Part 3

TERPENOIDS and STEROIDS

Introduction: Biogenetic Generalities

Elaborated from common precursors, terpenoids and steroids undoubtedly constitute the largest known group of plant secondary metabolites. The vast majority of terpenes are specific to the vegetable kingdom, but they also occur in animals: sesquiterpenoid insect pheromones and juvenile hormones, diterpenes of marine organisms (Coelenterata, Spongiae). Triterpenes are also specific to the vegetable kingdom. Plant steroids arise, like triterpenes—*via* squalene—from mevalonate: the mechanism of their formation is slightly different from that of triterpenes, and most often, their structure is perfectly specific to a plant group: this is true for cardenolides, steroidal alkamines, saponins, and phytosterols.

All terpenes and steroids, and this is an essential common point, may be considered formed by the assembly of a whole number of five-carbon units derived from 2-methylbutadiene: already in 1887, O. Wallach conjectured that terpenes must be constructed from isoprene units, and many years later (1953), Ruzicka, after more than thirty years of dedication to the study of terpenes, turned this hypothesis into a general rule, whose principle has since been confirmed experimentally.

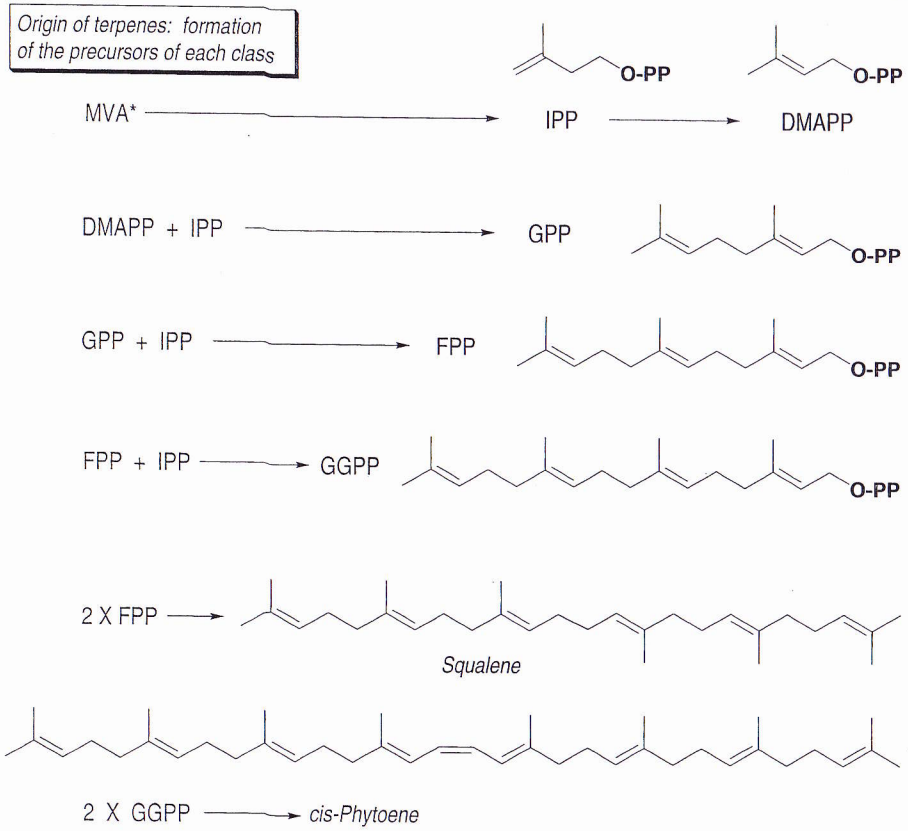
Each group of terpenes arises from the head-to-tail condensation of a variable number of isoprene units.

This theoretical rule (isoprene itself does not participate in the biogenesis) postulates that the structural diversity is only apparent: *in each group of terpenes, a unique precursor leads to the different known constituents* by a succession of classic reactions (cyclizations, functionalizations, or rearrangements).

