ACID DECOMPOSITION OF THE ANTIMALARIAL BETA-ARTEETHER[**]

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Abstract- The antimalarial agent β-arteether was reacted with aqueous ethanolic HCl. Four compounds (11, 12, 13, and 14) were isolated. Two, 11 and 12, have the same structure as analogs recently reported by Baker and Chi. Compound (13) is spectroscopically nearly identical with another compound isolated by Baker and Chi, but facile electrochemical reduction of this material suggests that the compound is in fact a peroxide.

β-Arteether (1) a derivative of the Chinese traditional medicine antimalarial artemisinin (2) (qinghaosu) is undergoing pre-clinical studies under the auspices of the World Health Organization and the U. S. Army's Walter Reed Army Institute of Research. The methyl ether analog is commercially available in many parts of the world.[1] There have been few reports of adverse side effects from the use of this drug in human beings, but recent *in vitro* and *in vivo* toxicity studies have revealed areas for concern for this drug.[2,3] In an effort to assign the agent(s) responsible for toxicity, we were interested in investigations of β-arteether decomposition products.

[**]*This work is dedicated to Professor Charles W. Jefford on the occasion of his 65th birthday.*
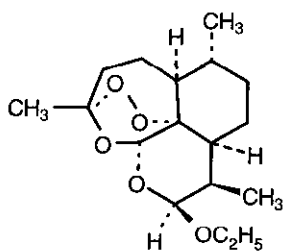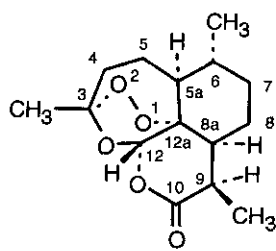
1   2

Baker *et al.* recently reported the formation of a new endoperoxide (**3**) which was obtained when **1** was allowed to stand in 0.01M HCl at 37°C, conditions which mimic stomach acid. On a preparative scale, treatment of **1** with 5M HCl in aqueous ethanol afforded a 25% yield of **3**.[4] The structure of **3** was based on extensive nmr analysis as well as on comparison with the artemisitene analog (**4**) a compound whose structure had been confirmed by X-Ray crystallography, and is thus well established.[5] An earlier paper by Idowu *et al.* reported the formation of several other compounds on treatment of **1** with 5M HCl: an α,β-unsaturated ketone (**5**) and two pairs of epimers (**6,7** and **8,9**).[6] These compounds were not isolated. Structures were apparently based entirely on mass spectroscopy of the liquid chromatography peaks.
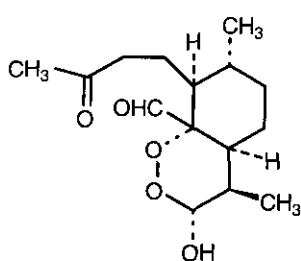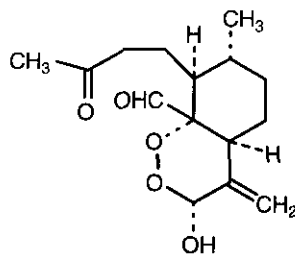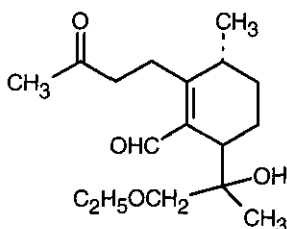

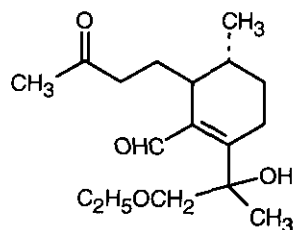3   4
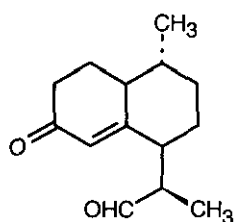
5   6,7   8,9

