The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms

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Recent studies have uncovered the existence of an alternative, non-mevalonate pathway for the formation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate, the two building blocks of terpene biosynthesis.

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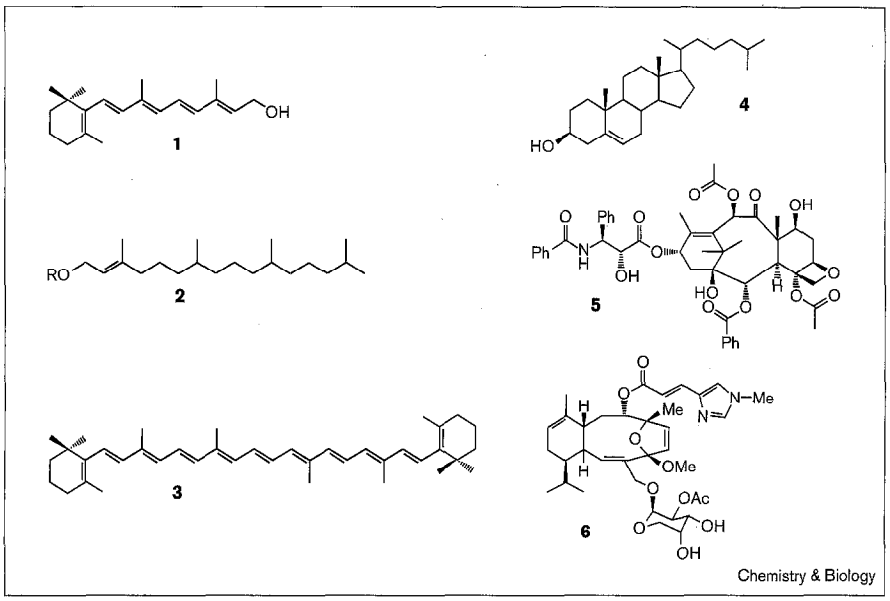
Introduction

Isoprenoid derivatives are one of the largest groups of natural products; more than 30,000 naturally occurring terpenes and terpenoids have been reported, and their number is growing steadily. Terpenoids have a wide variety of biological functions and many have potential medicinal applications. For example, vitamin A (**1**, Figure 1) acts as a photoreceptor in animals and as the chromophoric element of a light-driven proton pump in certain bacteria. The phytol sidechain (**2**) of chlorophyll, one of the most abundant organic molecules, serves to anchor the photoreceptor molecule in lipophilic environments. Carotenoids, such as β-carotene (**3**), act as light-protecting pigments in plants and have been claimed to have anticarcinogenic activity, and steroids, such as cholesterol (**4**), are essential components of eucaryotic cell membranes. The post-translational modification of some regulatory proteins, such as Ras, by terpenoid alcohols plays important roles in cellular signaling in vertebrates. In plants, the attraction of insects and defence against herbivores involve numerous terpenoids. Paclitaxel (**5**, Taxol®), a taxane-type diterpene from the yew tree, and the semi-synthetic Taxotere® have rapidly achieved a central position in the chemotherapy of malignant tumours such as ovarial and mammary carcinoma. Eleutherobin (**6**), a cladiellane-type diterpene from a soft coral, might also have potential as a future cytostatic agent.

All terpenoids are assembled biosynthetically from only two precursors: isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Initial observations by Folkers, Tavormina and coworkers [1,2] indicated that the isoprenoid monomers are biosynthetically derived from mevalonate. Subsequent work by Bloch, Cornforth, Lynen, Popjak and coworkers elucidated in detail the steps of the mevalonate pathway in animal cells and yeasts (for reviews see [3–6]). Briefly, a Claisen condensation of two acetyl CoA molecules (**7**, Figure 2) yields acetoacetyl CoA (**8**). An aldol addition of yet another acetyl CoA molecule yields 3-hydroxy-3-methylglutaryl CoA (**9**), which is reduced to mevalonic acid (**10**) in the subsequent step. Mevalonate is then phosphorylated, yielding 3-phospho-5-pyrophosphomevalonate (**11**), and the subsequent elimination of phosphate and CO_2 yields IPP (**12**), which can be converted to DMAPP (**13**) by an isomerase. On the basis of these seminal studies, the rate-limiting enzyme of the mevalonate pathway, 3-hydroxy-3-methylglutaryl CoA reductase, became a major drug target, because cardiovascular mortality and morbidity can be reduced by normalization

Figure 1

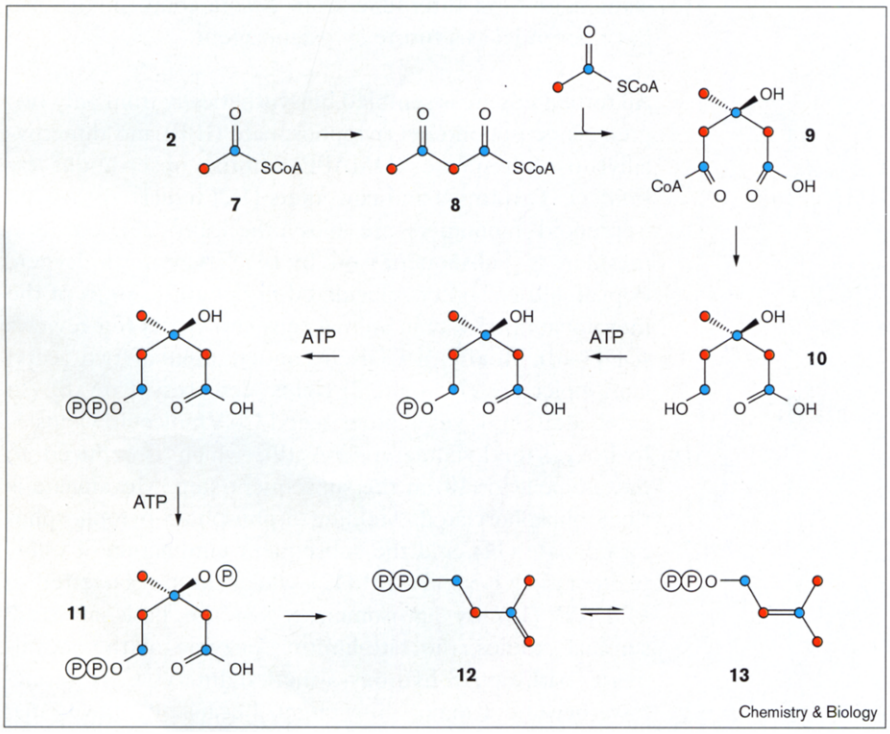


Structures of the terpenoids retinol (**1**); phytol (**2**); β-carotene (**3**); cholesterol (**4**); paclitaxel (Taxol®; **5**) and eleutherobin (**6**).

of elevated cholesterol levels, potentially achieved by manipulating the mevalonate pathway.

