Biosynthesis of Natural Products

Biosynthesis of Isoprenoids

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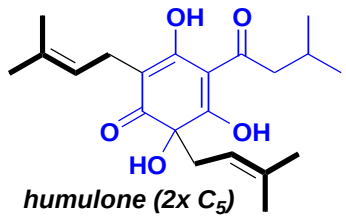
Format & Scope of Lecture

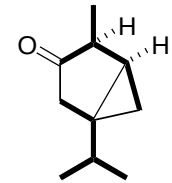
- ***What are isoprenoids?***
  - n × C_5 diversity: terpenes, steroids, carotenoids & natural rubber
  - 'the isoprene rule'
  - mevalonate pathway to IPP & DMAPP
- ***Monoterpnes (C_{10})***
  - regular ('head-to-tail') *via* geranyl pyrophosphate
  - apparently irregular 'iridoids' (*e.g. seco*-loganin)
- ***Sesquiterpenes (C_{15})***
  - farnesyl pyrophosphate derived metabolites
- ***Diterpenes (C_{20})***
  - taxol
- ***Triterpenes (C_{30})***
  - steroids (2,3-oxidosqualene → lanosterol → cholesterol → estrone)
  - ring-opened 'steroids': vitamin D_2 & azadirachtin

Isoprenoids

- ***isoprenoids*** are widely distributed in the natural world
  - particularly prevalent in plants and least common in insects; >30,000 known
  - composed of integral numbers of C_5 'isoprene' units:
    - ***mono***terpenes (C_{10}); ***sesqui***terpenes (C_{15}); ***di***terpenes (C_{20}); ***ses***terpenes (C_{25}, *rare*); ***tri***terpenes (C_{30}); ***carotenoids*** (C_{40})

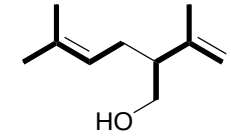


humulone (2x C_5)

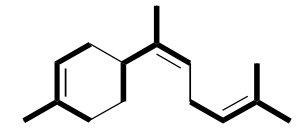


thujone (C_{10})

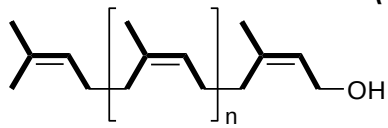
borneol (C_{10})



lavandulol (C_{10})

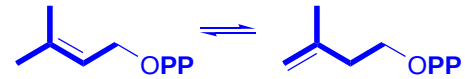


(Z)-α-bisabolene (C_{15})



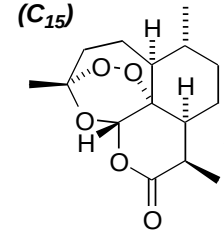
natural rubber (~10^5x C_5)

ISOPRENOIDS

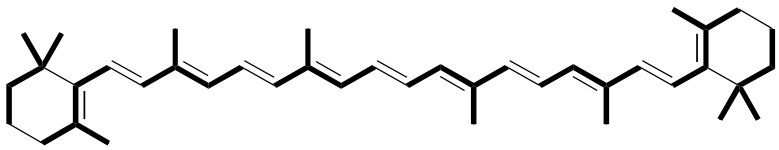


dimethylallyl pyrophosphate (DMAPP)

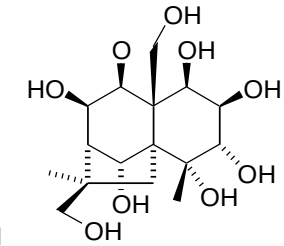
isopentenyl pyrophosphate (IPP)



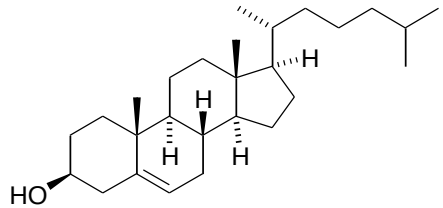
artemisinin (C_{15})



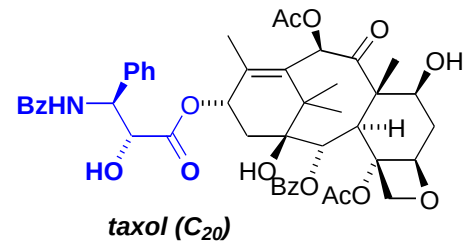
β-carotene (C_{40})



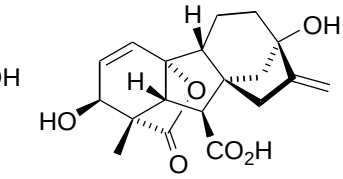
euonyminol (C_{15})



cholesterol (C_{27} but C_{30}-derived)



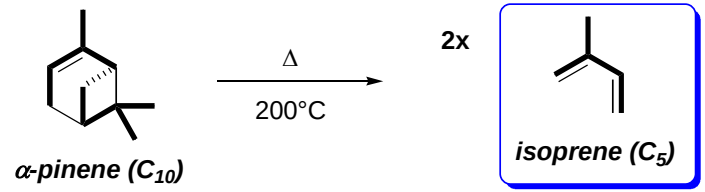
taxol (C_{20})



gibberellic acid (C_{20}) (gibberellin A_3)

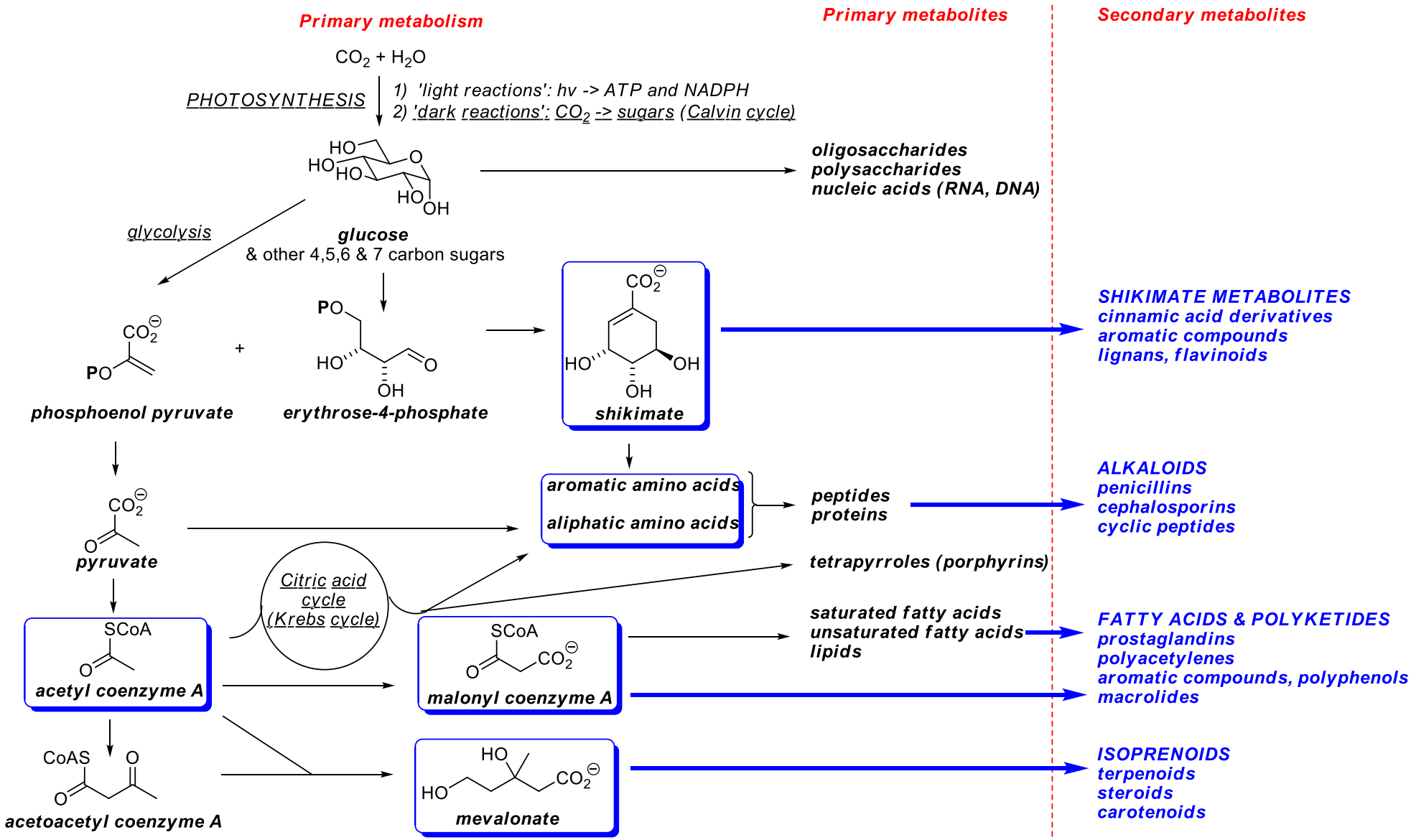
Historical Perspective – *'The Isoprenoid Rule'*

- ***Early 1900s:***
  - common ***structural feature*** of terpenes – ***integral*** # of ***C_5 units***
  - ***pyrolysis*** of many monoterpenes produced two moles of ***isoprene***:



- ***1940s:***
  - ***biogenesis*** of terpenes attributed to oligomers of isoprene – ***'the isoprene rule'***
- ***1953:***
  - ***Ruzicka*** proposes '***the biogenetic isoprene rule***' to accomodate 'irregular' terpenoids:
    - *i.e.* that terpenes were derived from a number of ***biological equivalents of isoprene*** initially joined in a '***head-to-tail***' manner & sometimes subsequently modified enzymatically to provide greater diversity of structure
- ***1964:***
  - ***Nobel prize*** awarded to ***Bloch, Cornforth*** & ***Popjak*** for elucidation of biosynthetic pathway to ***cholesterol*** including the first steps:
    - ***acetate*** → ***mevalonate*** (***MVA***) → ***isopentenylpyrophosphate*** (***IPP***) & ***dimethylallyl pyrophosphate*** (***DMAPP***)
- ***1993:***
  - ***Rohmer, Sahm*** & ***Arigoni*** elucidate an additional pathway to ***IPP*** & ***DMAPP:***
    - ***pyruvate + glyceraldehyde-3-phosphate → 1-deoxyxylulose-5-phosphate → IPP*** & ***DMAPP***

