Qinghaosu (Artemisinin): An Antimalarial Drug from China

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Summary. The herb *Artemisia annua* has been used for many centuries in Chinese traditional medicine as a treatment for fever and malaria. In 1971, Chinese chemists isolated from the leafy portions of the plant the substance responsible for its reputed medicinal action. This compound, called *qinghaosu* (QHS, artemisinin), is a sesquiterpene lactone that bears a peroxide grouping and, unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. The compound has been used successfully in several thousand malaria patients in China, including those with both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. Derivatives of QHS, such as dihydroqinghaosu, artemether, and the water-soluble sodium artesunate, appear to be more potent than QHS itself. Sodium artesunate acts rapidly in restoring to consciousness comatose patients with cerebral malaria. Thus QHS and its derivatives offer promise as a totally new class of antimalarials.

The first and last time a natural product gained wide acceptance as a treatment for malaria was in the 16th century. It was then that the therapeutic action of the bark of the cinchona tree was disclosed by the natives of Peru to Jesuit missionaries, who, in turn, brought the word of its utility to Europe. Quinine, the alkaloid isolated in 1834 from cinchona bark by the French chemist Pelletier, became the main treatment for malaria until the 1930's when synthetic antimalarials were developed. Plants, in addition to cinchona, that have been used against fevers and malaria include *Dichroa febrifuga*, which grows in China. However, its alkaloid, febrifugine, was found to be too toxic for use in humans. Recently, again in China, a naturally derived antimalarial compound has been investigated that may, eventually, rival quinine in importance. In view of the number of chloroquine-resistant strains of *Plasmodium falciparum* emerging in Asia, South and Central America, and Africa, the need for new antimalarials cannot be overemphasized (*1*).

In 1967 the government of the People's Republic of China embarked on a systematic examination of indigenous plants used in traditional remedies as sources of drugs (*2*). One such plant, a pervasive weed with a long history of use, is known as *qing hao* (*3*) (*Artemisia annua* L., sweet wormwood, annual wormwood). Its earliest mention occurs in the *Recipes for 52 Kinds of Diseases* found in the Mawangdui Han dynasty tomb dating from 168 B.C. In that work, the herb is recommended for use in hemorrhoids. This plant is mentioned further in the *Zhou Hou Bei Ji Fang* (Handbook of Prescriptions for Emergency Treatments) written in 340 A.D. The author, Ge Hong, advised that to reduce fevers one should soak one handful of *qing hao* in 1 sheng (about 1 liter) of water, strain the liquor, and drink it all (*4*). Later, Li Shizhen, the famous herbalist, whose death 390 years ago was recently commemorated in China (*5*), wrote in his *Ben Cao Gang Mu* (Compendium of Materia Medica) of 1596, that chills and fever of malaria can be combated by *qing hao* preparations (*6*, *7*). A decoction of *A. annua* and *Carapax trionycis* was suggested in the *Wenbing Tiaobian* in 1798 as a treatment for malaria (*7*).

Attempts to confirm the antipyretic and antimalarial activity of a hot-water extract of *A. annua* were disappointing. In 1971, it occurred to an investigator that low-temperature extraction of the plant, that is, with ethyl ether, should be tried (*2*). Crude ether extracts produced encouraging results in mice infected with the malaria parasite *Plasmodium berghei*. Further refinement of the antimalarial fractions led in 1972 to the isolation of a plant constituent that had not been reported previously in the chemical literature. The Chinese named the crystalline compound *qinghaosu* (*8*) [QHS, *qing hau sau* (*9*), arteannuin (*6*)], meaning "active principle of *qing hao*," and the more Western sounding name, "artemisinine." Because the material is a terpene, rather than an alkaloid or amine, which the "ine" suffix suggests, the name "artemisinin" (*10*) is preferred by *Chemical Abstracts*.

Chinese researchers have reported, without giving specific details, that 30 other species of *Artemisia* have been examined but that none yield extracts with antimalarial activity (*11*). American workers (*12*) have extracted *A. ludoviciana, A. vulgaris, A. schmidtiana, A. pontica, A. arbuscula*, and *A. dracunculus*, and none of these show the presence of QHS.

Plant species of the genus *Artemisia*, a member of the Compositae family, that are better known than *A. annua* include *A. absinthium*, used until the 1920's to prepare the narcotic and now illegal drink, absinthe; *A. dracunculus*, also known as tarragon, used as a spice in cooking and to flavor vinegar; and *A. tridentata*, called sagebrush in the western United States.

