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## *Biosynthesis*

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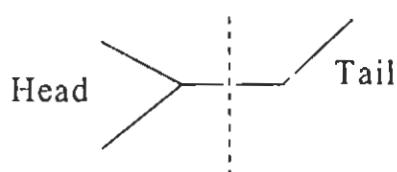
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## *Terpenes*

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### 3.1.1 Introduction

*Terpenes* are one of the most widespread and chemically interesting group of natural products, possessing a carbon framework comprised of five-carbon isoprene unit arrangements. The monomeric unit is called isoprene unit indicated by the symbol,  $C_5$ . This monomeric isoprene unit is considered as one of the Nature's favorite building block in terpene biosynthesis [1].



An isoprene unit ( $C_5$ )

Most terpenes possess carbon content in multiples of  $C_5$  arrangement and are classified into the following groups:

i.	Hemiterpenes	( $C_5$ )	1 $C_5$	Units
ii.	Monoterpenes	( $C_{10}$ )	2 $C_5$	Units
iii.	Sesquiterpenes	( $C_{15}$ )	3 $C_5$	Units
iv.	Diterpenes	( $C_{20}$ )	4 $C_5$	Units
v.	Sesterpenes	( $C_{25}$ )	5 $C_5$	Units
vi.	Triterpenes	( $C_{30}$ )	6 $C_5$	Units
vii.	Tetraterpenes	( $C_{40}$ )	8 $C_5$	Units
viii.	Polyterpenes	( $C_{>40}$ )	>8 $C_5$	Units

Isoprene units can be easily recognized through “head to tail” bonds as well as supplementary bonds, and are the common biosynthetic origin of the terpenes [2,3]. Biosynthesis of polycyclic triterpenes involves three major steps, which are:

- i. Biosynthesis of the active isoprene ( $C_5$ ) units, **IPP** and **DMAPP**

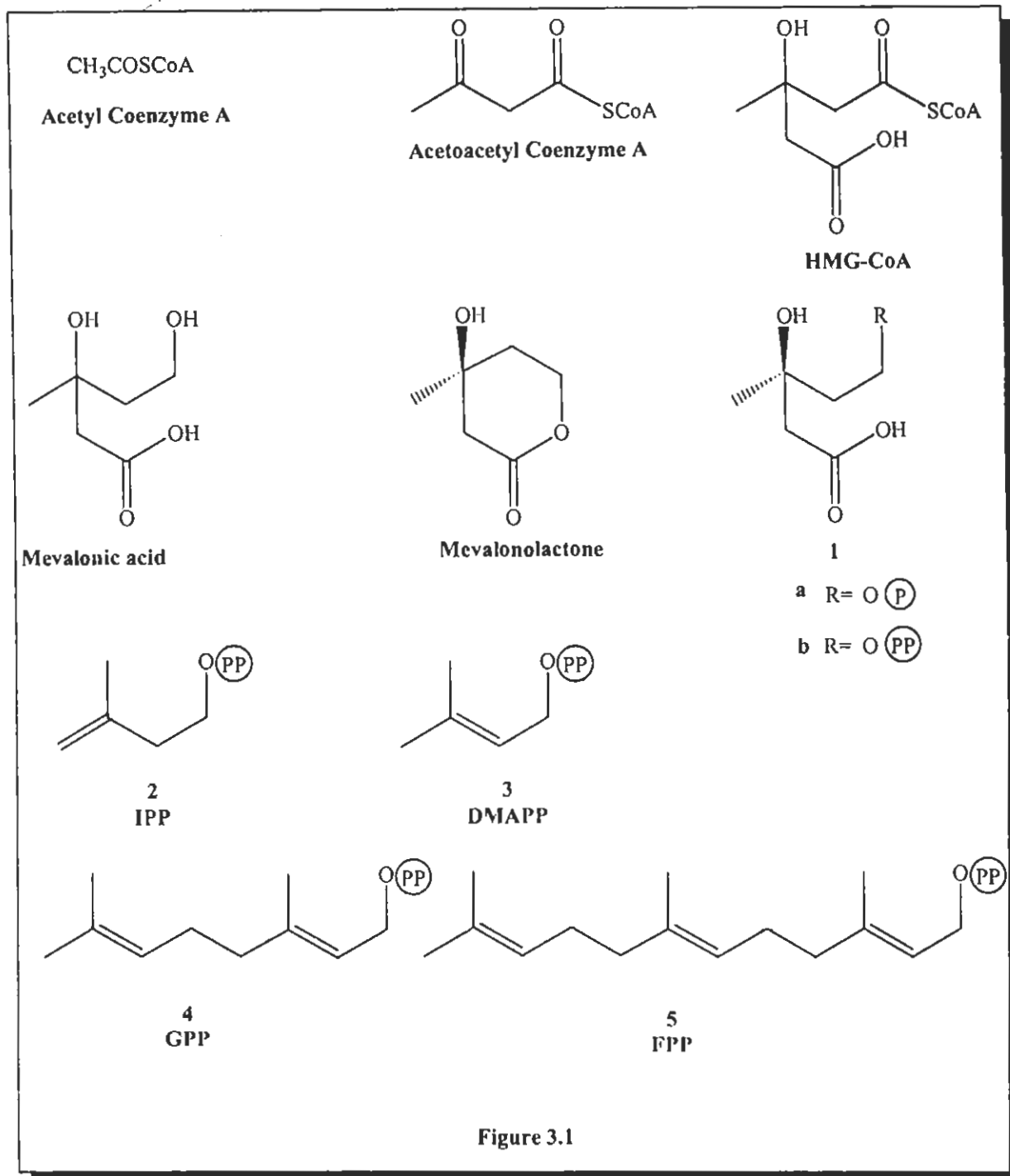
- i. Biosynthesis of the prenylogues, Squalene and Epoxysqualene
- ii. Cyclization and rearrangements to form polycyclic triterpenes.