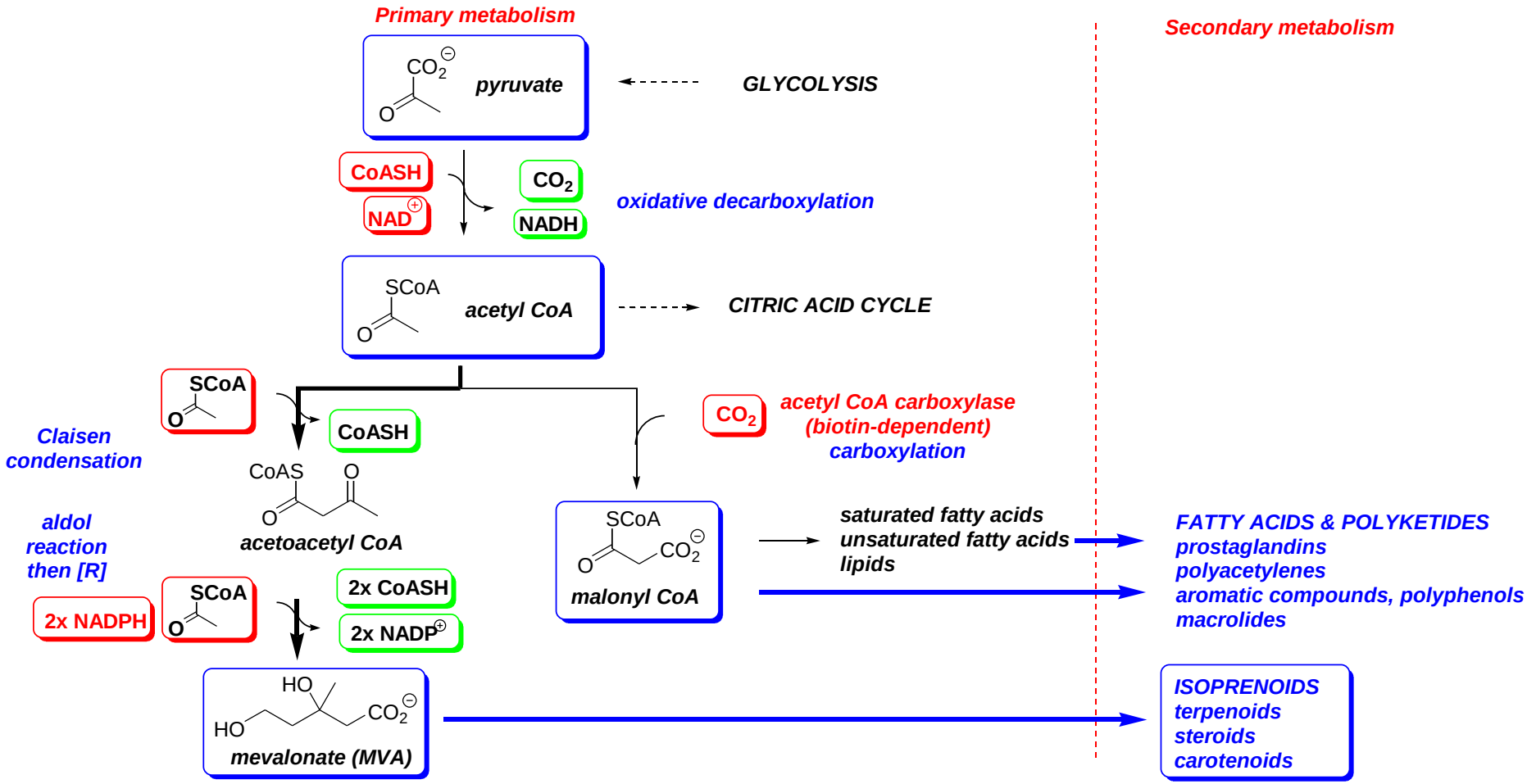
Primary Metabolism - *Overview*



For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

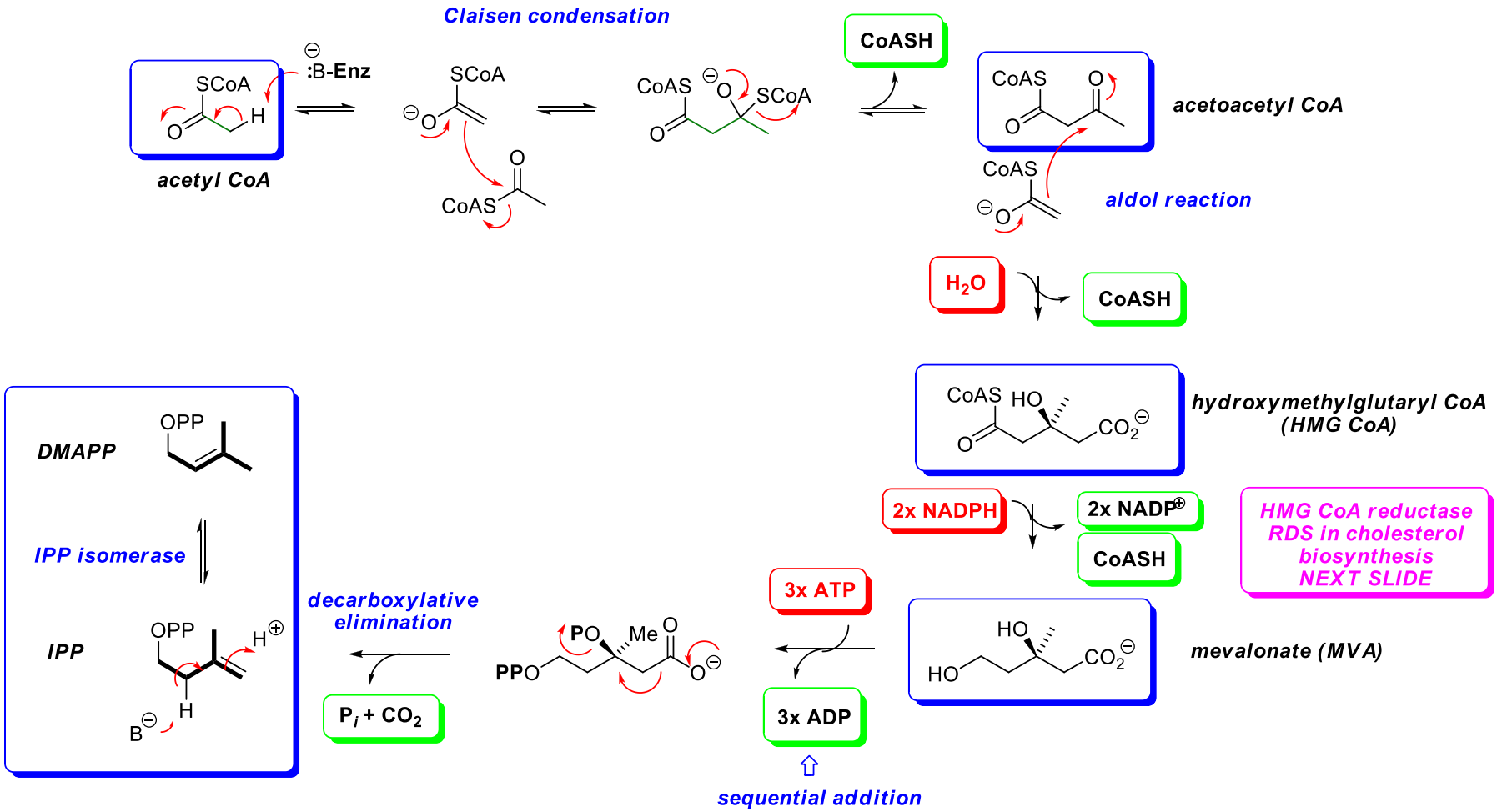
Biosynthesis of Mevalonate

- ***Mevalonate (MVA)*** is the first committed step of ***isoprenoid biosynthesis***
  - this key 6-carbon metabolite is formed from three molecules of ***acetyl CoA*** *via* ***acetoacetyl CoA:***



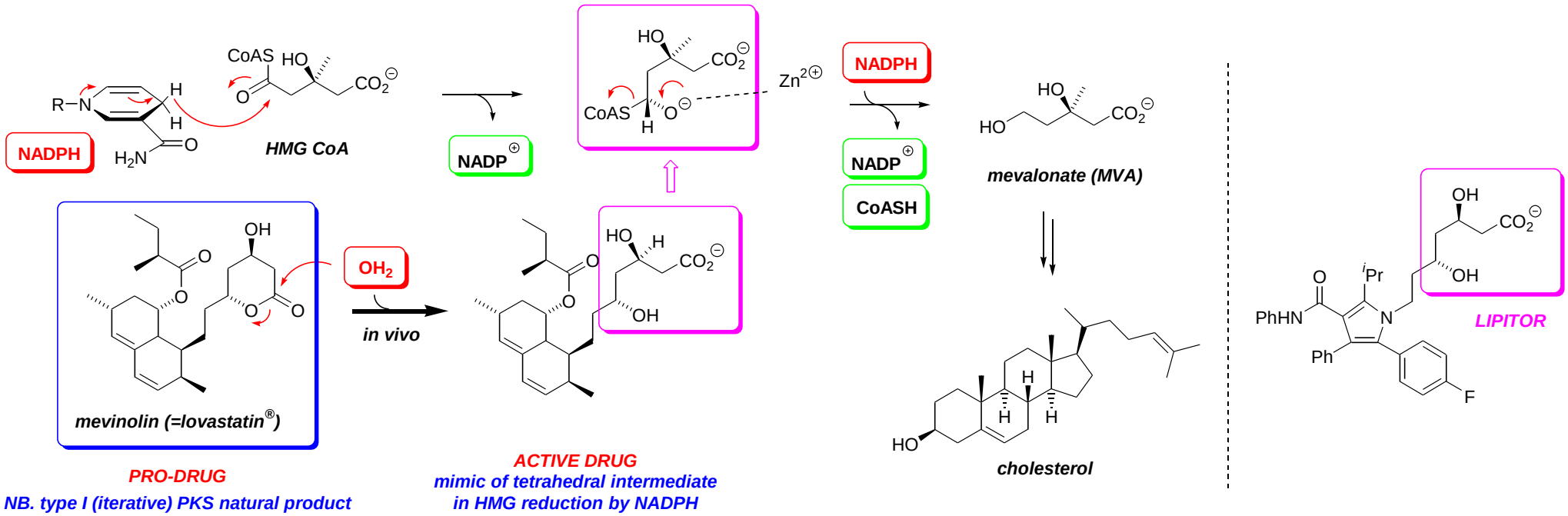
Biosynthesis of IPP & DMAPP - *via Mevalonate*

- ***IPP*** & ***DMAPP*** are the key ***C_5 precursors*** to ***all isoprenoids***
  - the ***main pathway*** is *via*: ***acetyl CoA → acetoacetyl CoA → HMG CoA → mevalonate → IPP → DMAPP:***



