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Artesunate and Dihydroartemisinin (DHA): Unusual Decomposition Products Formed under Mild Conditions and Comments on the Fitness of DHA as an Antimalarial Drug

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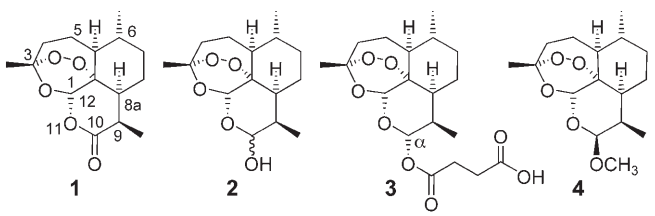
Artesunate drug substance, for which a rectal capsule formulation is under development for the treatment of severe malaria, when heated at 100 °C for 39 h gives β-artesunate, artesunate dimers, 9,10-anhydrodihydroartemisinin (glycal), a DHA β-formate ester, and smaller amounts of other products that arise via intermediate formation of dihydroartemisinin (DHA) and subsequent thermal degradation. Solid DHA at 100 °C provides an epimeric mixture of a known peroxyhemiacetal, arising via ring opening to a hydroperoxide and re-closure, smaller amounts of a 3:1 mixture of epimers of a known tricarbonyl compound, and a single epimer of a new dicarbonyl compound. The latter arises via homolysis of the peroxide and an ensuing cascade of α-cleavage reactions which leads to loss of formic acid incorporating the C10 carbonyl group of DHA exposed by this 'unzipping' cascade. The tricarbonyl compound that arises via peroxide homolysis and extrusion of formic acid from a penultimate hydroxyformate ester incorporating C12 of the original DHA, is epimeric at the exocyclic 1''-aldehyde, and not in the cyclohexanone moiety. It is converted into the dicarbonyl compound by peroxide-induced deformylation. The dicarbonyl compound is not formed during anhydrous ferrous bromide mediated decomposition of DHA at room temperature, which provides the 1''-R epimer of the tricarbonyl compound as the dominant product; this equilibrates at room temperature to the 3:1 mixture of epimers of the tricarbonyl compound obtained from thermolysis. Each of artesunate and DHA decomposes readily under aqueous acidic conditions to provide significant amounts of the peroxyhemiacetal, which, like DHA, decomposes to the inert end product 2-deoxyartemisinin under acidic or basic conditions. DHA and the peroxyhemiacetal are the principal degradants in aged rectal capsule formulations of artesunate. TGA analysis and thermal degradation of DHA reveals a thermal lability which would pose a problem not only in relation to ICH stability testing guidelines, but in the use of DHA in fixed formulations currently under development. This thermolability coupled with the poor physicochemical properties and relative oral bioavailability of DHA suggests that it is inferior to artesunate in application as an antimalarial drug.

Introduction

The isolation of artemisinin **1** and the discovery of its antimalarial activity by Chinese scientists represents one of the great events in medicine in the latter half of the 20th century. Now, the derivatives dihydroartemisinin (DHA, **2**), a mixture of epimers,[1] artesunate **3**[2–4] with α configuration at the ester linkage,[4–7] and artemether **4**, are routinely used for treatment of malaria. Because artemether requires fractional crystallisation to separate it from the α epimer,[3] DHA **2** and artesunate **3** are the most accessible derivatives.[8]



The artemisinins are the most potent and rapidly acting of all antimalarial drugs. However, their short half-lives results in

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recrudescence, a problem recognised by Chinese investigators.[9] To counter the problem, Li, Arnold, and co-workers[10] first used artemisinin itself in combination with the longer half-life antimalarial drug, mefloquine, which had the function of killing the recrudescing parasites once the artemisinin cleared from the circulation. The practice of combining an artemisinin with a longer half-life drug is now generally accepted as the best means of controlling recrudescence;[11,12] indeed, the clinical use of artemisinins in combination therapy is now mandatory.[13] Whilst the demand for the fixed combination (co-formulation) of artemether **4** with lumefantrine consumes by far the largest amount of artemisinin starting material used for preparation of the current clinically used artemisinins,[14] the clinically more effective artesunate[15] is the most widely used as a combination partner. Originally employed as a dual combination with mefloquine,[12] artesunate is being developed, or is now employed, in fixed formulations with each of mefloquine,[16] pyronaridine,[17] amodiaquine,[18] and a triple fixed combination with chlorproguanil–dapsone.[19] For the latter, concerns of toxicity and selection of parasite strains resistant to chlorproguanil–dapsone raise doubts over its justification.[20] The combination of DHA **2** with piperaquine phosphate, known as artekin, was introduced by the Chinese, and has been used in clinical trials;[21,22] a fixed combination is in development under a public–private partnership in the West.[23] The parent artemisinin **1** is now used in a fixed combination known as artequick with piperaquine free base. The combination is relatively inexpensive and has the additional advantage that the piperaquine free base does not cause gastric irritation, which characterises the use of piperaquine phosphate. The combination is as effective as artekin for the treatment of non-severe malaria.[24] Artesunate was selected by the Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization (WHO) for development as rectal capsules for emergency treatment of patients with acute malaria who cannot take oral medication and are not able to access injectable medication.[25] Rectal administration avoids the 'first pass' metabolism in the liver.

The peroxide within the 1,2,4-trioxane of artemisinin and its derivatives confers antimalarial activity,[26,27] but it also renders artemisinins more difficult to handle in a drug-development setting than conventional antimalarials. The peroxide is both a chemically and heat-sensitive group. A formulated drug product must have a shelf-life sufficient to cover the time taken for transportation from the site of manufacture to protracted storage at the site of clinical use under storage conditions recommended for the product. Artemisinins are used in countries where malaria is prevalent, that is, within climatic zones III and IV as classified by the International Conference on Harmonization (ICH). Climatic zone III has a climate that is hot and dry, and zone IV regions are hot and humid, with an average temperature of $>24\,^{\circ}C$.[28,29] To provide an estimate of shelf-life and to define storage conditions, drugs are normally assessed for stability, including thermal and humidity stress testing. For drugs destined for use in climatic zones III and IV, the WHO has draft guidelines that prescribe long-term thermal stress testing by heating the drug at $30\pm2\,^{\circ}C$ at a relative humidity (RH) of $65\pm5\%$ for 12 months.[30] Alternatively, accelerated thermal stress testing can be carried out at $40\pm2\,^{\circ}C$ and $75\pm5\%$ RH for six months. The ICH recommended threshold of unknown decomposition products based on a minimum daily dose of 100 mg should not exceed 0.2%, and there should be $<1.5\%$ decomposition to known degradants, the toxicity and efficacy profiles for which have been quantified as for the parent drug. Controlled thermal decomposition data for formulated artemisinins appears to be unavailable in the primary literature, although anecdotal evidence indicates that under thermal stress, artemisinins undergo substantial decomposition. There is anecdotal evidence of DHA tablets manufactured in Asia and sold over-the-counter in certain African countries that contain $<50\%$ of the specified active ingredient, and recent publications attest to the magnitude of the decomposition problem of formulated artemisinins used in tropical countries.[31,32] Analysis of DHA tablets purportedly manufactured in Europe, India, or China and purchased in various African countries shows that the content of active drug substance is substantially less than the claimed dose, with recorded values as low as 78%. On the other hand, just one sample of the formulated artesunate products examined and purportedly manufactured in India had an active drug content appreciably below the claimed amount.[32]

The rectal artesunate capsules under development for clinical use in emergency malaria treatment consist of 100-mg capsules for pediatric use and 400-mg capsules for adult use. The fill material contains glycerides and hard fat, the gelatin shell contains glycerin with about 15% water, and the coating contains polyethylene glycol. The capsule active ingredient, artesunate, is blended with the other fill material and then impressed into sheets of wet gelatin film, and the capsules are then dried by storing at 20% RH. The final water content of the capsule shell is approximately 5%. Thermal stress testing of 100-mg capsules according to the ICH/WHO guidelines results in approximately 7% decomposition at $40\,^{\circ}C$ over six months to provide 4% DHA, whereas at $25\,^{\circ}C$ for 24 months, 6% decomposition of active material to provide some 2% of DHA **2** takes place (Table 1).

The aim of the current work is to identify and quantify the levels of degradation products so that the changes in assay of

Table 1. Stress testing results for 100-mg active artesunate capsules.

T [°C]	RH [%]	t [months]	**3** [%][a]	**2** [%][b]
50	75	0.5	96.2	1.7
50	75	1	93.7	2.4
40	75	2	95.8	1.8
40	75	4	93.8	2.7
40	75	6	92.2	3.7
30	60	3	97.7	1.3
30	60	6	95.8	1.5
30	60	12	95.0	2.2
30	60	24	88.6	2.6
25	60	3	98.0	0.9
25	60	12	97.1	1.4
25	60	24	93.8	1.9

[a] Amount of artesunate **3** remaining. [b] Amount of DHA **2** formed.

artesunate in stored rectal capsules are fully accounted for, so that there is a mass balance as required in the ICH stability guidelines. The analytical methods are based on HPLC analyses of the capsule fill material in a commercial laboratory and on analyses of individual HPLC fractions by LC–MS, high-resolution MS, and NMR spectroscopy at Hong Kong University of Science and Technology (HKUST). Attention was also accorded to the capsule shell material and capsule coating in the overall attempt to locate any active substance or its degradants, which may migrate into the shell and coating over time. We do not describe herein the analytical methods, but rather focus on the putative degradants. Artesunate **3** is a hemiester of succinic acid and of DHA **2**, and has a free carboxylic acid end group. Thus, it is potentially susceptible to both hydrolysis by the water present in the capsule and to transesterification and other reactions involving fill material. The principle degradant DHA is also susceptible to thermal degradation as it is formed from artesunate under the thermal-stress-testing conditions.