### 3.1.2 Biosynthesis of the active isoprene ( $C_5$ ) units, IPP and DMAPP

Triterpenes are a group of natural products having thirty-carbon skeleton, which can be considered as being derived from **mevalonic acid (MVA)** through **squalene** and in most cases, *via* **2,3-epoxysqualene**. Their various structures depend on the tendency of squalene with its double bonds to undergo multiple cyclizations. These cyclizations mediated by enzymes (**cyclases**), are capable of exerting rigorous stereochemical controls. Biosynthetic studies have led to the formulation of the biogenetic isoprene rule, which was originally proposed by Ruzicka [4] in 1953. The rule has been subsequently improved and extended to cover the increasing number of terpenic constituents isolated from natural sources. In 1959, J. W. Cornforth characterized two active forms of isoprene, isopentenyl pyrophosphate (**IPP; 2**) and dimethylallyl pyrophosphate (**DMAPP; 3**), which are obligatory for the synthesis of plant terpenes. Various enzymes catalyze the incorporation of these units and their intermediates into terpenes.

**(+)-Mevalonic acid (MVA)** is considered as the starting material for the biosynthesis of squalene, and hence terpenoids [5,6]. **Mevalonic acid (MVA)** is derived from the reduction of  **$\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme A (HMG-CoA)** with **nicotinamide adenine dinucleotide phosphate (NADPH)** while **HMG-CoA** is derived from **acetylcoenzyme A** through **acetoacetyl coenzyme A**. (*Fig 3.1*).

**Mevalonolactone** and **mevalonic acid** [7] provide **R-5-diphosphomevalonic acid (1b)** through formation of **5-phosphomevalonic acid (1a)**, which then provides **isopentenyl diphosphate** or **isopentenyl pyrophosphate (IPP; 2)** through decarboxylative elimination. The conversion of **1a** into **1b** [8] is catalyzed by **phosphomevalonate kinase** while the conversion of **1b** into **IPP** is



catalysed by **diphosphomevalonate decarboxylase**. The isomerisation of **IPP** (2) leads to **dimethylallylpyrophosphate** (**DMAPP**; 3), catalysed by enzyme **isopentenyl-diphosphate  $\Delta$ -isomerase**.

### 3.1.3 Biosynthesis of the prenylogues, squalene and epoxy-squalene

**IPP** on coupling with **DMAPP** provides **geranyl diphosphate** or **geranyl pyrophosphate** (**GPP**; 4) and its isomer, while **farnesyl diphosphate** or **farnesyl pyrophosphate** (**FPP**; 5) is obtained by the coupling of **GPP** with another molecule of **IPP**. Both these condensations are catalysed by **dimethylallyl transferase**. The enzyme-catalysed condensation [8-15] involves superficial alkylation-deprotonation of **IPP**. In principle, the reaction could involve a concerted addition-elimination sequence or a *trans* addition process followed by *trans* elimination.

The formation of **squalene** from **farnesylpyrophosphate** (**FPP**) can be considered a reductive dimerization (tail to tail). The addition of two hydrogen atoms to the **FPP** molecules with liberation of two molecules of **pyrophosphoric acid** provides one molecule of **squalene**. This reaction proceeds through **presqualene alcohol pyrophosphate** (**PSAPP**) intermediate [15]. This conversion is an asymmetric process. In most organisms **squalene** is formed and immediately transformed into **2,3-epoxide**. The conversion of **squalene** into **(3S)-2,3-oxidosqualene** is catalysed by the enzyme **squalene epoxidase** (**squalene monooxygenase**).

