Redefined role of mevalonate–isoprenoid pathway in terpenoid biosynthesis in higher plants

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The terpenoids constitute the largest group of natural plant products which play a significant role in plants, human health and commerce[1]. The terpenoids have diverse functional role in plants as structural components of membranes (phytosterols), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastoquinone), hormones (gibberellins, abscisic acid) and mediators of polysaccharide assembly (polyprenyl phosphates). The essential oils and/or their constituents (mainly monoterpenoids and sesquiterpenoids) are important flavouring and fragrance agents in food, cosmetics and perfumes. Turpentine (principally monoterpene C_{10}), conifer resin (principally diterpenoid C_{20}) and rubber (a polyisoprenoid C_{5000}–C_{150000}) are some of the other plant terpenoids frequently used in industry. The terpenoid group also includes some of the very important naturally occurring pharmaceuticals (artemisinin (antimalarial), taxol (anticancer), *Digitalis* sterol glycosides (prescribed for congestive heart diseases) and steroidal saponins from yam (starting material in the synthesis of progesterone-like compounds for birth control pills)), vitamins (vitamin A, D, E and K) and agrochemicals (pyrethrin, azadirachtin, etc.).

Despite the remarkable diversity of plant isoprenoid compounds, the various pathways that direct the synthesis of these metabolic end products were thought to emerge from a single common biosynthetic pathway – the mevalonate–isoprenoid pathway, named after its best known intermediate mevalonic acid[1]. The mevalonate–isoprenoid pathway was first discovered in yeast and animals through investigation of sterol biosynthesis, but all of the steps have now been characterized in plants[2].

The mevalonate–isoprenoid pathway (Figure 1) involves at first the synthesis of the biological C_5 isoprene unit – the isopentenyl pyrophosphate from three molecules of acetyl CoA via acetoacetyl CoA and hydroxymethylglutaryl CoA (HMG CoA). HMG CoA is reduced to mevalonic acid which gets phosphorylated in two steps to form mevalonate pyrophosphate, and is subsequently decarboxylated to yield isopentenyl pyrophosphate (IPP). In the second stage, IPP is isomerized to dimethylallyl pyrophosphate (DMAPP), and then these two isomers combine to yield geranyl pyrophosphate (GPP, C_{10}). Further condensation with additional IPP units forms successively larger acyclic prenyl pyrophosphates, e.g. farnesyl pyrophosphate (FPP, C_{15}), geranyl geranyl pyrophosphate (GGPP, C_{20}), etc. which might undergo cyclization, coupling and/or rearrangement to produce the parent carbon skeleton of each class. GPP (C_{10}) and FPP (C_{15}) yield monoterpene and sesquiterpene skeletons, respectively. FPP (C_{15}) can also dimerize in a head to tail fashion to form squalene (C_{30}) – the precursor of triterpenes. Similarly GGPP (C_{20}) can dimerize to phytoene (C_{40}) – the precursor of the tetraterpenoids. These major structural conversions may be followed by oxidation, reduction, isomerization, hydration, conjugation and/or other transformations that eventually give rise to a variety of terpenoid metabolites[1,2].

HMG CoA in plants is formed not only during the process of terpenoid biosynthesis but also as an intermediate in leucine catabolism[3]. Thus leucine could serve as a direct progenitor of

