NOVEL REARRANGEMENTS OF THE TRIOXANE RING SYSTEM OF THE ANTIMALARIAL ARTEETHER UPON TREATMENT WITH ACID IN AN AQUEOUS METHANOL SOLVENT SYSTEM

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Abstract- While arteether does not have a uv-chromophore, the treatment of arteether with a HCl, water, methanol system has been previously proposed as a method of generating a uv-chromophore upon which to base a quantitative analysis. The present study demonstrates that the originally proposed structure for the product was not correct and that the reaction is far more complex than originally proposed. It was found that the reaction yields 5 different α,β-unsaturated ketones (**A-1**, **A-2**, **A-3**, **A-4**, and **A-5**; whoses strucutres are reported here for the first time) that have strong uv chromophores and 4 additional products (new compounds **B-1** and **B-2**; previously reported α-artemether and β-artemether) that do not have any significant chromophores. The structure and stereochemistry of these novel reaction products were determined using thermospray hplc/ms, ^{1}H-nmr, ^{13}C-nmr, and two-dimensional nmr methods.

Arteether[1,2] is a semi-synthetic 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* . Since arteether does not have a uv-chromophore, simple hplc/uv methods of analysis have been precluded, but recently a procedure using HCl treatment of arteether to generate the

chromophore necessary for hplc/uv analysis has been proposed.[3] In a more detailed study of the rearrangement of arteether in 2.5 M HCl (1:1, methanol: water solvent), electron impact mass spectral data and uv spectral data was used to show that the major product for this reaction was **1** as shown in Figure 1.[4] However as a result of the present study, the substance that had been thought to be **1** was actually found to be **A-2** (Figure 2).

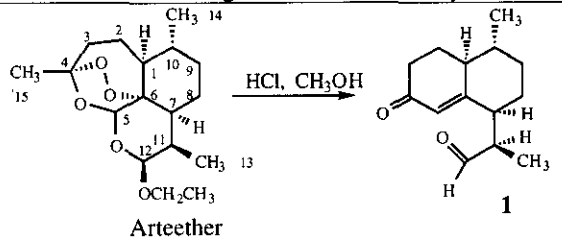

Figure 1: Previously proposed product from the acid treatment of arteether.[3,4]

Using the identical reaction conditions (yield shown in Table 1), it was found in the present study that arteether produced a series of five compounds (**A-1**, **A-2**, **A-3**, **A-4**, and **A-5**; Table 2) using monitoring at 254 nm of the hplc effluent. The hplc/uv results (Table 2) was essentially identical to that previously reported.[4] In addition to the five compounds that could be detected by hplc/uv, there were four additional compounds (**B-1**, **B-2**, **B-3**, **B-4**, Table 2, mass spectral section) that could only be detected by hplc/ms. The thermospray mass spectra of these nine compounds (Table 3) were not consistent with the structures that had been previously proposed based on EI-mass spectral and uv data. One of the compounds in the mixture was found to give the identical retention time and thermospray mass spectra of an authentic standard of **B-1** which had been previously prepared.[5] Two of the other compounds were also found to be identical to authentic reference standards of **B-3** (α-artemether) and **B-4** (β-artemether). In order to elucidate the structures of the remaining compounds, it was necessary to use preparative scale chromatographic isolation of the reaction products.

Table 1: Pre-workup yields of the rearrangement products from arteether in 2.5 N HCl (in 1:1 water:methanol) after 15 min at 53°C

Product	Percent	Product	Percent
A-1	9.4	B-1	33.4
A-2	19.1	B-2	20.5
A-3	4.3	B-3	6.4
A-4	3.1	B-4	3.2
A-5	0.6		

Table 2: Hplc/uv/ms chromatograms of the arteether, HCl, methanol reaction mixture

