SOME ASPECTS OF THE CHEMISTRY AND BIOLOGICAL ACTIVITY OF ARTEMISININ AND RELATED ANTIMALARIALS

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Abstract - Developments taken place in the field of artemisinin and related antimalarials during the last five years have been reviewed.

CONTENTS

1. INTRODUCTION

Malaria is one of the most widespread parasitic diseases caused by invasion on human body by the protozoan parasites of the class of *Plasmodium*. It is estimated that there are still 215 million people who are chronically affected and there are 150 million new cases reported every year.[1] In Africa alone more than 5.5 million cases of malaria were reported in 1980 and other hard hit areas are the Indian continent, east Asia and China. Due to the indiscriminate use of insecticides the vector mosquitoes have become resistant to these in some countries. Besides,

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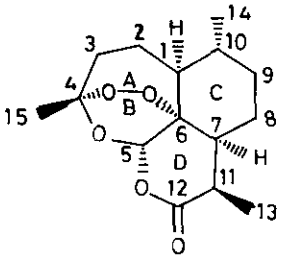
Dedicated to my revered teacher Prof M. S. Ahmad of AMU Aligarh on the occasion of his 60th birthday.

the resistance of *Plasmodium* species to chemotherapeutic agents, such as chloroquine, calls for development of newer drugs. From time to time, preparations from plants have achieved reputations as cures for the ubiquitous malaria. Some active constituents with diverse chemical structures from plants were reported to have significant antimalarial activity.[2-15] However, till the discovery of artemisinin by the Chinese workers from the Plant *Artemisia annua* in 1972, most of the compounds that were used as antimalarials were having the nitrogen-containing heterocyclic ring system.

Since ancient times, *A. annua* has been used as a traditional Chinese herbal medicine known as Qinghao for treating fever and malaria. The effective constituent was isolated by the Chinese investigators in 1972 and shown to be the sesquiterpene lactone(**1**),[16] named artemisinin[17] or arteannuin[18] or qinghaosu.[19] It was found to be a potent plasmodicidal agent and extensive clinical trials in China have revealed that **1** has considerable promise for the treatment of drug-resistant malaria.[16,20-23] The structure of this interesting sesquiterpene lactone endoperoxide has been fully confirmed by various methods.[24-37] Artemisinin is poorly soluble in water (0.46 mg/ml at 37°C) and oil but soluble in most aprotic solvents.[38] This unusual compound



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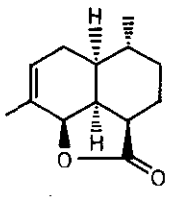
has a peroxide grouping but lacks a nitrogen containing heterocyclic ring system which is found in most antimalarial compounds. Artemisinin belongs to the amorphane sub-group of cadinenes.[39] It was obtained in *A. annua* ranging from 0.0 to a maximum of 0.1%, and the highest content was found about two weeks before flowering.[40] Several workers have investigated various *Artemisia* species in search for alternate sources of artemisinin but found that the species other than *A. annua* were devoid of this constituent.[10,23,41,42] However, in 1986 Liersch *et al.* surprisingly established the presence of artemisinin in *A. apiaceae* (0.08%).[40] Besides

A. annua that is the only other variety known to contain artemisinin. Several workers have tried improved techniques including tissue culture for the production of artemisinin in *A. annua*.[40-52]

Apart from artemisinin (**1**), sixteen closely related compounds and a few other compounds[20,24,44,50,53-66] that have been found in *A. annua* are : artemisinins A-F(**2-7**),[24,54,55,57,59] artemisinic acid (**8**),[55,57,58ii] 6,7-dehydroartemisinic acid (**9**),[62] artemisitene (**10**),[20,56] (+)-deoxyisoartemisinin B (**11**),[62] compounds (**12**) and (**13**),[24,65] 4,5-α-epoxyartemisinic acid (**14**),[64] deoxyartemisinin (**15**),[57] artemisinic acid methyl ester (**16a**),[58ii] artemisiniol (**16b**),[58ii] 1,8-cineole, borneol acetate, ß-pinene, cuminal, ß-fernesene, ß-caryophyllene, coumarin, stigmasterol, scopoletin, artemisia ketone, benzoyl isovalarate, 3'-methoxychryrosplenol, 6,7,4'-trimethoxy-3,5,3-trihydroxyflavone, α- and ß-myrcene hydroperoxide, chryrosplenol and artemetin.



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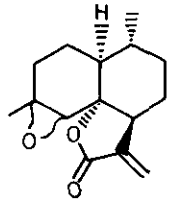
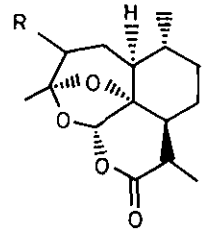
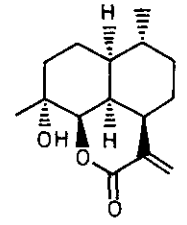


3 : α-epoxy
4 : β-epoxy
13 : 4,5-ene



5 : R = α-OH
15 : R = H



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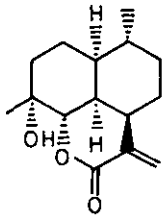

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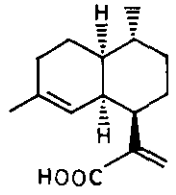

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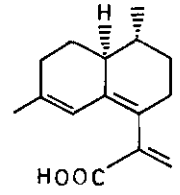

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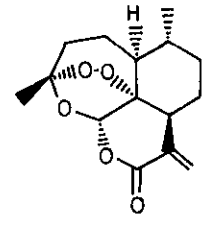

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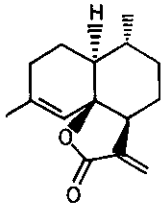

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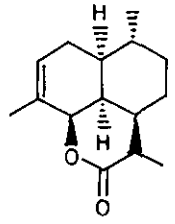

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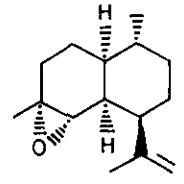

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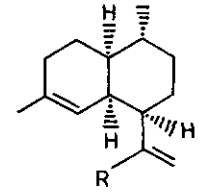
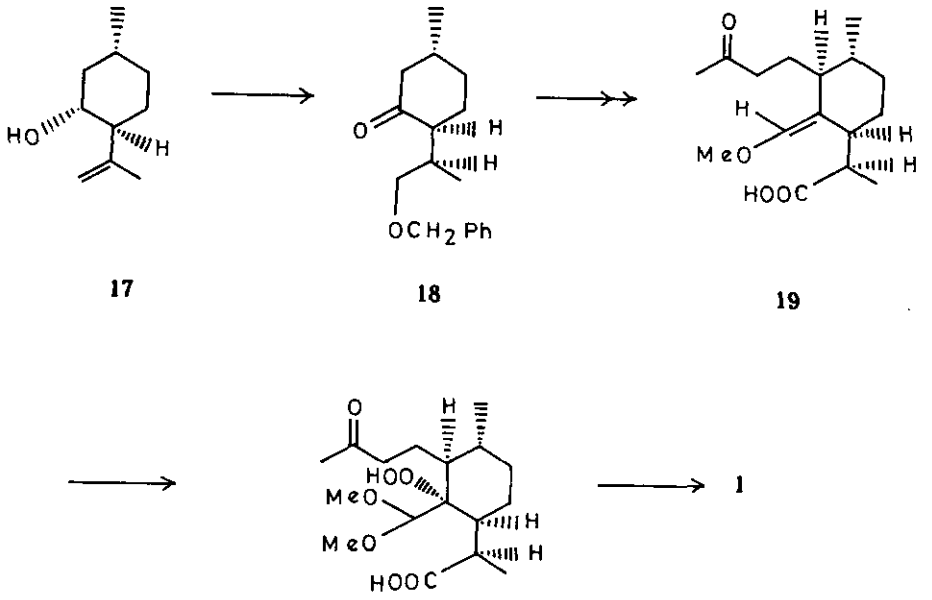


16 a : R = COOMe
b : R = CH_2OH

The combination of an interesting biological activity, a novel chemical structure and low yield of artemisinin from natural sources prompted the scientists all over the world to search for new synthetic routes of artemisinin and related compounds.[67-72]

II. TOTAL SYNTHESIS OF ARTEMISININ

The first total synthesis of artemisinin was reported in 1983 by Schimd and Hofheinz[73] who began their construction of the molecule with (-)-isopulegol(**17**)(Scheme 1). Compound (**17**) was converted to the benzyloxymenthone(**18**), which was elaborated to **19** in several steps. The final key step involved the irradiation of the methanol solution of **19** in the presence of singlet oxygen with Methylene Blue as sensitizer at -78°C to give the purported hydroperoxide intermediate (**20**). Treatment of the latter with formic acid in methylene chloride for 24 hours at 0°C gave 30% yield of artemisinin (**1**).



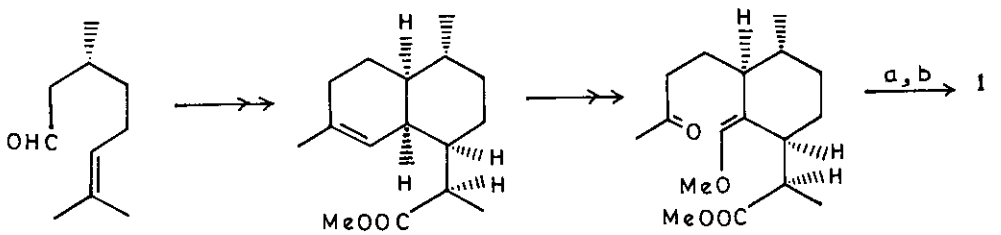
17 **18** **19**

20

Scheme 1

In 1986 Zhou *et al.*[74] also used a similar approach to synthesize artemisinin (**1**). They took R-(+)-citronellal(**21**) as the starting material and converted it into 12R-(-)-methyl dihydroartemi-

sinate (**22**) in 14 steps. The key intermediate (**23**) was obtained from compound (**22**) in 4 steps in 33% yield. Compound (**23**) on photo-oxidation and further treatment with 70% perchloric acid gave artemisinin (**1**). Hydroxylation of **23** with osmium tetroxide in ether at room temperature followed by treatment with hydrogen sulfide yielded deoxyartemisinin (**15**) (Scheme 2).



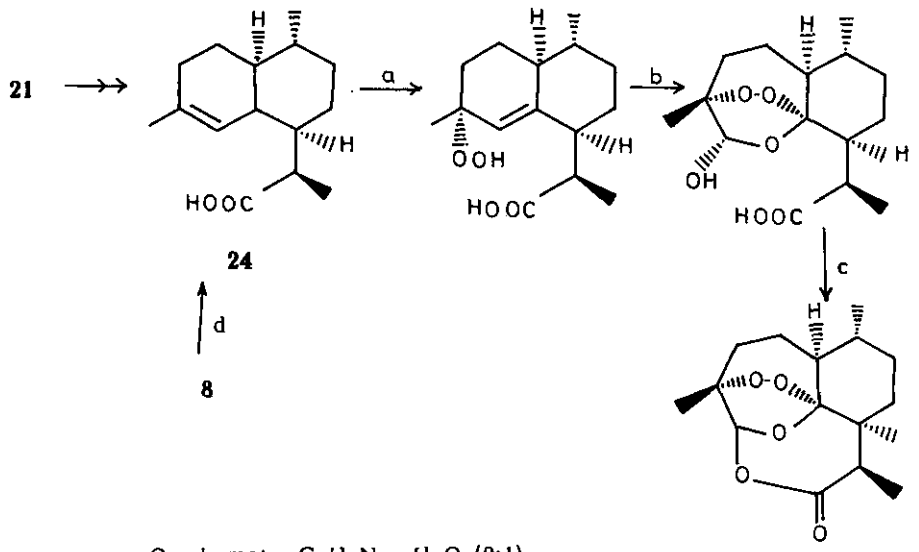
21 **22** **23**

a. O_2, Rose Bengal, $h\nu$
b. 70% $HClO_4$
c. OsO_4, H_2S

Scheme 2

In the same year Zhou[64] has also reported the synthesis of deoxyartemisinin (**15**), artemisinin A(**2**), artemisinin B (**3**), artemisinin E (**6**), artemisinin F (**7**) and another artemisinin analogue(**25**). Compound (**25**) has been synthesized from dihydroartemisinic acid (**24**) which was prepared from R-(+)-citronellal (**21**) as well as artemisinic acid (**8**) as shown in Scheme 3. Antimalarial tests showed that compound (**25**) possessed the same activity as artemisinin (**1**).

