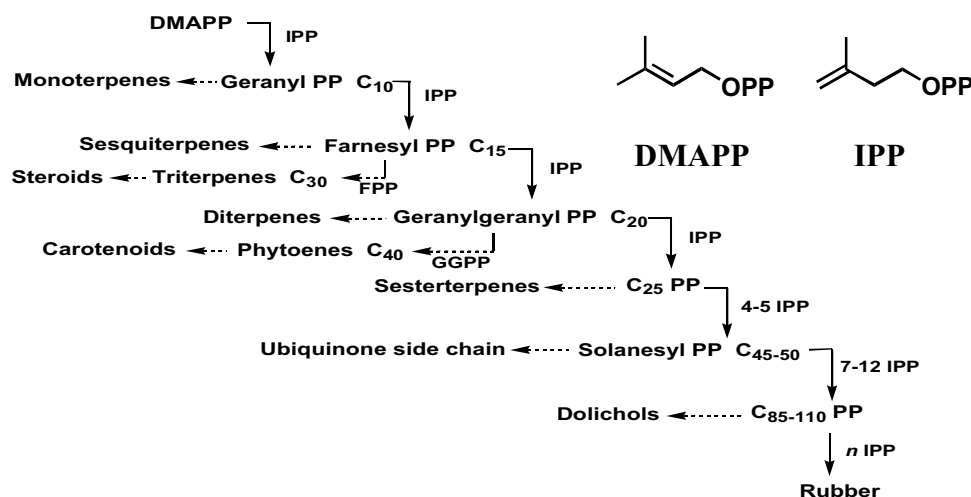


## Terpene Biosynthesis via the Mevalonate-Independent Pathway

Terpenes are a diverse family of compounds that are assembled from the basic unit *isoprene*. Members of this family include the monoterpenes, sesquiterpenes, diterpenes, steroids, carotenes, polyprenoids such as ubiquinone side chain, and the polymer rubber (Scheme 1). Nature uses two forms of “activated isoprene” to build these prenyl compounds, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Until ten years ago, only one pathway for the production of IPP and DMAPP was thought to exist. This pathway is known as the mevalonate (MVA) path and is the means by which mammalian cells synthesize terpenes.<sup>1</sup> The MVA pathway is also used by plant and bacterial cells, but not exclusively. The second terpene biosynthetic pathway was discovered after abnormal observations were noticed in a <sup>13</sup>C-labeling experiment.<sup>2</sup> Since that initial report, research in the area of terpene biosynthesis has increased dramatically.



Scheme 1. Terpene Family

Scientists study terpene biosynthesis for many reasons. Since nearly all organisms make and use terpenes, studying their biosynthesis allows us to gain a greater understanding of biological systems in general. Manipulation of these pathways, either by inhibiting the enzymes involved in the pathway<sup>3</sup> or by harnessing their reactivity to make nonnatural compounds is also a goal of this research. Finally, examining nature’s method to construct complex structures aids synthetic chemists in devising more efficient and elegant syntheses of their own.

### History

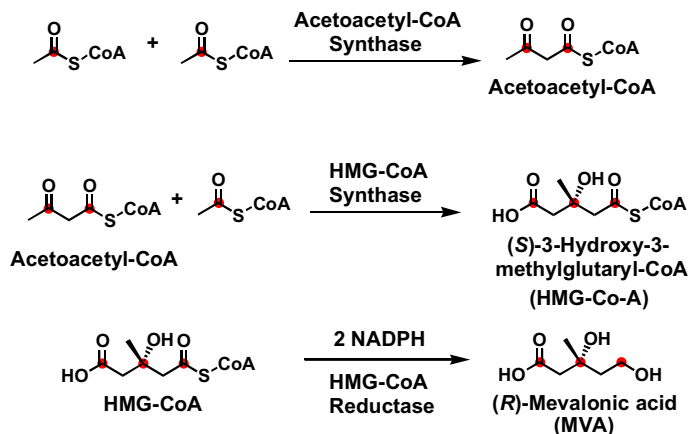
Chemists have studied terpenes for nearly two hundred years. Labillardière published some of the first work with terpenes in 1818, in which he determined that the oil of turpentine had a carbon to hydrogen ratio of 5:8. In 1826, Faraday correctly determined that the empirical formula of rubber was (C<sub>5</sub>H<sub>8</sub>)<sub>n</sub>. Further work was done by Williams in 1860, who, after pyrolyzing rubber, obtained a product with a formula of C<sub>5</sub>H<sub>8</sub> and named it *isoprene*. Boucharat then synthesized a compound with the formula

