Phytochemistry 69 (2008) 2881–2885

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Molecules of Interest

Making artemisinin ☆

Patrick S. Covello *

Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, Canada S7N OW9

ARTICLE INFO

Article history:
Received 29 September 2008
Accepted 2 October 2008
Available online 1 November 2008

Keywords:
Artemisinin
Artemisia annua
Biosynthesis
Synthesis

ABSTRACT

The possibilities for the production of the antimalarial artemisinin by biological and chemical means are explored. These include native biosynthesis, genetic modification of *Artemisia annua* and other plants, engineering of microbes, total and partial chemical synthesis and combinations of the above.

1. Sourcing artemisinin (1)

In the late 1960s, Chinese scientists began a search for new antimalarial drugs. Part of the motivation for this was the development of resistance by the malaria parasite to existing antimalarial drugs. Plants used in traditional Chinese medicine were tested, including *Artemisia annua* and extracts from this plant showed good antimalarial activity in mice. What ensued, over the decades since, was the isolation and characterization of the active sesquiterpenoid artemisinin (**1**), the development of artemisinin-derived semisynthetic drugs, the recommendation by the World Health Organization that artemisinin-based combination therapies (ACTs) be the treatment of choice for falciparum-type malaria in many countries, and the current annual worldwide demand for approximately 100 million ACT treatments (Haynes, 2006; Li et al., 2006; Weina, 2008).

The demand for ACTs has led to recent shortages of **1**. While such shortages can and have been alleviated by increased cultivation of *A. annua*, from a developing world perspective, plant-derived **1** remains relatively expensive. Recent prices for **1** have fluctuated in the US$150–1,600 range. A reliable supply at US$100/kg or less is desirable. Given the need for artemisinin (**1**) at the lowest possible cost for the developing world, this article explores the existing and potential methods for its production. The general possibilities for large scale production of **1** range from the purely biological to the purely chemical, with combinations of biological and chemical synthesis "in between" (see Table 1). At one extreme, total biosynthesis in the plant occurs from the ubiquitous isoprenoid intermediate farnesyl diphosphate (Fig. 1, solid arrows). On the other hand, schemes for total chemical syntheses typically start with monoterpenes (Fig. 1, open arrows). In this article, aspects of biosynthesis and chemical synthesis relating to **1** are explored. This leads to a discussion of the optimal combination of biological and chemical synthesis for large scale production.

2. Artemisinin (1) from plants

Initially, the only commercially viable source of **1** was plants. Three species in the genus *Artemisia* have been reported to contain **1**, *A. annua*, *Artemisia apiacea* and *Artemisia lanceolata* (Willcox et al., 2004). Most of the artemisinin-related interest has focused on *A. annua*. The cultivation of this plant is currently centered in Asia, with significant production in Africa (Kindermans et al., 2007). The simplest method for recovery of **1** from *A. annua* is its crystallization from extracts of dried plant material (Haynes and Vonwiller, 1994). Yields up to about 1.5% dry weight are typically reported. Indeed, the first attempts at improved production involved selection of high artemisinin genotypes (Delabays et al., 2001). Conventional breeding of *A. annua* continues and a program for developing high-artemisinin *A. annua* using advanced molecular breeding techniques is in progress at the University of York in the United Kingdom.

As with other plant metabolites of commercial interest, attempts have been made to produce **1** in cell and tissue cultures. Considerable effort has been put into the development of transformed root cultures (hairy roots) for artemisinin (**1**) production, for example. However, given the costs of tissue culture, the reported yields do not appear to be economical (Towler et al., 2007).

☆ NRCC Publication No. 50127.

* Tel.: +1 306 975 5269; fax: +1 306 975 4839.
E-mail address: Patrick.Covello@nrc-cnrc.gc.ca

0031-9422/$ - see front matter
doi:10.1016/j.phytochem.2008.10.001

Table 1
Possibilities for production of artemisinin (**1**) in decreasing order of chemical synthesis requirements.