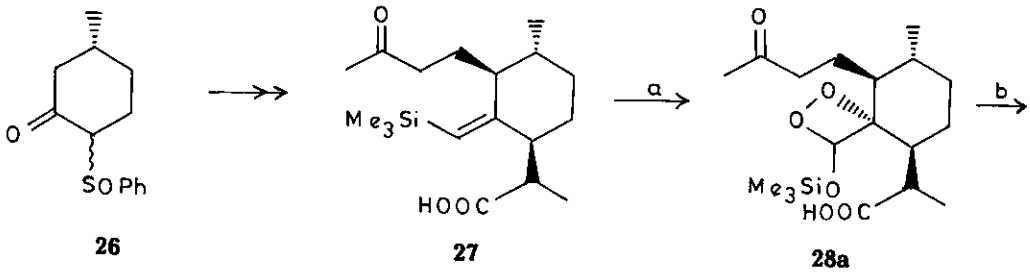
Artemisinin A(**2**) has also been prepared from artemisinic acid (**8**) through epoxidation, ozonolysis, lactonization and dehydration.[28,64,75]



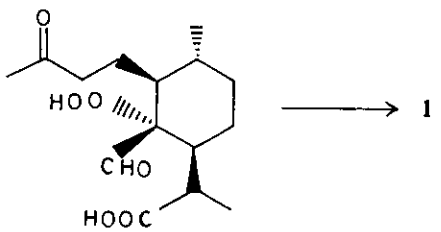
a. O_2, hemat., C_5H_5N - H_2O (9:1)
b. O_3, CH_2Cl_2 - C_5H_5N
c. TsCl - Et_3N, C_6H_6
d. $NaBH_4$ - $NiCl_2.6H_2O$, MeOH

Scheme 3

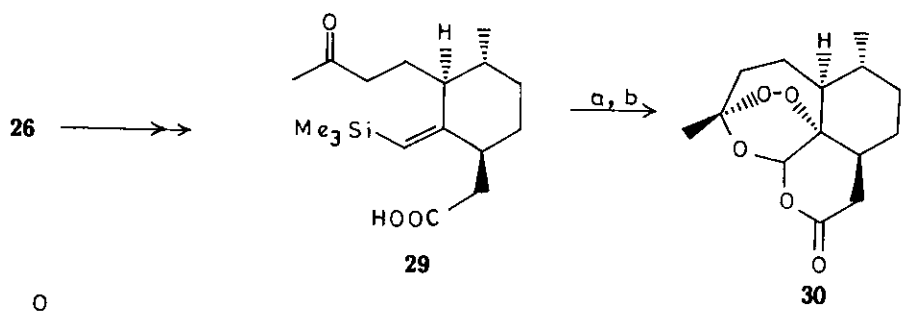
In 1987 Avery *et al.*[76] used the chiral sulfoxide derivative (**26**) (obtained from 3R-methylcyclohexanone) as the starting material and converted it into **27.** They have made use of the abnormal course of reaction of vinylsilanes with ozone which was discovered by Buchi *et al*.[77] Their key step involved the ozonoysis of **27** and the ring opening of the transient siloxydioxetane (**28a**) to labile α-hydroperoxyaldehyde (**28b**) with trifluoroacetic acid which undergoes further selective cyclization to give artemisinin (**1**) (37% yield). They have also reported the synthesis of (+)-11-desmethylartemisinin (**30**) (39% yield) and 11-isoartemisinin (**32**) from the same starting material via the intermediate (**29**) and (**31**), respectively (Scheme 4), but no mention has been made of their antimalarial activity.



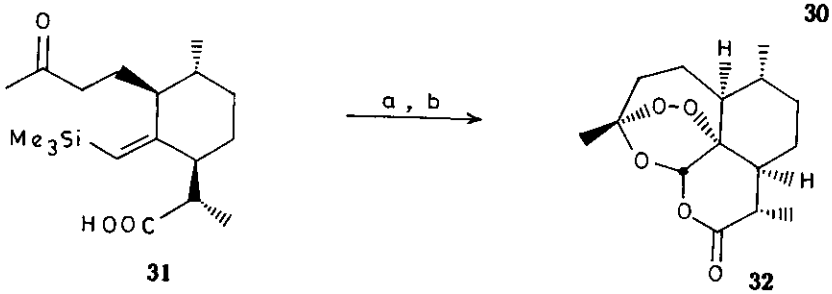
26 **27** **28a**



28b → **1**



29 **30**



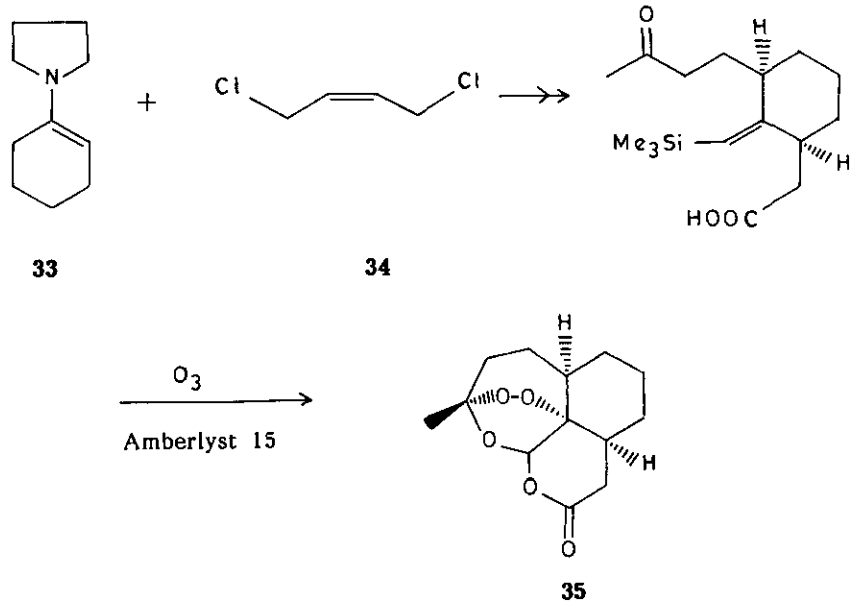
31 **32**

a. O_3, MeOH, -78°C

b. CF_3COOH, $CHCl_3$, H_2O

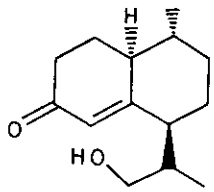
Scheme 4

In order to synthesize novel analogues of artemisinin, in 1989 the same group of workers[78] described the synthesis of a simplified analogue of artemisinin that does not have the C-10 and C-11 substituent methyl groups using a similar methodology as described above. They took pyrolidinocyclohexene (**33**) and *cis*-1,4-dichloro-2-butene (**34**) as starting materials and synthesized (+)-10,11-desmethylartemisinin (**35**) (53%) (Scheme 5). This analogue (**35**) displayed significant antimalarial activity against resistant strains of *P. falciparum*.



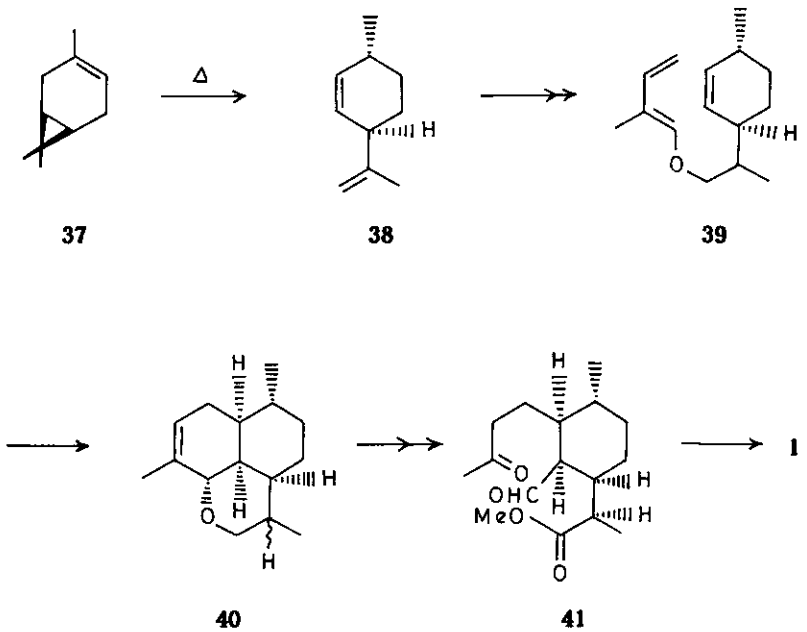
Scheme 5

In 1988, Kulkarni *et al.*[79] reported the synthesis of compound (**36**) which can be converted to artemisinin (**1**).



36

Recently, Ravindranathan *et al.*[80] have reported a stereoselective synthesis of artemisinin (**1**) from (+)-isolemonene (**38**) (Scheme 6) which can be easily obtained from (+)-car-3-ene (**37**), a cheap and abundantly available monoterpene. They prepared the intermediate (**39**) which underwent intramolecular Diels-Alder reaction to furnish the ether (**40**). The ether (**40**) was converted into compound (**41**) in seven steps which can be converted into artemisinin (**1**) as reported earlier[74](See Scheme 2). These workers have claimed that this synthesis proceeded without any



37 38 39

40 41

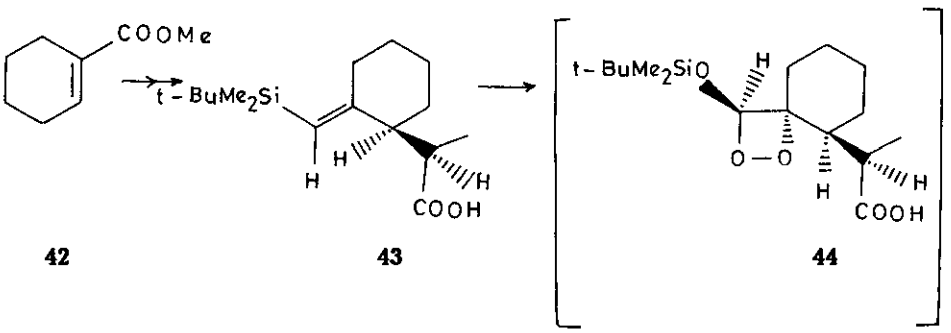
Scheme 6

stereoselective problems to generate the intermediate (**41**) in eight steps compared to fifteen steps involved in the earlier reported synthesis of Zhou *et al.*[74]

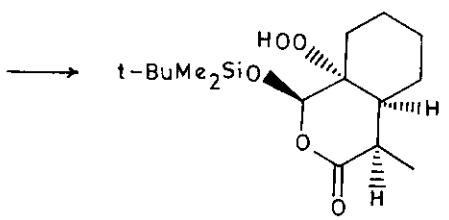
Total synthesis of artemisinin B(**3**) has been carried out in several steps by three research groups.[81-83] Photo-oxygenation of artemisinic acid (**8**) in pyridine-water mixture has been shown to provide artemisinin B(**3**) and (+)-deoxyisoartemisinin B(**11**).[84-87]

III. TOTAL SYNTHESIS OF ARTEMISININ ANALOGUES

Avery *et al.*[88] using their earlier methodology synthesized isolable structural portions to identify the essential structural features of a putative antimalarial pharmacaphore and designed improved analogues of artemisinin. They took commercially available carbomethoxycyclohexene (**42**) as the starting material (Scheme 7) and converted into carboxy vinylsilane (**43**) which is the key intermediate for this synthetic route. Treatment of **43** with ozone in methanol at -78°C gave the dioxetane (**44**) which upon standing at room temperature gave (+)-hexahydroisochroman-3-one (**45**) (57%) whose relative stereochemistry was determined by X-ray crystallographic analysis and was