Compound	ret. time	uv, 254 nm	m/z 281	m/z 316	m/z 302	m/z 284
A-1	5.2 min	35%[a]	0%[b]	0%[b]	0%[b]	25%[b]
A-2	9.8 min	100%	100%	0%	0%	0%
A-3	11.8 min	13%	21%	0%	0%	0%
A-4	18. 5 min	8%	14%	0%	0%	0%
A-5	20.1 min	3%	3%	0%	0%	0%
B-1	3.8 min	0%	0%	0%	100%	54%
B-2	6.7 min	0%	0%	100%	0%	100%
B-3	11.4 min	0%	0%	42%	0%	0%
B-4	23.7 min	0%	0%	5%	0%	8%

a: chromatographic peak height expressed as a percent of the largest peak detected at 254 nm.

b: chromatographic peak height expressed as a percent of the largest mass spectral peak at given m/z

Table 3: Thermospray mass spectral data for compounds not previously reported in the literature

A-1:	m/z 230 (71%), m/z 248 (48%), m/z 263 (38%), m/z 267 (100%), m/z 284 (43%, M+NH_4)
A-2:	m/z 281 (100%, M+H)
A-3:	m/z 281 (100%, M+H)
A-4:	m/z 281 (100%, M+H)
A-5:	m/z 281 (100%, M+H)
B-2:	m/z 267 (7%), m/z 281 (15%), m/z 300 (48%), m/z 316 (100%, M+NH_4)

After preparative scale isolation of the products, the thermospray mass spectrum (Table 3) and nmr spectrum (Table 4 and 5) of **A-2** indicated that the compound had a molecular weight of 280 with 16 carbons. This sample of **A-2** was also found to have the same electron-impact mass spectrum and uv spectrum previously assigned to the structure shown for **1**.[3,4] While the thermospray mass spectrum of **A-2** shows a very strong molecular ion, the electron-impact mass spectrum of **A-2** shows a very weak molecular ion (m/z 280 less than 3%) which mislead the earlier studies. As compared to the arteether starting material, the obvious differences in the ^{1}H-and ^{13}C-nmr spectra of **A-2** were the replacement of the ethoxy group with a methoxy, the addition of the H-5 olefinic proton, the addition of two olefinic carbons, and the addition of the carbonyl group. The ^{1}H-nmr spectrum also showed that H-5 was a multiplet ($J_{5,2\beta}$ = 1.6 Hz) and the coupling was readily observed in the 2-

dimensional COSY spectrum of **A-2**. The structure elucidation of the basic ring system for **A-2** was derived from a comparision to **3** and **2** (the latter sturcture derived by X-ray, Figure 3). The consistency between the ^{13}C-nmr of **A-2** and **3** (Table 5), was also strongly suggestive that the basic ring structure shown for **A-2** was correct. The most serious reservations about the structural assignment concerned the stereochemistry at C-12 and C-6. As an aid to the structure elucidation process, the $J_{H\text{-}H}$-values for key protons were calculated (the two sets of 6α and 6β isomers shown in Table 6) and compared to the experimental values (Table 4). It was clear that the oxygen at C-6 was not in the α-configuration. While the calculated values for **A-2** and **A-4** were in good agreement with the experimental values, it was difficult to distinguish between the α- and β- methoxy configurations because the values were close. However this ambiguity was removed through the observation of a very strong cross-peak between H-12 and the protons of the adjacent 13-methyl group in the 2-dimensional nuclear Overhauser spectrum (NOESY) of **A-2**. The stereochemistry of the 12-methoxy group also was observed to have consistent effects on the proton chemical shift of the adjacent methyl (α-methoxy, 13-CH_3 = 1.07 ppm for both **A-2** and **A-3**; β-methoxy, 13-CH_3 = 0.99 ppm for **A-4** and **A-5**).

