Decomposition of Arteether in Simulated Stomach Acid Yielding Compounds Retaining Antimalarial Activity

John K. Baker,[1,3] James D. McChesney,[2] and Hsien Tao Chi[1]

Received August 19, 1992; accepted November 13, 1992

In simulated stomach acid (aqueous 0.01 *M* HCl, 37°C) β-arteether decomposed (half-life, 441 ± 17 min) to dihydroartemisinin, which subsequently rearranged to a new compound (1) having an endoperoxide group and an aldehyde group. The *in vitro* antimalarial activity of dihydroartemisinin is similar to that of β-arteether, whereas compound 1 had approximately 1/10th the activity of β-arteether. Compound 1 was prepared in sufficient quantities to afford samples for biological evaluation and a complete chemical characterization with ^{1}H- and ^{13}C-NMR and mass spectrometry. While β-arteether would be somewhat unstable in the stomach, if the drug were administered on an empty stomach (emptying time, ≈30 min) as a suspension or tablet, sufficient quantities of intact arteether may reach the small intestines, where it would be stable and readily absorbed. Its decomposition products, dihydroartemisinin and 1, may also contribute to the antimalarial activity of the administered drug following oral administration.

KEY WORDS: thermospray mass spectrometry; high-performance liquid chromatography; carbon- and proton-nuclear magnetic resonance; trioxanes; ketals; endoperoxide; oral administration.

INTRODUCTION

Arteether is a new semisynthetic antimalarial drug derived from the natural product artemisinin (also known as Qinghaosu) which is particularly effective against either the chloroquine-resistant or the mefloquine-resistant strains of *Plasmodium falciparum* (see Refs. 1–3 for extensive reviews of chemical and pharmacological properties). Compared to most peroxides, the endoperoxide functionality of both artemisinin and arteether are relatively stable. Artemisinin, which also contains a lactone group, easily undergoes hydrolysis under acid conditions and especially under alkaline conditions, leading to the irreversible formation of rearrangement products. Primarily because of better resistance to base-catalyzed hydrolysis, arteether has been extensively investigated as a potentially useful agent for the treatment of malaria. Most frequently, the arteether dosage forms have been sesame oil (or other vegetable oils) solutions intended for intramuscular injection.

Though arteether is more resistant to hydrolysis than artemisinin, arteether does contain an acetal functional group which would be expected to be susceptible to acid-catalyzed hydrolysis. Indeed, under strongly acidic conditions extensive hydrolysis and ring rearrangements have been reported (1–4). However, there have been no reports on the stability of arteether under mildly acidic conditions such as would be encountered in the stomach following oral administration of the drug. The primary objective of the present study was to identify the decomposition products of arteether under simulated gastric conditions and to measure the kinetics of the process.

MATERIALS AND METHODS

Chemicals

HPLC-grade methanol and ammonium acetate were obtained from Fisher Scientific (Fair Lawn, NJ). Arteether and dihydroartemisinin were synthesized from artemisinin using a previously reported procedure (5).

Preparation of 1

For the synthesis of 1, a 100-mg portion of β-arteether in 50 mL of ethanol and 50 mL of 5 *N* HCl was stirred at room temperature for 3 hr. The reaction mixture was then extracted three times with 150 mL of methylene chloride. The CH_2Cl_2 extract was dried with 20 g anhydrous Na_2SO_4 and evaporated to a 2-mL final volume. Using TLC to monitor column fractions (silica gel, 1:9 v/v EtOAc:CH_2Cl_2; arteether R_f = 0.94, 1 R_f = 0.40, dihydroartemisinin R_f = 0.31), the products were flash chromatographed on a silica gel (Merck Cat. No. 9385) column using 10% EtOAc in CH_2Cl_2 to obtain 25 mg of compound 1: infrared in KBr, 1712 and 1724 cm^{-1} (aldehyde and ketone). NMR data (Varian VXR-300 spectrometer) are shown in Tables I and II; the thermospray mass spectrum in Fig. 2.