42 **43** **44**



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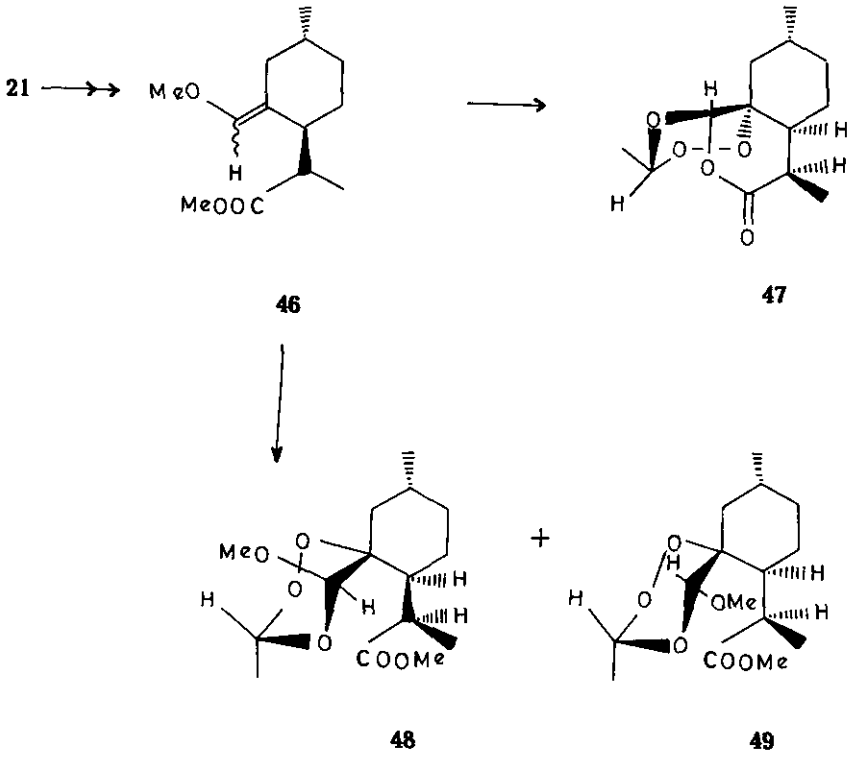
Scheme 7

found to resemble the C, D ring portion of the antimalarial artemisinin (**1**). Compound (**45**) does not show substantial *in vitro* antimalarial activity compared to artemisinin.

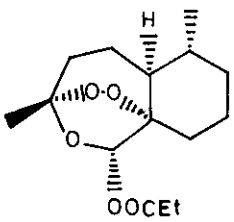
Keeping in mind the hypothesis from the studies on structure activity relationships on artemisinin and other related compounds that a requirement for the cyclic peroxide function to display antimalarial activity might involve the unique C-O-O-C-O-C-O-C-O-C=O moiety, Lee *et al.*[89] synthesized a simple analogue, desethanoartemisinin (**47**) (15% yield) and two other related compounds (**48**) and (**49**). R-(+)-Citronellal (**21**) was converted into an enol methyl ether (**46**) in five steps which on photo-oxygenation in the presence of acetaldehyde and THF as a solvent at -70 to -78°C gave compounds (**48**) and (**49**)(35%). However, photo-oxygenation of a methanolic solution of **46** in presence of acetaldehyde followed by HCl treatment and acidic hydrolysis with 60%



Scheme 8

$HClO_4$ furnished compound (**47**) (15%)(Scheme 8). The complete structure and stereochemistry of compounds (**47**),(**48**) and (**49**) were established by single crystal X-ray analysis by McPhail *et al*.[89]

Synthesis of (+)-7,11-secoartemisinin (**50**), a ring-D cleaved tricyclic analogue of (+)-artemisinin (**1**) has been reported by Avery *et al*.[90i] Ozonolysis of vinylsilane (**51a**) in methanol furnished dioxetane (**51b**) which on treatment with acid gave the stable bicyclic peroxyaldehyde (**51c**) readily transformable to **50**.



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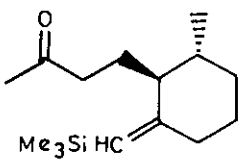
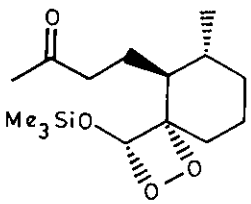


51a



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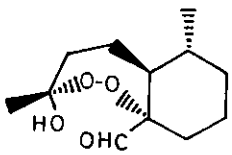
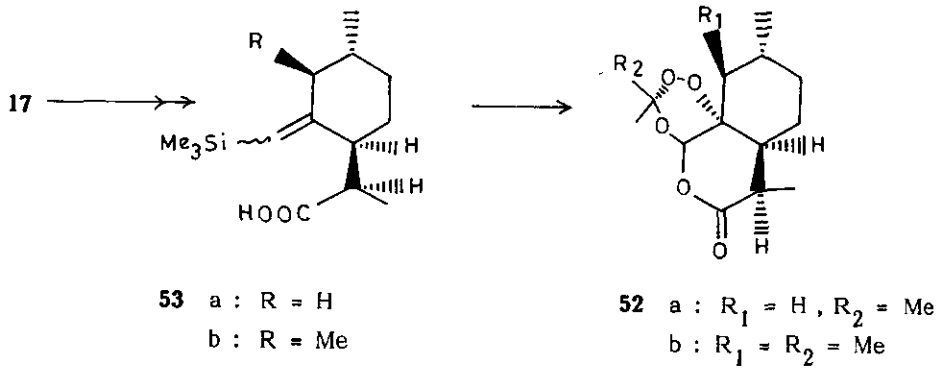


51c

Working on the same line Avery *et al.*[90ii] have also reported the synthesis of (-)-2-nor-2,3-secoartemisinin (**52a**) (desmethanoartemisinin) and (+)-2,3-secoartemisinin (**52b**) to study the effects on activity of different relative orientations of the peroxy grouping compared to that in artemisinin (**1**). The intermediates (**53a**) and (**53b**) were treated with ozone at -78°C followed by warming and addition of acetone and Amberlyst-15 (acid source) led to the production of compounds (**52a**) and (**52b**) respectively in good yield (Scheme 9). These analogues showed IC_{50} values ranging from 3 to 6 ng/ml in comparison to IC_{50} of (±)-artemisinin as 0.2-0.8 ng/ml when tested *in vitro* against drug resistant strains of *P. falciparum*.



53 a : R = H
b : R = Me

52 a : R_1 = H , R_2 = Me
b : R_1 = R_2 = Me

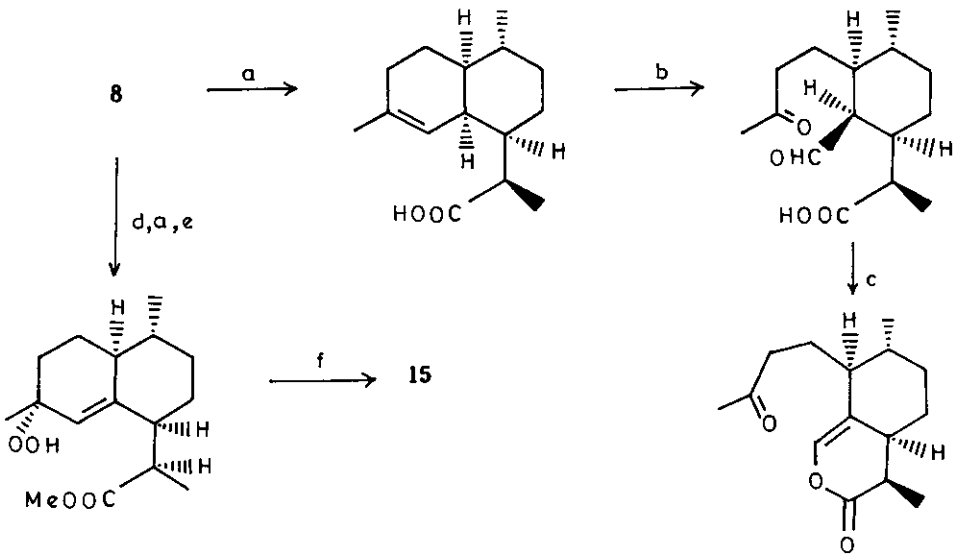
Scheme 9

IV. PARTIAL SYNTHESIS OF ARTEMISININ AND ITS ANALOGUES FROM ARTEMISINIC ACID

A. annua has been found to contain approximately 8-10 times more artemisinic acid (**8**) than artemisinin (**1**).[91] Several total synthesis of artemisinic acid (**8**) have been reported and biosynthetic studies have shown that it is the biogenetic precursor of artemisinin (**1**).[50,61,63,92-99]

Therefore, several workers have attempted the conversion of artemisinic acid (**8**) into artemisinin (**1**) and related compounds.

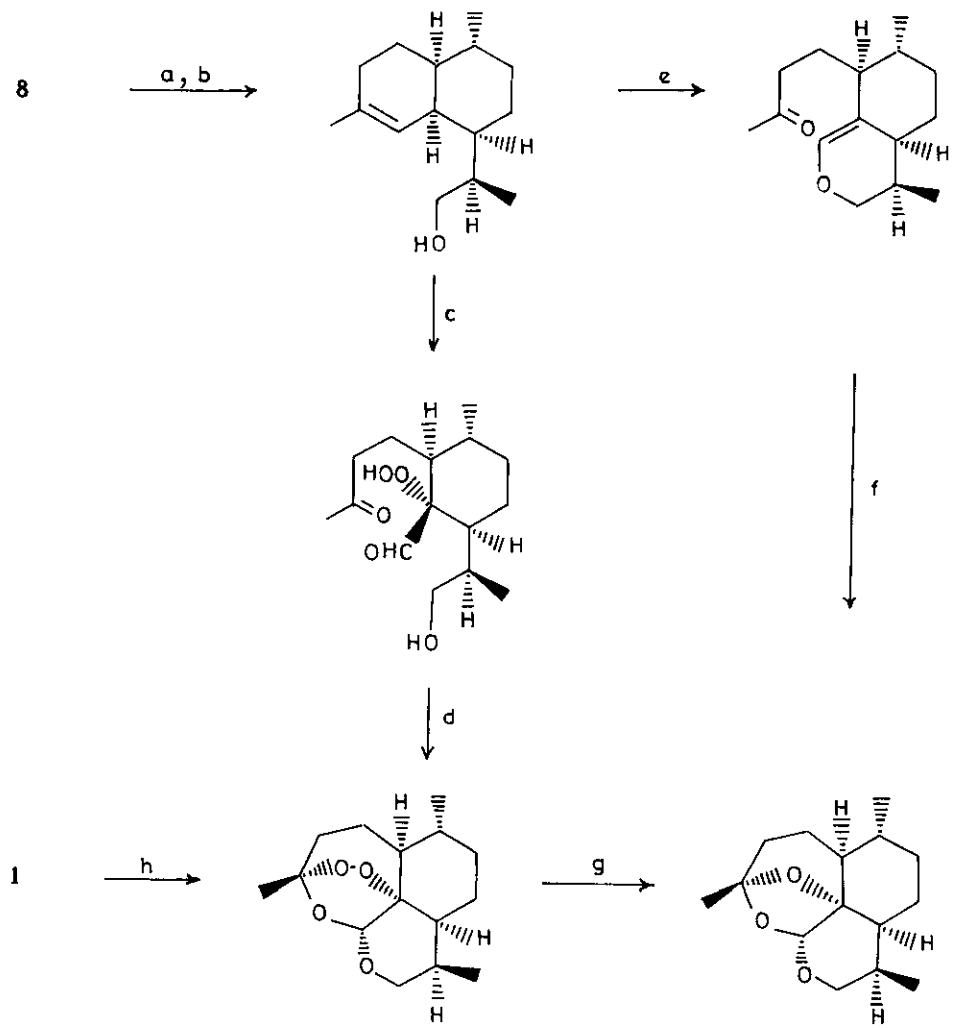
In 1986, Jung *et al.*[91] synthesized the enol ether (**54**) from **8** in 52% yield, but failed to photooxidize **54** into **1** as shown in Scheme 10. According to them, it might be due to decreased electron density of the double bond attached to the electron withdrawing ester group within the enol lactone ring. But they were able to convert artemisinic acid (**8**) into deoxyartemisinin (**15**) in natural configuration (42% yield). Compound (**15**) shows no antimalarial activity.



