This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
The fungal and bacterial transformation of terpenoids derived from plant essential oils, especially the sesquiterpenoid artemisinin from *Artemisia annua*, has produced several new candidate drugs for the treatment of malaria. Obtaining new derivatives of terpenoids, including artemisinin derivatives with increased antimalarial activity, is an important goal of research in microbial biotechnology and medicinal chemistry.

1. Introduction

The majority of people in many tropical countries are at risk for parasitic diseases, such as malaria (Snow et al., 2005), which may cause the death of one to three million people annually (Klassen, 2009). Malaria parasites are protozoa in the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes; the most dangerous species for humans are *P. falciparum*, *P. vivax*, and in some cases *P. malariae* and *P. ovale* (Chaturvedi et al., 2010). Although these parasites have been treated for many years with quinine, chloroquine and several other drugs (Chaturvedi et al., 2010; Vroman et al., 1999), multiple drug resistance is now commonly found in *P. falciparum* and *P. vivax* (Harinasuta et al., 1965; Kain, 1995). Due to the great need in many countries, innovative and cost-effective drug development programs to deal with malaria and other parasitic diseases are urgently required.

Descriptions of the use of the herbal drug *qinghao* for the treatment of febrile diseases appeared in Chinese texts as early as 341 A.D. and in several later works (Hien and White, 1993; Luo and Shen, 1987). The active component of *qinghao* for treatment of malaria was identified in China in 1972 by You-You Tu and her co-workers; it was named *qinghaosu* and shown to be a sesquiterpenoid lactone, which is now also known as artemisinin (Liao, 2009). For medicinal use and for chemical and biosynthetic manipulations, the most affordable source of artemisinin is the cultivated Asian plant *Artemisia annua* (wormwood) of the Asteraceae, although other species of *Artemisia* produce lesser amounts (Mannan et al., 2010).

Terpenoids are components of the essential oils of plants; they are derivatives of terpene hydrocarbons, which are combinations of five-
carbon isoprene units (Dewick, 2001; Newman, 1972). They have been classified into hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids, and polyterpenoids. Several of these groups of terpenoids are found in the essential oils of plants that are used in traditional medicine to treat malaria and other fevers (Kaur et al., 2009; Khamsan et al., 2011; Tikanji et al., 2008). The large variety of terpenoids, mostly derived from plants, that have been purified and shown to have antimalarial activity in vitro has been described extensively in recent review articles (Batista et al., 2009; Bero et al., 2009; Chaturvedi, 2011; Kaur et al., 2009; Nogueira and Lopes, 2011). Information about the comparative activities of most of these natural terpenoids and their derivatives in different Plasmodium spp., however, is difficult to obtain because of data security practices for potential commercial drugs.

Among the sesquiterpenoids, artemisinin and its derivatives are useful and effective drugs against most chloroquine-resistant strains of P. falciparum (Klayman, 1985). However, problems associated with artemisinin, including low solubility in water and even in oil (Vroman et al., 1999), have prompted scientists to seek new artemisinin derivatives. Some of these artemisinin-derived drugs have been reported to be neurotoxic to animals when injected (Gordi and Lepist, 2004; Medhi et al., 2009). Increasing resistance of malaria parasites to currently used drugs, including P. vivax resistance to chloroquine and primaquine in parts of New Guinea, Asia, and Africa (Price et al., 2011) and P. falciparum resistance to artemisinin in western Cambodia, eastern Thailand, and some nearby areas (Dondorp et al., 2010; Noedl et al., 2008; O’Brien et al., 2011; Wongsrichanalai and Meshnick, 2008), is another important reason for developing new antimalarial drugs. Some artemisinin analogs may be obtained by semisynthetic processes; for example, artemisinin can be easily reduced chemically to the more effective, but neurotoxic, dihydroartemisinin (Avery et al., 2002; Klayman, 1985; Vroman et al., 1999). Other structural changes in artemisinin remain a challenge for chemists because of the difficulty of introducing specific functional groups by conventional synthetic methods.