HMG CoA reductase inhibitors - *Statins*

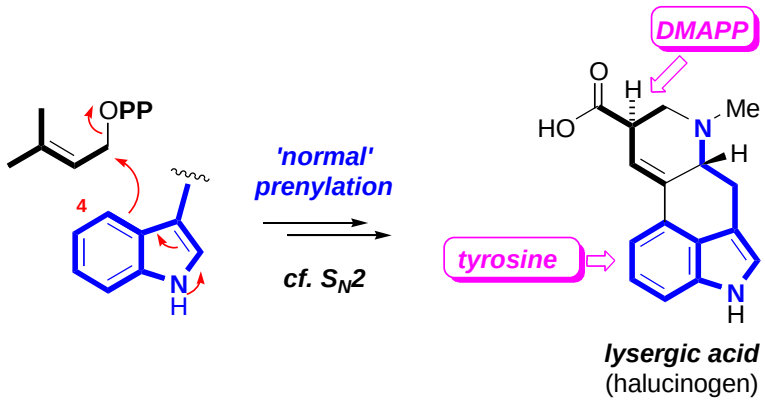
- ***HMG CoA → MVA*** is the ***rate determining step*** in the biosynthetic pathway to ***cholesterol***
  - 33 enzyme mediated steps are required to biosynthesise cholesterol from acetyl CoA & in principle the inhibition of any one of these will serve to break the chain. In practice, control rests with HMG-CoA reductase as the result of a variety of biochemical feedback mechanisms
- ***'Statins'*** inhibit HMG CoA reductase and are used clinically to treat ***hypercholesteraemia*** - a causative factor in ***heart disease***
  - *e.g.* ***mevinolin*** (=lovastatin®, Merck) a polyketide natural product from *Aspergillus terreus is a* competitive inhibitior of HMG-CoA reductase



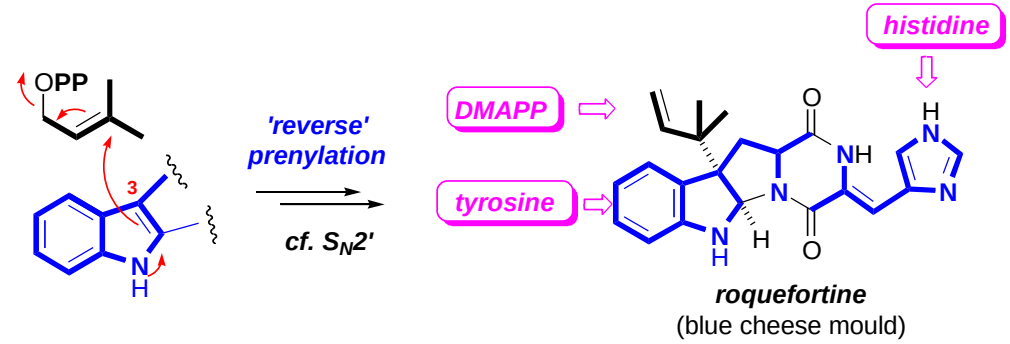
Hemi-Terpenes – *'Prenylated Alkaloids'*

- ***DMAPP*** is an excellent ***alkylating agent***
- ***C_5 units*** are frequently encountered as part of ***alkaloids*** (& ***shikimate metabolites***) due to 'late-stage' alkylation by ***DMAPP***
  - the transferred ***dimethyl allyl unit*** is often referred to as a ***'prenyl group'***
  - ***'normal prenylation' – 'S_N2'-like*** *alkylation;* ***'reverse prenylation' – 'S_N2'-like*** *alkylation*

    - *e.g.* ***lysergic acid*** (an ***ergot alkaloid***) – a 'normal prenylated' alkaloid (with significant subsequent processing)
    - *e.g.* ***roquefortine*** – a 'reverse prenylated' alkaloid



lysergic acid
(halucinogen)

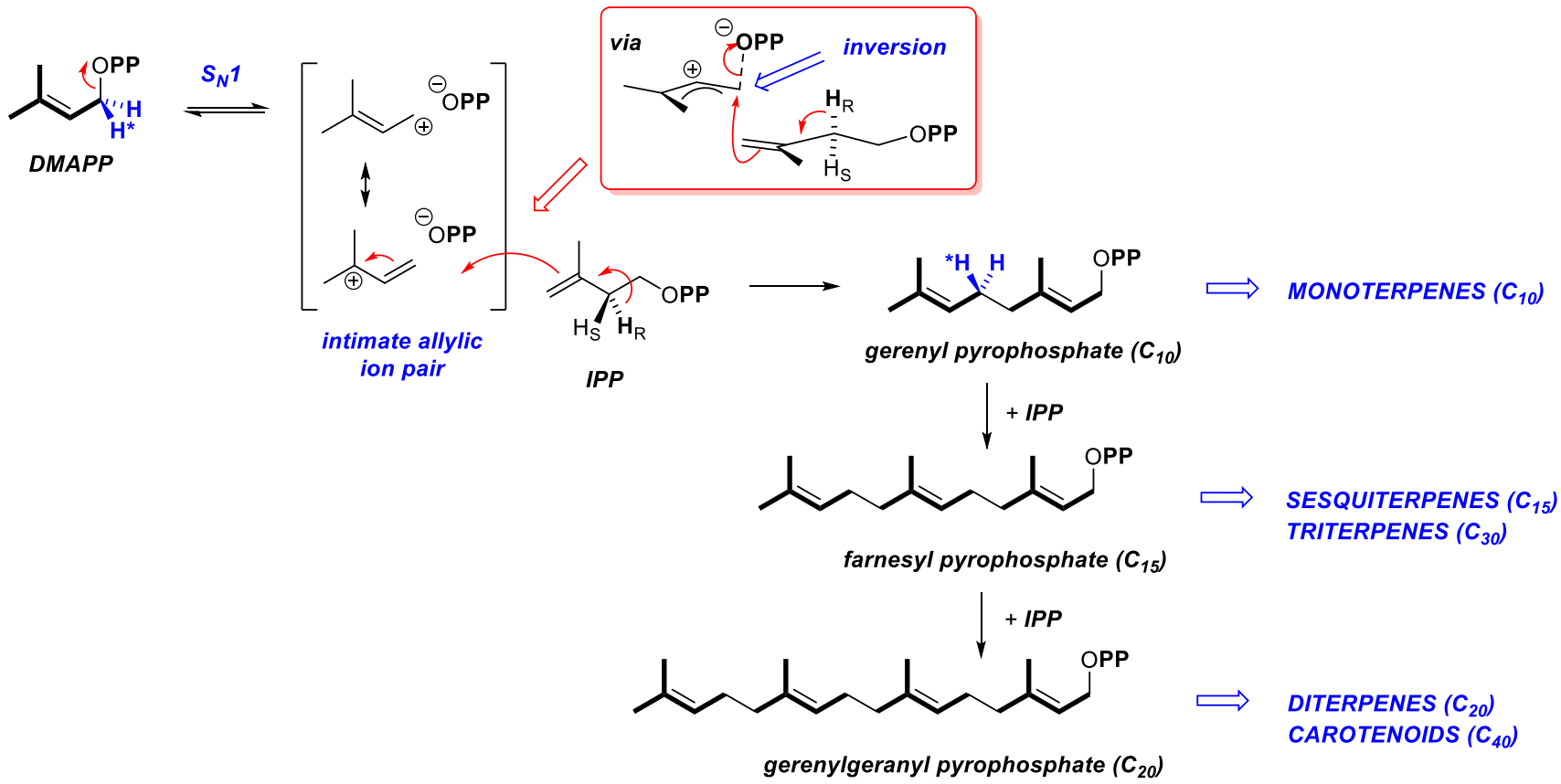


roquefortine
(blue cheese mould)

  - ***review:*** R.M. Williams *et al. 'Biosynthesis of prenylated alkaloids derived from tryptophan' Top. Curr. Chem.* **2000**, *209*, 97-173 (DOI)

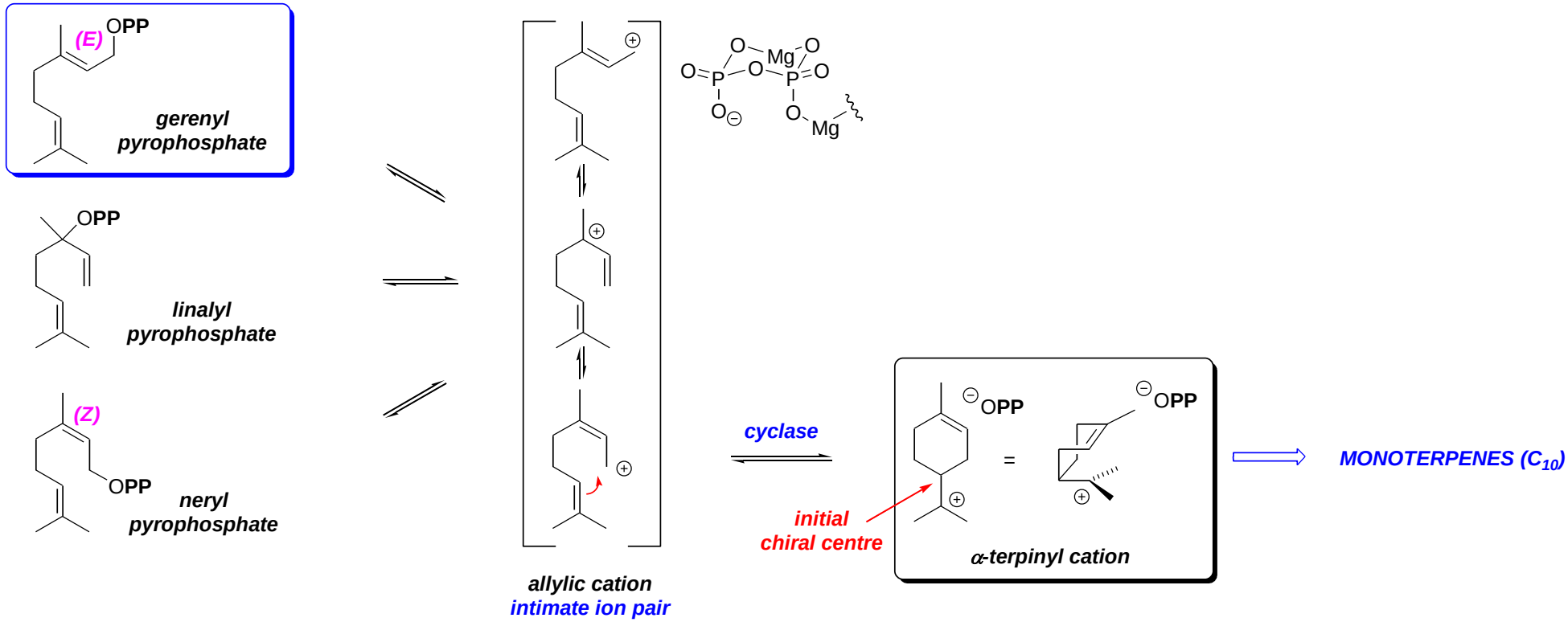
Linear C_{5n} 'head-to-tail' Pyrophosphates