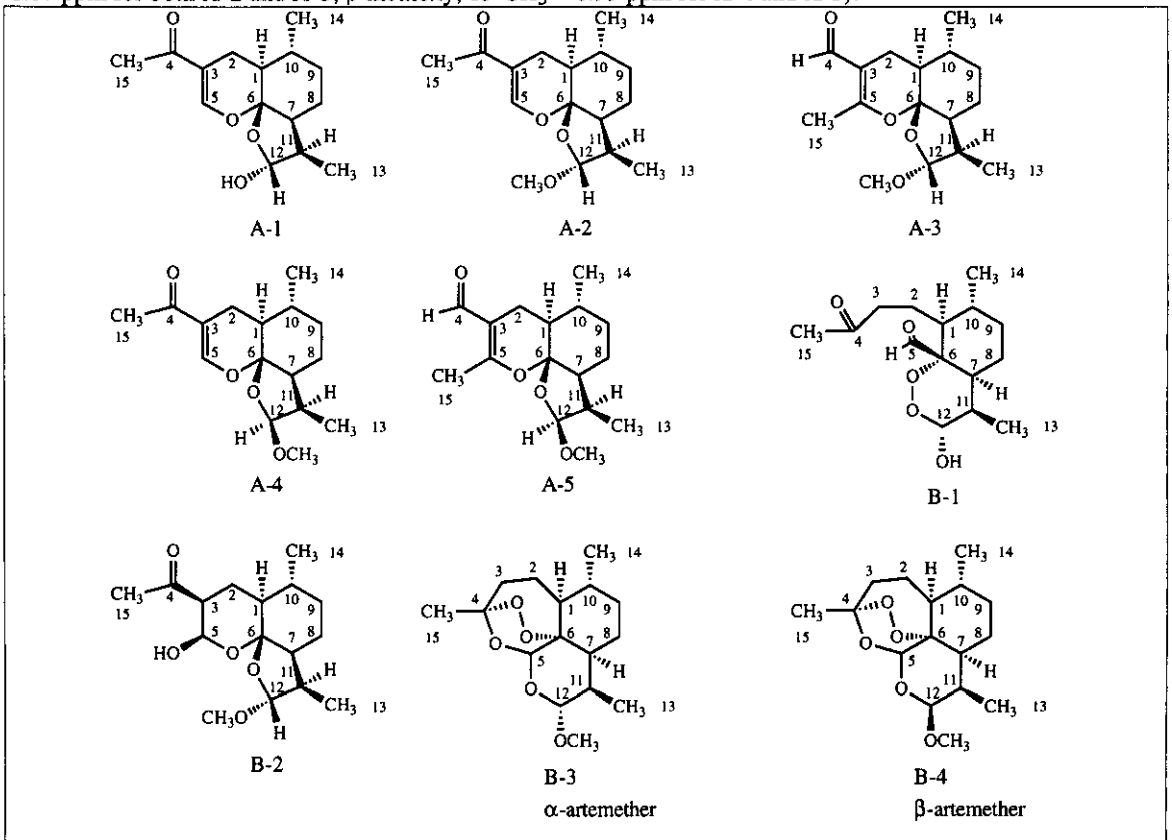

Figure 2: Rearrangement products formed from arteether

The most distinguishing features of the ^{1}H-nmr spectra of aldehyde series of products (**A-3**, **A-5**) were the presence of the H-4 aldehyde (9.86 and 9.84 ppm) and the triplet of H-15 (2.17 and 2.16 ppm). The H-15 methyl (which was a singlet in all of the other series) appears as a triplet in **A-3** and **A-5** with very small coupling (J = 1.2 and 1.0 Hz) and very strong cross peaks appears between H-15 and H-2α and between H-15 and H-2β in the COSY spectrum.

Table 4: ^{1}H-Nmr assignments of the arteether rearrangement products

Chemical Shifts (ppm units):