a. $LiBH_4$, $NiCl_2.2H_2O$, MeOH, room temperature
b. O_3, CH_2Cl_2 - MeOH, -78°C, 1 h
c. 70% $HClO_4$, THF-H_2O, room temperature, 7 h
d. CH_2N_2
e. $^1O_2$, MeOH
f. MCPBA, 0°C, 1 h

Scheme 10

However, in 1989 Jung *et al.*[100] successfully reported a short and stereospecific synthesis of (+)-deoxoartemisinin (**55**) (18% yield) and (-)-deoxodesoxyartemisinin (**56**) (22% yield) from

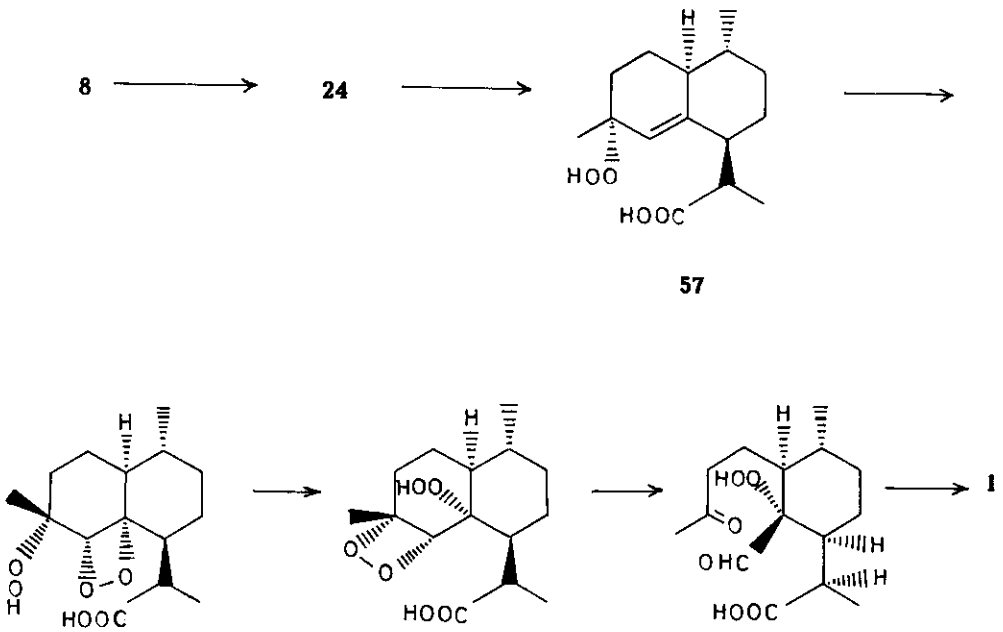


a. CH_2N_2, Et_2O, room temperature b. LAH, $NiCl_2.6H_2O$, MeOH, room temperature, 3 h c. $^1O_2$, Methylene Blue, CH_2Cl_2, -78°C, 2 h d. Dowex-resin, hexane, room temperature, 4 h e. O_3, CH_2Cl_2, -78°C, 2.5 h, p-TsOH, room temperature, 2 h f. MCPBA, $CHCl_3$, 0°C, 2 h g. H_2, 5% $Pd/CaCO_3$, EtOH, room temperature, 1 h then p-TsOH, toluene h. $NaBH_4$, $BF_3.OEt_2$, THF, 0°C, 1 h then reflux.

Scheme 11

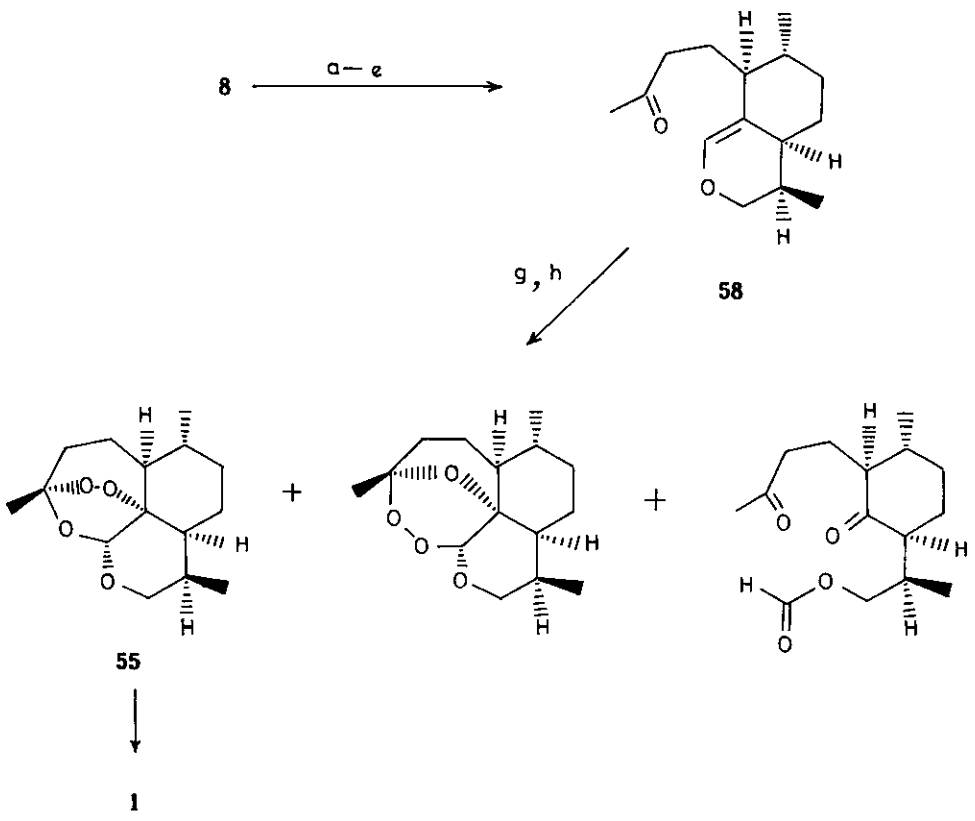
artemisinic acid (**8**) as shown in the Scheme 11. They have also converted **1** into **55** directly. (+)-Deoxoartemisinin (**55**) was found to show approximately eight times the antimalarial activity of artemisinin *in vitro* against chloroquine resistant malaria.[101]

At the same time Roth and Acton,[102] unaware of Jung *et al.*'s report,[100] converted artemisinic acid (**8**) into artemisinin (**1**) in two steps *via* reduction of the exocyclic methylene group and photo-oxidation of the resulting dihydroartemisinic acid (**24**). These workers have suggested that photo-oxygenation proceeds via the allylic hydroperoxide (**57**) as shown in Scheme 12.



Scheme 12

Ye and Wu[103] independently converted **8** into deoxoartemisinin (**55**) and artemisinin (**1**) through cyclic enol ether (**58**) (Scheme 13) which was earlier synthesized by the same workers.[104]

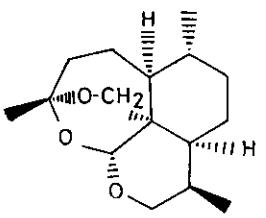


a. CH_2N_2 b. $NiCl_2.6H_2O$, $NaBH_4$ c. LAH d. O_3/MeOH-CH_2Cl_2 e. Me_2S f. Xylene, p-TsOH g. O_2, Sodium lamp, Methylene Blue, CH_2Cl_2, -70--80°C h. TfOTMS i. $RuCl_3$-$NaIO_4$/MeCN-H_2O-CCl_4.

Scheme 13

In order to find a better antimalarial medicine, Ye and Wu argued that in the synthetic studies of prostaglandin when one of the oxygen atom of the peroxide functional group is replaced by the methylene group, it still has biological activity.[104,105] So, they synthesized the corresponding carba-analogues of artemisinin (**1**), such as **59**, **60**, **61** and **62** starting from artemisinic

acid (**8**). However, in their report, no mention of the pharmacological activity of these compounds have been made.



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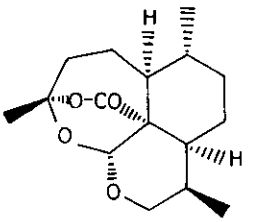
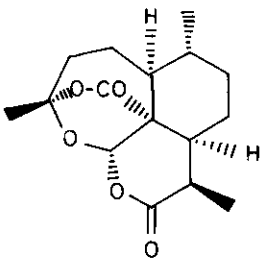
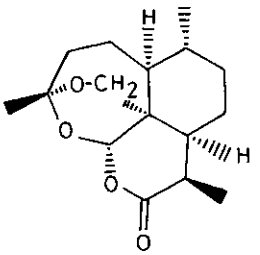


60

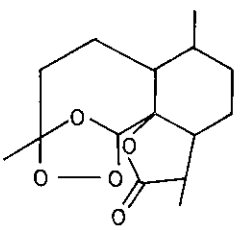


61

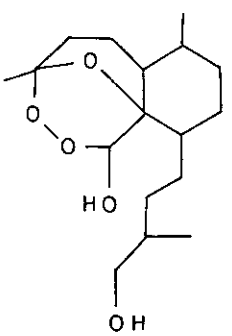


62

In 1988, Zhang *et al.*[106] synthesized new analogues of artemisinin i.e. **63** and **64** starting from the artemisinic acid (**8**) via ozonization. Compounds (**63**) and (**64**) showed poor antimalarial activity.

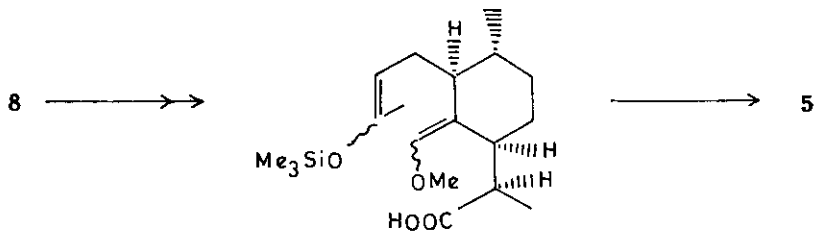


63



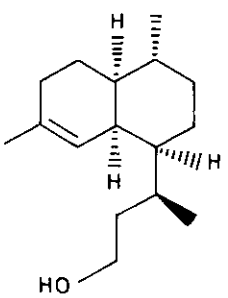
64

In 1989, Zhou *et al.*[107] synthesized artemisinin D(**5**) from artemisinic acid (**8**) *via* silyl enol ether (**65**). Lee *et al.*[108] have converted artemisinin (**1**) into artemisinin D(**5**) but it was found to be inactive.

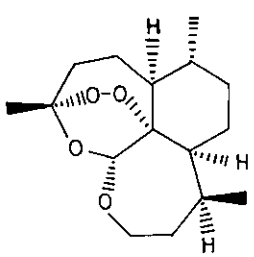


65

In 1989, Jung *et al.*[109] for the first time, investigated the effect of the size of D-ring of artemisinin analogues on the antimalarial activity and were successful in stereospecific conversion of artemisinic acid (**8**) via the alcohol (**66**) into a seven membered ring analogue of deoxoartemisinin (**55**) (7.6% overall yield). They named it as (+)-homodeoxoartemisinin (**67**). It was found to show 20 times less *in vitro* antimalarial activity compared to artemisinin (**1**) against chloroquin-resistant malaria. According to them enlargement of the D-ring allows greater flexibility of the overall ring system including the biologically active endoperoxide and hence decrease in the *in vitro* antimalarial activity. The increased flexibility of the polycyclic structure may lead to poor receptor fit or more probably decreased reactivity of the endoperoxide.



