ANALYSIS AND PRODUCTION OF ARTEMISININ, AN ANTIMALARIAL FROM *Artemisia annua* L.

by

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MIKROFIS
5221

Specially dedicated to :

Ama, Tata,

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&

Dexter

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ANALISIS DAN PENGHASILAN ARTEMISININ, SUATU DRUG ANTIMALARIAL DARIPADA *Artemisia annua* L.

ABSTRAK

A. annua daripada populasi bijibenih heterogenus, yang diperolehi dari Viet Nam Utara, tumbuh dengan baik di dalam persekitaran tempatan, tetapi ia tidak dapat menghasilkan bijibenih bila berbunga. Melalui teknik kultur tisu, ia berjaya dicambah dengan baik. Terdapat tiga jenis plantlet, yang mempunyai kandungan artemisinin tinggi, sederhana dan rendah. Plantlet dengan kandungan artemisinin yang tinggi didapati mempunyai purata artemisinin sebanyak 0.41 % ± 0.02 SD, manakala plantlet dengan kandungan artemisinin yang sederhana, mengandungi purata sebanyak 0.25 % ± 0.02 SD dan plantlet dengan kandungan artemisinin yang rendah, mempunyai purata sebanyak 0.13 % ± 0.03 SD. Apabila ditumbuhkan, plantlet-plantlet dengan kandungan artemisinin rendah dan tinggi seterusnya akan menghasilkan pokok-pokok yang juga mempunyai kandungan purata artemisinin yang rendah dan tinggi iaitu sebanyak 0.13 % ± 0.01 SD dan 0.33 % ± 0.01 SD masing-masing.

Kaedah kromatografi cecair prestasi tinggi dengan pengesanan elektrokimia yang menggunakan elektrod karbon kaca, telah dibangunkan dan didapati peka dan selektif untuk mengesan artemisinin di dalam plasma. Nilai purata perolehan semula artemisinin daripada ekstraksi artemisinin dari julat 25-800 ng mL^{-1} didapati berada di antara 88.86-97.68 % dengan koefisi variasi di antara 1.57-9.31 % (n=6). Had pengesanan untuk artemisinin ialah 12.5 ng mL^{-1} dengan nisbah isyarat kepada bisingan sebanyak 5:1.

Artemisinin yang dihablurkan semula dengan etanol akues 50 % (ArtaqEth) dan sikloheksana (ArtCyc) mempunyai bentuk polimorf yang berbeza. Hablur ArtaqEth kelihatan padat dan tebal manakala hablur daripada ArtCyc adalah nipis dan lutsinar. Keterlarutan ArtaqEth dan ArtCyc pada 37 °C adalah 0.0177 mg mL^{-1} ± 0.0010 SD dan 0.0443 mg mL^{-1} ± 0.0012 SD masing-masing, dengan ArtCyc yang mempunyai kadar pelarutan yang lebih cepat berbanding dengan ArtaqEth. Apabila dikaji secara *in vivo* dengan sukarelawan yang sihat, didapati bahawa tahap penyerapan ArtCyc adalah 3.4 kali lebih tinggi daripada ArtaqEth. Tambahan pula, pemonitoran transit gastrousus suspensi artemisinin dengan menggunakan sulfasalazine menunjukkan tiada penyerapan berlaku di dalam kolon.

ABSTRACT

Artemisia annua derived from a heterogeneous seed population obtained from North Viet Nam, grew well in the local climate but did not produce seeds upon flowering. The plant was successfully propagated using a tissue culture technique. The artemisinin content varied among the plantlets. The plantlets were selected and cloned to give 3 major yielding lines of high, moderate and low artemisinin content. The high yielding plantlets, were found to have a mean artemisinin content of 0.41 % ± 0.02 SD, the moderate yielders, a mean artemisinin content of 0.25 % ± 0.02 SD, whereas the low yielders, a mean artemisinin content of 0.13 % ± 0.03 SD. Low and high yielding plants in the field contained a mean artemisinin content of 0.13 % ± 0.01 SD and 0.33 % ± 0.01 SD, respectively.

A high performance liquid chromatography method with electrochemical detection using a glassy carbon electrode was developed and was found to be sensitive and selective for the detection of artemisinin in plasma. The mean recovery values of artemisinin from the extraction, over a range of 25-800 ng mL^{-1} were 88.86-97.68 % with a coefficient variation of 1.57-9.31 % (n=6). The detection limit was 12.5 ng mL^{-1} for artemisinin at a signal to noise ratio of 5:1.

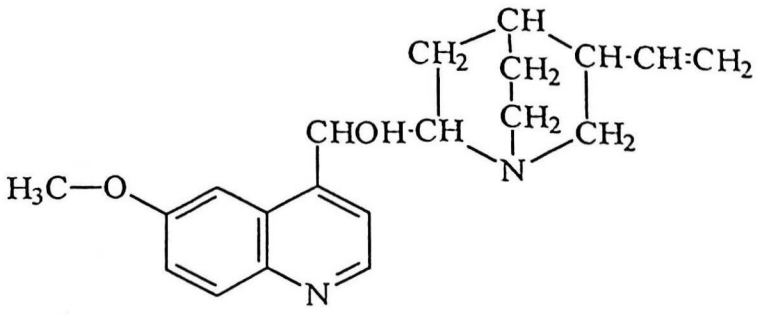
Artemisinin recrystallized from 50 % aqueous ethanol (ArtaqEth) and cyclohexane (ArtCyc) was found to be of different polymorphic forms. ArtaqEth showed a dense and thicker appearance than the ArtCyc, which was thin and transparent. The solubility of ArtaqEth and ArtCyc at 37 °C were 0.0177 mg mL^{-1} ± 0.0010 SD and 0.0443 mg mL^{-1} ± 0.0012 SD, respectively, with the latter also having a faster rate of dissolution. When evaluated in *in vivo* using healthy human volunteers, the extent of absorption of ArtCyc was 3.4 times higher than that of ArtaqEth. In addition, monitoring the gastrointestine transit of the drug administered as a suspension using sulfasalazine showed that no absorption occurred in the colon.