C<sub>10</sub>H<sub>16</sub> by heating isoprene. He noted that it was the same chemical found in turpentine. In 1880, Ballach recognized the pattern unfolding with various natural products and proposed the “isoprene rule,” which states that chemicals can be considered terpenes if their molecular formulas have a carbon to hydrogen ratio of 5:8.<sup>1</sup>

Cholesterol and the sterols were not recognized as terpenes until the 1920’s because their empirical formulas are not multiples of isoprene. Heilbron first suggested that cholesterol might come from squalene, a compound that did fit the definition of a terpene. Systematic studies on polyterpenes by Ruzicka, led him to propose the “biogenetic isoprene rule.” After World War II, the ability to isotopically enrich substances allowed more in depth studies on the biosynthetic pathway of terpenes.<sup>1</sup>

### Mevalonate Pathway

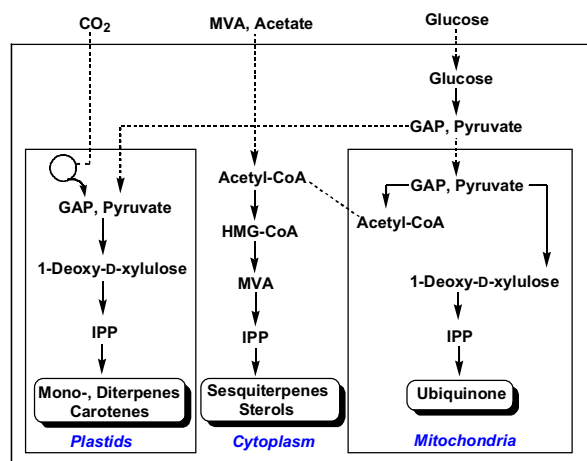
From studies performed with <sup>13</sup>C-labeled acetate, the mevalonate pathway was uncovered. After cellular uptake of the acetate, it is converted to acetyl-CoA and condensed with another unit of acetyl-CoA to generate acetoacetyl-CoA (Scheme 2). Addition of a third acetyl-CoA yields (S)-3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA), which is subsequently reduced by the enzyme HMG-CoA reductase to (R)-mevalonic acid (MVA).<sup>4</sup> MVA then undergoes a series of phosphorylations, loss of CO<sub>2</sub> and inorganic phosphate to give IPP, which can be isomerized by IPP-DMAPP isomerase (*idi*) to DMAPP.<sup>5</sup>



**Scheme 2.** Biosynthesis of mevalonic acid

Throughout the years studying the MVA pathway, some abnormal observations were noted.<sup>6,7</sup> In plants and bacteria, labeled acetate and mevalonate were poorly incorporated into some of the terpenes synthesized by those organisms. This observation was even more curious because labeled IPP was incorporated into all terpenes equally. Scientists suspected <sup>13</sup>C-labeled acetate and mevalonate possessed poor permeability, resulting in small amounts of labeled compounds. A second aberration was found when a known inhibitor of the MVA pathway, mevinolin, an HMG-CoA reductase inhibitor, did not inhibit production of the same terpenes that were unable to incorporate labeled-acetate and mevalonate. Lack of permeability of the inhibitor was again used to explain these results. Even unexpected labeling patterns were often dismissed as poor