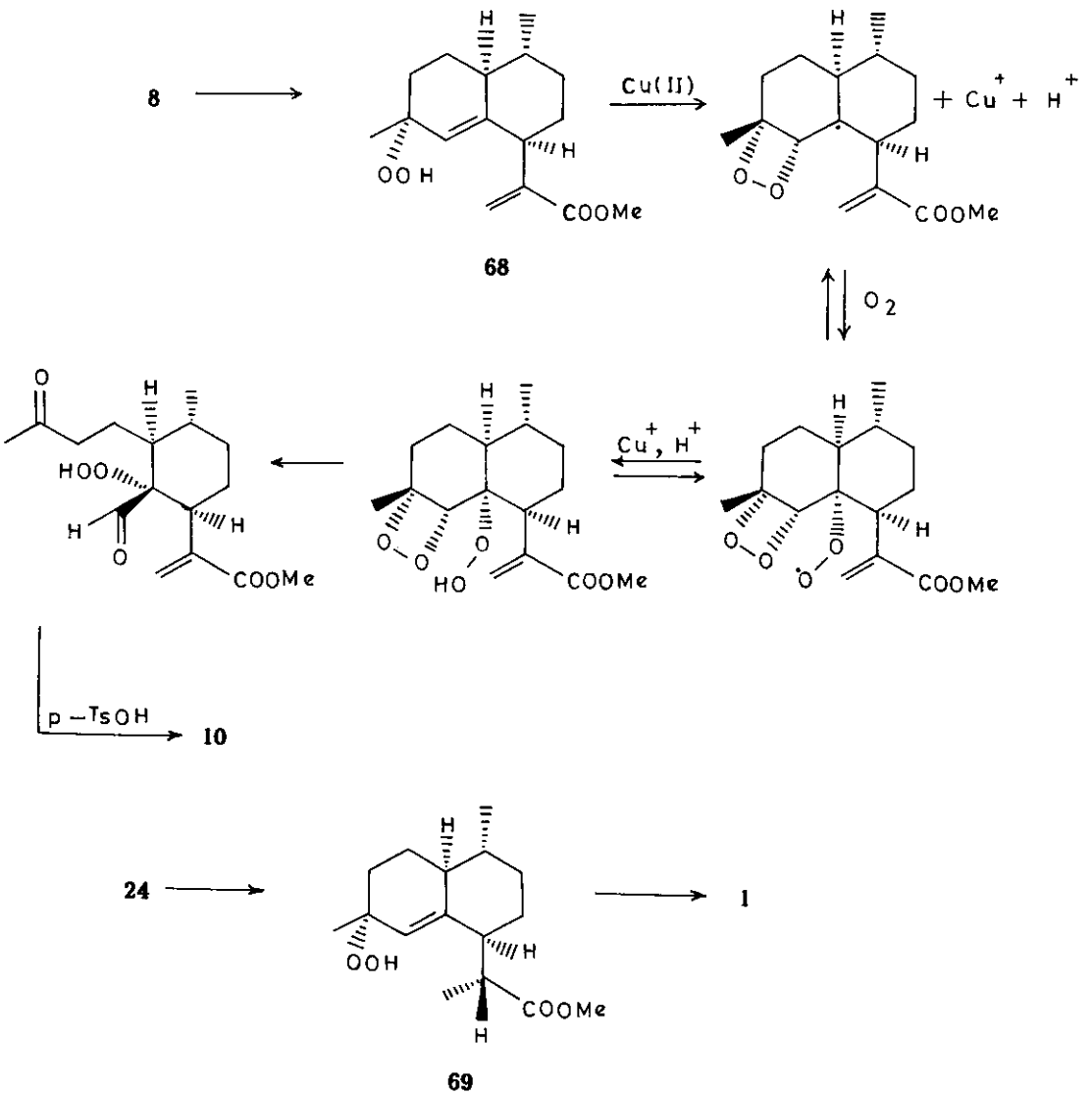
66



67

Recently, Haynes *et al.*[110] have converted artemisinic acid (**8**) and its dihydroanalogue (**24**) into artemisitene (**10**) and artemisinin (**1**) using a different strategy. Photo-oxidation of **8** and **24** in acetonitrile form the corresponding allylic hydroperoxides (**68**) and (**69**) which on treatment with a catalytic amount of copper(II) trifluoromethanesulfate and or without an iron(III) co-catalyst

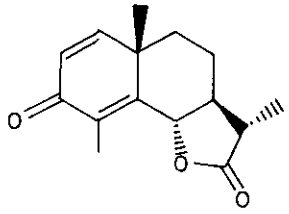
in dichloromethane under oxygen furnished compounds (**10**) and (**1**) respectively. The mechanism of these transformations has been described in Scheme 14.



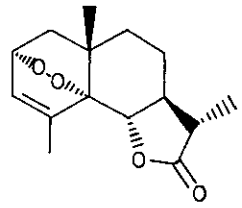
Scheme 14

V. SYNTHESIS OF PEROXIDES AND TRIOXANES

The demand for the antimalarial drug is too high that the plant source and other synthesis could not supply sufficient amount to the medicinal market to combat malaria infected with *P. vivax* and *P. falciparum*. So the search for discovering other antimalarial drugs related to **1** is being continued vigorously. In this connection, Lee *et al.*[111-116] screened different compounds with cyclic peroxy ring lacking sesquiterpene lactones and also a number of sesquiterpene lactones were examined for antimalarial activity but none was found to be active. Later on, Lee *et al.*[117] examined further the sesquiterpene lactones with the introduction of a cyclic peroxide function as potential antimalarial agents. They took α-santonin (**70**) as the starting material and synthesized its peroxide derivative (**71**) (60%) using Barrett and Buchi's method.[118] Compound (**71**) was tested for antimalarial activity *in vitro* against the chloroquin-resistant

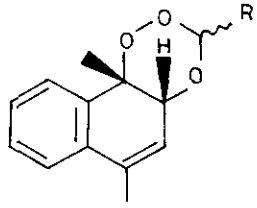


70

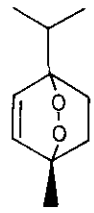


71

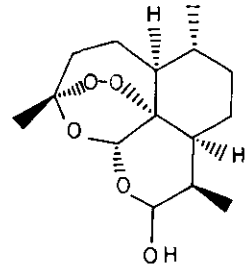
isolates of *P.falciparum* as well as *in vitro* against *P. berghei* in mice and was found to be very less active as compared to **1**.[23] According to them these results could seem to indicate that the requirement of the cyclic peroxide function for potent antimalarial activity among the



72 a : R = Me
b : R = CH_2OH
c : R = $-(CH_2)_6OH$



73

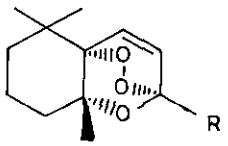


74

sesquiterpene lactones might be quite specific and could involve a unique C-O-O-C-O-C-O-C-O

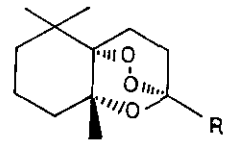
linkage as found in artemisinin. Therefore, in 1988 Jefford *et al.* reported the preparation of various benzopyranotrioxanes[119] and dihydronaphtho[1,2,4]trioxanes, their carbamates and ester derivatives (**72**).[120] Compounds (**72**) and ascaridole (**73**) were tested for antimalarial activity against the sensitive N-strains of *P. berghei* in mice. Modest activity was found in comparison to artemisinin (**1**) and dihydroartemisinin (**74**). The most active **72** were 12-18 times less potent than artemisinin (**1**).

Recently, Kepler *et al.*[121] undertook an investigation in an attempt to determine the minimum requirements for antimalarial activity of artemisinin like molecules that focuses on the unique 5-oxygen substituted 1,2,4-trioxane ring of artemisinin (**1**). As such, they synthesized some compounds containing a 1,2,4-trioxane ring, **75** - **83**. These compounds (**75** - **83**) were assayed in an



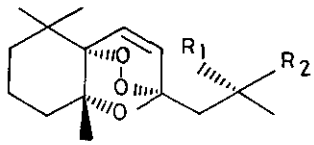
75 a : R = Me

b : R = CH_2COOMe



76 a : R = Me

b : R = CH_2COOMe

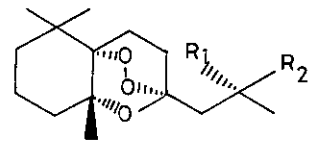


77 : R_1=H, R_2=OH

78 : R_1=OH, R_2=H

80 : R_1=H, R_2=OCOPh

81 : R_1=OCOPh, R_2=H



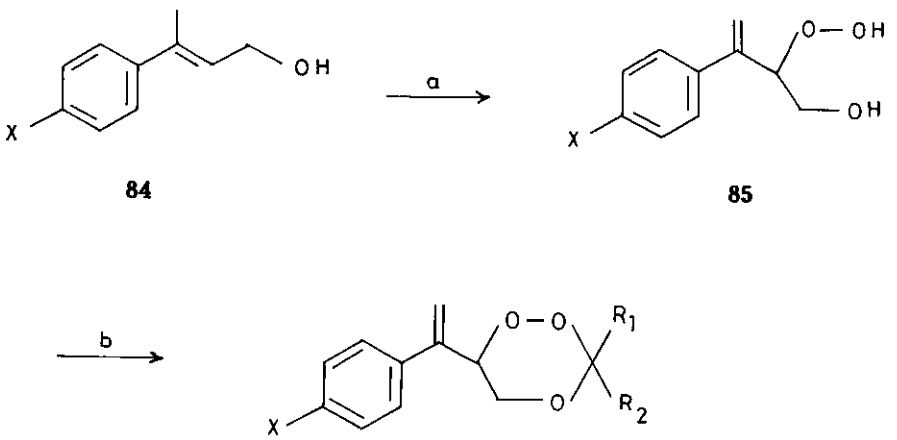
79 : R_1=OH, R_2=H

82 : R_1=H, R_2=OCOPh

83 : R_1=OCOPh, R_2=H

in vitro system for antimalarial activity against chloroquin-susceptible and chloroquin-resistant strains of *P. falciparum*, using normal method.[122,123] The most highly active compounds are **75b** and **82** which showed an IC_{50} of (96 and 39 ng/ml) and (24 and 99 ng/ml) for the chloroquin susceptible and resistant strains respectively. These compounds are inactive against the standard *P. berghei* in mice. The general lack of high activity indicates that the 1,2,4-trioxane ring system alone is not sufficient for antimalarial activity.

Most recently, Singh[124] has prepared a series of 1,2,4-trioxanes from 3-aryl-2-butenols (**84**) which on photo-oxygenation furnished 3-aryl-1-hydroxybut-3-ene-2-hydroperoxides (**85**). Compounds (**85**) were condensed with aldehydes and ketones to give 1,2,4-trioxanes (**86,87** and **88**)(Scheme 15).



84 **85**

86 $R_1 = R_2 = Me$

87 $\begin{matrix} R_1 \\ R_2 \end{matrix}\!\!>$ =

88 $\begin{matrix} R_1 \\ R_2 \end{matrix}\!\!>$ = OMe

X = H, Me, OMe, F, Cl

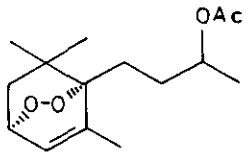
a. $^1O_2$, EtOH, Methylene Blue, O - 10°C, 10 - 16 h

b. Ketones or aldehydes, H^+/CH_2Cl_2, O°C, 12 h

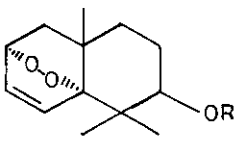
Scheme 15

Most of these compounds (**86–88**) were found to show antimalarial activity when tested *in vitro* against chloroquin-resistant *P. falciparum*; IC_{50} ranges from 2.86 to 222.46 ng/ml (IC_{50} of **1** is 0.65 ng/ml under same assay).