CHAPTER 1. INTRODUCTION

1.1 Malaria and therapy

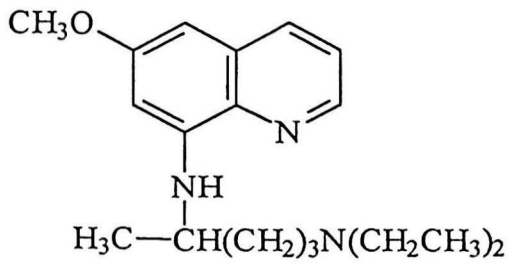
Malaria is caused by the bite of a female anopheles mosquito, carrying the sporozoites of the parasite *Plasmodium*. Four species are primarily responsible for infection in man : *P. falciparum* causing falciparum malaria (malignant tertian); *P. vivax* causing vivax malaria (benign tertian); *P. ovale* causing ovale malaria (mild benign tertian) and *P. malariae* causing quartan malaria. Patients may sometimes be classified as non-immune if they have not previously or recently been exposed to *Plasmodium* infection and as semi-immune or immune if they have a history of prolonged exposure (Atlas, 1989).

In the 16th century, the bark of the cinchona tree, *Cinchona officinalis* gained wide acceptance as a treatment for malaria (Richardson & Gangolli, 1994). The therapeutic action of the bark was disclosed by the natives of Peru to Jesuit missionaries, who, in turn, brought the word of its utility to Europe. The French chemist Pelletier, isolated quinine **(1)**, an alkaloid, from the cinchona bark in 1834. It became the main treatment for malaria until the 1930's when synthetic antimalarials were developed. The advent of the First World War in Germany forced its researchers to produce synthetic antimalarial drugs, due to the shortage of quinine from Peru (Schuleman *et al.*, 1932).

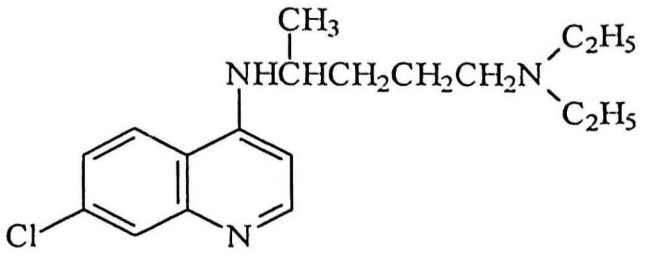


(1)

1.2 Synthetic antimalarials



(2)

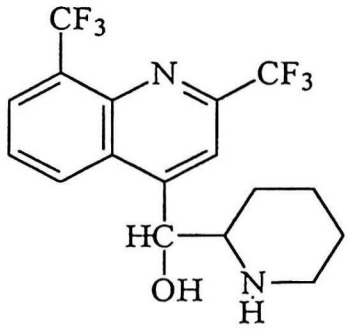


(3)

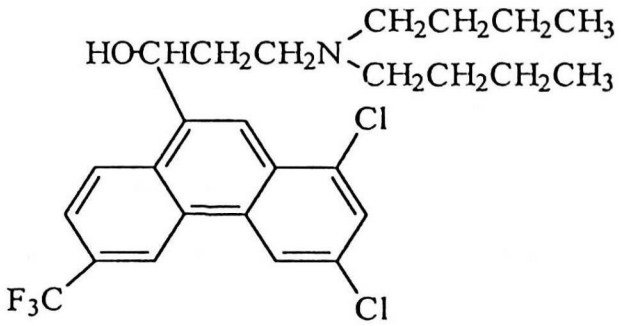
Pamaquine (2) was the first synthetic antimalarial drug to be synthesized by Schuleman in 1924 and its structure was determined in 1932 (Schuleman *et al.*, 1932). The success of pamaquine as an antimalarial drug inspired extensive research globally into synthetic derivatives of 8-aminoquinoline. Pamaquine was effective as a prophylactic agent in destroying *P. falciparum* gametocytes and reducing the rate of *P. vivax* relapses. High doses were required to maintain its efficacy and had caused severe side-effects (Elderfield *et al.*, 1955).

Chloroquine (3) was synthesized from the 4-aminoquinoline in 1934 and was widely used because it did not cause yellow discolouration of the skin (Bruce-Chwatt *et al.*, 1981). Chloroquine was effective at the asexual stage for all the four species of the

malarial parasites except in places where resistance have occurred. It was also effective as a gametocyticidal agent against *P. vivax*, *P. ovale* and *P. malariae*, but it is not effective for immature gametocyte of *P. falciparum*. Chloroquine was also used as a prophylactic agent for all the malarial parasites when given continuously for 4-6 weeks (Bruce-Chwatt *et al.*, 1981). The administration of chloroquine in large doses (300-600 mg) for more than three months caused neuroretinitis. Pigmentation of the skin and pruritis disappeared when chloroquine treatment was stopped (Baker, 1966).



(4)

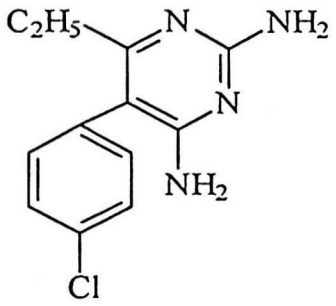


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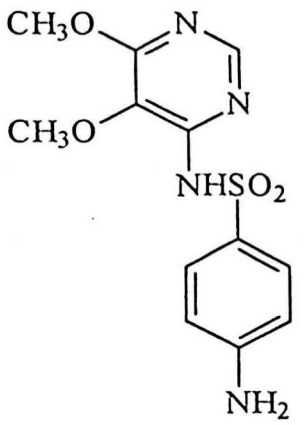
Mefloquine **(4)** was effective against the intraerythrocytic phase of all the malarial parasites. Absorption of mefloquine was rapid, with a half-life from 0.36 to 2 hours. The concentration of mefloquine in the plasma after administration of 250 mg of mefloquine as the first dose, was 1 mg L^{-1} after 2-12 hours. Its volume of distribution was 27-50 L kg^{-1}, which indicated that the drug was widely distributed after it has been absorbed. The administration of a single dose of mefloquine may cause abdominal pain to certain individuals (Desjardins *et al.*, 1979; Schwartz *et al.*, 1980; 1982).

Halofantrine **(5)** was a schizonticidal agent which was not phototoxic. Field studies in Thailand in 1982 indicated low bioavailability when given as an oral preparation (WHO, 1984). *In vitro* research on the susceptibility of halofantrine showed that the inhibition dose at 50 % (ID_{50}) was 2.5 ng mL^{-1} against *P. falciparum* Africa

Uganda I and 3.9 ng mL^{-1} against the Smith strain from Viet Nam (Desjardins *et al.*, 1979).