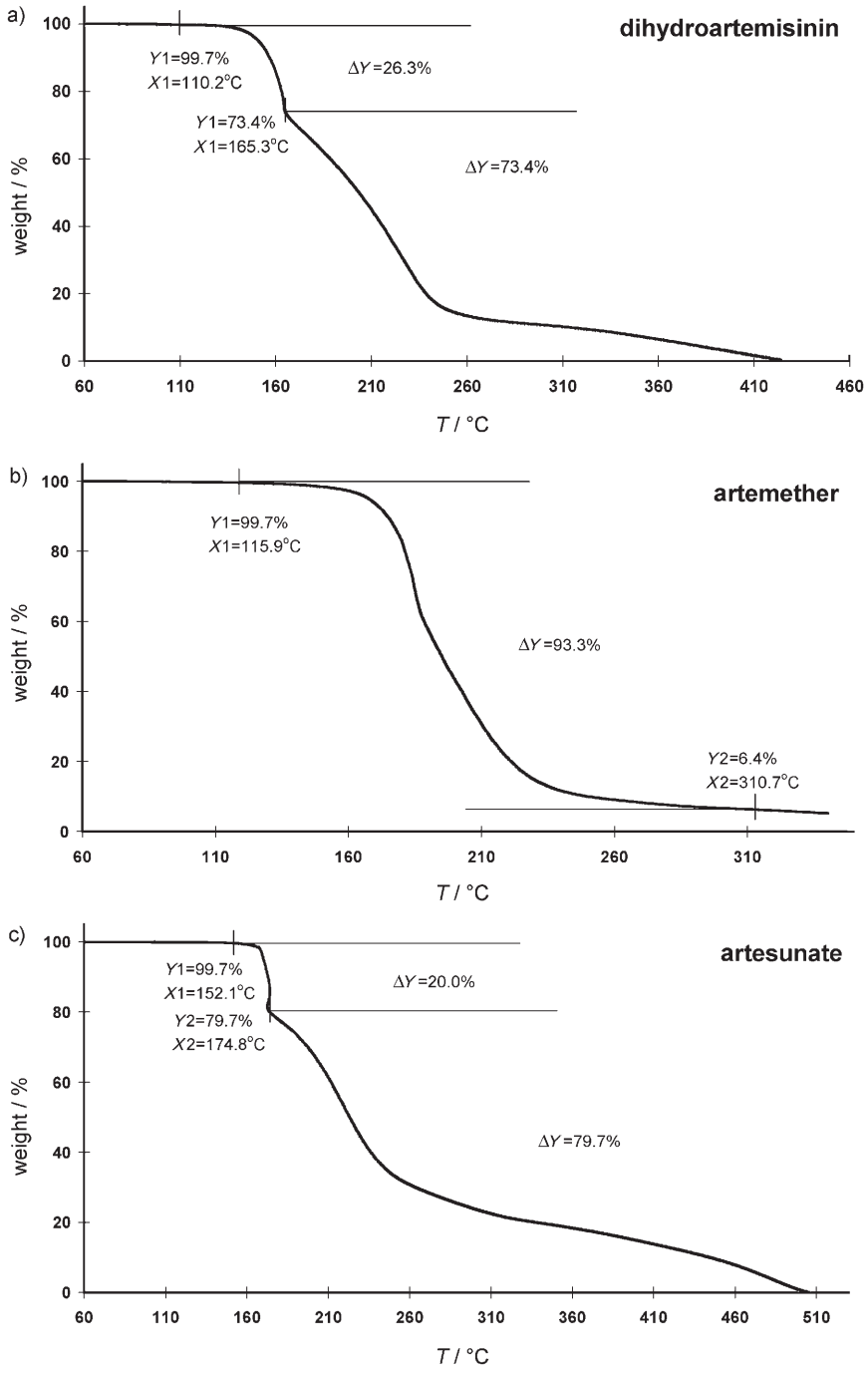


Figure 1. Results of TGA of a) dihydroartemisinin **2**, b) artemether **4**, and c) artesunate **3** heated under N_2. Values *X*1 and *Y*1 respectively refer to temperature and weight of the sample at the incipient decomposition event; ΔY represents the percent weight loss of sample between the designated temperatures.

Results and Discussion

a. Thermal decomposition

Thermogravimetric analysis (TGA) is a technique normally applied to determine thermal stability of a material by monitoring the temperature increase (*x* axis) as a function of change in weight (*y* axis). The onset of loss of volatiles such as a recrystallisation solvent, or of decomposition associated with the loss of volatile decomposition products is thereby recorded. Thus, DHA **2** was shown to possess a decomposition threshold of 110 °C; this was followed by loss of volatile components of approximately 26% by weight to a second decomposition threshold of 165 °C (Figure 1). Artemether **4** is slightly more robust in displaying a decomposition threshold of 116 °C, and artesunate **3** is the most stable, with a decomposition threshold of 152 °C (Figure 1). The facile decomposition of DHA in particular is associated with a distinct decomposition pathway that involves an "unzipping" process, discussed below. Discrete loss of material is partially apparent for DHA and artesunate. As shown below, products obtained from thermal decomposition of DHA, but not artesunate, can be correlated with the relative amounts of material lost according to the TGA results.

At 40 °C and 70% RH, solid artesunate decomposes, albeit at a rate slower than that of the formulated artesunate within the

capsules (cf. Table 1). Thus, approximately 2% decomposition is observed at four months, and at six months, approximately 5%. Approximately 25% decomposition is observed in a toluene solution at 100 °C for 24 h. In line with the TGA results, DHA is considerably less stable. At 40 °C and 70% RH, solid DHA undergoes 2% decomposition after one month and 2.9% after three months. At 100 °C in a toluene solution for 24 h, DHA decomposes completely, giving products whose identification is described in detail below.

i. Artesunate 3

To expedite identification of thermal degradation products, solid artesunate **3** was heated neat under nitrogen at a bath temperature of 100 °C for 39 h to give a mixture of artesunate and the products shown in Figure 2.

β-Artesunate **5** was identified through independent preparation from the equatorial trichloroacetimidate of DHA and succinate according to the Schmidt reaction.[7,8] The β-artesunate **5** is not present as an impurity in the starting sample of **3**. ^{1}H NMR spectroscopic analyses at 750 MHz of **3** prepared according to published procedures or obtained from Knoll AG (Basel, Switzerland) was carried out by spiking samples of artesunate **3** with β-artesunate **5** and constructing calibration curves to the established detection limits of **5**. It was established that β-artesunate was not present in samples of **3** above the limit of detection at 0.04%.[7] The stereochemistry of the novel artesunate dimers is revealed by their ^{1}H NMR spectra. The signal due to H10 in the α,β or equatorial–axial dimer **6** at $\delta = 5.78$ ppm has a trans-diaxial coupling of 9.9 Hz between H9 and H10. For the signal due to H10′ at $\delta = 6.25$ ppm, a vicinal equatorial–axial coupling with H9′ of 3.4 Hz is apparent.[5–8] The symmetrical dimers **7** and **8** have one set of signals for each of H10 and H12. The signal due to H10 at $\delta = 6.27$ ppm in the β,β or diaxial dimer **7** displays a vicinal equatorial–axial coupling of 3.4 Hz with H9, whereas the signal due to H10 at $\delta = 5.78$ ppm in the α,α or diequatorial dimer **8** displays a trans-diaxial coupling with H9 of 9.7 Hz. X-ray crystallography confirms the presence of the axial C10–O ester bonds in the dimer **7** (see below). The α,β dimer **6** was prepared in 51% yield by treatment of DHA trichloroacetimidate with artesunate **3**. Similarly, the β,β dimer **7** was prepared in 12% yield by treating DHA trichloroacetimidate with β-artesunate **5**. An attempt to prepare the α,α dimer **8** via activation of the free carboxyl group of artesunate with DCC,[7] and treatment with DHA was unsuccessful. Dimers **6** and **7** were also prepared simply by treating artesunate with boron trifluoride etherate in dichloromethane at −78 °C, albeit in low yields; a large amount of artesunate remained unchanged.

The success of the latter process indicates how β-artesunate **5** and the dimers are formed during thermolysis of artesunate. Protonation of the equatorial ester oxygen atom followed by loss of succinic acid generates oxonium ion **13**. This undergoes axial addition, thermodynamically favoured by the anomeric effect,[7,8] with succinate to generate β-artesunate **5** or with the free carboxyl group in artesunate to produce the α,β dimer **6** (Scheme 1). The β,β dimer **7** arises from **6** by equilibration with artesunate via protonation at the equatorial ester followed by reaction of the incipient β-artesunate **5** with the oxonium ion. The formation of the small amounts of α,α dimer **8** may be due to the addition of α-artesunate to the oxonium ion **13** from the α or *Si* face, because there is a steric effect involving a 1,3-diaxial interaction with the axial C8–C8a bond, which works against the anomeric effect by partially destabilising the axial product with its bulky β substituent at C10. Alternatively, a direct S_N2 displacement of β-succinate in β-artesunate or the α,β dimer **6** by α-artesunate **3** may take place. The stereochemistry of addition reactions of oxygen nucleophiles involving the oxonium ion **13** has been discussed elsewhere.[8]

9,10-Anhydrodihydroartemisinin (glycal) **9** arises via proton loss from the stabilised oxonium ion **13**,[8] or through a stereochemically favoured concerted thermal *syn* elimination of suc-

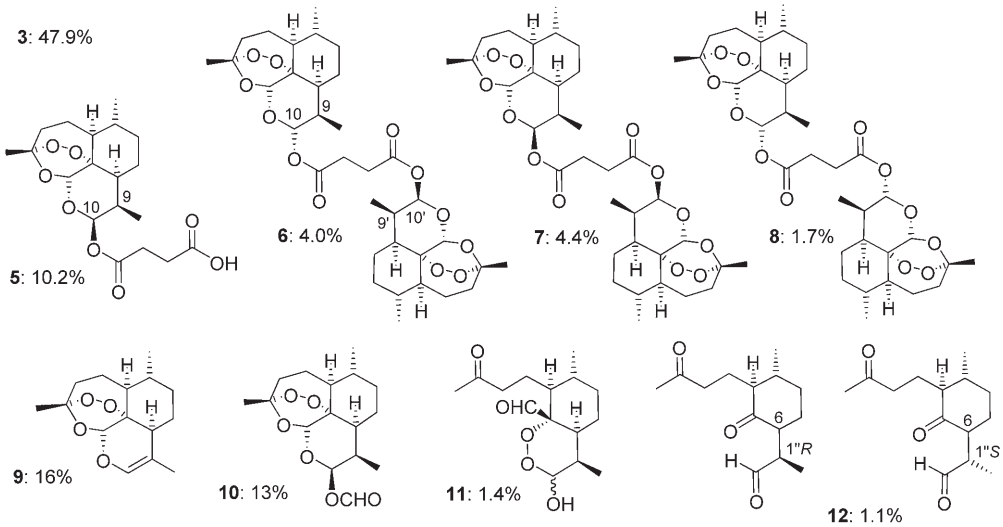
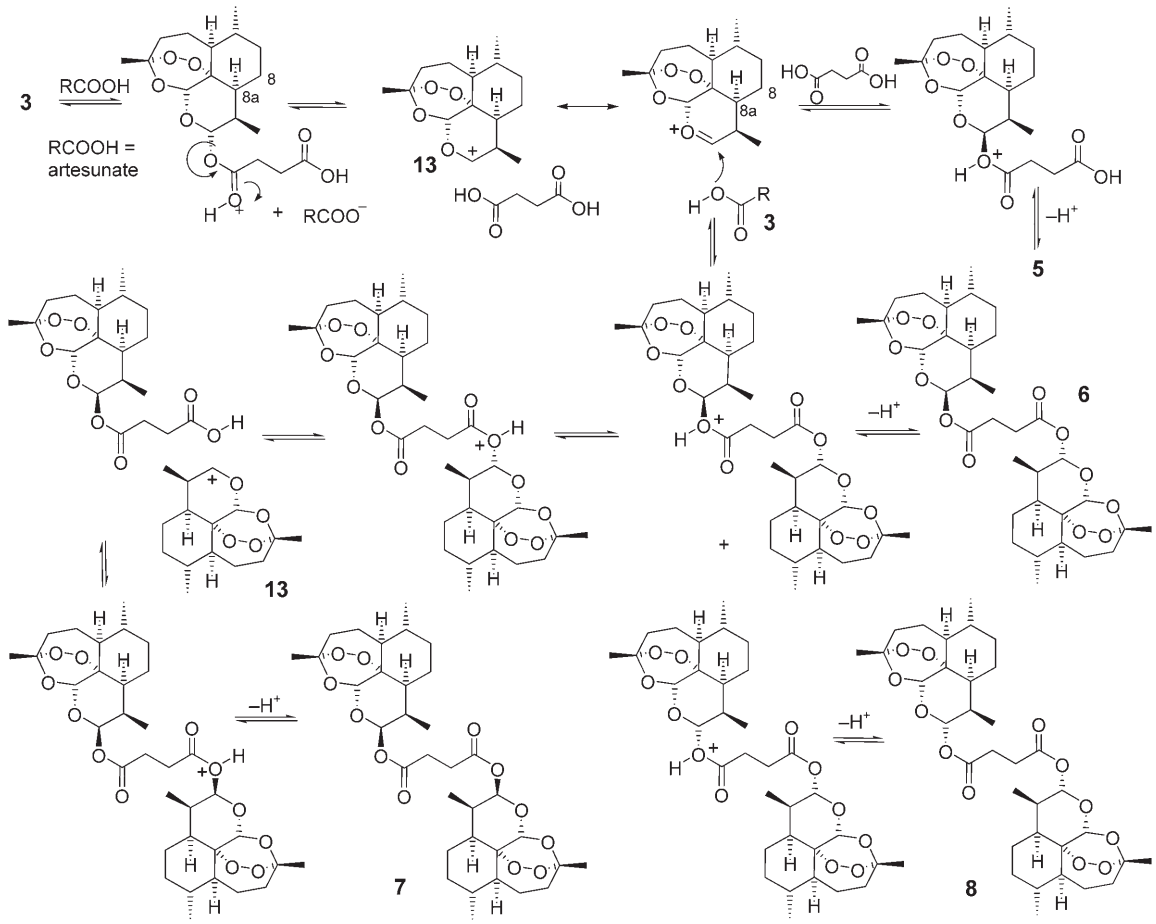
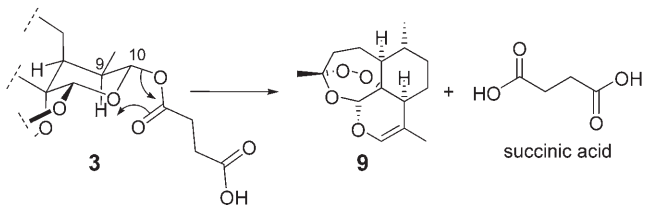


Figure 2. Products obtained from heating solid artesunate **3** at 100 °C for 38 h under N_2; the crude product mixture was separated into acidic and neutral fractions, and the indicated relative amounts of the components in each fraction were determined by ^{1}H NMR spectroscopy.