The precursors for the chief classes of terpenes are formed by reactions catalyzed by enzymes, and are phosphoric esters of $(C_5)_n$ alcohols formed by the sequential addition of a C_5 unit, *isopentenyl pyrophosphate* (IPP) onto a starter molecule, namely an allylic prenyl pyrophosphate, with the first unit in the series being *dimethylallyl pyrophosphate* (DMAPP):

- *geranyl pyrophosphate* (GPP), precursor of C_{10} monoterpenes;
- *farnesyl pyrophosphate* (FPP), precursor of C_{15} sesquiterpenes;
- *geranylgeranyl pyrophosphate* (GGPP), precursor of C_{20} diterpenes;
- *geranylfarnesyl pyrophosphate* (GFPP), precursor of C_{25} sesterterpenes;
- the formation of C_{30} triterpenes (and indirectly of steroids) and of C_{40} carotenes does not completely escape the rule: they arise from squalene and phytoene, two hydrocarbons resulting, respectively, from the reductive coupling of two FPP units ($2 \times C_{15} = C_{30}$), and of two GGPP units ($2 \times C_{20} = C_{40}$);
- in the case of polyprenols (rubber and related compounds), the addition of C_5 units takes place a great number of times.

Origin of terpenes: formation of the precursors of each class



* MVA = Mevalonic acid ; IPP = isopentenylpyrophosphate ; DMAPP = dimethylallylpyrophosphate ; FPP = farnesylpyrophosphate ; GPP = geranylpyrophosphate ; GGPP = geranylgeranylpyrophosphate.

In a few cases, the isoprene rule does not seem to be followed: "irregular" monoterpenes (e.g., pyrethric acid, santolinatriene) arise from the coupling of two DMAPP units, by a mechanism similar to the one which leads to triterpenes and carotenes.

At first glance, steroidal structures seem to disobey the isoprene rule, but a closer review of their structure and biogenesis reveals how a series of degradations and rearrangements of the triterpenoid skeleton is the origin of the apparent anomalies. The same statement applies to highly degraded compounds, such as limonoids or quassinoids.

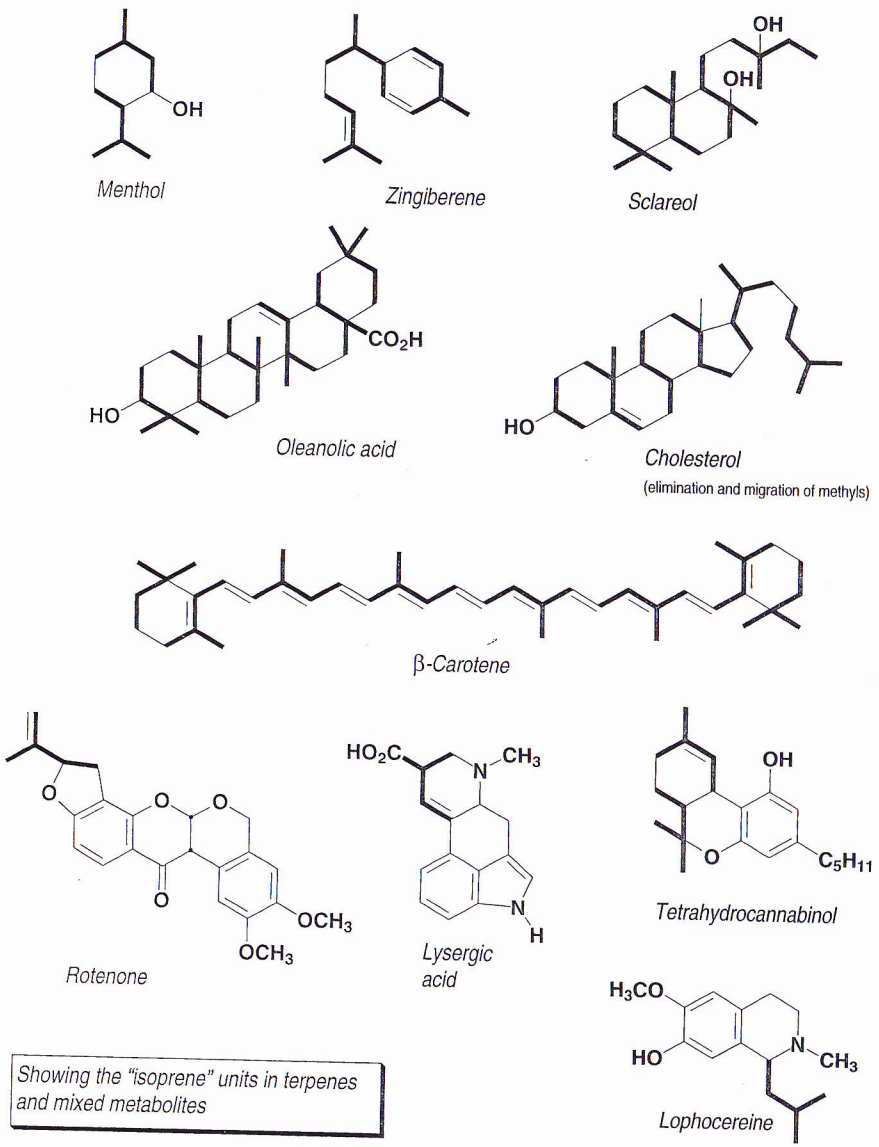
Although naturally-occurring "hemiterpenoid" (C_5) structures are rather exceptional (volatile hydrocarbons, free and glycosylated alcohols), the isoprene moiety is fairly often involved in elaborating the structure of a large number of secondary metabolites of so-called *mixed origin*: this is a result of the reactivity of DMAPP, an efficient alkylating agent. This is observed among polyphenols, for example coumarins such as bergapten, isoflavonoids such as rotenone, and naphthoquinones such as shikonin. This is also the case for some alkaloids: the C5 unit is apparent in some Cactaceae alkaloids (e.g., lophocereine), it is found again in the furan or pyran ring which characterizes several quinoline derivatives from Rutaceae, and although at first it is not obvious, it is present in the ergoline nucleus (ergot alkaloids). Examples of structures drawn especially to visualize the five-carbon pattern appear below.