Many microorganisms, especially certain fungi, have the ability to transform terpenoids regioselectively and stereoselectively (Carvalho and Fonseca, 2006; Simóe and Sinisterra, 2009; Sutherland, 2004). We have attempted in this review to outline some of the great variety of modifications that can be expected from the use of microorganisms for the transformation of antimalarial terpenoids, but no attempt has been made to be exhaustive. The biochemical mechanisms have scarcely been investigated, but it seems likely that cytochromes P450 and perhaps dioxxygenases will be found to be involved in many of the transformations (Klings et al., 2009; Martin et al., 2008). It is our hope that further developments in microbial biotechnology, including the discovery of new strains with unique enzyme systems for the transformation of terpenoids, may make it possible to derive a variety of newer and more useful drugs from those now available.

2. Transformation of monoterpenoids

In the leaves of lemon grass, Cymbopogon citratus, there is an essential oil that inhibits the growth of Plasmodium berghei, a species which does not infect humans, with 86.6% of the activity of chloroquine (Tchoumboungang et al., 2005). This oil contains several monoterpenoids, with citral (geranial and neral), β-myrcene, geraniol, nerol, citronellal and limonene as the main components (Schaneberg and Khan, 2002). Limonene has been shown to have antimalarial activity because it inhibits the isoprenylation of proteins in P. falciparum (Moura et al., 2001).

Fungi of the genera Aspergillus and Penicillium may transform citral and other monoterpenoids to various products (Demyttenaere and De Pooter, 1998; Demyttenaere et al., 2000; Esmaeili and Tavassoli, 2010). For example, a Penicillium sp. transformed citral (I) in 21 days to a mixture of six different monoterpenoids with a total yield of 67.4%, including thymol (II, 21.5%), limonene (III, 3.1%), α-pinene (IV, 3.7%), geraniol (V, 6.8%), geranial (VI, 18.6%) and nerol (VII, 13.7%) (Esmaeili and Tavassoli, 2010):

In the transformation of myrcene (VIII) by the bacterium Pseudomonas aeruginosa PTCC 1074, formation of the products depended on the time of transformation. After 1.5 days the products found were dihydroartemisol (IX, 79.5%) and 2,6-dimethylcane (X, 9.3%), whereas after 3 days they were α-terpinol (XI, 7.7%) and 2,6-dimethylcane (X, 90.0%) (Esmaeili and Hashemi, 2011):

The bacterium Rhodococcus sp. GR3 regioselectively transformed geraniol (XII) to geric acid (XIII) in 12.5 h (Chatterjee, 2004):

The bacterium Rhodococcus sp. GR3 regioselectively transformed geraniol (XII) to geric acid (XIII) in 12.5 h (Chatterjee, 2004):
The yeast *Rhodotorula minuta* in only 8 h reduced L-(−)-citronellal (XIV) to L-(−)-citronellol (XV) with a yield of 78.3% (Velankar and Hebble, 2003):

![Diagram](image1)

The fungus *Fusarium verticillioides* in 12 h converted R-(+)−limonene (XVI) to R-(+)−perillyl alcohol (XVII) with a yield of 12% (Oliveira and Strapasson, 2000):

![Diagram](image2)

Of the microbial transformations of monoterpenoids, those of greatest interest are those producing hydroxylated derivatives (Abraham and Arfmann, 1992; Khor and Uzir, 2011) that can be used in the stereospecific synthesis of valuable compounds, including potential antimalarial drugs.