Group	A-1	A-2	A-3	A-4	A-5	B-2
1		≈1.3	≈1.3	≈1.4		
2α	2.63	2.59	2.55	2.60	2.57	≈2.1
2β	1.90	1.93	1.91	1.95	1.92	1.85
3	-	-	-	-	-	2.95
4	-	-	9.86	-	9.84	-
5	7.45	7.45	-	-	-	3.81
7	2.11	2.08		≈2.1		≈2.1
8α		≈1.7	2.09	≈2.1		
8β		≈1.7	1.70	1.67		
9α		≈0.9	0.87	0.86	0.87	0.94
9β		≈1.7	1.15	≈1.2	≈1.2	1.64
10		≈1.3	≈1.3	≈1.4		≈1.2
11	2.69	2.71	2.73	2.86	2.88	≈2.1
12	5.18	4.75	4.74	4.89	4.90	4.35
13	1.11	1.07	1.07	0.99	0.99	1.05
14	0.97	0.97	0.96	0.96	0.96	0.98
15	2.23	2.23	2.17	2.22	2.16	2.23
OCH_3	-	3.36	3.32	3.35	3.35	3.40

H, H- Coupling Constants (Hz units)

Group	A-1	A-2	A-3	A-4	A-5	B-2
1, 2α	5.0	5.0	5.2	4.2	3.7	
1, 2β	12.5	12.3	12.2	12.8	≈12	8.0
2α, 2β	16.1	16.4	16.2	16.2	16.5	12.3
2α, 3	-	-	-	-	-	6.3
2β, 3	-	-	-	-	-	14.2
2α, 5	0.0	0.0	-	0.0	-	-
2β, 5	1.9	1.6	-	1.4	-	-
2α, 15	-	-	1.2	-	1.0	-
2β, 15	-	-	1.2	-	1.0	-
3, 5	-	-	-	-	-	5.8
10, 14	6.2	6.2	6.2	6.2	6.1	6.5
11, 12	6.2	6.2	6.1	6.3	6.3	7.3
11, 13	7.2	7.2	7.3	7.3	7.3	6.8

For **B-2**, the stereochemistry at C-3, C-5, and C-12 was determined through a comparison of the observed J values and molecular modeling. For the structure shown for **B-2** in Figure 2, the two fused six membered rings

form a *trans*-decalin that was only slightly distorted (the C-3 - C-5 bond is twisted about 12°) where H-5 was *equatorial*, H-3 was *axial*, and H-2β was *axial*. The observed values [($J_{3,5}$ = 5.8; *a,e*), ($J_{2\beta,3}$ = 14.2, *a, a*), ($J2_{\alpha,3}$ = 6.3, *e,a*)] were consistent with the acetyl group being β (*equatorial*) and the hydroxyl group being β (*axial*). Having the hydroxy group in what might be thought to be a high energy configuration was unexpected, but molecular modeling calculations (Table 7) revealed that this was actually the lower energy structure.

Table 5: ^{13}C-Nmr assignments of the Arteether rearrangement products

Chemical Shifts (ppm units):

Group	A-2	A-3	B-2	3[a]	Arteether[b]
1	42.87	42.68	37.79	42.5	52.8
2	20.05	19.64	28.19	19.8	24.8
3	118.49	115.06	57.02	119.5	36.2
4	196.73	189.44	208.91	195.9	104.0
5	154.41	168.45	75.77	152.3	87.9
6	108.34	108.43	93.71	105.3	81.2
7	46.10	46.02	47.27	43.4	44.7
8	24.57	24.61	23.71	25.0	24.6
9	33.09	33.04	32.19	32.2	34.8
10	33.24	33.29	33.11[c]	32.7	37.6
11	40.80	40.86	33.22[c]	38.7	31.0
12	111.97	111.75	105.67	178.0	101.7
13	10.49	10.56	14.75	8.7	13.1
14	18.99	18.93	21.10	*d*	20.4
15	24.66	24.61	28.71	24.8	26.3
OCH_3	56.75	56.47	55.52	-	-

a: Chemical shifts and multiplicities taken from ref. 5, but the assignments have been made here in the present study.
b: ref. 10
c: interchangeable assignments
d: The original paper reported only 14 of the 15 carbon chemical shifts.

Table 6: $J_{H\text{-}H}$-Values calculated using an extended Karplus equation[6] and molecular modeling for the C-6 and C-12 isomers of **A-2** and **A-4** (both 6β) and the two other potential 6α-isomers.