Kinetic Studies

A 60-μL portion of a 1.0 mg/mL arteether stock solution (in ethanol) was added to 3.0 mL of a freshly prepared 0.01 *N* HCl aqueous solution (preheated to 37°C). The resulting mixture was sealed to prevent water evaporization and maintained at 37°C in a water bath. Samples of the reaction mixture were taken at time intervals of 0, 30, 60, 120, 240, 480, 960, and 1440 min. The samples were stored in a dry ice–acetone bath and analyzed (in triplicate) as quickly as possible.

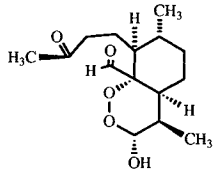
Thermospray LC/MS. The thermospray LC/MS spectra were used to identify the decomposition products, and HPLC/EC was used for quantitation. The thermospray mass spectrometer (Vestec Model 201, Houston, TX) was operated in the positive ion mode with the filament at 400 μA. The tip temperature of the thermospray vaporizer probe was maintained at 210°C, and the source block temperature was set to 190°C. These temperatures were chosen to give the highest absolute intensity for the $(M + NH_4)^+$ ions for arteether and 1.

HPLC/EC. The electrochemical detector was used to quantify the peroxide compounds: arteether, dihydroartemisinin, and 1. The detector (LC-3A, Bioanalytical Sys-

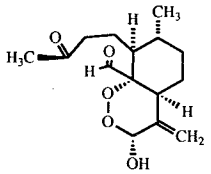
[1] Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, Mississippi 38677.
[2] Research Institute of Pharmaceutical Sciences; School of Pharmacy, University of Mississippi, University, Mississippi 38677.
[3] To whom correspondence should be addressed.

0724-8741/93/0500-0662$07.00/0

Table I. Carbon NMR Assignments (ppm in $CDCl_3$)



1



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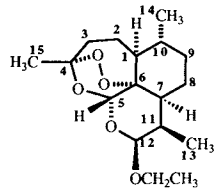

Arteether

Carbon	1	2	Arteether
1	50.4	49.0[a,b]	52.8[c]
2	21.7[d]	21.7[b]	24.8
3	43.7	43.6[b]	36.6
4	208.5	208.5	104.0
5	207.4	204.3	87.9
6	89.6	92.0	82.2
7	41.3	42.8[b]	44.7
8	23.7[d]	21.3[b]	24.6
9	35.3	34.2[b]	34.8
10	34.8[e]	33.8[b]	37.6
11	33.9[e]	140.5	31.0
12	101.5	100.3	101.7
13	13.9	111.3	13.1
14	20.4	20.3	20.4
15	30.0[e]	30.0	26.3

[a] Data from Ref. 6. In the literature report, the NMR data were for the major component of a mixture of α- and β-isomers at C-12, but the single-crystal X-ray was only for the α-isomer.
[b] Specific assignments were not made in Ref. 6.
[c] From Ref. 8.
[d] Interchangeable assignments.
[e] Interchangeable assignments.

tems Inc.) was used in the reductive mode, with the potential of the mercury/gold electrode set to −0.9 V. The column was a Partisphere C-18 (5-μm particle size, 12.-5 cm length) column used with a mobile phase (1.0 mL/min) which was completely deoxygenated by flowing argon gas (mobile phases continually refluxed in a distillation/condenser system at 40°C with a constant 30-mL/min flow or argon). Two mobile phase systems were used, where system 1 (for the assay of arteether) consisted of 0.1 M ammonium acetate with 65% methanol in water (arteether t_r = 24.6 min). System 2, used for dihydroartemisinin (t_r = 13.4 min) and 1 (t_r = 6.1 min), consisted of 0.1 *M* ammonium acetate with 50% methanol in water. Two internal standards were used: artemether (t_r = 15.5 min) for HPLC system 1 and artemisinin (t_r = 20.3 min) for system 2. For the assay, 20 μL of the appropriate internal standard (20 μg/mL) was mixed with 80 μL of the sample; this solution was degassed for 1 min and then transferred directly into a 20-μL sample injector loop by means of pressurized argon gas to avoid the introduction of atmospheric oxygen into the injector loop.

