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Artemisinin, a Novel Antimalarial Drug: Biochemical and Molecular Approaches for Enhanced Production

Review

Abstract

Artemisinin, a sesquiterpene lactone containing an endoperoxide bridge, has been isolated from the aerial parts of *Artemisia annua* L. plants. It is effective against both drug-resistant and cerebral malaria-causing strains of *Plasmodium falciparum*. The relatively low yield (0.01 – 0.8 %) of artemisinin in *A. annua* is a serious limitation to the commercialization of the drug. Therefore, the enhanced production of artemisinin either in cell/tissue culture or in the whole plant of *A. annua* is highly desirable. It can be achieved by a better understanding of the biochemical pathway leading to the synthesis of artemisinin and its regulation by both exogenous and endogenous factors. Furthermore, genetic engineering tools can be employed to overexpress gene(s) coding for enzyme(s) associated with the rate limiting step(s) of artemisinin biosynthesis or to inhibit the enzyme(s) of other pathway competing for its precursors. These aspects which may be employed to enhance the yield of artemisinin both *in vitro* and *in vivo* are discussed in this review.

Key words
Artemisinin · *Artemisia annua* · Asteraceae · tissue culture · biotransformation · antimalarial drug

Introduction

Each year, 100 million people are infected with malaria and over 1 million people die [1]. The parasite responsible for the vast majority of fatal malaria infections is *Plasmodium falciparum*, which can kill patients in a matter of hours.

The first effective antimalarial drug was quinine, which was isolated from the bark of *Cinchona*. Since then, malaria has been treated with quinoline-based drugs such as chloroquine, quinine, mefloquine and primaquine, and with antifolates such as Fansidar (Sulphadoxinepyrimethamine). Unfortunately, many *P. falciparum* strains have now become resistant to chloroquine and some, such as those in South-East Asia, have also developed resistance to mefloquine and halofantrine [2]. Hence, with the problem of resistance on the one hand and multiple side effects on the other, it becomes inevitable to look for an alternative drug that would cure the deadly disease. Artemisinin is showing very strong potential as a non-conventional antimalarial drug. It is a sesquiterpene lactone with an endoperoxide bridge and has been isolated from *Artemisia annua* L., a plant belonging to the family Asteraceae. It is an annual herb native to China and known as Quinghao (*A. annua*) with a long history of use. This plant has now become naturalized in many countries including Argentina, Bulgaria, France, Hungary, Romania, Italy, Spain, Yugoslavia and India [3], [4], [5]. Phenotypically the plant is 50 – 150 cm tall and may appear as small, prostrate to tall erect specimens with a woody stem. The maximum attained height is 2 m (studies at Hamdard University, New Delhi, 1993 – 2000). The earliest report on the use of the extract of Quinghao (*A. annua*) was in the preparation of a cure for 52 kinds of “diseases” that was found in the Mawanhgolui Han dynasty Tombs dating to 168 BC, which recommended its use for hemorrhoids. The use of the extract for fever including malaria was first reported in Zhon Hon Bei ji Jang (Handbook of Prescriptions for Emergency Treatments), written in 340 AD by Ge Hong [6].

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Artemisinin (Fig. **1**) was isolated in pure form in 1972, and its structure was determined in 1979 [7]. Artemisinin and its derivatives (artemether, arteether and sodium artesunate) are effective against both chloroquine-resistant and cerebral malaria. The concentration of artemisinin in *A. annua* is very low, in the range of 0.01 – 0.8 % [8]. Its chemical synthesis is possible [9], [10], [11], [12], but is complicated and economically unviable due to very poor yields [13]. The relatively low yield of artemisinin in the *A. annua* is a serious limitation to the commercialization of the drug [14]. Therefore, the enhanced production of artemisinin either in cell/tissue culture or in the whole plant of *A. annua* is the long cherished dream of many research groups. This can only be achieved by a better understanding of the biochemical pathway leading to the synthesis of artemisinin and its regulation by both exogenous and endogenous factors. Furthermore, genetic engineering tools can be employed to overexpress gene(s) coding for enzyme(s) associated with the rate limiting step(s) of artemisinin biosynthesis or to inhibit the enzyme(s) of other pathway(s) competing for its precursors.

Approaches to Enhance Artemisinin Content

Phytochemical and physiological approach

A. annua appears to be the only *Artemisia* species that contain appreciable amounts of artemisinin. Chinese scientists have reported that extracts from 30 other different species of *Artemisia* did not show antimalarial activity [15]. American scientists have failed to detect artemisinin in various species endemic to America [16]. In India, Balachandran et al. [17] also did not find artemisinin in various *Artemisia* species of Indian origin.

Artemisinin was first isolated from the aerial parts of *A. annua* by Chinese scientists and later characterized by others [7], [16]. But, the details of isolation procedure were not published for long time [4]. Researchers at the Walter Reed Army Institute of Research, USA spotted some *A. annua* growing in the neighborhood of Washington D.C. and extracted its various air-dried parts with a number of apolar organic solvents. The petroleum ether extraction proved most satisfactory for the isolation of artemisinin and its derivatives [4].

Artemisinin has been reported to accumulate in leaves, small green stems, buds, flowers and seeds [18], [19], [20], [21]. Its content was found more in leaves and inflorescence, but neither artemisinin nor its precursors were detected in roots [6], [22]. Duke et al. [23] showed that artemisinin is sequestered in the glandular trichomes of *A. annua.* Artemisinin contents in full-bloom flowers were 4 – 5 times higher than in leaves [19]. The artemisinin yield estimated at different steps of development reveals a possible correlation between plant age and artemisinin content. This is assumed to be due to both an increase in leaf yield and artemisinin content with the progressive increase in plant growth [24]. Our own observations have revealed that the artemisinin content was highest at the full vegetative stage. Some researchers reported that artemisinin content is highest just prior to flowering [20], [25], [26], [27], [28], others found an artemisinin peak at full flowering stage [29], [30], [31].