- head-to-tail ***C_5 oligomers*** are the key precursors to isoprenoids
  - ***geranyl*** pyrophosphate (C_{10}) is formed by ***S_N1 alkylation*** of ***DMAPP*** by ***IPP*** → ***monoterpenes***
  - ***farnesyl*** (C_{15}) & ***geranylgeranyl*** (C_{20}) pyrophosphates are formed by ***further S_N1 alkylations*** with ***IPP***:



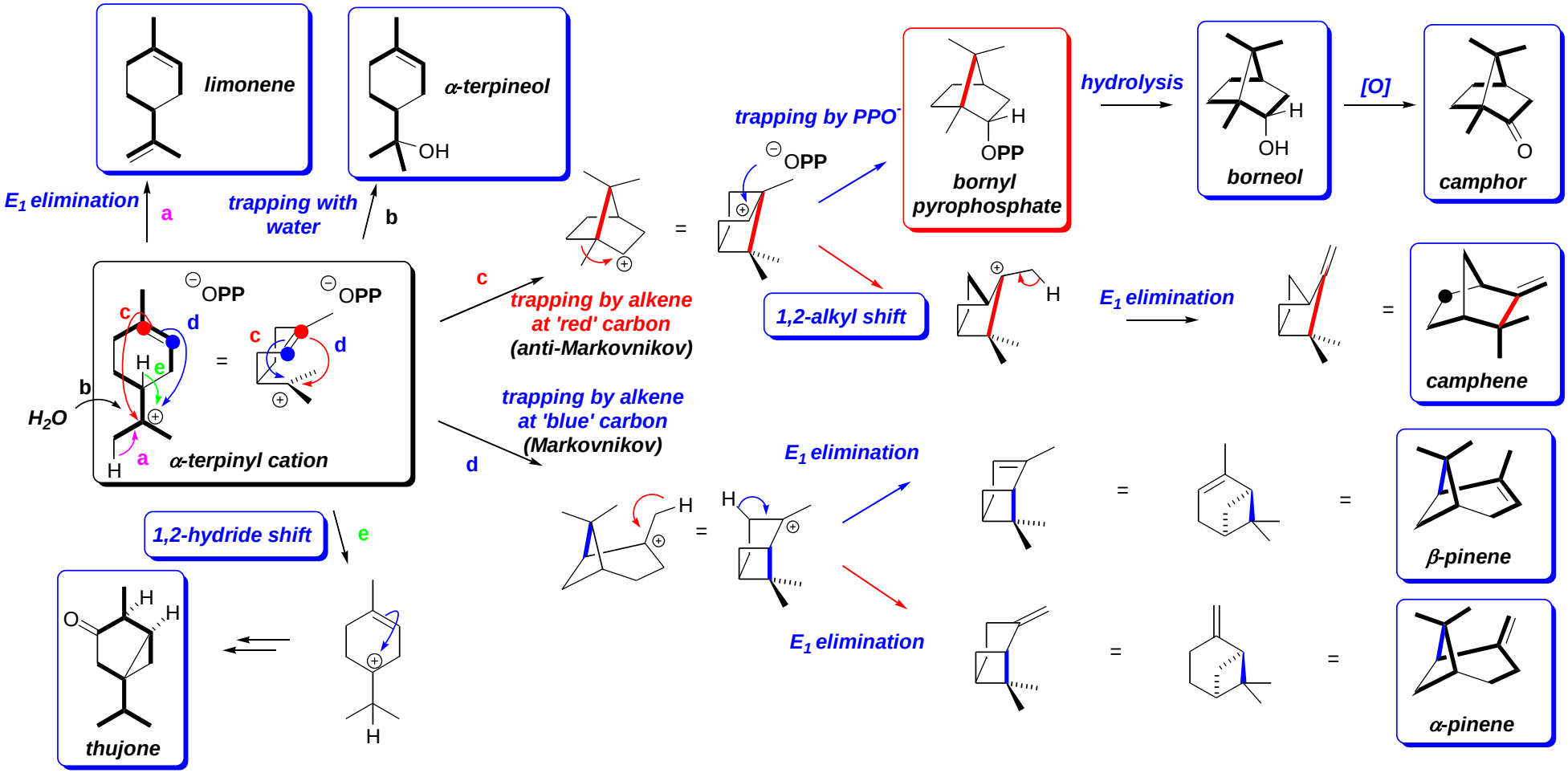
Monoterpenes – *α-Terpinyl Cation Formation*

- ***geranyl*** pyrophosphate isomerises readily via an allylic cation to ***linalyl*** & ***neryl*** pyrophosphates
  - the leaving group abilty of pyrophosphate is enhanced by coordination to Mg^{2+} ions
  - all three pyrophosphates are substrates for ***cyclases*** *via* an ***α-terpinyl cation***:



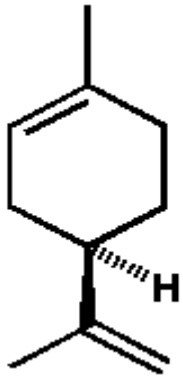
Monoterpenes – *Fate of the α-Terpinyl Cation*

- The ***α-terpinyl cation*** undergoes a rich variety of further chemistry to give a diverse array of ***monoterpenes***
- Some important enzyme catalysed pathways are shown below
  - *NB. intervention of* ***Wagner-Meerwein 1,2-hydride-*** & ***1,2-alkyl shifts***



Limonene & Carvone

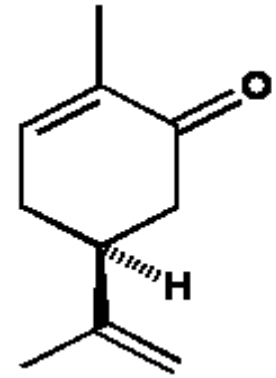
Chiroscience plc. (now Dow Inc.)



1. *S*-(-)-limonene (lemon)

2. *R*-(+)-limonene (orange)

3. *RS*-(±)-limonene (pleasant)



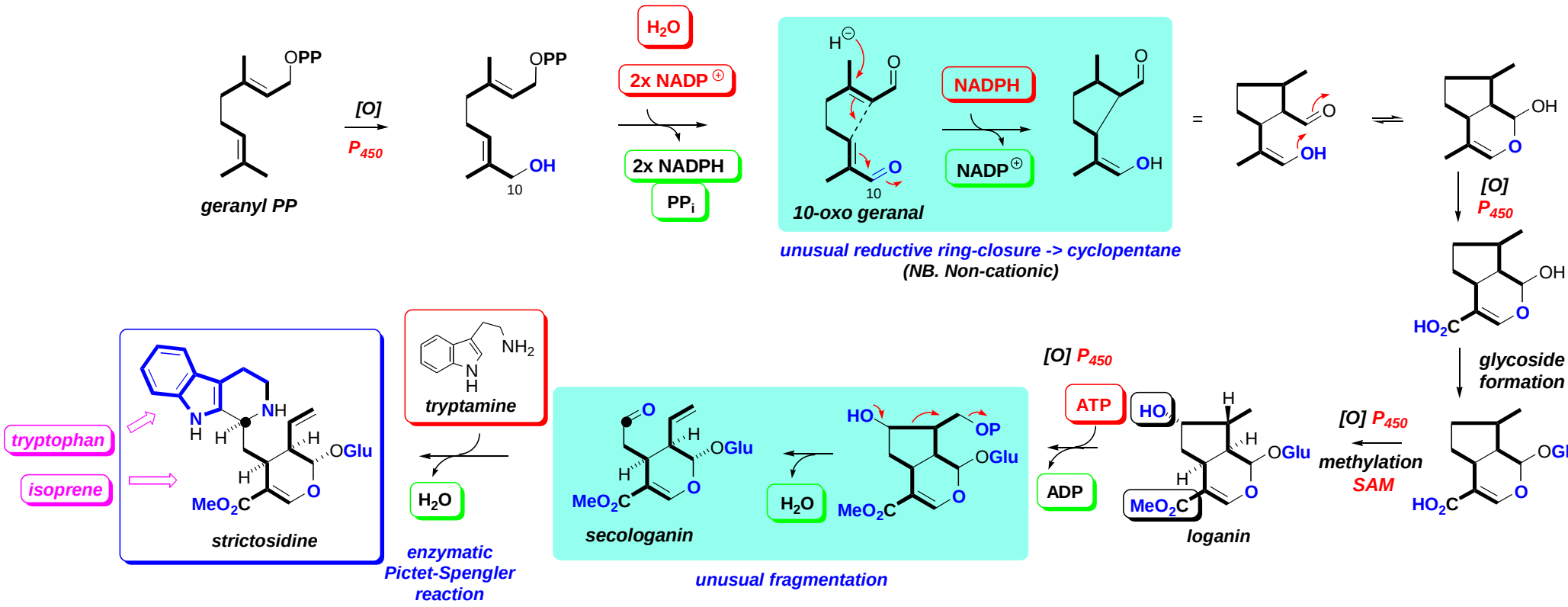
4. *R*-(-)-carvone (spearmint)

5. *S*-(+)-carvone (caraway)

6. *RS*-(±)-carvone (disgusting)