Table II. Proton NMR Assignments (ppm in $CDCl_3$)

Proton	1	2	Arteether
1	1.22	—[a]	1.25
2	≈1.6[b]	—[a]	1.88, 1.51
3	≈2.5[b]	—[a]	2.37, 2.03
5	10.28[c]	9.96[d]	5.41
7	2.28	—[a]	1.41
8	≈1.4[b]	—[a]	1.83, 1.74
9	≈1.8[b]	—[a]	1.63, 0.91
10	≈1.8[b]	—[a]	1.33
11	≈1.7[b]	—	2.62
12	5.07	5.52	4.79
13	0.82	5.03, 4.88	0.90
14	0.95	0.94	0.95
15	2.15	2.14	1.43

[a] Chemical shift not reported in Ref. 7.
[b] Approximate chemical shift determined by COSY and/or HETCOR two-dimensional NMR spectra.
[c] Doublet of doublets ($J_{5,1}$ = 1.7 Hz, $J_{5,7}$ = 1.7 Hz).
[d] Doublet of doublets.

RESULTS AND DISCUSSION

Preliminary HPLC/MS studies of the decomposition of arteether in simulated stomach acid had shown that arteether was transformed fairly rapidly into dihydroartemisinin (retention time and thermospray mass spectrum identical to authentic standard) and more slowly into a relatively polar product (1; Fig. 1) which had a molecular weight of 284 (thermospray mass spectrum; Fig. 2). Using thermospray mass spectrometry with ammonium acetate as the thermospray reagent in the HPLC mobile phase, one would anticipate that neutral or acidic compounds would produce $(M + NH_4)^+$ pseudo molecular ions, while basic or amphoteric compounds generally produce $(M + H)^+$ pseudomolecular ions. As shown in Fig. 2, the pseudomolecular ion readily dehydrated (to give *m/z* 284) and then lost CO (to give *m/z* 256). By observing the changes in the thermospray mass spectra by replacing the H_2O with D_2O in the mobile phase, one can remove some of the ambiguities in the interpretation of the spectra (i.e., NH_4 and H_2O are both 18). This deuterium exchange technique has been used in these laboratories to study the fragmentation of a number of arteether derivatives that have exhibited fragmentation which was essentially the same as shown in Fig. 2 (9). In order to characterize this new compound additionally, 1 was prepared on a larger scale as detailed. The ^{13}C-NMR spectrum of 1 (Table I) revealed that the ethoxy group at the 12 position had been lost, and the product contained 15 carbons with chemical shifts which were somewhat similar to the starting material, but the most remarkable feature of the spectrum was the presence of an aldehyde (207.4 ppm) and a ketone (208.5 ppm) which were not present in either arteether or dihydroartemisinin. The ^1H-NMR spectrum of 1 (Table II) also showed the presence of an aldehyde (10.28 ppm) and the C-15 methyl group had shifted from 1.43 ppm in arteether to 2.15 ppm (characteristic of $CH_3-C=O$).

The structure elucidation of 1 was greatly facilitated by a recent literature report (6) of a very similar compound, 2, whose structure had been determined using ^1H- and ^{13}C-NMR spectroscopy along with X-ray crystallography. The aldehyde and ketone carbon-NMR chemical shifts of 1 and 2 were found to be essentially the same, the C-15 methyl group had moved from 26.3 to 30.0 ppm (aliphatic methyl to a methyl ketone), and the remainder of the peaks for 1 and 2