We have reinvestigated the acid decomposition of **1** as well as of dihydroartemisinin (**10**). Compound (**10**), which is the synthetic precursor of **1**, is also formed when **1** is treated with acid. It is therefore not surprising that we find that treatment of either **1** or **10** in aqueous ethanolic HCl gives rise to identical products, albeit in somewhat different ratios. In addition to compound (**3**), we have isolated compounds to which we assign structures (**11, 12, 13** and **14**).[7]
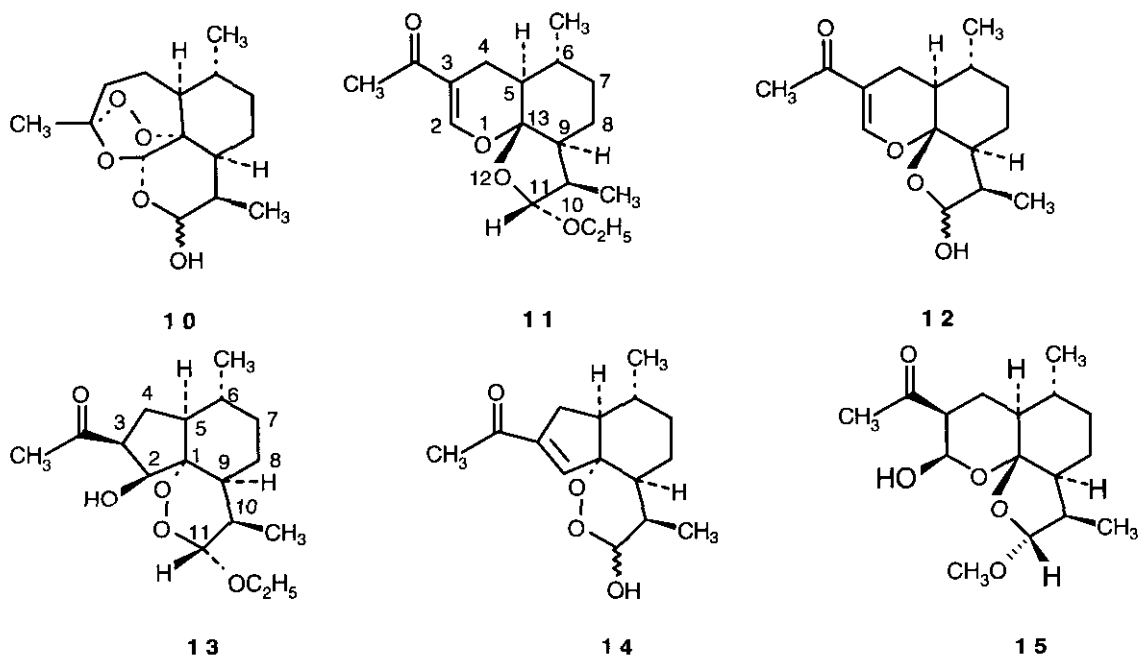
After this work was completed, Baker and Chi reported additional decomposition products formed from β-arteether on treatment with aqueous methanolic HCl.[8] They isolated the methyl ether analog of **11** (α– and β– methoxy isomers), **12**, and several other products, and they argued convincingly that Idowu's **5** is actually **11** (α-methyl ether).

Compound (**11**) was characterized by ms, ir, ^{1}H nmr, and ^{13}C nmr as well as DEPT, COSY, and HETCOR nmr experiments. We isolated a single epimer of this material. The stereochemistry at position 11 is assigned as shown because of a cross peak in the NOESY spectrum between H-11 and the 10-CH$_3$ group. The stereochemistry at the ring junction, position 13, is tentatively assigned as

shown because of a cross peak in the NOESY spectrum between the olefinic H at position 2 and the methyl triplet of the ethoxy group. Dreiding models indicate that these two groups can be closer together in the isomer shown here than they would be in the opposite configuration. A similar compound was isolated by Liang *et al.* on treatment of artemisinin (**2**) with aqueous base followed by acidification.[9,10] Compound (**12**) was an epimeric mixture in solution (*ca.* 5:1 based on nmr integration). It was also characterized by ms, ir, and ^{1}H, ^{13}C nmr, DEPT, COSY, and HETCOR nmr experiments. The structure was assigned by virtue of the near identity of the nmr spectra (especially the ^{13}C) with those of **11**, with the obvious exception of the missing ethyl peaks.