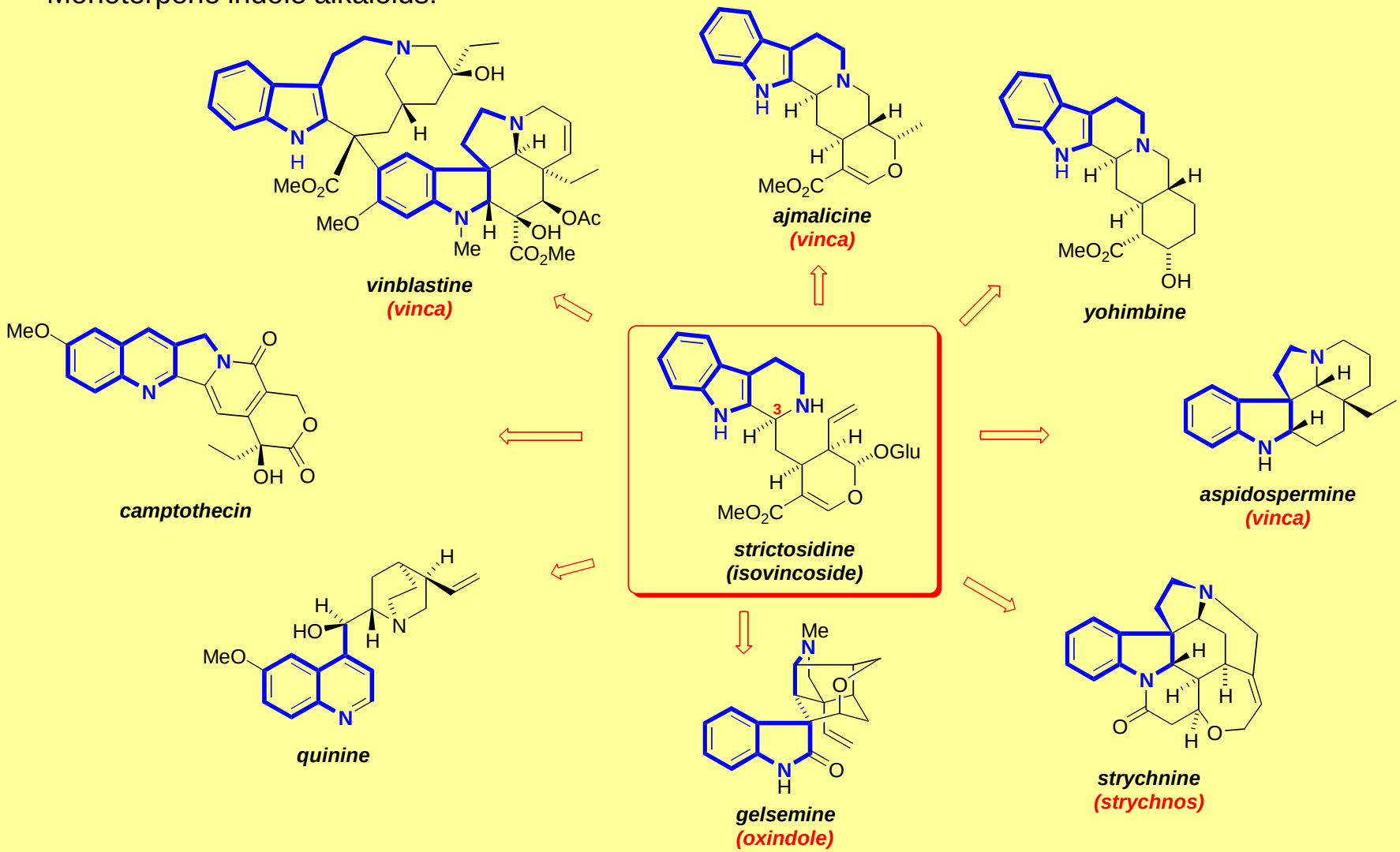
Apparently Irregular Monoterpenes

- Apparently irregular monoterpenes can also occur by ***non-cationic cyclisation*** of ***geranyl PP*** derivatives followed by ***extensive rearrangement***
  - *e.g.* ***iridoids*** – named after *Iridomyrmex* ants but generally of plant origin and invariably glucosidated
    - *e.g.* ***seco-loganin*** (recall ***indole alkaloids***) is a key component of ***strictosidine*** - precorsor to numerous complex medicinally important alkaloids:



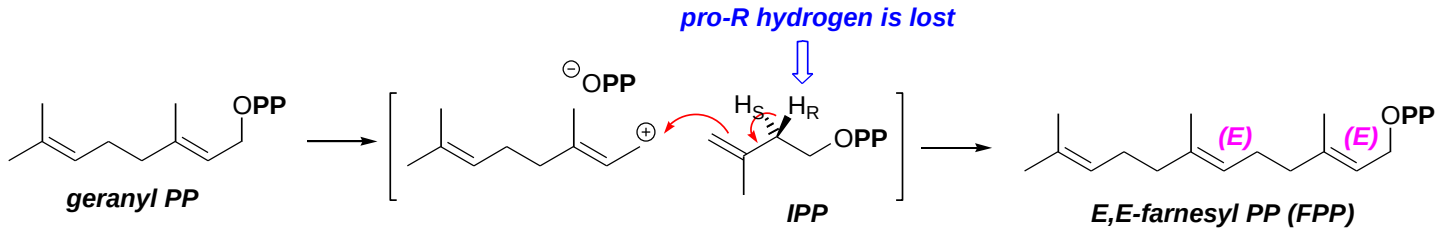
Strictosidine → *Vinca, Strychnos, Quinine etc.*

- The diversity of alkaloids derived from ***strictosidine*** is stunning and many pathways remain to be fully elucidated:
  - Monoterpene indole alkaloids:

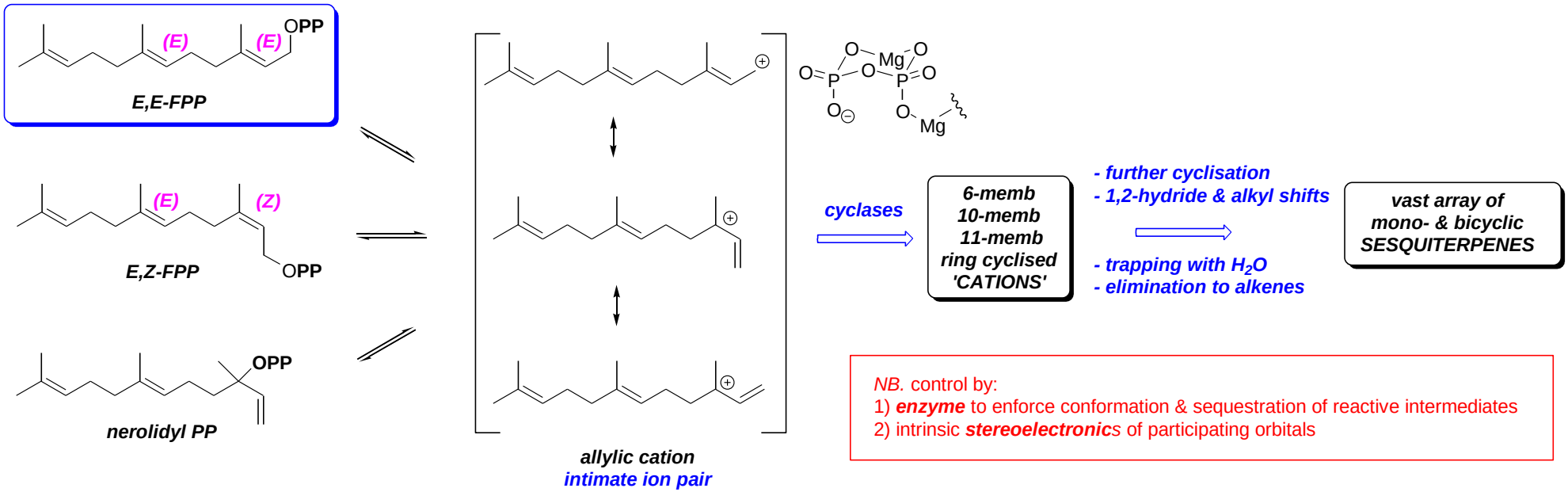


Sesquiterpenes – *Farnesyl Pyrophosphate (FPP)*

- ***'S_N2'-like alkylation*** of ***geranyl PP*** by ***IPP*** gives ***farnesyl PP***:

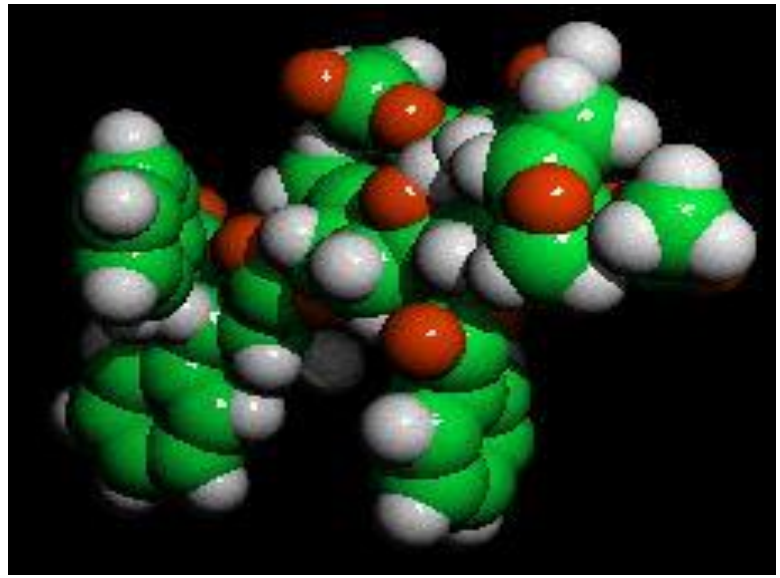


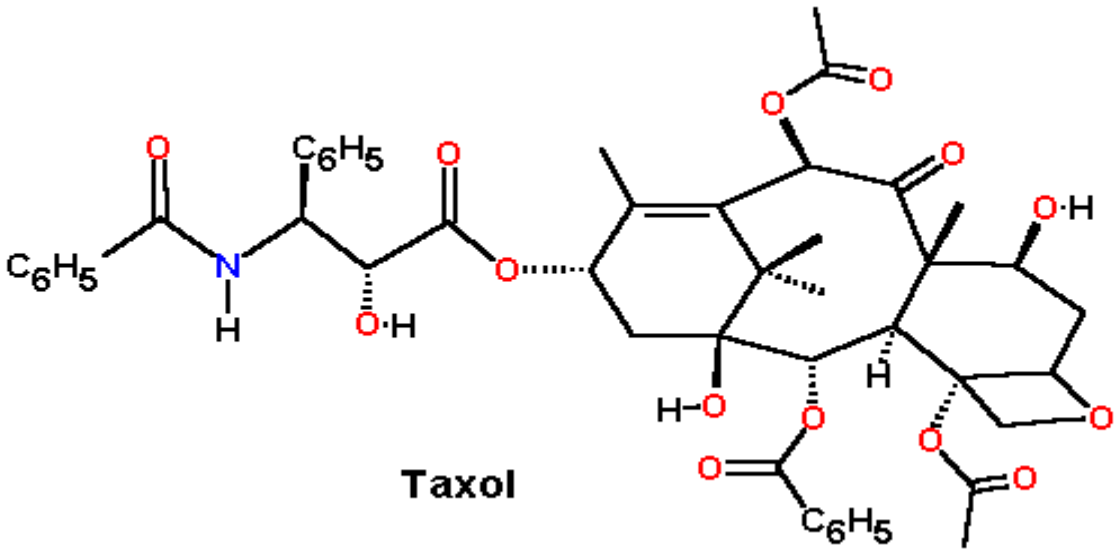
- just as ***geranyl PP*** readily isomerises to neryl & linaly PPs so ***farnesyl PP*** readily isomerises to equivalent compounds – allowing many modes of cyclisation & bicyclisation