(6)



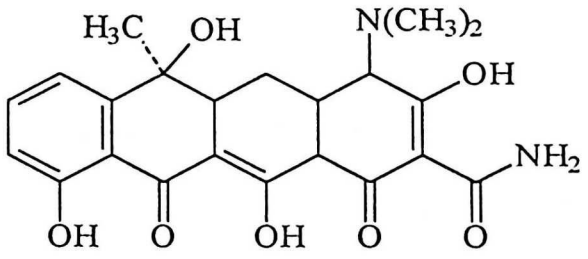
(7)

The chief value of pyrimethamine **(6)** is as a suppressant. It usually kills the primary exoerythrocytic parasites of *P. vivax* and if used regularly will therefore prevent relapses of most strains of benign tertian malaria. Pyrimethamine also affects gametocytes, rendering them incapable of complete development in the mosquito. Pyrimethamine is given together with chloroquine for the treatment of malarial fever. The chloroquine rapidly eliminates asexual parasites and the pyrimethamine helps to prevent transmission by the mosquito (Jaffe, 1972). Pyrimethamine has caused foetal abnormalities in animals, but pregnancy is not considered a contra-indication to the prophylactic use of pyrimethamine in malaria. High doses should be avoided during pregnancy unless folinic acid supplements are given. These do not interfere with the antimalarial action (Bruce-Chwatt *et al*, 1981).

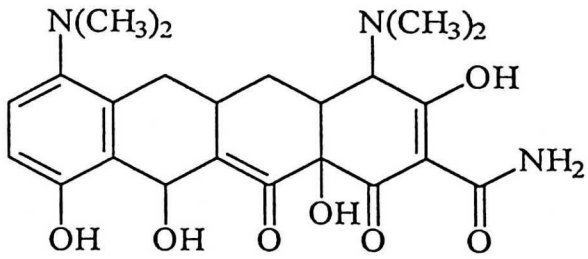
Sulphadoxine (7) is used in conjuction with pyrimethamine in the treatment of malaria due to *P. falciparum* which is resistant to other therapy. The recommended dosage regimen is a dose of one gram of sulphadoxine, followed by 500 mg on the

following day and then a single dose of 50 mg of pyrimethamine is given (Hudson, 1995).

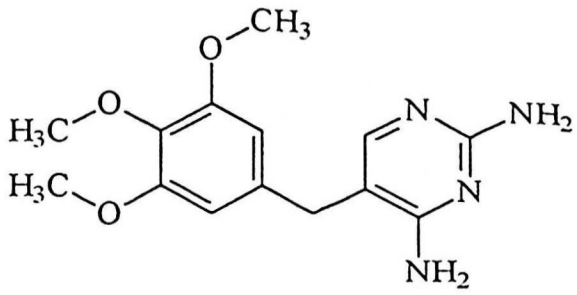
Other drugs with antimalarial activity include some antibiotics such as tetracycline **(8)**, minocycline **(9)** and trimethoprim **(10)**. Tetracycline **(8)** is active against both pre-erythrocytic and blood stages of malaria and it has been suggested for prophylactic use in tourists. Minocycline **(9)** is the most potent of the tetracyclines against strains of *P. berghei.* Trimethoprim **(10)** is effective against *P. falciparum* when given in combination with sulfadoxine **(7)** (Glasby, 1992).



(8)



(9)

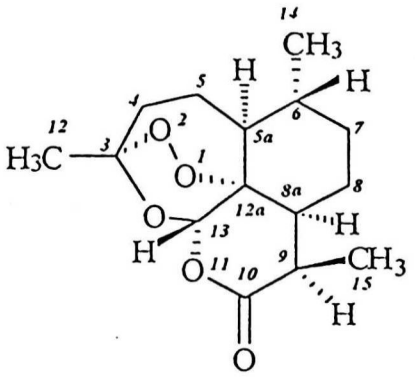


(10)

P. falciparum, the most insidious of all the malarial parasites, are fast developing resistance to currently used antimalarial drugs including chloroquine **(3)**, sulpha-pyrimethamine combinations and quinine **(1)** (WHO, 1994). Mefloquine **(4)** has been the treatment of choice for uncomplicated falciparum malaria in Thailand since 1984. High prevalence of resistance to mefloquine, the only alternative drug available in control programmes has recently been found along the Thai/Cambodian and the Thai/Myanmar borders (Fontanet *et al*, 1993). Cure rates on the eastern and western borders have declined rapidly over the past 3 years. There has been an alarming increase in high grade resistance, that is initial failure to respond to mefloquine therapy, despite increasing the dose from 15 mg kg^{-1} to 25 mg kg^{-1} (Nosten *et al.*, 1991; Ter Kuile *et al.*, 1992; White, 1992). The search for alternative antimalarial agents have been intensified in many laboratories in order to prevent possible future occurrence of non effective antimalarial drugs.

A strategy that is increasingly advised is to initiate treatment only after an infection has occurred. This is to prevent the development of resistance due to prophylactic treatment. Drugs advised for this approach include pyrimethamine **(6)** combined with sulphadoxine **(7)** (Fansidar®), mefloquine **(4)** or halofantrine **(5)** (Hudson, 1995).

1.3 Artemisinin in malarial therapy



(11)

The activity of artemisinin (**11**) has been shown to be potent and rapid against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum in vitro*. The acute toxicity of artemisinin was found to be considerably less than that of chloroquine (Klayman, 1985). Artemisinin cleared parasitaemia more rapidly than oral mefloquine or intramuscular quinine in a study involving forty patients with falciparum malaria. Oral artemisinin acted more quickly than intramuscular artemisinin in aqueous suspension (Jiang *et al.*, 1982). The addition of artemisinin to treatment with mefloquine and pyrimethamine-sulfadoxine greatly enhanced the rate of parasite clearance produced by mefloquine alone or pyrimethamine-sulfadoxine incombination (Li *et al.*, 1984).

The most striking clinical results with artemisinin and its derivatives are seen in the treatment of cerebral malaria, although high recrudescence rates necessitated the use of additional drugs such as sulfadoxine (**7**). Artemisinin (**11**) is reported to have a shorter half-life than some other antimalarial drugs, and this may account for the high rate of recrudescence seen in some studies (Klayman, 1985).

The poor solubility of artemisinin has given rise to problems in formulation as well as in its mode of administration. The tablet form produced rapid clearance of parasitaemia of both *P. vivax* and *P. falciparum*, but also a higher rate of recrudescence than intramuscular formulations (Qinghaosu Antimalarial Coordinating Research Group, 1979).