Biological host	Starting material or intermediate for chemical synthesis	Reference	Comments
None	Monoterpenes	Schmid and Hofheinz, 1983	Expensive; low yields in >8 steps
E. coli	Amorphadiene (**4**)	Martin et al., 2003	High biosynthetic yields; >3 chemical steps
Tobacco	Amorphadiene (**4**)	Wu et al., 2006	Low yield
E. coli	Artemisinic acid (**2**)	Chang et al., 2007	Efficient cytochrome P450 expression is a challenge
Yeast	Artemisinic acid (**2**)	Ro et al., 2006	High yields
Yeast	Dihydroartemisinic acid (**3a**)	Zhang et al., 2008	Yields not optimized, simple chemistry
Chicory	Artemisinic acid (**2**)/dihydroartemisinic acid (**3a**)	http://www.dafra.be	In progress
A. annua hairy roots	None	Towler et al., 2007	Low yields
A. annua	None	Delabays et al., 2001	US$150–1600/kg; expensive compared to other malaria drugs

3. Biosynthesis and plant genetic engineering

3.1. Biosynthesis

Genetic engineering of plants is also a possibility for improving artemisinin production. This engineering could involve *A. annua* or other plant species. Clearly, such metabolic engineering requires a fairly complete understanding of artemisinin biosynthesis. In the last decade, and especially, in the last 4 years, excellent progress has been made in elucidating the biosynthetic pathway to this sesquiterpene lactone **1** (Bertea et al., 2005; Covello et al., 2007; Zhang et al., 2008; see Fig. 1). There has been a certain amount of confusion in the literature regarding artemisinin biosynthesis (Li et al., 2006). This has centered around the possible roles of artemisinic acid (**2**) and dihydroartemisinic acid (**3a**) as precursors of **1** (Brown and Sy, 2007; Dhingra and Narasu, 2001). However, as recent data have emerged the pathway is beginning to "solidify". As indicated in Fig. 1, artemisinin (**1**) biosynthesis is thought to proceed via the cyclization of FDP to amorphadiene (**4;** by amorphadiene synthase), oxidation to artemisinic aldehyde (**5;** by amorphadiene oxidase; CYP71AV1), reduction to dihydroartemisinic aldehyde [by artemisinic aldehyde Δ11(13) reductase], oxidation to dihydroartemisinic acid (**3a**), possibly by a trichome-specific aldehyde dehydrogenase (Teoh et al., 2007) and, finally, a possibly non-enzymatic conversion to **1**.

In a "dehydro" branch of the pathway, artemisinic acid (**2**) is formed from artemisinic aldehyde (**5**), a reaction catalyzed by amorphadiene oxidase (Ro et al., 2006; Teoh et al., 2006; Zhang et al., 2008). Artemisinic acid is thought to be transformed, possibly nonenzymatically, into a number of compounds including arteannuin B (**6;** Brown and Sy, 2007). It is worth noting that *A. annua* leaf extracts have been reported to catalyze the conversion of **6** to **1** (Dhingra and Narasu, 2001). However, given the measured K_m of 0.5 mM for the enzyme activity and the complexity of the transformation, it is not clear whether this is relevant *in vivo*.

Of course, genetic engineering requires knowledge of the genes involved in artemisinin (**1**) biosynthesis and very good progress has been made in isolating cDNAs encoding the relevant enzymes. Three groups reported the molecular cloning of amorphadiene synthase around the turn of the century (Chang et al., 2000; Mercke et al., 2000; Wallaart et al., 2001) and two groups isolated clones for amorphadiene oxidase (CYP71AV1; Ro et al., 2006; Teoh et al., 2006). The latter cytochrome P450 not only hydroxylates amorphadiene (**4**) to artemisinic alcohol, but it is also capable of oxidizing the alcohol to the corresponding aldehyde **5** and acid **2**. The recent cloning of artemisinic aldehyde Δ11(13) reductase provides strong support for the position of dihydroartemisinic acid (**3a**) as a true biosynthetic precursor to **1;** artemisinic acid (**2**) appears to be in the "dehydro" branch based at **5** (Zhang et al., 2008). Indeed, given its chemistry (see below), **3a** may be the last artemisinin intermediate in *A. annua* that is formed with enzyme involvement (Brown and Sy, 2004). From a genetic engineering point of view, it is also notable that artemisinic aldehyde reductase may be very important in determining artemisinin (**1**) content in *A. annua*. High artemisinin chemotypes of *A. annua* typically have high ratios of **3a** to **2** (Wallaart et al., 2000), suggesting that double bond reduction may limit artemisinin (**1**) accumulation.