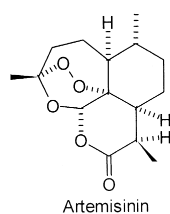

Fig. **1** Structure of artemisinin.

Artemisinin yields reported from plants in China range from 0.01 to 0.5 % (w/w), varieties growing in Siachuan Province showing the highest content. Klayman et al. [16] reported a 0.06 % (w/w) yield from *A. annua* growing wild in Washington D.C. Other reports claim the yield to be 0.09 – 0.17 % [20], [31], [32]. The yields of the related sesquiterpenes, i. e., artemisinic acid and arteannuin B also show variation in their contents. In USA, the artemisinic acid content is 8 – 10 times more than the artemisinin content [33], [34] followed by arteannuin B [5]. In India the yield of arteannuin B (0.27 %) is relatively higher than those of the other two sesquiterpenes [31], [35].

A study on effect of levels of nitrogen (0, 50, and 100 kg ha^{-1}), phosphorus (0 and 50 kg ha^{-1}) and potassium (0 and 50 kg ha^{-1}) on growth, oil and artemisinin yield revealed that application of 50 and 100 kg N/ha increased herbage, oil and artemisinin yield by 26.2 and 40.1 %, respectively, compared with control (no nitrogen) [36]. A study of the influence of micro-nutrient imbalance on growth and artemisinin contents shows that *A. annua* is very sensitive to boron [B] deficiency. Boron-deficient plants did not show flowering and there was approximately 50 % reduction in artemisinin content. Similarly, artemisinin content declined by 25 – 30 % in Fe-, Mn-, Zn- and Cu-deficient plants [37]. The effect of plant growth regulators on yield, oil composition and artemisinin content of *A. annua* under temperate conditions was studied in 1998 by Yaseen [38]. Foliar application of indole-3-acetic acid (IAA) at 100 ppm produced significantly higher herb and oil yields than the control, due to increases in plant height, leaf/stem ratio and delayed leaf senescence. Although the Artemisia ketone in the oil was highest following application of gibberillic acid (GA_3) and IAA at 150 ppm, and the artemisinin content was higher in the plants treated with 6 ppm triacontanol. The effect of bio-regulators, chlormequat and triacontanol, was studied for artemisinin content, growth parameters and leaf yield. Plants treated with chlormequat were found to have more herbage yield, but the effect of higher doses was not statistically significant. The level of abscisic acid (ABA) in chlormequat-treated plants was higher than in control plants, whereas treatment with triacontanol lowered the abscisic acid level [39]. In contrast, application of triacontanol increased the level of endogenous GA_3-like components, while chlormequat caused a reduction in their concentration [40]. According to Liersch et al. [20] chlormequat was able to increase the artemisinin content by 30 %. Local climatic conditions, the time of planting and season of harvesting as well as the post-harvest handling seem to play an important role in the yield of essential oils and artemisinin content [19], [21], [31], [41], [42]. Moreover, environmental stress such as light, temperature, water and salt significantly alter artemisinin yields [43], [44].

Genetic studies on *A. annua* have confirmed that the diploid plants are 2n = 18 [45]. The average artemisinin level in tetra-

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ploids was 38% higher than that of the wild type (diploid) as measured over the whole vegetation period [46]. A hybrid form of *A. annua* containing 0.63 – 0.7% aremisinin was successfully cultivated in Central Africa [47].

Synthetic Approach

It is a major challenge for organic chemists to develop a process for the total synthesis of artemisinin due to its complex structure. Various attempts for the chemical synthesis of artemisinin have been reported. Schmid and Hofheinz [11] converted (–)-isopulegol to artemisinin in 13 synthesis steps with an overall yield of 5% (Fig. **2**). Xing-Xiang Xu et al. [12] reported a scheme for artemisinin synthesis, where the starting material was (+)-citronellal (Fig. **3**). Avery et al. [9] used a 10-step synthetic route with *R*-(+)-pulegone as the starting compound, which involves the ozonolysis of a vinylsilane (Fig. **4**). These syntheses are very complex and not feasible for the large-scale production of artemisinin. Ravindranathan et al. [13] described a stereoselective synthesis of artemisinin from (+)-isolimonene. Lin et al. [48] gave another, different synthesis approach, starting from the (–)-*β*-pinene using an intramolecular Diels-Alder approach. It overcomes the disadvantages of complex organic synthetic reactions in the preparation of artemisinin from its closely related biosynthetic precursors. Artemisinin could be obtained from artemisinic acid, which is 8 – 10 times more abundant, in an overall yield of about 40% [49], [50]. The photo-oxidation of artemisinic acid has been studied by several groups [12], [51] in the search for a route for artemisinin synthesis from this relatively abundant constituent of *A. annua*. Jung et al. [52] proposed the synthesis of deoxoartemisinin from artemisinic acid (Fig. **5**). Roth and Acton [53] proposed the conversion of artemisinic acid to artemisinin with a 30% yield and a 2-step synthesis from dihydroartemisinic acid (Fig. **6**) [54]. Lansbury and Nowak [55] converted artemisinic acid and arteannuin B into a precursor leading