1.3.1 Resistance

Artemisinin resistance has been induced under laboratory conditions in *P. falciparum* and rodent malaria parasites. Resistance of *P. falciparum* to artemisinin, of five to sixfold has been induced *in vitro* at Walter Reed Army Institute of Research, by subjecting various parasite clones to step-wise increasing concentrations of artemisinin over a twelve month period. Such resistance has remained stable for six months without drug pressure(Chawira *et al.*, 1986). In a chloroquine resistant Indochina clone, which was resistant to artemisinin, there was a fourfold decrease in susceptibility to halofantrine and mefloquine, but an apparent increase in susceptibility to both quinine and chloroquine. Studies conducted with a mefloquine resistant (chloroquine-susceptible) clone revealed a twofold increase in susceptibility to artemisinin and mefloquine, with no changes in susceptibility to halofantrine, chloroquine or quinine. These findings suggest that the patterns of cross-resistance are independent of chemical class (WHO, 1986). Artemisinin resistant lines of *P. falciparum* have also been produced *in vitro* by prior exposure of the parasites to the chemical mutagen, *N*-methyl-*N*^{1}-nitronitrosoquanidine (Inselberg, 1985). Liu & Ren (1987) have produced a line of *P. berghei* resistant to sodium artesunate by increasing drug pressure with each successive passage in mice. In the absence of drug pressure, resistance was gradually lost. Cross-resistance with mefloquine and quinine has been observed following *in vivo*

induction of resistance to artemisinin in chloroquine resistant strains of *P. yoelii* in rodent models (Chawira *et al.*, 1986; 1987). There is no good evidence of true resistance to artemisinin in humans. WHO has proposed the following policy recommendations for artemisinin and its derivatives in order to suppress the development of resistance (WHO, 1994).

1.3.2 Registration and certification (WHO, 1994).

1. The use of artemisinin and its derivatives should be restricted to areas where multidrug resistance is prevalent, where chloroquine, sulpha-pyrimethamine combination and quinine are ineffective.

2. Artemisinin and its derivatives should be regulated in countries where it is the most effective antimalarial known.

3. The importation of artemisinin and its derivatives should be controlled by national governments through a governmental health authority, a central drug procurement agency or by a limited number of licensed importers.

4. WHO should alert drug regulatory authorities that most artemisinin group drugs are not produced to the standards of Good Manufacturing Practice (GMP), and that in some countries drugs of substandard quality are being promoted and used.

5. Artemisinin and its derivatives should only be available on prescription and not over-the-counter. Therefore, storage and its distribution should be in accordance with national policies and guidelines.

6. Governments need to evaluate carefully the necessity for oral forms of artemisinin and its derivatives and target their usage to high risk populations only. National registration of oral formulations of these drug may bring special problems. These include lack of quality assurance, difficulties in patient compliance with an effective radical cure requiring prolonged dosage, difficulties in controlling distribution, sales, importation and over-the-counter use resulting in overuse and misuse.

7. Routine sampling of the drugs must be carried out to monitor their quality.

1.3.3 Monitoring and post-marketing surveillance (WHO, 1994).

1. Post-marketing surveillance to identify problems related to drug quality, stability and efficacy, adverse reactions and the development of drug resistance should be established.

2. The indiscriminate use and misuse of these drugs may encourage the development of parasite resistance. Monitoring of resistance is a responsibility of national malaria control programmes.

3. It is desirable that the long term results of treatment be followed with regard to delayed toxicity and curative efficacy, particularly among pregnant women and individuals who received repeated treatments. An exhaustive enquiry should be undertaken if death might be drug related.

1.3.4 Information (WHO, 1994).

1. The authorities must give clear guidelines on the use of artemisinin and its derivatives to all prescribers. This includes national drug formulary and pharmacy guidelines. Information should also be given to health workers to ensure correct prescribing in respect of clinical indication and dosage.

2. It is important that the general public as potential patients, be educated of the importance of compliance and correct use of artemisinin and its derivatives.

3. Donor agencies and pharmaceutical companies should be discouraged from promoting or importing these drugs except through officially authorized channels.

1.3.5 Adverse effects

Acute toxicity studies in animals indicated that artemisinin have higher LD_{50} and better chemotherapeutic indices than chloroquine (WHO, 1994). A minority of patients (<6 %) had tenesmus, abdominal pain and diarrhoea with administration of artemisinin suppositories (Qiao, 1990; Hien & White, 1993).

1.3.6 Pregnancy

A report by Wang (1989) indicated that six children of mothers treated with artemisinin during the second and third trimester were followed up during childhood showed no abnormalities. Another report by Qiao (1990) of 17 children exposed to artemisinin between the sixteen and thirty eight weeks gestation who were followed for periods from three months to ten years showed no abnormalities.

1.4 Objective of this study

1. To look into the viability of growing *A. annua,* a temperate plant in a tropical climate by propagation via tissue culture

2. To select and clone high artemisinin yielding plantlets.

3. To develop a sensitive and selective measurement of artemisinin in plant extracts and blood plasma.

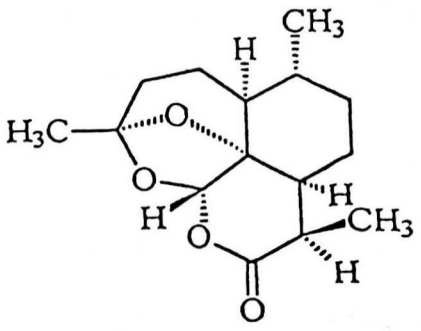
4. To investigate the reasons behind the variation in bioavailability of some artemisinin preparations

CHAPTER 2. LITERATURE REVIEW

2.1 Artemisinin : A new antimalarial

Artemisinin **(11)** is the active principle isolated from the Chinese medicinal plant qinghao, *Artemisia annua* L. (Asteraceae). It has been used for over a thousand years as a herbal cure for malaria. The structure of artemisinin has been characterized in 1972 as an endoperoxide sesquiterpene lactone, by Chinese scientists (Coordinating Research Group on the Structure of Qinghaosu, 1977). Artemisinin is unique in possessing a 1,2,4-trioxane ring which is novel among known antimalarial compounds, and lacks a nitrogen-containing heterocyclic ring system (Webster & Lehnert, 1994). Artemisinin is a rapidly acting blood schizontocide with good activity against chloroquine and multidrug-resistant strains of *Plasmodium falciparum* parasites and is effective in the treatment of cerebral malaria (Schmid & Hofheinz, 1983).