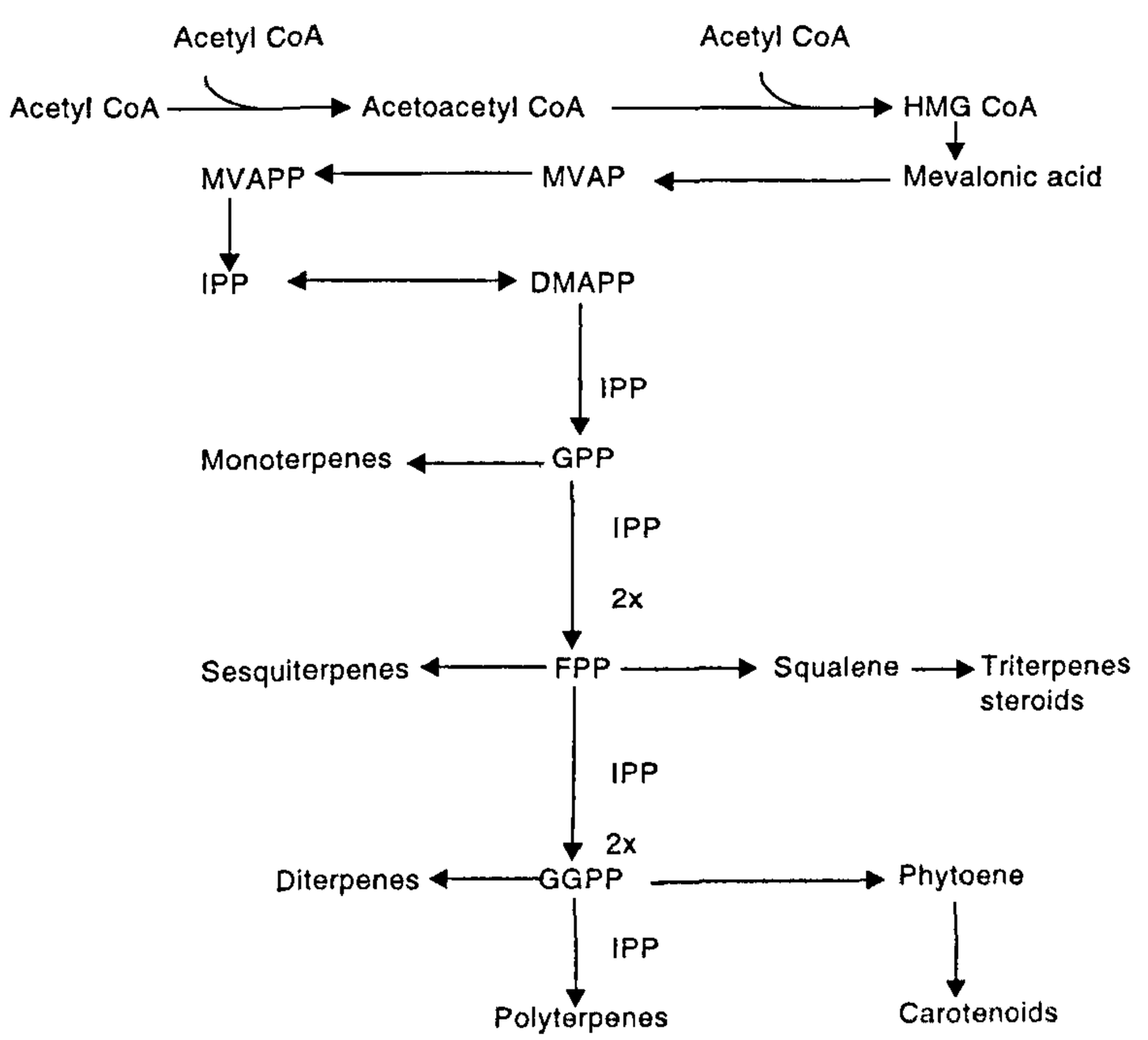

Figure 1. Outline of the conventional mevalonate–isoprenoid pathway in higher plants. MVAP, MVAPP and HMGCoA denote mevalonate phosphate, mevalonate pyrophosphate, hydroxymethylglutaryl CoA, respectively. IPP, DMAPP, GPP, FPP and GGPP are the pyrophosphate esters of isopentenol, dimethylalyl alcohol, germinol, farnesol, geranyl geraniol, respectively.

terpenoids. In pea seeds, the incorporation of branched chain amino acids leucine and valine into squalene and β-amyrin has been reported. The label from these amino acids was equally distributed over the DMAPP and IPP derived parts. In *Cinnamomum camphora* and *Pelargonium roseum* plants, however, the DMAPP derived part of monoterpenes showed higher incorporation than the IPP derived part. The DMAPP part was about 65% labelled with ^{14}C-labelled valine or leucine but with labelled mevalonic acid (MVA), only 32% incorporation was found. These results indicated that the 5-C unit might be derived from the leucine degradative pathway. However, experiments with *Andrographis paniculata* cell cultures have shown that the isotopically labelled leucine is only incorporated into terpenoids after first being degraded to acetyl CoA and acetoacetyl CoA, thereby suggesting that the pathway from leucine to HMG CoA is not an important route to terpenoids in plants. Similar results were obtained when leucine was fed to isolated spinach chloroplasts[2,3].