Isolation and Structure Determination of *Qinghaosu*

Other than indicating that ethyl ether extraction was used to prepare QHS from the aerial portions of *A. annua*, the Chinese literature provides no details of the isolation method. Investigators at the Walter Reed Army Institute of Research (*12*) located some *A. annua* (see Fig. 1) growing in the Washington, D.C., area (*13*), and extracted various air-dried components of the plant with several nonprotic solvents, of which petroleum ether (30° to 60°C) was the most satisfactory (*14*). The extract was then chromatographed on silica gel with chloroform-ethyl acetate being used as the eluant. Fractions rich in QHS crystallize readily, and recrystallization was effected from cyclohexane (*12*) or 50 percent ethanol (*15*) to give fine, colorless needles, melting point 156° to 157°C (*6*) and 158° to 159°C (*12*). QHS was obtained in 1983 from the dried leaves or flowers in 0.06 percent yield from plants growing in the Washington, D.C., vicinity. By comparison, yields in China (*11*) range from 0.01 to 0.5 percent with the varieties growing in Sichuan province, which are apparently the richest in QHS (*16*).

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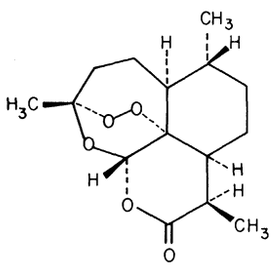
The amount of QHS in the extract can be measured by high-performance liquid chromatography and the use of an electrochemical detector (*17*). Benzoyl peroxide is used as an internal standard. Another method is based on thin-layer chromatography with visualization being achieved by means of *p*-dimethylaminobenzaldehyde (*16*). Studies aimed at determining the cellular location of QHS in the leaves of *A. annua* have been performed by Chen *et al.* (*18*).

Other terpenes and related compounds that have been found in *A. annua* are: 1,8-cineole, borneol acetate, 1-β-pinene, cuminal, β-caryophyllene, coumarin, stigmasterol, camphene, cadinene, arteannuin B, camphor, β-farnesene, arteannuin A, hydroarteannuin, scopolin, scopoletin, artemisia ketone, artemisic acid (artemisininic acid, qing hao acid), and benzyl isovalerate (*19–22*).

The empirical formula for QHS, $C_{15}H_{22}O_5$, arrived at through elemental analysis and high-resolution mass spectrometry (*7, 9, 23*), suggests that the compound is a sesquiterpene (Fig. 2). Its infrared spectrum shows an intense peak at 1745 cm^{-1} (δ-lactone) and peaks at 831, 881, 1115 cm^{-1} (peroxide). The presence of the unusual peroxy group was verified by the quantitative reaction of QHS with triphenylphosphine. The ^1H 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 (*7, 23*). The structure of QHS, **1**, as well as its



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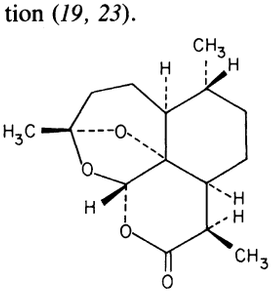
relative configuration, was determined unambiguously by x-ray diffraction (*7, 15*). Its absolute configuration has also been ascertained (*15, 24*), and the *trans* configuration of the lactone ring was arrived at by comparison of its ORD spectrum (*23*) with that of arteannuin B, a structurally related sesquiterpene isolated from *A. annua* in Yugoslavia (*19*).

Thus, QHS joins the terpene ascaridole, and the sesquiterpenes yingzhaosu A (*25*) and benghalensin A (*26*), as one of the few naturally occurring endoperoxides (*27*). The compound is related to the cadinane or amorphane class of sesquiterpenes which are defined by their *cis*-decalin skeleton.

Qinghaosu is poorly soluble in water (*28*) and decomposes in other protic solvents, probably by the opening of the lactone ring. It is soluble in most aprotic solvents, is unaffected by them up to 150°C (*29*), and is poorly soluble in oil. Unlike ascaridole, which is very sensitive to heat (*31*), QHS shows a remarkable thermal stability. It does not explode at its melting point, as one might expect, but rather can exist unchanged for about 3 minutes at 50°C above its melting point and can be purified by sublimation (*32*).

Chemical Reactions of QHS

Hydrogenation of QHS in the presence of a $Pd/CaCO_3$ catalyst causes loss of one of the peroxide oxygen atoms to give the epoxide, desoxyartemisinin, **2** (*19, 23*). The peroxy group is unaffected, however, when reduction is carried out instead with sodium borohydride. Here, the product dihydroqinghaosu (**3**, R=H, dihydroartemisinin, DHQHS) is produced, in which the lactone group is converted to a lactol (hemiacetal) function (*19, 23*).