**Figure 1.** Terpene biosynthesis in plants.

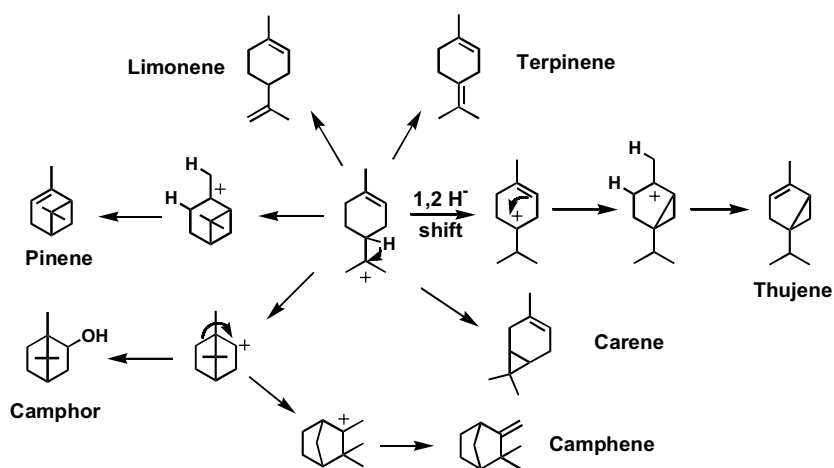
### Coupling of IPP and DMAPP

Both the mevalonate and the non-mevalonate pathways lead to the synthesis of IPP and DMAPP, which can then be coupled together by prenyltransferases to give polyprenoid chains.<sup>5</sup> The prenyltransferases that carry out these condensations can be classified into four categories based on stereochemistry and chain length of the product and accessory proteins required. The first class contains the short chain transferases that catalyze the coupling of IPP and DMAPP. The second and third classes, medium and long chain prenyltransferases, respectively, are able to extend intermediates synthesized by class I transferases. The long chain transferases need a protein factor to facilitate turnover of the enzyme. The fourth class of prenyltransferases catalyzes the formation of *Z*-polyprenoids.<sup>19, 20</sup>

The coupling of IPP to either DMAPP or a growing prenyl chain begins with the enzyme positioning the two groups using their pyrophosphate moieties. In farnesyl pyrophosphate synthase (FPPS), the homodimer has two conserved DDxxD motifs in each subunit that presumably chelate Mg<sup>2+</sup>, which is used to anchor the pyrophosphate groups of the substrates. The double bond of IPP then displaces the diphosphate of the growing prenyl chain as the allylic proton is removed.<sup>21, 22</sup> The chain continues to grow until it hits the “floor” of the active site, which consist of aromatic residues five and six amino acids before the first DDxxD motif. Mutations of these residues to smaller amino acids have allowed chain elongation to continue several prenyl units beyond the normal length catalyzed by this enzyme.<sup>23</sup> Artificial substrates have also been used with prenyl transferases to stereospecifically generate biologically active compounds.<sup>24</sup>

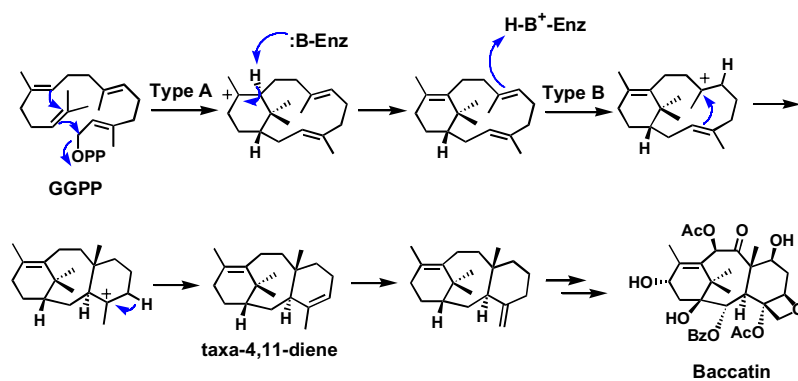
## Cyclization of Mono- and Diterpenes

Polyprenoid pyrophosphates, specifically geranyl pyrophosphate (GPP) and geranylgeranyl pyrophosphate (GGPP) are cyclized and further manipulated to generate the mono- and diterpenes, respectively. GPP must undergo a net isomerization in order to be able to cyclize. This is postulated to occur by dissociation of the diphosphate group and rearrangement to linalyl pyrophosphate. The C2-C3 bond can then rotate 180° and reionize to give the allylic cation. The pendant double bond is then able to add to the cation and generate the  $\alpha$ -terpinyl cation.<sup>25</sup> From this intermediate, deprotonations, double bond additions, and cationic rearrangements yield the diverse family of monoterpenes (Scheme 4).<sup>26</sup> Enzymes at various locations in the cell carry out subsequent oxidations and reductions demonstrating how complex terpene biosynthesis and trafficking can be.<sup>27</sup>



**Scheme 4.** Cyclization of monoterpenes.

Diterpene cyclizations are classified into two types, A and B. Type A cyclizations are initiated by dissociation of the pyrophosphate group with ensuing attack by a nearby double bond. Protonation of a double bond to generate a carbocation for attack by another double bond is a type B cyclization.<sup>28</sup> Cembrene A is a fourteen-membered carbocycle generated from a type A cyclization followed by deprotonation.<sup>29</sup> The labdane and clerodane families are formed from a type B cyclization of GGPP to a decalinic intermediate.<sup>28</sup> Often, both cyclizations can occur in the same molecule and could be performed by the same enzyme. A well-studied example of this combination of steps is the cyclization of the taxanes (Scheme 5). A type A cyclization occurs to yield a tertiary cation, which is then deprotonated at the adjacent carbon to install a unit of unsaturation between C11 and C12. This proton is subsequently used to initiate the type B cyclization, which after cyclization and elimination yields the intermediate taxa-4,11-diene.<sup>30, 31</sup>