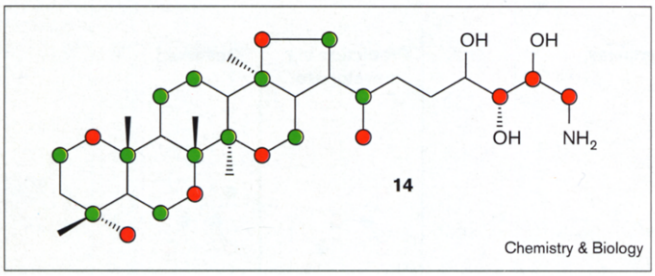
Since the discovery of the mevalonate pathway, many studies on the biosynthesis of terpenoids in a wide variety of species have been published. In many cases, however, the experimental data on the biosynthesis of specific terpenoids in plants and microorganisms could not be explained easily by the mevalonate pathway. Specifically, it was often found that mevalonate and acetate were not incorporated efficiently into the terpenoids under study. Complex hypotheses involving the compartmentalization

Figure 2



Mevalonate pathway of terpenoid biosynthesis.

Figure 3



Labelling pattern of aminobacteriohopanetriol (**14**) from *Rhodopseudomonas palustris* supplied with [1-^{13}C] acetate [7]. Carbon atoms expected to be ^{13}C-enriched following the mevalonate pathway are indicated by green dots; ^{13}C-enriched carbon atoms observed experimentally are indicated by red dots.

of metabolites and poor access of extrinsic metabolites to specific cellular compartments were developed in an effort to harmonize these data with the paradigm of a universal mevalonate pathway as the source of all terpenoids in all organisms. Some attempts were also made to explain the conflicting data by alternative hypotheses, however, such as linking the biosynthesis of certain terpenoids with the biosynthesis of branched-chain amino acids. These historical developments have been reviewed repeatedly (for example, [4,6]) and will not be discussed in detail here. Instead we will describe the discovery of a new pathway in terpenoid biosynthesis, how this pathway relates to the mevalonate pathway and what recent genomics research shows us about the evolution of the pathways.

Discovery of the deoxyxylulose pathway

The reassessment of the isoprenoid biosynthesis in plants and microorganisms was initiated in the late 1980s by studies performed independently by Rohmer, Sahm, Arigoni and their respective coworkers. Experiments by Flesch and Rohmer [7], incorporating ^{13}C-labelled acetate into bacterial hopanoids such as **14** (Figure 3), revealed unexpected labelling patterns in the terpenoid moiety of the molecules under study. Originally, the existence of distinct acetyl CoA pools was proposed to explain how the anomalous labelling patterns could arise via the mevalonate pathway [7], but other possibilities were also considered. Later, the data were definitely reinterpreted in terms of a novel pathway, the deoxyxylulose pathway discussed below [8].

Independently, Arigoni and his research group studied the incorporation of various ^{13}C-labelled glucose samples into the isoprenoid sidechain of ubiquinone by the bacterium *Escherichia coli* [9] and into ginkgolides in seedlings of the tree *Ginkgo biloba* [10]. The labelling pattern of ginkgolide A (**15**) observed after feeding with [U-$^{13}C_6$] glucose (as a mixture with unlabelled glucose) is shown in Figure 4. Five blocks of two contiguous ^{13}C atoms and one block of three contiguous ^{13}C atoms (indicated in red in Figure 4) were shown to be transferred together from the ^{13}C-labelled carbohydrate to the ginkgolide. The formation of the hexacyclic diterpene from the linear precursors involves a sequence of skeletal rearrangements corresponding to reshuffling of the original C5-moieties [10,11]. As a result of one of these rearrangement processes, C-4 of IPP (**12**), shown in red in Figure 4, becomes directly connected to C-2. The observed block of three contiguous ^{13}C atoms therefore indicated that carbon atoms 1, 2 and 4 of the corresponding IPP molecule stem from a single, universally ^{13}C-labelled glucose molecule. One of the original direct linkages between the carbon atoms is disrupted during the formation of IPP, and subsequently reestablished in the rearrangement process (Figure 4).

Figure 4

Incorporation of D-[U-$^{13}C_6$] glucose (diluted with unlabelled D-glucose at a ratio of 1:10) into ginkgolide A (**15**) in seedlings of *G. biloba* [10,56]. Blocks of carbon atoms that were jointly diverted to the ginkgolide from individual, universally ^{13}C-labeled glucose molecules are connected by bold lines. For details see text.

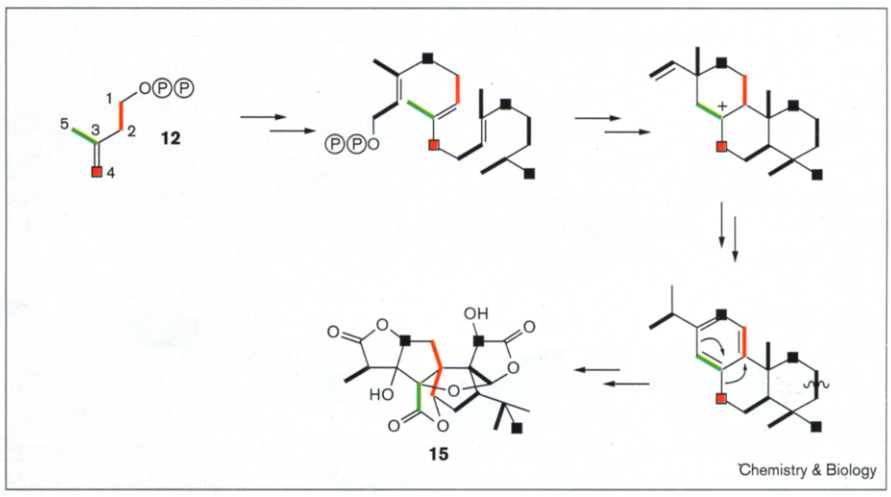
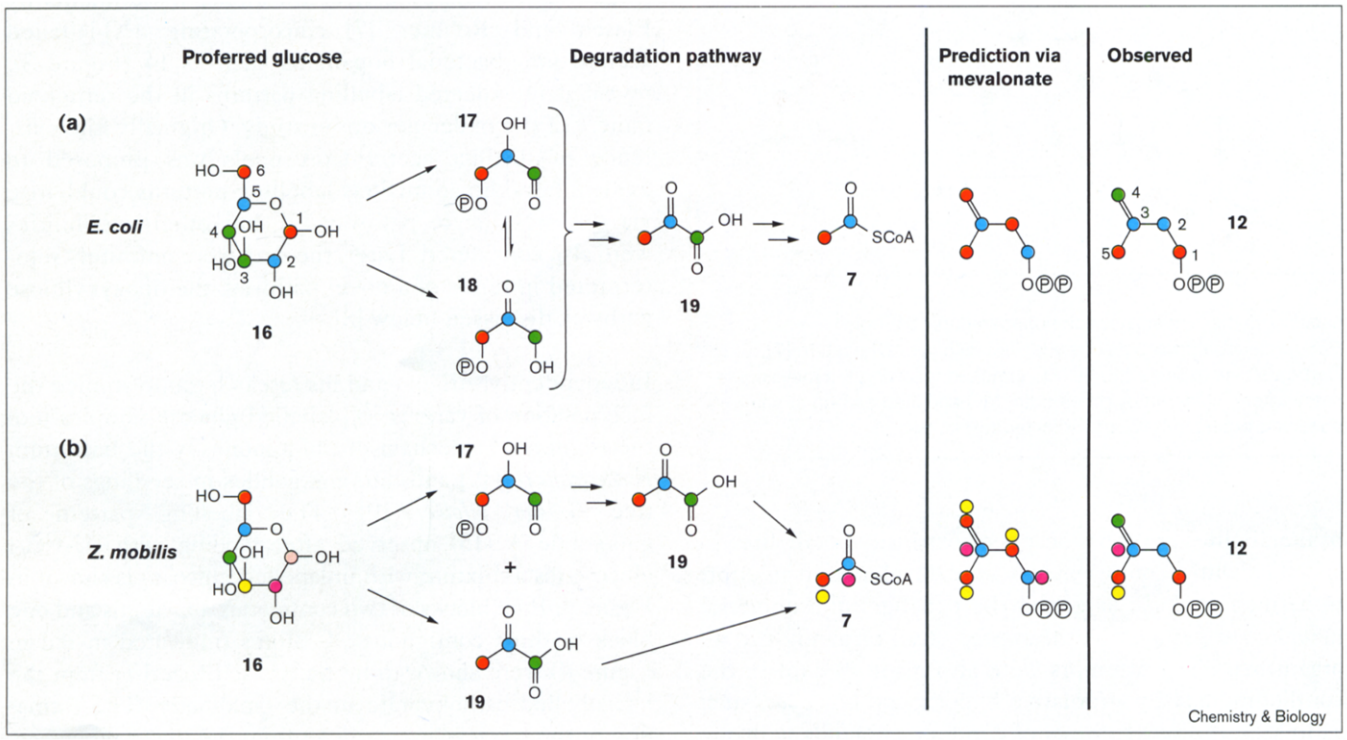


