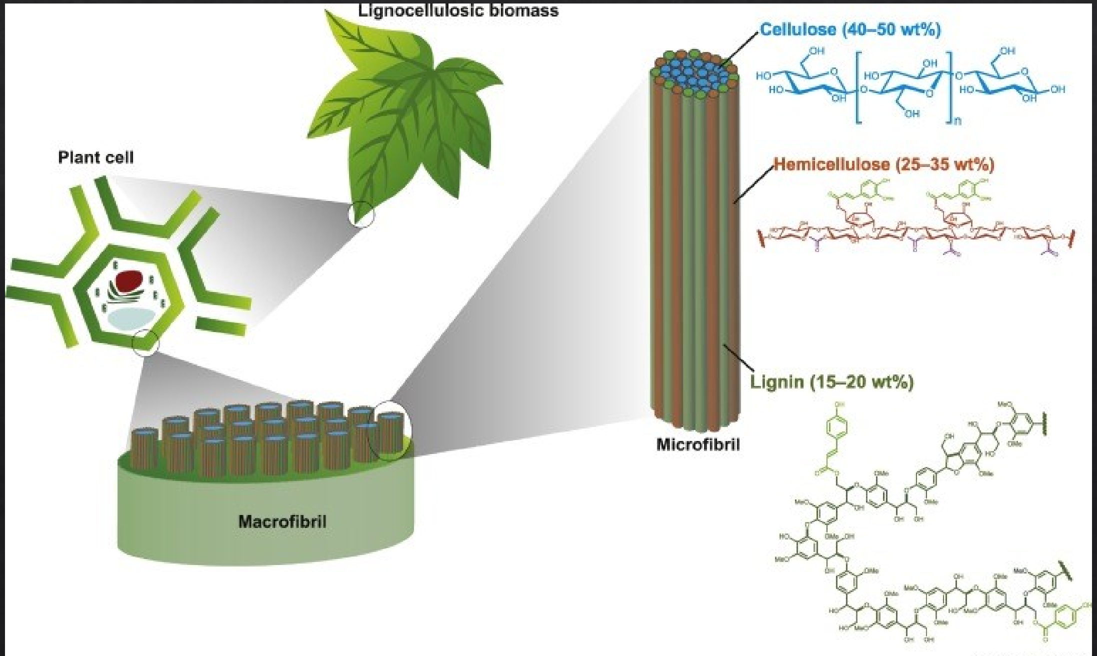
α-Tocopherol, $R^1 = R^2 = CH_3$, vitamin E
β-Tocopherol, $R^1 = H$, $R^2 = CH_3$
γ-Tocopherol, $R^1 = CH_3$, $R^2 = H$



Example of plastoquinone (derives from tyrosine)

Lignin constituents

- Lignin is a polymeric network of aromatic building blocks present in all woody tissues.
- It acts as a matrix for the cellulose fibers and allows strength and stability of the cell wall.



Trends in Chemistry

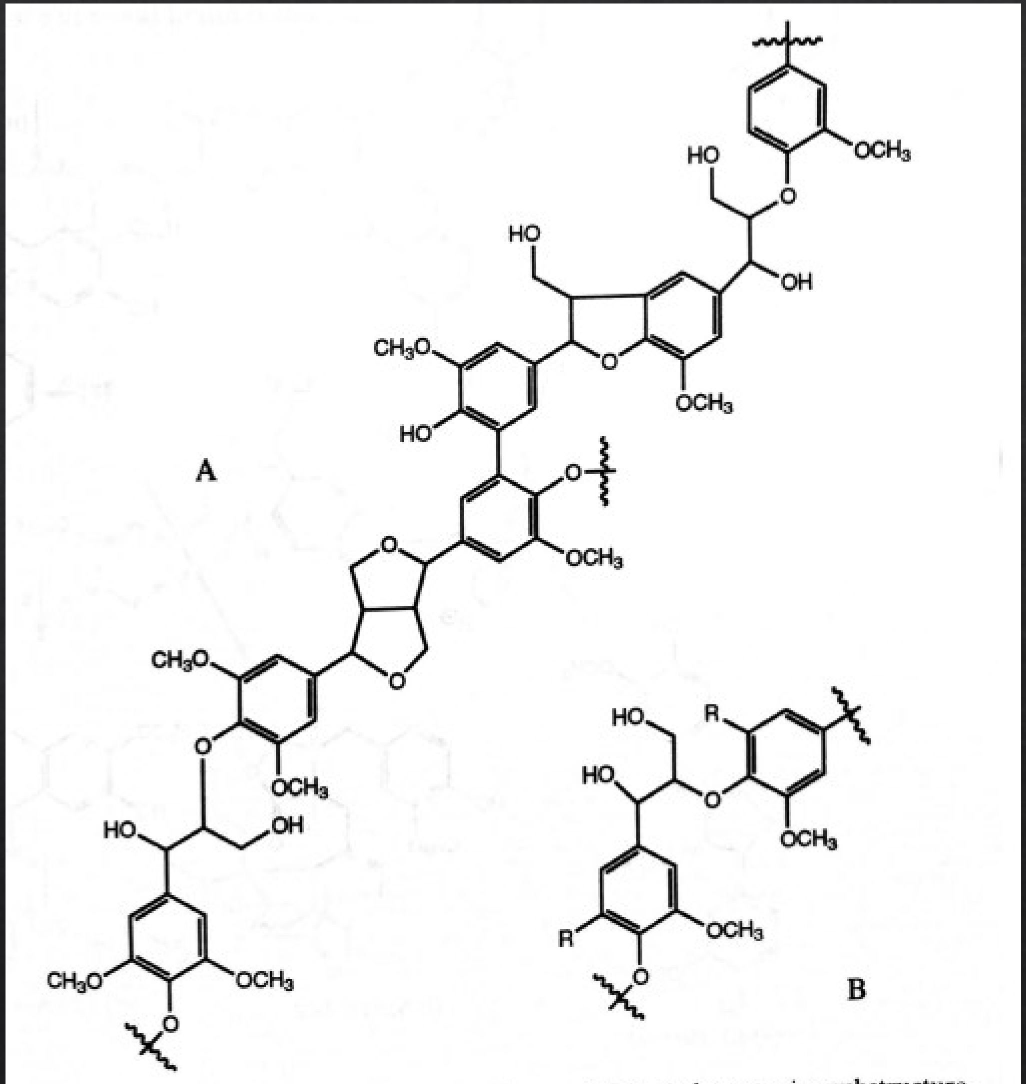
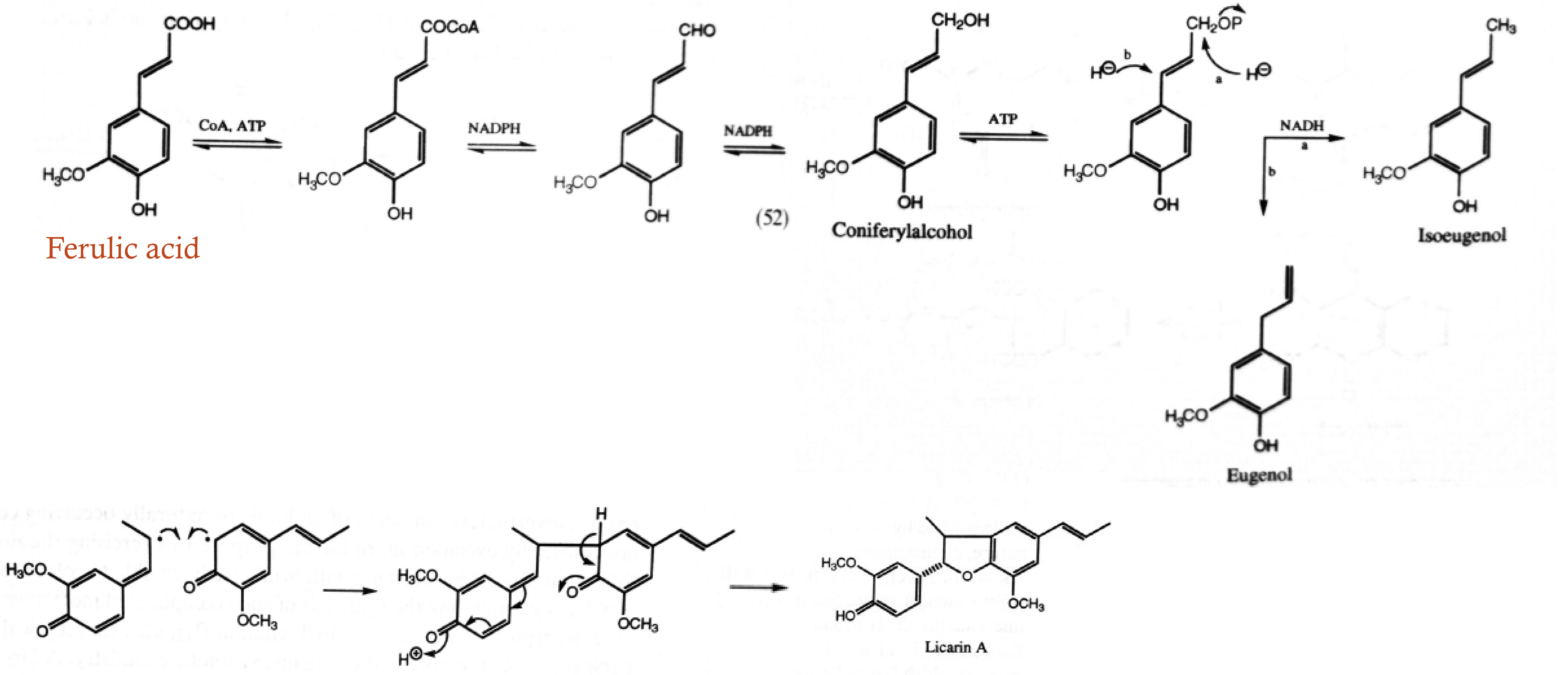


Fig. 26 A. Hypothetical lignin model. B. The most frequently occurring substructure. R = H, OCH_3

Lignin constituents



Synthesis of natural products in the lab

- The use of cheap optically active starting material, like sugars, is of importance.

- Shikimik acid can be synthesized from the easily available D-arabinose.
- Shikimik acid contains 3 asymmetric centres, with the same absolute configuration as the 3 asymmetric centres of D-arabinose.

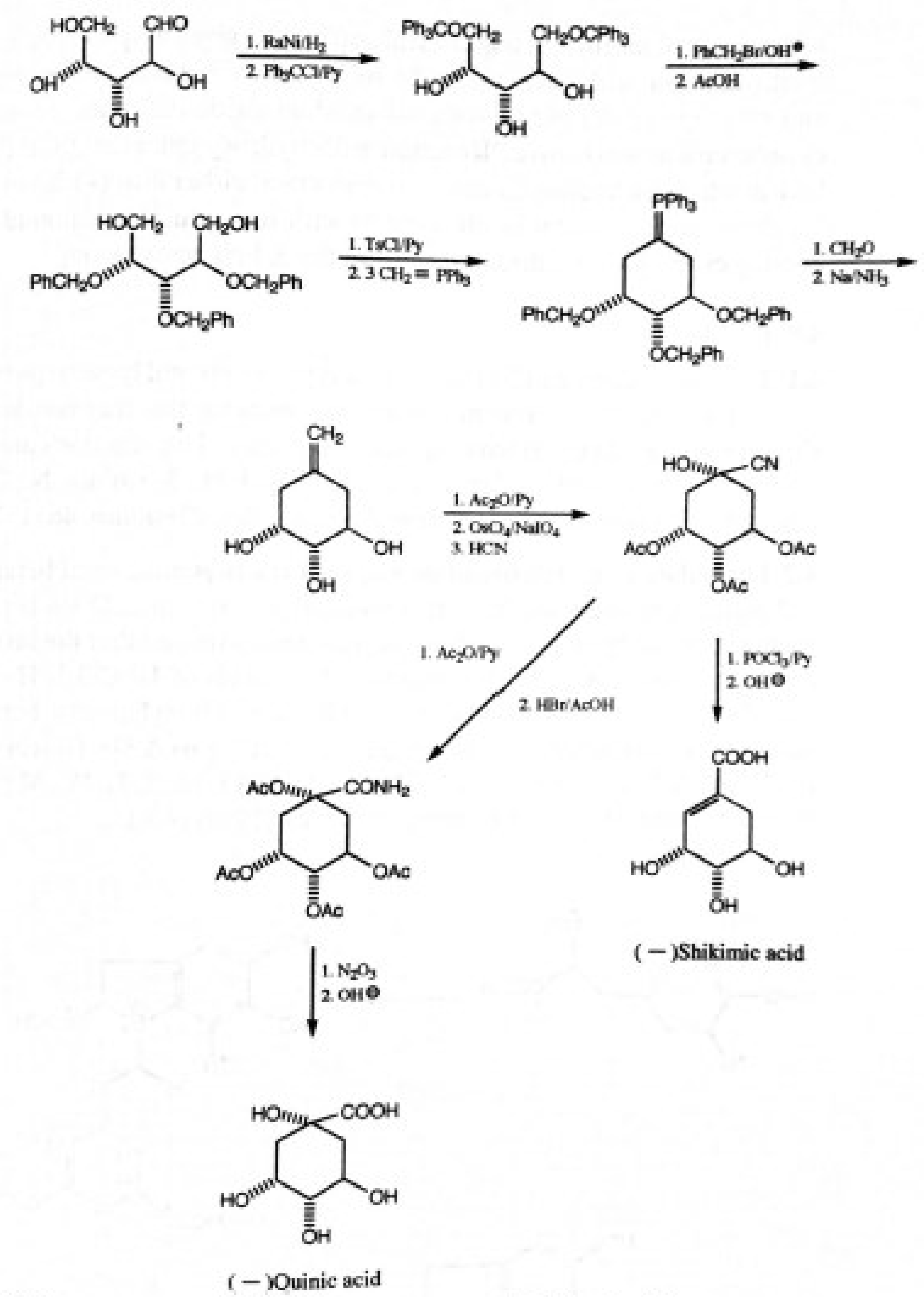


Fig. 29 Total synthesis of (−)quinic acid and (−)shikimic acid

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Chapter 5-2
The polydetide pathway

Elisabeth Jacobsen and Lucas Boquin, NTNU

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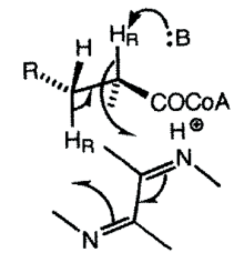
β-Oxidation:
How the body uses the energy stored in fatty acids