**Scheme 5.** Cyclization of the taxane skeleton.

Work continues to elucidate mechanistic details of the non-MVA pathway. This information will allow better design of enzyme inhibitors such as fosmidomycin, which shows great promise as an antimalarial antibiotic.<sup>3</sup> It will also allow additional manipulation of the enzymes to produce a variety of complex molecules, similar to the use of artificial substrates with prenyltransferases to create other biologically active compounds.<sup>24</sup>

## References

- (1) S. L. Spurgeon and J. W. Porter In *Biosynthesis of Isoprenoid Compounds*; S. L. Spurgeon and J. W. Porter, Eds.; Wiley-Interscience: New York, 1981; Vol. 1, pp 1-46. "Introduction."
- (2) G. Flesch and M. Rohmer *Eur. J. Biochem.* **1988**, *175*, 405-411. "Prokaryotic hopanoids: the biosynthesis of bacteriohopane skeleton."
- (3) H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Turbachova, M. Eberl, J. Zeidler, H. K. Lichtenthaler, D. Soldati and E. Beck *Science (Washington, D. C.)* **1999**, *285*, 1573-1576. "Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs."
- (4) D. A. Bochar, J. A. Friesen, C. V. Stauffacher and V. W. Rodwell In *Comprehensive Natural Products Chemistry*; D. E. Cane, Ed.; Elsevier: New York, 1999; Vol. 2, pp 15-44. "Biosynthesis of Mevalonic Acid from Acetyl-CoA."
- (5) T. Koyama and K. Ogura In *Comprehensive Natural Products Chemistry*; D. E. Cane, Ed.; Elsevier: New York, 1999; Vol. 2, pp 69-96. "Isopentenyl diphosphate isomerase and prenyltransferases."
- (6) M. Rohmer *Nat. Prod. Rep.* **1999**, *16*, 565-574. "The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants."
- (7) W. Eisenreich, M. Schwarz, A. Cartayrade, D. Arigoni, M. H. Zenk and A. Bacher *Chem. Biol.* **1998**, *5*, R221-R233. "The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms."

- (8) M. Rohmer, M. Knani, P. Simonin, B. Sutter and H. Sahn *Biochem. J.* **1993**, *295*, 517-524. "Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentyl diphosphate."
- (9) M. Schwarz and D. Arigoni In *Comprehensive Natural Products Chemistry*; D. E. Cane, Ed.; Elsevier: New York, 1999; Vol. 2, pp 367-400. "Ginkgolide biosynthesis."
- (10) M. Rohmer, M. Seemann, S. Horbach, S. Bringer-Meyer and H. Sahn *J. Am. Chem. Soc.* **1996**, *118*, 2564-2566. "Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis."
- (11) J.-F. Hoeffler, D. Tritsch, C. Grosdemange-Billiard and M. Rohmer *Eur. J. Biochem.* **2002**, *269*, 4446-4457. "Isoprenoid biosynthesis via the methylerythritol phosphate pathway - mechanistic investigations of the 1-deoxy-D-xylulose-5-phosphate reductoisomerase."
- (12) D. Arigoni, S. Sagner, C. Latzel, W. Eisenreich, A. Bacher and M. Zenk *Proc. Nat. Acad. Sci., USA* **1997**, *94*, 10600-10605. "Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement."
- (13) G. A. Sprenger, U. Schorken, T. Wiegart, S. Grolle, A. A. de Graff, S. V. Taylor, T. P. Begley, S. Bringer-Meyer and H. Sahn *Proc. Nat. Acad. Sci., USA* **1997**, *94*, 12857-12862. "Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose-5-phosphate precursor to isoprenoids, thiamin, and pyridoxol."
- (14) S. Herz, J. Wungsintaweeikul, C. A. Schuhr, S. Hecht, H. Luttgen, S. Sagner, M. Fellermeier, W. Eisenreich, M. Zenk, A. Bacher and F. Rohdich *Proc. Nat. Acad. Sci., USA* **2000**, *97*, 2486-2490. "Biosynthesis of terpenoids: YgbB protein converts 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate to 2C-methyl-D-erythritol-2,4-cyclodiphosphate."
- (15) M. Seemann, B. T. Bui, M. Wolff, D. Tritsch, N. Campos, A. Boronat, A. Marquet and M. Rohmer *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 4337-4339. "Isoprenoid biosynthesis through the methylerythritol phosphate pathway: the (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) is a [4Fe-4S] protein."
- (16) D. Arigoni, W. Eisenreich, C. Latzel, S. Sagner, T. Radykewicz, M. H. Zenk and A. Bacher *Proceedings of the National Academy of Sciences of the United States of America* **1999**, *96*, 1309-1314. "Dimethylallyl pyrophosphate is not the committed precursor of isopentenyl pyrophosphate during terpenoid biosynthesis from 1-deoxyxylulose in higher plants."
- (17) F. Rohdich, F. Zepeck, P. Adam, S. Hecht, J. Kaiser, R. Laupitz, T. Grawert, S. Amslinger, W. Eisenreich, A. Bacher and D. Arigoni *Proceedings of the National Academy of Sciences of the United States of America* **2003**, *100*, 1586-1591. "The deoxyxylulose phosphate pathway of isoprenoid biosynthesis: Studies on the mechanisms of the reactions catalyzed by IspG and isph protein."
- (18) J.-F. Hoeffler, A. Hemmerlin, C. Grosdemange-Billiard, T. J. Bach and M. Rohmer *Biochem. J.* **2002**, *366*, 573-583. "Isoprenoid biosynthesis in higher plants and in *Escherichia coli*: on the branching in the methylerythritol phosphate