Figure 5



Catabolism of ^{13}C-labelled glucose in **(a)** *E. coli* via the Embden–Meyerhof pathway and **(b)** *Zymomonas mobilis* via the Entner–Doudoroff pathway. The hypothetical labelling patterns of IPP/DMAPP that could have been obtained via the mevalonate pathway differ substantially from the observed labelling patterns in both microorganisms. Coloured dots indicate the metabolic fate of carbon atoms [9,12].

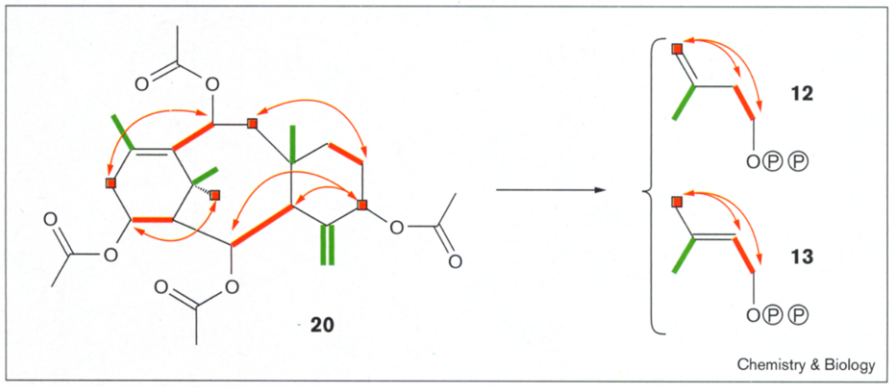
The detailed analysis of specimens of ginkgolides generated from various specifically labelled ^{13}C isotopomers of glucose indicated that the formation of the isoprenoid precursors involved a rearrangement reaction resulting in the fragmentation of a three-carbon moiety introduced from the glucose precursor into a two-carbon moiety and an isolated carbon atom forming part of the same precursor moiety. For the isoprenoid unit shown in red in Figure 4, the rearrangement mechanism had reversed the separation between the two-carbon block and the isolated carbon atom, thus resulting in the three-carbon unit observed. These results established that the formation of the isoprenoid monomers had proceeded by the contribution of a three-carbon and a two-carbon fragment from glucose. Clearly, this could not be explained via the mevalonate pathway, which had been shown long ago to utilize exclusively two-carbon building blocks (i.e. acetyl CoA).

As mentioned above, Rohmer, Sahm and their coworkers [12] studied the biosynthesis of hopanoids and ubiquinones in the bacteria *Zymomonas mobilis*, *Methylobacterium fujisawaense*, *E. coli* and *Alicyclobacillus acidoterrestris*. The biosynthetic relationship between glucose and isoprenoid carbon atoms deduced from these studies is shown in Figure 5. To rationalize the results of the studies by Rohmer, Sahm and their coworkers [12], it is important to note that different pathways of glucose catabolism are used in the microorganisms studied. In *E. coli*, degradation of glucose (**16**) by the glycolytic pathway yields equimolar amounts of the triose phosphates glyceraldehyde phosphate (**17**) and dihydroxyacetone phosphate (**18**). As the two triose phosphates are rapidly interconverted by triose phosphate isomerase, each triose carbon can acquire label from the top as well as from the bottom part of the glucose molecule (Figure 5a). Incorporation of [1-^{13}C] or [6-^{13}C] glucose into ubiquinone by *E. coli* [9,12] and into hopanoids by *A. acidoterrestris* [12] resulted in labelling of C-1 and C-5 of IPP (**12**) via the glycolytic pathway. If the mevalonate pathway was operating, incorporation of [1-^{13}C] or [6-^{13}C] glucose should have resulted in labelling of C-2, C-4 and C-5 of IPP (Figure 5a).

On the other hand, the Entner–Doudoroff pathway used predominantly in *Zymomonas* generates glyceraldehyde/dihydroxyacetone phosphate (**17**, Figure 5b) from the bottom half of glucose and pyruvate (**19**) from the top part. In a subsequent step, the triose phosphates can also be converted to pyruvate (**19**). Hence, the top, as well as the bottom, carbon atoms of glucose can contribute label to pyruvate (**19**), whereas glyceraldehyde phosphate and

Figure 6

The labelling pattern of taxuyunnanine C (**20**) from a cell culture of *T. chinensis* grown with [U-$^{13}C_6$] glucose (diluted with unlabelled glucose at a ration of 1:20). Pairs of adjacent ^{13}C atoms are shown by bold colored lines. Multiple-bond ^{13}C couplings are shown by arrows [13].



dihydroxyacetone phosphate can acquire label with high efficacy from the bottom part but not the top part of the glucose molecule (Figure 5).