The peroxide moiety appears to be responsible for its blood schizonticidal activity. The reduction of artemisinin with hydrogen under the catalytic action of palladium in calcium carbonate yields deoxyartemisinin **(12)**. Deoxyartemisinin as well as other nonperoxy metabolites and other sesquiterpenes lacking the peroxide bridge are devoid of antimalarial activity (Luo & Shen, 1987).



(12)

2.1.1 Isolation and structure determination of artemisinin

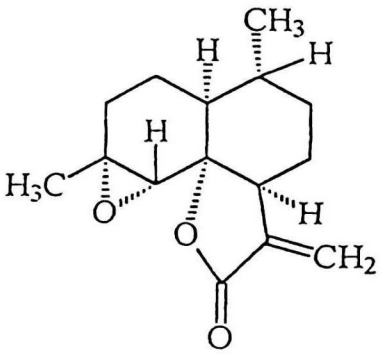
2.1.1.1 Isolation of artemisinin

The Chinese literature indicated that ethyl ether extraction was used to isolate artemisinin from the aerial portions of *A. annua* (Klayman, 1985). Scientists at the Walter Reed Army Institute of Research extracted various air-dried components of the plant with several nonprotic solvents, of which, petroleum ether (30 ° to 60 °C) was the most satisfactory. The extract was then chromatographed on silica gel with chloroform-ethyl acetate being used as the eluent (Klayman *et al.*, 1984). Fractions rich in artemisinin, crystallized readily, and recrystallization was effected from cyclohexane or 50 % ethanol to give fine, colourless needles with a melting point of 156 ° to 157 °C and 158 ° to 159 °C (Klayman *et al.*, 1984; Qinghaosu Research Group, 1980; Qinghaosu Antimalarial Coordinating Research Group, 1979).

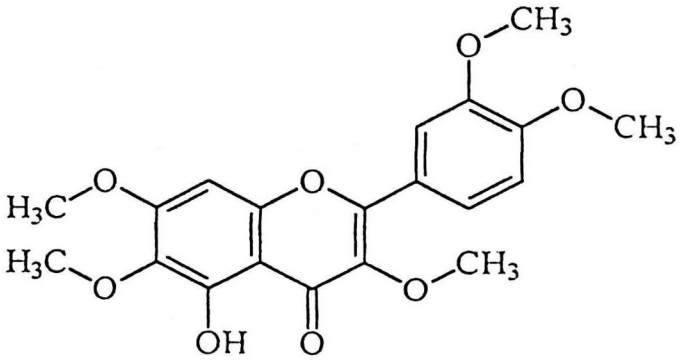
A. annua appears to be the only *Artemisia* species that contain appreciable amounts of artemisinin. Extracts from thirty other species of artemisinin did not show antimalarial activity according to Chinese scientists (WHO, 1981). American scientists have failed to detect artemisinin in *A. arbuscula, A. dracunculus, A. ludoviciana, A. pontica, A. schmidviana* and *A. vulgaris* (Klayman *et al.*, 1984). Small amounts of artemisinin have been detected in extracts of *A. scoparia, A. abrotanum* and *A. caucasica* from Turkey (Trigg, 1989) and *A. apiacea* from Japan (Liersch *et al.*, 1986).

Other terpenes and related compounds that have been found in *A. annua* were : arteannuin B **(13)**, artemetin **(14)**, artemisia ketone **(15)**, artemisinic acid **(16)**, benzyl isovalerate **(17)**, borneol acetate **(18)**, cadinene **(19)**, camphene **(20)**, camphor **(21)**, β-caryophyllene **(22)**, casticin **(23)**, 1,8-cineole **(24)**, coumarin **(25)**, cuminal **(26)**,

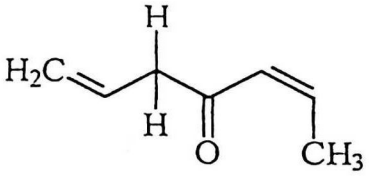
β-farnesene **(27)**, β-pinene **(28)**, scopoletin **(29)**, scopolin **(30)** and stigmasterol **(31)** (Jeremic *et al.*, 1973; Tu *et al.*, 1981a, Tu *et al.*, 1981b; Tian *et al.*, 1982; Elford *et al.*, 1987). Some of the methoxylated flavones found in *A. annua* such as casticin **(23)** and, to a lesser extent, artemetin **(14)**, have shown to possess synergistic effect on the activity of artemisinin against *P. falciparum in vitro* (Elford *et al.*, 1987).