### 3.1.4 Cyclization and rearrangements to form polycyclic triterpenes

The fundamental or primary triterpenes are those triterpenes, which are derived from the cyclisation of **squalene** or of its **2,3-epoxide**; they can be divided into sub-groups according to their origin from:

- a) Oxidative cyclization
- b) Non-oxidative cyclization
- c) Oxidative and non-oxidative cyclisation

#### 3.1.4.1 Oxidative Cyclisation

Oxidative cyclization of **squalene** (*Scheme 3.1*) gives rise to the tetracycles, euphol, tirucallol, lanosterol, dammarenediols, cycloartenol and protosterol, which differ slightly from each other in their cyclisation mechanisms. The **dammarenediols**, **euphol** and **tirucallol** are derived from **2,3-epoxy squalene** molecule, held in active site of the enzyme in “chair-chair-chair-boat” conformation, while **lanosterol** and **cycloartenol** are derived from a “chair-boat-chair-boat” conformation.

#### 3.1.4.2 Non-oxidative Cyclisations

The Non-oxidative cyclisations (e.g. in **tetrahymanol**) are rare, and occur from a proton attack at the **2,3-double bond** of **squalene** (*Scheme 3.1*). In the non-oxidative cyclisations, the formation of the various skeletons could be derived from the “chair-chair-chair-chair-chair” squalene conformation.

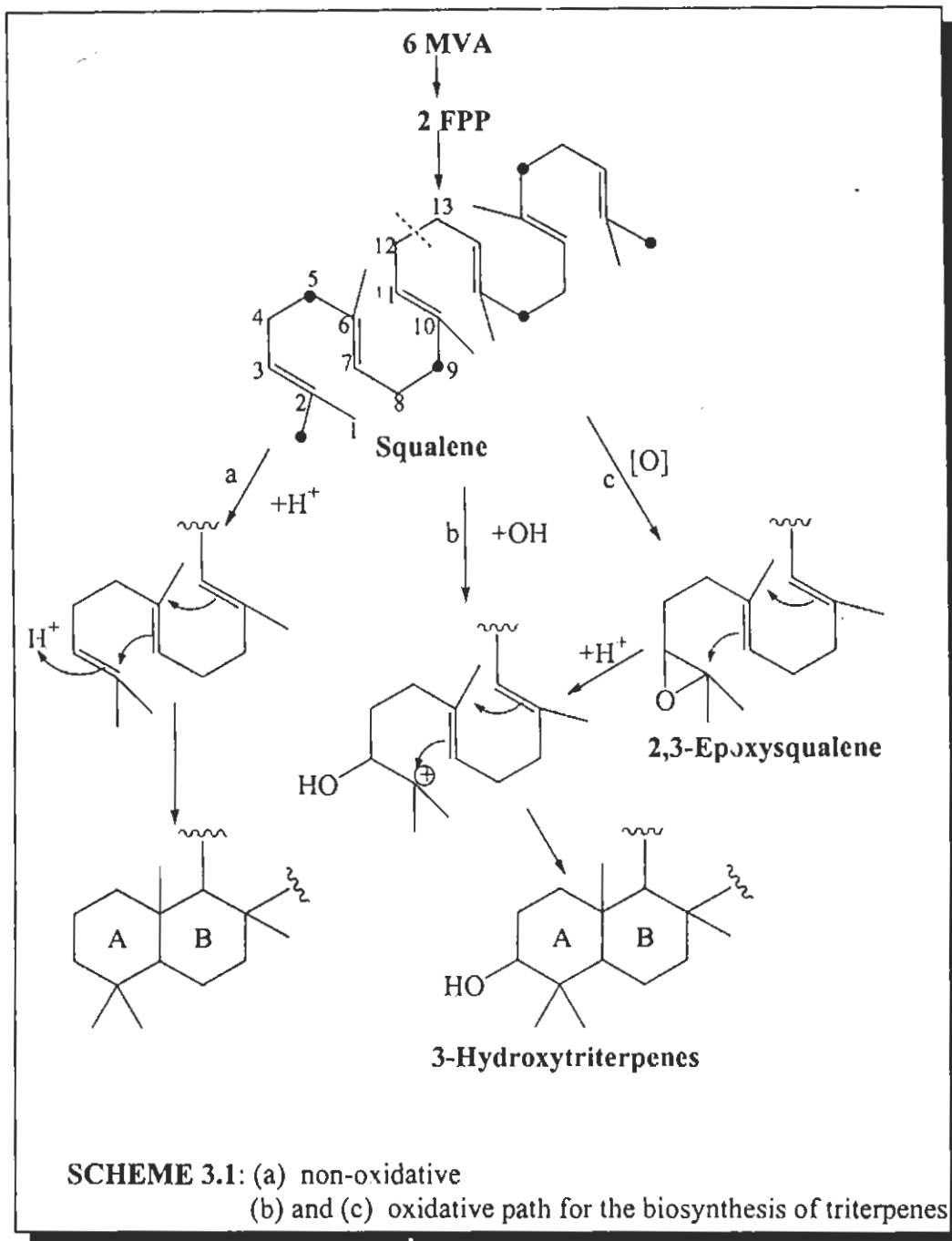
#### 3.1.4.3 Oxidative and Non-oxidative Cyclisations