The diversion of ^{13}C atoms to IPP (**12**) from different positions of glucose are summarized in Figure 5. The data suggest the contribution of a three-carbon unit supplying C-1, C-2, and C-4 of IPP from C-6, C-5, and C-4 of glucose and the contribution of a two-carbon unit supplying C-3 and C-5 of IPP from C-2/C-5 and C-3/C-6 of glucose, respectively. These data are supported by more recent studies using labelling with [U-$^{13}C_6$] glucose to investigate the formation of taxoids, such as taxuyunnanine C (**20**; Figure 6), in cell cultures of the yew, *Taxus chinensis* [13]. The incorporation of a three-carbon moiety from the carbohydrate into the isoprenoid precursors **12** and **13** with concomitant disruption of the connectivity caused by a rearrangement, could be observed directly via the analysis of long range ^{13}C–^{13}C coupling (Figure 6) [13].

Intermediates and mechanism of the novel pathway

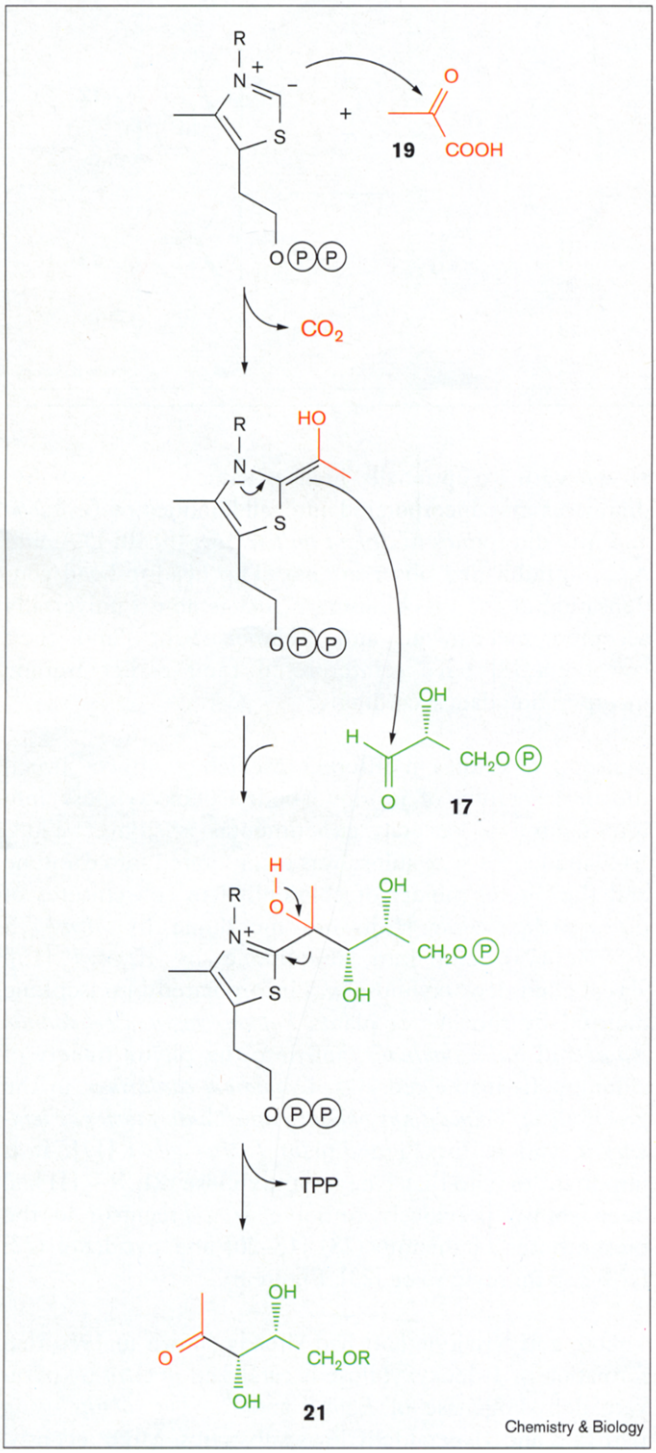
From the glucose-labelling data, it was concluded that the diversion of ^{13}C to isoprenoids had occurred via intermediates in the triose phosphate pool. Initially, the groups of Rohmer and Sahm [12] proposed that dihydroxyacetone phosphate could undergo an acyloin-type condensation with 'activated acetaldehyde'. The resulting branched-chain carbohydrate was assumed to undergo a rearrangement resulting in the observed reshuffling of the three-carbon moiety.

Arigoni's group [9,10], however, suggested a head-to-head condensation of glyceraldehyde 3-phosphate (**17**) and 'activated acetaldehyde' generated from pyruvate (**19**) by a thiamine-pyrophosphate-dependent decarboxylation (Figure 7). The decisive evidence in favor of this hypothesis was the incorporation of the putative condensation product 1-deoxy-D-xylulose (**21**, R = H), which was shown to occur into the isoprenoid sidechain of ubiquinone in *E. coli* with exceptionally high efficiency. The carbohydrate was also incorporated into ginkgolides of *G. biloba* and into diterpenes of *Salvia miltiorrhiza* ([9,10] D.A. and A.C., unpublished observations). The head-to-head condensation of the two building blocks is now a universally accepted mechanism, and Rohmer, Sahm and their coworkers [8] have reinterpreted their earlier isotope incorporation data accordingly.

Subsequent studies in different research groups confirmed the incorporation of isotope-labelled deoxyxylulose into terpenoids in several experimental systems. Thus, ^{13}C-labelled deoxyxylulose was incorporated into carotene and the phytol moiety of chlorophyll in cell cultures of *Catharanthus roseus* [14], into menthone by *Mentha x piperita* [15], and into ubiquinone by *E. coli* [16]. ^{2}H-labelled deoxyxylulose was incorporated into isoprene emitted by the higher plants *Populus nigra*, *Chelidonium majus* and *Salix viminalis*, and into the phytol moiety of chlorophylls in the red alga, *Cyanidium caldarium*, in the green algae, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*, and in the higher plant *Lemna gibba* [17]. It is important to note that 1-deoxy-D-xylulose (**21**, R = H) had been shown previously to serve as a precursor in the biosyntheses of thiamin (**23**) [18–20] and pyridoxol (**22**) ([20–22]; for review see [23]; Figure 8).