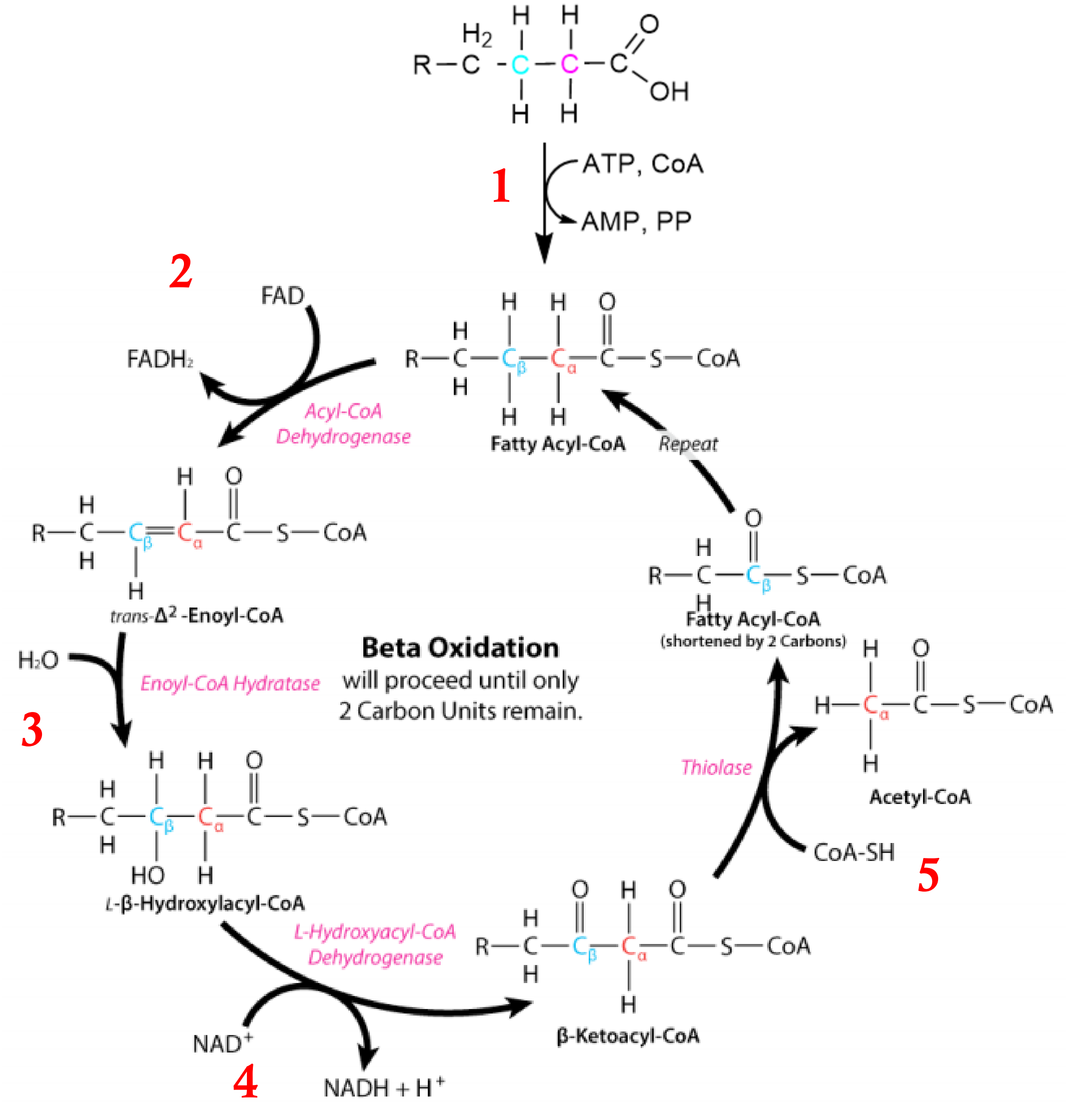
- 1- The fatty acid in transported into the mitochondria and is esterified with CoA
- 2- Dehydrogenation (anion formation, oxidation by FAD, final H abstraction)



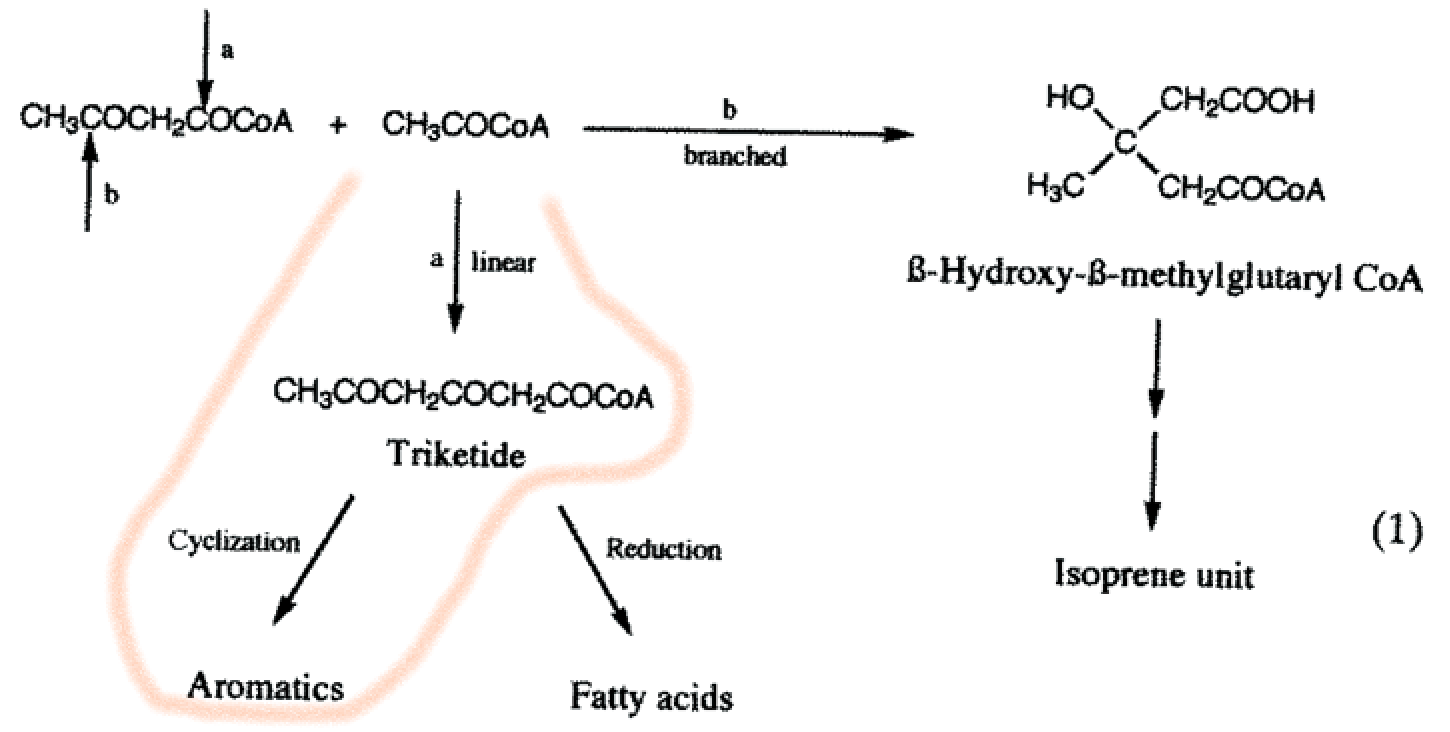
- 3- Stereospecific hydration
- 4- Oxidation by NAD+ to a keto fatty acid
- 5- Thiolysis by another CoA molecule

Overall equation (for one cycle):

$$C_{15}H_{31}COCoA + FAD + NAD^{\oplus} + CoA + H_2O \rightarrow$$
$$C_{13}H_{27}COCoA + FADH_2 + NADH + CH_3COCoA + H^{\oplus} \qquad (30)$$



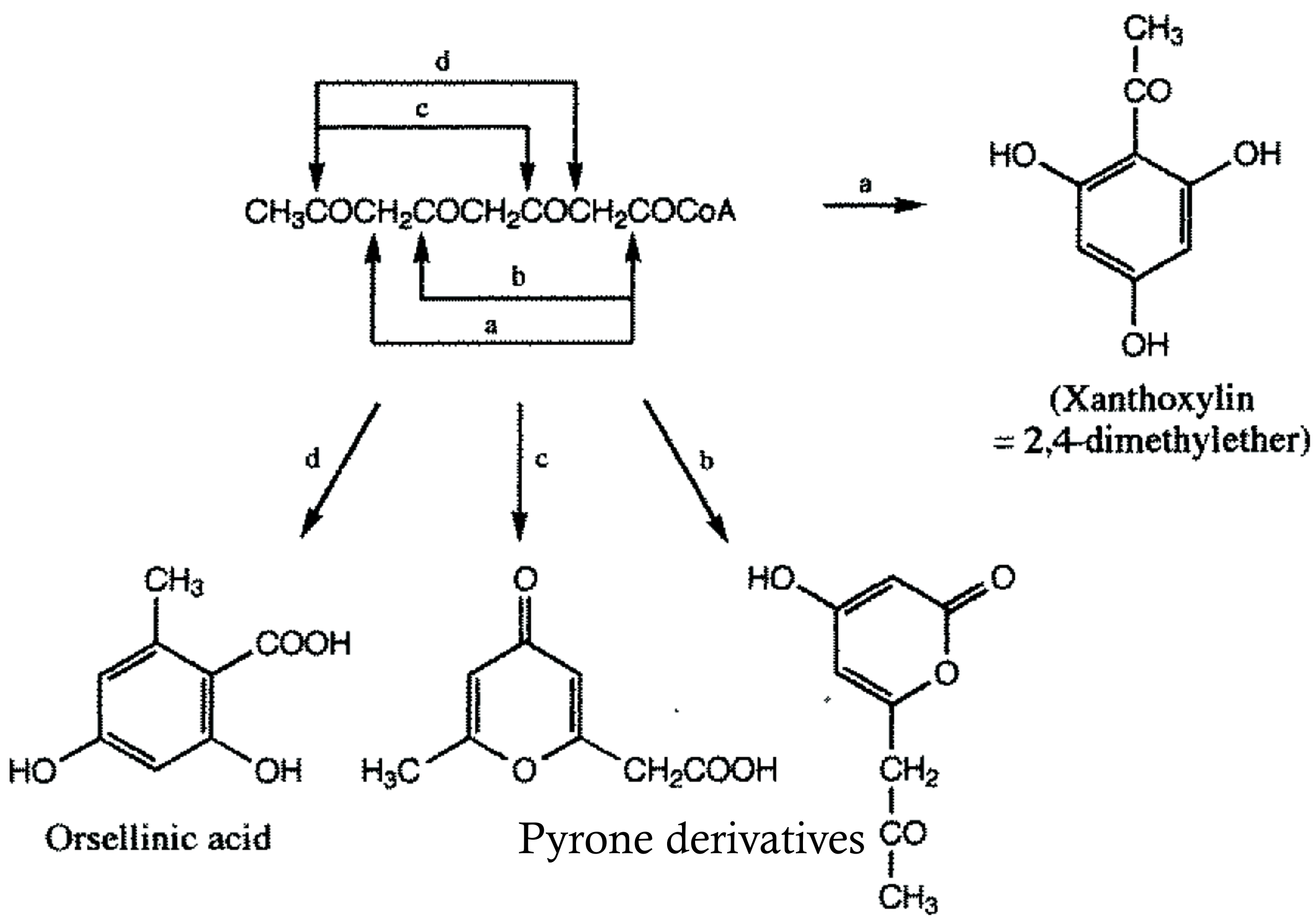
Formation of aromatics from polyketides



- Further condensations before reduction lead to the very reactives β-polyketoesters. They can be temporarly stabilized by bonding on an enzyme for further building.
- They undergo cyclization, leading to aromatics.

Formation of aromatics from polyketides

- Tetraketides can cyclize in different ways:

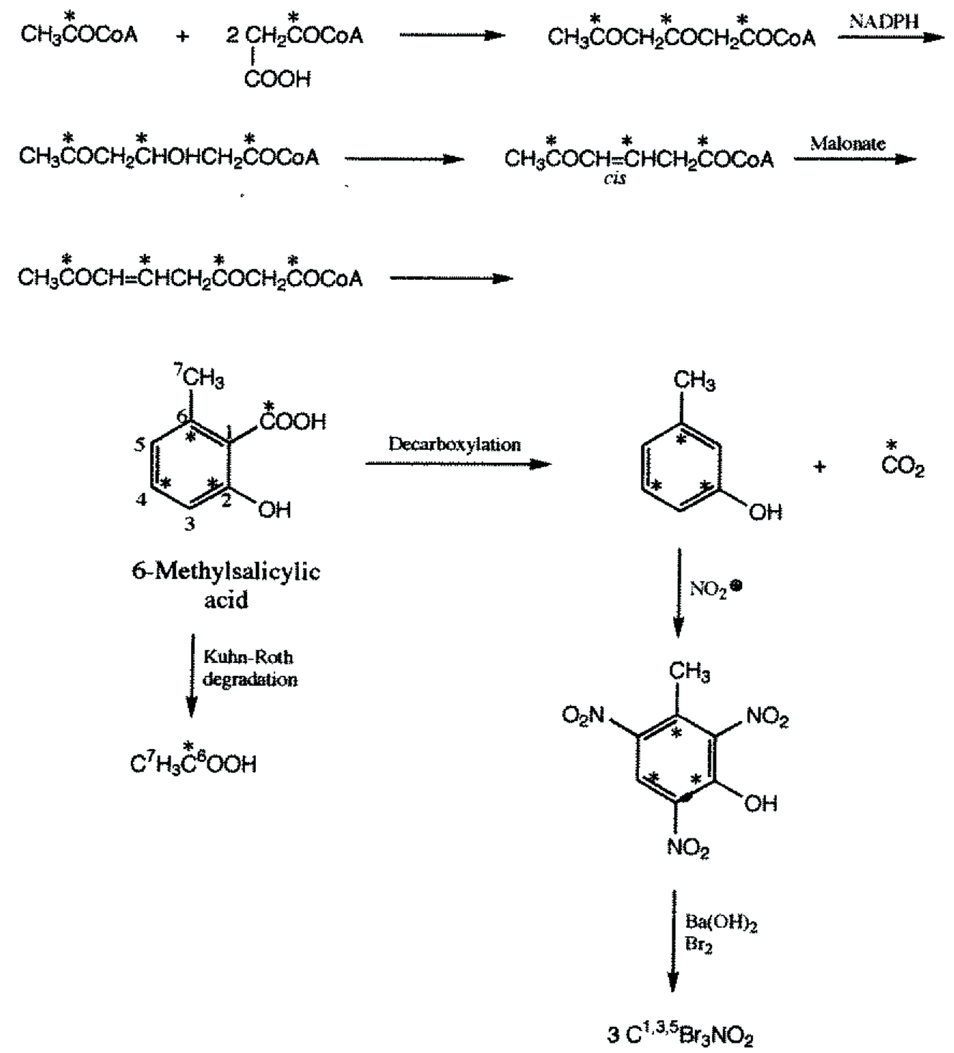


A lot of metabolites can be synthesized, depending on:

- The chain initiating unit
- The number of acetyl CoA involved
- The mode of cyclization
- The condensation of separately synthesized polyketides
- The secondary processes (halogenation, alkylation, redox reactions,...)

