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DECOMPOSITION REACTIONS OF ARTEETHER, A SEMISYNTHETIC DERIVATIVE OF QINGHAOSU (ARTEMISININ)

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ABSTRACT

Arteether, an antimalarial derivative of qinghaosu, is extensively decomposed in acid aqueous media. The decomposition products include an α,β-unsaturated decalone and α,β-unsaturated aldehydes. Formation of the latter involves the unusual partial hydrolysis of acetal ring of arteether to an ether function. The endoperoxide group of arteether is also readily attacked by sodium dithionite in an alkaline medium to give a product in which both peroxidic oxygens have been eliminated while the acetal ring is unaffected. In a neutral medium, however, the product of reduction is a diol.

Qinghaosu (Artemisinin; QHS) is a sequiterpene endoperoxide (Figure 1) and is the antimalarial principle of Artemisia annua, a herb used in traditional Chinese medicine[1].

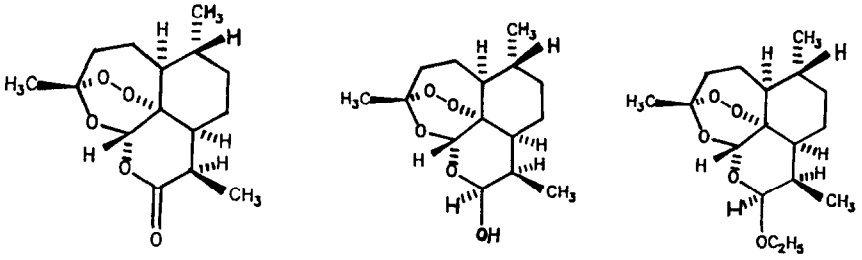


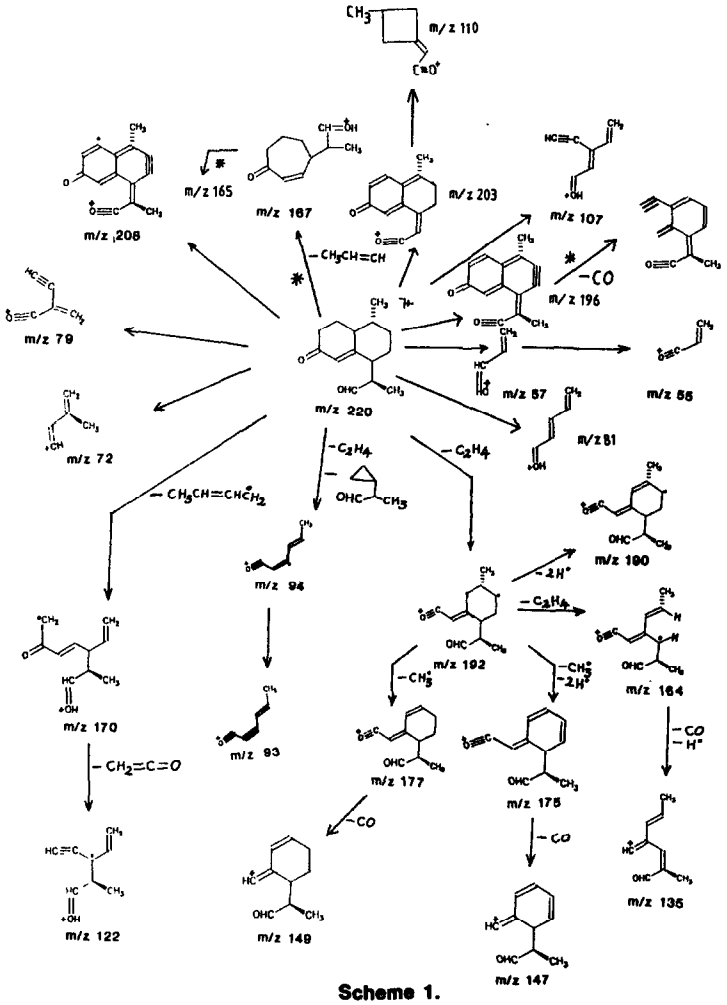
Figure 1. Structures of artemisinin (qinghaosu; QHS) dihydroqinghaosu (QHS) and arteether (ART).