Scheme 1. Proposed formation of β-artesunate **5** and the artesunate dimers **6**–**8** from artesunate **3** under thermolysis conditions at 100 °C.

cinic acid involving the ester and H9 (Scheme 2). Interestingly, loss of succinic acid in this manner is not reflected in the TGA analysis (Figure 1); succinic acid is presumably relatively nonvolatile at the temperature of incipient decomposition, and may convert into succinic anhydride (see below). The formate ester **10** arises via axial addition of formate to the oxonium ion **13**. The compound is unstable and undergoes decarbonylation to DHA on attempted recrystallisation. It was independently prepared by stirring artesunate **3** with anhydrous formic acid in dichloromethane at gentle reflux. Under such conditions, a small amount of the α epimer is also formed. The source of the formic acid in the thermal decomposition of artesunate is DHA formed in situ as discussed below. Although DHA was not detected, both the peroxyhemiacetal **11** and tricarbonyl compound **12** are markers for its intercession. The tricarbonyl compound **12**, previously believed to be a mixture of epimers isomeric at C6,[33] is shown to be epimeric about C1″, as discussed below. The inability to detect DHA after thermolysis of artesunate at 100 °C for 39 h indicates the conditions are too severe for it to remain intact. Loss of volatile fragments from artesunate may be formic acid and possibly succinic anhydride (and water), accounting for 12 and 23% loss, respectively, of material under conditions of TGA (cf. Figure 1).



Scheme 2. Illustration of the possible thermal concerted *syn* elimination of succinic acid from artesunate **3** to produce glycal **9**.

ii. Dihydroartemisinin 2

To expedite identification of thermal degradation products, solid DHA was heated under nitrogen at a bath temperature of 100 °C over a period of 14 h. The following were obtained from DHA: glycal **9**, a 3:2 mixture of epimers of the peroxyhemiacetal **11**, a 2:1 mixture of epimers of the tricarbonyl compound **12**, the new dicarbonyl compound **14**, 2-deoxyartemisinin **15**, and a trace amount of the furanose acetal **16**. Repetition of the reaction under air resulted in formation of all products in about the same yields. At longer reaction times, product mixtures became more complex, presumably as a result of secondary reactions involving the initial decomposition prod-

ucts. Previously, it was reported that thermolysis of DHA **2** at 190 °C for 3 min gave 2-deoxyartemisinin **15** (30%) and the tricarbonyl compound **12** (50%) as a mixture of stereoisomers assumed to be epimeric at C6.[33] In a solution in toluene under nitrogen at 100 °C for 24 h, DHA decomposed completely to give largely the peroxyhemiacetal **11** and the tricarbonyl compound **12** (Figure 3).

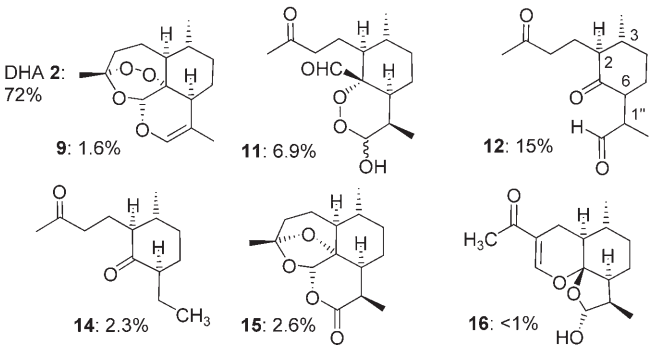
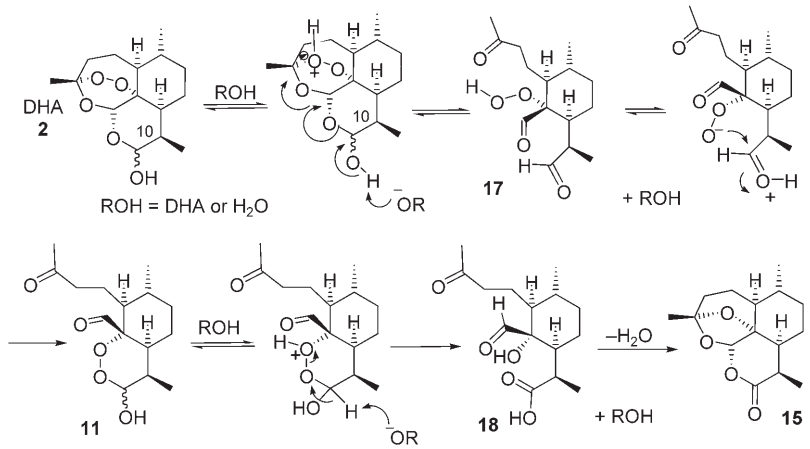


Figure 3. Products obtained from heating solid DHA **2** at 100 °C for 14 h under N_2; relative amounts of each were determined by ^{1}H NMR spectroscopy of the reaction mixture.

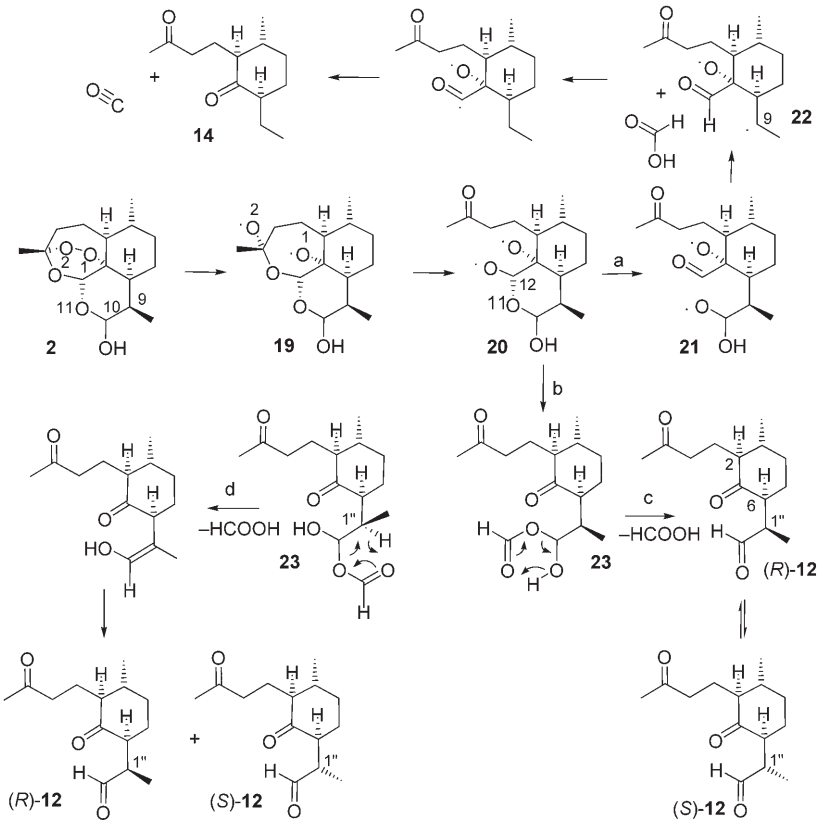
2-Deoxyartemisinin **15** was originally obtained by treatment of artemisinin with triphenylphosphine, and acid-induced closure of the intermediate alcohol.[1] The peroxyhemiacetal **11** was previously obtained from arteether and aqueous hydrochloric acid in ethanol.[34] Its formation under thermal conditions is likely to proceed via unzipping triggered by protonation of the peroxide and re-closure by reaction of the pendant hydroperoxide with the aldehyde in **17** (Scheme 3). Compound **11** is converted into **15** via the Kornblum–de la Mare process characteristic of secondary dialkyl peroxides.[35] This involves abstraction of H10, collapse to the carboxylic acid **18** and closure with loss of water.[36] The stereochemistry associated with both the unzipping of DHA and the Kornblum–de la Mare process has been thoroughly discussed.[8] The proton and base sources for the thermal reactions can be the hydroxy group of DHA or water produced as the thermolysis proceeds. 2-Deoxyartemisinin **15** therefore serves as a marker for the intercession of the peroxyhemiacetal.

Formation of the dicarbonyl compound **14** requires a decarbonylation. Because aldehydes can be decarbonylated by oxygen-centred radicals generated from di-*tert*-butyl peroxide or benzophenone,[37] it is likely that a related process involving alkoxyl or peroxyl radicals occurs here. Homolysis of the peroxide provides the O1 and O2 alkoxyl biradical **19**. Radical O2 is electronically distinct from radical O1, as the former has an adjacent oxygen atom that predisposes it to rapid α cleavage to generate a new alkoxyl biradical **20**. The latter undergoes a second α-cleavage reaction of the C12–O11 bond (Scheme 4, path a). The resulting O11 radical **21**, again with an adjacent O atom, undergoes a third α-cleavage reaction resulting in expulsion of formic acid and generation of the C9 alkyl radical **22**. H-atom abstraction from the pendant formyl group results in expulsion of carbon monoxide with formation of the dicarbonyl compound **14**. Radical scission via α cleavage has been invoked previously to explain the course of ferrous iron mediated decomposition of artemisinin.[38]

The tricarbonyl compound **12** arises via cleavage of the trioxane, formally a [2+2+2] cycloreversion characteristic of six-membered saturated endoperoxides.[39] However, it is depicted here as peroxide bond homolysis to provide the incipient biradical **19**, as homolysis of peroxides to produce alkoxyl radicals may compete with heterolytic pathways during thermolysis.[37,40] α Cleavage occurs to give biradical **20**, which, via the ensuing cleavage of the C12–C5b bond, provides hydroxyformate ester **23** (Scheme 4, path b). This undergoes extrusion of formic acid via one of two possible thermally allowed [1,5]-H shifts involving either the OH group proton (Scheme 4, path c) or H1″ (Scheme 4, path d). The route involving path c should only give the C1″ *R* epimer (*R*)-**12**, and path d should lead to a mixture of the C1″ *R* and C1″ *S* epimers (*R*)-**12** and (*S*)-**12**. The tricarbonyl compound **12** is obtained as a mixture of epimers under either thermal conditions as described herein and elsewhere,[33] or via ferrous iron catalysed decomposition of derivatives of DHA.[41] However, treatment of DHA itself with 0.4 equivalents of ferrous bromide in anhydrous THF at room temperature for 45 min followed by immediate chromatography provides the tricarbonyl compound **12** in 62% yield as essentially a single epimer, presumably the C1″ *R* epimer (*R*)-**12** (Scheme 4). If this epimer is allowed to stand as a neat liquid, it equilibrates to the 3:1 mixture of epimers found in the thermal decomposition of DHA described above. Therefore path c or d would be permitted, in that the product of the former pathway, the C1″ *R* epimer (*R*)-**12**, equilibrates to provide a mixture containing the C1″ *S* epimer (*S*)-**12**.



Scheme 3. Rearrangement of DHA into peroxyhemiacetal **11** and conversion into 2-deoxyartemisinin **15** via the Kornblum–de la Mare process.



Scheme 4. Proposed formation of a) dicarbonyl compound **14** and b) tricarbonyl compound **12** from DHA via homolysis of the peroxide and sequential α-cleavage reactions c) and d).