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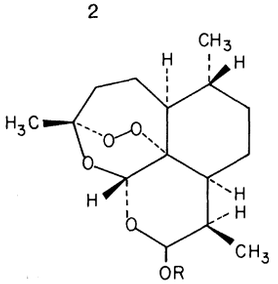

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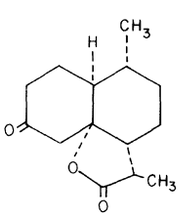
Treatment of QHS with a mixture of 10 percent sulfuric acid in glacial acetic acid at room temperature for 1 hour causes the fission of the peroxide linkage and the loss of the elements of formaldehyde (*24, 33*). The newly formed compound, **4**, is a decalone with a γ-lactone ring fused *cis* to it. A mechanism for its formation has been proposed (*29*) and, recently, its synthesis from (R)-(+) citronellal has been reported (*30*).



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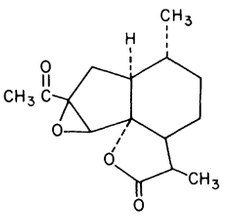
The action of potassium carbonate in methanol at room temperature on QHS initially causes disruption of its lactone ring. This leads to further dismantling of the molecule and, finally, to a series of complex condensation reactions. Only two compounds, obtained in low yields, namely **5** (*24*) and **6** (*29*), have been identified thus far.



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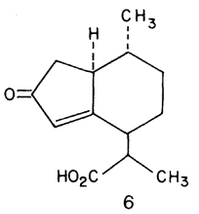

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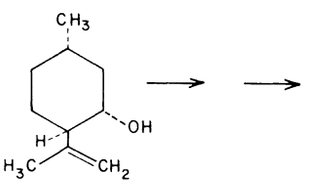
Attempts have been made to synthesize QHS (*34, 35*). In 1983, a total synthesis of QHS was reported in a note by Schmidt and Hofheinz (*36*), who began their construction of the molecule with (−)-isopulegol, **7**. The final key steps involved the irradiation of **8** in the presence of singlet oxygen at −78°C to give the purported hydroperoxide intermediate, **9**. Treatment of the latter with formic acid in methylene chloride for 24



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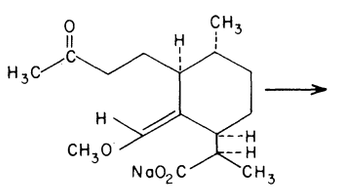

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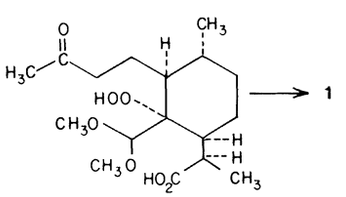

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hours at 0°C gave a 30 percent yield of QHS. Recently, another synthesis of 1 was reported from the People's Republic of China (*37*). These complex syntheses, while being of great interest, do not provide feasible methods for the large-scale production of QHS.

Fig. 1. *Artemisia annua* growing in the Washington, D.C., area in early August (left) and flowering in mid-September (right). Flowers are small (1 to 2 mm in diameter) and pale yellow in color. Leaves and flowers have a pleasant aromatic odor.

Testing for Antimalarial Activity

Assay of QHS against *P. falciparum* in vitro revealed that its potency is comparable to that of chloroquine in two Hainan strains (*38, 39*) and of mefloquine in the Camp (chloroquine-susceptible) and Smith (chloroquine-resistant) strains (*12*). Milhous *et al.* (*40*) compared the activity of QHS in vitro with that of other well-known antimalarials and reported that the compound is both potent and rapidly acting. The efficacy of QHS relative to some of its derivatives has also been evaluated (*41, 42*).

In mice infected with *P. berghei* in the erythrocytic stage, clearance of parasites could be accomplished by the oral administration of 50 milligrams of QHS per kilogram of body weight daily for 3 days (*6*). The median effective dose (ED_{50}) was 138.8 mg/kg. In a later study (*4*), QHS was given to mice orally as a water suspension or intramuscularly as either a water or oil suspension. The oil suspension was most effective and exhibited activity of the order of that shown by chloroquine.