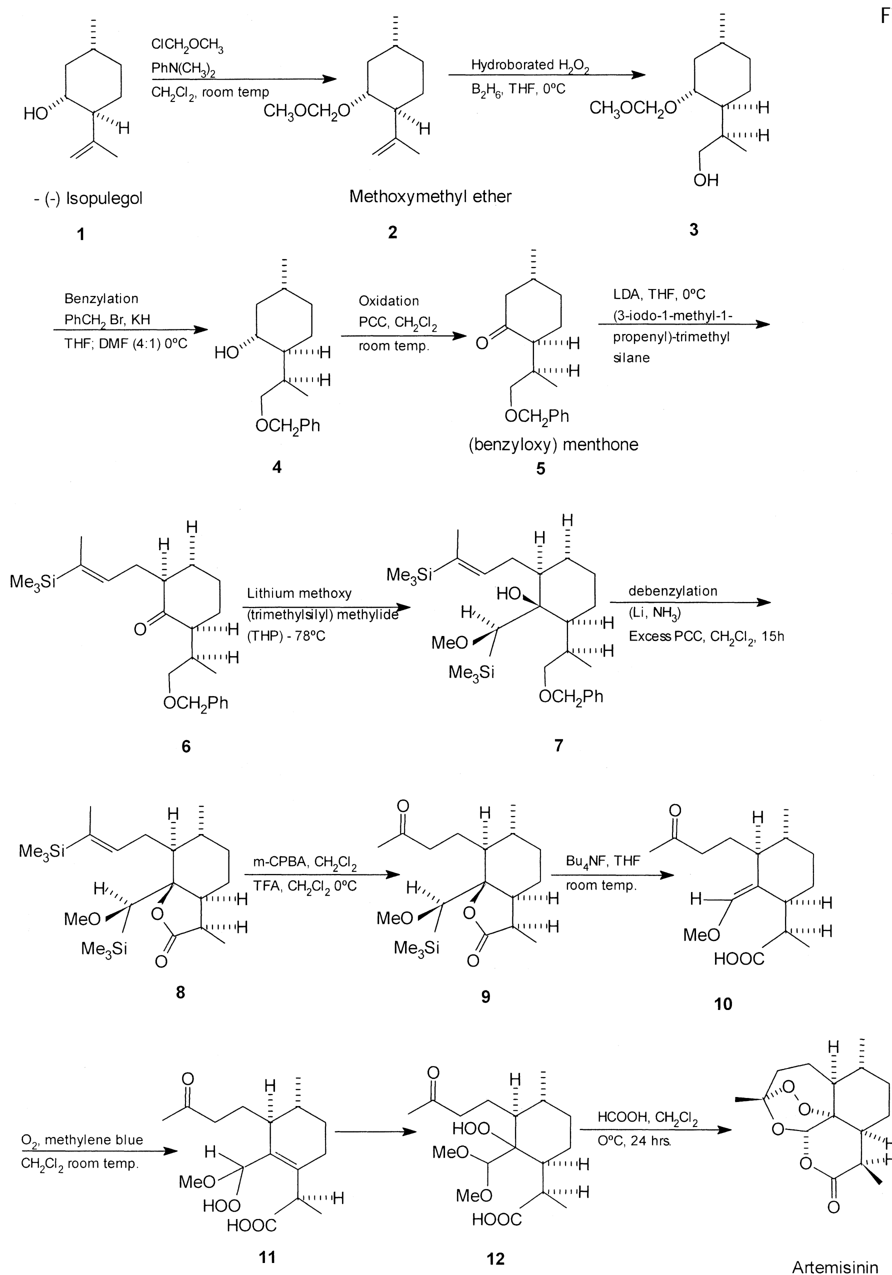

Fig. **2** Synthesis of artemisinin from (–)-isopulegol.

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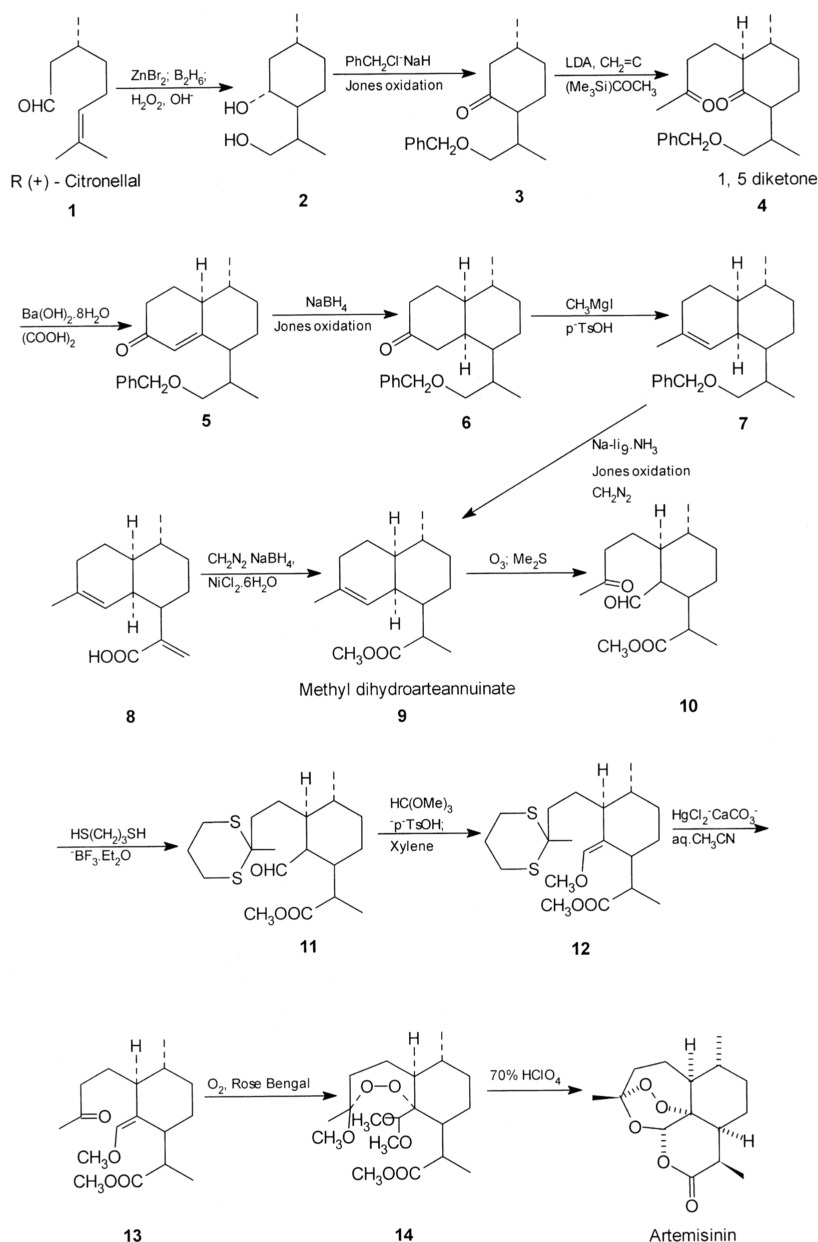