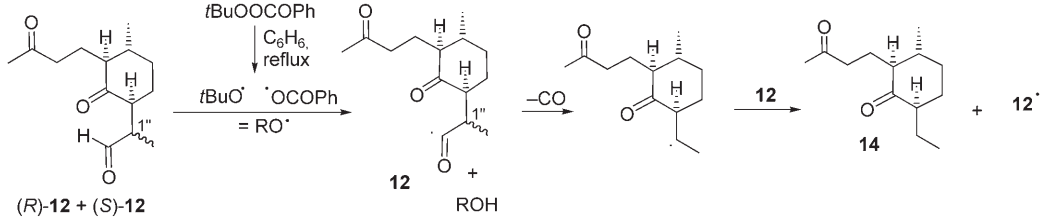
It is unlikely that the site of epimerisation in the tricarbonyl compound is at C6.[33] The dicarbonyl compound **14** and a related tricarbonyl compound obtained from 10-deoxoartemisinin[41] are each single, stable epimers. To establish the site of epimerisation in **12**, the epimer mixture was heated in benzene at reflux under nitrogen with *tert*-butylperoxybenzoate for 4 h (Scheme 5). These conditions are related to, but rather milder than, those recorded for decarbonylation of aldehydes induced by di-*tert*-butyl peroxide.[37] Whilst a relatively large amount (72%) of the tricarbonyl compound was recovered, dicarbonyl compound **14** was obtained cleanly in 17% yield based on reacted starting material. Thus, the site of epimerisation in the tricarbonyl compound is at C1″.

In the thermal decomposition of DHA, the carbonyl compounds **12** and **14**, formed in a ratio of 88:12, are generated from a common intermediate, the diradical intermediate **20** (or its ring-closed equivalent, the dioxetane), as depicted in Scheme 4. Therefore, it is likely that both compounds will appear among the decomposition products formed from DHA when the latter is thermally stressed. The volatile fragments lost from DHA under conditions of TGA are thus likely to be formic acid together with carbon monoxide, accounting for 26% of material as indicated in Figure 1. The dicarbonyl compound **14** is not formed from ferrous iron catalyzed decomposition of DHA. Clearly, the presence of iron influences the reactivity of the intermediate radicals (cf. Scheme 4).

b. Decomposition under aqueous conditions

i. Artesunate 3

Artesunate **3** is hydrolytically unstable under both acidic and neutral conditions. At pH 1.2, the conversion into DHA **2** is rapid, with a half-life ($t_{1/2}$) of 26 min.[42] At pH 7.4, the $t_{1/2}$ is about 10 h at 23 °C. Within the artesunate capsules, water will induce hydrolysis to DHA. As listed in Table 1, DHA is the principal degradant when artesunate capsules are thermally stressed. Treatment of artesunate with a 1:1 mixture of 5 M aqueous hydrochloric acid–ethanol at room temperature for 1.5 h gave DHA **2** (12%), the peroxyhemiacetal **11** (30%), the furanose acetal **16** (1.2%), β-arteether **25** (34%), and α-arteether **26** (15%; Figure 4). No artesunate was recovered from this reaction. The arteether epimers were readily identified.[3,43,44] Three more products, glycal **9**, 2-deoxyartemisinin **15**, and the furanose acetal **24** were obtained when the reaction was repeated in 1:1 2 M aqueous hydrochloric acid–acetonitrile at room temperature for 17 h (Figure 4). Identification of furanose acetal **24** (Figure 4) follows from comparison of spectroscopic data with the other furanose acetals discussed below, and by its clean conversion into unsaturated furanose acetal **16** by treatment with 5 M hydrochloric acid in THF at room tempera-



Scheme 5. Conversion of tricarbonyl compound **12** into dicarbonyl compound **14** by free-radical decarbonylation.

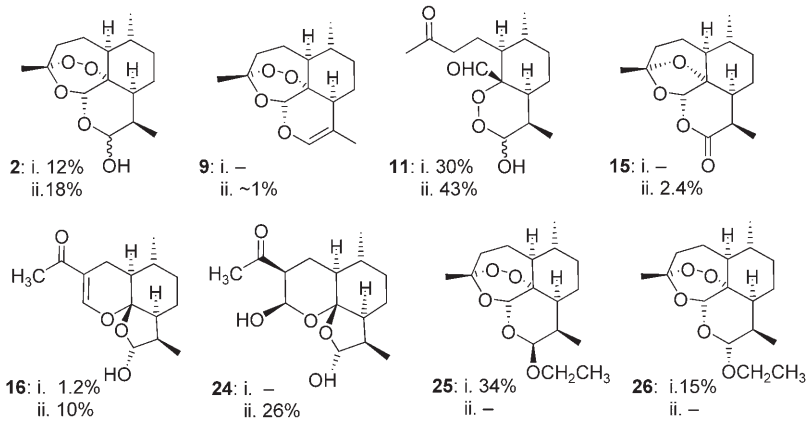


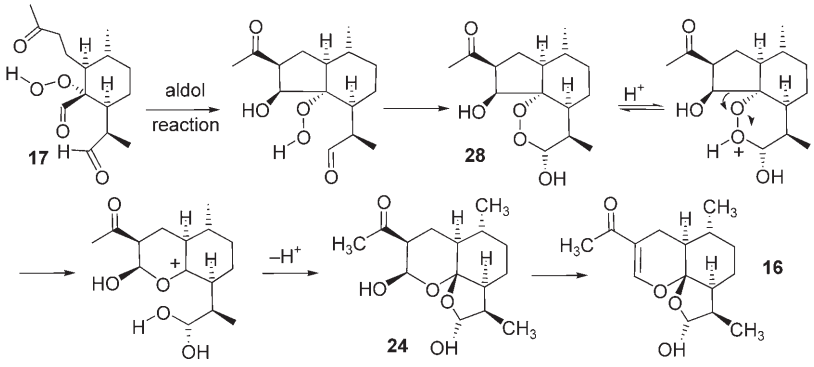
Figure 4. Products obtained from treatment of artesunate **3** with aqueous HCl in EtOH or CH_3CN. Conditions: i) 5 M aqueous HCl in EtOH, 1.5 h, room temperature; ii) 2 M aqueous HCl in CH_3CN, 17 h, room temperature. Relative amounts of each were determined by ^1H NMR spectroscopy of the reaction mixture.

ture for 4 h. The isolation of such large quantities of the peroxyhemiacetal **11** is noteworthy.

ii. Dihydroartemisinin 2

Under the conditions used for acid hydrolysis of arteether,[34,45] DHA **2** in 5 M aqueous hydrochloric acid–ethanol (1:1) at room temperature for 2 h gave the peroxyhemiacetal **11** (48%), the two furanose acetals **16** and **27**, and the arteether epimers **25** and **26** (Figure 5). The furanose acetal **27**, a single epimer, is identified by comparison of its spectroscopic data with that of the known methoxy analogue.[45] Furanose acetal **16** was obtained in higher yield by treatment of DHA **2** with aqueous 5 M HCl in THF (Figure 5). Originally reported as an oil,[45] it forms a crystalline hydrate for which the results of an X-ray crystallographic study are given below. Treatment of peroxyhemiacetal **11** with 5 M aqueous hydrochloric acid in THF at room temperature for 2 h largely returned unchanged peroxyhemiacetal and a small amount (10%) of **16**. Therefore, it is unlikely that **11** is an important intermediate in formation of **16** from DHA. We propose that the ring-opened hydroperoxide **17** (Scheme 3) first undergoes aldolisation to peroxide **28**, which, via proton-initiated migration to electron-deficient oxygen, is transformed into **24** (Scheme 6). A related aldolisation involving the acetyl and aldehyde without disruption of the hydroperoxide has been reported elsewhere.[46]

The behaviour of DHA in the presence of acids and bases is relevant to the stability of DHA in fixed formulation with piperaquine phosphate.[21,22] When DHA **2** was heated at reflux with excess triethylamine in ethanol for 21 h, or stirred in a 25:15 ethanol/dichloromethane solution containing silica gel,[47] triethylamine, and acetic acid at room temperature for 5 days, the only compound obtained was 2-deoxyartemisinin **15** in 76–80% yield. 2-Deoxyartemisinin was also obtained by heating DHA in benzene in the presence of silica gel.[44,47] Treatment of the peroxyhemiacetal **11** at reflux with excess triethylamine in ethanol for 21 h or with equimolar amounts of triethylamine and acetic acid in dichloromethane at room temperature also provided 2-deoxyartemisinin. No reversion to DHA was detected, although this has been proposed to occur.[48]



Scheme 6. Proposed formation of compounds **16** and **24**.

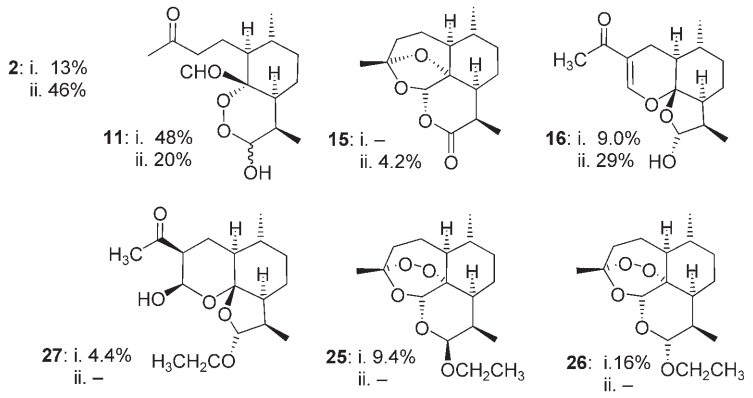


Figure 5. Products obtained from treatment of DHA **2** with aqueous HCl in EtOH or THF. Conditions: i) 5 M aqueous HCl in EtOH, 2 h, room temperature; ii) 5 M aqueous HCl in THF, 2 h, room temperature. Relative amounts of each were determined by ^1H NMR spectroscopy of the reaction mixtures.

c. Analysis of rectal capsules

Batches of new and aged capsules, that is, capsules that had been stored for at least one year at 25 °C and 60% RH (cf. Table 1) containing 400, 100, or 0 (placebo) milligrams of artesunate were rinsed with ethanol to remove the polyethylene coating, then frozen in liquid nitrogen and crushed, and treated with a mixture of hexane and acetonitrile. Hexane removes the glycerides and hard fats. The acetonitrile layer was treated with standard phosphate buffer (pH 3.8) and then analyzed by HPLC. For the NMR spectroscopic analysis, the acetonitrile–buffer solution was extracted with organic solvents, and the resulting solution was evaporated prior to NMR analysis. The shell material and coating of the capsules were also processed to provide extracts suitable for analysis by HPLC and NMR spectroscopy. The hexane layer was also examined to cover the possibility that lipophilic degradants such as glycal **9** had been removed in this phase. It was established that the peroxyhemiacetal **11** is an important degradant, which accounts for up to 1.2% of artesunate degradation. In addition, glycal **9** and 2-deoxyartemisinin **15** were also found, although in quantities $<0.2\%$. β-Artesunate **5** and/or the dimers **6** and **7** were detectable at levels $<0.2\%$ in aged 400-mg capsules, but not in 100-mg capsules. Extracts of shell material from the aged 100-mg capsules contain artesunate (0.2%) and DHA (0.35%). Artesunate was not found in the coating material, but DHA may be present up to approximately 0.4%. No artesunate or DHA was found in the capsule shell or coating of new capsules. HPLC chromatograms of aged capsules contain significant fractions that are present in neither new nor placebo capsules. Analysis of these fractions by ^{1}H NMR, ^{31}P NMR, high-resolution CIMS and FABMS spectra indicate that they are composed of medium-chain fatty acids and triglycerides. Neither succinic acid, an obvious hydrolysis product of artesunate, nor possible products arising via transesterification of artesunate with fatty acids in glycerides were in these fractions. Full details are given elsewhere.[49]

Conclusions

Comments on the fitness of artesunate and DHA as antimalarial drugs: use of fixed vs. dual combinations

Irrespective of the actual mechanisms of the thermal decomposition pathways, the very formation of the isomeric β-artesunate **5**, the novel dimers **6**–**8**, and the glycal **9** highlights the multifaceted chemical reactivity of artesunate. Artesunate is a dicarboxylic acid hemiester with the normal acid–base reactivity of the free carboxyl group compounded by the reactivity of the ester group at C10. This is made labile by O11 such as to undergo ester *O*-alkyl cleavage to provide oxonium ion **13** as the active intermediate, which leads to compounds **5**–**8**. The oxonium ion can accept water to provide DHA, amounting to a hydrolysis of the ester by *O*-alkyl cleavage under S_N1 conditions. Therefore, artesunate is readily hydrolyzed to DHA under both acidic and neutral conditions. The *cis* relationship between the succinyl residue and H9 also allows for intercession of a thermally concerted *syn*-elimination reaction to produce the glycal **9**.