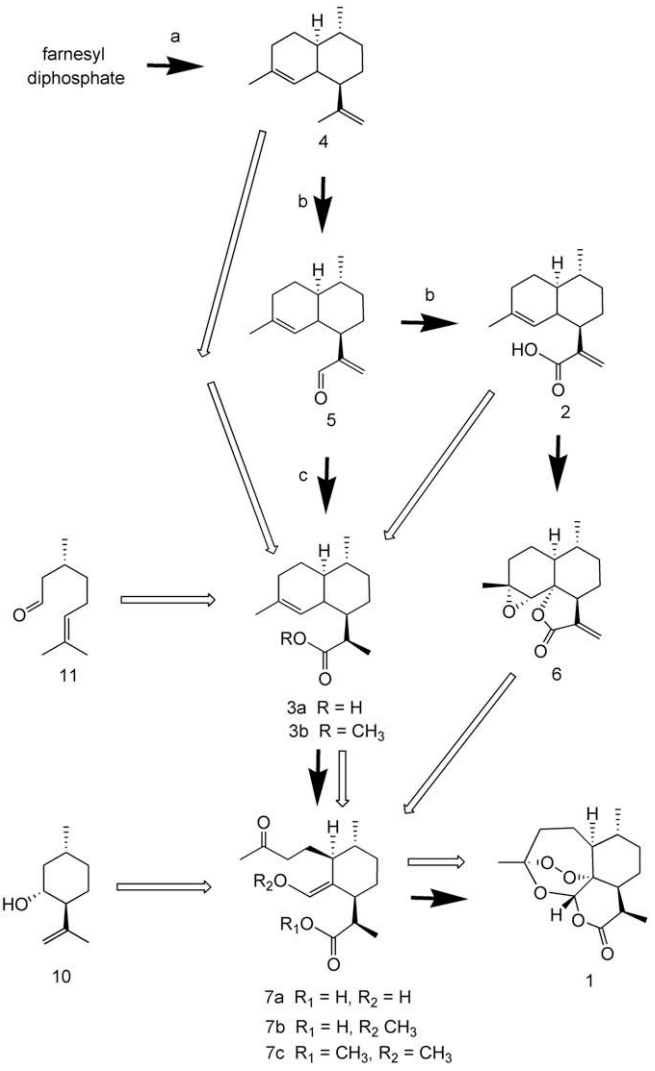

Fig. 1. Biosynthesis and chemical synthesis of artemisinin. Biosynthetic steps are shown as solid arrows; chemical synthetic steps are shown as open arrows. a, amorpha-4,11-diene synthase; b, amorpha-4,11-diene oxidase (CYP71AV1); c, artemisinic acid Δ11(13) reductase and a dehydrogenase.

3.2. Oxidation of dihydroartemisinic acid – is there an enzyme?

A discussion of the chemistry of dihydroartemisinic acid (**3a**) is relevant to both the biosynthesis and chemical synthesis of **1**. At first glance, the formation of the polycyclic peroxide lactone structure of artemisinin (**1**) from **3a** appears complicated. However, this can be broken down into individual reactions which appear to be reasonably straightforward. In this regard, it may be useful to think of **1** as a pair of linked modified acetals, in which one acetal group includes a lactone and one acetal includes a peroxide group. With this in mind, Brown and others have developed the scheme shown in Fig. 2 for the oxygen-dependent conversion of **3a** to **1** (Brown and Sy, 2004). The keto-enol **7a** can be formed from **3a** in the presence of singlet (excited state molecular) oxygen formed by UV absorption or visible light in the presence of photosensitizers, or from hydrogen peroxide. The tertiary allylic hydroperoxide intermediate **8** is formed in a singlet oxygen ene reaction (Frimer, 1984; Wasserman and Ives, 1981) followed by Hock cleavage to give **7a** (Frimer, 1984; Hock and Schrader, 1936). A second hydroperoxide **9** forms in the presence of triplet (ground state) oxygen under mild conditions. What follows is the formation of the acetal peroxide and the acetal lactone groups. Thus, biosynthetically, **1** is thought to be formed from **3a** in what may be a set of spontaneous rather than enzyme-catalyzed reactions. It turns out that equivalents of **7a** are also important from a chemical synthesis point of view (see below; Sy and Brown, 2002).