Fig. **3** Synthesis of artemisinin from *R*-(+)-citronellal.

to artemisinin in four and five reaction steps, respectively (Fig. **7**). The *de novo* synthesis of artemisinin is complex, uneconomical and gives low yields but higher yield may be achieved by using simple precursors and optimizing the reaction conditions.

Biotransformation Studies

Studies have been conducted in different laboratories to elucidate the biochemical pathway of artemisinin and its regulation with an aim to improve artemisinin content of the plants (Fig. **8**). Akhila et al. [56], [57] proposed a biosynthetic pathway for artemisinin. The following biosynthetic sequence is suggested: farnesyl pyrophosphate (FPP) $\rightarrow$ germacrane skeleton $\rightarrow$ dihydrocostunolide $\rightarrow$ cadinanolide $\rightarrow$ arteannuin B $\rightarrow$ artemisinin. But their studies did not indicate artemisinic acid as a precursor of artemisinin. In another study, Brown in 1993 [58] isolated two compounds, secocadinane and dihydroxycadinanolide, and postulated an alternative route for artemisinin biosynthesis. According to his postulates, arteannuin B gets converted into a dihydroxycadinanolide, which then undergoes Grobs fragmentation to yield an enolic form of a sesocadinane. This then undergoes enzymatic oxygenation to yield artemisitene, which is finally reduced to artemisinin. The biochemical evidences obtained so far, however, do not support this pathway [59]. Sangwan et al. [60] achieved *in vitro* and *in vivo* transformation of artemisinic acid to arteannuin B and artemisinin with an overall yield of 4.0%, suggesting that artemisinic acid is a common precursor for both arteannuin B and artemisinin. This was confirmed by several studies employing crude and semipurified cell-free extracts of leaf homogenates of *A. annua* where arteannuin B was found to be an intermediate in the bioconversion of artemisinic acid to artemisinin [51], [56], [57], [61], [62], [63]. Kim and Kim [64] reported the transformation of dihydroartemisinic acid into artemisinin by cell-free extracts from *A. annua.* The cell-free extract used in these studies, however, failed to

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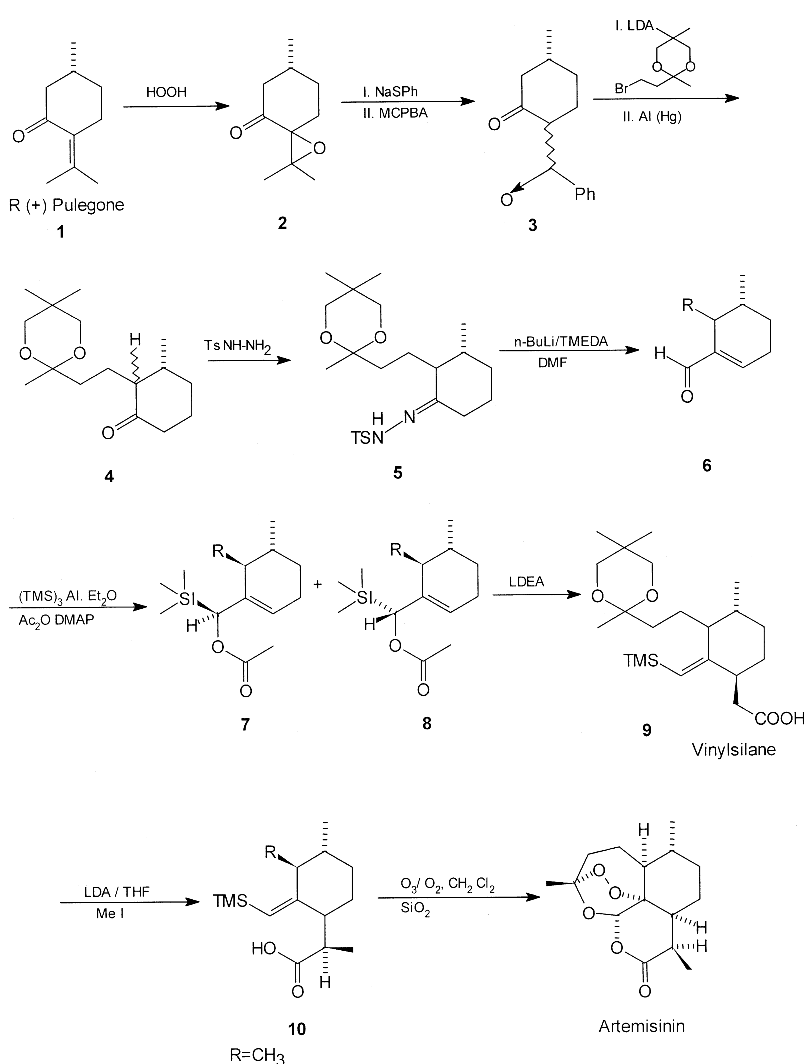