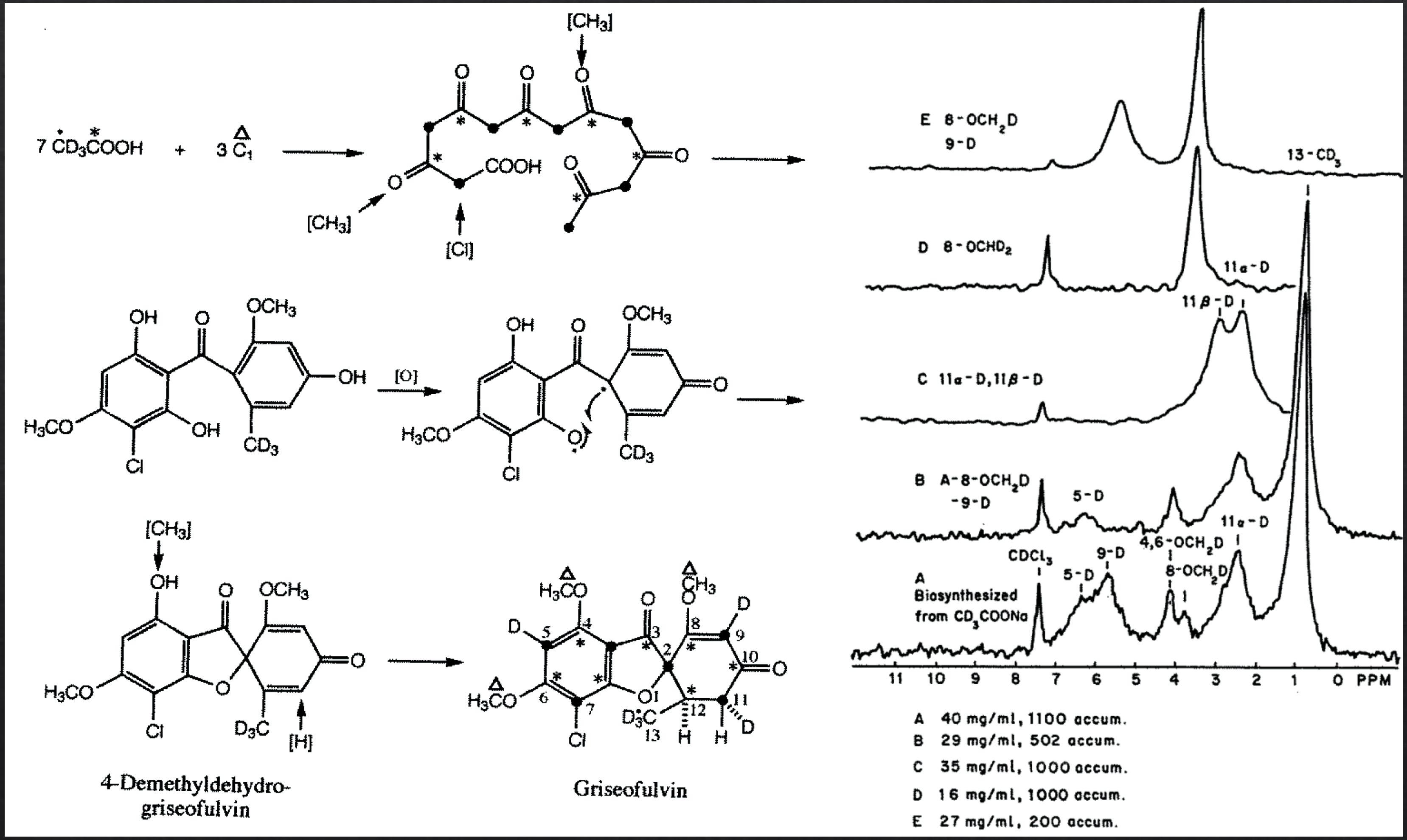
Confirmation of the acetate hypothesis

- Birch was the first to formulate the acetate hypothesis.
- First confirmations:
- By feeding the plant with marked starting materials, and then chemically degrading the product.
  - The Kuhn-Roth degradation allow us to analyze C6 and C7 activity.
  - The other pathway allow us to verify the absence of activity on carbons 1,3 and 5, and the activity of the CO_2 released.



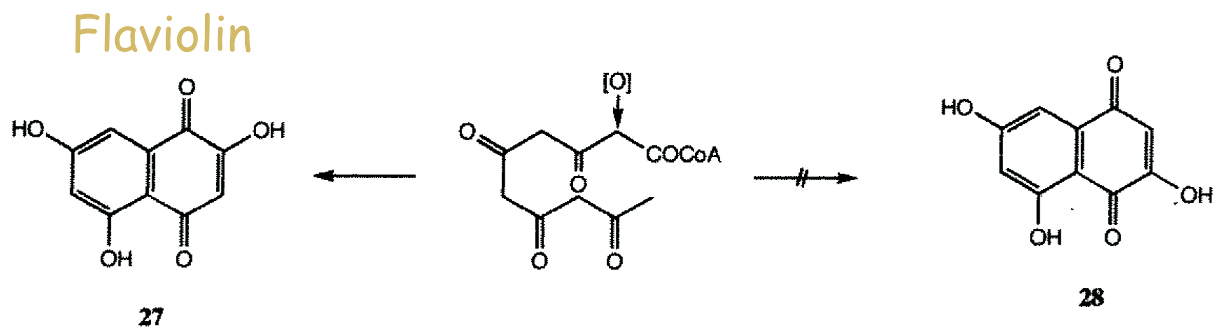
Confirmation of the acetate hypothesis

- Chemical degradation is a very complex process, especially for big molecules.
- Today, ^{2}H-,^{3}H- and ^{13}C- labelled compounds can be synthesized, and analyzed by NMR.
  - ^{3}H-NMR: Slightly negative NOE effect, which can allow us to measure the isotopic content in a molecule.
  - ^{2}H-NMR: Inexpensive, stable, low natural abondance (shorter relaxation time), no NOE, but low sensitivity
  - ^{13}C-NMR: Stable, but higher natural abondance and low sensitivity. Some techniques can increase signal intensity and maintain some hydrogen coupling information (off-resonance technique)



Derivation of structure

- Usually, aromatic products coming from polyketide have a pattern of meta-hydroxy substitution. Those coming from shikimic acid have ortho-hydroxy substitution. Structure can often help to determine the origin of a molecule, even if it is not always accurate.
- Two structures of Flaviolin were considered, and one intermediate was known. Structure 28 is not likely to be the good one.

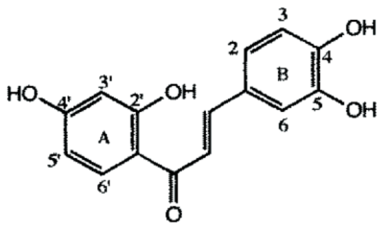


Flavonoids

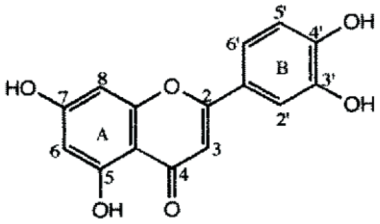
Flavonoids are coloring substances present in flowers and fruits.


Dahlia

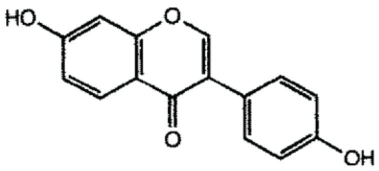

Soybeans (daidzu (大豆) in japanese)



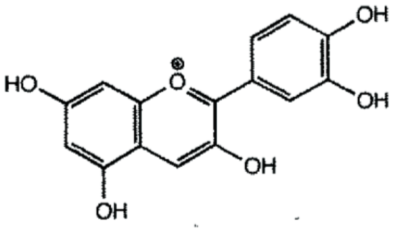
Butein
(Chalcone)



Luteolin
(Flavone)



Daidzein
(Isoflavone)



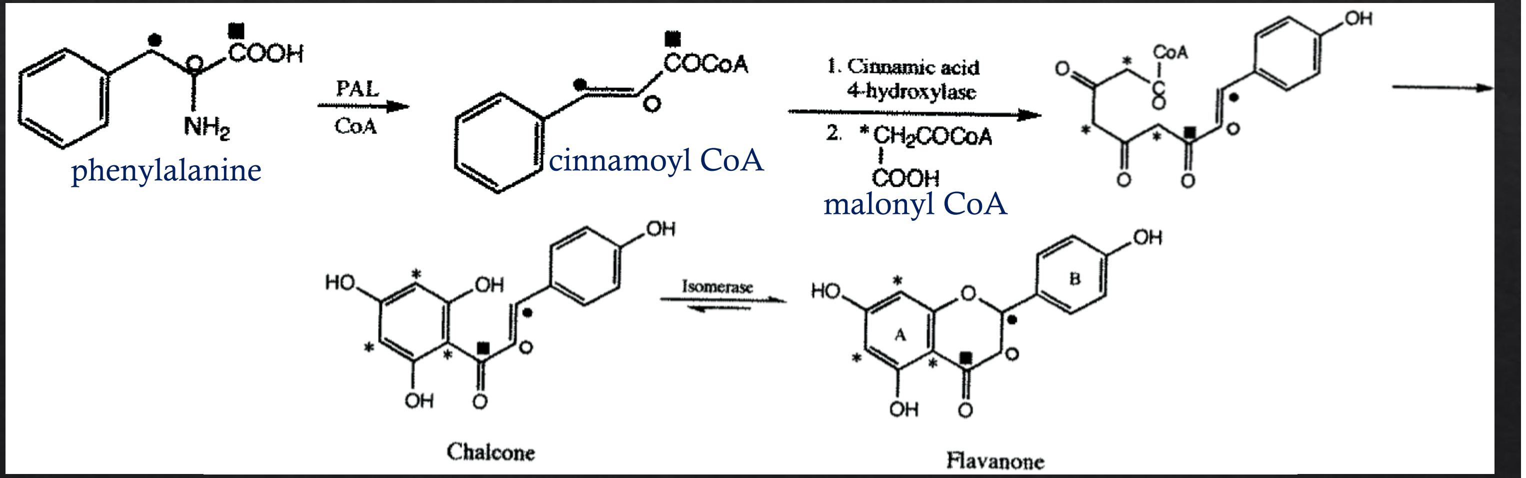
Cyanidin
(Anthocyanidin)


Reseda luteola

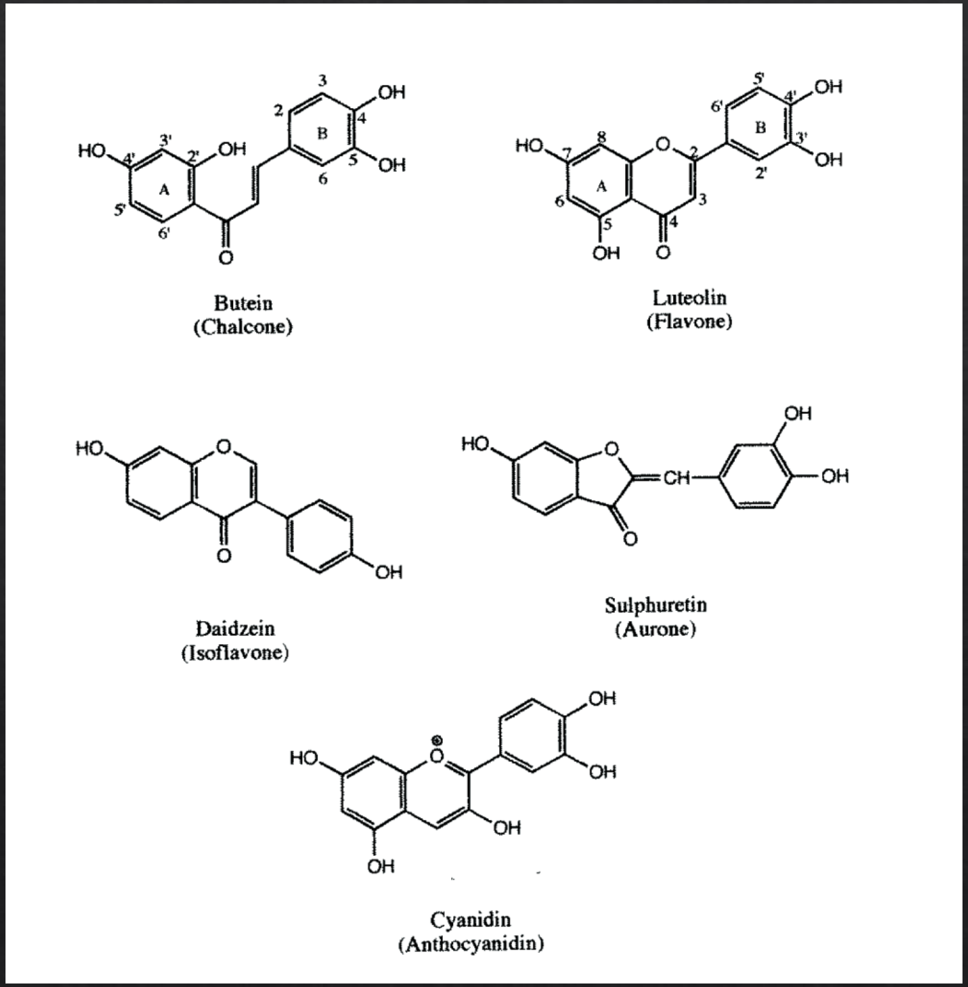

Ipomoea purpurea

Flavonoids

- Flavonoids are coloring substances present in flowers and fruits.
    - Flavones: yellow or orange
    - Antocyanidins: Red, purple or blue
- They are composed of two hydroxylated aromatic rings, A and B, joint by a three carbon fragment.