- pathway and the independent biosynthesis of isopentenyl diphosphate and dimethylallyl diphosphate."
- (19) K. Ogura and T. Koyama *Chem. Rev.* **1998**, *98*, 1263-1276. "Enzymatic aspects of isoprenoid chain elongation."
  - (20) P. H. Liang, T. P. Ko and A. H. J. Wang *Eur. J. Biochem.* **2002**, *269*, 3339-3354. "Structure, mechanism and function of prenyltransferases."
  - (21) J. W. Cornforth, R. Cornforth, H., C. Donninger and G. Popjak *Proc. R. Soc. London, B* **1965**, *163*, 492-514. "Studies on the biosynthesis of cholesterol."
  - (22) J. W. Cornforth, R. Cornforth, H., G. Popjak and L. Yengoyan *J. Biol. Chem.* **1966**, *241*, 3970-3987. "Studies on the biosynthesis of cholesterol."
  - (23) L. C. Tarshis, P. J. Proteau, B. A. Kellogg, J. C. Sacchettini and C. D. Poulter *Proc. Nat. Acad. Sci., USA* **1996**, *93*, 15018-15023. "Regulation of product chain length by isoprenyl diphosphate synthase."
  - (24) T. Koyama, K. Ogura, F. C. Baker, G. C. Jamieson and D. A. Schooley *J. Am. Chem. Soc.* **1987**, *109*, 2853-2854. "Synthesis and absolute configuration of 4-methyl juvenile hormone I (4-MeJH I) by a biogenetic approach: a combination of enzymic synthesis and biotransformation."
  - (25) R. Croteau and F. Karp *Arch. Biochem. Biophys.* **1979**, *198*, 512-522. "Biosynthesis of monoterpenes: preliminary characterization of bornyl pyrophosphate synthetase from sage (*Salvia officinalis*) and demonstration that geranyl pyrophosphate is the preferred substrate for cyclization."
  - (26) L. Ruzicka *Experientia* **1953**, *9*, 357-396. "The isoprene rule and the biogenesis of terpenic compounds."
  - (27) M. L. Wise and R. Croteau In *Comprehensive Natural Products Chemistry*; D. E. Cane, Ed.; Elsevier: New York, 1999; Vol. 2, pp 97-153. "Monoterpene biosynthesis."
  - (28) J. MacMillan and M. H. Beale In *Comprehensive Natural Products Chemistry*; D. E. Cane, Ed.; Elsevier: New York, 1999; Vol. 2, pp 217-243. "Diterpene biosynthesis."
  - (29) W. Dauben, G., W. E. Thiessen and P. R. Resnick *J. Org. Chem.* **1965**, *30*, 1693-1698. "Cembrene, a fourteen-membered ring diterpene hydrocarbon."
  - (30) A. E. Koepp, M. Hezari, J. Zajicek, B. S. Vogel, R. E. LaFever, N. G. Lewis and R. Croteau *Journal of Biological Chemistry* **1995**, *270*, 8686-8690. "Cyclization of Geranylgeranyl Diphosphate to Taxa-4 (5),11 (12) -diene Is the Committed Step of Taxol Biosynthesis in Pacific Yew."
  - (31) X. Lin, M. Hezari, A. E. Koepp, H. G. Floss and R. Croteau *Biochemistry* **1996**, *35*, 2968-2977. "Mechanism of Taxadiene Synthase, a Diterpene Cyclase That Catalyzes the First Step of Taxol Biosynthesis in Pacific Yew."