NB. control by:
1) ***enzyme*** to enforce conformation & sequestration of reactive intermediates
2) intrinsic ***stereoelectronic***s of participating orbitals

Diterpenes - *Taxol*



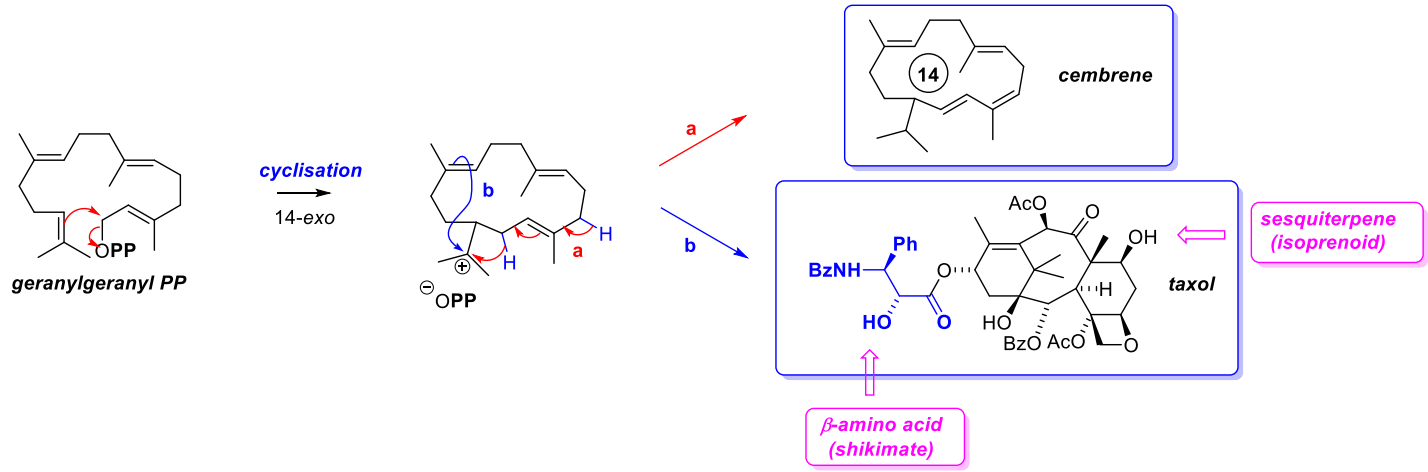


Taxol

Mode of action – binds to tubulin and interferes with microtubule disassembly thus preventing cell division

Diterpenes – *Geranylgeranyl PP → Taxol*

- ***Taxol*** is a potent ***anti-cancer agent*** used in the treatment of ***breast*** & ***ovarian cancers***
  - comes from the bark of the ***pacific yew*** (*Taxus brevifolia*)
  - binds to tubulin and intereferes with the disassembly of microtubules
- biosynthesis is from ***geranylgeranyl PP:***



  - for details see: http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/terp/taxadiene.html
  - home page is: http://www.chem.qmul.ac.uk/iubmb/enzyme/
    - recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzyme-Catalysed Reactions
    - based at Department of Chemistry, Queen Mary University of London

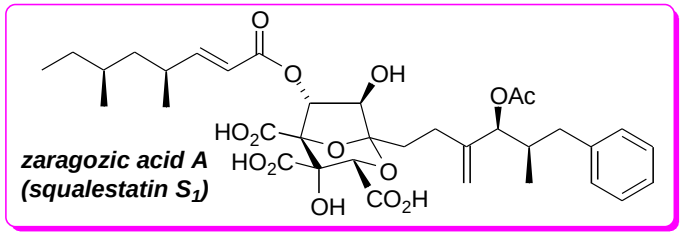
Triterpenes – *FPP → Squalene*

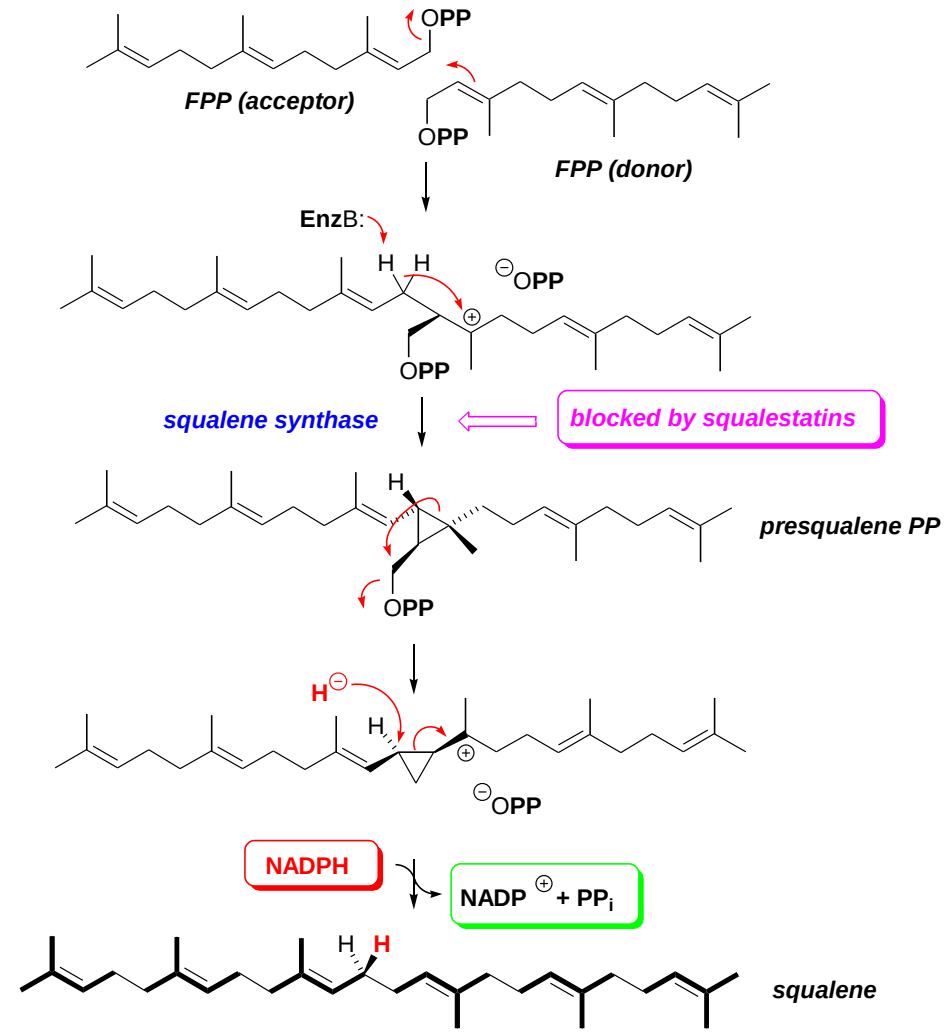
- ***triterpenes*** (C_{30}) arise from the ***'head to head' coupling*** of ***two fanesyl PP units*** to give ***squalene*** catalysed by ***squalene synthase:***

  – squalene was first identified as a steroid precursor from ***shark liver oil***

  – the dimerisation proceeds *via* an unusual mechanism involving electrophilic cyclopropane formation - rearrangement to a tertiary cyclopropylmethyl cation and reductive cyclopropane ring-opening by NADPH (*NB.* exact mechanism disputed)

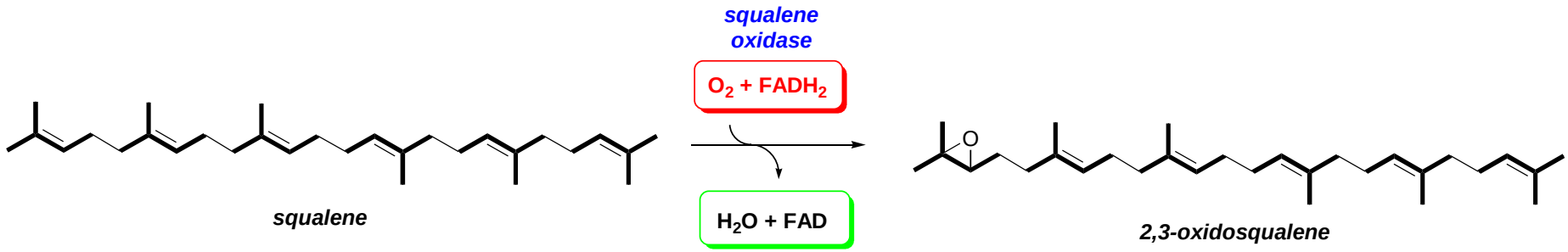
  – ***Zaragozic acids*** (***squalestatins***) mimic a rearrangement intermediate and inhibit squalene synthase. They constituted interesting leads for development of new treatments for ***hypercholesteraemia*** & ***heart disease*** (*cf.* statins)



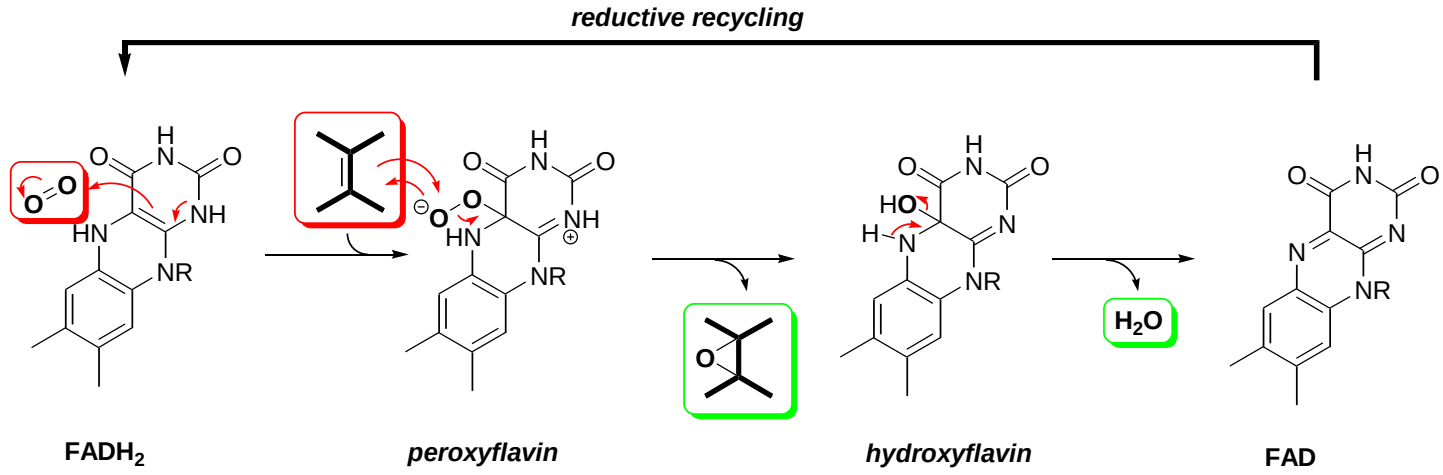


Triterpenes – *Squalene → 2,3-Oxidosqualene*

- ***squalene*** is oxidised to ***2,3-oxidosqualene*** by ***squalene oxidase*** – which is an ***O_2/$FADH_2$-dependent*** enzyme:

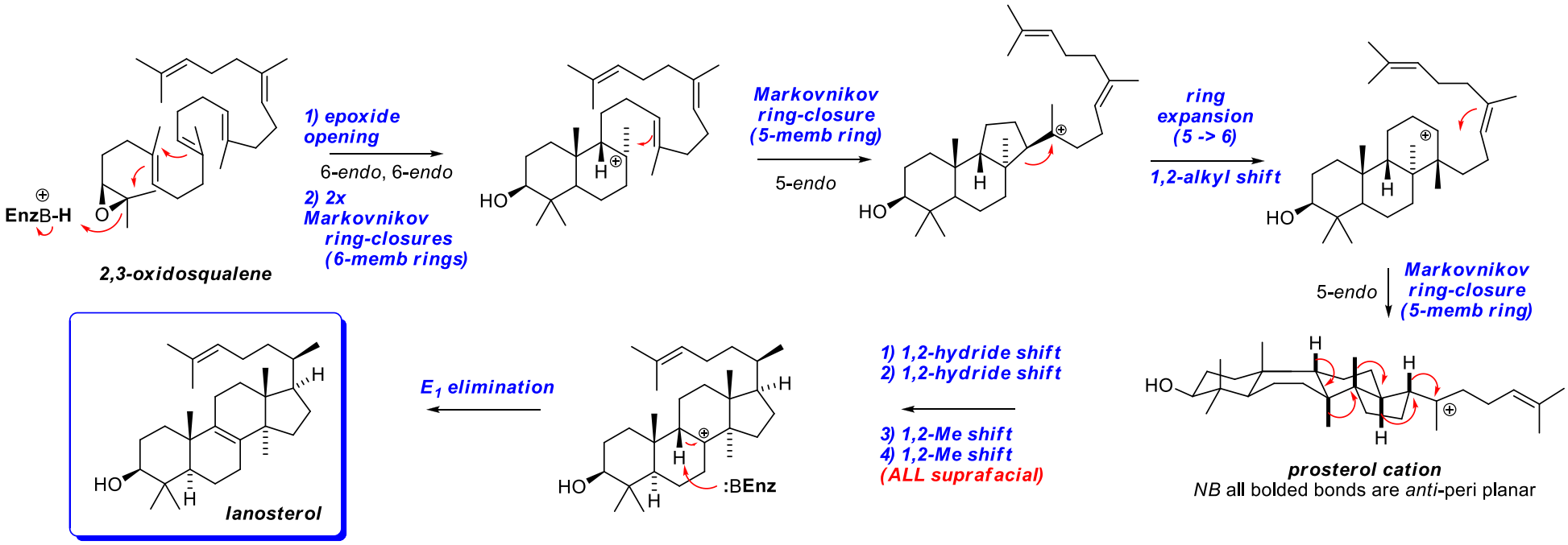


- the key oxidant is a ***peroxyflavin***:



Oxidosqualene-Lanosterol Cyclase – *Mechanism*

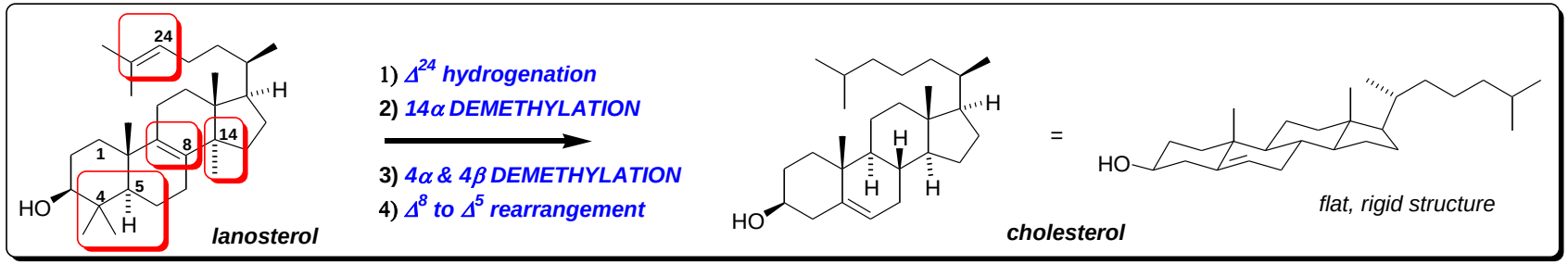
- ***oxidosqualene-lanosterol cyclase*** catalyses the formation of ***lanosterol*** from ***2,3-oxidosqualene***:
  - this cascade establishes the characteristic ring system of *ALL* ***steroids***
  - ring-expansion sequence to establish the C ring
  - the process is ***NOT concerted***, discrete ***cationic intermediates*** are involved ***& stereoelectronics dictate*** the ***regio- & stereoselectivity*** although the enzyme undoubtedly plays a role in pre-organising the ~chair-boat-chair conformation



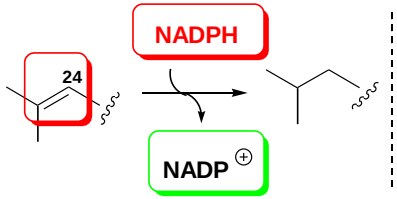
  - "*The enzyme's role is most likely to shield intermediate carbocations… thereby allowing the hydride and methyl group migrations to proceed down a thermodynamically favorable and kinetically facile cascade*"
    - Wendt *et al. Angew. Chem. Int. Ed.* **2000**, *39*, 2812 (DOI) & Wendt *ibid* **2005**, *44*, 3966 (DOI)

Lanosterol → Cholesterol – *Oxidative Demethylation*

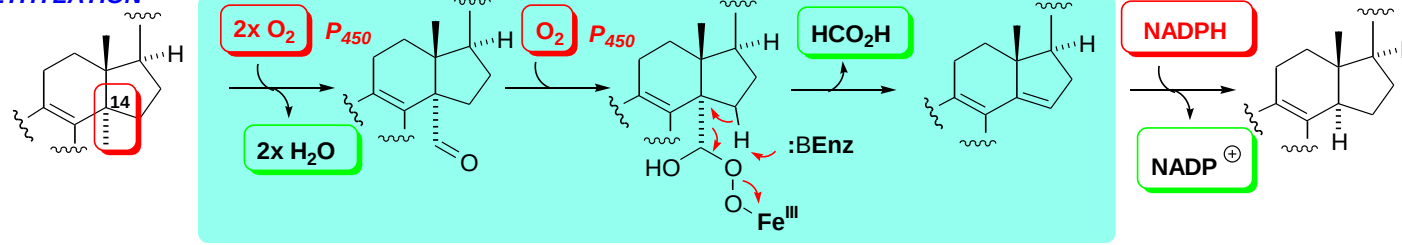
- Several steps are required for conversion of ***lanosterol*** to ***cholesterol:***