Groups	12α, 6β (**A-2**)	12β, 6β (**A-4**)	12α, 6α	12β, 6α
11, 12	8.5 Hz	4.6 Hz	2.4 Hz	5.9 Hz
1, 2α	4.6	4.5	2.9	3.0
1, 2β	15.0	15.0	0.6	0.6

Table 7: Calculated energies for the various possible configurations of **B-2**.

5-OH	3-acetyl	12-methoxy	Energy
β	β	α	36.80 kcal
α	β	α	37.23
β	α	α	38.80
α	α	α	39.28
β	β	β	39.51

While a computer substructure search of the *Chemical Abstracts* database revealed that the dioxygenated-fused ring system of the **A**-series of compounds was a new series, a very close analog was found in **3** (Figure 3). Compound (**2**) had been derived from artemisinin with a multiple-step reaction leading to **2** (whose structure was determined by X-ray analysis) and **2** was subsequently transformed to **3** by acid treatment.[7]

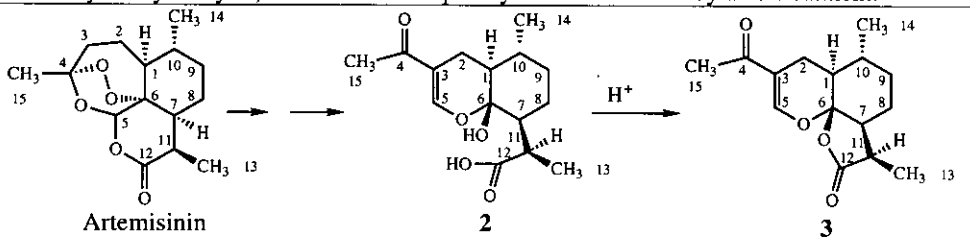

Figure 3: Conversion of artemisinin to **2** (structure determined by X-ray) with subsequent conversion to **3**.[7]

It was not immediately obvious how one might account for the apparent shift of the 15-CH_3 from the C-4 position (**A-2**) to the C-5 position (**A-3**). A possible mechanism for this transformation is shown in Figure 4. The inter conversion of **A-2** and **A-3** essentially is the result of the pivoting of the C-2 - C-3 bond of the ring-opened form and observed ratio of **A-2** and **A-3** (6:1) in the reaction mixture may reflect the relative tendency of the aldehyde and the ketone groups to cyclize.

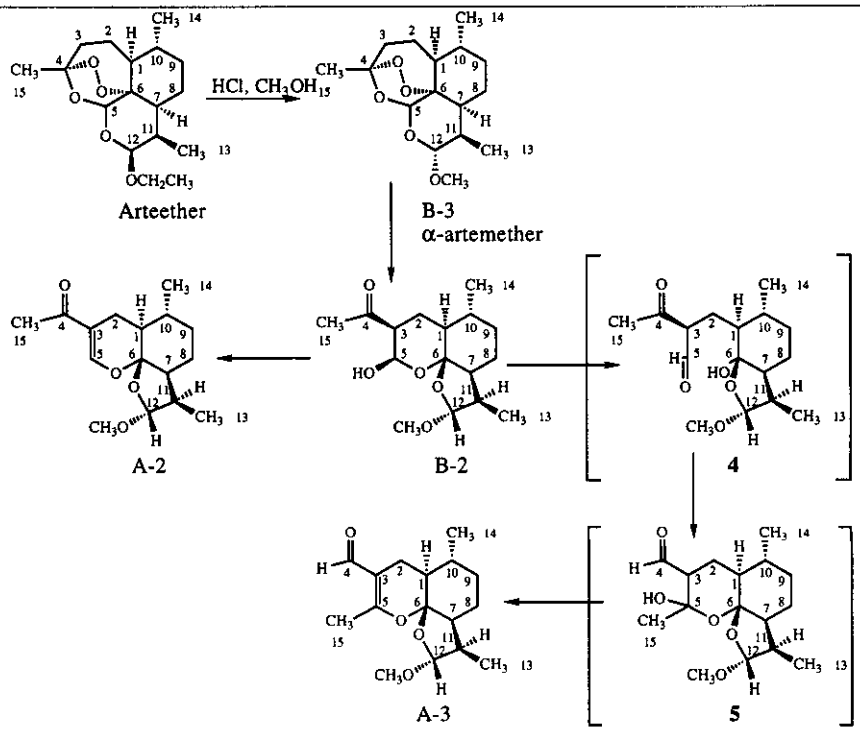

Figure 4: Proposed mechanism for the formation of the A- and B-series of Arteether products