Monkeys infected with *P. cynomolgi* sporozoites and treated with QHS daily (200 mg/kg) by intubation for 3 days, were cleared of the parasitemia completely in 2 to 3 days. However, there was relapse in five of the seven test animals (*6*). In a continuation of this work, four monkeys infected with *P. cynomolgi* sporozoites developed a parasitemia on days 7 or 8 after infection despite their being given a prophylactic regimen (100 mg/kg) of QHS daily for 6 days starting either on day 1 or day 6 after infection. Controls also developed a parasitemia on day 8 after infection. These studies suggest that QHS has no practical effect on either the early or persistant exoerythrocytic tissue stage at these dosages. In chickens infected with sporozoites of *P. gallinaceum* and treated with QHS, 200 mg/kg daily for 6 days after infection, parasitemia developed on days 8 and 9 as it did also in the controls. Again, QHS had no detectable prophylactic effects on the preerythrocytic stages (*6*).

Early reports of the toxicity of QHS suggested that the compound was almost incapable of producing ill effects (*6*). A later and more detailed study (*43*) verified the compound's low toxicity. Median lethal doses were as follows: mouse (oral administration) 4228 mg/kg; mouse (intramuscular oil suspension), 3840 mg/kg; rat (oral), 5576 mg/kg; and rat (intramuscular, oil suspension), 2571 mg/kg. Two dogs given two intramuscular doses of 400 mg/kg (oil suspension) survived the 10-day observation period; however, 15 minutes after administration of QHS they developed tonic and clonic convulsions which subsided after about 30 minutes. Doses leading to acute toxic manifestations generally caused some restlessness, tremors, and incoordination, followed by diminished activity, slower respiration, and loss of the righting reflex. These symptoms were not seen in smaller animals. Surviving animals apparently returned to the normal state in 10 to 24 hours. Rats given high doses (600 mg/kg) for seven consecutive days survived and showed no gross pathological changes except for some engorgement and slight degenerative changes in heart, liver, spleen, lung, and kidney. Dogs given oral doses of 100 mg/kg for 7 days all survived with minimal clinically observable physiological effects (*43*). Subacute oral doses of QHS given daily for 14 days to rats produced no pathologic changes. QHS administered intramuscularly as an oil suspension to rhesus monkeys at a dose of 192 mg/kg per day for 14 consecutive days caused the death of 75 percent of the animals within 3 days after the final dose. Cardiac and blood abnormalities (including extensive inhibition of hemopoietic function) were seen. A diminution of circulating reticulocytes was the only change noted in the group given the lower dose of 24 mg/kg per day. Recently, the ultrastructure of the myocardium of rhesus monkeys given daily doses of 96 or 192 mg/kg of QHS for 14 days was studied (*44*). Severe changes in the myocardium were observed at postmortem on day 3 after the last injection, but not after day 35. Overall, the acute toxicity of QHS is considerably less than that of chloroquine (*43*).

When pregnant rats were given QHS in doses up to 1/25th of the LD_{50} within the first 6 days of gestation, the majority had normal fetuses. However, when QHS was given to pregnant rats beyond day 6 of gestation, no fetuses survived and extensive of organogenesis (fetal absorption) was observed. While similar effects were seen in mice, it is not known if the teratogenic effect is limited to rodents (*43*). QHS does not appear to be mutagenic in bacterial assays (*43*).

Antimalarial Activity of QHS in Humans

In 1979, the Qinghaosu Antimalaria Coordinating Research Group reported (*6*) that they treated 2099 cases of malaria (*P. vivax* and *P. falciparum* in a ratio of about 3:1) with different dosage forms of QHS, leading to the clinical cure of all patients. In addition, 143 cases of chloroquine-resistant falciparum malaria and 141 cases of cerebral malaria were treated with "good" results. The prime criteria for acceptable therapeutic activity were the return to normal body temperature within 72 hours and the elimination

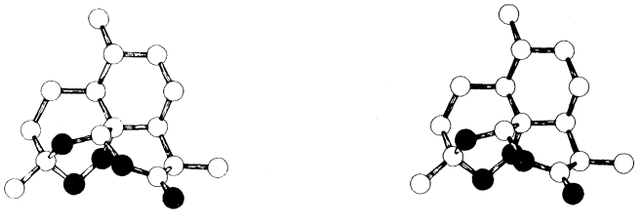


Fig. 2. Stereoscopic view of the structure of *qinghaosu* [ORTEP (Oak Ridge Thermal Ellipsoid Program) presentation]. Kindly provided by J. Flippen-Anderson, Naval Research Laboratories, Washington, D.C. 20375.

of the asexual parasite forms from blood films within 120 hours after beginning treatment.