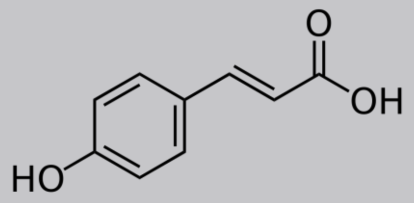


Biosynthesis of the basic flavone skeleton

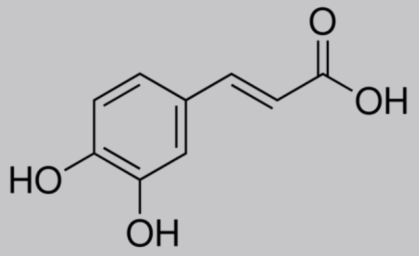


Flavonoids

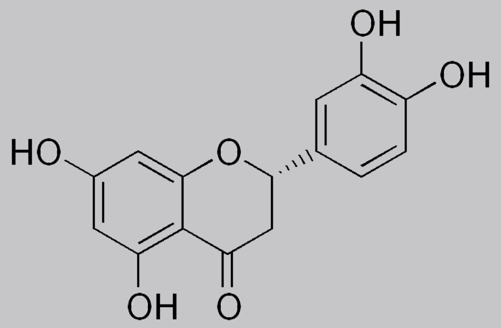
- In *Haplopappus gracilis*, p-hydroxycinnamic acid is used efficiently at pH 8 to synthesize eriodictyol, but at pH 7, caffeic acid is preferred.



p-Hydroxycinnamic acid



Caffeic acid

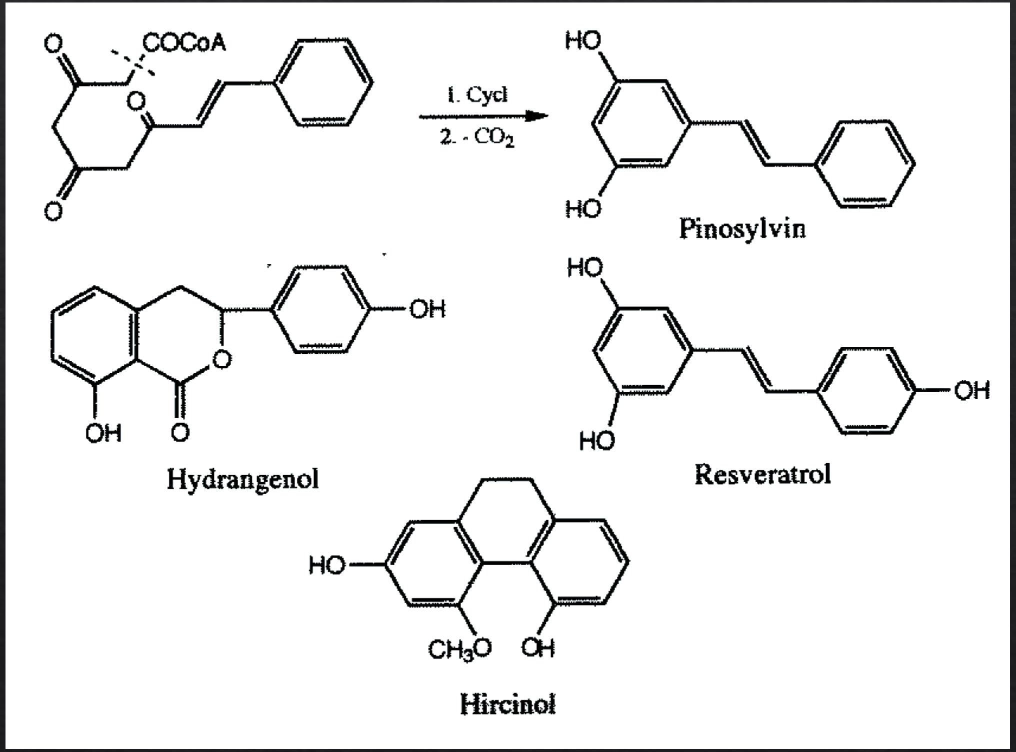


Eriodictyol

Stilbenes

- Stilbenes are structurally close to flavonoids, and their biosynthesis results from a slightly different folding of the polyketide. They are often formed by plants in response to microbial attack or stress.

Hydrangea macrophylla (hortensia)

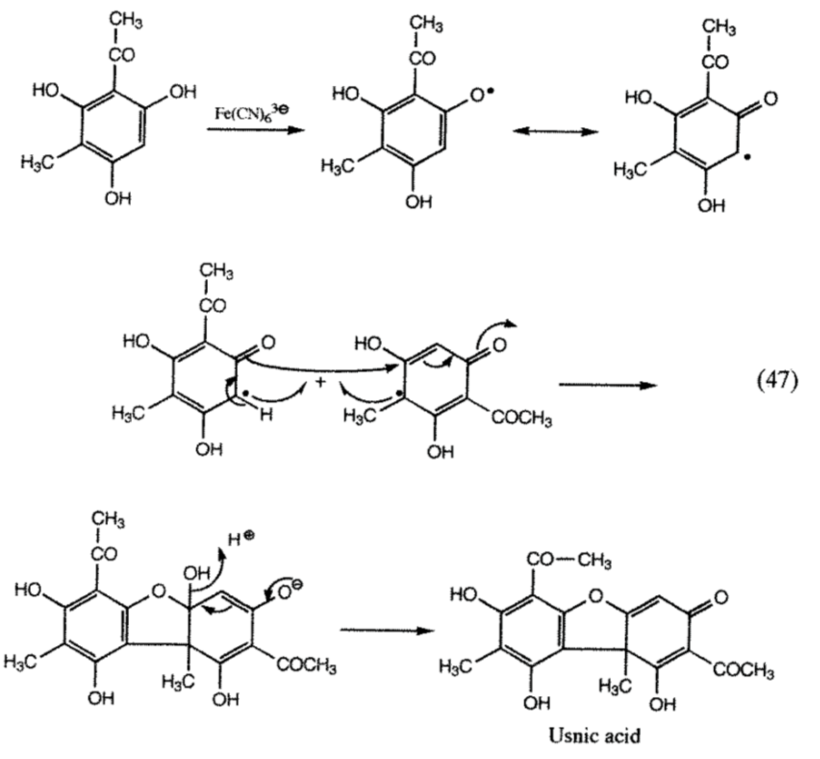


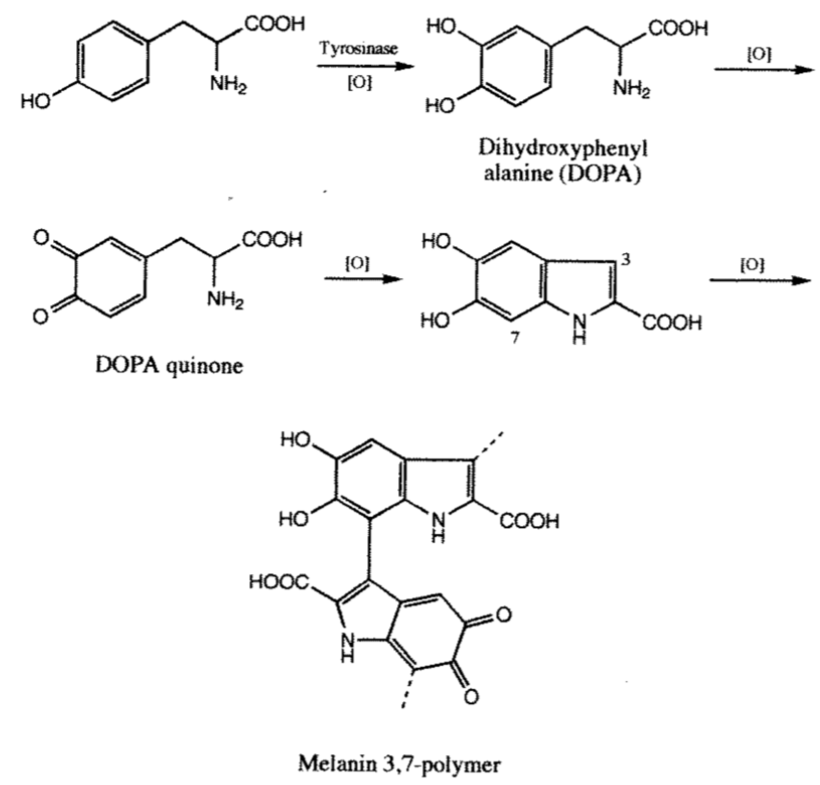
Norway spruce (Gran på norsk)

Pinosylvin: Present in pine trees. Fungitoxin protecting the wood from fungal infection.

Oxidative coupling of phenols

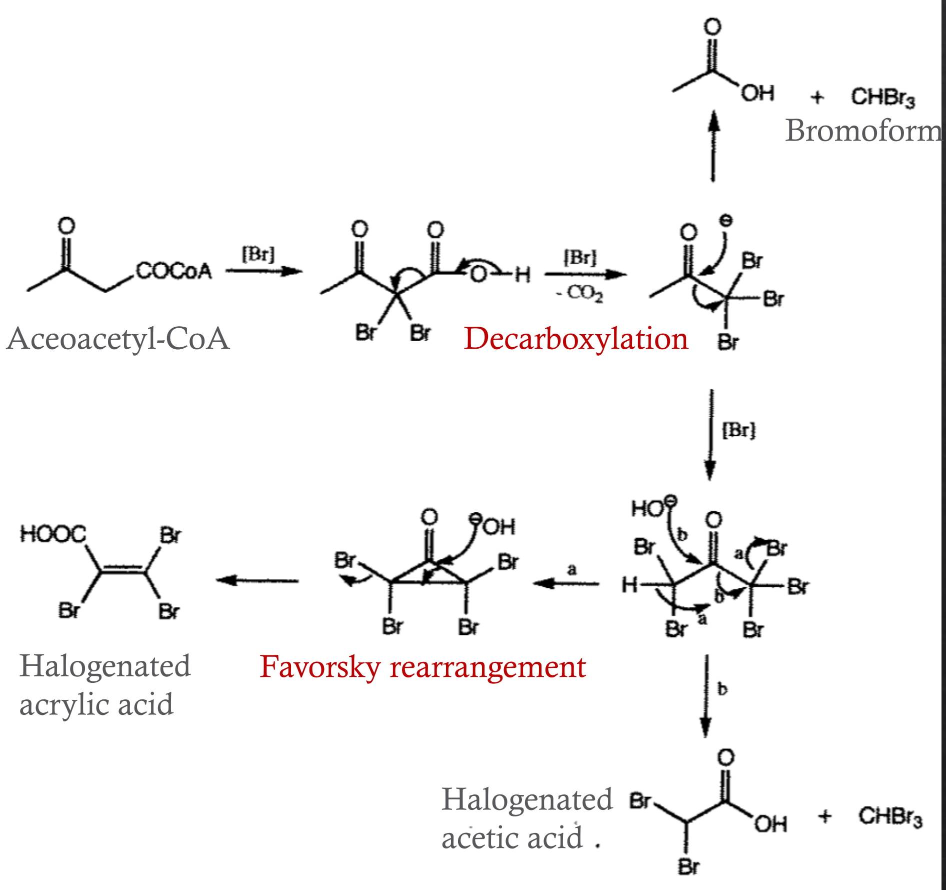
- Some enzymes can catalyze oxidative coupling of phenols, by one-electron transfers. For instance, melanine is formed by enzymatic oxidation of tyrosine.





Halogen compounds

- Iodine, fluorine and bromine compounds are extremely rare in nature.
- Chloro compounds are more common, biosynthesized mainly by microorganisms and marine organisms.
- Halogenation has a potentiating effect on biological activities of a lot of compounds.
    - Chloromycetin has a 100-times more antibacterial effect than the acetyl analogue.
- The capability of seaweeds to accumulate bromine and iodine, and the low oxidation potential of these halogens, explains the occurrence of some iodine and bromide-containing metabolites.