3. Transformation of sesquiterpenoids

Artemisinin (XVIII) is the most important antimalarial sesquiterpenoid obtained from plants (Klayman, 1985; Liao, 2009; Luo and Shen, 1987), although several others have been described (Chaturvedi et al., 1995; Elmarakby et al., 1987; Rustaiyan et al., 2011). Biotransformation of artemisinin has been aided by studies of QSAR (quantitative structure-activity relationships), which suggest modifications of artemisinin that are likely to increase antimalarial activity (Avery et al., 2002). Although many terpenoid biotransformations produce metabolites with less antimalarial activity, the products nevertheless may be useful for further modification (Liu et al., 2006). Occasionally, inactive compounds may be transformed to active metabolites by microbial processes (Musharraf et al., 2010).

The bacterium *Nocardia corallina* ATCC 19070 transformed artemisinin to deoxyartemisinin (XIX, yield 24%), which lacks antimalarial activity, in 14 days (Lee et al., 1989). Cultures of *Aspergillus flavus* in 48 h transformed artemisinin to deoxyartemisinin with a yield of 30.5% (Srivastava et al., 2009):

![Diagram](image3)

Semisynthetic derivatives of artemisinin also have interested researchers seeking possible microbiological modifications. For example, *U. ramanniana* 1839 transformed the semisynthetic antimalarial drug 10-deoxoartemisinin (XXVII) to the inactive 4α-hydroxydeoxy-10-deoxoartemisinin (XXIX, yield 7.0%) and the partially active 7β-hydroxy-10-deoxoartemisinin (XXX, yield 10.9%) in 14 days (Khalifa et al., 2009). Madeiros et al. (2002) optimized the conditions and obtained a 45% yield of XXX, which despite its lower antimalarial activity may be useful for further transformations, in 14 days. *Aspergillus niger* hydroxylated 10-deoxoartemisinin (XXVII) to 7β-hydroxy-10-deoxoartemisinin (XXX, yield 69%) and 15-hydroxy-10-

The fungus *Cunninghamella elegans* ATCC 9245 transformed artemisinin to four different hydroxylated derivatives, 7β-hydroxy-9α-artemisinin (XX, yield 6.0%), 4α-hydroxydeoxyartemisinin (XXI, yield 5.4%), 7β-hydroxyartemisinin (XXII, yield 21.0%) and 6β-hydroxyartemisinin (XXIII, yield 6.5%). The 7β-hydroxyartemisinin product (XXII), which cannot be produced chemically, is valuable for further synthesis of candidate antimalarial compounds (Parshikov et al., 2004b):

![Diagram](image4)

Penicillium chrysogenum ATCC 9480 transformed artemisinin to two inactive compounds, deoxyartemisinin (XIX, yield 1.0%) and 4α-hydroxydeoxyartemisinin (XXI, yield 3.6%) in 13 days (Lee et al., 1989). *Cunninghamella echinulata* AS 3.3400 and *Aspergillus niger* AS 3.795 in 4 days transformed artemisinin to 6β-hydroxyartemisinin (XXIII, yield 50%) and 4α-hydroxydeoxyartemisinin (XXI, yield 15%), respectively (Zhan et al., 2002a), and *Mucor polymorphus* AS 3.3443 produced 7β-hydroxyartemisinem (XXII) and two other hydroxylated products (Zhan et al., 2002b).

Three strains of *Umbelopsis ramanniana* (Mucor ramannianus) hydroxylated artemisinin in 14 days to 7β-hydroxyartemisinem (XXII, yield 51–88%), 6β-hydroxyartemisinem (XXIII, yield 1–51%), and two other isomers (Parshikov et al., 2005). *Aspergillus niger* VKM F-1119 hydroxylated artemisinin to 5β-hydroxyartemisinem (yield 80%) and 7β-hydroxyartemisinem (XXII, yield 19%) (Parshikov et al., 2006).