The nature of dihydroartemisinic acid chemistry raises the question of enzyme involvement in the last steps of artemisinin biosynthesis. While it has been suggested that no enzymes are involved in conversion of **3a** to **1** (Brown and Sy, 2004), the possibility certainly exists. In this regard, it is notable that perhaps the least spontaneous step in **3a** oxidation, first $^1O_2$-dependent hydroperoxidation and resulting ring cleavage is very reminiscent of other enzyme reactions. In particular, carotenoid cleavage dioxygenases come to mind (Bugg, 2003). Similar to the ring cleavage of **3a**, these enzymes catalyze the cleavage of double bonds, including trisubstituted double bonds, to give two carbonyl compounds. Thus, the possibility for dioxygenase involvement in artemisinin biosynthesis remains an open question, one with technological relevance. However, there doesn't appear to be any positive evidence for enzyme involvement in oxidation of **3a**. In fact, there is circumstantial evidence against enzyme involvement in **3a** oxidation. This is based on the fact that **3a** typically accumulates to high levels in *A. annua* and its level is correlated with high artemisinin (**1**) levels (Wallaart et al., 2000). This suggests that the conversion of the intermediate **3a** is relatively slow. From a more technological point of view, even if no enzyme exists for this transformation in *A. annua*, it may be possible to find or develop an enzyme capable of oxidative ring cleavage of **3a**.

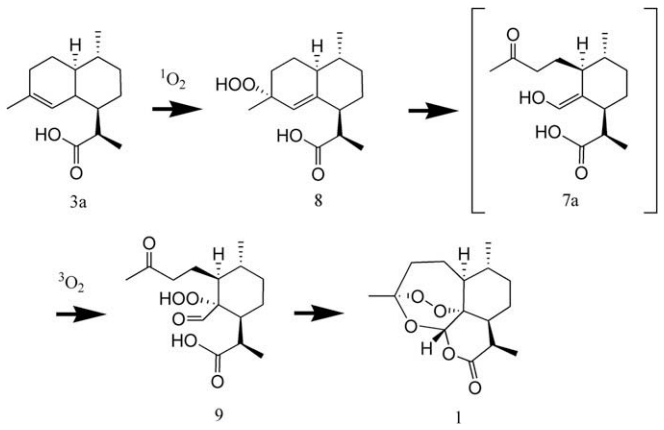

Fig. 2. Non-enzymatic oxidation of dihydroartemisinic acid (**3a**) to yield artemisinin (**1**).

3.3. Plant genetic engineering

Some attempts have been made to genetically-modify *A. annua per se* for the purpose of increasing artemisinin (**1**) content. This has included the overexpression of farnesyl diphosphate synthase (Chen et al., 2000) and the suppression of squalene synthase (Zhang et al., 2008). However, the results of these efforts have not been dramatic. Efforts in this area are hampered by the lack of efficient protocols for genetic transformation of *A. annua*.

Other plants also represent hosts for possible artemisinin production. Bouwmeester and coworkers investigated the biochemistry of chicory, a sesquiterpene lactone-producer in the same family (*Cichorium intybus*, *Asteraceae*). They found that wild type chicory extracts showed amorphadiene oxidase activity (De Kraker et al., 2003). Thus, expression of amorphadiene synthase in chicory would be predicted to give rise to artemisinic acid (**2**). This concept appears to form the basis for a commercial approach to artemisinin (**1**) production based on genetically-modified chicory. If **2** can be produced, it can be converted chemically to **1** (see below). Along similar lines, lettuce was engineered to overexpress amorphadiene synthase; no artemisinin (**1**) was detected, although the presence of related precursors doesn't appear to have been investigated (Cho et al., 2005).