Fig. 4 Synthesis of artemisinin from *R*-(+)-pulegone.

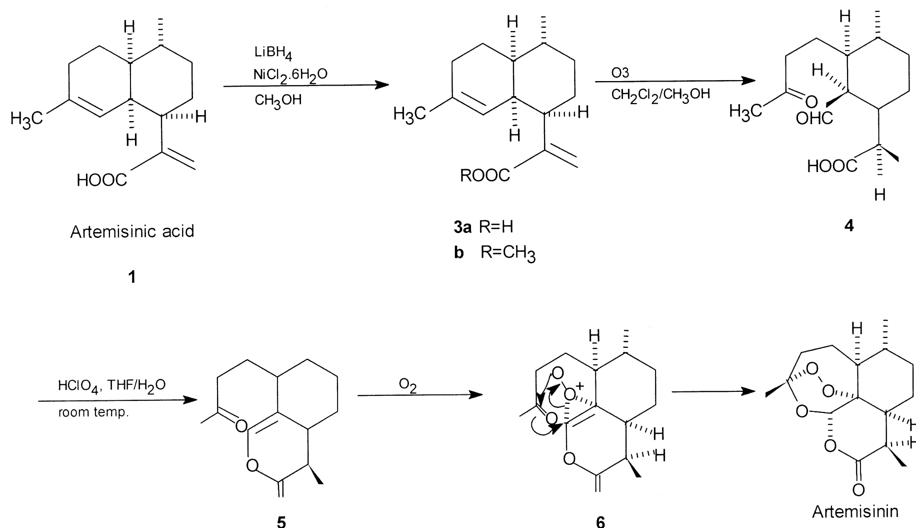

Fig. 5 Synthesis of artemisinin from artemisinic acid.

transform artemisinic acid into dihydroartemisinic acid, both of which were found in crude plant extracts. Wallaart et al. has isolated dihydroartemisinic acid from *A. annua* and photochemically converted it into artemisinin with dihydroartemisinic acid hydroperoxide as an intermediate with an overall yield of 27% [65], [66]. These studies thus indicate that artemisinic acid is biotransformed into artemisinin either via arteannuin B or dihydroartemisinic acid. The biotransformation studies conducted in

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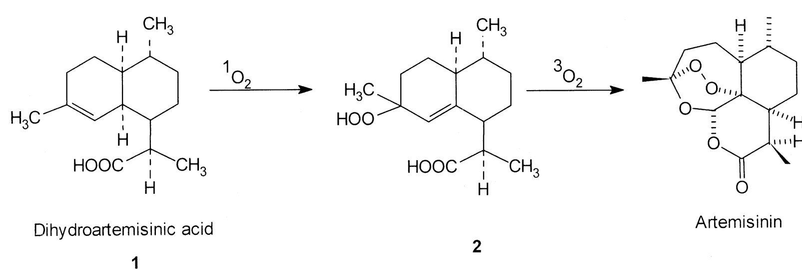

Fig. 6 Synthesis of artemisinin from dihydroartemisinic acid.