The bacterium *Streptomyces griseus* ATCC 13273 oxidized artemisinin to a less active ketone, artemisitone (XXIV, yield 12.5%), in 3.5 days (Liu et al., 2006). *Penicillium simplicissimum* modified artemisinin to produce 4β-acetoxy and 4α-hydroxy derivatives (Goswami et al., 2010). A few other natural sesquiterpenoids have been investigated for possible biotransformations. Arteenanin B (XXV), another terpenoid produced by *Artemisia annua*, is transformed by the fungi *Aspergillus flavipes* and *Beauveria bassiana* to three different products (Elmarakby et al., 1987). A *Microbacterium trichotoconematocytum* extract transformed arteenanin B to artemisinin (Tatineni et al., 2006). Artedifusin (XXVI), a recently discovered sesquiterpene lactone produced by *Artemisia diffusa* (Rustaiyan et al., 2011), has antimalarial activity and may also be amenable to biotransformation.

![Diagram](image5)
deoxoartemisinin (yield 26%) (Parshikov et al., 2004a). Cunninghamamella elegans ATCC 9245 transformed 10-deoxoartemisinin (XXVII) to three hydroxylated derivatives, 5β-hydroxy-10-deoxoartemisinin (XXVIII), yield 8.8%, 4α-hydroxydeoxy-10-deoxoartemisinin (XXIX, yield 4.6%) and 7β-hydroxy-10-deoxoartemisinin (XXX, yield 83.9%) (Parshikov et al., 2004c):

Cultures of Aspergillus niger ATCC 16404 regioselectively transformed the diterpenoid imbricatolic acid (XI) to 1α-hydroxyimbricatolic acid (XII) in 15 days, but Rhizopus stolonifer UBA6 transformed it instead to 15-hydroxy-8,17-epoxylabdan-19-oic acid (XLI) (Schmeda-Hirschmann et al., 2007):

The same strain of A. niger also regioselectively converted jatrophone (XLV), a known antiprotozoal compound, to 9β-hydroxyisabellione (XLVI, yield 0.65%) in 25 days (Pertino et al., 2007):

Some mulinane derivatives from the medicinal plant Azorella compacta have been shown to have antipROTOZOAL activity (Loyola et al., 2004). Mucor plumbeus IMI 116688 transformed mulin-11,13-dien-20-oic acid (XLVI) to two metabolites, 16-hydroxymulin-11,13-dien-20-oic acid (XLVII, yield 0.8%) and 7α,16-dihydroxymulin-11,13-dien-20-oic acid (XLVIII, yield 0.75%) in 15 days (Areche et al., 2008):

4. Transformation of diterpenoids

Many diterpenoids from medicinal plants have antimalarial activity (García et al., 2007; Kaur et al., 2009; Titani et al., 2008). Cultures of the fungus Cephalosporium aphidicolum CCT 2163 hydroxylate the kaurane diterpenoid ent-kaur-16-en-19-ol (XXXI) with formation of two products, ent-kauran-16b,19-diol (XXXII, yield 54%) and ent-kauran-16b,17,19-triol (XXXIII, yield 18.6%), in 13 days (Rocha et al., 2009):

Because plants containing pimarane diterpenoids, such as Kaempferia marginata, have been used as antimalarials in traditional medicine, the pimaranes have also been investigated for antimalarial activity (Thongnest et al., 2005). Although the reduction of specific carboxyl groups to alcohols is not always possible by chemical methods, the fungus Glomerella cirrata regioselectively transformed ent-pimara-8(14),15-dien-19-oic acid (XXXIV) to ent-8(14),15-pimaradien-19-ol (XXXV, yield 18.3%) in 10 days (Severiano et al., 2010), Mucor rouxii converted XXXIV to ent-pimara-7,15-dien-19-oic acid (XXXVII, yield 2.8%) and 7-keto-ent-pimara-8,15-dien-19-oic acid (XL, yield 2.1%) in 7 days (Severiano et al., 2010):