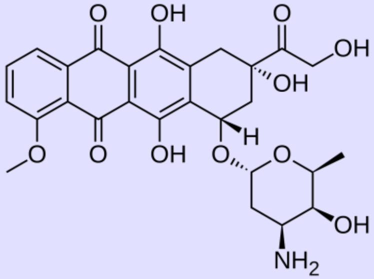
Chapter 5-1
The Polyketide Pathway

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

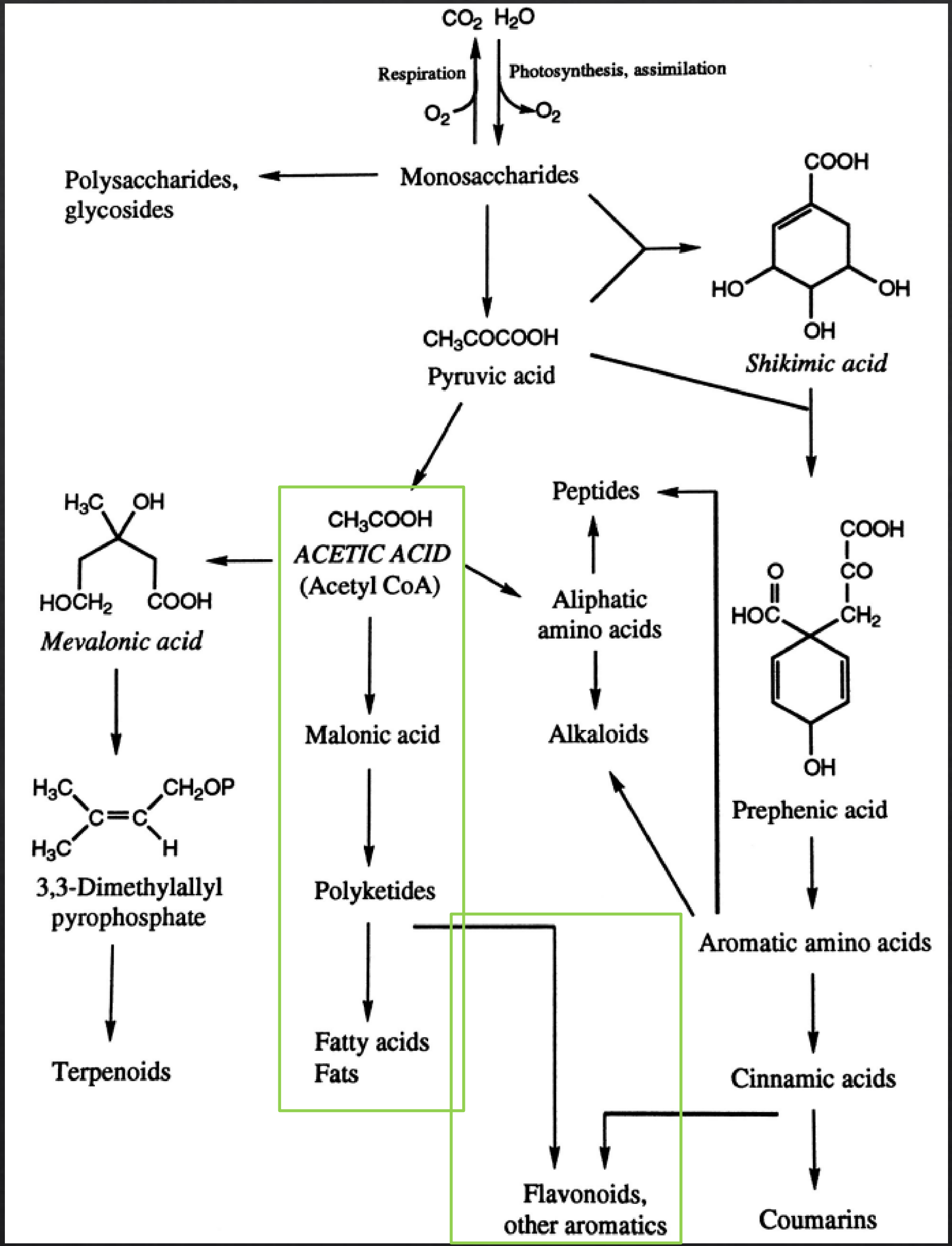
Spring 2022

Polyketide synthesis:

- In bacteria, fungi and plants, some marine animals
- Polyketide synthases (PKSs)
- Large group of molecules with very diverse properties

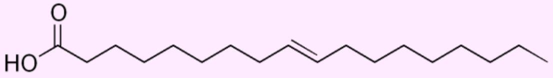


Doxorubicin



Fatty acid synthesis:

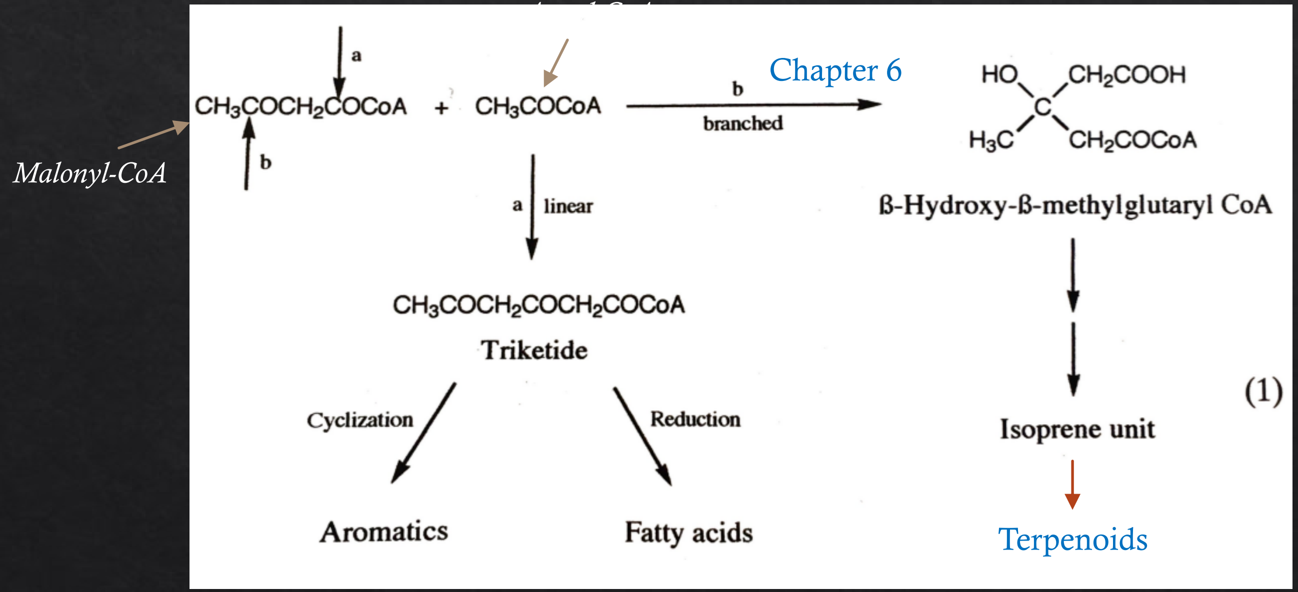
- In animals, bacteria, fungi, plants
- Fatty acid synthases (FASs)
- Relatively small group of molecules

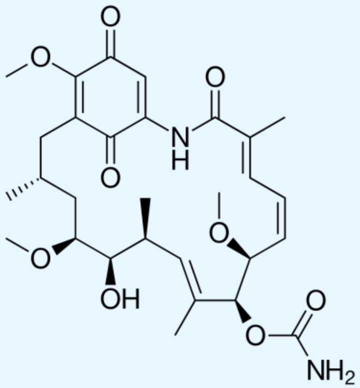


Oleic acid

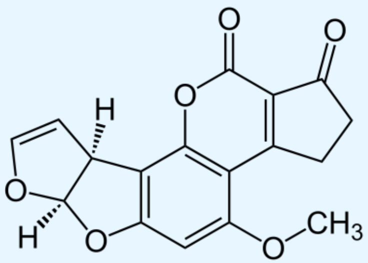
Polyketides

- Natural compounds containing alternating carbonyl and methylene groups (-CO-CH$_2$-)
- Secondary metabolites
- Derived from repeated condensation of acetyl coenzyme A (via malonyl coenzyme A)

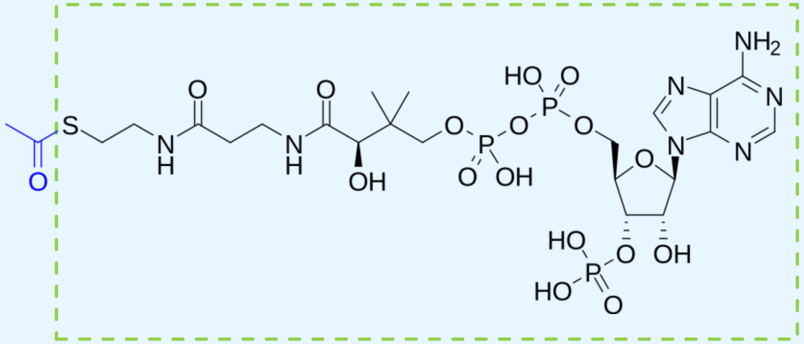




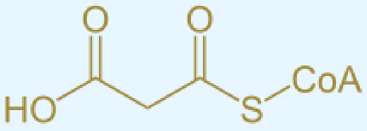
Geldanamycin
antibiotic



Aflatoxin B1
carcinogenic



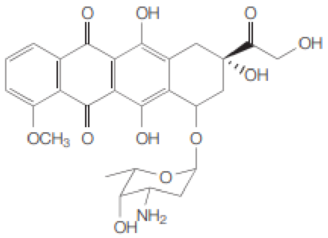
Acetyl CoA



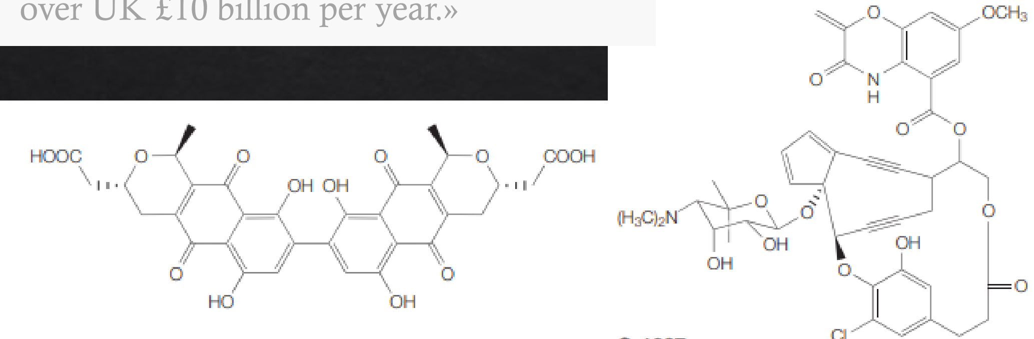
Malonyl-CoA

Polyke

«Polyketide natural products are among the most important microbial metabolites in human medicine, targeting both acute and degenerative diseases.
They are in clinical use as antibiotics (erythromycin A, rifamycin S), anticancer drugs (doxorubicin, epothilone), cholesterol-lowering agents (lovastatin), antiparasitics (avermectin), antifungals (amphotericin B), insecticides (spinosyn A) and immunosuppressants (rapamycin). Polyketide-derived pharmaceuticals comprise **20% of the top-selling drugs**, with combined worldwide revenues of over UK £10 billion per year.»

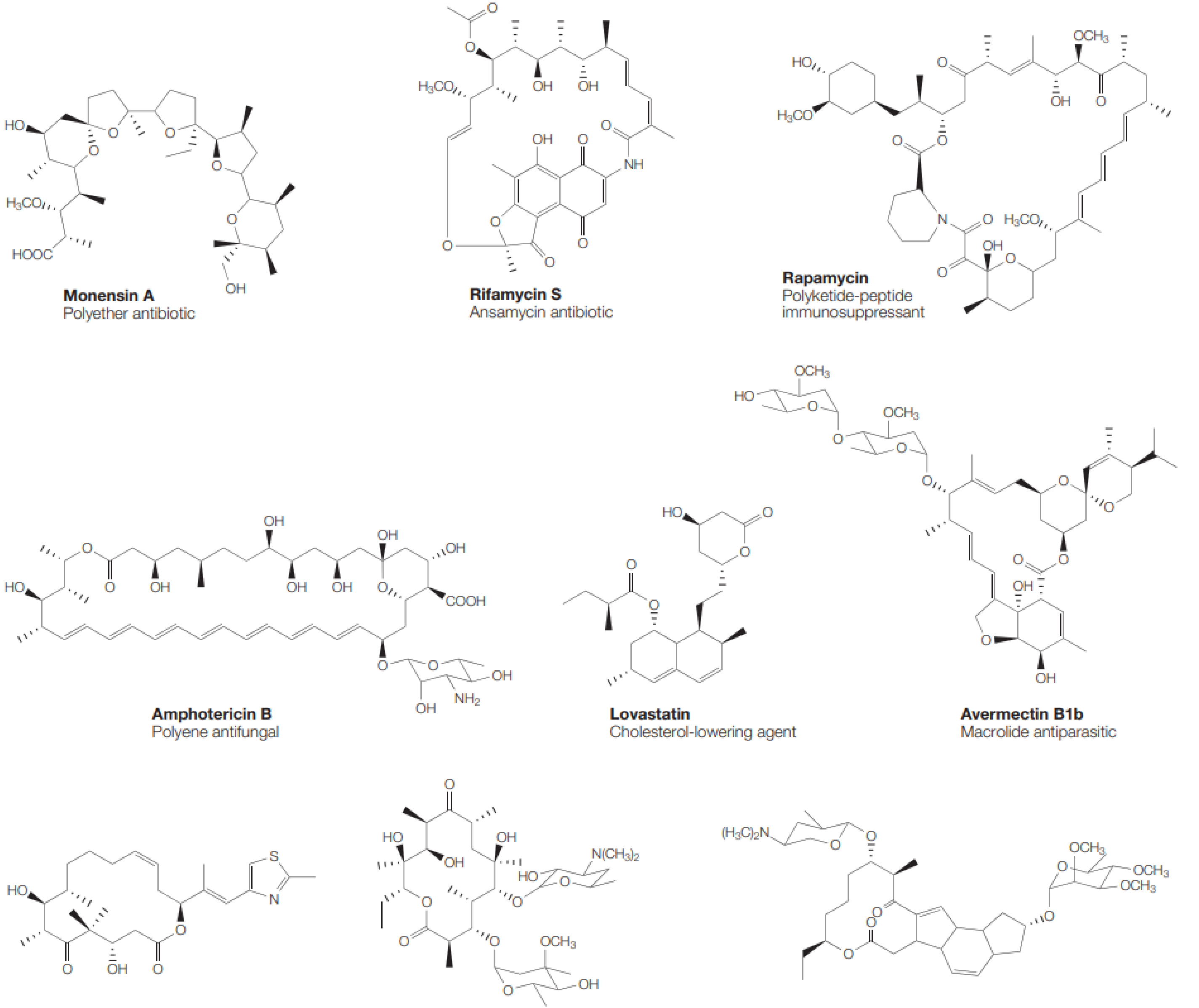


Doxorubicin
Aromatic antitumour agent



Actinorhodin
Aromatic antibiotic

C-1027
Enediyne antitumour agent



Monensin A
Polyether antibiotic

Rifamycin S
Ansamycin antibiotic

Rapamycin
Polyketide-peptide immunosuppressant

Amphotericin B
Polyene antifungal

Lovastatin
Cholesterol-lowering agent

Avermectin B1b
Macrolide antiparasitic

Epothilone
Polyketide-polypeptide anticancer agent

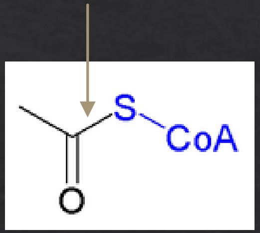
Erythromycin A
Macrolide antibiotic

Spinosyn A
Macrolide insecticide

Nature Reviews Microbiology, 2005, volume 3, pp. 925–

Fatty acids and fats

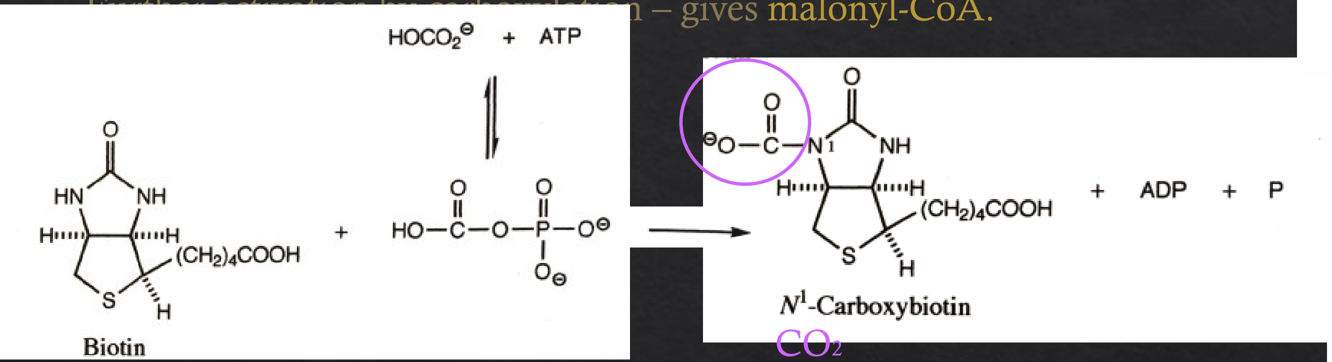
High-energy bond, makes acetyl-CoA more active in condensations.