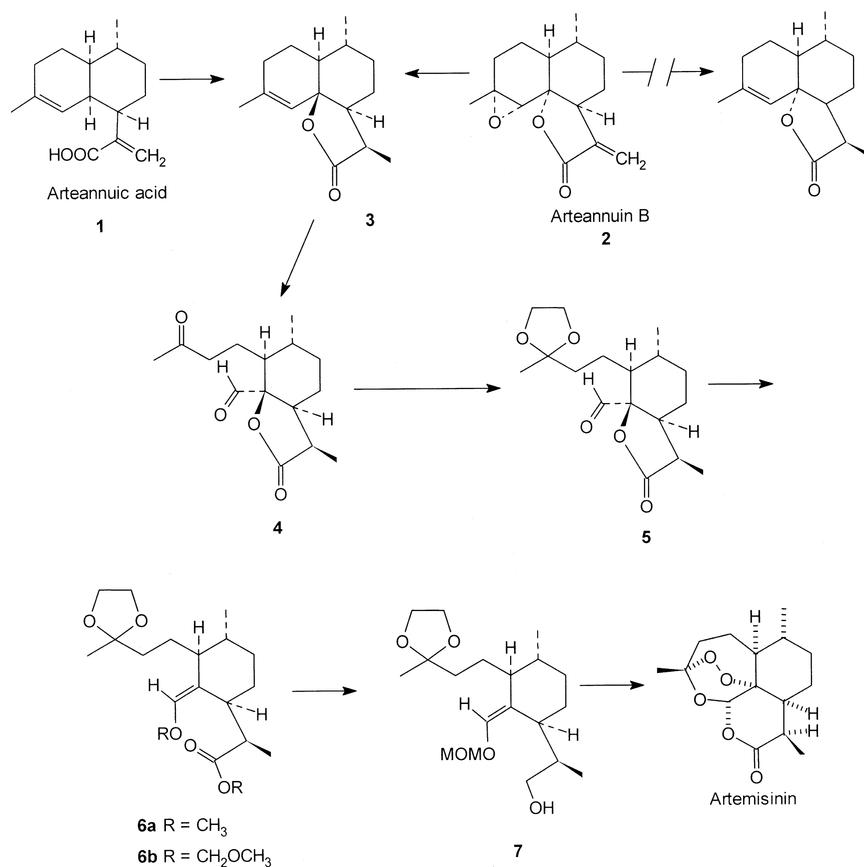

Fig. 7 Synthesis of artemisinin from arteannuic acid and arteannuin B.

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our laboratory with a new substrate, dihydroarteannuin B, suggest that, before being converted into artemisinin, the arteannuin B is converted into this compound, which is then rapidly converted into artemisinin. Consequently, it does not exist *in vivo* and *in vitro*. It has also been found that HEPES is a better buffer for bioconversion studies as it has higher buffering capacity and is less prone to variations in pH with change in temperature. Furthermore, the cell-free extracts developed by us yielded 7.2% artemisinin with artemisinic acid, and 6.5% with arteannuin B as substrates [67]. These values are much higher than those obtained by Wang et al. [61], [68] and Sangwan et al. [60] using artemisinic acid as substrate.

Recently we have partially purified and characterized the enzyme(s) involved in artemisinin biosynthesis. Two proteins of 12 and 14.5 kDa have been identified using SDS-PAGE from the 75 – 100% $(NH_4)_2$ SO_4 saturated protein fraction with very high biotransformation activity. It is believed that these proteins may be involved in the bioconversion of artemisinic acid and arteannuin B into artemisinin [69].

Currently the biosynthetic pathways postulated by various groups are contradictory. The biochemical reactions and enzymes involved after FPP synthesis have not been fully understood and well characterized as yet. Therefore, further studies are required to understand the whole pathway and its regulation by both endogenous and exogenous factors in order to optimize artemisinin content in plant.

Biotechnological Approach

Cell and tissue culture

In view of low concentrations of artemisinin detected in the plants, tissue culture systems have also been used with great interest as alternative methods for the production of this drug. However, the results so far are not very encouraging. The artemisinin content that has been detected so far in shoot cultures of *A. annua* is lower than that in intact plants. The first trial of using a tissue culture technique for artemisinin production came from He et al. [70]. The yield was 0.008% in shoots from the callus of