Our conventional view of the synthesis of all terpenoids through the mevalonate isoprenoid pathway is recently being questioned. Besides the ubiquitous MVA pathway, there are now reports of another completely different pathway that leads to the formation of IPP, and from these to hopane type triterpenes and ubiquinone (in certain bacteria) and to monoterpenes, diterpenes, carotenoids and phytol chain of chlorophyll (in certain higher plants). The non-mevalonate pathway is thought to be more or less similar to the valine biosynthetic route involving glyceraldehyde-3-phosphate and pyruvate as precursors for the 5-C isoprene unit[4] (Figure 2).

The IPP biosynthetic pathway followed to form a specific terpenoid can be identified by incorporation of (1-^{13}C) glucose into the desired compound. Depending on the pathway followed, glucose is metabolized accordingly resulting in a specific labelling pattern of the isoprene unit which can be determined by ^{13}C NMR spectroscopy[5]. The procedure involves measurement of ^{1}H decoupled ^{13}C NMR spectra of a biosynthetic sample and of a sample with natural ^{13}C abundance under identical conditions. The relative ^{13}C abundance of individual carbon atoms is then calculated from the integrals of biosynthetic samples by comparison with the natural abundance sample.

The label from (1-^{13}C) glucose is diverted to C-3 of triose phosphate/pyruvate and C-2 of acetyl CoA by glycolysis and action of pyruvate dehydrogenase. IPP synthesized from three C-2 labelled acetyl CoA via the mevalonate pathway should have labels at C-2, C-4 and C-5 positions, whereas IPP biosynthesized from C-3 labelled pyruvate should divert the labels to C-1 and C-5 position of IPP[5]. By comparison with the observed labelling pattern, one can conclude whether the IPP/DMAPP units are biosynthesized via the mevalonate pathway or the alternative triose phosphate/pyruvate pathway. The feeding of ^{13}C labelled glucose to *Ginkgo bioloba* embryo led to a labelling pattern in the diterpene ginkgolide A, which is incompatible with its mevalonate origin[6]. Similarly, feeding of (U-$^{13}C_6$) and (1-^{13}C) glucose to a cell culture of *Taxus chinensis* indicated that the diterpene taxuyunnanine C is also not of mevalonate origin[7]. Recent studies on chlorophyll and carotenoids using *Lemna gibba*, *Hordeum vulgare* and *Daucus carota* showed similar results[8]. Analysis of the isolated phytol from the cyanobacterium *Synechocystis* sp. UTEX 2470 using deuterium and ^{13}C NMR also showed labelling patterns consistent with incorporation of labelled glucose via the non-mevalonate pathway to terpenes[9]. Another set of labelling studies concerning the formation of simple monoterpenes such as menthone (*Mentha piperita*), pulegone (*Mentha pulegium*) and thymol (*Thymus vulgaris*) have shown that these terpenoids are synthesized through triose phosphate/pyruvate pathway rather than by the classical mevalonate–isoprenoid pathway[5]. The chemical structures of an array of plant terpenoids, illustrating diversity of terpenoids synthesized through non-mevalonate pathway, are depicted in Figure 3.

The non-mevalonate pathway (Figure 2) most likely involves a free or phosphorylated intermediate of 1-deoxyxylulose, resulting from a condensation of pyruvate with glyceraldehyde 3-phosphate. The role of 1-deoxyxylulose or its 5 phosphate as a 5-C precursor of IPP was shown by the successful incorporation of deuterium labelled 1-deoxyxylulose into the