EXPERIMENTAL

Arteether was synthesized from artemisinin using a previously reported procedure.[8] Authentic reference standards of α-artemether and β-artemether were also prepared using a similar procedure.[9] An authentic reference standard of **B-1** was also prepared.[5]

Preparation of **A-2**, **A-4**, and **A-5**: 100 mg (0.32 mmol) of arteether in 100 ml of methanol was mixed with 26 ml (0.31 mol) of 12 N HCl in water, then brought to 53°C for 15 min. After cooling, the mixture was extracted with 3 x 200 ml of methylene chloride, then the combined extract was washed with 100 ml of water. The extract was dried with 15 g of Na_2SO_4 then evaporated under vacuum to yield 92 mg of residue. The residue was loaded onto a silica gel column (1 x 60 cm, Merck cat. no. 9385) and eluted with 4:96 ethyl acetate:methylene chloride (3.8 ml/ min) to yield 34 mg of a residue containing **A-2** and **A-3** and 15 mg of a residue containing **A-4** and **A-5**. Using a reversed-phase semi-preparative hplc column (9.4 mm x 25 cm, Whatman Magnum 9, C-18), the first residue was separated using 60:40 methanol:water as the mobile phase to yield 14 mg of pure **A-2** (λmax = 251 nm in methanol, thermospray mass spectrum Table 3, nmr shown in Tables 4 and 5). This sample of **A-2** was also found to have the same electron-impact mass spectrum and uv

spectrum previously assigned to the structure shown for **1**.[3,4] The second residue from the silica gel column was separated using 50:50 methanol:water on the semi-preparative reversed-phase column to yield 2.0 mg pure **A-4** and 1.0 mg of pure **A-5**.

Preparation of **A-1**, **A-3**, and **B-2**: 300 mg (0.96 mmol) of arteether in 300 ml of methanol was mixed with 300 ml (1.5 mol) of 5 N HCl in water then brought to 53°C for 15 min. After cooling the mixture was extracted with 3 x 200 ml of methylene chloride, the extract washed with 100 ml of water, the extract was dried with 15 g of Na_2SO_4, and then the extract was evaporated under vacuum to give 273 mg of residue. The residue was chromatographed on a silica gel column (1 x 60 cm, eluted with 200 ml 2%, then 100 ml 7%, then 300 ml of 10% ethyl acetate in methylene chloride, followed by 100 ml pure ethyl acetate) to give 34 mg of pure **B-2**, 33.6 mg of a mixture of **A-2** and **A-3** and 39 mg of a residue containing primarily **A-1** along with other minor products. The **A-2**, **A-3** residue was loaded onto the reversed-phase column and eluted with 60:40 methanol:water to yield 1.5 mg pure **A-3**. The 39 mg residue obtained from the silica gel column was then chromatographed on the reversed-phase column (40:60 methanol:water) to yield 2.0 mg of pure **A-1** (λmax = 253 nm in methanol). Using tlc analysis (silica gel, 10:90 ethyl acetate:methylene chloride) of the pure products, the following R_f values were obtained: **A-1**, 0.08; **A-2**, 0.46; **A-3**, 0.45; **A-4**, 0.60; **A-5**, 0.58; **B-1**, 0.28; **B-2**, 0.23; **B-3**, 0.71; and **B-4**, 0.85.

ACKNOWLEDGMENT

This investigation received the financial support of the UNDP/World Bank/World Health Organization Special Program 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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Received, 6th December, 1993