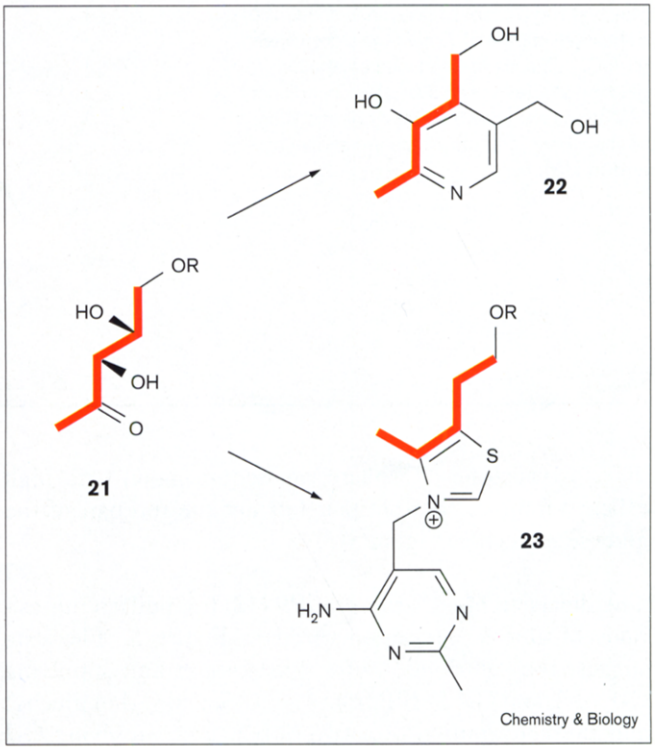
Yokoto and Sasajima [24] had already shown in 1986 that formation of 1-deoxyxylulose is catalyzed *in vitro* by pyruvate dehydrogenase of *E. coli* and *Bacillus subtilis* using pyruvate and glyceraldehyde as substrates. More recently, an *E. coli* enzyme catalyzing the formation of 1-deoxy-D-xylulose 5-phosphate (**21**, R = PO_3H_2; Figure 7) from pyruvate (**19**) and glyceraldehyde 3-phosphate (**17**) was reported independently by the research groups of Sahm [25] and Boronat [26,27]. The enzyme-catalyzed reaction involves the transfer of an activated aldehyde moiety, obtained by decarboxylation of pyruvate, to glyceraldehyde 3-phosphate. The gene of this enzyme shows sequence similarity to those of transketolases and pyruvate

Figure 7



The proposed mechanism for the formation of 1-deoxyxylulose 5-phosphate formation [**21**, R = PO_3H_2] catalyzed by 1-deoxyxylulose 5-phosphate synthase.

Figure 8



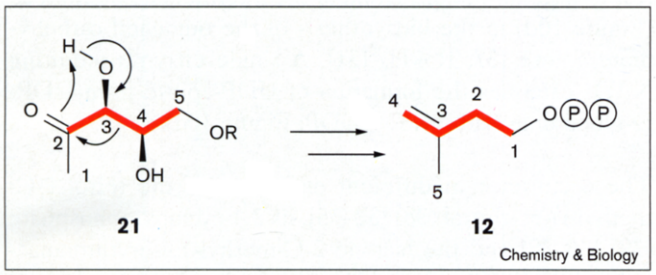
The biosynthesis of pyridoxol (**22**) and thiamine pyrophosphate (**23**) from 1-deoxy-D-xylulose (**21**, R = H) or 1-deoxy-D-xylulose 5-phosphate (**21**, R = PO_3H_2).

decarboxylase [25] and the enzyme requires thiamin pyrophosphate as cofactor. The 65 kDa enzyme is encoded by the *dxs* gene, which is located directly adjacent to the *ispA* gene encoding farnesylpyrophosphate synthase at 9 min of the *E. coli* chromosome, and it is likely that *dxs* and *ispA* form a single transcriptional unit. A gene encoding 1-deoxy-D-xylulose 5-phosphate synthase has also been cloned from peppermint (*Mentha x piperita*) and expressed in *E. coli* [28]. The gene contains a plastid-targeting sequence and the gene product is approximately 71 kDa. On the basis of sequence comparison, putative orthologs of the *dxs* gene have been observed in various plant and microbial species [25,26,28].

Although the enzymes catalyzing the subsequent biosynthetic steps have yet to be identified, it is plausible that 1-deoxyxylulose 5-phosphate is the committed precursor in the alternative terpenoid pathway, and that exogenous 1-deoxyxylulose can enter the pathway via phosphorylation by carbohydrate kinases with broad substrate specificity. This would imply that the introduction of one additional phosphate residue is required for the biosynthesis of IPP and DMAPP. Similarly, 1-deoxyxylulose 5-phosphate (**21**, R = PO_3H_2; Figure 8) rather than 1-deoxyxylulose might be the committed precursor in the pathways of thiamin and pyridoxal phosphate biosynthesis.

In vivo experiments documented unequivocally that the linear carbohydrate, 1-deoxyxylulose, is converted to the branched isoprenoid precursor by an intramolecular

Figure 9



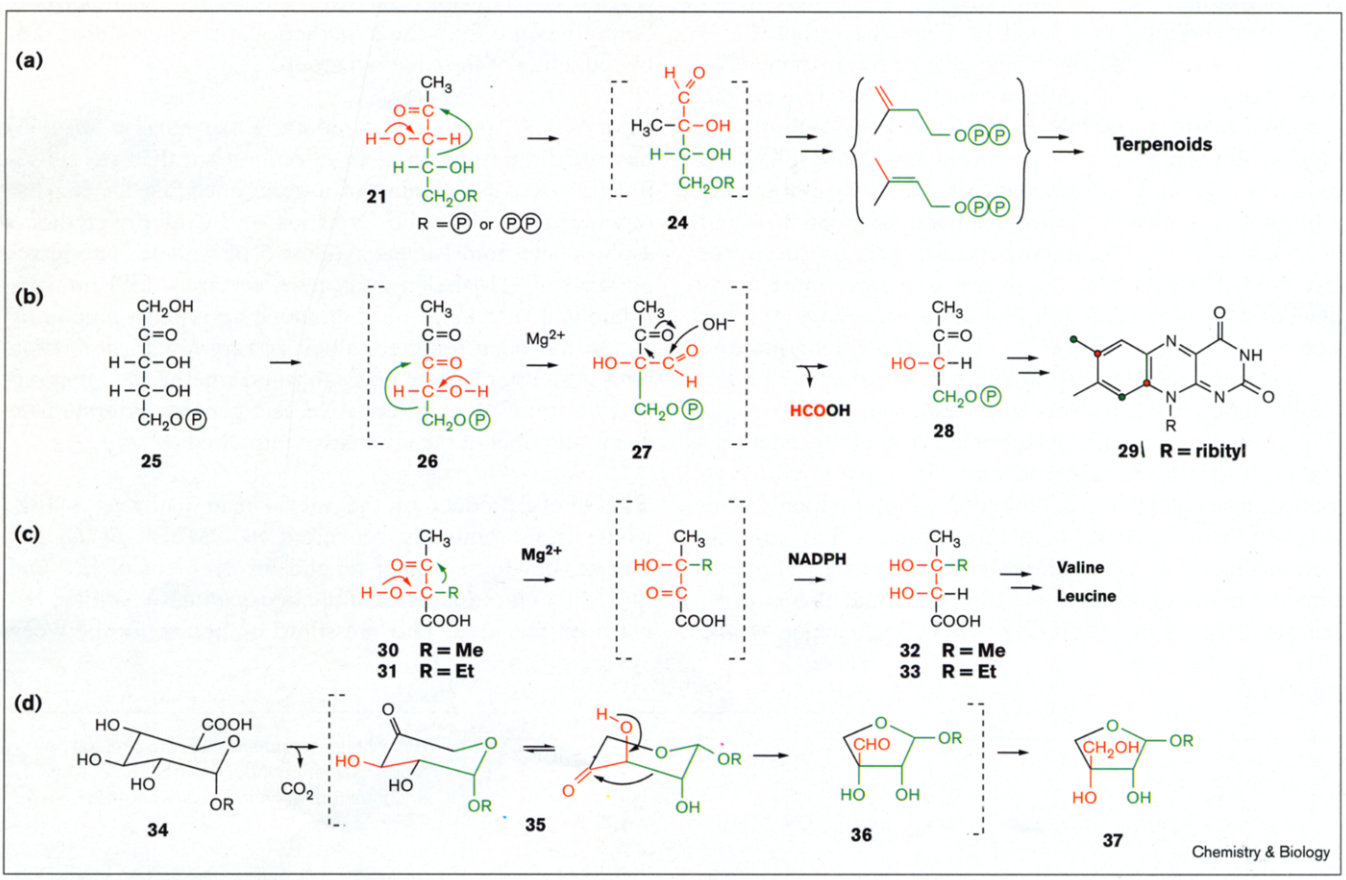
Skeletal rearrangement of 1-deoxyxylulose conducive to IPP formation. Carbon atoms are numbered according to IUPAC convention. Contiguous labelling from [2,3,4,5-$^{13}C_4$]-**21** into IPP as reconstructed from phytol, β-carotene and lutein in *C. roseus* is indicated by bold lines [14].