Because QHS is essentially water insoluble, experimentation was required to determine the most suitable vehicle and route for its administration. Four kinds of pharmaceutical formulations were tested (total weight of drug): tablets (2.5 to 3.2 g), in oil (usually tea seed, 0.5 to 0.8 g), as an oil suspension (0.8 to 1.2 g), and as a water suspension (1.2 g). Each preparation was given in three divided doses, once daily for three successive days. Except for the tablets, administration was by intramuscular injection. The order of rapidity of action of each pharmaceutical form to effect fever subsidence and parasite clearance in *P. vivax* patients was: tablets > oil > oil suspension > water suspension. The frequency of recrudescence in 1 month also followed in the same order, that is, 31, 19, 13, and 9 percent, respectively. In another study, parasite clearance occurred with chloroquine controls in about 56 hours, whereas with QHS (tablet) the effect was more rapid, that is, about 40 hours. However, the recrudescence rate in the QHS group was 21 percent within 1 month but was 0 in the chloroquine control group (*6*).

In patients with *P. falciparum* malaria, QHS in oil was the most rapid acting of the dosage forms; however, the average 1-month recrudescence rate was 25 percent and ranged from 10.5 percent (oil suspension) to 85 percent (tablets).

From a study of the effects of QHS administered orally or intramuscularly and mefloquine administered orally to malaria patients with chloroquine-resistant *P. falciparum* on Hainan Island (*45*), it was concluded that clearance of parasitemia was more rapid with QHS, but that mefloquine afforded a much higher cure rate (*46*).

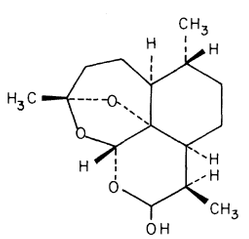
Neither obvious adverse reactions nor noticeable side effects were seen in the 2089 (*47*) patients given the QHS preparations (*6*). In 139 cases that were studied before and after treatment, no abnormalities were observed in serum aminotransferase activity, nonprotein nitrogen, or electrocardiogram. QHS also appears to be safe in cases complicated by heart, liver, and renal diseases of pregnancy. Some patients did, however, experience temporary local pain at the site of injection of aqueous suspensions (*6, 45*).

Metabolism of QHS

When QHS was incubated with rat liver slices, only 8.3 percent of the parent compound was recovered after 1 hour, suggesting the liver as the most probable site for the metabolism of the compound. Kidney and lung were less active; gut and whole blood, totally inactive (*48*).

In pharmacokinetic studies performed on mice, ^{3}H-labeled QHS given orally was rapidly absorbed giving peak blood levels in about 1 hour. The half-life of QHS in serum is about 4 hours. Distribution of the drug is widespread and excretion of about 80 percent of the oral dose occurs within 24 hours (*48*).

Extraction with ethyl acetate of urine from patients given QHS orally yielded four metabolites. The structures of three have been identified: desoxyartemisinin, **2**, dihydrodesoxyartemisinin, **10**, and so-called Crystal-7, **11** (*48, 49*). As is typical of compounds in this group that lack the peroxide moiety, these metabolites are inactive against *P. berghei* in the mouse.



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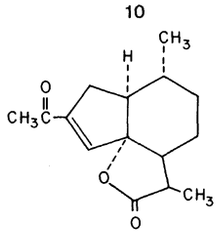

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An additional metabolite, 9,10-dihydroxyhydroartemisinin, was identified recently in the urine of patients treated with QHS (*50*). Nanomolar quantities of QHS and **10** are detectable and separable as their diacetylfluorescein derivatives (*51*).

Mechanism of Action

Mice infected with *P. berghei*, with a parasitemia exceeding 10 percent, were given QHS orally, and blood samples were examined at regular intervals by electron microscopy. After 8 hours the trophozoites (the asexual erythrocytic forms of the malaria parasite) began to show morphologic changes such as swelling and spiral deformation of the membrane of the food vacuole. By 12 to 14 hours, most trophozoites showed whirls of the food vacuole and limiting membranes and swelling of the outer mitochondrial and nuclear membranes. After 20 to 24 hours, the trophozoites showed extensive degeneration of their inner structure (*6, 39*).

When a chloroquine-sensitive strain of *P. falciparum* grown in human erythrocytes was treated with QHS in vitro, similar morphologic changes, as described above, were seen in the trophozoites except that no alterations appeared in the mitochondrial membrane (*4*). The minimum concentration of QHS required to effect changes on the parasite in vitro is $10^{-7}M$ (*39*). QHS appears to inhibit, to a maximum of about 50 percent, chloroquine-induced pigment clumping, further suggesting a difference in mode and site of action of the two drugs (*4, 52*). These findings may explain the antagonistic drug interaction observed by Milhous *et al.* (*40*) in drug combination studies of QHS and chloroquine with various *P. falciparum* isolates in vitro. QHS has been reported by Whaun and co-workers (*53*) to affect polyamine metabolism in cultures of *P. falciparum*-infected red blood cells. Putrescine levels became depressed, whereas spermine and spermidine levels became elevated.