Compounds (**13** and **14**) are assigned the structures shown. That they are either endoperoxides or hydroperoxides is evident from the very large response of the compounds on reductive electrochemical hplc (ECLC). The alcohol (**13**) was obtained as a single epimer, whereas the nmr spectra of **14** revealed that in solution **14** exists as an epimeric mixture (*ca.* 3:1) at carbon 11. Both were characterized by ms, ir, and nmr including DEPT, COSY and HETCOR experiments. The stereochemistry at position 11 of **13** was assigned because of a NOESY cross peak between H-11 and the 10-CH$_3$ doublet. Assigning stereochemistry at positions 2 and 3 was more difficult. H-3 correlated with H-2 in the NOESY nmr spectrum indicating that H-2 and H-3 are cis to each other. However, there appeared to be cross peaks between H-3 and both H-4α and H-4β. The stereochemistry at C-2 (and hence C-3) was tentatively assigned as shown because of a NOESY correlation between H-2 and overlapping multiplets which included protons 4α, 5, 9, and 10, all of which are α. Examination of the nmr spectral data in the paper by Baker and Chi reveals that our compound (**13**) is identical with one reported by them and assigned the isomeric structure (**15**) (methyl instead of ethyl ether).[8] It is difficult to distinguish spectroscopically between peroxides and isomeric ethers such as **13** and **15**. However, we have found that a response on reductive ECLC is a good diagnostic tool for the peroxide group.[11] Formation of **13** requires only ring closure of the keto - aldehyde (**3**). Compounds (**11** and **12**) can be derived from **13** as shown below. In fact, allowing samples of either **3** or **13** to stand overnight in 5M HCl/ethanol produced mixtures of **11** and **12**.

$$3 \longrightarrow 13 \longrightarrow \left[\text{structure} \right] \longrightarrow 11 + 12$$
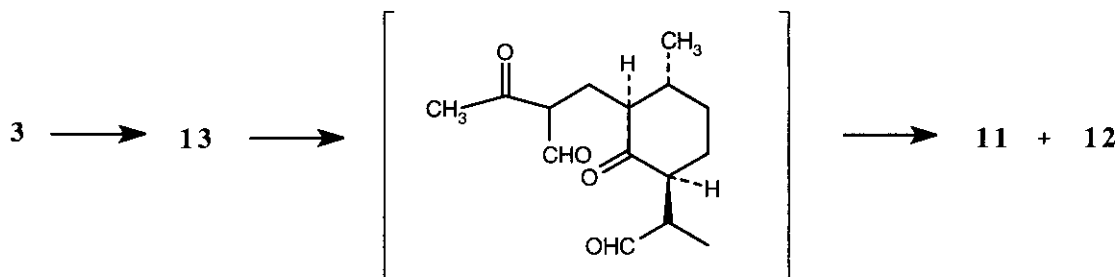
Keto - aldehyde (3) was reported by Baker and Chi to have significant antimalarial activity in an *in vitro* antimalarial assay.[4] When tested in the same assay, the peroxide compounds (13) and (14) have some activity, whereas 11 and 12 have none (Table I).[12] Compounds (3, 11, and 13) were also subjected to a recently developed *in vitro* neurotoxicity assay.[13] All three compounds were found to have similar IC_{50} values (*ca.* 10^{-5}M) and were less toxic than dihydroartemisinin (10) (IC_{50} *ca,* 10^{-7}M), a known *in vivo* metabolite of β-arteether and of other artemisinin analogs and a material with demonstrated neurotoxicity.[13]

Table I	Antimalarial activities against *Plasmodium falciparum*	
	IC_{50} in ng/ml	
cmpd	Sierra Leone clone (D-6)	Indochina clone (W-2)
11	>500	>500
12	>500	>500
13	340	139
14	47	14

EXPERIMENTAL

All nmr spectra were run in CDCl3 using a Bruker 300 AC spectrometer. Ir spectra are of neat oils. ECLC conditions were those described in reference 11.