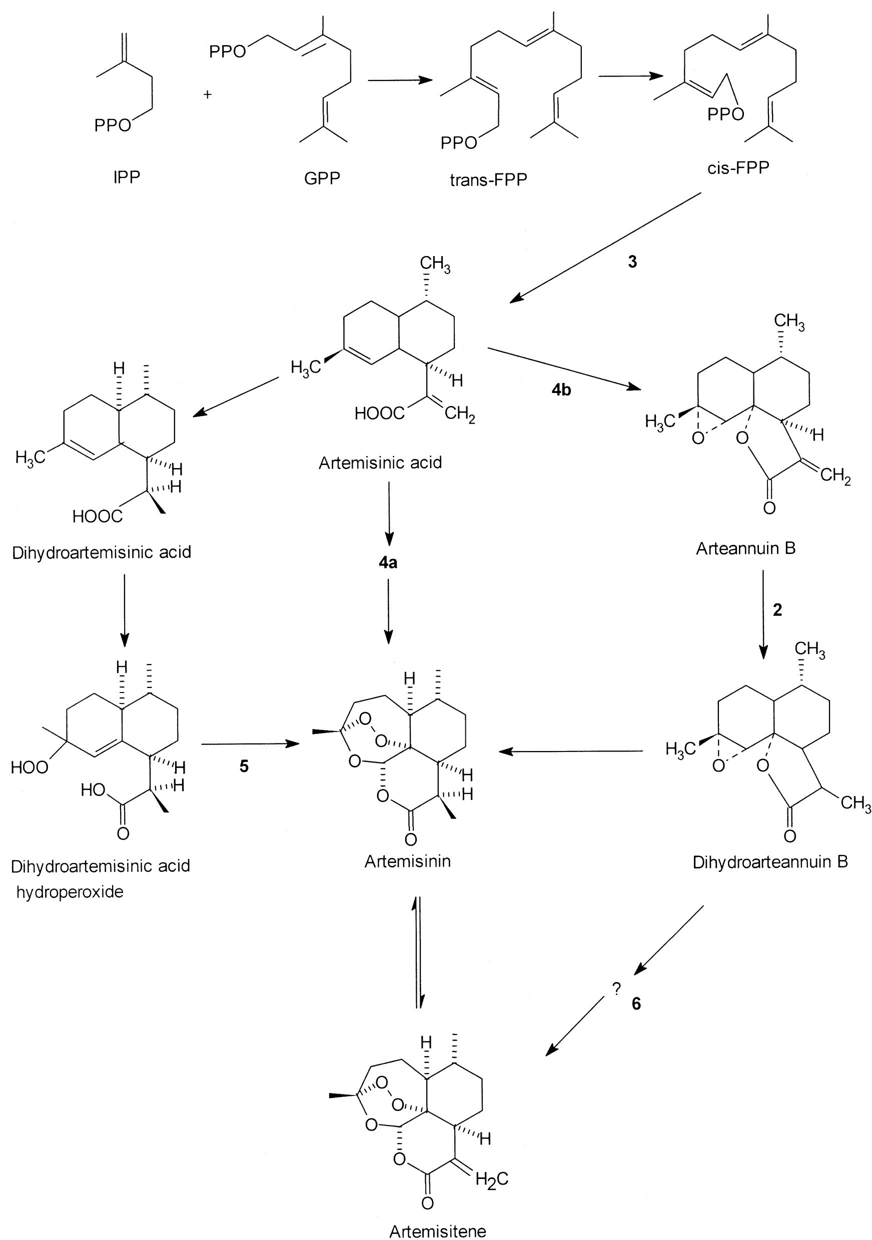

Fig. **8** Proposed biosynthetic pathway for artemisinin.

A. annua plants of Chinese origin. Martinez and Staba [21] did extensive monitoring with shoot cultures and were able to isolate high (0.3 mg/g DW) and low (0.03 mg/g DW) artemisinin synthesizing lines. On the other hand, Simon et al. [71] reported concentrations ranging from 0.03% to 0.05% on a dry weight (DW) basis. The presence of artemisinin in roots, unrooted shoots and callus was shown by Jha et al. [72]. They also induced shoot regeneration from callus that could be transplanted to soil. Yields as high as 0.4 to 0.7 mg/g DW have been reported by Whipkey et al. [73]. Results from experiments with undifferentiated callus and cell suspension cultures of *A. annua* are disappointing with respect to artemisinin production [21], [27], [62], [69], [71], [74]. We have obtained *in vitro* flowering from various explants by supplementing the medium with GA_3, where artemisinin content reached up to 0.1% [75]. There have been contradictory reports indicating both the presence and absence of trace amounts of artemisinin in callus and cell cultures. No artemisinin or related sesquiterpenes could be found in callus cultures initiated from the leaves of selected high artemisinin producing clones [76]. No artemisinin was detected in suspension grown cells and in the spent medium. When root initiation occurred from shoot cultures, active synthesis of arteannuin B and artemisinin was observed, suggesting that a certain degree of differentiation is required for the production of artemisinin [21], [58], [77]. The reason for obtaining only trace amounts of artemisinin in cell cultures has been attributed to high peroxidase activity in undifferentiated cultures [78]. Paniego and Giuletti [79] reported the establishment of dedifferentiated and differentiated cultures of *A. annua*. In the primary callus, 1.13 and 0.78 mg artemisinin/g DW were obtained. They failed to detect artemisinin in cell suspension cultures and only trace amounts were found in the multiple shoot cultures. The shoot cultures showed better growth and produced more artemisinin on 2% sucrose [78]. Kumar and Narasu [80] reported that substitution of sucrose with other carbohydrates or combination of sucrose and other sugars resulted in differences in growth and artemisinin production. Best growth

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could be observed in the medium supplemented with sucrose or a combination of sucrose and maltose. Least growth was observed when lactose was used instead of sucrose. Use of maltose alone as the carbohydrate source resulted in lower biomass production [81]. Woerdenbag et al. [28] reported a high percentage of artemisinin content (0.16% of dry weight) from shoot cultures using MS medium supplemented with 0.05 mg /L NAA, 0.2 mg/L BAP and 1% sucrose. Most workers [21], [64], [74], [75], [82] did not detect artemisinin in roots. Roots do not contain artemisinin, but evidently enhance its production in cultured shoots. Removal of roots from shoots cultured in hormone free liquid medium reduced artemisinin content in shoot by 53% [82].

Attempts were also made to improve the artemisinin production by omittance or addition of medium components like plant growth regulators, casein hydrolysate, by inclusion of precursors of artemisinin biosynthetic pathway and elicitors and by addition of sterol synthesis inhibitors as well as mutagens. Casein hydrolysate, a source of amino acids and oligopeptides, in low concentration enhances the artemisinin production, but prolonged exposure to it negatively affects the biomass production and growth of shoots. Kumar [81] reported that incorporation of boron, casein hydrolysate and gibberillic acid enhances artemisinin production by 25%, 36% and 65%, respectively. No effect on growth was however, observed. Fulzele et al. [83] reported the stimulation of terpenoid synthesis in plantlet cultures from *A. annua* by addition of GA_3. However, other scientists found only a slight effect on biomass production of plants treated with 40 and 80 mg/L GA_3 [21], [83], [84]. Smith et al. [85] reported that GA_3 (0.01 mg/L) increased the growth rate of hairy roots of *A. annua* by 25% with a slight increase in artemisinin levels as compared to control. A combination of benzylaminopurine (BAP, 1 mg/L) and kinetin (KN, 10 mg/L) increased the yields of artemisinin *in vitro* by 3.6- and 2.6-fold, respectively due to increase in dry matter production which can overcome a concurrent decrease in the artemisinin content [73].

Improvement of artemisinin production by precursor feedings has also been tested. Addition of artemisinin precursors to the medium used for tissue cultures of *A. annua* resulted in a fourfold increase of artemisinin in the tissue and an eleven-fold increase of artemisinin in the spent medium [43]. The feeding of mevalonic acid alone, however, did not induce an enhancement of artemisinin production [28].