In mice infected with *P. berghei*, *p*-aminobenzoic acid (PABA) did not suppress the antimalarial effect of QHS, leading to the conclusion that QHS does not interfere with the folic acid metabolism of the parasites (*4, 6*).

It is of interest that *tert*-butyl hydroperoxide was found to inhibit the development of *P. falciparum* in culture and that hydrogen peroxide or *tert*-butyl hydroperoxide suppresses parasitemias of several other *Plasmodia* in vivo (*54*).

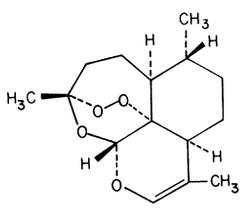
Derivatives of QHS

Although QHS rapidly suppresses the parasitemias of *P. vivax* and *P. falciparum*, the problems encountered with recrudescence, as well as the desire to improve on the natural product, led to efforts to modify its chemical structure (*55, 56*). 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, as are the deoxy form of QHS, **2**, and the nonperoxides, **4** and **5** (*7*).

The borohydride-reduction product of QHS, namely DHQHS, **3**, retains the peroxide function and is more potent than QHS [compare **3**, with an SD_{90} (concentration at which the parasitemia is 90 percent suppressed) of 3.65 mg/kg and **1**, SD_{90} of 6.20 mg/kg in mice infected with *P. berghei*] (*41*). The secondary hydroxy group in **3** provides the only site in an active QHS-related compound that has been used for derivatization. Many of the semisynthetic compounds that have been made are more potent than QHS (*56*).

Treatment of DHQHS with an unspecified dehydrating agent yielded both the olefinic compound, **12**, and the DHQHS ether, **13**, formed on addition of DHQHS to 12 (*57*). The former compound is inactive, whereas the latter has an SD_{90} of 10 mg/kg in *P. berghei*-infected mice.

Three types of derivatives of DHQHS have been made (listed in order of their overall antimalarial efficacy): (QHS) < ethers (**3**, R=alkyl) < esters [**3**, R=C(=O)-alkyl or -aryl] < carbonates [**3**, R=C(=O)O-alkyl or -aryl] (*56*).



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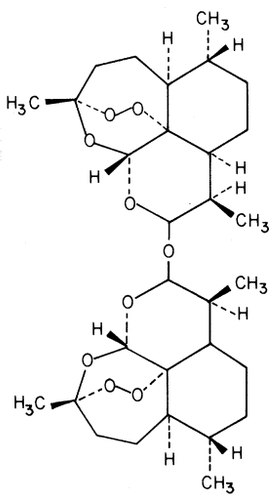

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The ethers, which have the advantage of being more oil soluble than QHS, are made by treating DHQHS with an alcohol in the presence of boron trifluoride etherate (*56, 57*). Of some 32 ether derivatives, which include 13 epimeric pairs, the most active is the methyl ether (β-epimer), called artemether (**3**, R=CH_3; SM224). The compound exhibits an SD_{90} of 1.02 mg/kg in mice infected with *P. berghei* (*41, 55*). Artemether has been studied clinically in the treatment of pernicious malaria (*58*). The acute toxicity of artemether in laboratory animals, while lower than that of chloroquine (*59*), is considerably greater than that of QHS (*43*). In mice, for example, the LD_{50} of artemether administered intramuscularly in oil is 263 mg/kg. The $^{14}CH_3O$-derivative (*55*) of artemether, administered intramuscularly, and a tritiated form (*60*) were metabolized in mice in 24 hours to return 15 percent of the radiolabel as CO_2. The ether demethylation could be accelerated by giving the mice phenobarbital and was more extensive if the drug was administered intravenously.