There have been attempts to prepare semi-synthetic derivatives of qinghaosu with enhanced antimalarial potency and improved pharmacological properties. Arteether, (ART) is one such recent derivative and is prepared by the sodium borohydride reduction of QHS to dihydroqinghaosu (DQHS) followed by etherification of DQHS with ethanol, using boron trifluoride etherate as catalyst[2]. In the course of developing an assay method for arteether based on high performance liquid chromatography with ultraviolet detection, attempts were made to convert arteether to ultra-violet absorbing derivatives through its decomposition in acidic or alkaline media. This communication reports on some of the products formed when arteether is decomposed in aqueous acidic media and also when the compound is treated

with a reducing agent in alkaline or neutral media.

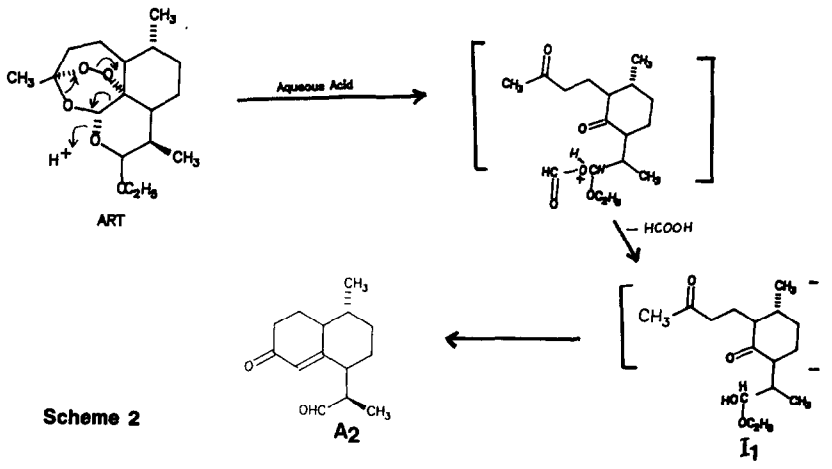
RESULTS AND DISCUSSION

Unlike QHS which has been reported to be unstable in alkaline media[3], arteether was found to be stable in alkali, the compound remaining unchanged after heating an ethanolic solution with 5M sodium hydroxide solution for 2h at 53°C. Arteether was, however, found to be highly unstable in aqueous solutions of mineral acids as may be expected from the presence of an acetal ring in the molecule. When a methanolic solution of arteether was heated for 5h at 70°C with 5M hydrochloric acid, extraction of the reaction mixture and chromatography of the extract showed the presence of many UV-detectable products (Figure 2). Under milder conditions (reaction mixture kept at room temperature for 1h) only one product could be detected by HPLC-UV. This product was identified as an α,β-unsaturated decalone (Compound A_2, Scheme 1) following mass spectrometry of the eluate fraction containing this compound. The EI mass spectrum is