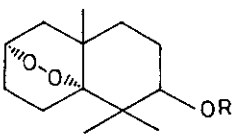
Kepler *et al.*[125] prepared a set of cyclic peroxides, analogues of the 5-oxygen substituted 1,2,4-trioxane ring structure of artemisinin, to get the information about the necessity of the artemisinin molecule. They prepared compounds (**89–97**) and assayed in an *in vitro* system for



89



90 : R = PhCO
91 : R = MeCO
92 : R = H
93 : R = BuOCO



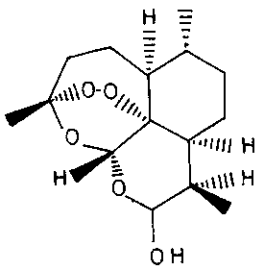
94 : R = PhCO
95 : R = BuOCO
96 : R = MeCO
97 : R = H

antimalarial activity against chloroquin-susceptible and chloroquin-resistant strains of *P. falciparum*. Compound (**93**) which showed an IC_{50} of 100 and 57 ng/ml respectively for the susceptible and resistant strains, was the most active of the compounds assayed. Compounds (**89–97**) were also tested for blood schizonticidal activity against *P. berghei* in mice and all were found to be inactive. The absence of significant activity indicates that the peroxide linkage alone is insufficient for activity.

VI. SYNTHESIS OF ETHER AND ESTER DERIVATIVES OF DIHYDROARTEMISININ

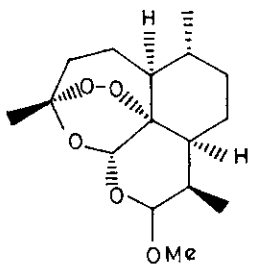
Although artemisinin rapidly supresses the parsitemias of *P. vivax* and *P. falciparum*, the problems encountered with recrudesence, as well as the desire to improve on the natural product, led to efforts to modify its chemical structure and to synthesize new artemisinin derivatives and related compounds.[126,127] The essentiality of the peroxy group became readily apparent on testing of seven other sesquiterpenes isolated from *A. annua* which lack that particular moiety. All are devoid of antimalarial activity. So, the main emphasis was given not to disturb the peroxy-linkage in the synthesis of artemisinin derivatives and related compounds.

Hydrogenation of artemisinin in presence of a $Pd/CaCO_3$ catalyst causes loss of one of the peroxide oxygen atom to give the epoxide desoxyartemisinin (**15**).[24,25,91] However, the peroxy group is uneffected when reduction is carried out with sodium borohydride and dihydroartemisinin (**74**) is produced in which the lactone group is converted to a lactol (hemiacetal) function.[24,25,128-130] Compound (**74**) retains the peroxide function and is more potent than artemisinin whereas deoxyartemisinin (**15**) does not show antimalarial activity.[91,131]



74

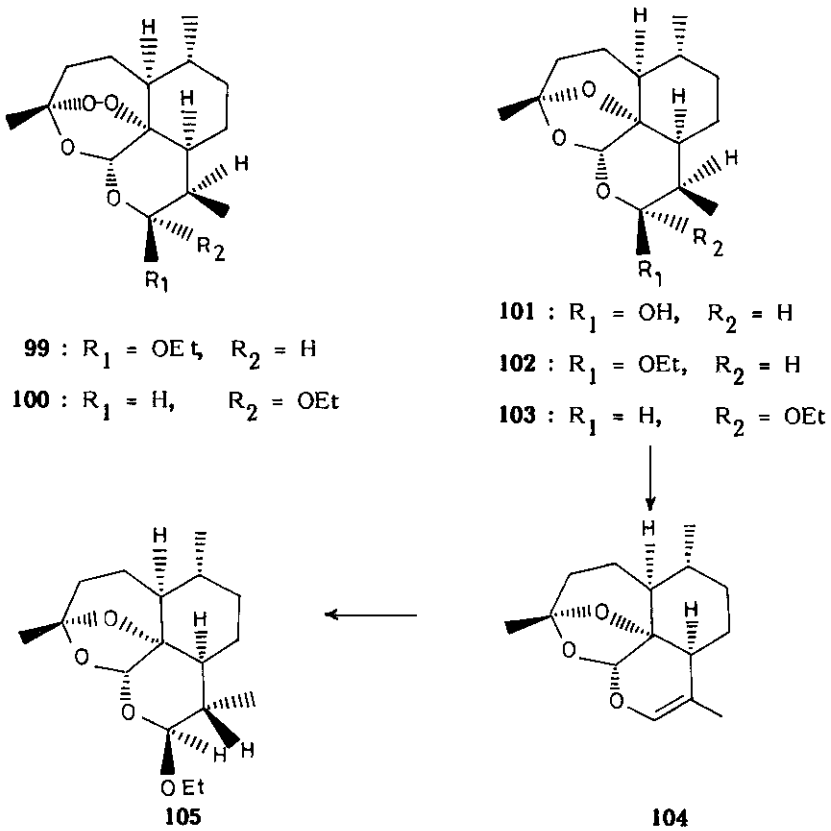
The ethers which have the advantage of being more oil soluble than artemisinin, are made by treating dihydroartemisinin (**74**) with an alcohol in the presence of boron trifluoride etherate.[132-134] Among the ether derivatives, the most active compound is the methyl ether (ß-epimer) (**98**) called artemether which exhibits significant antimalarial activity in mice infected with *P. berghei*.[131]



98

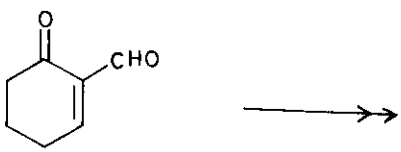
Arteethers (**99**) and (**100**) were prepared from **74** by etherification with ethanol in the presence of Lewis acid.[135] Absolute stereochemistry of **99** at C-12 was also determined and it is 2-3 times more potent than artemisinin;[136] but deoxy compound (**102**) was 100-300 times less potent *in vitro* than its peroxy precursor (**99**). Flippen-Anderson *et al.*[37] have shown that ethanol

saturated with hydrochloric acid converts deoxydihydroartemisinin (**101**), deoxyarteethers (**102**) and (**103**) and the enol ether (**104**) into 11-epi-deoxyarteether (**105**)(Scheme 16) which is less potent than artemisinin. Recently, Brossi *et al.*[130] have reported that ß-arteether (**99**) can be prepared from the α-isomer (**100**) via isomerisation with several reagents such as boron trifluoride etherate, p-toluenesulphonic acid, hydrochloric acid, boron tribromide, hydrobromic acid and trifluoroacetic acid at 50-80°C.



Scheme 16

In 1989, Wallace *et al.*[138] tried unsuccessfully to synthesize an analogue of arteether (**99**) from compound (**107**) which was synthesized by them from 2-formylcyclohex-2-en-1-one (**106**). However photo-oxygenation of **107** followed by acid treatment furnished compounds (**108**) and (**109**) only.



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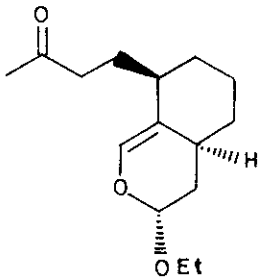

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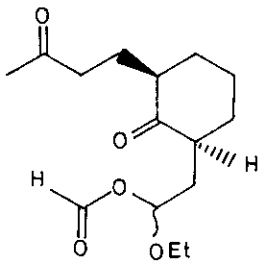

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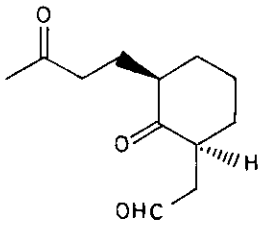
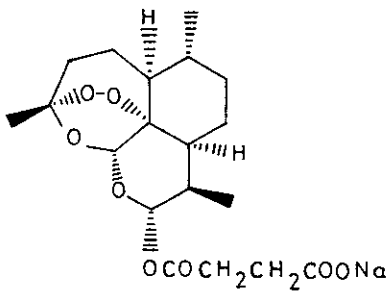


109

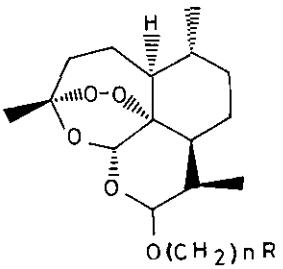
Among the artemisinin derivatives the greatest promise is the dihydroartemisinin half-ester of succinic acid,[139] whose water soluble sodium salt known as sodium artesunate (**110**) can be administered intravenously. Sodium artesunate exhibits potent antimalarial activity. When administered intravenously in saline to mice, the compound was about 5.2 times more potent



110

than artemisinin against both chloroquin-resistant and chloroquin-sensitive strains of *P. berghei*.[140,141] Compound (**110**) is more toxic than artemisinin; nevertheless, it is only about

1/50th as toxic to the cardiovascular system of rabbits as chloroquin[140] and less toxic than artemether. It is particularly effective against cerebral malaria. The compound is stable in an ampoule as a freeze-dried powder but in about 1 week in solution at physiological pH it loses the succinate moiety through hydrolysis. Lee *et al.*[141] reported the inhibition by the sodium artesunate (**110**) of cytochrome oxidase activity as a possible target for drug action within the intraerythrocytic trophozoite of mice infected with *P. berghei*. Artemisinin (**1**) and sodium artesunate (**110**) have also been shown to have a different mechanism of action from the other class of antimalarial agents,[141] such as sulfonamides, trimethoprim, and pyrimethamine, which are inhibitors of folic acid metabolism.[142] The utility of sodium artesunate, however, is impaired by its poor stability in aqueous solution due to the ease of hydrolysis of ester linkage.[143] In order to overcome the stability problem Lin *et al.*[144] prepared a series of water soluble, stable



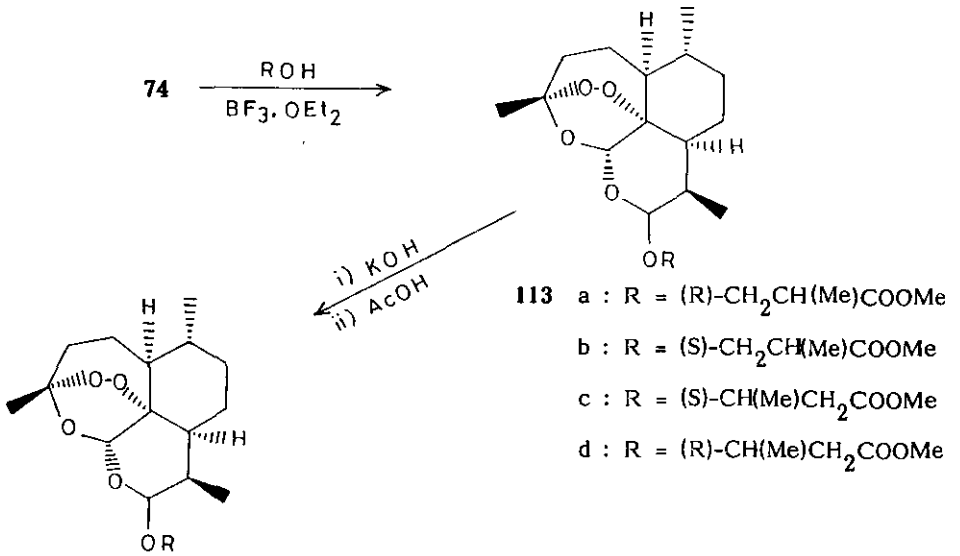
	111	**112**
a : n = 1	R = COOEt	R = COOH
b : n = 2	R = COOMe	R = COOH
c : n = 3	R = COOMe	R = COOH
d : n = 1	R = p-C_6H_4COOMe	R = p-C_6H_4COOMe