Thermogravimetric analysis indicates that DHA is appreciably less stable than artesunate; indeed it is thermally labile. The facile formation of the peroxyhemiacetal **11** indicates how easily DHA can "unzip", either thermally, or under aqueous conditions. Of the current clinically used artemisinins, DHA elicits the highest neurotoxicity in cellular and animal assays.[7] It is tempting to speculate that the facile unzipping coupled with unmasking of the hydroperoxide and aldehyde groups, as in compound **11**, capable of either providing reactive oxygen species in the presence of ferrous iron (which may damage mitochondrial membranes)[50] or of interacting with biogenic amines, may be responsible. This aside, homolysis of the peroxide bridge, induced either thermally or by ferrous iron,[41] enables a remarkable cascade of α-cleavage reactions to intercede, terminating in the formation of the tricarbonyl and dicarbonyl compounds **12** and **14**. Whilst the peroxyhemiacetal **11** has antimalarial activity, the other non-peroxide degradation products, including 2-deoxyartemisinin **15**, do not.[34]

Clinically, artesunate serves essentially as a prodrug for DHA. Measurement of plasma C_{max} drug levels in healthy subjects administered oral artesunate shows a DHA/artesunate ratio of approximately 3.5:1.[51] The dose-corrected relative oral bioavailability of directly administered DHA compared with DHA arising from administerd artesunate is 43%; this difference is ascribed to first-pass metabolism, wherein a substantial amount of the administerd DHA is converted into the glucuronide and excreted.[52] Artesunate is more resistant to such metabolism, but once in the bloodstream, it is hydrolyzed to DHA. Nevertheless, in malaria patients administered either oral DHA or artesunate, both drugs evince approximately the same bioavailability.[15,53] Hence, DHA has been recommended as the preferred drug on the basis of its lower cost, but, as we record herein, it is thermally and chemically labile, and storage in tropical countries is problematic.[32] Artesunate has other advantages over DHA. The former is an easily crystallised single isomer; DHA is a mixture of epimers[8] which apparently deposits as a poorly crystalline mass from methanol. Aqueous solubility of DHA in solution at physiological pH is unknown, as attempts to measure this results in its decomposition.[8] As alluded to above, decomposition products of DHA, in particular the peroxyhemiacetal **11** and 2-deoxyartemisinin **15**, are easily formed. It is not yet known if such compounds may form during the preparation or storage of the fixed formulation with piperaquine phosphate (artekin),[23] or appear as metabolites in patients treated with artesunate or DHA. Piperaquine is chemically very stable, and its metabolic robustness is reflected in a very long biological half-life;[22] thus the pharmacokinetic and chemical mismatch with the fragile DHA must serve to complicate the development of the fixed combination product. The Chinese artequick combination, which employs artemisinin and piperaquine free base, does not suffer the instability problems attending the use of artekin.[24]

The thermal lability of the clinically used artemisinins is incompatible with ICH/WHO requirements for formulated drugs destined for use in climatic zone III and IV countries. Yet the ar-

temisinins are by far the most effective drugs for the treatment of malaria, and alternatives with the rapidity of cure of the artemisinins are simply not available. In light of the current recommendation of using a fixed combination of an artemisinin with another antimalarial drug, emphasis should be placed on providing dose regimens that make allowance for attrition through thermal decomposition of the artemisinin partner up to a designated expiry date. That is, in such a fixed formulation, it is critically important that the amount of active artemisinin present does not fall below the designated effective therapeutic dose before the expiry date. The rate of decomposition under thermal stress testing will need to be carefully established such that the initial loading of artemisinin in the fixed formulation can be evaluated, although toxicity concerns associated with a higher initial loading of the artemisinin will have to be addressed. A fixed-formulation tablet has the advantage of simplified administration, which would potentially enhance patient compliance.[13] However, given the problems that must emerge through co-formulating a thermally labile and chemically reactive artemisinin with a substantially more robust and basic quinoline or guanidine antimalarial, the use of a dual combination in which the tablets of each drug are either formulated in a manner that prevents direct interaction or are individually packaged for separate administration must also be considered.

Finally, emphasis must be placed on infrastructural improvements involving transportation and storage facilities, so that these thermally fragile yet absolutely essential drugs can be delivered to malarious areas and stored under appropriately controlled conditions, such as through the use of solar-powered refrigerators.[54]

X-Ray Crystallography

Single-crystal structure determinations were carried out on suitable specimens of compounds **7** and **16**, which were found to crystallise in chiral space groups $P2_1$ and $P2_12_12_1$, respectively, consistent with enantiomeric purity. Data were collected at 100 K on a Bruker Smart APEX CCD diffractometer. Structure solution and refinement was carried out using the SHELXTL suite of X-ray crystallography programs. Absolute structure determination was not possible because the radiation used was $Mo_{K\alpha}$. However, the handedness of the artemisinin framework was established previously by numerous absolute structure determinations. Both crystal structures refined successfully to low-discrepancy R indices and with low residual electron-density peaks and holes in the final difference Fourier map.

The single-crystal X-ray structure determination confirmed the structure of the β,β diaxial dimer **7**, for which a thermal ellipsoid plot (40% probability) is shown in Figure 6. Although the molecule has potential twofold symmetry, in the solid state, two independent molecules are found in the asymmetric unit. The molecular configurations and geometry of the two independent molecules are similar. The molecular parameters in **7** are similar to those found for other artemisinin derivatives: the four independent O–O peroxy bond lengths are 1.477(3) and 1.478(3) Å in molecule A, and 1.474(3) and 1.482(3) Å in molecule B, showing no significant variation.

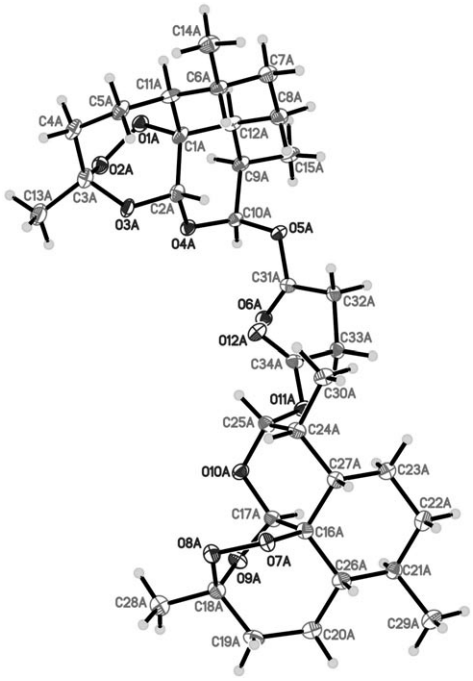


Figure 6. X-ray crystal structure of the β,β′ or diaxial artesunate dimer **7**.

Compound **16** obtained by acid treatment of DHA in a mixed aqueous THF solvent system crystallises as a monohydrate. The molecular geometry and labelling scheme is shown in Figure 7. The structure of the rearranged product is as expected, and the bond length of 1.348(3) Å for C2–C3 confirms the alkenyl double bond between these carbon atoms and its anti-conformation to the conjugated keto group C12–O12. This is indicated by the torsion angle C2–C3–C12–O12 of 168.7°. The water of crystallisation forms three hydrogen bonds to the main molecule. It serves as an acceptor from the hydroxy group at O10–H10 and as an H-bond donor to the

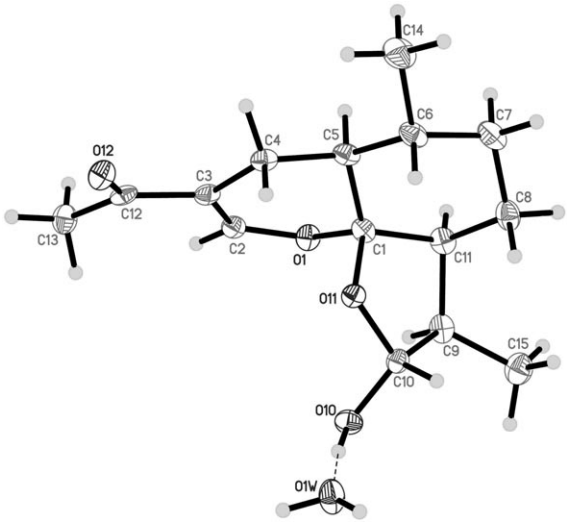


Figure 7. X-ray crystal structure of the furanose acetal **16**.

Table 2. Crystallographic data and structure refinement for compounds **7** and **16**.

	7	**16**
CSD deposition number	633301	633302
empirical formula	$C_{34}H_{50}O_{12}$	$C_{15}H_{24}O_5$
formula weight [Da]	650.74	284.34
T [K], λ [Å]	100(2), 0.71073	100(2), 0.71073
crystal system, space group	monoclinic, $P2_1$	orthorhombic, $P2_12_12_1$
a [Å]	15.892(2)	5.2484(5)
b [Å]	6.4937(8)	14.2412(13)
c [Å]	31.237(4)	19.7618(18)
β [°]	92.143(3)	90
V [Å^3]	3221.3(7)	1478.7(2)
Z, D_c [Mgm^{-3}]	4, 1.342	4, 1.277
μ [mm^{-1}]	0.101	0.095
crystal size [mm]	0.5×0.08×0.05	0.50×0.15×0.10
$2\Phi_{max}$, completeness [%]	50, 99.4	52, 98.0
transmission [max/min]	1.00/0.81	1.00/0.84
data, restraints, parameters	6177, 1, 829	1689, 0, 181
R_1 (obs), wR_2 (all)	0.0404, 0.0739	0.0329, 0.0752
GoF	0.985	1.011
peak/hole [e–Å^{-3}]	+0.19/−0.19	+0.22/−0.13

O10 hydroxy oxygen atom and O12 keto oxygen of different neighbour molecules.

Crystal data and structure refinement for the compounds are given in Table 2.

Experimental Section

All reactions were carried out under N_2 atmosphere. Dihydroartemisinin was obtained either from the Kunming Pharmaceutical Corporation, Kunming (China), or from Haphacen, Hanoi College of Pharmacy (Vietnam), and used without further purification. Artesunate **3** was supplied by Dr. Robert Carter, Knoll AG, Liestal (Switzerland) or prepared according to the Chinese procedure.[55] The following solvents were dried prior to use: ethyl acetate from $MgSO_4$, hexane ($CaCl_2$), CH_2Cl_2 (CaH), triethylamine (CaH and stored over KOH pellets), and THF (sodium in benzophenone). TLC was performed with Merck Kieselgel 60 F_{254} plates and visualised with UV light (254 nm) and/or heating after treatment with 5% ammonium molybdate in 10% concentrated sulfuric acid. Column chromatography was performed with Merck silica gel 60 (0.04–0.063 mm).

NMR spectral data, unless otherwise stated, were obtained from spectra of samples in $CDCl_3$. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury spectrometer operating at 300 and 75 MHz, respectively. Melting points were carried out on a Leica Hot Stage DME E compound microscope and are corrected. MS data were obtained on a Finnigan TSQ 7000 mass spectrometer (CI+, methane), on an API QSTAR high-performance triple quadrupole TOF mass spectrometer with electrospray ionisation, and on a Waters Micromass GCT premier TOF high-resolution mass spectrometer (CI+, methane). IR spectra were recorded either on a PerkinElmer PC 16 or a PerkinElmer Spectrum One spectrometer. Optical rotations were performed on a PerkinElmer model 241 spectrometer. Elemental analyses were obtained from MEDAC Ltd., Surrey (UK). Thermal analysis was carried out on a PerkinElmer thermogravimetric analyzer TGA7, with samples under N_2 and heated at a rate of 10 °C min^{-1}. Single-crystal X-ray structure measurements were carried out on a Bruker Smart-APEX CCD four-circle diffractometer. All computations in structure determination and refinement were performed on a Silicon Graphics Indy computer using programs of the Siemens SHELXTL PLUS (Version 5) package.