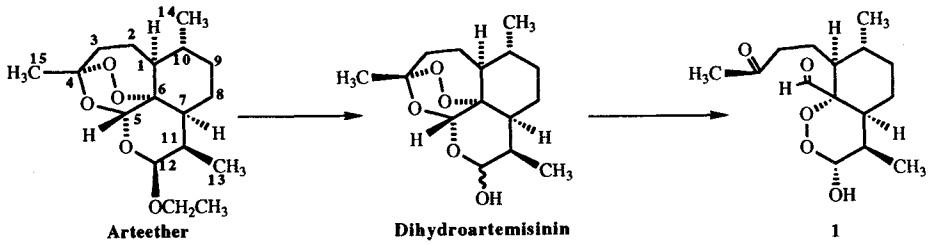

Fig. 1. Decomposition of arteether in aqueous 0.01 *N* HCl at 37°C.

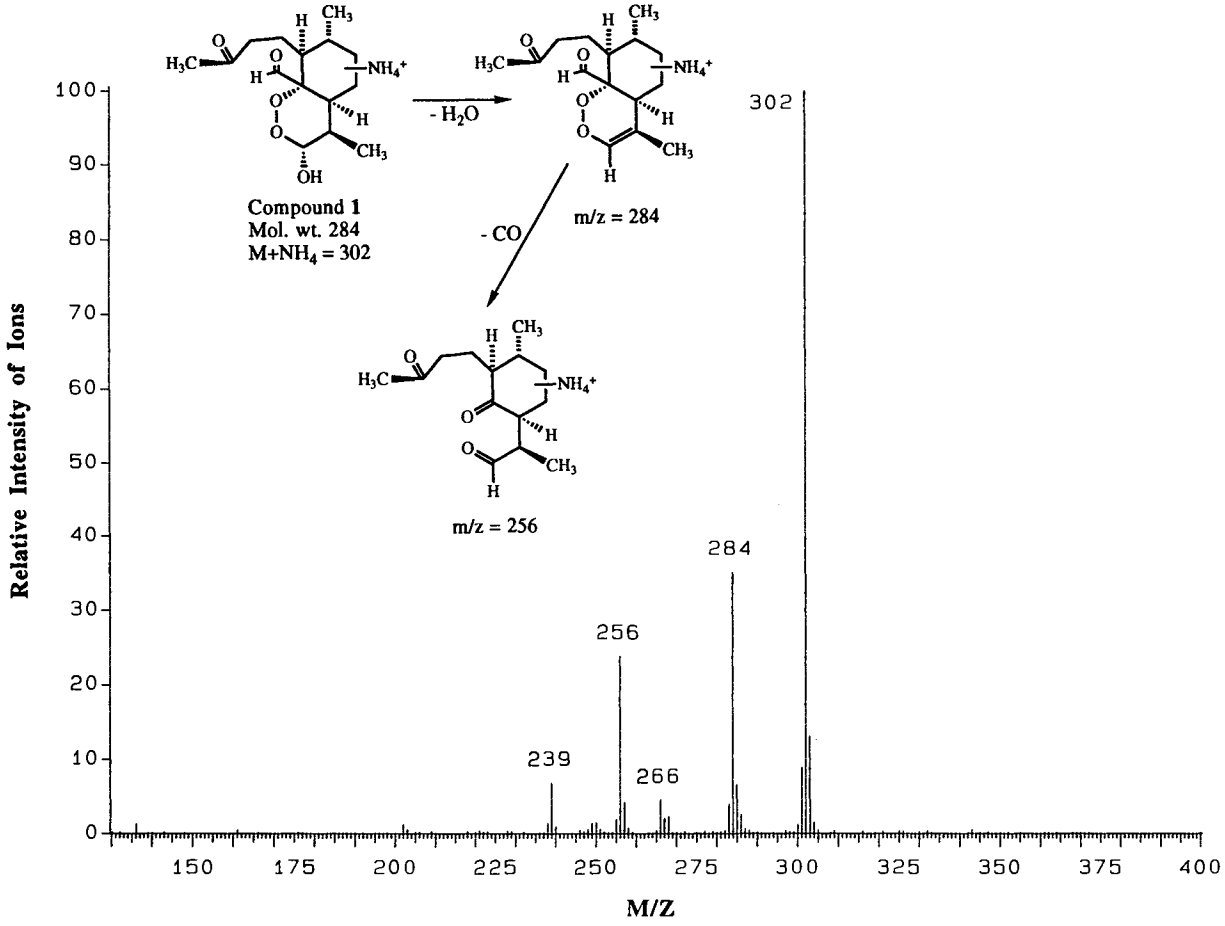

Fig. 2. Thermospray mass spectrum of 1.

Table III. Molecular Modeling and Calculation of the $J_{H11,H12}$ Proton NMR Coupling of the 12-Hydroxy Isomers of 1

Compound	12-OH	Energy (kcal)	Calc. dihedral angle	Calc. $J_{H11,H12}$ (Hz)	Obs. $J_{H11,H12}$ (Hz)
1	α	35.46	−72.8°	0.90 Hz	<1.0 Hz[a]
3	β	37.92	+37.9°	2.62 Hz	2.5 Hz

[a] The coupling constant could not accurately be determined because of the limitations of the spectral resolution (0.3 Hz), but judging from the peak shape in the 1-D spectrum and the intensity of the cross-peak in the 2-D COSY spectrum, the $J_{H11,H12}$ value was approximately 1.0 Hz.

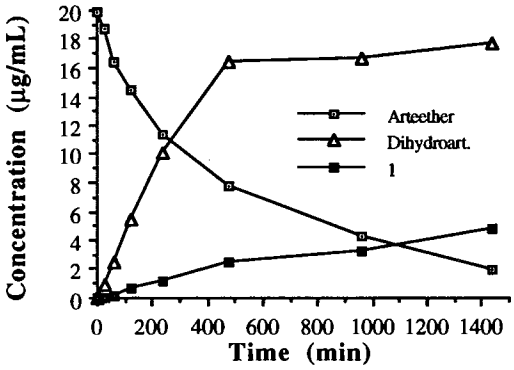

Fig. 3. Kinetics of the hydrolysis and rearrangement of arteether in aqueous 0.01 *N* HCl at 37°C.

were very similar, except for the obvious differences between C-11 and C-13 (olefinic vs aliphatic).

The proton-NMR spectral data (Table II) also showed that the aldehyde of **1** (10.28 ppm) and the aldehyde of **2** (9.96 ppm) were very similar as well as the characteristic shift of the methyl ketone (2.15 and 2.14 ppm). One rather unusual aspect of the aldehyde proton of **1** was the long-range proton–proton coupling ($J_{H5,H1}$ = 1.7 Hz and $J_{H5,H7}$ = 1.7 Hz), which was verified by the presence of strong cross-peaks in the two-dimensional proton–proton correlation spectrum (COSY). The aldehyde proton of **2** also appeared as a deceptively simple triplet with a very small coupling constant. Thus, it would appear that the stereochemistry at the C-6 position of **2** determined by X-ray crystallography is probably the same in **1**.