Scheme 17

derivatives of dihydroartemisinin (**74**) in which the solubilizing group, carboxylate, is on a moiety that is joined to dihydroartemisinin by an ether rather than an ester linkage. The new derivatives (**111a–d**) were prepared in good yield by treatment of **74** with an appropriate alcohol under boron trifluoride etherate catalysis at room temperature. All major condensation products are the ß-isomer. Hydrolysis of the esters (**111b–d**) with 2.5% KOH/MeOH solution gave the

corresponding potassium salts which were converted to free acids (**112b–d**) by acidification (Scheme 17). The derivatives were tested *in vitro* against two clones of human malaria, *P. falciparum* D-6 (Sierra Leone Clone) and W-2 (Indochina clone). No cross-resistance to the antimalarial agents mefloquin, chloroquin, pyrimethamine, sulfadoxine and quinine was observed. In general, the new compounds are more active against the W-2 than the D-6 strains. Esters (**111a–d**) possess activity comparable to that of the parent compounds (**1**) and (**74**), however, conversion of the esters to their corresponding carboxylates or acids (**112b–d**) with the exception of artelinic acid (**112d**) drastically decreases the antimalarial activities in both cell lines. Artelinic acid (**112d**) which is both soluble and stable in 2.5% K_2CO_3 solution, possesses superior *in vivo* activity against *P.berghei* and artemisinin.[144]

In continuation of their[144] efforts to prepare compounds with activity superior to that of artelinic acid (**112d**), Lin *et al.*[145] prepared a new series of hydrolytically stable and water soluble

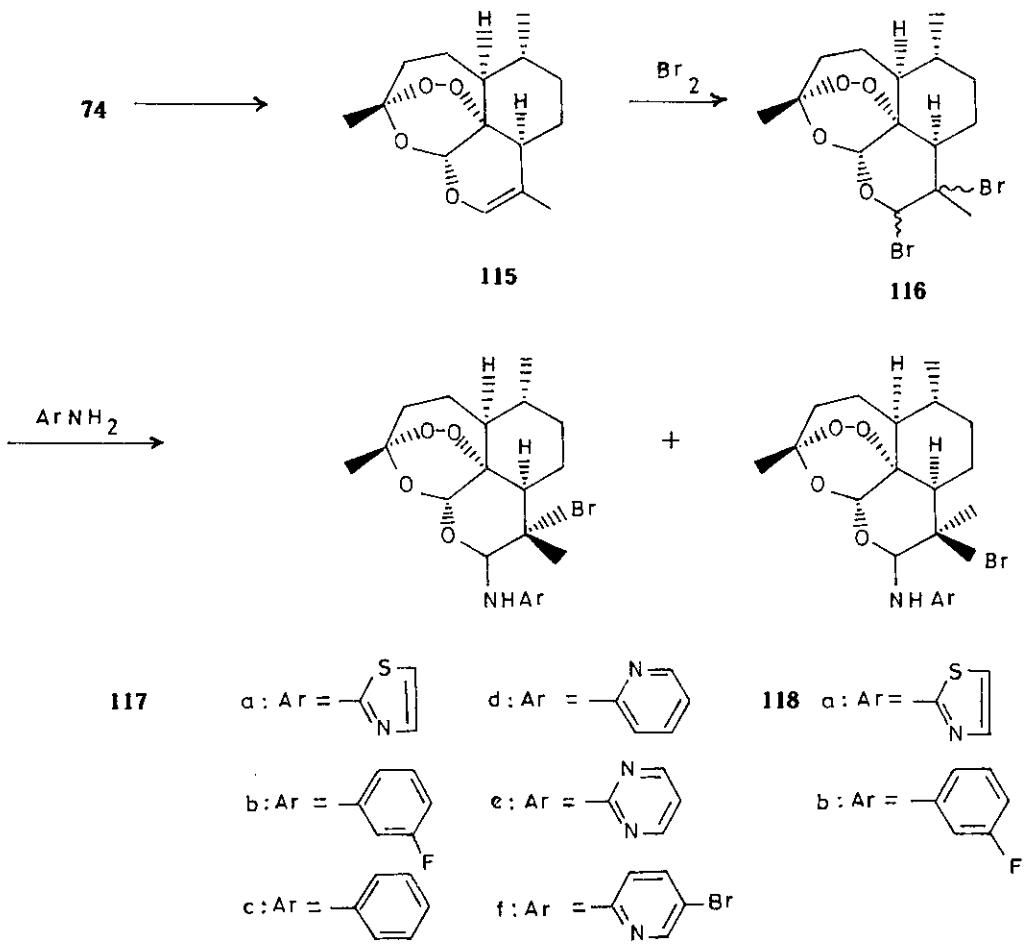


113 a : R = (R)-CH_2CH(Me)COOMe
b : R = (S)-CH_2CH(Me)COOMe
c : R = (S)-CH(Me)CH_2COOMe
d : R = (R)-CH(Me)CH_2COOMe

114 a : R = (R)-CH_2CH(Me)COOH
b : R = (S)-CH_2CH(Me)COOH
c : R = (S)-CH(Me)CH_2COOH
d : R = (R)-CH(Me)CH_2COOH

Scheme 18

dihydroartemisinin derivatives with optically active side chains and examined the impact of the stereospecificity of the intoduced alkyl side chain on biological properties. Dihydroartemisinin (**74**) was converted into ester derivatives (**113a-d**) which on saponification with 2.5% KOH/MeOH solution followed by acidification with cold dilute hydrochloric acid gave the corresponding carboxylic acids (**114a-d**) (Scheme 18). These water soluble dihydroartemisinin derivatives were tested *in vitro* against human malaria parasite *P. falciparum* D-6 (Sierra Leone clone) and W-2 (Indochina clone) and (**113a-d**) were found, in general, to possess superior activity to that of artemisinin (**1**), arteether (**99**) and artemether (**98**), but their corresponding carboxylic acids (**114a-b**) reduced their antimalarial activity by 10-100 fold. So far, the sodium salt of artelinic acid (**112d**) remains the most active of the water soluble derivatives of dihydroartemisinin.[145]

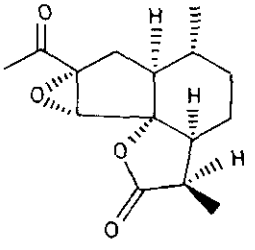


Scheme 19

Recently, the same group of workers[146] prepared a series of artemisinin derivatives containing bromo and heterocyclic or aromatic amine functions in the search for analogues with good water solubility and high antimalarial activity. Dihydroartemisinin (**74**) upon treatment with boron trifluoride etherate at room temperature gave a key intermediate 11,12-dehydrodihydroartemisinin (**115**) which is more active than artemisinin and is as active as dihydroartemisinin (**74**).[145] Treatment of **115** with bromine at low temperature gave the corresponding dibromides (**116**) which on condensation with various amine at $<-10^{\circ}C$ in anhydrous dichloromethane furnished the desired products (**117**) and (**118**) in 25-55% yield (Scheme 19). The new derivatives were tested *in vitro* against *P. falciparum* and found to be more effective against W-2 than D-6 clones and were not cross-resistant with existing antimalarials. The 3'-fluoroaniline derivative (**118b**) was the most active of the series with $IC_{50} \leq 0.16$ ng/ml, making it several fold more potent than **1** (IC_{50}=0.56-0.61 ng/ml). However, no significant *in vivo* antimalarial activity against *P. berghei* was observed in any of the new compounds tested.

VII. CHEMICAL TRANSFORMATIONS OF ARTEMISININ AND ITS ANALOGUES

Zhou *et al.*[147] have reported that artemisinin (**1**) on treatment with potassium carbonate in aqueous methanol at room temperature gave an intramolecular epoxidation product, α-epoxide (**119**) and a new peroxidic product (**120**) and established their structures. The antimalarial activity of these compounds have not been reported.



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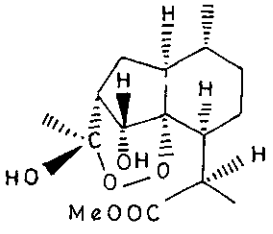
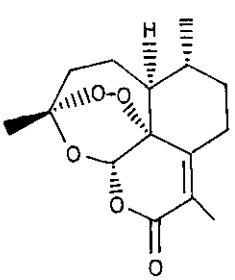
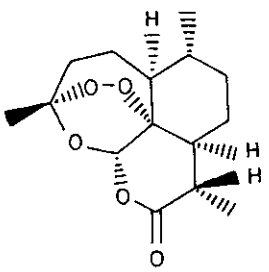


120

Acton *et al.*[148] have reported the conversion of artemisinin (**1**) into iso-artemisitene (**121**) and 11-epi-artemisinin (**32**). Bromination of **1** with NBS furnished a mixture of two isomeric bromides (**122**) which on treatment with DBU gave **121**. Isomerization of **1** to **32** was effected with base. An *in vivo* assay for antimalarial activity shows that artemisitene (**10**), compounds (**121**) and (**32**) are considerably less active than artemisinin (**1**).



121



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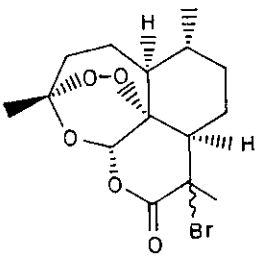
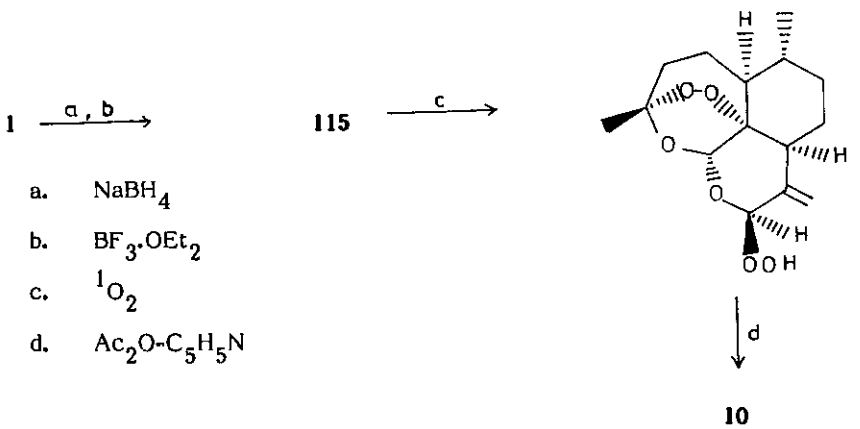


122

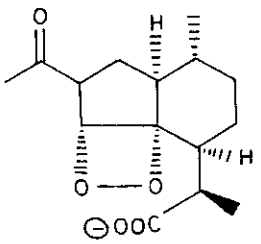
Artemisinin (**1**) has been converted into artemisitene (**10**) through different routes as shown in Scheme 20.[149-151]



Scheme 20

Artemisinin (**1**) is known to give a plethora of products in alkaline media.[147] recently, Liang *et al.*[152] reported that in alkaline media of K_2CO_3 or NaOH, artemisinin suffers lactonic ring opening with simultaneous unlocking of all the latent functionalities to give an important

intermediate (**123**) with a dioxetane ring. Rupture of the dioxetane ring in aqueous solution gives different derivatives of artemisinin such as **120, 124, 125** and **126** (0.08%). The stereochemistry of these products has been established.[147,152,153]



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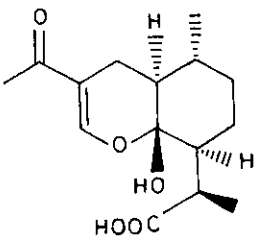

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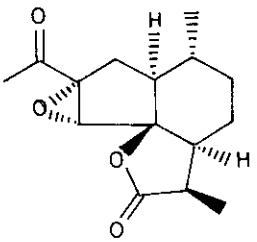
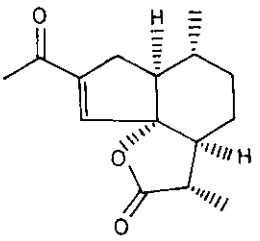
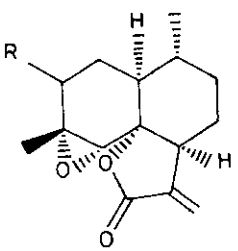


125



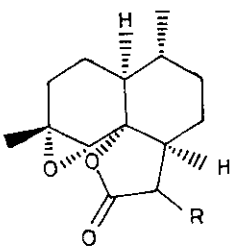
126

Several workers[154-156] have tried the microbial transformation techniques to isolate useful metabolites or artemisinin (**1**). Hufford *et al.*[157] examined the microbial transformations of artemisinin-B(**3**)and used *Aspergillus flavipes* to produce dihydroartemisinin-B(**127**) as the main



3 : R = H

128 : R = ß-OH

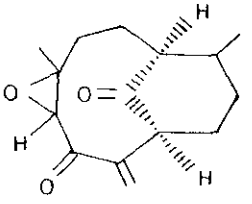


127 : R = ß-Me

129 : R = α - CH_2OH

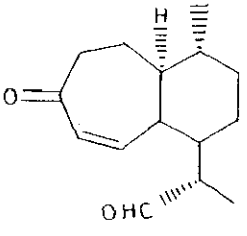
transformation product. Preparative-scale fermentation of **3** with *Beauveria bassiana* on the other hand had resulted in the production of two metabolites, 3-hydroxyartemisinin B (**128**) and 13-hydroxy-11-epidihydroartemisinin-B (**129**). The structures of these metabolites, all of which are new compounds, were established using chemical and spectroscopic techniques. The anti-malarial activity of these compounds have not been reported so far.