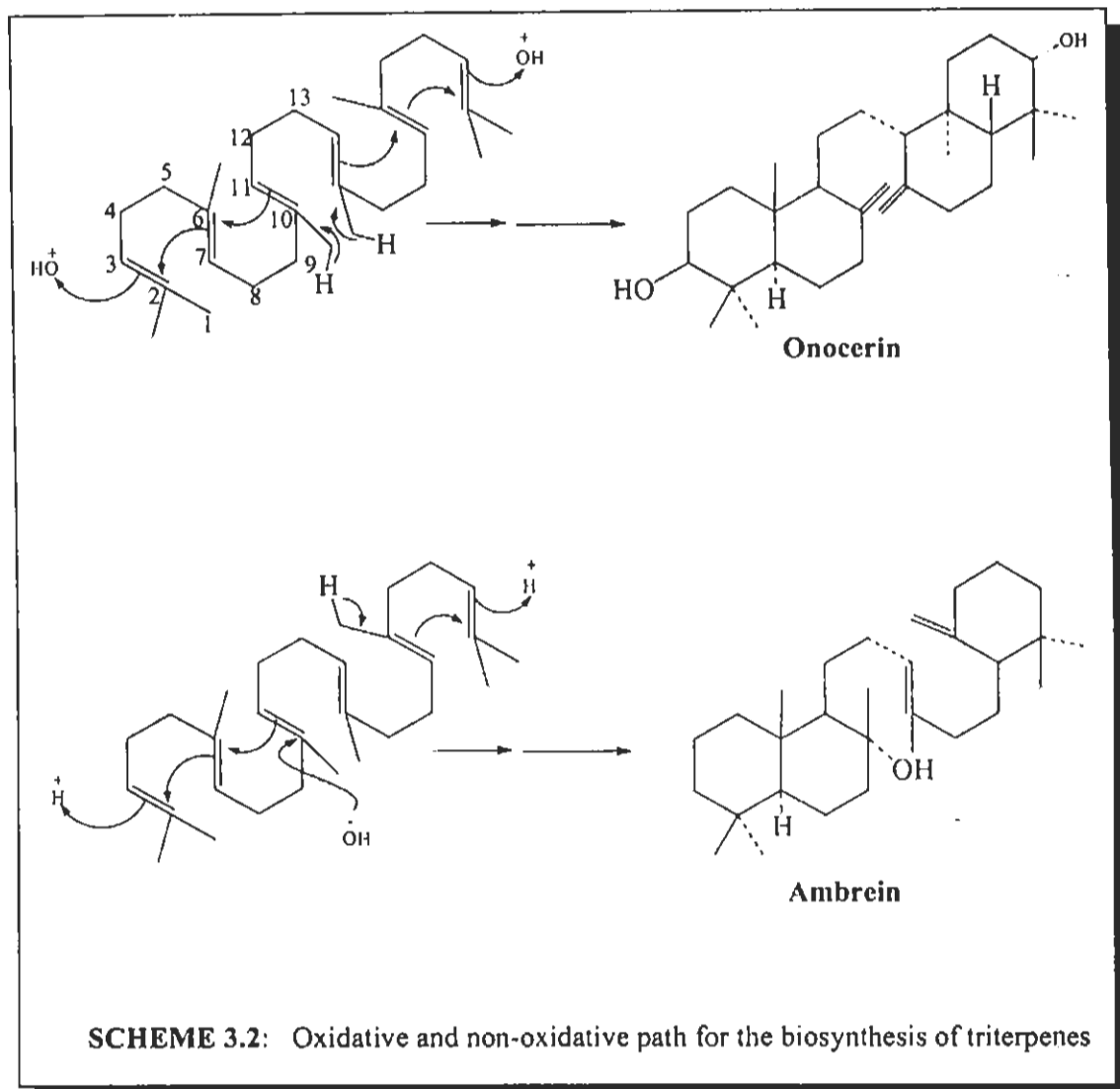
Both terminals of the **squalene** molecule are involved in these cyclisations. The biosynthesis of some triterpenes is explained by independent **electrophilic attacks** at both terminals of the **squalene** molecule [16]. These two attacks are both oxidative (e.g. **onocerin**) and non-oxidative (e.g. **ambrein**) (*Scheme 3.2*).