5. Transformation of triterpenoids

Several triterpenoids from plant essential oils are potential antimalarial drugs (Kaur et al., 2009). The lupanes are a group of pentacyclic triterpenoids that contain compounds with antimalarial activity (Kaur et al., 2009; Suksamarn et al., 2006). Aspergillus ochraceus converted lupeol (XLIX) to two metabolites, L (yield 19.0%) and LI (yield 11.1%) in 10 days (Carvalho et al., 2010):
Also, *M. rouxii* transformed lupeol (XLIX) to two metabolites, LII (yield 26.5%) and LIII (yield 16.0%) in 10 days (Carvalho et al., 2010):

![Chemical structure of LII](image1)

![Chemical structure of LIII](image2)

The triterpenoids betulinic acid and betulonic acid are known to have antimalarial activity (Sá et al., 2009). Several fungi have been investigated for their ability to biotransform these compounds. For instance, *Colletotrichum* sp. transformed betulinic acid (LIV) to 3-oxo-15α-hydroxylup-20(29)-en-28-oic acid (LV, yield 2.34%) (Bastos et al., 2007):

![Chemical structure of LIV](image3)

![Chemical structure of LV](image4)

Some oleanolic acids from medicinal plants have been reported to be antimalarial (Cimanga et al., 2006; Kaur et al., 2009). The fungus *Absidia glauca* CGMCC 3.67 transformed 3-oxo-oleanolic acid (LVI) to three new derivatives, 1β-hydroxy-3-oxo-olean-11-eno-28,13-lactone (LVII, yield 0.74%), 1β,11α-dihydroxy-3-oxo-olean-12-en-28-oic acid (LVIII, yield 2.3%) and 1β,11α,21β-trihydroxy-3-oxoolean-12-en-28-oic acid (LIX, yield 0.23%) (Guo et al., 2010):

![Chemical structure of LVI](image5)

![Chemical structure of LVII](image6)

![Chemical structure of LVIII](image7)

![Chemical structure of LIX](image8)

The triterpenoid ursolic acid, from the medicinal plant *Morinda lucida*, has been shown to have antimalarial activity (Cimanga et al., 2006). The soil fungus *Umbelopsis isabellina* converted ursolic acid (LX) to three metabolites, 3β-hydroxy-urs-11-eno-28,13-lactone (LXI, yield 0.69%), 3β,7β-dihydroxy-urs-11-eno-28,13-lactone (LXII, yield 0.5%) and 1β,3β-dihydroxy-urs-11-eno-28,13-lactone (LXIII, yield 0.88%) (Fu et al., 2011):

![Chemical structure of LX](image9)

![Chemical structure of LXI](image10)

![Chemical structure of LXII](image11)

![Chemical structure of LXIII](image12)

Transformation of another triterpenoid, senegenin (LXIV), by *Nocardia* sp. NRRL 5646 was accompanied by the formation of senegenic acid 28-methyl ester (LXV) (Zhang et al., 2005):

![Chemical structure of LXIV](image13)

![Chemical structure of LXV](image14)

6. Transformation of tetraterpenoids

Carotenoids, an important group of tetraterpenoids found in nearly all plants, are often biotransformed for preparation of food additives and flavorings (Uenojo and Pastore, 2010). Biotransformation of β-carotene may produce retinoids, which can be used as raw materials for drugs and cosmetics (Jang et al., 2011), and some retinoids, including retinol, have antimalarial activity (Hamzah et al., 2003). A recombinant strain of *Escherichia coli* expressing β-carotene 15,15'-monooxygenase and the mevalonate pathway transformed β-carotene (LXVI) to retinal (LXVII),

![Chemical structure of LXVI](image15)

![Chemical structure of LXVII](image16)
the antimalarial retinol (LXVIII) and retinyl acetate (LXXIX) (Jang et al., 2011):

For the biotransformation of β-carotene, over 300 strains of microorganisms (bacteria, yeasts and filamentous fungi) were tested and seven unidentified strains showed transformation activity (Uenojo and Pastore, 2010). The isoprenoid chain of β-carotene (LXVI) was cleaved with the formation of several products, including the principal product β-ionone (LXX), β-damascene (LXXI), β-damascenone (LXXII), pseudoionone (LXXIII) and probably 1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (LXXIV) (Uenojo and Pastore, 2010):