Chappell and coworkers have engineered amorphadiene (**4**) production in tobacco (Wu et al., 2006). Interestingly, it was found that expression of FDP synthase and amorphadiene synthase targeted to plastids gave much better yields than cytosolic (untargetted) enzymes. The tobacco platform offers the basis for additional engineering. Alternatively, **1** could be obtained from plant-derived **4** by semisynthesis (Reiling et al., 2006; see below).

Given the biosynthetic pathway and the known relevant genes, in principle, it should be possible to engineer any plant, or almost any other organism for that matter, to produce **3a** using 3–4 genes encoding amorphadiene synthase, amorphadiene oxidase (CYP71AV1), artemisinic aldehyde reductase and possibly a dihydroartemisinic aldehyde dehydrogenase (Teoh et al., 2007). In practice, the real challenge of such engineering endeavours is typically the optimization of yield and it will be interesting to see what is possible in plants in the future.

4. Microbial engineering

In recent years, the production of **1** via microbial engineering has been investigated (Keasling, 2008). Metabolic engineering, particularly in microbes, has advanced to such a point that new terminology has been applied to it – synthetic biology. Artemisinin (**1**) production has become a prime example of the capabilities of this parts-list-and-systems-design approach to microbial genetic engineering. For **1**, emphasis has been on the engineering of *E. coli* and yeast. Initially, the production of the FDP precursor was optimized and the enzyme catalyzing the first committed step in the artemisinin pathway, amorphadiene synthase, was expressed in *E. coli* (Martin et al., 2003). This gave rise to good yields of amorphadiene (**4**). In parallel, a scheme for chemical conversion of **4** to **1** was developed (Reiling et al., 2006).

In further work, the isolation of cDNA encoding amorphadiene oxidase (CYP71AV1) allowed the engineering of artemisinic acid (**2**) in both *E. coli* (Chang et al., 2007) and yeast (Ro et al., 2006), with yeast being the better producer. Again, this compound can be converted chemically to **1**. Indeed, Amyris Biotechnologies, Inc. and the Institute for One World Health have announced an

arrangement with sanofi-aventis to produce semisynthetic artemisinin from a microbial source developed at the University of California at Berkeley. In a related development, the recent cloning of artemisinic aldehyde Δ11(13) reductase from *A. annua* has allowed the production in yeast of **3a** (Zhang et al., 2008), which is readily converted to **1** (see above).

5. Chemical synthesis of artemisinin (1)

The first total syntheses of natural artemisinin (**1**) from the monoterpenoids (−)-isopulegol (**10;** Schmid and Hofheinz (1983)) and (+)-citronellal (**11;** Xu et al. (1986)) were reported in the 1980s. Since then, other total syntheses from (+)-pulegone, (+)-isolimonene and (−)-β-pinene and partial syntheses have been reported (Abdin et al., 2003; Webster and Lehnert, 1994). As pointed out by Brown and Sy (Sy and Brown, 2002), essentially all syntheses to date involve a late intermediate which is equivalent to **7**. For example, **7b** is a late intermediate in the synthesis from **10** (Schmid and Hofheinz, 1983) and synthesis from **11** includes **7c** (Xu et al., 1986). The major difference in the various synthetic schemes is the approach to compound **7.** As indicated in Fig. 1, in a number of cases, the starting point for total synthesis is a monocyclic monoterpenoid which is elaborated to an equivalent of **7** (Avery et al., 1992; Liu et al., 1993; Ravindranathan et al., 1990; Schmid and Hofheinz, 1983; Yadav et al., 2003). Alternatively, synthesis proceeds first via dihydroartemisinate **3** and then **7** (Haynes and Vonwiller, 1990; Nowak and Lansbury, 1998; Roth and Acton, 1989; Xu et al., 1986). This is the case for total synthesis from **11** (Xu et al., 1986), (−)-β-pinene (Liu et al., 1993) and semi-synthesis from **2** (Roth and Acton, 1989) and from **6** (Nowak and Lansbury, 1998), for example. Recently, a family of processes for chemical synthesis of **1** from **4**, again via dihydroartemisinate **3** has appeared in the patent literature (Reiling et al., 2006).