#### 3.1.5 Triterpenoids of *Azadirachta Indica*

The major group of isoprenoids of neem is triterpenoid, which include protolimonoids (apo-protolimonoids), mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids (limonoids),

pentanortriterpenoids, hexanortriterpenoids, octanortriterpenoid and nonanortriterpenoid. Other isoprenoids reported from neem include sterols (modified isoprenes) and diterpenoids. The biosynthetic aspects of these are discussed along with the chemistry of neem terpenoids.





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## *Flavonoids*

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### 3.2.1 Introduction

*Flavonoids* are another diverse and widespread group of natural products, which have prominent position among the natural phenols. The name “flavonoid” is derived from Greek word “flavus” (yellow) [17,18]. Flavonoids are the colouring co-pigment of the plants, which are also called anthoxanthins [19]. Flavonoids can be divided into sub-classes according to their structural variations. The parent nucleus comprises fifteen carbon atoms and share the common structural feature of two phenyl rings linked by a three-carbon chain (diphenyl propane derivatives). The compounds possessing **1,3-diphenylpropane** skeletons are regarded as **chalconoids**. The tricyclic system is generated by the ring (five or six membered) formation of oxygen of one of these phenyl rings with one of the carbons of three-carbon chain. Those tricyclic compounds, which possess a five membered heterocyclic ring are called **auronoids**, whereas those possessing a six membered heterocyclic ring are designated as **flavonoids**. The tricyclic compounds derived from **1,2-diphenylpropane** system, are known as **isoflavonoids** and **3-phenylcoumarins**, whereas those derived from **1,1-diphenylpropane** are called **neoflavonoids**. **Homoflavonoids** are the class of flavonoids, which contain an additional carbon in their skeleton. A large number of flavonoids occur as *O*-glycosides in which one or more of the hydroxyl groups of the flavonoid are bound to a sugar or sugars *via* an acid labile hemiacetal bond. In *C*-glycoside flavonoids, the sugar is linked with carbon atom and this linkage is acid resistant [20,21]. The effect of glycosylation is to render the flavonoid less reactive and more water-soluble.

The biological effects of the flavonoids have been reviewed by Willaman [22], who listed thirty-three different manifestations of activity under the heading “Bioflavonoids”. Rutin and hesperdin, also called vitamin P or permeability factors, are used in the treatment of various diseases, like capillary bleeding, increased capillary fragility, diabetes, allergic manifestation and hypertension. Phenolic

substances are well known to have anti-inflammatory activity. Some flavonoids like myricetin and kaempferol-3-glucoside have an anti HIV-1 potency at non-toxic concentration [23]. It has also been observed that the position of the substitution affects the activity. The flavonols containing two *ortho* or *para* hydroxyls in ring-B have anti-oxidant properties, while free hydroxyl at the 5,7-positions have a pro-oxidant effect [19]. Chalcones have been thoroughly investigated by G. Michiro and it was found that these have anti-microbial activity, which is enhanced by bromination [24].