Attempts were made to enhance the artemisinin production by modulating the biosynthesis route, i.e., by addition of sterol synthesis inhibitors and mutagenic compounds. Addition of other compounds such as naphtiphine (inhibitor of the enzyme squalene epoxidase) to the medium improved the artemisinin production. Other additions such as 5-azacytidine (a gene regulator), colchicine (a gene regulator), miconazole (inhibitor of sterol desmethylase), terbinaphine (inhibitor of the enzyme squaline epoxidase), were too toxic for the cultures and did not induce an enhancement of the artemisinin production [28]. Kudakasseril et al. [62], however, reported a concentration-dependent increase in the levels of artemisinin and growth of shoot cultures with miconazole. Other sterol inhibitors such as AMO-1618 [2-isopropyl-4-(trimethylammonium chloride)-5-methylphenylpiperidinecarboxylate], CCC (chlorocholine chloride), and MER-29 [4-chloro-2-(2-diethylaminoethoxyphenyl)-2-(4-methylphenyl)-benzene-ethanol] increased both the incorporation of ^{14}C-IPP into artemisinin by cell-free extracts and the production of artemisinin in shoot culture of *A. annua* [62].

Several attempts have been made for selection and breeding of high-yielding strains of *A. annua*. Artemisinin, which is present mainly in the leaves, varies in concentrations from 0.01% to 0.5% (based on dry weight) in China. A clone, however, has been found to contain up to 1.1% artemisinin [86]. In USA, strains of *A. annua* have been detected with a mean artemisinin concentration ranging from 0.05% to 0.42% at full flowering stage [19]. As *A. annua* is a strongly out-crossing species, hybridization has been made between the Chinese clone and pollen obtained from the strains of Italian, Yugoslavian and Spanish origins. These hybrids produced on average 0.64, 0.73 and 0.95% artemisinin, respectively [86]. Pras et al. [30] observed that plants that are high yielding in the laboratory continue to remain so in the field. The high artemisinin producing clones are characterized as tall with long internodes, open branching, dense leaves and thick stems [76]. The superior clones can be later intercrossed under short days to achieve genetic gain, while tissue culture may be used to store and preserve clones.

Genetic engineering of *A. annua*

The breakthrough in production of artemisinin by means of cell or tissue culture has not been achieved as yet. It would, therefore, be interesting to produce transgenic plants of *A. annua* which ensure a constant high production of artemisinin by over-expressing the enzyme(s) of the terpene biosynthetic pathway or by inhibiting an enzyme(s) of another pathway competing for its precursor.

Attempts to transform the *A. annua* plants using *Agrobacterium rhizogenes* [43], [87], [88], [89], resulted in transformed hairy roots, but no transgenic plants. Vergauwe et al. [90] has developed an *A. tumefaciens*-mediated transformation system for *A. annua* L. plants with high transformation rates (75% regenerants harboring foreign gene). Artemisinin content in leaves of regenerated plant was 0.17% DW, a little higher than that present in leaves of normal *in vitro* grown plants (0.11% DW). Ghosh et al. [91] transformed organ cultures of *A. annua* with two wild-type nopaline strains of *A. tumefaciens*. The artemisinin content in shoot teratomas was 0.083 ± 0.002 g per100 g DW. Non-transformed shoots, however, were found to synthesize only 0.0179 ± 0.002 g per 100 g DW of the shoot. In 1998, Chen et al. [92] developed an efficient transformation system. They transformed leaf explants of *A. annua* using *A. rhizogenes* with a cotton cadinene synthase cDNA, cad-14. In another study, seven hairy root lines with the properties of fast growth and high artemisinin contents were selected from 747 hairy roots induced by transformation of *A. annua* L. with *A. rhizogenes* ATCC 15834. The difference of growth rates and artemisinin contents among the seven selected hairy root lines were extremely significant. Among these lines, HR-9 gave the highest yield of artemisinin [93]. Chen et al. [94] expressed a chimeric farnesyl diphosphate synthase gene in *A. annua*. The resulting transformed plants have 2 – 3 times higher artemisinin contents than that of the control.

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The effect of isopentenyl transferase (IPT) gene expression on the physiological and biochemical characteristics of *A. annua* L. was studied by Sa et al. [95]. The content of cytokinin was elevated 2- to 3-fold, chlorophyll increased 20 – 60% and artemisinin increased 30 – 70%, respectively when compared with the control plants.

Conclusions

Artemisinin and its derivatives are effective against both drug-resistant and -sensitive strains of *Plasmodium* including *P. falciparum*, the causal organism of cerebral malaria. Furthermore, it has no side effect and is safe in pregnancy, but high doses of artemisinin derivatives have been found to be neurotoxic in experimental animals. Unfortunately, the relatively low yields of artemisinin in *A. annua* and non-availability of economically viable synthetic protocols are the major hurdles for its commercial production and clinical use. Efforts, therefore, are being made to enhance the production of artemisinin both *in vivo* and *in vitro*. Breeding of high artemisinin yielding plants as well as the manipulation of culture conditions, growth media, and hormone levels to increase the yield of artemisinin in tissue and cell culture have not been successful. It is, therefore, essential to look for non-conventional, alternate strategies, which are economically viable for the commercial production of artemisinin. Two approaches can be used to achieve this goal. The first approach could be the use of a semisynthetic route for the synthesis of artemisinin from its simple precursors such as artemisinic acid and arteannuin B. The second approach could involve the use of genetic engineering to over-express enzyme(s) catalyzing the rate-limiting steps of artemisinin biosynthesis or by using anti-sense RNA technology to inhibit the enzyme(s) of other pathways competing for its precursors. These approaches are currently being used in our laboratory to enhance the production of artemisinin.

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