The diversity of natural terpenoid metabolites will lead us to present the reactions and mechanisms behind the chief skeletons only when their structure and distribution is described in the corresponding, successive chapters:

- regular monoterpenes (essential oils, oleoresins, iridoids);
- irregular monoterpenes (pyrethrins);
- sesquiterpenes (essential oils, sesquiterpenoid lactones);
- diterpenes;
- triterpenes and steroids (saponins, cardiac glycosides, phytosterols, modified triterpenes);
- carotenes;
- polyisoprenes.

In contrast, we shall elaborate here on the three fundamental reaction sequences which account for the existence of all terpenes and steroids:

- formation of the reactive C_5 units from acetate, *via* mevalonate;
- head-to-tail coupling of isoprene units involved in the formation of mono-, sesqui-, di-, sester-, and polyterpenes;



Showing the "isoprene" units in terpenes and mixed metabolites

- tail-to-tail coupling of C_{15} and C_{20} units, leading to the precursors of triterpenes (squalene) and carotenes (phytoene).

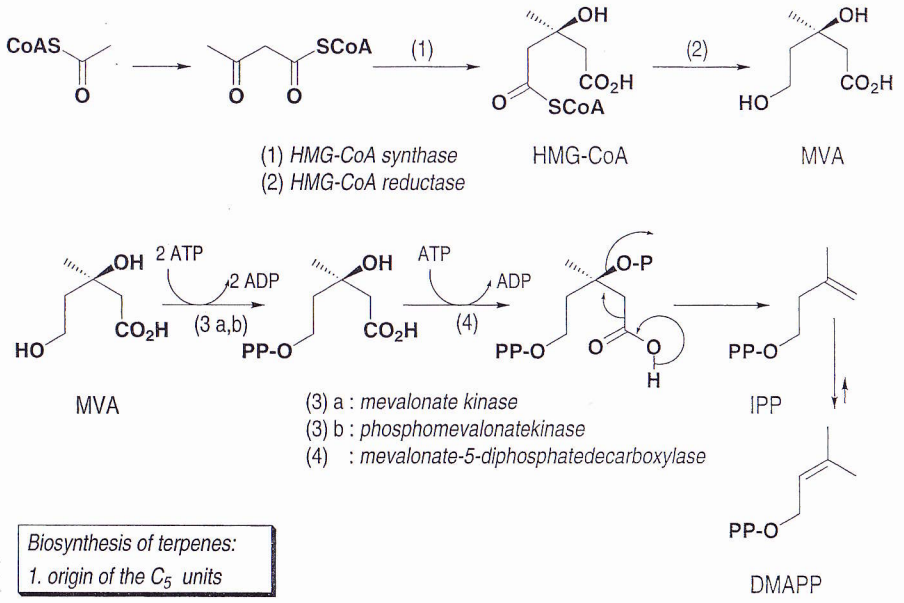
Outside of these three principal sequences, the remainder of the biosynthesis is easy to interpret as a series of very classic reactions: formation of carbonium ions by solvolysis of allylic pyrophosphates, epoxide ring openings, double bond protonations, electrophilic cyclizations, Wagner-Meerwein rearrangements, and so forth.