In 1988, Flippen-Anderson *et al.*[158] prepared lumiartemisinin B (**130**) by photochemical rearrangement of artemisinin B (**3**).

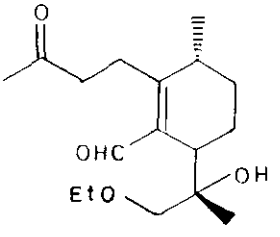


130

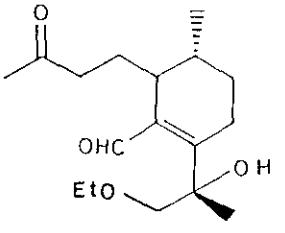
Most recently, the acid decomposition products of arteether (**99**) have been isolated.[159] The main products include α,β-unsaturated aldehydes (**131**) and (**132** or **133**). Formation of the latter involves the unusual partial hydrolysis of the acetal ring of arteether to an ether function.



131

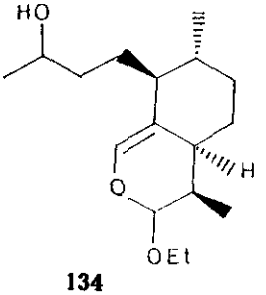


132



133

The endoperoxide group of arteether is also readily attacked by sodium dithionite in an alkaline



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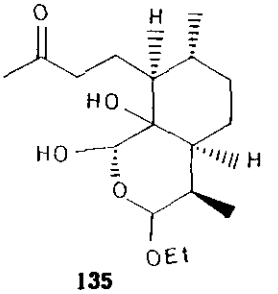
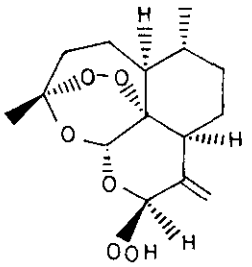


135

medium to give a product (**134**) in which both peroxidic oxygens were eliminated, while the acetal ring is unaffected. In a neutral medium, however, the product of reduction is a diol(**135**). The hydroperoxide (**136**) on reduction with polymer bound triphenylphosphine furnishes **137** whereas **138** is formed when free Ph_3P was used. Silica gel has been reported to convert **137** into **138**.[160]



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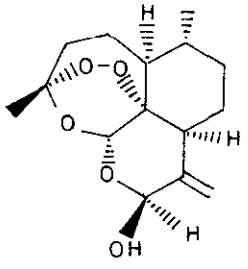

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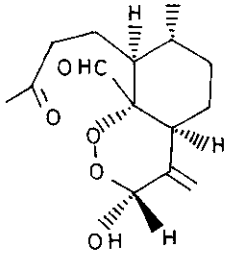
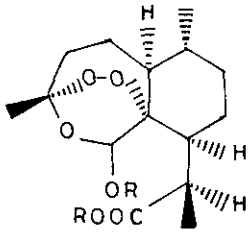


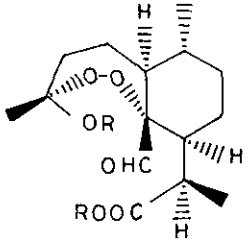
138

Earlier, Lee *et al.*[89] reported the synthesis of artemisinin related compounds including the 1',2',4'-trioxane lactone (**47**), 1',2',4'-trioxanes (**48** and **49**) and all were found to be 30-100 times less active than **1** in the *in vitro* antimalarial assay against the chloroquin-resistant *P. falciparum*, which indicates that the antimalarial activity of **1**-related analogues may be



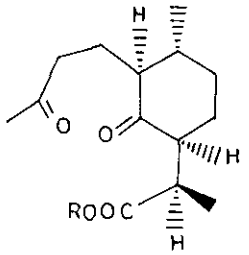
139 : R = Me

142 : R = Et



140 : R = Me

143 : R = Et



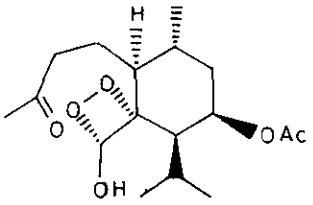
141 : R = Me

144 : R = Et

affected significantly by the steric environment of the 1',2',4'-trioxane ring among these molecules. It was considered that the ethane bridge between C-1 and C-4 of **1** might play an important role in this respect. To prove this hypothesis Lee *et al.*[161] synthesized C-1/C-4 ethane bridge-bearing 1',2',4'-trioxanes (**139**) and (**142**), endoperoxides (**140**) and (**143**), and diketones (**141**) and (**144**) by acidic degradation of **1**. Treatment of **1** with p-toluenesulphonic acid monohydrate or 14% hydrogen chloride in anhydrous methanol gave three products (**139**)(5.5%),

(**140**)(20.4%) and (**141**)(3.9%). On the other hand, treatment of **1** with p-toluenesulphonic acid monohydrate or 14% hydrogen chloride in anhydrous ethanol gave rise to **142** (9.9%), **143**(38.0%) and **144** (3.5%) respectively. The stereostructures of these compounds were established as 1',2',4'-trioxanes (**139**) and (**142**), endoperoxides (**140**) and (**143**) and diketones (**141**) and (**144**) respectively on the basis of ^{1}H and ^{13}C nmr and ms spectral data. The 1',2',4'-trioxanes (**139**) and (**142**) were found to be almost equipotent active with **1** in the *in vitro* antimalarial assay against the chloroquin-resistant *P. falciparum*. On the other hand, the endoperoxides(**140**) and (**143**) did not show noteworthy activity (about 100 times less active than **1**). Thus, it was proved that the steric environment of the 1',2',4'-trioxane ring system as found in **1**, **139** and **142** is vital for expressing antimalarial activity.[161]

Recently, Sharma *et al.*[162] have synthesized compounds (**145**), (**146**) and (**147**) from the naturally occurring cadinene (**148**) of authenticated stereochemistry.[163] Compound (**148**) was transformed into **149** which on photo-oxygenation in dichloromethene at -78°C furnished **145** and **146**. Trifluoroacetic acid converted **145** and **146** into **147**. Compound **145** was found to be five



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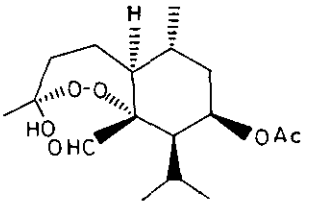
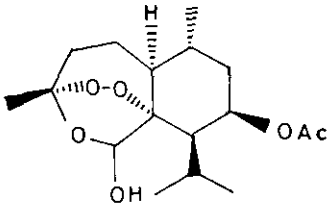
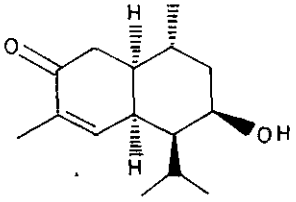


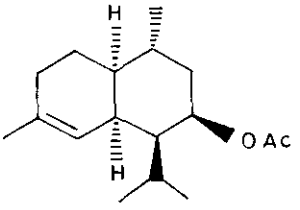
146



147



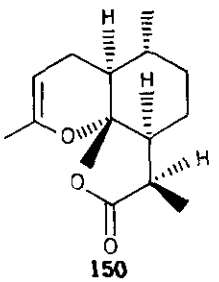
148



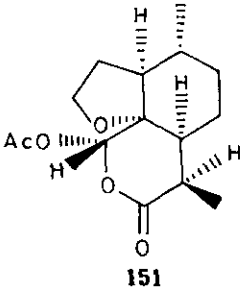
149

times more active than compound (**146**) when tested *in vitro* against African D-6 clone and Indochina W-2 clone of *P. falciparum* Bioassay of **147** as potential antimalarial agent is in progress.

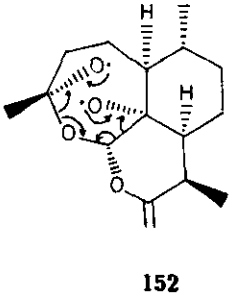
X-ray analysis of artemisinin showed that all the five oxygen atoms present in it are crowded on the same side of the molecule, and starting from O_5 an alternate carbon-oxygen chain of O_5-C_{12}-O_4-C_5-O_3-C_4-O_1-O_2-C_6 is formed. In this chain the carbon-oxygen bond distances, starting from C_{12}-O_4 are in sequence of short, long, short, long, short ....., but all lying well within the ranges of that of a normal single bond or a partial double bond. Probably, the lone pair of electrons of oxygen are no longer confined only on the oxygen atom and a variation of bond type has occurred. This may tend to make the entire molecule more stable.[64]. but in the course of developing a gc/ms assay method to be used in pharmacokinetic studies, it was necessary to examine the thermal stability of the compound and to identify its degradation or rearrangement products. Artemisinin has been reported to be labile to acidic or basic treatment, but unexpectedly stable in neutral solvents heated upto 150°C.[38] Twenty per cent of artemisinin was destroyed when treated with ethanol for 48 h as indicated by the appearance of a new spot at the origin.[23] Lin *et al.*[164] found that artemisinin is unaffected by heating neat for about 2.5 min at 200°C, i.e. about 50°C above its melting point;[18,23] but when heated with stirring in a flask without solvent in a silicon oil bath at 190°C for 10 min, artemisinin was decomposed to give three products (**150**)(4%), (**151**)(12%) and artemisinin D(**5**)(10%). The structures of these products were characterised by ir spectroscopy, cims, ^{1}H nmr, ^{13}C nmr and X-ray crystallography. The mechanism that accounts for the formation of these products involves the homolytic cleavage of epidioxide to generate a free radical intermediate (**152**) which



150



151

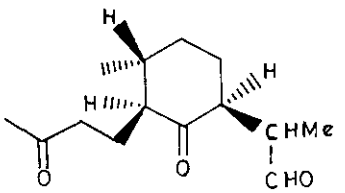


152

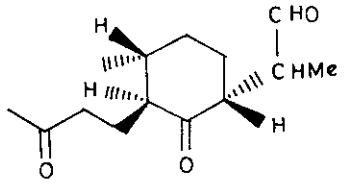
rearranges or decomposes to give the observed products. Compound (**5**) was reported to be a

constituent of *A. annua*[57] and was also synthesized from artemisinin. It does not show any antimalarial activity.[108]

In 1986, Lin *et al.*[165] again reported on the thermal decomposition products of dihydroartemisinin (**74**). Dihydroartemisinin (**74**) when heated neat in a round bottom flask for three minutes in an oil bath preheated to 190°C gave a mixture of deoxyartemisinin (**15**)(30%) and another product (**153**) (50%) consisting of two epimers (**153a**) and (**153b**). The structures of these compounds were established by spectral analysis.



153(a)



153(b)

In recent years artemisinin and its analogues have been put to various kind of pharmacological screening studies.[66,136,166-186]

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