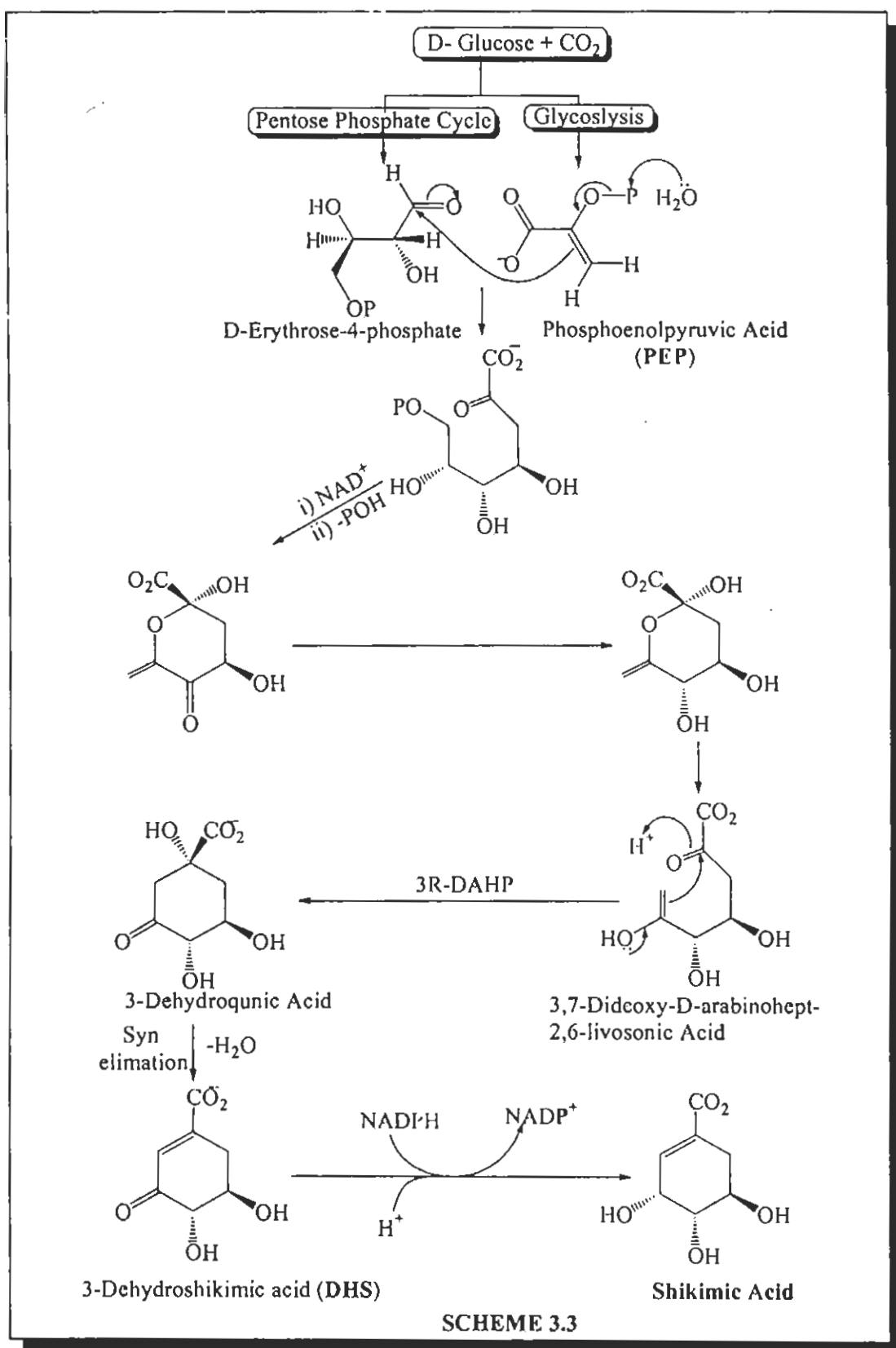
### 3.2.2 Biosynthesis of Chalcones

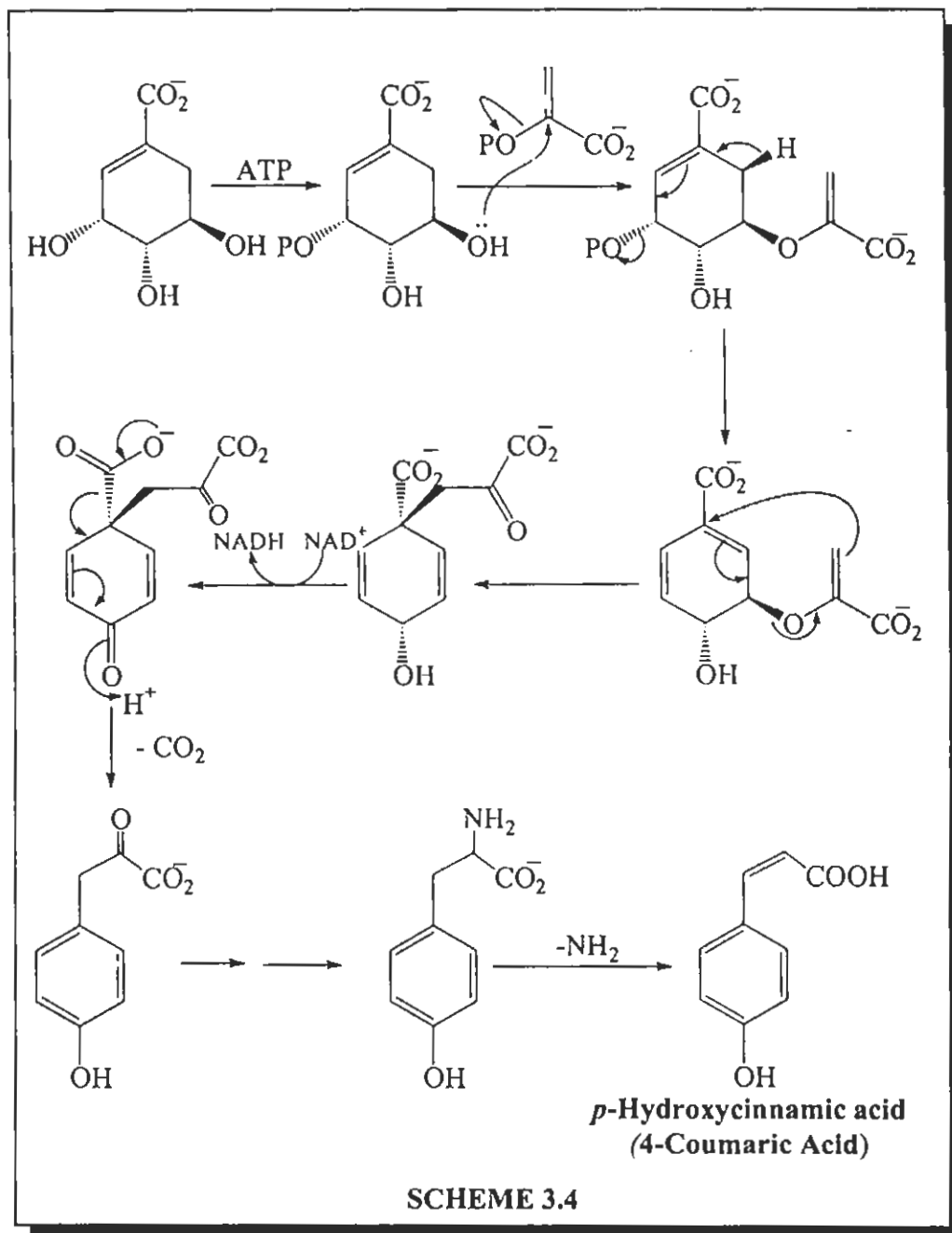
The biosynthesis of all flavonoids involves the formation of central C-15 intermediate known as **chalcone**. The central role of chalcones in flavonoid biosynthesis has been attested in a number of investigations. The precursors for **chalcone** formation are **malonyl-CoA** and **4-coumaroyl-CoA** (*p*-hydroxycinnamic acid CoA ester). **Malonyl-CoA** is synthesized from the glycolysis intermediate **acetyl-CoA** and **carbon dioxide**, the reaction being catalyzed by **acetyl-CoA carboxylase**. The supply of **4-coumaroyl-CoA** is more complex and involves the **shikimate pathway**, which is the main route to **aromatic amino acid**, **phenylalanine** and **tyrosine** in higher plants [25]. The biosynthesis of **shikimic acid** (*Scheme 3.3*) begins with the condensation between **D-erythrose-4-phosphate** and **phosphoenolpyruvic acid**. **Shikimic acid** is converted into *p*-hydroxycinnamic acid (*Scheme 3.4*) following a series of reactions. **Chalcone** is obtained by the coupling of condensation product of **malonyl-CoA** and **4-coumaroyl-CoA** followed by the cyclization catalyzed by **chalcone synthase** (*Scheme 3.5*).