1. ORIGIN OF THE C5 UNITS

A. Mevalonate pathway

Initially, isotopic labeling experiments showed that the terpene carbon skeleton arises from acetate. Subsequently, it was shown that mevalonic acid had to be a universal precursor for these compounds. The initial step in the process is the Claisen condensation of two molecules of acetic acid thioester, and the resulting acetoacetate formation. Next is the aldol condensation of the latter with a molecule of acetyl coenzyme A; the reaction is catalyzed by an enzyme, hydroxymethylglutaryl-coenzyme A synthase. Another enzyme, hydroxymethylglutaryl-coenzyme A reductase, catalyzes the NADPH-dependent reduction of the resulting *3-hydroxy-3-methylglutaryl-coenzyme A* (HMG-CoA) to *(3R)-mevalonic acid* (MVA) (that the other isomer of this acid is not incorporated has been verified).

The conversion of mevalonic acid into hemiterpenoid structures begins with a double phosphorylation. A new phosphorylation introduces a good leaving group (the pyrophosphate group), the elimination of which will assist the decarboxylation: mevalonate-5-diphosphate decarboxylase induces the formation of isopentenyl pyrophosphate (IPP).



Biosynthesis of terpenes: 1. origin of the C_5 units

Isopentenyl pyrophosphate is isomerized by isopentenyl diphosphate-Δisomerase to dimethylallyl pyrophosphate (DMAPP). The allylic rearrangement involves the addition of a proton from the medium and the elimination of the pro-(2*R*) hydrogen). This DMAPP is highly reactive: it is susceptible to nucleophilic attack at C-1, with simultaneous departure of the pyrophosphate group; the attack may be by an IPP molecule (see below), or any other reactive molecule (hence the existence of "mixed" *C*- or *O*-alkylated metabolites as mentioned above).

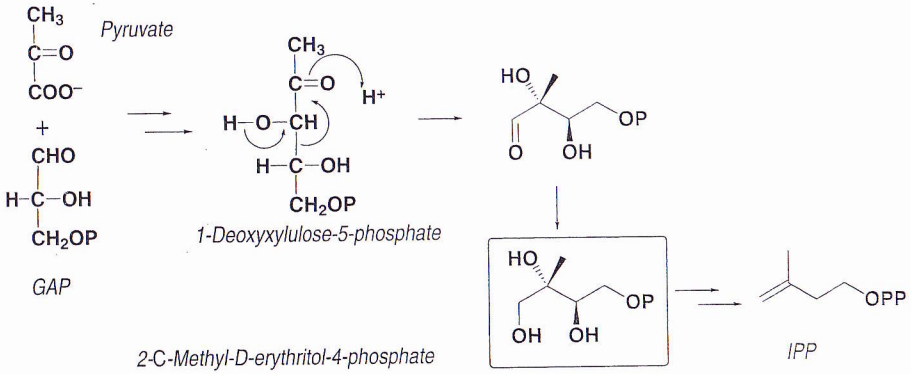
B. Alternate pathway to IPP (GAP/pyruvate)

In the late 1980s, it was discovered that bacteria (*Rhodopseudomonas* spp., *Methylobacterium* spp.) could elaborate, from labeled precursors, hopanoids (i.e., triterpenes) labeled in positions other than the expected ones. This led to the elucidation, in various bacteria and in one green alga (*Scenedesmus obliquus*), of the principal steps of a novel biosynthetic pathway for isopentenylpyrophosphate (IPP). This pathway was also shown to lead, *in the plastids* of higher plants (*Daucus*, *Lemna, Hordeum*), to polyprenols (plastoquinone, carotenoids, phytols).

This novel pathway (GAP/pyruvate) includes the following reaction sequence:

- condensation of a two-carbon unit arising from the decarboxylation of a pyruvate molecule onto the carbonyl carbon of glyceraldehyde phosphate (GAP);
- transposition and reduction of the first C_5 intermediate, 1-deoxy-D-xylulose-5-phosphate, to form 2-*C*-methyl-D-erythritol 4-phosphate, the likely precursor of IPP.

Recent (^{13}C NMR) incorporation experiments with different ^{13}C-labeled intermediates showed that mevalonate is not the precursor of ginkgolide A in *Ginkgo biloba,* taxayunnanin C in *Taxus chinensis*, the monoterpenes in *Mentha piperita,* or marrubiin in *Marrubium vulgare*. Although the 1-deoxy-D-xylulose pathway has not been formally demonstrated for all of these examples, it may not be that exceptional.