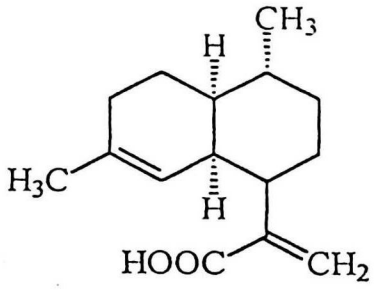
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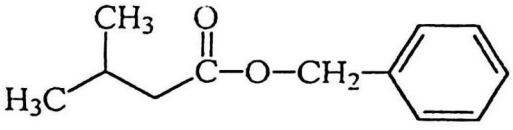
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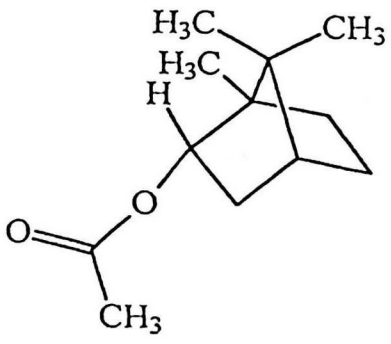
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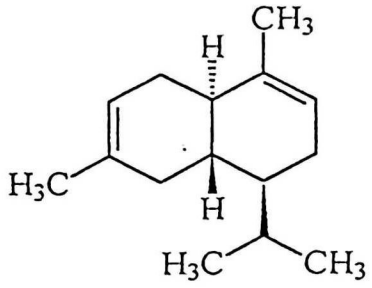
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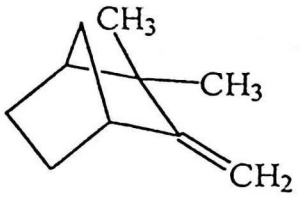
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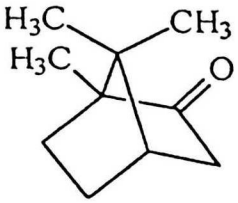
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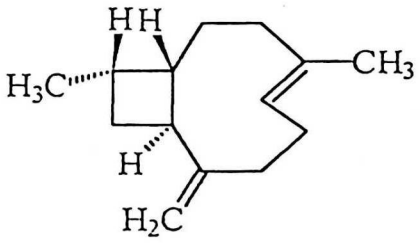
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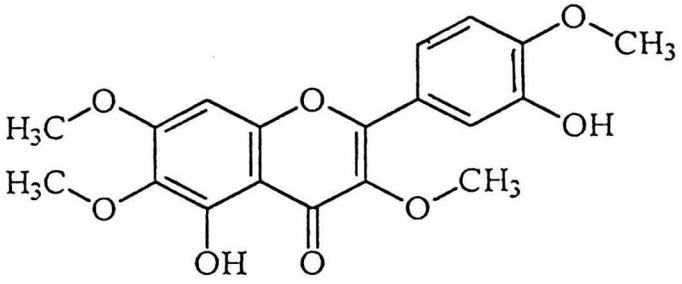
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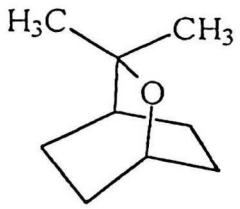
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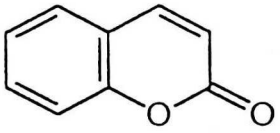
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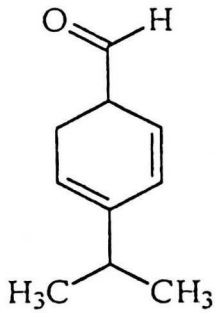
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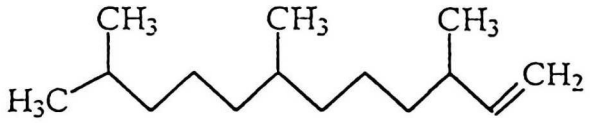
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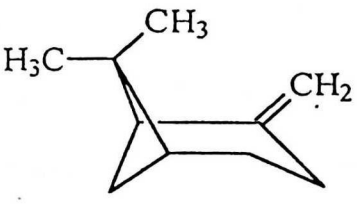
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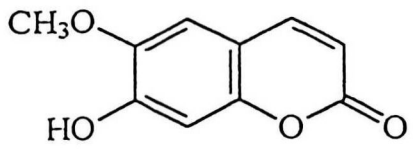
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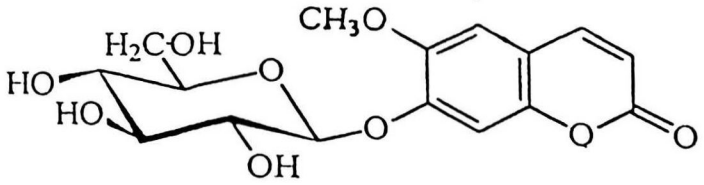
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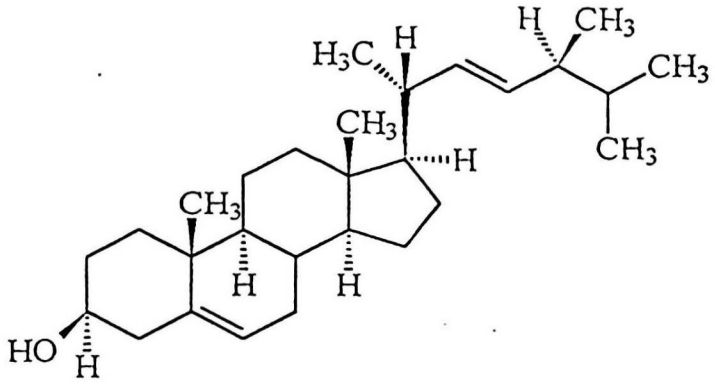
(28)



(29)



(30)



(31)

2.1.1.2 Structural determination

The empirical formula for artemisinin, $C_{15}H_{22}O_5$ was assigned through elemental analysis and high-resolution mass spectrometry, and suggests that the compound is a sesquiterpene. Its infrared spectrum shows an intense peak at 1745 cm^{-1} (υc = o, for lactone) and peaks at 831, 881, 1115 cm^{-1} (peroxide). The presence of this unusual peroxy group can also be verified by the quantitative reaction of artemisinin with triphenylphosphine (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982). The ^{1}H and ^{13}C nuclear magnetic resonance spectra indicated the presence of three methyl groups (one tertiary and two secondary), an acetal function, and several kinds of aliphatic carbon atoms (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982; Coordinating Group for Research on the Structure of Qinghaosu, 1977; Liu *et al.*, 1979; Hu *et al.*, 1991).