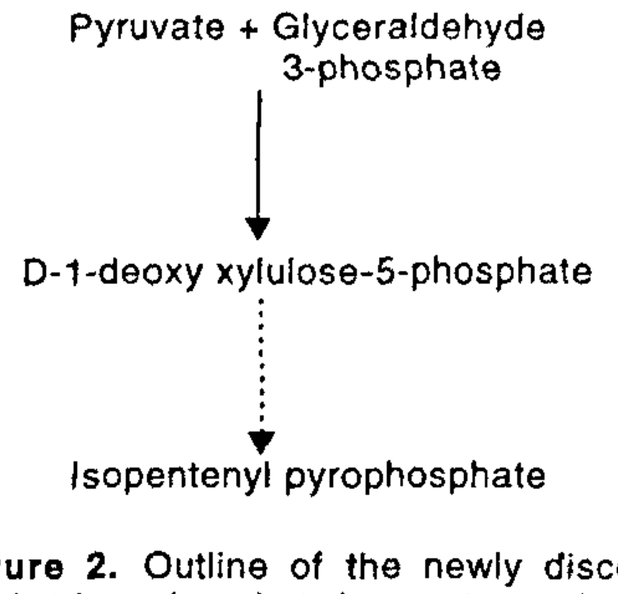

Figure 2. Outline of the newly discovered triosephosphate/pyruvate pathway for the synthesis of isopentenyl pyrophosphate (IPP). IPP is utilized for the synthesis of specific terpenoids as described in Figure 1.

isoprenoids of *Escherichia coli*[10], and more recently of the mixture of deuterium labelled methyl α and β-1-deoxy-D-xylulosides into isoprenes emitted by leaves of higher plants[11]. Experiments with 2-C-methyl-D-erythritol in *E. coli* have shown that it is incorporated into the isoprenoid side chain of ubiquinone and menaquinone[12]. These results strongly support the proposed intermediate role of this branched sugar derivatives in the mevalonate independent pathway for isoprenoid biosynthesis. It has been proposed that the branching is introduced by a rearrangement of 1-deoxyxylulose-5-phosphate. Lange *et al.*[13], Lois *et al.*[14] and Bouvier *et al.*[15] have recently described cloning and characterization of a gene encoding 1-deoxy-D-xylulose-5-phosphate from peppermint, *E. coli* and pepper fruits, respectively. This gene defines a unique family of transketolases that are highly conserved between bacteria and plants, but absent in animals, which rely entirely on the classical mevalonate pathway for isoprenoid biosynthesis.

Recent literature search indicates that in higher plants monoterpenes, diterpenes, carotenoids and phytol chain of chlorophyll are formed via the triose phosphate/pyruvate pathway and not via the classical mevalonate route. Therefore, the role of the mevalonate pathway in the synthesis of terpenoids in higher plants has to be redefined. One can presume that some terpenoids may be synthesized through the mevalonate pathway and others by the triose phosphate/pyruvate pathway. This presumption fits in with the observation that in tobacco transgenic plants, the total sterols appear to be limited by HMG