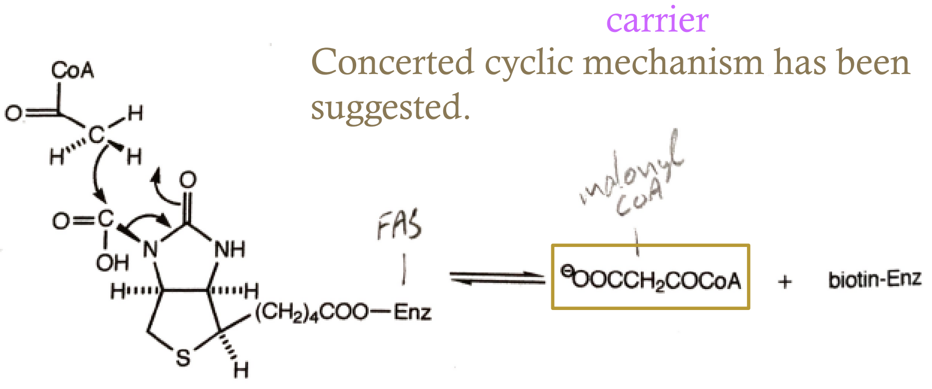
This happens in animals, bacteria, fungi, plants

But it is not reactive enough for **fatty acid synthase (FAS)** to use.

Further activation by carboxylation – gives malonyl-CoA.

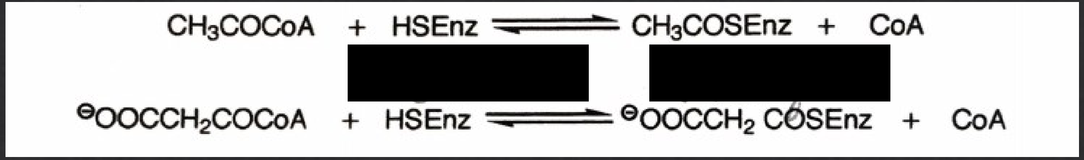


CO2 carrier



Concerted cyclic mechanism has been suggested.

Acetyl-CoA and malonyl-CoA attaches to the acyl carrier protein on FAS.

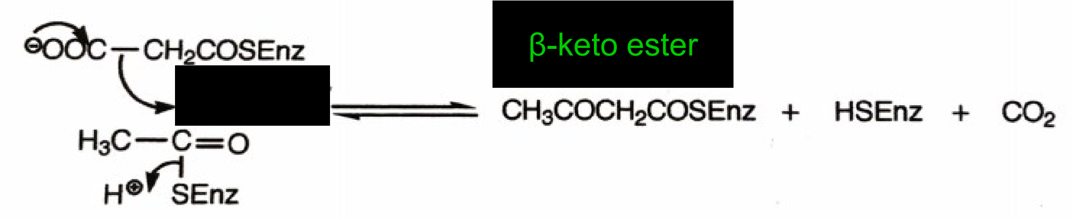


$CH_3COCoA + HSEnz \rightleftharpoons CH_3COSEnz + CoA$

[redacted] [redacted]

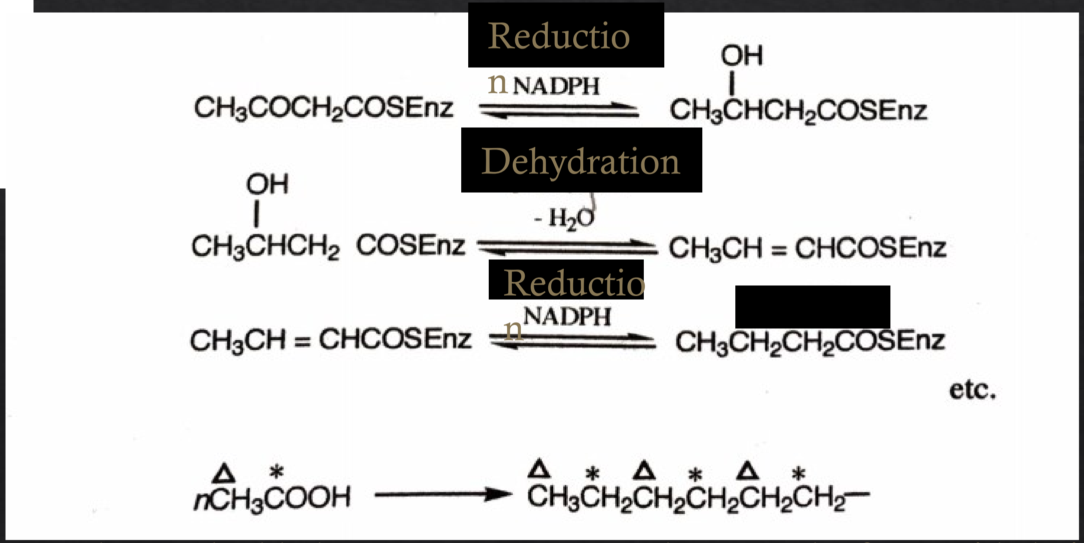
${}^{\ominus}OOCCH_2COCoA + HSEnz \rightleftharpoons {}^{\ominus}OOCCH_2COSEnz + CoA$

Claisen condensation



[redacted]

$\rightleftharpoons CH_3COCH_2COSEnz + HSEnz + CO_2$



Reduction

$CH_3COCH_2COSEnz \xrightleftharpoons{NADPH} CH_3\overset{OH}{C}HCH_2COSEnz$

Dehydration

$CH_3\overset{OH}{C}HCH_2COSEnz \xrightleftharpoons{-H_2O} CH_3CH=CHCOSEnz$

Reduction

[redacted]

$CH_3CH=CHCOSEnz \xrightleftharpoons{NADPH} CH_3CH_2CH_2COSEnz$

etc.

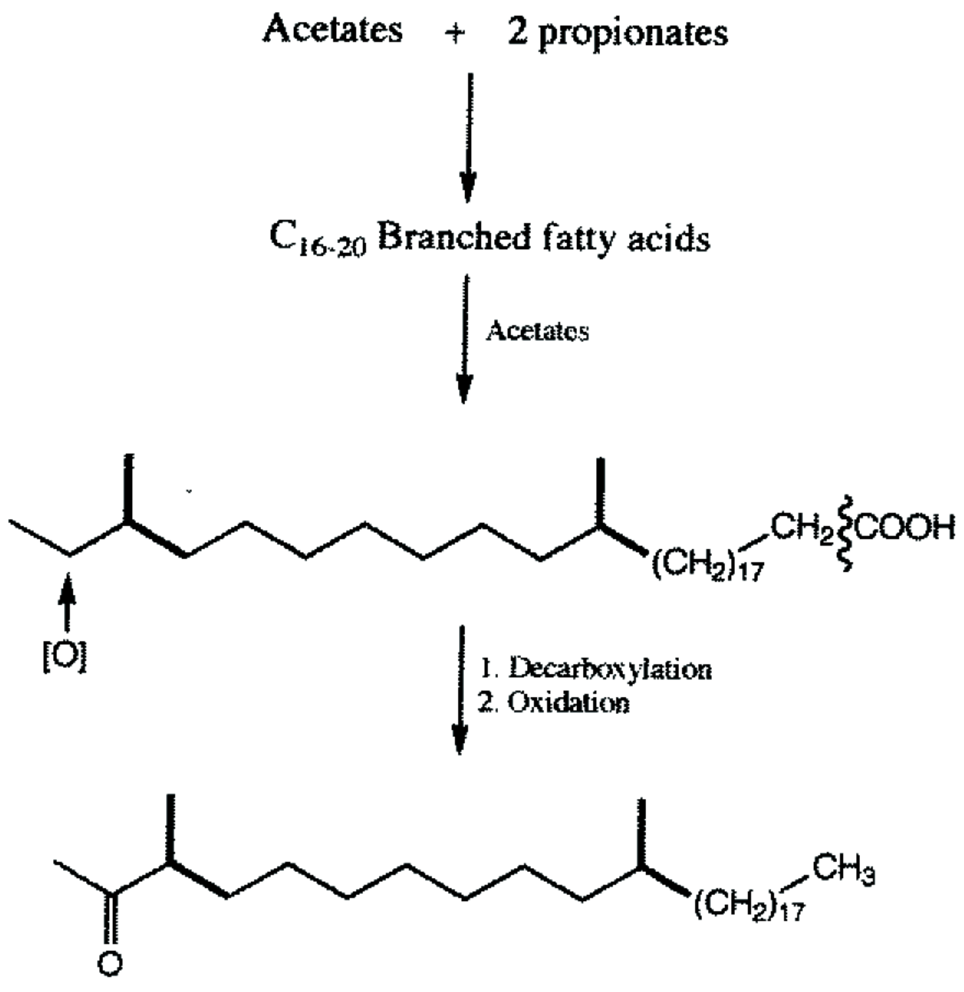
$n\overset{\triangle}{C}H_3\overset{*}{C}OOH \longrightarrow \overset{\triangle}{C}H_3\overset{*}{C}H_2\overset{\triangle}{C}H_2\overset{*}{C}H_2\overset{\triangle}{C}H_2\overset{*}{C}H_2—$

For each acetyl added, we get two more carbons on the fatty acid chain.

Fatty acids usually have an even number of carbons.

Long-chain fatty acids and hydrocarbons

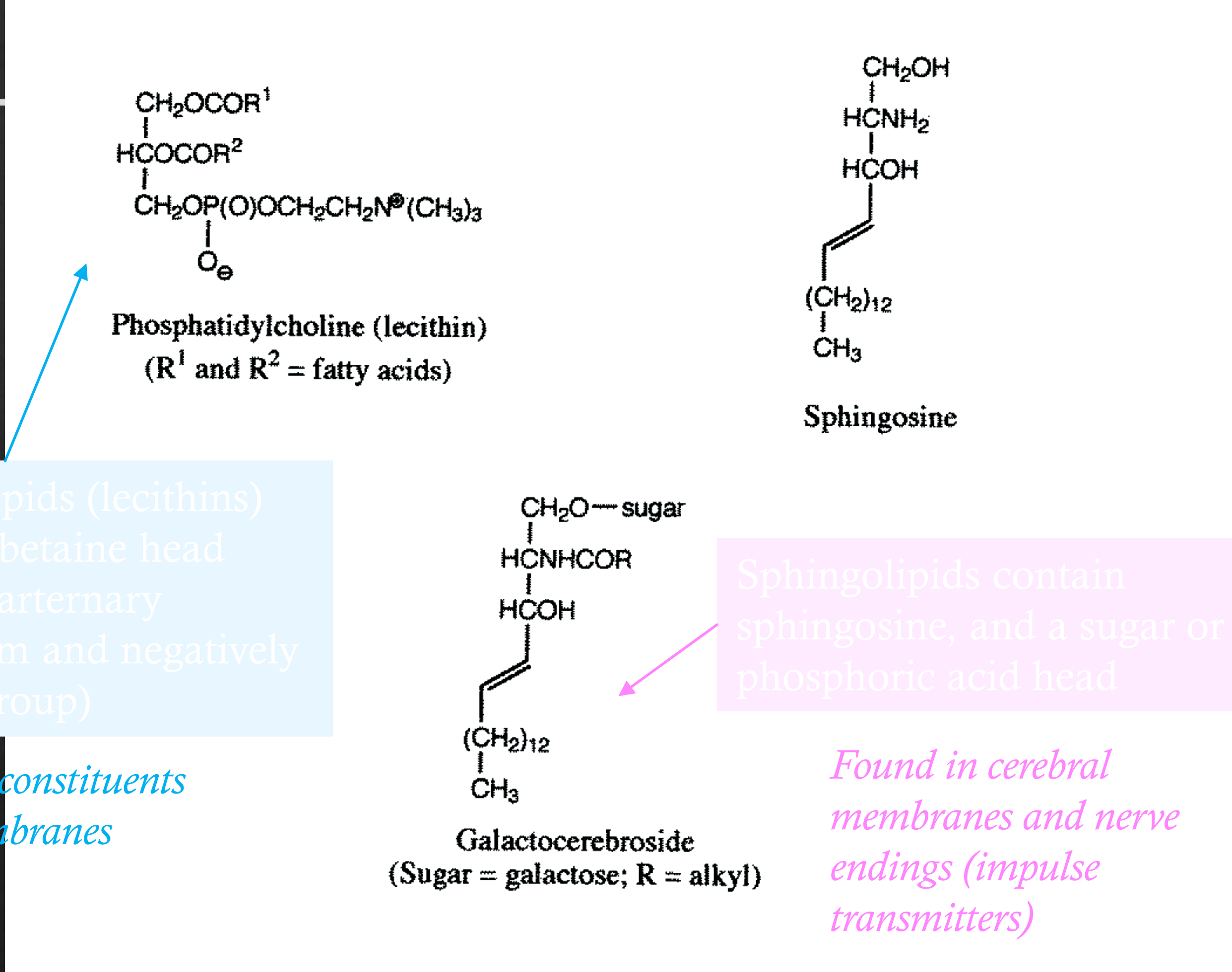
- Branched fatty acids have been identified as intermediates in synthesis of long-chain fatty acids and hydrocarbons
- 3,11-dimethylnonacosan-2-one is the pheromone of the female German cockroach