While it is difficult to determine the cost of various synthetic and semisynthetic chemical schemes, the economics suggest that relatively few chemical steps and losses can be tolerated. All of the published total syntheses required at least 8 steps and provide yields of less than 10%. With this in mind, and with starting materials such as β-pinene, for example, selling for about US$15/kg, it is not practical to produce **1** from total synthesis at $100/kg.

6. Mixed mode production systems

The above discussion highlights some general possibilities for the large scale production of a given chemical. Traditionally, such production has been from natural sources, or from chemical synthesis from readily available starting materials. With advances in plant genetic engineering and synthetic biology, another option is a mixture of biological and chemical synthesis. The production of **1** via **4** is a good example of this. Amorphadiene (**4**) is not at all readily available. However, it could be made available through synthetic biology in *E. coli* (Chang et al., 2007) and **1** could be produced from it by chemical synthesis (Reiling et al., 2006). In principle, much of the technology to genetically engineer amorphadiene (**4**) production has been available for a quarter century. However, only recently have the inventory of genetic parts and the knowledge of microbial genetic and biochemical systems reached a stage to allow for the design of commercially viable systems in plants and microbes.

7. Concluding remarks

In the sense that *A. annua* cultivation is the only current commercial source of **1**, it is economical. However, as discussed, there is a need for greater economy in the production of artemisinin-based drugs. Certainly any improvements by conventional breeding are likely to lower the cost of artemisinin (**1**) production. Depending on regulatory and related costs, genetically modified plants may also help and it is encouraging to see commercial interest in this area in the form of Dafra Pharma International. For microbially-derived ACTs, involving semisynthesis from artemisinic acid, Hale et al. (2007) suggest a 30–60% reduction in cost is possible. It is encouraging to see this approach pursued for large scale production by sanofi-aventis.

Acknowledgements

The author wishes to thank Sue Abrams, John Balsevich and Peter Buist for reviewing the manuscript prior to publication, Darwin Reed for technical assistance and numerous colleagues for teaching him things relevant to artemisinin.

References

Abdin, M.Z., Israr, M., Rehman, R.U., Jain, S.K., 2003. Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production. Planta. Med. 69, 289–299.

Avery, M.A., Chong, W.K.M., Jennings-White, C., 1992. Stereoselective total synthesis of (+)-artemisinin, the antimalarial constituent of *Artemisia annua* L. J. Am. Chem. Soc. 114, 974–979.

Bertea, C.M., Freije, J.R., van der Woude, H., Verstappen, F.W., Perk, L., Marquez, V., de Kraker, J.W., Posthumus, M.A., Jansen, B.J., de Groot, A., Franssen, M.C., Bouwmeester, H.J., 2005. Identification of intermediates and enzymes involved in the early steps of artemisinin biosynthesis in *Artemisia annua*. Planta. Med. 71, 40–47.

Brown, G.D., Sy, L.-K., 2004. *In vivo* transformations of dihydroartemisinic acid in *Artemisia annua* plants. Tetrahedron 60, 1139–1159.

Brown, G.D., Sy, L.-K., 2007. *In vivo* transformations of artemisinic acid in *Artemisia annua* plants. Tetrahedron 63, 9548–9566.

Bugg, T.D.H., 2003. Dioxygenase enzymes: catalytic mechanisms and chemical models. Tetrahedron 59, 7075–7101.

Chang, Y.J., Song, S.H., Park, S.H., Kim, S.U., 2000. Amorpha-4, 11-diene synthase of Artemisia annua: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis. Arch. Biochem. Biophys. 383, 178–184.