Artesunate 3

a. Thermal decomposition: Artesunate **3** (500 mg) was heated for 39 h under N_2 in an oil bath at a temperature of 100 °C. Then, ethyl acetate (10 mL) was added to dissolve the cooled solid. The resulting solution was treated with saturated sodium hydrogen carbonate (10 mL), and the aqueous layer was separated and extracted with ethyl acetate (3×10 mL). The extracts were combined with the original ethyl acetate organic layer, and the combined solution was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure left a pale-yellow residue (365 mg) whose components were verified by ^{1}H NMR spectroscopic analysis. The glycal **9** (41 mg, 16%), the formate ester **10** (37 mg, 13%), the β,β-artesunate dimer **7** (27 mg, 4.4%), the α,β-artesunate dimer **6** (25 mg, 4.0%), the α,α-artesunate dimer **8** (10.5 mg, 1.7%), the tricarbonyl compound **12** (2.5 mg, 1.1%), and the peroxyhemiacetal **11** (3.8 mg, 1.4%) were shown to be present. The residue was submitted to chromatography with ethyl acetate–hexane (30:70) to give the compounds which were characterised as described below. The aqueous sodium hydrogen carbonate layer was treated with saturated aqueous sodium hydrogen sulfate until it became acidic (pH <4). The resulting aqueous mixture was extracted with ethyl acetate (3×10 mL). The aqueous layer was treated with aqueous sulfuric acid (1 M) until the pH was ~2. It was also extracted with ethyl acetate (3×10 mL). The ethyl acetate extracts from the two extraction operations were combined, washed with H_2O, then dried ($MgSO_4$). Filtration and concentration of filtrate under reduced pressure left β-artesunate **5** (37 mg, 10%) and unchanged artesunate **3** (175 mg, 48%), which were separated by chromatography on silica gel with ethyl acetate–hexane (60:40). The artesunate **3** crystallised from ethyl acetate as prisms; mp: 135–136 °C (reference [3]: 134–137 °C). It was identical to a sample prepared by treatment of DHA **2** with succinic anhydride in CH_2Cl_2 in the presence of 1 equiv triethylamine or *N*,*N*-dimethylaminopyridine (DMAP) according to the Chinese procedure.[7,55] The β-artesunate **5** crystallised from ethyl acetate–hexane as colourless rectangular plates; mp: 97.6–98.2 °C, identical to an authentic sample prepared according to the published procedure.[7]

The α,β-artesunate dimer **6** was obtained as a foam; $[\alpha]_D^{22} = +67.8°$ (c = 1.1, $CHCl_3$); ^{1}H NMR: δ = 0.84–0.88 (m, 6H, 9-Me), 0.96–0.99 (m, 6H, 6-Me), 1.23–1.36 (m, 4H), 1.42 (d, J = 5.0 Hz, 6H, 3-Me), 1.47–1.66 (m, 4H), 1.68–1.82 (m, 6H), 1.87–1.97 (m, 2H), 2.04 (s, 3H), 2.19–2.28 (m, 1H), 2.32–2.42 (m, 2H), 2.52–2.82 (m, 6H), 5.46 (d, J = 8.8 Hz, 2H, H12), 5.79 (d, J = 9.7 Hz, 1H, H10α), 6.255 (d, J = 3.5 Hz, 1H, H10β); ^{13}C NMR: δ = 12.50 (C15), 12.95 (β, C15′), 20.65 (C14), 22.38 (C8), 24.49 (C8′), 25.00 (C5), 26.33 (C13), 29.56 (C16), 30.24 (C9′), 32.16 (C9), 34.44 (C7), 34.83 (C7′), 36.60 (C4), 37.63 (C6), 37.76 (C6′), 44.23 (C8a′), 45.57 (C8a), 51.89 (C5a), 52.71 (C5a′), 80.41 (C12a), 80.83 (C12a′), 89.00 (C12′), 91.77 (C12), 92.50 (C10), 95.36 (C10′), 104.60 (C3′), 104.70 (C3), 170.83 (C=O), 170.98 (C=O); IR

(film): $\tilde{\nu}_{max}$ = 2938, 2927, 2874, 1747 (C=O), 1559, 1508, 1492, 1452, 1412, 1376, 1362, 1307, 1279, 1260, 1228, 1207, 1199, 1159, 1132, 1119, 1076, 1035, 1017, 989, 925, 908, 875, 859, 824, 802, 735, 703 cm^{-1}; MS (CI, CH_4) *m/z* (%) = 221.2 (87), 249.1 (24), 339.2 (32), 441.3 (46), 487.3 (21), 546.3 (9), 559.3 (24), 605.3 (100), 651.3 (8); MS (ESI) calcd 668.3408 [M^++H_2O], found 668.3410.

The α,α-artesunate dimer **8** was obtained as viscous oil; $[\alpha]_D^{22}$ = +26.4° (*c* = 0.41, $CHCl_3$); ^{1}H NMR δ = 0.86 (d, *J* = 7.3 Hz, 6H, 9-Me), 0.96 (d, *J* = 5.9 Hz, 6H, 6-Me), 1.03–1.22 (m, 4H), 1.24–1.40 (m, 3H), 1.43 (s, 6H, 3-Me), 1.47–1.70 (m, 4H), 1.74–1.93 (m, 5H), 1.99–2.45 (m, 6H), 2.46–2.63 (m, 2H), 2.64–2.83 (m, 4H), 5.43 (s, 2H, H12), 5.79 (d, *J* = 9.7 Hz, 2H, H10); ^{13}C NMR δ = 12.48 (C15), 20.61 (C14), 22.39 (C8), 24.97 (C5), 26.34 (C13), 29.24 (C16), 32.15 (C9), 34.47 (C7), 36.58 (C4), 37.64 (C6), 45.59 (C8a), 51.90 (C5a), 80.40 (C12a), 91.75 (C12), 92.45 (C10), 104.71 (C3), 171.18 (C=O); MS (ESI) calcd 668.3408 [M^++H_2O], found 668.3533.

The β,β-artesunate dimer **7** crystallised from ethyl acetate–hexane as colourless needles; mp: 170–171 °C; $[\alpha]_D^{22}$ = +143.6° (*c* = 0.58, $CHCl_3$); ^{1}H NMR: δ = 0.87 (d, *J* = 7.3 Hz, 6H, 9-Me), 0.98 (d, *J* = 6.2 Hz, 6H, 6-Me), 1.23–1.36 (m, 4H), 1.42 (s, 6H, 3-Me), 1.45–1.58 (m, 4H), 1.66–1.74 (m, 6H), 1.78–2.09 (m, 8H), 2.31–2.42 (m, 2H), 2.58–2.81 (m, 4H), 5.47 (s, 2H, H12), 6.27 (d, *J* = 3.5 Hz, 2H, H10); ^{13}C NMR δ = 12.86 (C15), 20.65 (C14), 24.50 (C8), 25.02 (C5), 26.30 (C13), 29.58 (C16), 30.28 (C9), 34.81 (C7), 36.59 (C4), 37.84 (C6), 44.22 (C8a), 52.69 (C5a), 80.84 (C12a), 89.06 (C12), 95.46 (C10), 104.66 (C3), 170.94 (C=O); MS (ESI) calcd 668.3408 [M^++H_2O], found 668.3485; $C_{34}H_{50}O_{12}$ calcd C 62.75, H 7.74; found C 62.11, H 7.67.

The glycal **9** was obtained as a white solid, which was recrystallised from hexane to give colourless needles; mp: 95–97 °C (reference [3]: 96–98 °C); $[\alpha]_D^{22}$ = +158.7° (*c* = 1.22, $CHCl_3$); ^{1}H NMR δ = 0.98 (d, *J* = 5.3 Hz, 3H, 6-Me), 1.02–1.26 (m, 3H), 1.43 (s, 3H, 3-Me), 1.59 (s, 3H, 9-Me), 1.63–1.73 (m, 2H), 1.86–1.98 (m, 1H), 1.99–2.10 (m, 2H), 2.35–2.45 (m, 1H), 5.54 (s, 1H, H12), 6.18 (d, *J* = 1.1 Hz, 1H, H10).

The formate ester **10** was obtained as needles by concentration under reduced pressure of the eluate from chromatography; attempts to recrystallise this compound resulted in its decomposition; mp: 153–154 °C; $[\alpha]_D^{22}$ = +95.6° (*c* = 0.55, $CHCl_3$); ^{1}H NMR: δ = 0.91 (d, *J* = 7.3 Hz, 3H, 9-Me), 0.98 (d, *J* = 6.2 Hz, 3H, 6-Me), 1.22–1.40 (m, 2H), 1.43 (s, 3H, 3-Me), 1.47–1.59 (m, 2H), 1.67–1.84 (m, 3H), 1.88–1.95 (m, 1H), 2.01–2.09 (m, 2H), 2.33–2.46 (m, 1H), 2.82–2.87 (m, 1H), 5.49 (s, 1H, H12), 6.24 (d, *J* = 3.2 Hz, 1H, H10), 8.19 (s, 1H, H OCHO); ^{13}C NMR δ = 12.87 (C15), 20.65 (C14), 24.36 (C8), 24.99 (C5), 26.26 (C13), 30.16 (C9), 34.76 (C7), 36.55 (C4), 37.78 (C6), 44.02 (C8a), 52.66 (C5a), 80.75 (C12a), 89.09 (C12), 95.91 (C10), 104.74 (C3), 160.38 (C=O); IR (film): $\tilde{\nu}_{max}$ = 2986, 2947, 2924, 2869, 2853, 1546, 1539, 1532, 1495, 1447, 1377, 1350, 1308, 1280, 1260, 1227, 1208, 1187, 1176, 1159, 1134, 1092, 1060, 1024, 985, 969, 932, 895, 876, 847, 824; MS (ESI): calcd 267.1596 [M^+−OCHO], found 267.1594.