3,11-Dimethylnonacosan-2-one

L

- Lipids are compounds that are soluble in hydrocarbons
- Fats, waxes, phosphoglycerides
- Common fatty acids: palmitic, stearic and oleic acid



Phosphatidylcholine (lecithin)
(R^1 and R^2 = fatty acids)

Sphingosine

Galactocerebroside
(Sugar = galactose; R = alkyl)

Fig. 2 Structures of a phosphoglyceride and a sphingolipid

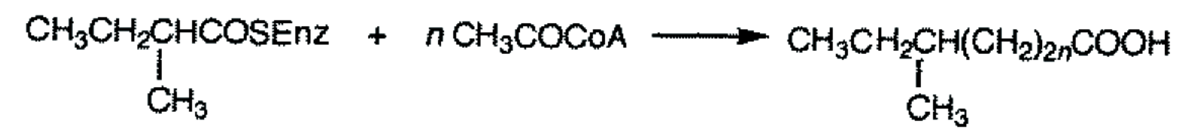
Phospholipids (lecithins) contain a betaine head group (quarternary ammonium and negatively charged group)

Important constituents of cell membranes

Sphingolipids contain sphingosine, and a sugar or phosphoric acid head

Found in cerebral membranes and nerve endings (impulse transmitters)

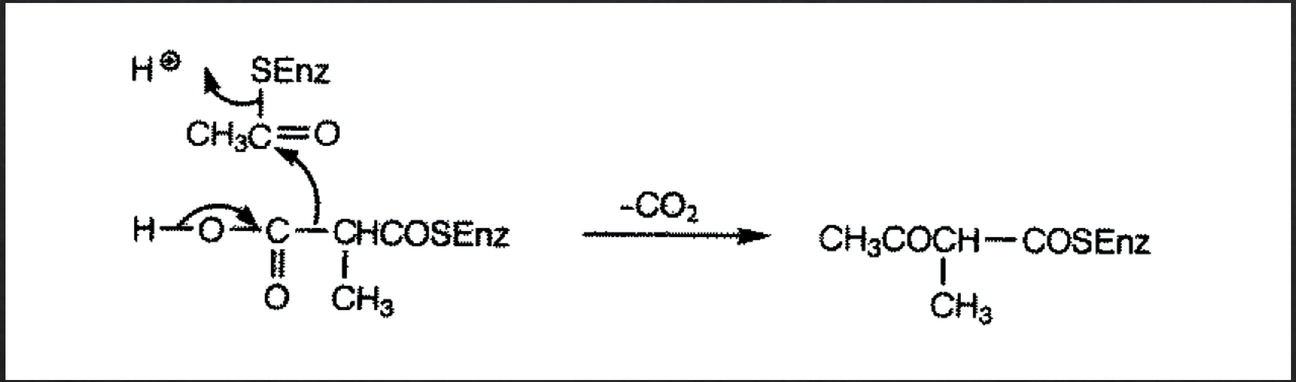
Branched fatty acids



$$CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCOSEnz + n\,CH_3COCoA \longrightarrow CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}H(CH_2)_{2n}COOH$$

Branched starting material
Isobuturyl-CoA or α-metylbutyryl-CoA

OR



Condensation of alkylated malonyl-CoA

Methylmalonyl CoA

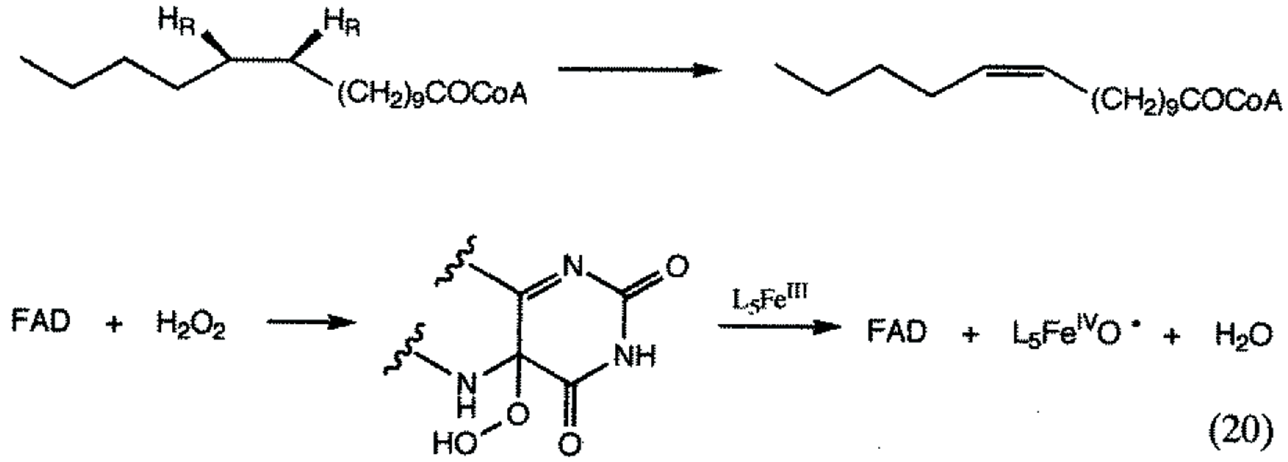
Alkylation of malonyl-CoA occurs by carboxylation of acetic acid homologues

Unsaturated fatty acids & prostagladins

- Unsaturation is introduced differently in aerobic and anaerobic environments

Aerobic
Double bond is introduced into the saturated fatty acid

Requires oxygen, FAD, NADPH and a dehydrogenase



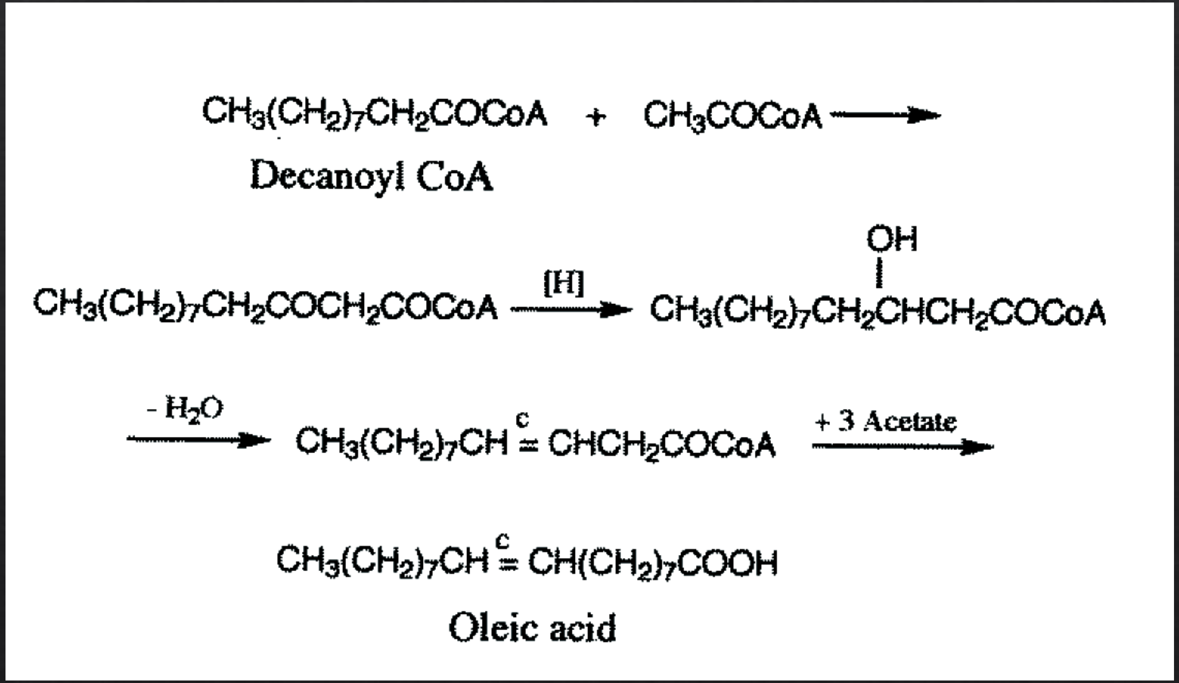
(20)

Unsaturated fatty acids & prostagladins

- Unsaturation is introduced differently in aerobic and anaerobic environments

Anaerobic
Double bond comes from dehydrated hydroxyalkanoylthioesters (intermediates in the fatty acid synthesis)

More «economic»



$$\underset{\text{Decanoyl CoA}}{CH_3(CH_2)_7CH_2COCoA} + CH_3COCoA \longrightarrow$$

$$CH_3(CH_2)_7CH_2COCH_2COCoA \xrightarrow{[H]} CH_3(CH_2)_7CH_2\overset{\overset{OH}{|}}{C}HCH_2COCoA$$

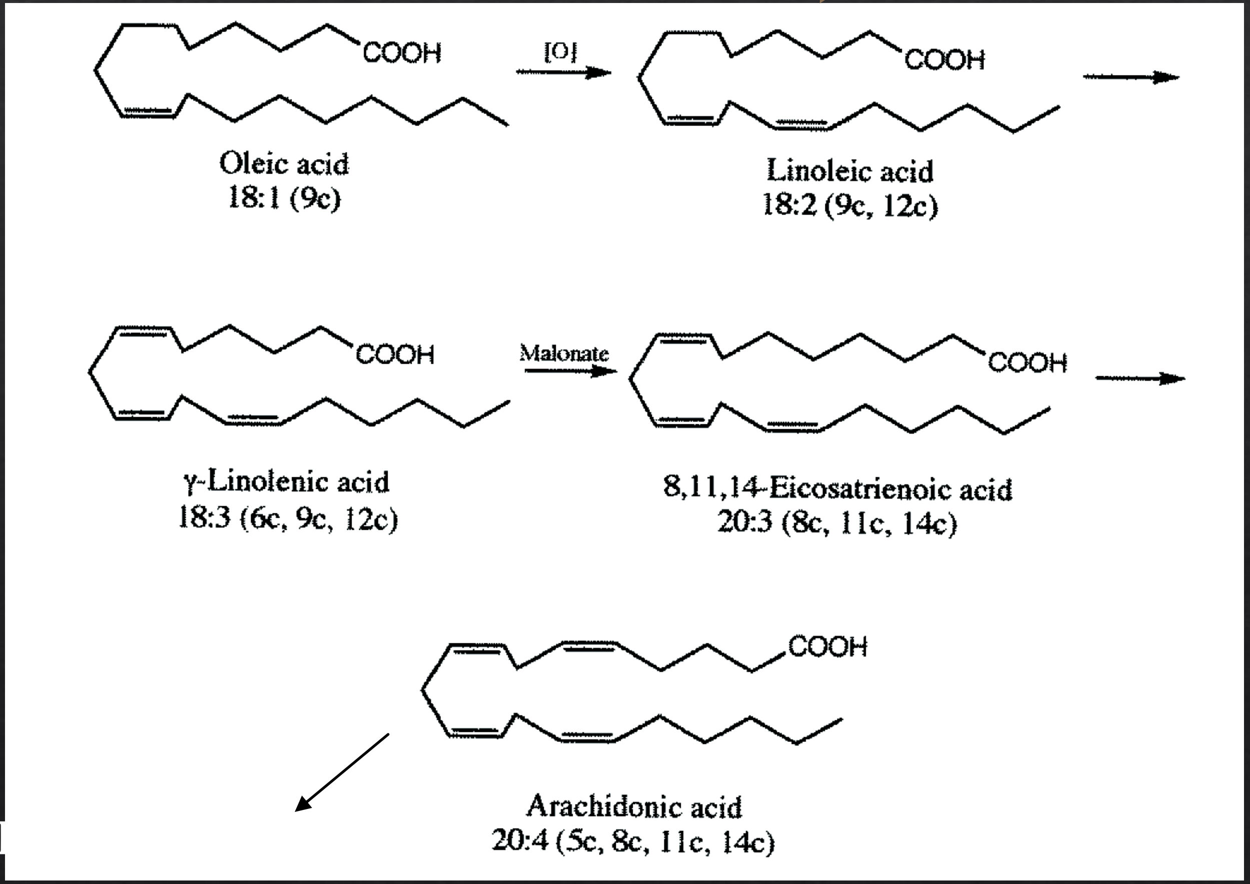
$$\xrightarrow{-H_2O} CH_3(CH_2)_7CH \overset{c}{=} CHCH_2COCoA \xrightarrow{+3\ \text{Acetate}}$$