process [14,16]. Thus, it was shown that all ^{13}C atoms of [2,3,4,5-$^{13}C_4$] deoxyxylulose (**21**, R = H) were incorporated into individual isoprenoid molecules by analysis of phytol and carotenoids biosynthesized by *C. roseus* cells under conditions where isoprenoid monomers were predominantly formed from unlabeled glucose supplied in large excess (Figure 9) [14].

Although the intermediates between 1-deoxy-D-xylulose 5-phosphate and IPP/DMAPP have yet to be characterized, the overall transformation must involve a skeletal rearrangement as well as three reduction steps and at least one phosphorylation step.

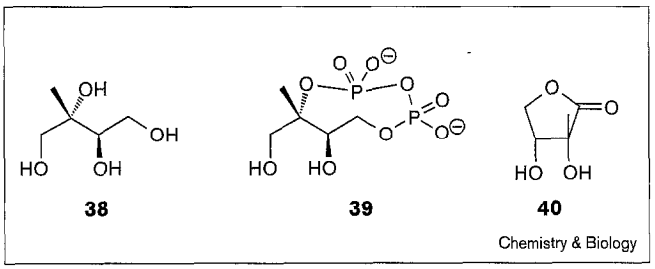
The rearrangement reaction can be expected to occur with 1-deoxyxylulose phosphate (**21**, R = PO_3H_2). The presence of an α-hydroxyketo moiety fulfils the requirements for the rearrangement step, which would result via breaking of the C-3–C-4 bond and closure of a new bond between C-2 and C-4 in the formation of the branched carbohydrate 2-C-methyl-D-erythrose 4-phosphate (**24**, Figure 10a). The chemical literature is replete with examples of sigmatropic rearrangements of α-hydroxyketones. Moreover, there is ample evidence for enzyme catalysis of similar sigmatropic rearrangements involving carbohydrate-type substrates. Thus, a rearrangement reaction has been shown to be

Figure 10



1,2-Ketol intermediates in the biosynthesis of **(a)** terpenoids via the deoxyxylulose pathway, **(b)** riboflavin (**29**), **(c)** valine and leucine and **(d)** apiose (**37**, R = H).

Figure 11



Natural products structurally related to hypothetical intermediates of the deoxyxylulose pathway.

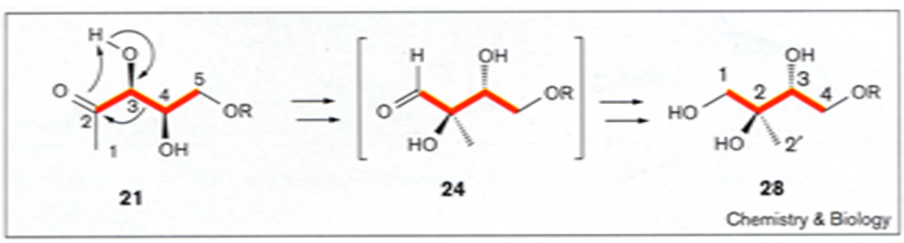
implicated in the formation of 3,4-dihydroxy-2-butanone 4-phosphate (**28**), the precursor of riboflavin (**29**), from ribulose 5-phosphate (**25**), a reaction catalysed by a single, homodimeric 46 kDa enzyme requiring only Mg^{2+} as cofactor (Figure 10b) [29]. The substrate of 3,4-dihydroxy-2-butanone 4-phosphate synthase, ribulose 5-phosphate, differs from 1-deoxyxylulose 5-phosphate by the presence of an additional hydroxyl group at C-1 and by the configuration at C-3. The initial reaction steps are expected to yield the labile diketone (**26**) by a sequence of dehydration and tautomerization reactions. Breaking of the C-4–C-5 bond and generation of a new bond between the carbonyl C-3 and C-5 results in the branched-chain carbohydrate (**27**), which then undergoes a deformylation reaction to yield the 4-carbon product, 3,4-dihydroxy-2-butanone 4-phosphate (**28**), a *nor* analogue of 1-deoxyxylulose 5-phosphate. It should be noted, however, that 3,4-dihydroxy-2-butanone 4-phosphate synthase breaks the bond between C-4 and C-5 of its carbohydrate intermediates, whereas the hypothetical 1-deoxyxylulose phosphate isomerase must break the bond between C-3 and C-4 of its substrate. Another well-documented example is the rearrangement reaction catalyzed by acetohydroxy acid isomeroreductase in the biosynthetic pathways of branched-chain amino acids (Figure 10c), a reaction in which acetolactate (**30**) and acetohydroxybutyrate (**31**) are converted into 2,3-dihydroxy-3-methylbutyrate (**32**) and 2,3-dihydroxy-3-methylpentanoate (**33**), respectively, via 1,2-ketol intermediates. The enzymic rearrangement steps require Mg^{2+} as cofactor. The presumed intermediates do not dissociate from the enzyme but are reduced in a NADPH-dependent reaction to the 1,2-diol intermediates [30]. A similar rearrangement is also responsible for the formation of the intermediate **36** from **35** (Figure 10d) in the biosynthesis of the branched carbohydrate apiose (**37**, R = H) [31]. A single enzyme requiring NAD^+ catalyses the formation of UDP-apiose from UDP-D-glucuronic acid (**34**, R = UDP; Figure 10d).