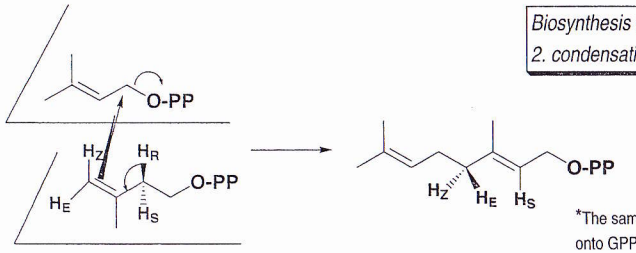


2. HEAD-TO-TAIL COUPLING OF ISOPRENE UNITS

The addition of DMAPP to the double bond of IPP is catalyzed by a prenyltransferase, GPP synthase. The reaction involves the ionization of the allyl pyrophosphate *via* the departure of the pyrophosphate group as a leaving group. This allows the concomitant and stereoselective electrophilic attack of the resulting allylic carbocation on the double bond of isopentenyl pyrophosphate (on the *si-si* face); the condensation takes place with the elimination of the *pro*-2*R* proton of IPP.

The same prenylation reaction can continue: the addition of GPP onto IPP yields farnesyl pyrophosphate (FPP), and so on and so forth to form the series of

homologous allylic prenyl pyrophosphates. The elongation is catalyzed by prenyl-transferases, some of which are specific to a given chain length.

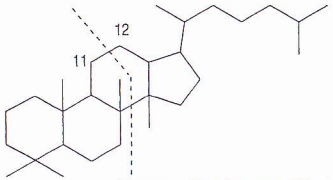


Biosynthesis of terpenes:
*2. condensation of the C_5 units **

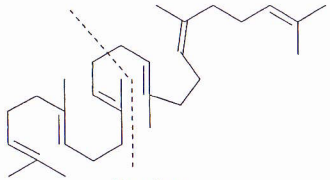
*The same mechanism allows the addition of IPP onto GPP (–> FPP), then onto FPP (–> GGPP), and so forth (–> polyprenols).

3. TAIL-TO-TAIL COUPLING OF C_{15} AND C_{20} UNITS

Upon thorough consideration of the structure of triterpenes, it appears that there is a symmetry about the C-11-C-12 bond, and that these molecules may arise from precursors such as squalene, a C_{30} hydrocarbon initially isolated from shark (= Squali) liver and which, like triterpenes, seems to correspond to a "doubling" of farnesyl pyrophosphate.



Tetracyclic triterpenoid



Squalene

The mechanism of this "tail-to-tail" coupling has been elucidated only recently, with the isolation of another intermediate, presqualene pyrophosphate. The cyclopropane structure of the latter suggests that the C-2-C-3 bond in FPP is alkylated by another molecule of FPP, and that the elimination of a proton leads to the formation of the cyclopropane ring (see opposite page). If the medium is deficient in NADPH, squalene pyrophosphate accumulates; in the opposite case, it rearranges to squalene, probably through a cyclopropane carbocation. An identical mechanism explains the formation of phytoene, the precursor of carotenes (doubling of GGPP).

4. BIBLIOGRAPHY

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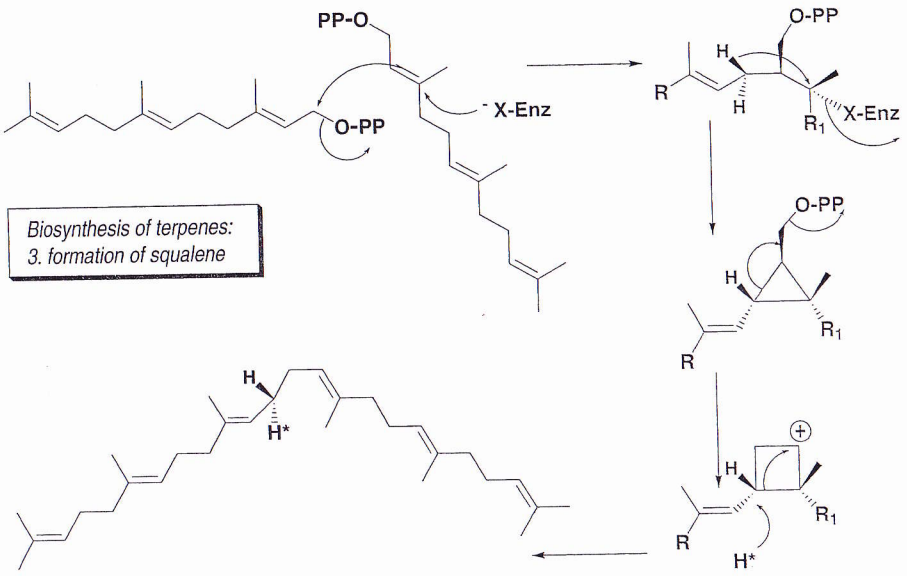

Biosynthesis of terpenes:
3. formation of squalene

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