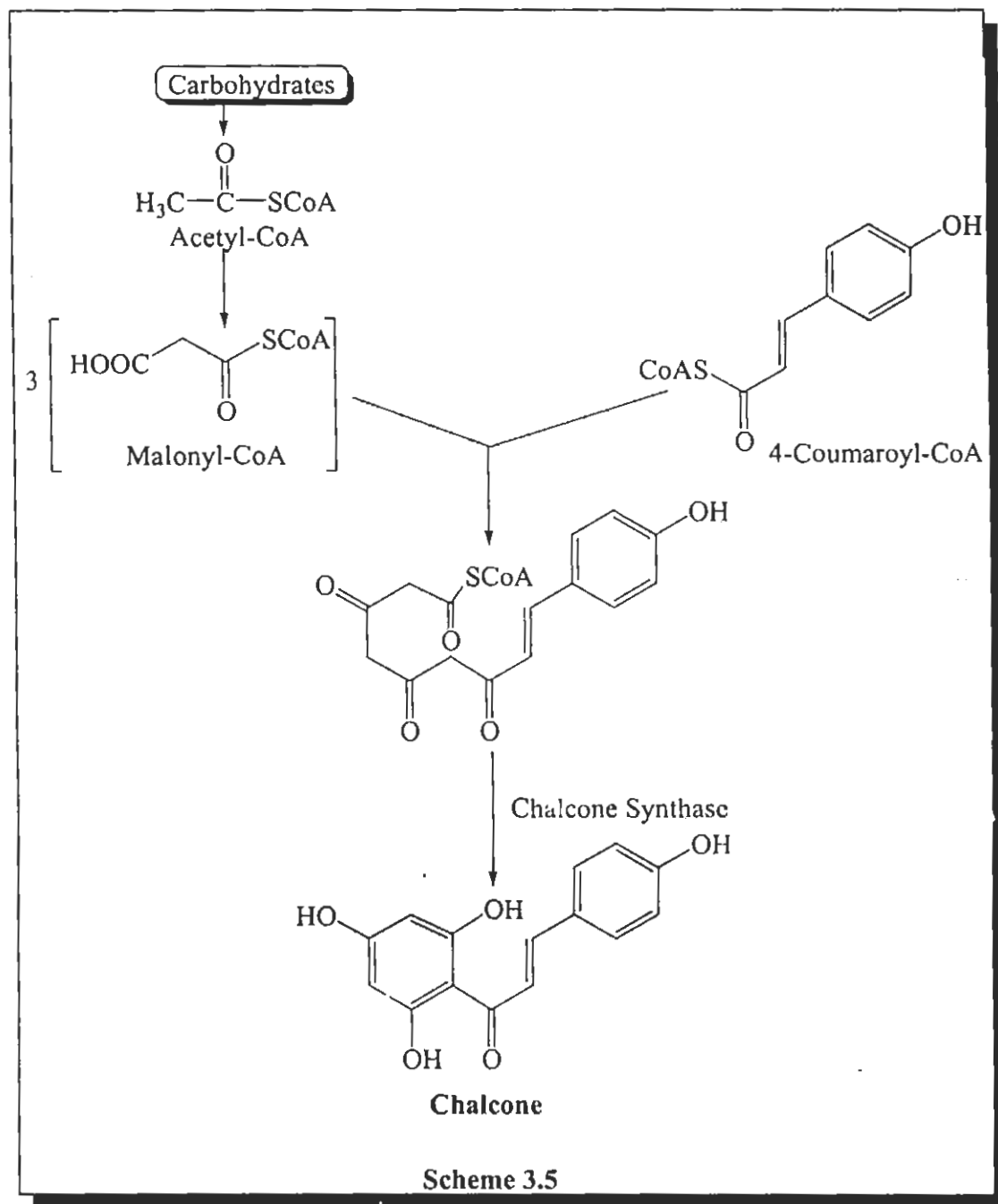
### 3.2.3 Biosynthesis of Flavanone

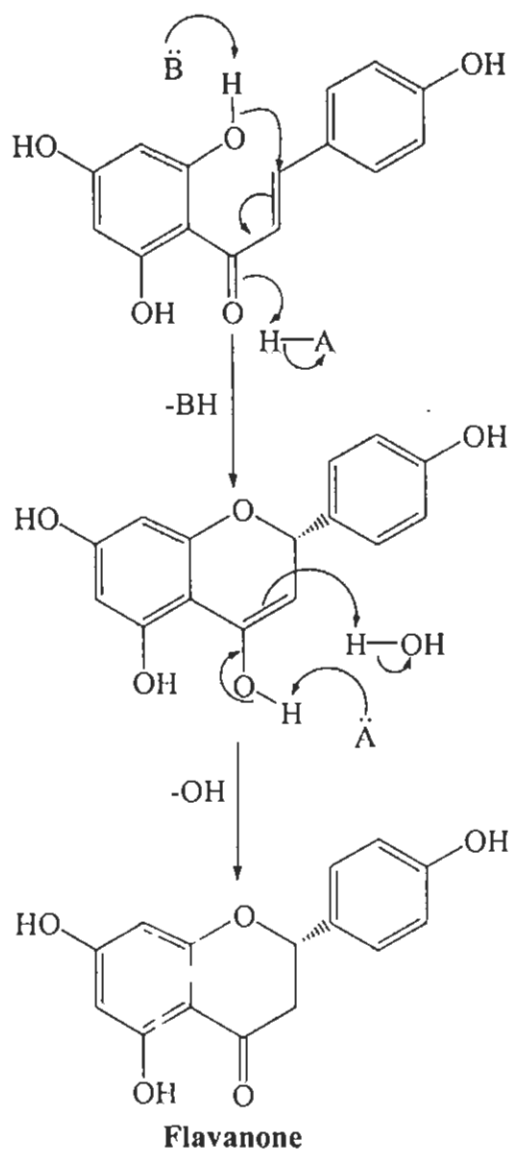
Flavanones are formed from **chalcones** by isomerization. There is a good evidence for the *in vitro* and *in vivo* existence of equilibrium between **flavanones** and the corresponding **chalcones**. The interconversion between **chalcones** and

**flavanones** is catalyzed *in vivo* by enzyme known as **chalcone isomerase**. The stereospecificity of this enzymatic reaction is apparent in the (*S*) chirality of C-2 in flavanone derivative. Therefore, it is not accidental that all the **flavanones** found in nature have the (*S*) configuration at C-2 and are levorotatory. With **chalcones** having at least two free hydroxyl groups at C-2 and C-6, the equilibrium in an aqueous solution is completely and rapidly shifted to the **flavanone** (*Scheme 3.6*). The stabilization energy of the strong hydrogen bond between the carbonyl group and the *ortho*-phenolic hydroxyl group greatly influences the position of equilibrium and the interconversion rate. When only one hydroxyl is available, either for the cyclization or for hydrogen bonding, the system tends to remain in the open form (**chalcone form**).









SCHEME 3.6

### 3.3 References

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