The peroxyhemiacetal **11** was obtained as a 2:1 mixture of epimers as a pale-yellow oil; ^{1}H NMR (major epimer) δ = 0.74 (d, *J* = 7.0 Hz, 1H), 0.83 (d, *J* = 7.3 Hz, 3H, 9-Me), 0.92 (d, *J* = 6.1 Hz, 3H, 6-Me), 1.18–1.28 (m, 2H), 1.51–1.72 (m, 2H), 1.81–1.91 (m, 2H), 2.13 (s, 3H, $COCH_3$), 2.23–2.44 (m, 2H), 2.45–2.67 (m, 3H), 5.05 (s, 1H, H10), 10.25 (s, 1H, CHO); ^{1}H NMR (minor epimer) δ = 0.73–0.75 (d, *J* = 7.0 Hz, 1H), 0.82–0.85 (d, *J* = 7.3 Hz, 3H, 9-Me), 0.91–0.93 (d, *J* = 6.1 Hz, 3H, 6-Me), 1.18–1.28 (m, 2H), 1.51–1.72 (m, 2H), 1.81–1.91 (m, 2H), 2.13 (s, 3H, $COCH_3$), 2.23–2.44 (m, 2H), 2.45–2.67 (m, 3H), 5.36 (s, 1H, H10), 10.15 (s, 1H, CHO); MS (ESI): *m/z* calcd 285.1697 [M^++1], found 285.1688. This data is in essential agreement with that previously recorded.[34]

The tricarbonyl compound **12** was obtained as a 3:1 mixture of the 1″*R*,2*S*,3*R*,6*S* and 1″*S*,2*S*,3*R*,6*S* epimers, as a viscous colourless oil. Attempts to separate the epimers by HPLC (Agilent G1311 A quaternary pump, G1313 A auto-sampler, G131 A diode array detector, column: reversed-phase YMC-Pack ODS-AQ 3 μm, 250×4.6 mm^2, eluting solvent: CH_3CN–phosphate buffer, pH 3.4, 5:95→35:65) was not successful; as shown below, the individual major 1″*R* epimer epimerises readily at room temperature to provide a mixture containing the 1″*S* epimer. ^{1}H NMR (major 1″*R* epimer) δ = 1.10 (d, *J* = 5.8 Hz, 3H, 6-Me), 1.17 (d, *J* = 7.0 Hz, 3H, 9-Me), 1.46–1.69 (m, 4H), 1.71–1.97 (m, 4H), 2.12 (s, 3H, H4′), 2.29–2.43 (m, 1H), 2.49–2.64 (m, 1H), 2.67–2.78 (m, 1H), 9.75 (s, 1H, CHO); ^{1}H NMR (minor epimer) δ = 1.10 (d, *J* = 5.8 Hz, 3H, 6-Me), 1.17 (d, *J* = 7.0 Hz, 3H, 9-Me), 1.46–1.69 (m, 4H), 1.71–1.97 (m, 4H), 2.12 (s, 3H), 2.29–2.43 (m, 1H), 2.49–2.64 (m, 1H), 2.67–2.78 (m, 1H), 9.74 (s, 1H, CHO); ^{13}C NMR 11.41, 11.44, 20.40, 20.90, 20.94, 29.84, 30.29, 30.35, 30.66, 34.78, 34.83, 39.65, 40.58, 41.45, 41.54, 45.64, 45.68, 52.02, 52.74, 56.59, 56.81, 203.92, 204.64, 209.08, 209.18, 211.12, 212.19; IR (CH_2Cl_2): $\tilde{\nu}$ = 3056.58, 1709.37, 1422.42, 1266.47, 896.73, 739.44, 705.27 cm^{-1}; MS (CI, NH_3) *m/z* = 239 [M^++1] (8%), 256 [M^++NH_4^+] (36%), 476 [2M^+] (12%), 494 [2M^++NH_4^+] (100%); MS (ESI) calcd 237.1491 [M^+−1], found 237.1506; calcd 239.1491 [M^++1], found 239.1644. The NMR data is in essential agreement with that reported in the literature.[33]

b. Independent preparation of degradation products

i. Artesunate dimers 6 and 7: *By Schmidt reaction from DHA*: Dihydroartemisinin (500 mg, 1.761 mmol) in CH_2Cl_2 (20 mL) containing 1,8-diazabicyclo[5.4.0]undecane (13.2 μL, 0.05 equiv) was treated with trichloroacetonitrile (194 μL, 1.1 equiv) at room temperature. After stirring for 2 h, the resulting solution was treated with artesunate (1.02 g, 2.64 mmol, 1.5 equiv). After a further 4 h, excess solvent was directly removed by evaporation under reduced pressure, and the residue was submitted to chromatography on silica gel with ethyl acetate–hexane (30:70) to give the dimer **6** as a foam (588 mg, 51%); $[\alpha]_D^{22}$ = +83.7° (*c* = 0.55 in $CHCl_3$), with spectroscopic data identical with that obtained above.

Dihydroartemisinin (250 mg, 0.88 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) containing 1,8-diazabicyclo[5.4.0]undecane (6.6 μL, 0.05 equiv) was treated with trichloroacetonitrile (97 μL, 1.1 equiv) at room temperature. After stirring for 2 h, the resulting solution was treated directly with β-artesunate (0.51 g, 1.32 mmol). After 4 h, excess solvent was directly removed by evaporation under reduced pressure, and the residue was submitted to chromatography on silica gel with diethyl ether–hexane (70:30) to give the crystalline dimer **7** (66 mg, 11.5%); $[\alpha]_D^{22}$ = +125.8° (*c* = 0.52 in $CHCl_3$), with spectroscopic data identical with that obtained above.

From artesunate: Boron trifluoride diethyletherate (123 μL, 1.00 mmol) was added to a solution of artesunate **3** (384 mg, 5.00 mmol) in CH_2Cl_2 (10 mL) cooled to −78 °C. This was stirred under N_2 atmosphere at −78 °C and warmed gradually to 20 °C for 12 h. It was poured into aqueous sodium hydrogen carbonate (2 M, 50 mL) and was then separated, and the aqueous phase was washed with CH_2Cl_2 (3×25 mL). The combined organic phase was dried over anhydrous sodium sulfate and evaporated in vacuo. It was purified by flash chromatography over silica gel (250 g), eluted with hexane–ethyl acetate (70:30) to yield initially the β,β-artesunate dimer **7** as a colourless crystalline solid (43.3 mg, 13.4%) and the α,β-artesunate dimer **6** as a white foam (9.7 mg, 3.0%).

ii. Formate ester 10: Formic acid (0.099 mL, 2.60 mmol, 2.0 equiv) was added to a stirred solution of artesunate **3** (500 mg, 1.30 mmol) in CH_2Cl_2 (10 mL). The resulting solution was heated at gentle reflux under N_2 in an oil bath at 40 °C for 22 h. The solution was cooled and treated with saturated aqueous sodium hydrogen carbonate (10 mL). The organic layer was separated. The aqueous layer was extracted with ethyl acetate (3×10 mL), and the combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave white foam, which after chromatography with ethyl acetate–hexane (20:80) gave the glycal **9** (3.7 mg, 1.1%), compound **10** (41.4 mg, 10.2%), identical to the compound obtained by thermal degradation of artesunate above, the epimeric α-formate ester (9.2 mg, 2.3%), and unreacted artesunate **3** (0.204 g, 41%). The epimeric α-formate ester was obtained as colourless oil; $[\alpha]_D^{22} = +47.8^\circ$ ($c = 0.25$ in $CHCl_3$); ^{1}H NMR $\delta = 0.89$ (d, $J = 7.3$ Hz, 3H, 9-Me), 0.97 (d, $J = 5.6$ Hz, 3H, 6-Me), 1.22–1.39 (m, 2H), 1.44 (s, 3H, 3-Me), 1.61–1.84 (m, 5H), 1.87–1.95 (m, 1H), 2.0–2.09 (m, 1H), 2.31–2.44 (m, 2H), 2.58–2.65 (m, 1H), 5.46 (s, 1H, H12), 5.86 (d, $J = 10.0$ Hz, 1H, H10), 8.13 (s, 1H, −OCHO); IR (film): $\tilde{\nu}_{max} = 2950$, 2925, 2870, 2853, 1559, 1547, 1493, 1450, 1377, 1279, 1260, 1227, 1208, 1186, 1176, 1159, 1133, 1124, 1093, 1024, 933, 896, 877, 846, 824, 803 cm^{-1}; MS (ESI) calcd 267.1596 [M^+−OCHO], found 267.1583.

c. Decomposition under aqueous conditions

i. Aqueous HCl–EtOH: Hydrochloric acid (5 M, 50 mL) was added to a stirred solution of artesunate **3** (500 mg, 1.30 mmol) in EtOH (50 mL) under N_2 at room temperature. After 1.5 h, the solution was quenched with saturated aqueous sodium hydrogen carbonate (100 mL), and the mixture was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a pale-yellow residue (496.8 mg), which after chromatography with ethyl acetate–hexane (30:70) gave five fractions: β-arteether **25** (137 mg, 34%), α-arteether **26** (59 mg, 15%), DHA **2** (45 mg, 12%), peroxyhemiacetal **11** (110.5 mg, 30%), and the furanose acetal **16** (4 mg, 1.2%). Saturated aqueous $NaHSO_4$ was added to the aqueous layer until it became acidic. The resulting mixture was extracted with CH_2Cl_2 (3×100 mL). The aqueous layer was treated with sulfuric acid (1 M) until the pH was ~2. It was extracted with CH_2Cl_2 (3×100 mL). The CH_2Cl_2 extracts from the two extraction operations were combined, washed with H_2O, then dried ($MgSO_4$). Filtration and concentration of filtrate under reduced pressure gave a small amount of an unrecognisable white solid (10 mg), but no artesunate was detected.

Recrystallisation of β-arteether **25** from ethyl acetate gave colourless hexagonal crystals; mp: 80–81 °C (references [3,43]: 81–83 °C). α-Arteether **26** was obtained as a colourless oil, identified by comparison with an authentic sample.[44] Compound **16** crystallised from ethyl acetate–hexane as colourless needles; mp: 112–113 °C; $[\alpha]_D^{22} = -23.1^\circ$ ($c = 0.52$, $CHCl_3$); this compound has not been reported previously as a solid; ^{1}H NMR: $\delta = 0.97$ (d, $J = 6.2$ Hz, 3H, 6-Me), 1.11 (d, $J = 7.0$ Hz, 3H, 9-Me), 1.26–1.42 (m, 3H), 1.65–1.77 (m, 2H), 1.85–1.95 (m, 2H), 2.04 (m, 1H), 2.14–2.28 (s, 3H, OCH_3), 2.57–2.7 (m, 2H), 5.18 (d, $J = 6.2$ Hz, 1H, H10), 7.44 (d, $J = 1.8$ Hz, 1H, =CH); ^{13}C NMR: $\delta = 10.79$ (C9-Me), 19.39 (C6-Me), 20.35 (C5), 24.85 (C8), 25.11 (C3-Me), 33.38 (C7), 33.56 (C6), 43.05 (C9), 43.82 (C5a), 46.84 (C8a), 105.75 (C10), 108.68 (C12a), 119.02 (=C), 154.10 (=CH), 196.85 (CO); IR (film): $\tilde{\nu}_{max} = 3475$, 3452, 3441, 3434, 2930, 2877, 1614, 1493, 1459, 1400, 1380, 1321, 1285, 1261, 1234, 1207, 1187, 1159, 1122, 1087, 1054, 980, 936, 924, 881, 863, 820 cm^{-1}; MS (CI, CH_4) *m/z* (%) = 181.1 (10), 208.1 (26), 249.1 (100), 250.1 (18), 267.1 (10); MS (CI) calcd 267.1596 [M^++1], found 267.1597. The NMR data is in essential agreement with that previously recorded.[45]

ii. Aqueous HCl–CH_3CN: A solution of HCl (2 M, 10 mL) was added to a stirred solution of artesunate **3** (500 mg, 1.30 mmol) in CH_3CN (10 mL), and the resulting solution was stirred for 17 h at room temperature. It was then quenched with saturated aqueous sodium hydrogen carbonate (20 mL), and the final mixture was extracted with CH_2Cl_2 (3×20 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure left a foam (469 mg), analysis of which by ^{1}H NMR spectroscopy indicated that it consisted of the glycal **9** (~2.6 mg, 1%), 2-deoxyartemisinin (8.9 mg, 2.4%), DHA **2** (73.3 mg, 18%), peroxyhemiacetal **11** (173.5 mg, 43%), and the furanose acetals **16** (38.9 mg, 10%) and **24** (104.9 mg, 26%). Compounds were isolated from the mixture by chromatography with ethyl acetate–hexane (30:70) and identified as described above, or as follows.

The 2-deoxyartemisinin **15** crystallised from ethyl acetate–hexane as colourless rectangular plates; mp: 114–115 °C (reference [1]: 105–107 °C); $[\alpha]_D^{22} = -144.9^\circ$ ($c = 0.65$, $CHCl_3$) (reference [1]: $[\alpha]_D^{23} = -133.7^\circ$ ($c = 0.98$, $CHCl_3$)); ^{1}H NMR: $\delta = 0.94$ (d, $J = 5.3$ Hz, 3H, 9-Me), 1.19 (d, $J = 7.3$ Hz, 3H, 6-Me), 1.23–1.27 (m, 3H), 1.53 (s, 3H, 3-Me), 1.56–1.67 (m, 2H), 1.74–1.81 (m, 3H), 1.89–1.94 (m, 2H), 1.97–2.04 (m, 1H), 3.14–3.22 (m, 1H, H9), 5.68 (s, 1H, H12); MS (CI, CH_4) *m/z* (%) = 151.2 (13) 164.2 (30), 193.2 (16), 203.2 (34), 222.3 (21), 267.2 (100); MS (ESI) calcd 267.1596 [M^++1], found 267.1605.