Compound (11): β-Arteether (1) (1.0 g. 3.2 mmol) was allowed to stand for 3 h at room temperature in 200 ml of a 1:1 mixture of 5N HCl and ethanol. Workup (extraction into CH2Cl2

followed by washing the organic extract with water and brine, then drying over sodium sulfate) followed by two silica gel flash chromatographies (10% ethyl acetate in CH_2Cl_2, then 10% ethyl acetate in hexane followed by 2:1 hexane/ethyl acetate) afforded **11** (65 mg, 7%) as an oil. It was obtained in somewhat lower amounts from **10** under similar conditions. Ms (EI): m/z 294 (3%), 208 (100%). Ir: υ 1714 (w), 1657 (s) and 1624 (s) cm^{-1}. [1]H Nmr: δ 7.4 ppm (1H, d, J = 1.5 Hz, H-2), 4.8 (1H, d, J = 6.3 Hz, H-11), 3.7 and 3.5 (2H, m's, OCH_2CH_3), 2.7 (1H, hextuplet, J = ca. 6.5 Hz, H-10), 2.6 (d of d, J = 16.5 Hz, J' = 5 Hz, H-4α or β), 2.2 (3H, s CH_3CO), 2.1 (1H, m H-9), 1.9 (1H, m, H-4α or β), 1.7 (2H, m, H-8α or 8β, 7α or β), 1.3 (1H, m, H-5), 1.2 (3H, t, J=7 Hz, OCH_2CH_3), 1.2 (2H, m, 7α or β, 8α or β), 1.1 (3H, d, J=7.2 Hz, 10-CH_3), 1.1 (1H, m, H-6), 1.0 (3H, d, J=6.2 Hz, 6-CH_3). [13]C Nmr: δ 196.7 (s, C=O), 154.5 (d, C-2), 118.4 (s, C-3 or 13), 110.9 (d, C-11), 108.3 (s, C-13 or 3), 65.0 (t, OCH_2CH_3), 45.9 (d, C-9), 42.8 (d, C-6 or 5), 40.8 (d, C-10), 33.2 (d, C-5 or 6). 33.1 (t, C-7 or 8), 24.6 (q, CH_3CO), 24.5 (t, C-7 or 8), 20.1 (t, C-4), 18.9 (q, 6-CH_3), 15.4 (q, OCH_2CH_3), 10.4 (q, 10-CH_3). *Anal.* Calcd for $C_{17}H_{26}O_4 \cdot 0.5 \ H_2O$: C, 67.30; H 8.97. Found: C, 67.87; H 8.76.

Compounds (12, 13 and 14): Dihydroartemisinin (**10**) (1.0 g, 3.5 mmol) was allowed to stand overnight in a 200 ml of a 1:1 mixture of 5N HCl and ethanol. Workup followed by flash chromatography (silica gel, 2:1 hexane/ethyl acetate) afforded **12** (80 mg, 8%) as an oil and **14** (35 mg, 3.5%) as a semicrystalline mixture of epimers. Rechromatography of early fractions (10:1 then 10:2 hexane/ethyl acetate)afforded **13** (89 mg, 8%) as an oil.

12: Ms (EI): m/z 266 (2%, parent ion), 208 (49%), 55 (100%). Ir: υ 3393 (s, OH), 1700 (w), 1645 (sh), 1619 (s) cm^{-1}. [1]H Nmr: δ 7.4 (1H, d, J=1.4 Hz, H-2), 5.2 (1H, d, J=6.4 Hz, H-11), 2.6 (1H, m, H-10), 2.2 (3H, s, CH_3CO), 1.2-2.2 (m's), 1.1 (3H, d, J=7.3 Hz, 10-CH_3), 1.0 (3H, d, J=6.2 Hz, 6-CH_3). [13]C Nmr: δ 196.7 (s, C=O), 154.5 (d, C-2), 118.6 (s, C-3 or C-13), 108.6 (s, C-13 or -3), 105.5 (d, C-11), 46.5 (d, C-10), 43.2 (d, C-6 or -5), 43.0 (d, C-9), 33.2 (d, C-5 or -6), 33.0 (t, C-7 or -8), 24.6 (q, $CH_3C=O$), 24.4 (t, C-7 or -8), 20.0, (t, C-4) 19.0 (q, 6-CH_3), 10.3 (q, 10-CH_3). *Anal.* Calcd for $C_{15}H_{22}O_4 \cdot H_2O$: C, 63.36; H, 8.51. Found: C, 63.02; H, 8.17.