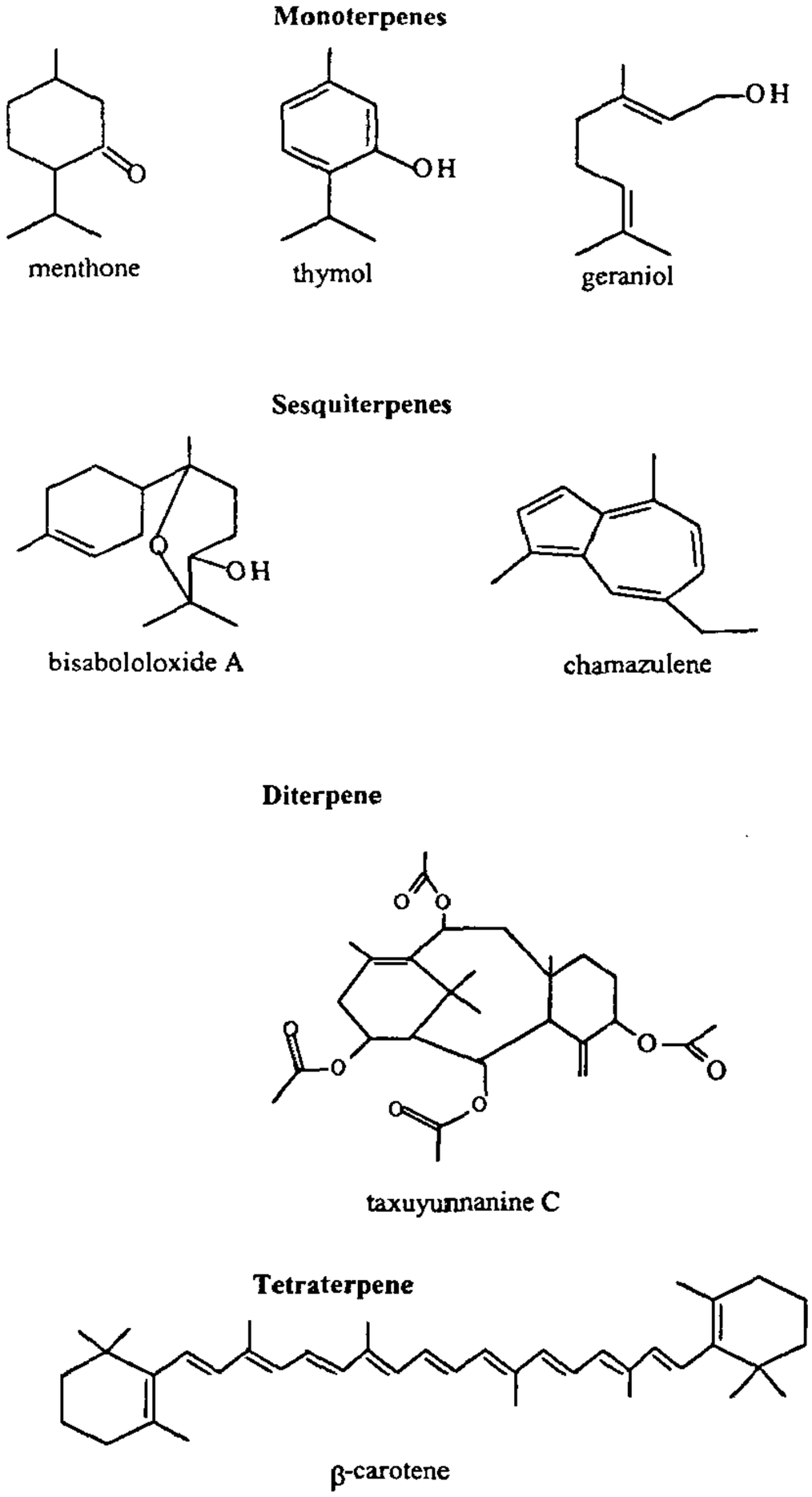

Figure 3. Chemical structures illustraing diversity of plant terpenoids synthesized through non-mevalonate pathway.

CoA reductase activity, the key enzyme involved in the regulation of isoprenoids through the mevalonate route, whereas the level of other isoprenoids such as carotenoids, phytol chain of chlorophyll and sesquiterpene phytoalexins were relatively unaltered, being synthesized through the non-mevalonate pathway[16]. Further, mevinolin, a highly specific inhibitor of mevalonate formation (i.e. of formation of IPP), strongly inhibits sterol biosynthesis in higher plants. However, the biosynthesis of chlorophyll, carotenoids and plastoquinone 9 (with a C_{45} prenyl side chain) was unaffected by mevinolin[17]. Analysis of the labelling patterns and absolute ^{13}C abundances using quantitative ^{13}C NMR spectroscopy of sesquiterpenes bisabololxide A and chemazulene, isolated from the hydrodistillate of the labelled *Matericaria recutita* (chamomile) flowers showed that two of the isoprene building blocks were predominantly formed via the triose phosphate/pyruvate pathway, whereas the third unit is of mixed origin, being derived from both the MVA pathway and the triose phosphate/pyruvate pathway[18]. Using inhibitors selective for each pathway, it was, however, possible to show that in *Marrubium vulgare*, isopentenyl pyrophosphate is not exchanged between the pathways suggesting strict separation[19]. These discoveries may point to a restricted role of the mevalonate–isoprenoid pathway, mainly for sterol synthesis, and a novel physiological role for the newly discovered triose phosphate/pyruvate pathway for the synthesis of a variety of terpenoids in higher plants.

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