The furanose acetal **24** was obtained as a white foam; $[\alpha]_D^{22} = -62.6^\circ$ ($c = 1.15$ in $CHCl_3$); ^{1}H NMR: $\delta = 0.97$–0.99 (d, $J = 6.1$ Hz, 3H, 6-Me), 1.09 (d, $J = 6.7$ Hz, 3H, 9-Me), 1.20–1.32 (m, 3H), 1.62–1.72 (m, 2H), 1.77–1.95 (m, 2H), 1.98–2.15 (m, 3H), 2.21 (s, 3H, OCH_3), 2.83 (s, 1H, OH), 2.89–2.98 (m, 1H), 4.00 (d, $J = 6.4$ Hz, 1H, H12), 4.85 (d, $J = 7.0$ Hz, 1H, H10), 7.99 (s, 1H, OH); ^{13}C NMR: $\delta = 15.30$, 21.60, 23.98, 29.33, 29.39, 32.61, 33.49, 35.12, 38.48, 48.08, 57.60, 75.93, 94.08, 98.83, 210.41; IR (film): $\tilde{\nu}_{max}$ 3367.0, 2933.4, 1701.1, 1458.4, 1364.7, 1093.3, 1024.4, 913.4, 854.5, 742.3 cm^{-1}; MS (CI, CH_4) *m/z* calcd 267.1596 [$M^+ - H_2O$], found 267.1596.

Hydrochloric acid (5 M, 5 mL) was added to a stirred solution of compound **24** (29.1 mg, 0.102 mmol) in THF (5 mL) under N_2 at room temperature. After 4 h, the solution was quenched with saturated aqueous sodium hydrogen carbonate (10 mL), and the mixture was extracted with CH_2Cl_2 (3×10 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a colourless oil, which after chromatography with ethyl acetate–hexane (40:60) gave compound **16** (8 mg, 29%) and the starting compound **24** (16.3 mg, 56%).

Dihydroartemisinin 2

a. Thermal decomposition: Finely powdered DHA **2** (501 mg) under N_2 in a round-bottom flask was heated for 14 h in an oil bath at 100 °C. After 14 h, ethyl acetate (5 mL) was used to dissolve the pyrolysate, analysis of which by ^{1}H NMR spectroscopy revealed the presence of the glycal **9** (7.66 mg, 1.6%), the dicarbonyl compound **14** (8.47 mg, 2.3%), 2-deoxyartemisinin **15** (12.3 mg, 2.6%), a 3:1 mixture of epimers of the tricarbonyl compound **12** (61.7 mg, 15%), DHA **2** (356 mg, 72%), and the peroxyhemiacetal **11** (34.4 mg, 6.9%). The pyrolysate was submitted to chromatography with ethyl acetate–hexane (30:70) to give the compounds characterised as described above. The dicarbonyl compound **14** (2*S*,3*R*,6*R*)-2-(3′-oxo-1′-butyl)-3-methyl-6-ethylcyclohexan-1-one was isolated as a colourless oil; $[\alpha]_D^{22} = -33.8^\circ$ ($c = 1.13$ in $CHCl_3$); ^{1}H NMR: $\delta = 0.85$–0.90 (m, 3H, H2″), 1.07 (d, $J = 5.9$ Hz, 3H, 6-Me),

1.12–1.29 (m, 2H), 1.40–1.48 (m, 2H), 1.51–1.60 (m, 2H), 1.70–1.88 (m, 4H), 1.99–2.10 (m, 1H), 2.13 (s, 3H, H4″), 2.31–2.42 (m, 1H), 2.52–2.62 (m, 1H); ^{13}C NMR: δ = 12.15, 20.57, 21.02, 22.49, 30.24, 33.62, 35.09, 40.74, 41.77, 52.74, 56.89, 209.32, 213.7; MS (ESI) calcd 211.1693 [M^++1], found 211.1602.

b. Independent preparation of carbonyl compounds

i. Tricarbonyl compound 12: A mixture of DHA (500 mg, 1.76 mmol) and anhydrous ferrous bromide (0.1898 g, 0.88 mmol, 0.5 equiv) in THF (15 mL) was stirred under a N_2 atmosphere for 45 min at room temperature. It was then quenched by the addition of saturated sodium hydrogen carbonate (100 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layer was washed with saturated ammonium chloride, and the aqueous layer was separated and extracted with ethyl acetate (3×30 mL). The organic extracts were combined, briefly dried ($MgSO_4$), and then filtered. The filtrate was concentrated by evaporation under reduced pressure to leave a pale-yellow residue, which was submitted to chromatography with ethyl acetate–hexane (10:90) to give nonpolar fractions (the identity of which will be described elsewhere), and then the tricarbonyl compound as a colourless viscous oil (238 mg, 57%). Examination of the tricarbonyl compound by ^{1}H NMR spectroscopy indicated that it consisted essentially of one epimer corresponding to the major fraction in the tricarbonyl epimer mixture obtained by pyrolysis as described above: ^{1}H NMR: δ = 1.09–1.10 (d, J = 5.8 Hz, 3H, 6-Me), 1.16–1.18 (d, J = 7.3 Hz, 3H, 9-Me), 1.48–1.68 (m, 4H), 1.71–1.96 (m, 4H), 2.13 (s, 3H, H4′), 2.30–2.43 (m, 1H), 2.48–2.65 (m, 2H), 2.70–2.81 (m, 1H), 9.74 (s, 1H, CHO).

ii. Dicarbonyl compound 14: A solution of the tricarbonyl compound (146 mg, 0.61 mmol) and *tert*-butyl peroxybenzoate (0.036 mL, 0.61 mmol, 0.4 equiv) in dry benzene (3 mL) under N_2 was heated under vigorous reflux for 4 h at an oil bath temperature of 140 °C. After 4 h, the resulting mixture was cooled to room temperature and filtered to remove a crystalline precipitate. The filtrate was concentrated under reduced pressure to leave a pale-yellow residue, which after chromatography with ethyl acetate–hexane (10:90) gave the dicarbonyl compound as a colourless oil (6.0 mg, 17%) and the starting tricarbonyl compound (105 mg, 72% unreacted).

c. Decomposition under aqueous conditions

i. Aqueous HCl–EtOH: Aqueous HCl (5 M, 50 mL) was added to a stirred solution of DHA **2** (500 mg, 1.76 mmol) in EtOH (50 mL) at room temperature. After 2 h, the reaction mixture was treated with saturated aqueous sodium hydrogen carbonate (100 mL), and the resulting suspension was extracted with CH_2Cl_2 (3×100 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a dark-green residue, analysis of which by ^{1}H NMR spectroscopy indicated the presence of β-arteether **25** (51.4 mg, 9.4%), α-arteether **26** (86.9 mg, 16%), the peroxyhemiacetal **11** (238 mg, 48%), the furanose acetal **27** (24 mg, 4.4%), the furanose acetal **16** (42.3 mg, 9.0%), and unchanged DHA (63.3 mg, 13%). The compounds were isolated by chromatography of the residue with ethyl acetate–hexane (30:70) and characterised as described above or as follows.

Compound **27** was obtained as a pale-yellow oil; ^{1}H NMR: δ = 0.97 (d, J = 6.5 Hz, 3H, 9-Me), 1.04 (d, J = 6.7 Hz, 3H, 6-Me), 1.14–1.23 (m, 3H, CH_3 of $-OCH_2CH_3$), 1.24–1.28 (m, 2H), 1.60–1.69 (m, 2H), 1.78–1.99 (m, 2H), 2.0–2.12 (m, 4H), 2.20 (s, 3H, $COCH_3$), 2.87–2.96 (m, 1H), 3.43–3.53 (m, 1H, CH_2 of $-OCH_2CH_3$), 3.73–3.81 (m, 1H, CH_2 of $-OCH_2CH_3$), 3.83 (d, J = 6.7 Hz, 1H, CH of –CHOH), 4.43 (d, J = 7.3 Hz, 1H, H10), 8.46 (s, 1H, OH); ^{13}C NMR: δ = 15.11 (C9-Me), 15.55 (CH_3 of $-OCH_2CH_3$), 21.51 (C6-Me), 24.12 (C8), 28.59 (C5), 29.04 ($-COCH_3$), 32.60 (C7), 33.48 (C6 or C9), 33.60 (C9 or C6), 38.14 (C5a), 47.65 (C8a), 57.35 (C4), 63.64 (OCH_2), 76.94 (CHOH), 94.10 (C12a), 104.20 (C10), 208.82 (C=O); MS (CI, CH_4): *m/z* (%) = 163.3 (44), 209.2 (72), 233.2 (100), 249.2 (44), 267.3 (18), 295.3 (6); MS (ESI) *m/z* calcd 295.1909 [M^+ –OH], found 295.1933.

ii. Aqueous HCl–THF: Aqueous hydrochloric acid (5 M, 50 mL) was added to a stirred solution of DHA **2** (500 mg, 1.76 mol) in THF (50 mL) at room temperature. After 2 h, the reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (100 mL), and extracted with ethyl acetate (3×80 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a yellow–green residue, analysis of which by ^{1}H NMR spectroscopy revealed the presence of the peroxyhemiacetal **11** (100 mg, 20%), the furanose acetal **16** (136 mg, 29%), 2-deoxyartemisinin **15** (20 mg, 4.2%), and unreacted DHA (230 mg, 46%). The compounds were isolated by chromatography of the residue with ethyl acetate–hexane (30:70) and characterised as described above.

d. Decomposition with amines

i. Triethylamine: A solution of DHA **2** (100 mg, 0.35 mmol) and triethylamine (0.123 mL, 0.88 mmol, 2.5 equiv) in EtOH (20 mL) was heated at gentle reflux under N_2 in an oil bath at 90 °C for 21 h. After 21 h, the solution was treated with saturated aqueous ammonium chloride (20 mL) and extracted with ethyl acetate (3×20 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of filtrate under reduced pressure left a pale-yellow residue, which was submitted to chromatography with ethyl acetate–hexane (30:70). 2-Deoxyartemisinin **15** (72 mg, 76%) and unreacted DHA **2** (1.5 mg, 0.3%) were obtained. Repetition of the procedure with thiomorpholine (42.5 μL, 0.423 mmol, 1.2 equiv) gave 2-deoxyartemisinin **15** (52 mg, 55%). Control experiments in which a solution of DHA (568 mg, 2 mmol) in CH_2Cl_2 (4 mL) or EtOH (4 mL) containing triethylamine (1.39 mL, 10 mmol) were stirred at room temperature for 24 h, or solutions of DHA (568 mg, 2 mmol) in CH_2Cl_2 (20 mL) or EtOH (20 mL) where heated at reflux for 24 h indicated negligible decomposition of DHA.

ii. Triethylamine-acetic acid-silica gel: Triethylamine (1.39 mL, 10 mmol), silica gel (Merck Kieselgel 60 9385, 230–400 mesh, 1 g), and acetic acid (min. 99.8%, 0.5 mL) were added to a solution of DHA (568 mg, 2 mmol) in EtOH–CH_2Cl_2 (25:15, 10 mL) at room temperature. The mixture was stirred at room temperature under N_2 for 5 days. Filtration of the mixture and evaporation of the filtrate under reduced pressure left a crystalline residue, analysis of which by ^{1}H NMR spectroscopy indicated that it consisted essentially of 2-deoxyartemisinin **15** (430 mg, 80%).

Peroxyhemiacetal **11**: A solution of the peroxyhemiacetal **11** (100 mg, 0.352 mmol) and triethylamine (0.123 mL, 0.88 mmol, 2.5 equiv) in EtOH (20 mL) under N_2 was heated at reflux for 21 h in an oil bath at 90 °C. It was then treated with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (3×20 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a pale-yellow residue, which after chromatography with ethyl acetate–hexane (30:70) gave crystalline 2-deoxyartemisinin **15** (62 mg, 66%).

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Keywords: artemisinins · artesunate · DHA · malaria · thermal instability

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