13: Ms (EI): m/z 312 (0.2%), 209 (100%). Ir: υ 3350 (s, OH), 1712 (s, C=O) cm^{-1}. [1]H Nmr: δ 4.4 (1H, d, J = 7.3 Hz, H-11), 3.8 (1H, d, J = 6.1 Hz, H-2), 3.5 and 3.7 (2H, m's, OCH_2CH_3), 2.9 (1H, m, H-3), 2.2 (3H, s,

CH3C=O), 2.1 (4H, m, H-4α or β, H-5, H-9, H-10), 1.9 (2H, m, H-4α or β, H-8α or β), 1.6 (1H, m, 7α or 7β), 1.2 (2H, m, 7β or 7α, 8α or 8β), 1.2 (3H, t, J = 7.0 Hz, OCH2CH_3), 1.0 d (3H, d, J = 6.8 Hz, 10-CH3), 1.0 (3H, d, J = 6.4 Hz, 6-CH3). ^{13}C Nmr; δ 209.0 (s, C=O), 104.0 (d, C-11). 93.7 (s, C-1), 75.8 (d, C-2), 63.3 (t, OCH_2CH3), 57.1 (d, C-3), 47.3 (d, C-5), 37.8 (d. C-10), 33.2 (d, C-9), 33.1 (d, C-6), 32.2 (t, C-7), 28.6 (q, CH_3C=O). 28.2 (t, C-4), 23.7 (t, C-8), 21.1 (q, 6-CH3), 15.1 (q, OCH2CH_3), 14.7 (q, 10-CH3). ECLC retention time: 8 min. *Anal.* Calcd for $C_{17}H_{28}O_5$·0.25 H_2O: C, 64.42; H, 9.06. Found: C, 64.06; H, 8.96.

14: Ms (CI): m/z 267 (M+1, 73%), 249 (85%), 233 (100%), 221 (44%). Ir: υ 3420 (s, OH), 1688 (s) cm^{-1}. ^{1}H Nmr: δ 7.1 (1H, d, J = 1.3 Hz, H-2), 5.1 (1H, s, H-11), 2.4 (3H, s, CH_3C=O), 1.2 (3H, d, J = 7 Hz, 10-CH3), 1.0 (3H, d, J = 5 Hz, 6-CH3). ^{13}C Nmr: δ 197.6 (s, C=O), 101.7 (d, C-11), 94.7 (s, C-1), 192.3 (d, C-2), 151.8 (s, C-3), 50.0 (d, C-5), 35.2 (d, C-10), 37.4 (d, C-9), 37.0 (d. C-6), 34.1* (t, C-7), 26.7 (q, CH_3C=O), 33.4 (t, C-4), 25.5* (t, C-8), 21.3 (q, 6-CH3), 13.6 (q, 10-CH3). ECLC retention time: 3 min.

Conversion of **3** into **11** and **12**: A sample of **3** (14 mg) was allowed to stand overnight in a 1:1 mixture of 5N HCl and ethanol (4 ml). The conversion to **11** and **12** was apparent from the appearance of hplc peaks (uv monitoring at 254 nm) corresponding to these compounds. Workup by extraction into methylene chloride, washing with water, and drying over sodium sulfate gave a solution with TLC spots corresponding to **11** and a trace amount of **12.** Nmr of the extract showed that the mixture consisted almost entirely of **11.**

Conversion of **13** into **11** and **12**: A sample of **13** (40 mg) was allowed to stand in a 1:1 mixture of 5N HCl and ethanol (4 ml). After 4 h, ECLC indicated that >90% of **13** was consumed. Hplc with uv monitoring at 254 nm showed the presence of **11** (major peak) and **12**. After workup as above, nmr showed that the crude mixture was almost entirely **11.**

antimalarial assays, and to Dr. David L. Wesche, Department of Pharmacology, WRAIR, for the *in vitro* neurotoxicity assays.

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