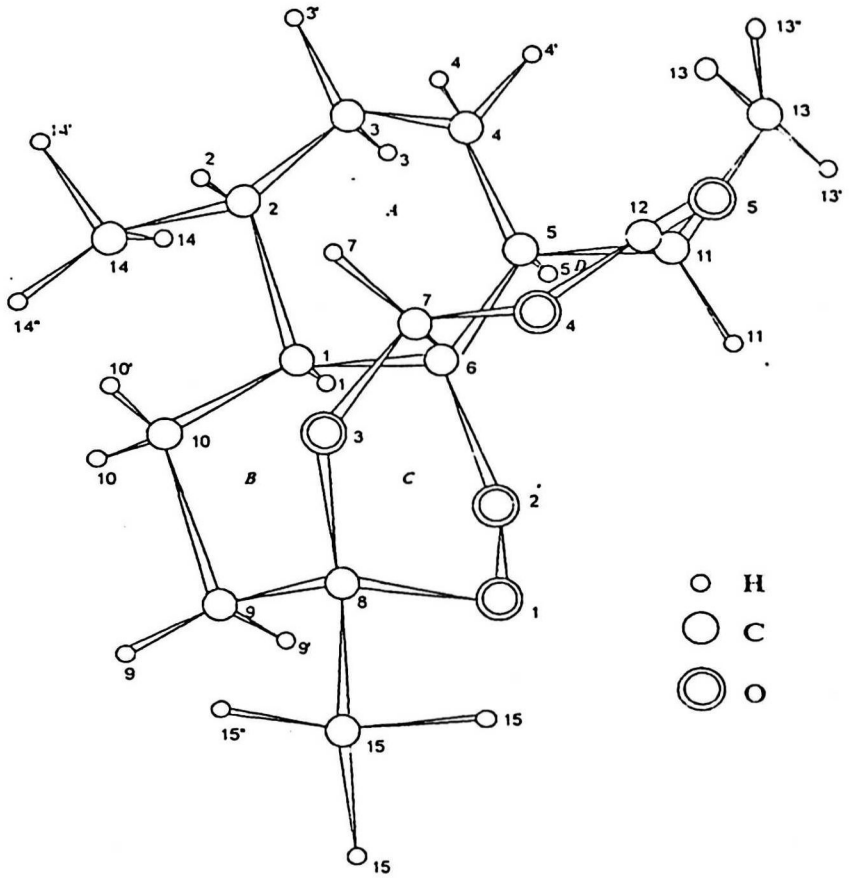


Figure 2.1. A perspective view of the molecule as viewed along the *c* axis

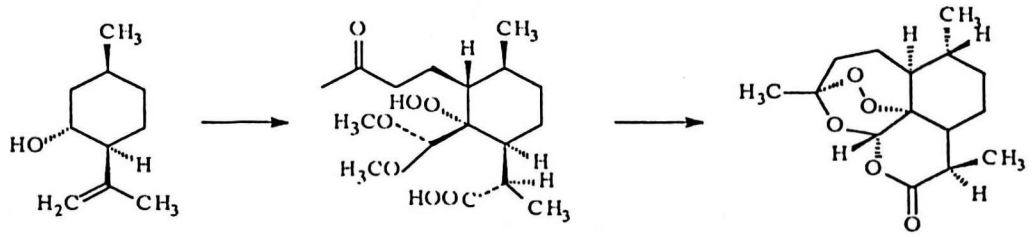
The structure of artemisinin **(11)** and its absolute configuration, was determined unambiguously by X-ray diffraction (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982; Qinghaosu Research Group, 1980) (**Figure 2.1**). The *trans* configuration of the lactone ring was also determined by comparison of its Optical Rotatory Dispersion (ORD) spectrum with that of arteannuin B **(13)**, a structurally related sesquiterpene isolated from *A. annua* in Yugoslavia (Qinghaosu Research Group, 1980; Liu *et al.*, 1979; Jeremic *et al.*, 1973).

2.1.2 Synthesis of artemisinin

Artemisinin, (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin-10(3*H*)-one **(11)**, is a member of the amorphane subgroup of cadinenes. It is soluble in most aprotic solvents but only sparingly in oil and water. Reduction of artemisinin, depending on conditions, yields deoxyartemisinin **(12)**, an epoxide which is devoid of antimalarial activity or dihydroartemisinin **(38)**, a lactol which is more active than the parent artemisinin. The peroxide moiety is thus essential for activity (Webster & Lehnert, 1994).

2.1.2.1 Complete synthesis

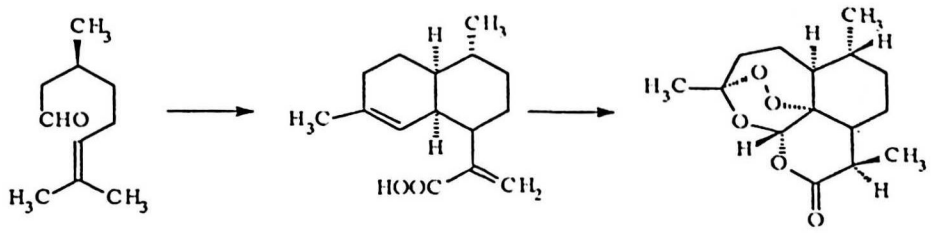
The complete synthesis of artemisinin **(11)** has been achieved by at least three routes. Schmid & Hofheinz (1983) reported the first total synthesis of **(11)** starting from (-)-isopulegol **(32)** (**Scheme 2.1**). It involved thirteen steps and gave 2.1 % total yield.



(32) **(33)** **(11)**

Scheme 2.1 : Total synthesis of **(11)** via **(32)**.

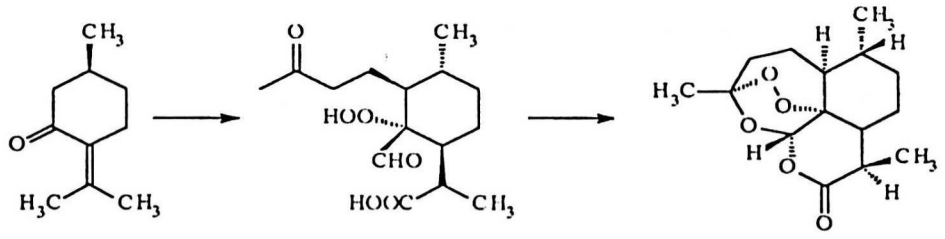
A second longer synthesis, starting from R-(+)-citronellal **(34)**, was reported by Xu *et al.* (1986) in China (**Scheme 2.2**). Twenty steps were involved with 0.25 % total yield. This synthesis proceeded through the apparent natural precursor of artemisinin, artemisinic acid **(16)**.



(34) (16) (11)

Scheme 2.2 : Total synthesis of **(11)** via **(34)** and **(16)**.

A third method (**Scheme 2.3**), which has the added the advantage of being selective for a single stereoisomer of artemisinin, starting from R-(+)-pulegone **(35)**, was developed by Avery *et al.* (1992). Ten steps were involved with 3.6 % total yield. Although these methods of total synthesis show yields higher than the plant, their labour-intensive nature and lack of potential for scaled-up production made them economically unfeasible (Webster & Lehnert, 1994).



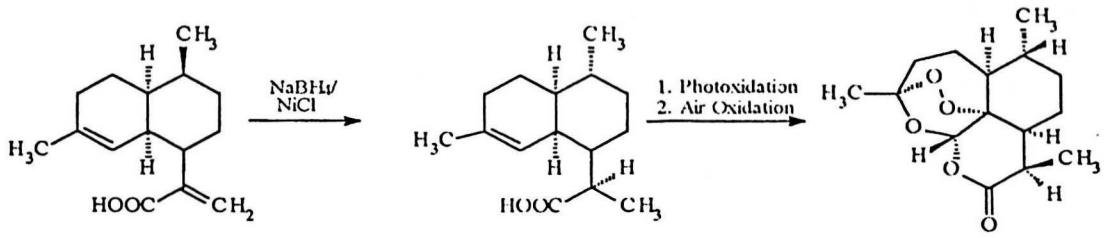
(35) (36) (11)

Scheme 2.3 : Stereospecific total synthesis of **(11)** via **(35)**.