The occurrence in different organisms of the tetrol 2-C-methyl-D-erythritol (**38**) [32–35], its 2,4-cyclopyrophosphate (**39**) [36–38] and the lactone 2-C-methyl-1,4-erythronolactone (**40**) [39,40] (Figure 11), all of which can be derived from the corresponding aldehyde (**24**), provides indirect evidence for the rearrangement of 1-deoxy-D-xylulose 5-phosphate, suggested in Figure 10a. Indeed, isotope-incorporation studies in *Corynebacterium ammoniagenes* using [1-^{13}C], [6-^{13}C], and [U-$^{13}C_6$] glucose showed that the 2,4-cyclopyrophosphate of 2-C-methyl-D-erythritol (**39**) has the same labelling pattern as the isoprenoid sidechains of dihydromenaquinones [41]. More recently, leaves of the tulip tree, *Liriodendron tulipifera*, were shown to form [1,2,3,4-$^{13}C_4$] 2-C-methyl-D-erythritol (**38**) from [2,3,4,5-$^{13}C_4$] 1-deoxy-D-xylulose (**21**, R = H) by the same type of skeletal rearrangement [42] expected to be involved in the biosynthesis of IPP and DMAPP via the novel pathway (Figure 12). This suggests that the branched-chain polyol is biosynthesized from the hypothetical, branched aldose (**24**) by reduction of the carbonyl group.

In a recent preliminary report, Kuzuyama *et al.* [43] described the isolation of an *E. coli* mutant that was subsequently used for cloning of a gene encoding an enzyme reported to catalyze the formation of 2-C-methylerythritol 4-phosphate from 1-deoxyxylulose 5-phosphate. The incorporation of ^{2}H-labeled 2-C-methylerythritol (**38**) into the isoprenoid sidechains of ubiquinone and menaquinone by *E. coli* has been reported, albeit at very low incorporation rates [44]. It remains to be established whether 2-C-methyl-D-erythritol (**38**), or a derivative, is a genuine intermediate or a by-product of the alternative terpenoid pathway.

The direct product of the mevalonate pathway is IPP, which is subsequently converted to DMAPP by an isomerase, but formal proof for the involvement of IPP and DMAPP as intermediates of the deoxyxylulose pathway has not been obtained. The 'crossflow' of metabolites between

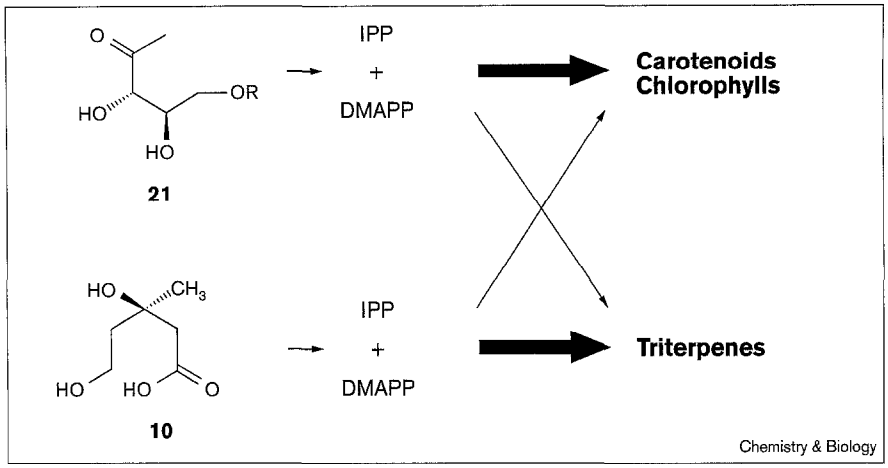
Figure 12



Hypothetical mechanism of the skeletal rearrangement conducive to 2-C-methyl-D-erythritol formation in *L. tulipifera* [42].

Figure 13

Crosstalk of mevalonate and deoxyxylulose pathway in cell cultures of *C. roseus* [14].



the two terpene pathways found in plants (see below) could well occur at the biosynthetic level of these isoprenoid monomers, however. In any case, it is worth noting that we do not know yet whether the initial product of the deoxyxylulose pathway is IPP or, alternatively, DMAPP.

Crosstalk between the deoxyxylulose and mevalonate pathways

Arigoni and coworkers had already noted in 1968 [45] that ^{14}C-mevalonate (**10**) is efficiently used as a precursor of triterpenes and sterols in the plant *Menyanthes trifoliata*. Mevalonate was also incorporated into the monoterpene alkaloid loganin, albeit with much lower efficiency. Similarly, ^{14}C mevalonate labelled sterols from various plants at high rates, whereas β-carotene and the isoprenoid sidechains of chlorophyll and plastoquinone acquired only low levels of the radiolabel [46]. Four decades later, these confusing data found a conclusive explanation when Arigoni's group [10] was able to show that both terpenoid pathways, the mevalonate and the deoxyxylulose pathways, operate in higher plants. In *G. biloba* seedlings, sterols were found to be formed preferentially via the mevalonate pathway, whereas ginkgolides are formed via the deoxyxylulose pathway, as described in detail above. More specifically, Arigoni and coworkers found evidence for compartmental separation of the two terpenoid machineries: the mevalonate pathway appears to operate in the cytoplasm, whereas the deoxyxylulose pathway appears to be located in plastids.

The compartmental separation of the two pathways is not absolute. Schwarz and Arigoni [10] found that approximately 1–2% of ginkgolides derive their isoprenoid precursors from the mevalonate pathway. More recently, experiments using [1-^{13}C]- and [2,3,4,5-$^{13}C_4$]-1-deoxy-D-xylulose (**21**, R = H) showed that *C. roseus* cells predominantly derive the monomers for formation of phytol and carotenoids from the deoxyxylulose pathway [14]. On the other hand, 6% of the building blocks used for sitosterol formation are derived in the same system from the deoxyxylulose pathway (Figure 13) [14]. Generally, it appears that, in higher plants, steroids are preferentially formed via the mevalonate pathway, whereas monoterpenes, diterpenes, carotenoids, phytol and plastoquinone are formed predominantly via the deoxyxylulose pathway (Table 1). The 'crosstalk' between the two metabolic pathways is best explained by the exchange of metabolic intermediates between the cytoplasm and the chloroplasts. The specific intermediate involved in the intercompartmental exchange and the regulation of the process is unknown.