Chang, M.C., Eachus, R.A., Trieu, W., Ro, D.K., Keasling, J.D., 2007. Engineering *Escherichia coli* for production of functionalized terpenoids using plant P450s. Nat. Chem. Biol. 3, 274–277.

Chen, D., Ye, H., Li, G., 2000. Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. Plant. Sci. 155, 179–185.

Cho, D.W., Park, Y.D., Chung, K.H., 2005. *Agrobacterium*-mediated transformation of lettuce with a terpene synthase gene. J. Korean Soc. Hort. Sci. 46, 169–175.

Covello, P.S., Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G., 2007. Functional genomics and the biosynthesis of artemisinin. Phytochem 68, 1864–1871.

De Kraker, J.-W., Scurink, M., Franssen, M.C.R., König, W.A., de Groot, A., Bouwmeester, H.J., 2003. Hydroxylation of sesquiterpenes by enzymes from chicory (*Cichorium intybus* L.) roots. Tetrahedron 59, 409–418.

Delabays, N., Simonnet, X., Gaudin, M., 2001. The genetics of artemisinin content in *Artemisia annua* L and the breeding of high yielding cultivars. Curr. Med. Chem. 8, 1795–1801.

Dhingra, V., Narasu, M.L., 2001. Purification and characterization of an enzyme involved in biochemical transformation of arteannuin B to artemisinin from *Artemisia annua*. Biochem. Biophys. Res. Commun. 281, 558–561.

Frimer, A.A., 1984. The effect of strain on the rearrangement of allylic hydroperoxides: a research account. J. Photochem. 25, 211–226.

Hale, V., Keasling, J.D., Renninger, N., Diagana, T.T., 2007. Microbial-derived artemisinin: a biotechnology solution to the global problem of access to affordable antimalarial drugs. Am. J. Trop. Med. Hyg. 77, 198–202.

Haynes, R.K., 2006. From artemisinin to new artemisinin antimalarials: biosynthesis, extraction, old and new derivatives, stereochemistry and medicinal chemistry requirements. Curr. Top. Med. Chem. 6, 509–537.

Haynes, R.K., Vonwiller, S.C., 1990. Catalysed oxygenation of allylic hydroperoxides derived from qinghao (artemisinic) acid. Conversion of qinghao acid into dehydroqinghaosu (artemisitene) and qinghaosu (artemisinin). J. Chem. Soc. Chem. Commun., 451–453.

Haynes, R.K., Vonwiller, S.C., 1994. Extraction of artemisinin and artemisinic acid: preparation of artemether and new analogues. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1), S23–S26.

Hock, H., Schrader, O., 1936. Der Mechanismus der Autoxydation einfacher Kohlenwasserstoffe als Beitrag zur Kenninis der Autoxydation von Brennstoffen. Angew. Chem. 49, 565–566.

Keasling, J.D., 2008. Synthetic biology for synthetic chemistry. ACS Chem. Biol. 3, 64–76.

Kindermans, J.M., Pilloy, J., Olliaro, P., Gomes, M., 2007. Ensuring sustained ACT production and reliable artemisinin supply. Malar. J. 6, 125.

Li, Y., Huang, H., Wu, Y.-L., 2006. Ginghaosu (artemisinin) - a fantastic antimalarial drug from a traditional Chinese medicine. In: Liang, X.-T., Fang, W.-S. (Eds.), Medicinal Chemistry of Bioactive Natural Products. Wiley Interscience, Hoboken, pp. 183–256.

Liu, H.-J., Yeh, W.-L., Chew, S.Y., 1993. A total synthesis of the antimalarial natural product (+)-qinghaosu. Tetrahedron Lett. 34, 4435–4438.

Martin, V.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat. Biotechnol. 21, 796–802.

Mercke, P., Bengtsson, M., Bouwmeester, H.J., Posthumus, M.A., Brodelius, P.E., 2000. Molecular cloning, expression, and characterization of amorpha-4, 11- diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua* L. Arch. Biochem. Biophys. 381, 173–180.