$$\underset{\text{Oleic acid}}{CH_3(CH_2)_7CH \overset{c}{=} CH(CH_2)_7COOH}$$

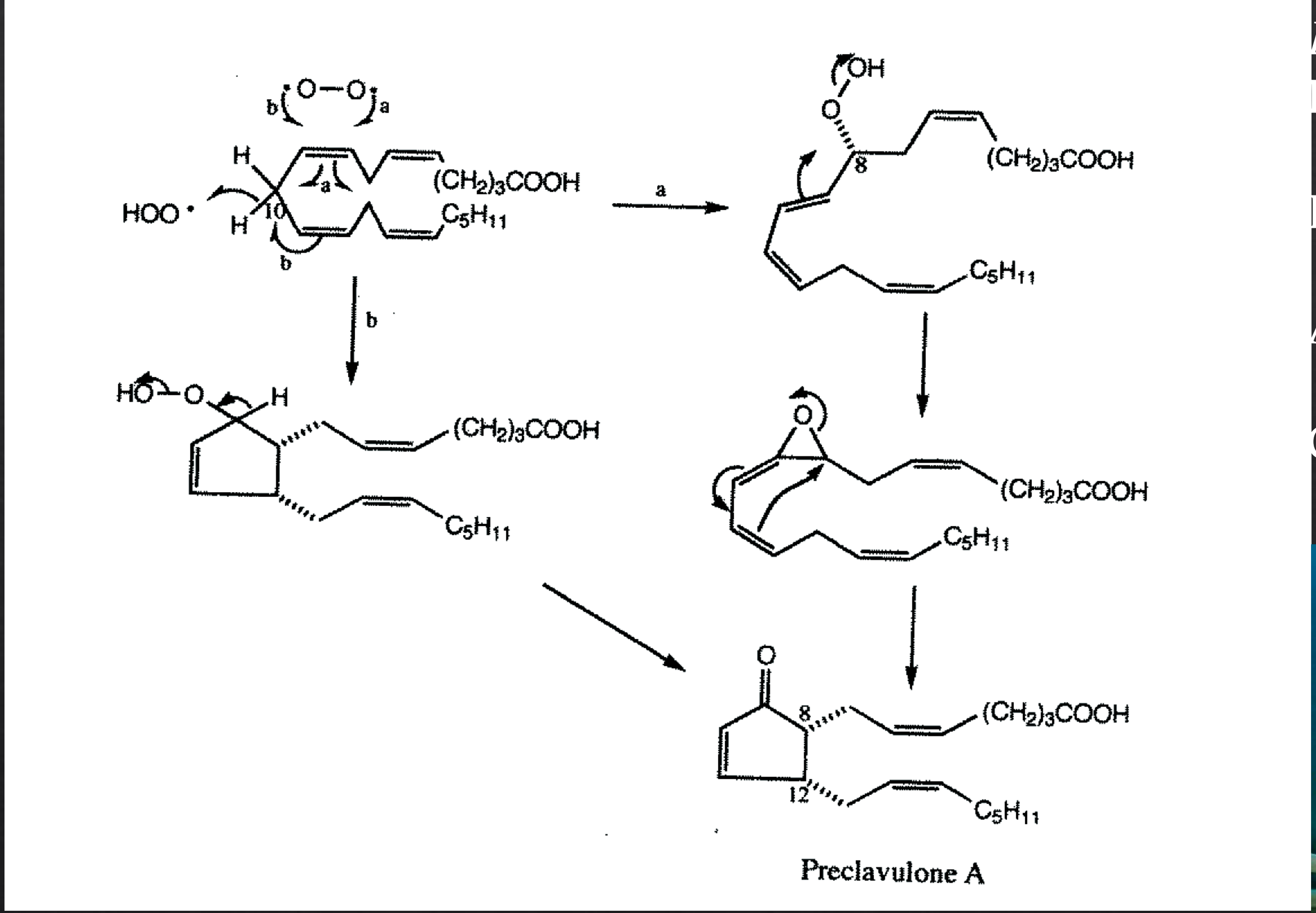
Further desaturation of oleic acid gives linoleic and linolenic acids

Unsaturated fatty acids & prostagladins

- **Arachidonic acid** is a precursor for prostagladin hormones
  - Biosynthesized from oleic acid
- Prostagladins
  - Control blood pressure, contractions of smooth muscle, gastric acid secretion, platelet aggregation
  - Medical properties



Essential fatty acid for mammals



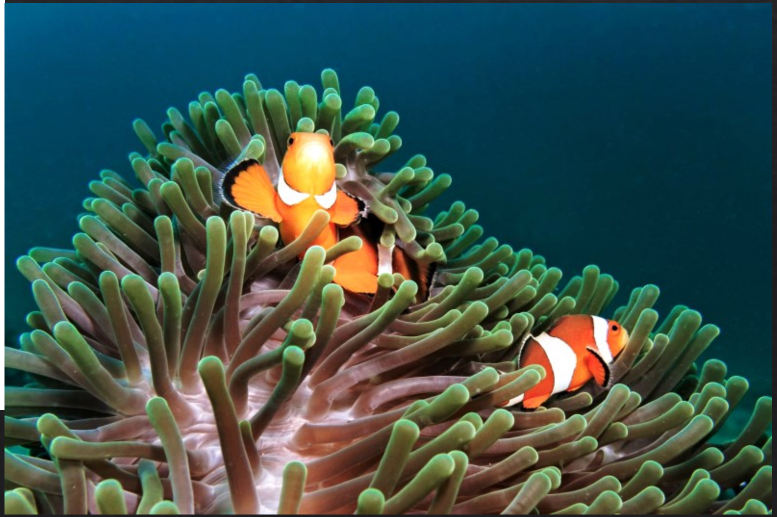
Example:

Biosynthesis of preclavulone A

In corals

Attack at C10 of arachidonic acid

Catalyzed by a lipooxygenase



Acetylene (ethyne) compounds

Sequential dehydrogenation catalyzed by desaturases

Umbrelliferae

Compositae

Basidiomycetes

- Often unstable, sensitive to light, heat and oxygen
- Formed from polyketide products
  - Further desaturation of fatty acids

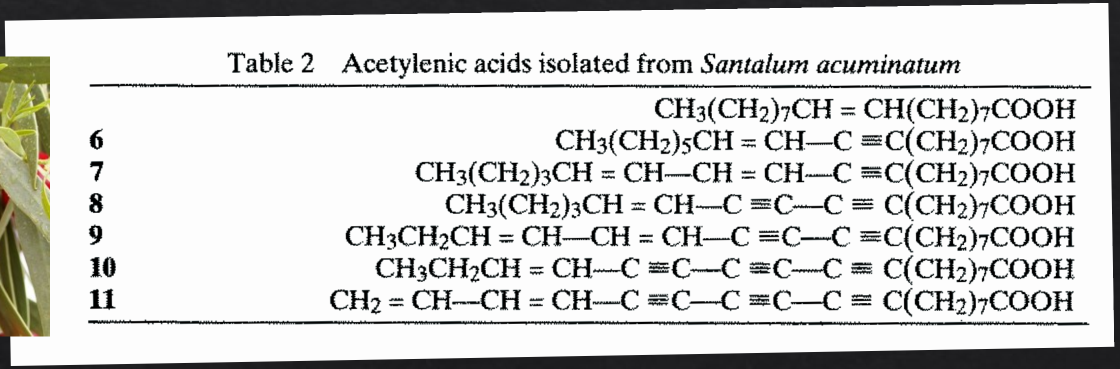
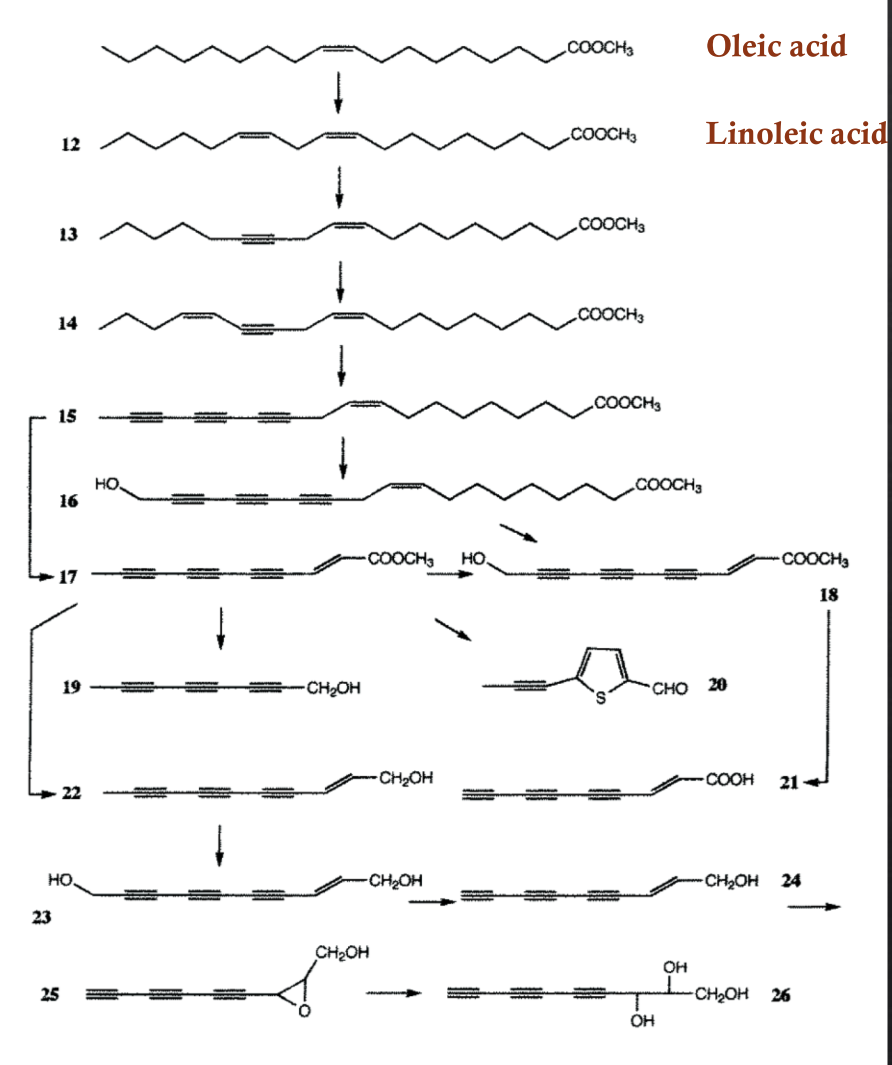


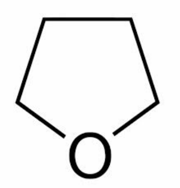
Table 2 Acetylenic acids isolated from *Santalum acuminatum*

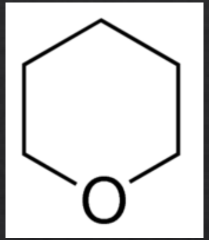
	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
6	$CH_3(CH_2)_5CH=CH{-}C\equiv C(CH_2)_7COOH$
7	$CH_3(CH_2)_3CH=CH{-}CH=CH{-}C\equiv C(CH_2)_7COOH$
8	$CH_3(CH_2)_3CH=CH{-}C\equiv C{-}C\equiv C(CH_2)_7COOH$
9	$CH_3CH_2CH=CH{-}CH=CH{-}C\equiv C{-}C\equiv C(CH_2)_7COOH$
10	$CH_3CH_2CH=CH{-}C\equiv C{-}C\equiv C{-}C\equiv C(CH_2)_7COOH$
11	$CH_2=CH{-}CH=CH{-}C\equiv C{-}C\equiv C{-}C\equiv C(CH_2)_7COOH$

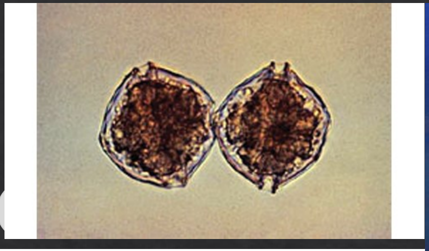


Polyeth

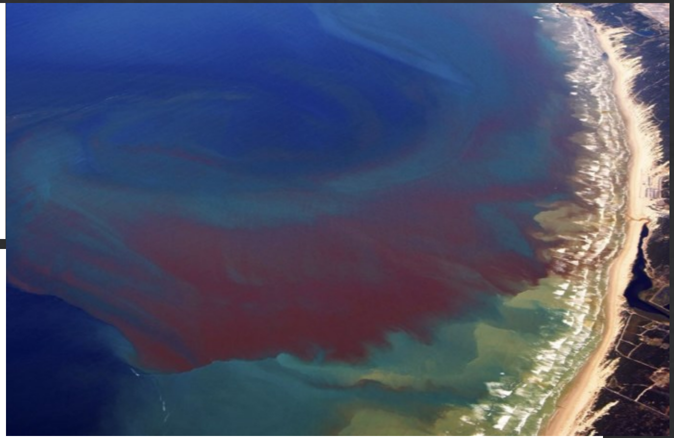
- Contain tetrahydrofuran ar tetrahydropyrane rings



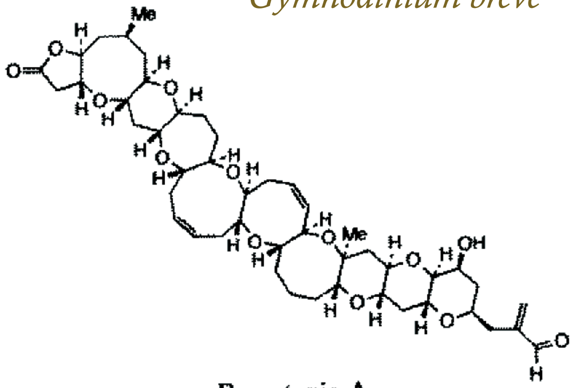




Gymnodinium breve



Brevetoxin A is a toxic compound that causes the red tide in the Gulf of Mexico

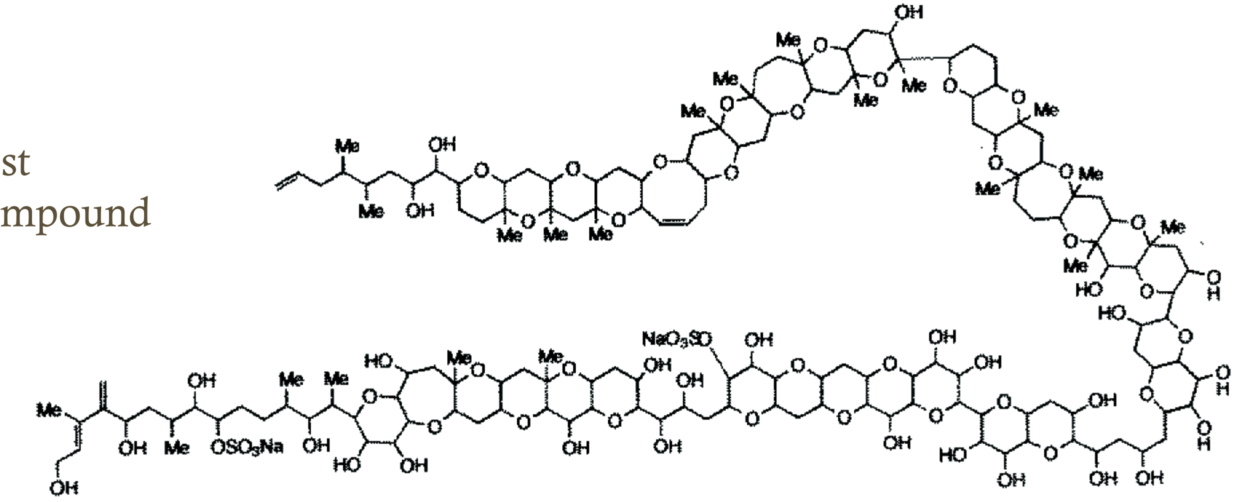


Brevetoxin A

Gambierdiscus toxicus

Maitotoxin is the largest monomeric organic compound known

Mw = 3422 g/mol



Maitotoxin