Esterification of DHQHS can be achieved by treatment with an acid halide (*57*) or an acid anhydride in the presence of pyridine (*61*) or preferably, 4-dimethylaminopyridine (*56, 62–64*). DHQHS has also been esterified by use of a carboxylic acid in the presence of dicyclohexylcarbodiimide (*55, 64*). The esters obtained are mainly in the α-epimeric form (*56*) and most are reported to have activity considerably greater than either QHS or the parent, DHQHS (*55*). The QHS derivative with the greatest promise is the DHQHS half-ester of succinic acid, whose water-soluble sodium salt, known as sodium artesunate (**3**, R=$COCH_2CH_2CO_2Na$; sodium DHQHS hydrogen succinate monoester, 804-Na), 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 than QHS against both chloroquine-resistant and chloroquine-sensitive strains of *P. berghei* (*65*). In monkeys given 6 to 12 mg/kg for 3 days, there was a faster disappearance of parasitemia than seen with QHS (*65*). The compound is more toxic than QHS; nevertheless, it is only about 1/50th as toxic to the cardiovascular system of rabbits as chloroquine (*65*) and less toxic than artemether. In the mouse it has an LD_{50} of 520 mg/kg (intravenously) 475 mg/kg (intramuscularly). Dogs suffered no ill effects when dosed orally with 100 mg/kg of QHS on 5 succeeding days (*43*). In a pharmacokinetic study in rats, the compound hydrolyzed rapidly to DHQHS (*66*) in 10 minutes and was then further metabolized. 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 *p*H, it loses the succinate moiety through hydrolysis.

There is evidence that malaria parasites obtain amino acids by proteolytic decomposition of host-cell hemoglobin. Therefore, one mode of action of antimalarial agents might be as hemoglobinase inhibitors. Artesunic acid, tested at a concentration of $1.4 \times 10^{-3} M$, however, lacked any such inhibitory activity (*67*).

The third group of derivatives, the carbonates, are prepared by the action of a chloroformic ester on DHQHS in the presence of triethylamine and 4-dimethylaminopyridine (*55, 56, 63*). Again, the α-epimer predominates in the product whose oil solubility is similar to that of the esters. Although the carbonates are the most potent derivatives in the *P. berghei* mouse test (*55*), no detailed study of their therapeutic properties has been published. QHS analogs have been subjected to Hansch quantitative structure-activity analysis (*68*).

Li *et al.* (*42*), studying the incorporation of [^{3}H]hypoxanthine by *P. falciparum* parasites in vitro, found that DHQHS, artemether, and the α-epimer of the propylcarbonate derivative of QHS (SM242) were >100 times more active in inhibiting incorporation than QHS itself. However, on observing the more rapid and extensive inhibition of incorporation of tritiated isoleucine (an essential exogenous amino acid required by malaria parasites for protein synthesis) by QHS, DHQHS, and artemether, it was suggested by Gu and co-workers (*69*) that the prevention of the construction of proteins may be the primary target of the QHS family of drugs, rather than the inhibition of nucleic acid synthesis. The latter mechanism is inferred from the experiments on hypoxanthine incorporation.

When QHS was reduced with $NaBH_3T$ to the lactol, the carbinol carbon atom acquired a tritium atom. Methylation of the resultant tagged DHQHS gave tritiated artemether (*70*). Sodium artesunate has been prepared in which the succinate moiety is tritiated (*70*).

The binding of QHS and its derivatives to blood plasma protein has been studied

(*71*). For the investigation of the distribution, metabolism, and toxicity of QHS and related compounds, various high-performance liquid chromatography procedures have been developed (*72–74*) in which cholesterol is generally used as an internal standard. Other quantitation techniques that have been used include infrared (*75*), ultraviolet (*76*), nuclear magnetic resonance (NMR) (*34, 77, 78*) spectrometry, optical rotatory dispersion (*79*), thin-layer chromatography (*80–82*), and colorimetry (*83*). The absolute configuration of DHQHS, artemether, and artesunic acid have been elaborated by NMR and x-ray determinations (*84*).

Cerebral Malaria and Other Therapeutic Applications of QHS

The most striking results achieved with QHS and its derivatives are seen in the treatment of cerebral malaria. This potentially fatal disease is an advanced form of *P. falciparum* malaria that can occur when >5 percent of erythrocytes are infected with parasites. When QHS was administered in the form of a tablet, in oil (intramuscularly), or in water suspension (intragastrically by means of a nasal catheter) on three successive days (600, 300, and 300 mg) to 106 patients with cerebral malaria, the cure rate averaged about 90 percent (*85*). The time for recovery from the coma was about 21 hours when the oil suspensions was used. Artemether and sodium artesunate gave about the same cure rate with the time for recovery from the coma being reduced to about 12 hours through the use of the latter compound. Chloroquine and quinine are considerably slower acting. On the negative side, however, sodium artesunate-treated patients showed a very high recrudescence rate, necessitating the use of a supplemental drug such as sulfadoxine. By comparison, quinine cured 97 percent of the patients but required about 49 hours of recovery from the coma.