The coupling between H-12 and H-11 has been used previously to assign the stereochemistry of the substituent at C-12 (5,7). For arteether, the coupling constant would be 3.4 Hz if the ethoxy substituent were in the β position and 9.2 Hz when the ethoxy group was in the α position. Because a $J_{H11,H12}$ value of approximately 1.0 Hz was observed for **1**, one might erroneously conclude that its 12-hydroxyl group was in the β configuration. However, in arteether the ring fusion is like a *cis*-decalin (which places the β-methyl at the 11 position in an equatorial orientation), whereas in **1** the ring fusion is like a *trans*-decalin (which places the β-methyl at the 11 position in an axial orientation). In order to analyze the spectral data more critically, molecular modeling[4] of **1** and the corresponding β 12-hydroxyl isomer, **3**, was used to calculate (7) the $J_{H11,H12}$ values using an extended Karplus equation as had been used for arteether (Table III). Because the acetal at the 12 position is easily epimerized under acidic conditions for the arteether family of compounds, it would be anticipated that the major product of this reaction, **1**, would be the more thermodynamically stable of the two isomers. The much greater yield of **1**, as compared to **3**, was consistent with molecular modeling calculations which showed that **1** would had a lower energy than **3** (Table III). The minor product of this reaction, **3**, was not produced in large enough quantities to characterize fully, but the proton NMR data for H-12 was readily determined (H-12 δ = 5.37 ppm, $J_{H11,H12}$ = 2.5 Hz). The agreement of the calculated and observed *J* values as well as the relative energies strongly suggested that the stereochemistry of the 12-hydroxyl of **1** was *alpha* (Fig. 1).

[4] Molecular modeling was conducted using a MM2-Plus program that is implemented in a commercially available program as Chem3D-Plus Version 3.0 by Cambridge Scientific Computing, Cambridge, MA.

Table IV. *In Vitro* Antimalarial Activity of β-Arteether and Hydrolysis Products

Compound	African clone[a] (ng/mL) IC-50[b]	African clone[a] (ng/mL) IC-90[b]	Indochina clone[a] (ng/mL) IC-50	Indochina clone[a] (ng/mL) IC-90
Arteether	0.05	0.33	0.03	0.29
Dihydroartemisinin	0.04	0.15	<0.04	<0.2
Compound **1**	2.2	7.39	0.99	4.05

[a] The *in vitro* antimalarial activity was determined by Dr. Wilbur K. Milhous at the Walter Reed Army Institute of Research, Washington, DC, using African (chloroquine-sensitive, mefloquine-resistant) and Indochina (chloroquine-resistant, mefloquine-sensitive) clones of *Plasmodium falciparum* in 1.5% hematocrit cultures.

[b] Concentrations giving 50 and 90% inhibition.

In the simulated stomach acid, arteether was found to disappear with pseudo-first order kinetics, with a half-life of 441 ± 17 min (Fig. 3). If the hydrolysis of arteether to dihydroartemisinin were to follow the acid-catalyzed hydrolysis of typical acetals, then one would anticipate a 10-fold increase in the rate if the pH were dropped by 1 unit (preliminary studies with arteether gave a half-life of 50 min using a slightly lower pH). From the data shown in Fig. 3, it could be seen that the concentration of dihydroartemisinin initially increased quickly, then reached a steady state as it was slowly being transformed to **1**. Though the rate of formation of **1** at this pH and temperature was fairly slow, it was found that the conversion of arteether to **1** was essentially complete within 3 hr using 5 *N* HCl at room temperature.

Both of the decomposition products of arteether were found to have significant antimalarial activity (Table IV). Using this *in vitro* model, where drug absorption and distribution were likely to be less important, dihydroartemisinin was found to be slightly more active than arteether. Compound **1** was found to have only about 0.1 the activity of arteether, but with an IC_{50} in the 1–2 ng/mL range, **1** had more activity than many of the totally synthetic endoperoxide antimalarials that have been reported (1–3).

In the simulated stomach acid used in the present study (0.01 *M* HCl, 37°C), arteether was found to be sufficiently stable ($t_{1/2}$ = 441 ± 17 min) so that little of the drug would be expected to be lost during the typical time of residence within the stomach. However, if the pH were to drop to 1.0, giving a half-life somewhere in the range of 50 min, one might expect that 25–75% of the arteether might decompose during this time. Clearly, in future bioavailability studies of orally administered arteether, one should consider the effect of gastric pH and emptying time in the design of the study.

ACKNOWLEDGMENTS

This investigation received the financial support of the UNDP/World Bank/World Health Organization Special Pro-

gram for Research and Training in Tropical Diseases. Financial assistance was also obtained from the Research Institute for Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

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