2.1.2.2 Partial synthesis

The partial synthesis of artemisinin (**Scheme 2.4**) through another of the plant's isolated compounds, artemisinic acid **(16)**, is an economically appealing alternative. Artemisinic acid exists in 8-10 times the concentration of artemisinin in the plant (Laughlin, 1994; Haynes & Vonwiller, 1994). Recent work by Acton & Roth (1992)

has simplified the method for the transformation of artemisinic acid to artemisinin by Xu *et al.* (1986) from eight steps to three steps and the total yield increased to more than 32 %.



(16) **(37)** **(11)**

Scheme 2.4 : Three step partial synthesis of **(11)** from **(16)**.

In addition to being a possible method of increasing the plant yield of artemisinin, this development also suggests a possible mechanism for the synthesis of artemisinin in the plant. Artemisinic acid have been shown by labelling experiments to be intermediates in the biosynthetic pathway of artemisinin (Webster & Lehnert, 1994).

2.2 Artemisinin derivatives

The activity and solubility of artemisinin could be improved by chemical modifications, so long as the peroxide linkage was preserved (Luo & Shen, 1987). Two of the dihydroartemisinin **(38)** derivatives that have received considerable attention are artesunate **(39)** [water soluble half-ester succinate derivative] and artemether **(40)** [methyl ether derivative] (Webster & Lehnert, 1994). Another derivative, arteether **(41)** [ß-ethyl ether] was developed based on its stability and formulation properties (WHO, 1986).

2.2.1 Significance of artemisinin derivatives

Artesunate **(39)** and artemether **(40)**, two derivatives of artemisinin, with greater antimalarial activity were synthesized from the reduction product of dihydroartemisinin **(38)**. Reports from Burma, Viet Nam, Thailand, Gambia (Shwe *et al.*, 1988; Arnold *et al.*, 1990; Looareesuwan *et al.*, 1992; White *et al.*, 1992), Nigeria, Ivory Coast, Zimbabwe, Malawi, Kenya and Brazil, have all confirmed that artemisinin or its derivatives were the most rapidly acting of all antimalarial drugs. The artemisinin derivatives have since replaced quinine for the treatment of falciparum malaria. The drugs were active by parenteral, oral or rectal routes of administration and there has been no toxicity reported in humans (Hien & White, 1993). Artesunate and artemether (oral and parenteral) are now available in China and Viet Nam. The suppositories have proven as a very simple, safe and rapidly effective treatment of severe malaria and were of particular value in children (Hien *et al.*, 1991). A preliminary study on arteether intramuscular injection in humans was conducted recently (Kager *et al.*, 1994). Artemisinin and its derivatives have proven to be effective in these situations :

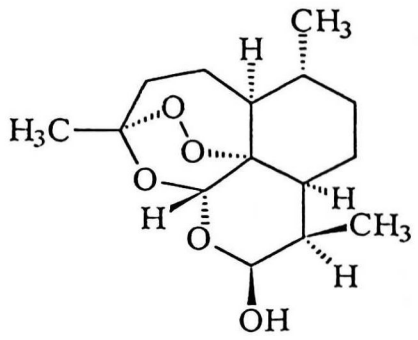
(i) Primary treatment of uncomplicated malaria in combination with mefloquine, has accelerated the clinical recovery rates and the risk of dangerous early failures was eliminated.

(ii) Treatment of recrudescent infections following mefloquine or quinine treatment, when failure rates were twice as high in primary treatment, has shown success especially in children, who have less background immunity.

(iii) The oral treatment of patients with high parasitaemias (≥4 %), but who have no symptoms or sign of severity.

(iv) Treatment of cerebral malaria (Nosten, 1994).

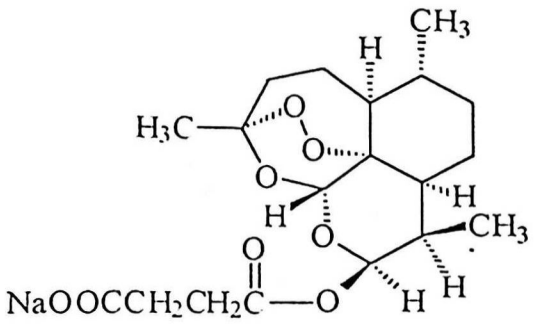
2.2.2 Dihydroartemisinin



(38)

Dihydroartemisinin **(38)** was prepared by reducing the carbonyl group of artemisinin with sodium borohydride (Li *et al.*, 1979; 1981), and was used to prepare various esters, ethers and succinyl derivatives. Under acidic conditions, dihydroartemisinin was primarily ß-epimer, whereas under alkaline conditions, the α-epimer was predominant. Major differences in antimalarial activity between α and ß epimers have not been observed (Webster & Lehnert, 1994). 120 mg of dihydroartemisinin was given once daily and 60 mg subsequently for the next 6 days for the treatment of uncomplicated malaria (Li *et al.*, 1994). The antimalarial efficacy of dihydroartemisinin is greater than that of artemisinin (Li *et al.*, 1981), but not widely used due to its rapid clearance. Studies in dogs, indicated that dihydroartemisinin has a half-life of about 2.5 hours (Theoharides *et al.*, 1988). The dihydroartemisinin concentration decreased to the detection limit in about 2 hours in rats (Edlund *et al.*, 1984).

2.2.3 Sodium artesunate



(39)

Sodium artesunate (39) is a water-soluble hemisuccinate derivative of dihydroartemisinin (Li *et al.*, 1990). Neutral aqueous solutions was unstable. The parenteral drug was therefore formulated separately as powdered artesunic acid and an ampoule of 5 % sodium bicarbonate in a single carton. The two were dispensed by mixing immediately before injection. Parenteral sodium artesunate could be given by intravenous or intramuscular injection; both were equally effective (Hien *et al.*, 1992). Oral artesunate was also rapidly effective and has been used either alone, for 5 to 7 days (Hien *et al.*, 1991) or with mefloquine, when the combination increased cure rates and accelerated symptomatic recovery (Looareesuwan *et al.*, 1992; Luxemburger *et al.*, 1994). The combination of artesunate with mefloquine remains very effective against even the highly multi-drug resistant strains of *Plasmodium falciparum* now prevalent on the eastern and western borders of Thailand. 100 mg of sodium artesunate was given immediately and subsequently 50 mg every 12 hours to a total dose of 300 mg, followed by 750 mg of mefloquine (Looareesuwan, 1994). This combination has optimum cure rates of more than 90 % (Looareesuwan *et al.*, 1992; Luxemburger *et al.*, 1994).