Because of its unusual structure, QHS, and some of its derivatives, is being examined for further therapeutic applications. When artemether was given subcutaneously to mice infected with *Schistosoma mansoni*, worm reduction rates approached 80 percent (*86*). The compound, administered to mice intragastrically or subcutaneously, appeared to have no effect on the ova of *S. japonicum*; however, subcutaneous doses of 225 to 435 mg/kg over 3 days gave worm reduction rates of 70 to 81 percent (*87*) and there were marked histologic changes in the worms residing in the liver (*88*). The oral administration of sodium artesunate (450 to 900 mg/kg for 1 to 3 days) to *S. japonicum*-infected mice led to worm reduction rates of 60 to 70 percent (*89*). Infected dogs showed *S. japonicum* worm reduction of 91 to 99 percent after administration of 150 to 250 mg/kg of artemether over 5 days (*86*). Several other derivatives of DHQHS are also active schistosomicides (*56*). It is of interest that both the terpene-peroxide ascaridole and the sesquiterpene α-santonin (from *A. maritima*) have been used as anthelmintics.

Another parasite affected by QHS and its derivatives is *Clonorchis sinensis*, which is acquired through the ingestion of raw infected fish. Various ether modifications of DHQHS administered to infected rats over 7 days had potent clonorchicidal activity while imparting no apparent deleterious effects to the test animals (*90*).

Investigators have also studied QHS in the treatment of systemic lupus erythematosus (*91*) and have reported that the compound has virustatic effect on influenza virus in chick embryo (*92*). It has an adjuvant effect on cell-mediated immunity, as well as a marked immunosuppressive effect on antigen-binding and antibody-forming spleen cells in mice (*92*). It is, however, less potent than cyclophosphamide. Sodium artesunate produced different immunological effects in mice depending on the quantity administered, that is, low doses increased, whereas high doses diminished, immunologic responses (*93*). When tested against clinical isolates of Gram-positive and Gram-negative organisms, QHS exhibited no antibacterial activity (*12*). The compound was also inactive against P388 leukemia (*94*).

Scientists in the People's Republic of China have not only contributed a structurally novel and well-tolerated class of rapidly acting antimalarial agents but have also encouraged the investigation of folk medicine. There is now a wide interest in QHS, and plans are being made to cultivate *A. annua* in the United States so that adequate supplies of the material will be available for study.

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RESEARCH ARTICLE

Model Structure for the Inflammatory Protein C5a

Jonathan Greer

C5a is a biologically active molecule produced during the course of serum complement activation (*1*). As an anaphylatoxin, it shares with C3a and C4a the ability to induce contraction of smooth muscle, increase vascular permeability and release histamine from mast cells and basophilic leukocytes. However, C5a also has the ability to recruit monocytic and polymorphonuclear leukocytes to inflammatory sites by stimulating their locomotion (chemokinesis) and directing their migration (chemotaxis). C5a also fosters the release from these inflammatory cells of free radicals and tissue-digesting enzymes that are believed to play a major role in the tissue injury accompanying inflammation. Consequently, inhibition of C5a activity is of considerable therapeutic interest for the treatment of inflammatory diseases such as rheumatoid arthritis.

Human C5a is a relatively small protein with a sequence of 74 amino acid residues (*2*). It contains one oligosaccharide (molecular size 2800 to 3000 daltons), which is attached to an asparagine at position 66[64] [see (*3*)]. The total molecular size of human C5a is around 11,000 daltons. Porcine C5a has also been sequenced (*4*); it consists of 74 residues but has a Glu rather than Asn at position 66[64] and therefore contains no carbohydrate. The sequences of C3a, C4a, and C5a are all clearly homologous, suggesting a common evolutionary origin for these molecules (*1*).

Studies have been performed on C5a in order to identify the parts of the molecule that are essential for receptor binding and biological activity. C5a desArg, from which the COOH-terminal Arg residue has been removed, shows a significant decrease in chemotactic activity (*5*). When the pentapeptide that corresponds to the COOH-terminal five residues of C5a is examined, no chemotactic or chemokinetic activity is observed (*6*). On the other hand, the balance of the molecule containing the remaining 69 NH_2-terminal residues, while chemotactically inactive, does compete with C5a for binding to the C5a receptor (*7*). Thus, it appears that the NH_2-terminal portion and the COOH-terminus of the C5a molecule interact with the receptor and are involved in producing biological activity. Similar modification and peptide studies on C3a (*1*) show that only the COOH-terminal residues of C3a are required or necessary for full biological activity.

A considerable increase in our ability

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