7. Concluding remarks

Currently, artemisinin derivatives appear to be the most promising sources of new terpenoid antimalarial drugs. The main route selected by most researchers for the preparation of derivatives begins with chemical reduction of the carbonyl at position 10 of artemisinin (XVIII) to produce the toxic antimalarial compound dihydroartemisinin (LXXV) (Chaturvedi, 2011; Klayman, 1985). The semisynthetic antimalarial drugs artemether (LXXVI), arteether (LXXVII), sodium artesunate (LXXVIII) and sodium artelinate (LXXIX) were developed by researchers using this process (Chaturvedi, 2011; Li et al., 1998).

Arteether (LXXVII) can be converted to several metabolites, not only by mammalian systems but also by fungi and bacteria (Vroman et al., 1999). Other chemical derivatives of artemisinin may be useful in the future for the microbial biosynthesis of new drugs with novel therapeutic properties. The combination of artemether (LXXVI) with the unrelated drug lumefantrine is one of five artemisinin-based combinations currently recommended by the World Health Organization (WHO) for treatment of malaria (O’Brien et al., 2011; Omari et al., 2004). Various laboratories now are conducting research on hybrid trioxaquine molecules that have two different modes of action (Chauhan et al., 2010), such as a drug combining the structures of artemisinin and quinine that is highly effective against P. falciparum (Walsh et al., 2007).

The mechanisms of action of artemisinin and its derivatives on malaria parasites have not been completely studied, but there is evidence that the endoperoxide group plays an important role in antimalarial activity (Fernández and Robert, 2011; Muraleedharan and Avery, 2009; Vroman et al., 1999). The endoperoxide linkage breaks down under the influence of heme iron, with formation of an oxy free radical and then a carbon free radical, which interacts with proteins of the parasite to cause its death (Chaturvedi et al., 2010).

Some of the artemisinin derivatives, especially the trioxane dimers, are selectively cytotoxic; they have been shown not only to target cancer cells by inducing apoptosis but also to prevent tumor growth by antiangiogenesis (Beekman et al., 1998; Nakase et al., 2008; Posner et al., 2006). The endoperoxide moiety required for antimalarial activity also appears to be required for cytotoxicity toward tumor cell lines (Beekman et al., 1998; Meunier and Robert, 2010). Therefore, in the development of microbial biotransformation processes for the derivatization of artemisinin, the endoperoxide group should be preserved.

Among the microbial biotransformation processes described here, the ones of greatest interest are those for the regiospecific and stereospecific hydroxylation of artemisinin and other antimalarial terpenoids because they increase solubility and provide sites for further modification (Medeiros et al., 2002; Parshikov et al., 2006). Microbial biotransformation procedures can be used to obtain terpenoid derivatives hydroxylated in almost any position, including some not obtainable by organic synthesis, such as 7β-hydroxyartemisinin (Khor and Uzir, 2011; Parshikov et al., 2004b). These metabolites may be used for further chemical or biological transformations that yield many potential candidate drugs from one compound.

Future research on antimalarial terpenoids should include studies of the biochemistry of the most useful biotransformations and of the antiplasmodial efficacy and toxicity of each of the metabolites. The compounds that are most effective against drug-resistant strains of P. falciparum or P. vivax may be produced in higher yields by the use of biotechnology. New biotransformations of terpenoids, perhaps combined with chemical derivatization, may provide ways to overcome parasite resistance to currently used antimalarial drugs.

Acknowledgments

We thank Drs. C. E. Cerniglia, B. D. Erickson, F. Rafii, and K. Sung for their kind help with reviewing this manuscript and Dr. V. V. Lashin for consultations in organic chemistry. The views presented in
References