Evolution of the two alternative pathways

The evolution of the two terpenoid pathways can be addressed via their distribution in the different taxonomic kingdoms (Table 1). Archaea appear to utilize the mevalonate pathway [47,48]; genes that are assumed to encode enzymes of the mevalonate pathway have been found in the genomes of several archaea (*Archaeoglobus fulgidus*, *Methanobacterium thermoautotrophicum*, and *Methanococcus jannaschii)* [49–51]. The complete genome of *Borrelia burgdorferi* has been published recently and was found to contain putative orthologs of all mevalonate pathway genes [52]. Thus, this microorganism, which is genetically similar to *Mycoplasma*, is also likely to utilize the mevalonate pathway. The photosynthetic bacterium *Chloroflexus aurantiacus*, representing the earliest branch in the development of eubacteria, was shown recently to form the diterpene verrucosan-2β-ol via the mevalonate pathway [53]. On the other hand, no genes with similarity to those of the mevalonate pathway are present in the genomes of the eubacteria *E. coli*, *Helicobacter pylori*, *Haemophilus influenzae*, *B. subtilis*, and in the blue green alga *Synechocystis* sp. These genomes contain putative orthologs of the *dxs* gene that are likely to encode 1-deoxyxylulose phosphate synthases. According to these data, it appears possible that

Table 1

Current knowledge about the occurrence of the alternative pathways for terpenoid biosynthesis.

Organism	Products of the deoxyxylulose pathway	Products of the mevalonate pathway	Reference
Bacteria			
Alicyclobacillus acidoterrestris	Hopanoids		[12]
Caldariella acidophila		Ether lipids	[48]
Chainia rubra		Napyradiomycin	[57]
Chloroflexus aurantiacus		Verrucosanol	[53]
Corynebacterium ammoniagenes	Menaquinone, 2-Methyl-D-erythritol		[41]
Escherichia coli	Ubiquinone, Menaquinone		[8,9,12,16,58]
Flavobacterium		Zeaxanthin	[59]
Halobacterium cutirubrum		Carotenoids	[47,60]
Kitasatosporia		Terpentecin	[61]
Methanobacterium thermoautotrophicum		Ether lipids	[48]
Methylobacterium fujisawaense	Ubiquinone, Hopanoids		[12]
Methylobacterium organophilum	Hopanoids		[7]
Rhodopseudomonas palustris	Hopanoids		[7]
Rhodopseudomonas acidophila	Hopanoids		[7]
Streptomyces aeriouvifer	Menaquinone	Furachinocin, Naphterpin	[62–64]
Streptomyces spheroides	Novobiocin		[65]
Streptomyces sp.	Pentalenolacton		[66]
Synechocystis sp.	Phytol, β-Carotene		[55]
Zymomonas mobilis	Hopanoids		[12,67]
Plants			
Catharanthus roseus	β-Carotene, Phytol, Lutein	Sitosterol	[14]
Chelidonium majus	Isoprene		[17,68]
Chlamydomonas reinhardtii	Phytol, Ergosterol, 7-Dehydroporiferasterol		[17]
Conocephalum conicum	Bornyl acetate		[69]
Cyanidium caldarium	Phytol	Ergosterol	[17]
Daucus carota	Phytol	Sitosterol, Stigmasterol	[70]
Euglena gracilis		Sterols, Phytol	[55]
Fossombronia alaskana	Epineoverrucosane		*
Ginkgo biloba	Ginkgolide A	Sitosterol	[10]
Heteroscyphus planus	Phytol	β-Carotene, Phytol, Heteroscyphic acid A,	[71–73]*
Hordeum vulgare	β-Carotene, Phytol, Plastoquinone	Sitosterol, Stigmasterol	[70]
Ipomoea parasitica	2-C-Methyl-D-erythritol		[42]
Lemna gibba	β-Carotene, Phytol, Plastoquinone, Lutein	Sitosterol, Stigmasterol	[17,70]
Liriodendron tulipifera	2-C-Methyl-D-erythritol		[42]
Lithospermum erythrorhizon		Shikonin	[74]
Lophocolea heterophylla		β-Carotene, Phytol	[72]
Marrubium vulgare	Marrubiin		[75]
Mentha piperita	Menthone		[15,76]
Mentha pulegium	Pulegone		[15,76]
Morus alba		Sitosterol	[77]
Nicotiana tabacum	Plastoquinone	Sterols, Ubiquinone	[78]
Pelargonium graveoleus	Geraniol		[15,76]
Populus nigra	Isoprene		[17]
Rauwolfia serpentina	Loganin		[79,80]
Ricciocarpos natans		Ricciocarpin A	[69]
Rubia tinctorum	Lucidin-3β-primveroside, Rubiadin-3β-primverosid		[80]
Salix viminalis	Isoprene		[17]
Salvia miltiorrhiza	Ferruginol		[56]
Scenedesmus obliquus	Phytol, Plastoquinone, β-Carotene, Lutein, Chondrillasterol, Ergost-7-enol		[17,81]
Taxus chinensis	Taxuyunnanine C		[13]
Thymus vulgaris	Thymol		[15,76]

*C. Rieder, C. Grammes, G. Heßler, K.-P. Adam, H. Becker, D.A., A.B. and W.E., unpublished observations.

archaea universally use the mevalonate pathway, whereas eubacteria may use either the mevalonate pathway or the deoxyxylulose phosphate pathway.

Both biosynthetic pathways appear to operate in different compartments of plant cells. It is generally accepted that chloroplasts originated by endocytosis and subsequent

endosymbiosis of cyanobacteria. The genes specifying the enzymes of the deoxyxylulose pathway could have been imported with the primitive endosymbionts and relocated subsequently to the nuclear genome. Recently, Martin and Müller [54] proposed that eukaryotes could have originated from a methanobacterial organism that had incorporated a eubacterium. This could imply that the cytoplasmic mevalonate enzymes could be the descendants of those of the methanogenic host, whereas the enzyme of the 1-deoxy-D-xylulose pathway could have been derived from a cyanobacterium-type endosymbiont. Indeed, cyanobacteria have been shown to utilise the 1-deoxy-D-xylulose pathway [55]. Additional research is required in order to examine this hypothesis.

Conclusions

The mevalonate pathway to isopentenyl pyrophosphate and dimethylallyl pyrophosphate has been considered, since its discovery in yeast and animals, the universal route for the formation of terpenes. In recent years, it was found that certain bacteria use an alternative pathway and that both pathways operate in higher plants. The alternative pathway starts with glyceraldehyde phosphate and pyruvate in a reaction that leads to 1-deoxyxylulose 5-phosphate as the first intermediate. The intermediates and enzymes involved in the subsequent conversion of 1-deoxyxylulose 5-phosphate into isopentenyl pyrophosphate and dimethylallyl pyrophosphate remain to be determined. There is evidence for a limited exchange of metabolites between the two pathways (at least one intermediate can be exchanged). This crosstalk explains in part why the alternative pathway went undetected for several decades despite numerous studies using mevalonate as a precursor. The rapid accumulation of genomic data will facilitate further studies on the occurrence and evolutionary aspects of the two pathways in different organisms.

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