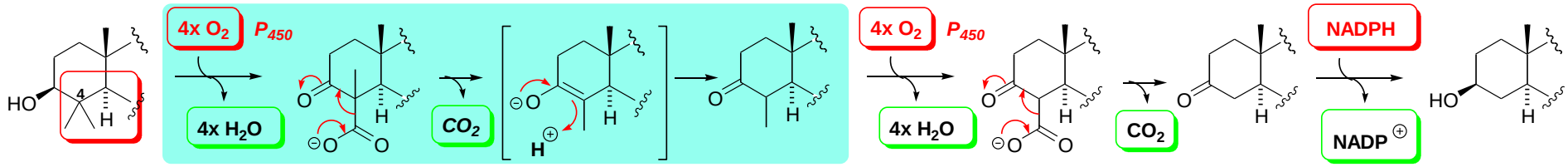
1) *Δ²⁴ hydrogenation*



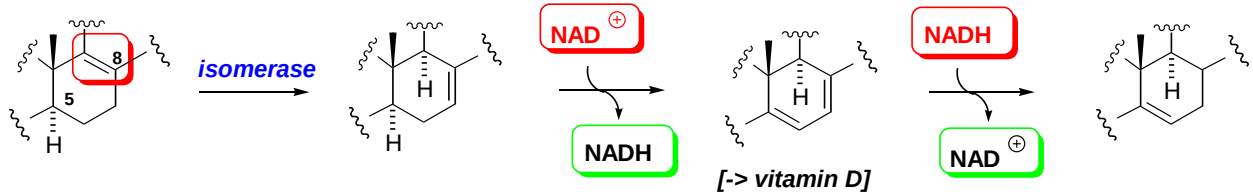
2) *14α DEMETHYLATION*



3) *4α & 4β DEMETHYLATION*

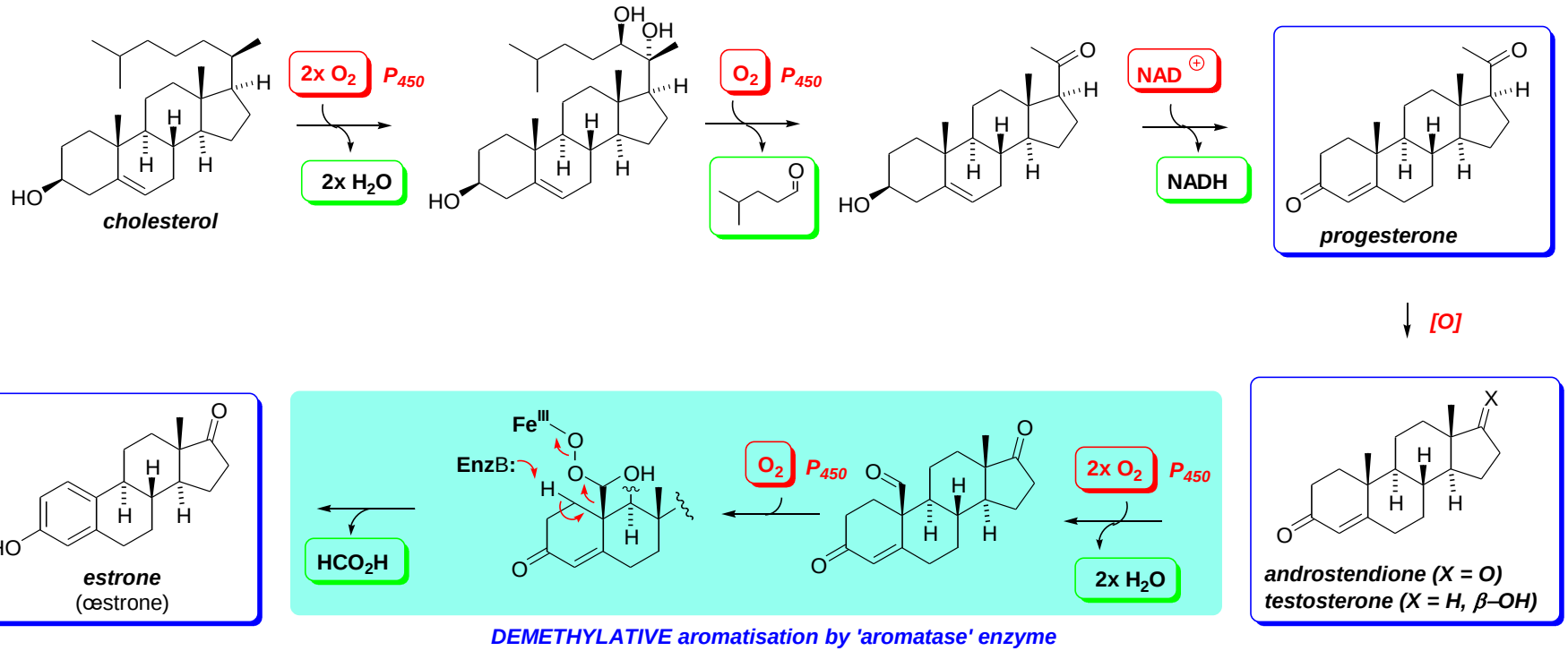


4) *Δ⁸ to Δ⁵ rearrangement*



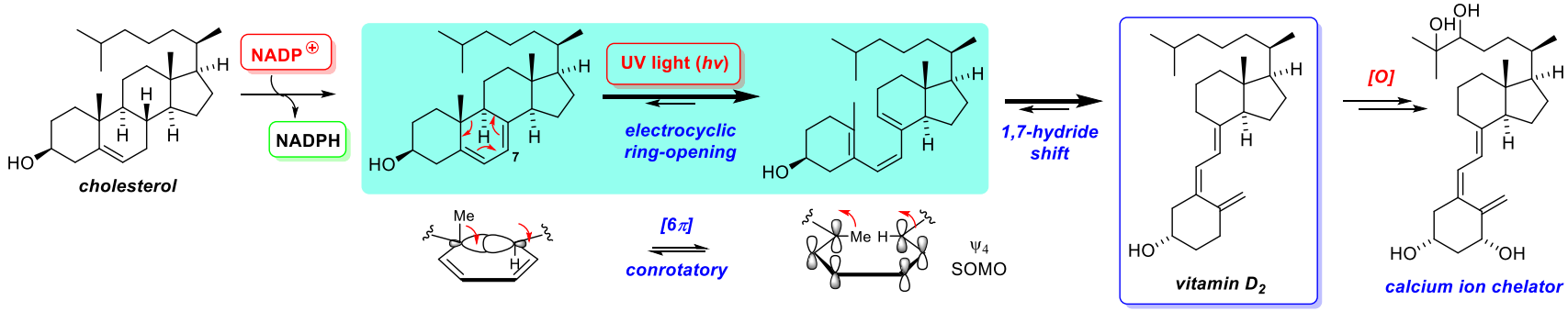
Cholesterol → Human Sex Hormones

- ***cholesterol*** is the precursor to the human sex hormones – ***progesterone, testosterone*** & ***estrone***
  - the pathway is characterised by ***extensive oxidative processing*** by ***P_{450} enzymes***
  - ***estrone*** is produced from ***androstendione*** by ***oxidative demethylation*** with ***concomitant aromatisation:***



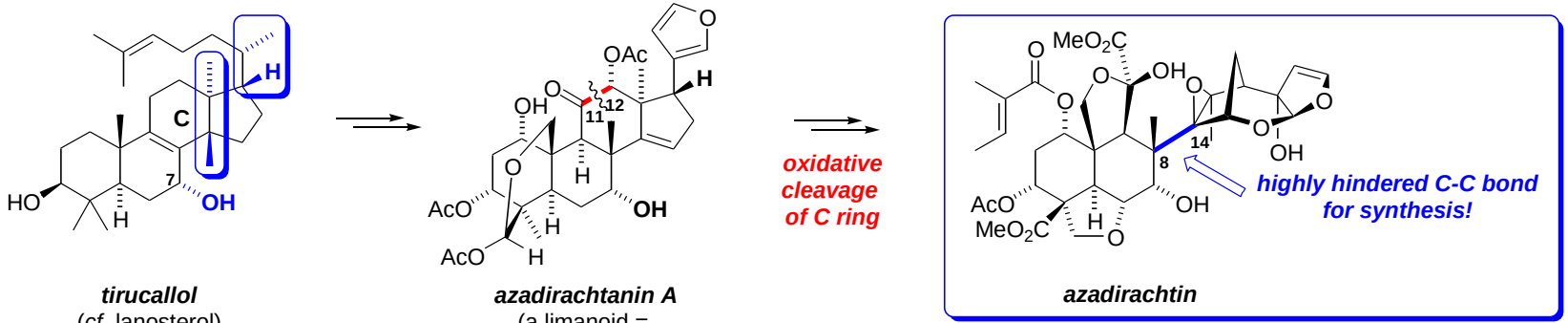
Steroid Ring Cleavage - *Vitamin D & Azadirachtin*

- ***vitamin D_2*** is biosynthesised by the ***photolytic cleavage*** of ***Δ^7-dehydrocholesterol*** by UV light:
  - a classic example of ***photo-allowed, conrotatory electrocyclic ring-opening:***

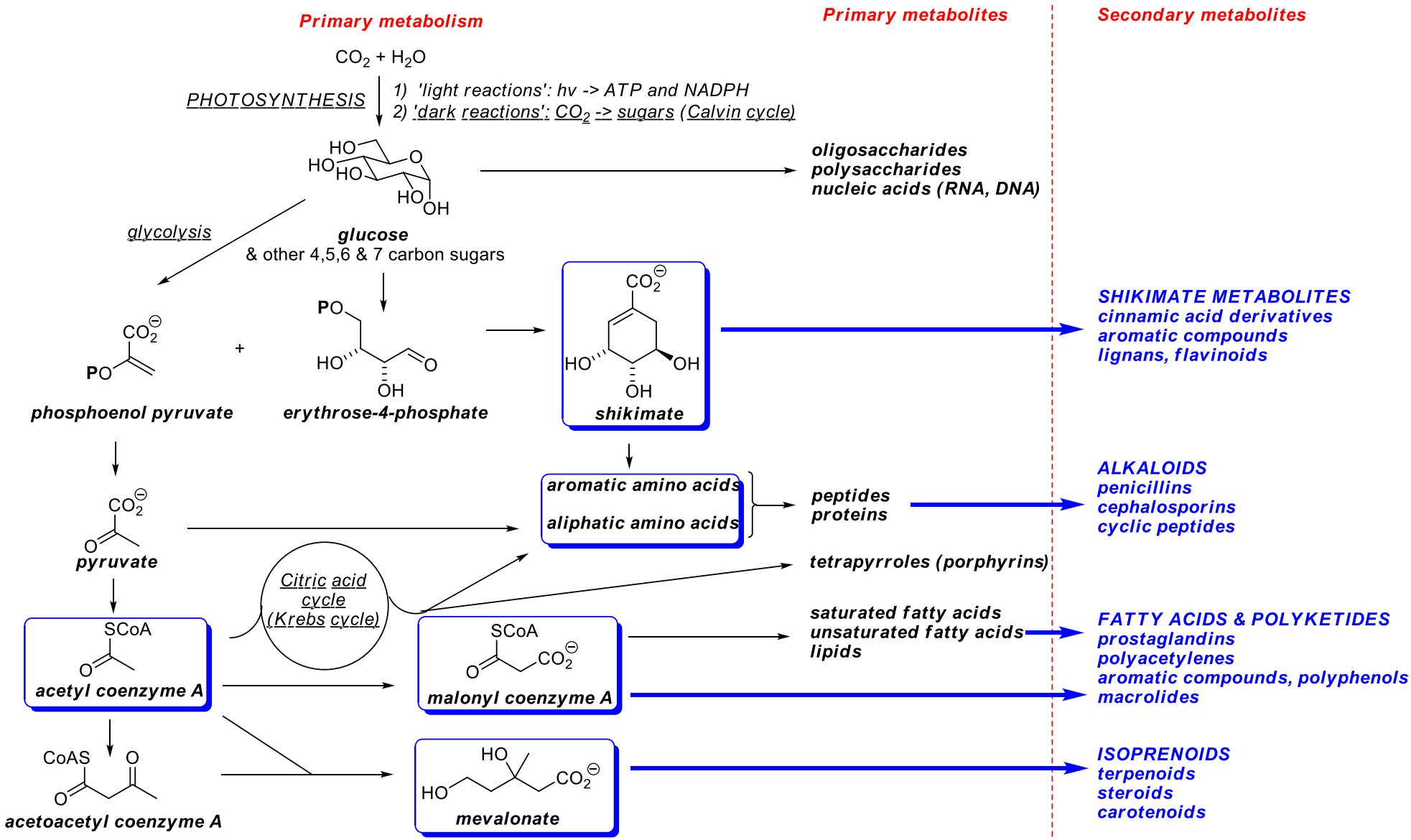


  - D vitamins are involved in ***calcium absorption; defficiency*** leads to ***rickets*** (brittle/deformed bones)

- ***Azadirachtin*** is a potent ***insect anti-feedant*** from the Indian ***neem tree:***
  - exact biogenesis unknown but certainly *via* steroid modification:



Primary Metabolism - *Overview*



For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**