Nowak, D.M., Lansbury, P.T., 1998. Synthesis of (+)-artemisinin and (+)-deoxyartemisinin from arteannuin B and arteannuic acid. Tetrahedron 54, 319–336.

Ravindranathan, T., Kumar, M.A., Menon, R.B., Hiremath, S.V., 1990. Stereoselective synthesis of artemisinin. Tetrahedron Lett. 31, 755–758.

Reiling, K.K., Renninger, N.S., McPhee, D.J., Fisher, K.J., Ockey, D.A., 2006. Conversion of amorpha-4,11-diene to artemisinin and artemsisinin precursors. PCT Application Patent No. WO 2006/128126.

Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., Chang, M.C., Withers, S.T., Shiba, Y., Sarpong, R., Keasling, J.D., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440, 940–943.

Roth, R.J., Acton, N., 1989. A simple conversion of artemisinic acid into artemisinin. J. Nat. Prod. 52, 1183–1185.

Schmid, G., Hofheinz, W., 1983. Total synthesis of qinghaosu. J. Am. Chem. Soc. 105, 624–625.

Sy, L.K., Brown, G.D., 2002. The mechanism of the spontaneous autoxidation of dihydroartemisinic acid. Tetrahedron 58, 897–908.

Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G., Covello, P.S., 2006. *Artemisia annua* L. (*Asteraceae*) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. FEBS Lett. 580, 1411–1416.

Teoh, K.T., Reed, D.W., Polichuk, D.R., Covello, P.S., 2007. Nucleotide sequences encoding enzymes in biosynthesis of dihydroartemisinic acid. Patent Application No. PCT/CA2007/000614.

Towler, M.J., Wyslouzil, B.E., Weathers, P.J., 2007. Using an aerosol deposition model to increase hairy root growth in a mist reactor. Biotechnol. Bioeng. 96, 881–891.

Wallaart, T.E., Pras, N., Beekman, A.C., Quax, W.J., 2000. Seasonal variation of artemisinin and its biosynthetic precursors in plants of *Artemisia annua* of different geographical origin: proof for the existence of chemotypes. Planta. Med. 66, 57–62.

Wallaart, T.E., Bouwmeester, H.J., Hille, J., Poppinga, L., Maijers, N.C., 2001. Amorpha-4, 11-diene synthase: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin. Planta 212, 460–465.

Wasserman, H.H., Ives, J.L., 1981. Singlet oxygen in organic synthesis. Tetrahedron 37, 1825–1852.

Webster, H.K., Lehnert, E.K., 1994. Chemistry of artemisinin: an overview. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1), S27–S29.

Weina, P.J., 2008. Artemisinins from folklore to modern medicine–transforming an herbal extract to life-saving drugs. Parassitologia 50, 25–29.

Willcox, M., Bodeker, G., Bourdy, G., Dhingra, V., Falquet, J., Ferreira, J.F.S., Graz, B., Hirt, H.-M., Hsu, E., Melillo de Magalhaes, P., Provendier, D., Wright, C.W., 2004. *Artemisia annua* as a traditional herbal antimalarial. In: Willcox, M., Bodeker, G., Rasoanaivo, P. (Eds.), Traditional Medicinal Plants and Malaria. CRC Press, Boca Raton, pp. 43–59.

Wu, S., Schalk, M., Clark, A., Miles, R.B., Coates, R., Chappell, J., 2006. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nat. Biotechnol. 24, 1441–1447.

Xu, X.-X., Zhu, J., Huang, D.-Z., Zhou, W.-S., 1986. Total synthesis of arteannuin and deoxyarteannuin. Tetrahedron 42, 819–828.

Yadav, J.S., Babu, R.S., Sabitha, G., 2003. Stereoselective total synthesis of (+)-artemisinin. Tetrahedron Lett. 44, 387–389.

Zhang, Y., Teoh, K.H., Reed, D.W., Maes, L., Goossens, A., Olson, D.J., Ross, A.R., Covello, P.S., 2008. The molecular cloning of artemisinic aldehyde Delta11(13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. J. Biol. Chem. 283, 21501–21508.