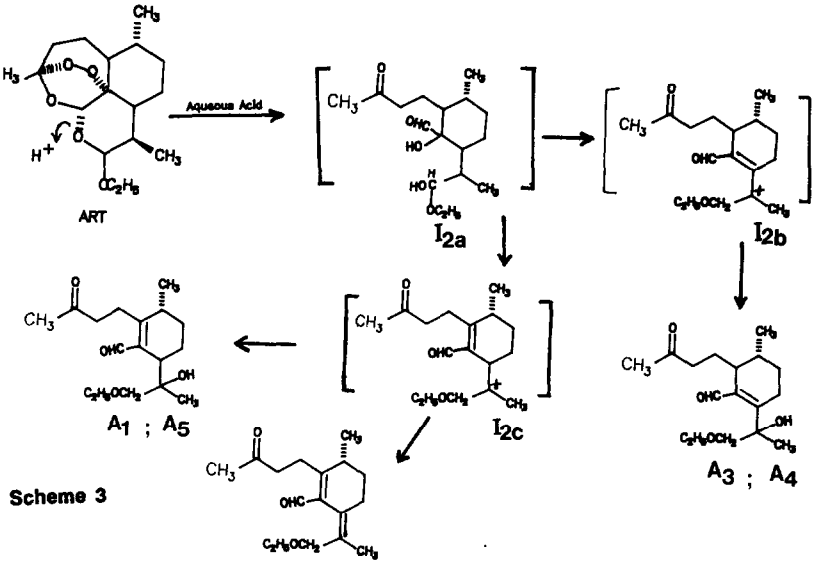
Scheme 1.
*metastable transition

presented in table 1. The proposed fragmentation pattern is shown in Scheme 2. The ultraviolet spectrum of an extract of the reaction mixture showed a maximum at about 254nm. Under optimum conditions for the formation of the α,β-unsaturated decalone (5M HCl; 53°C; 15 min) the formation of the four main side products was observed. A chromatogram of an extract of the reaction mixture obtained under these conditions is shown in Figure 2. The four main side products were identified as two epimeric pairs of α,β-unsaturated aldehydes (structures A_1, A_5; A_3 and A_4 as shown in Scheme 3). The C1 spectra of these compounds are presented in Tables 2 and 3.



Scheme 2

The compounds A_1, A_3, A_4 and A_5 have similar CI spectra. The proposed fragmentation pattern for A_3 (and A_4) is presented in Scheme 5. A similar fragmentation pattern for A3 and A4 can readily be derived for compounds A1 and A5 based on the processes shown in Scheme 5. However, the mass spectra of compounds A_1 and A_5 contain a number of peaks which are absent from the spectra of compounds A_3 and A_4 (see Table 3). These differences in the mass spectra of the two pairs of compounds are readily rationalised on the basis of the different positions of the olefinic bond in the two pairs of compounds. The proposed fragmentation pattern to account for those peaks which are present only in the spectra of A_1 and A_5 is presented in Scheme 6.



Scheme 3

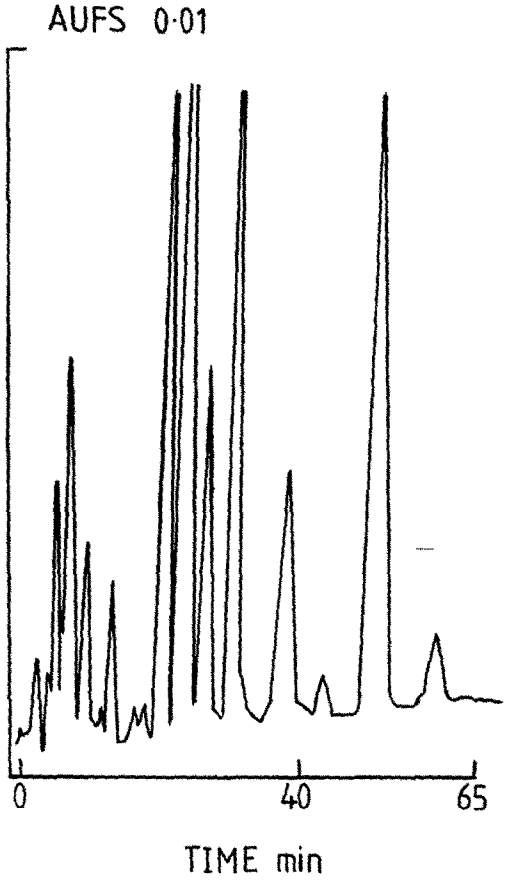


Figure 2 Chromatogram of the reaction mixture obtained after heating ART with 5M HCl for 5h at 70°C Mobile phase: CH_3CN+H_20 (40:60 v/v); flow rate, 0.5ml/min.

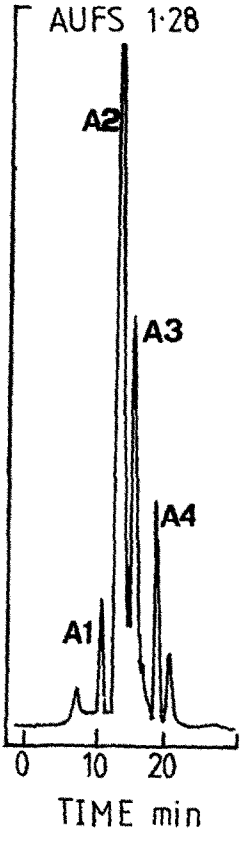


Figure 3 Chromatogram of the reaction mixture obtained after heating ART with 5M HCl for 15 min at 53°C. (Chromatographic conditions as in the text).

TABLE 1

Mass Spectrum of Compound A2

M/Z	20	208	191	190	168	167	149	147	
Relative Intensity (%)	11.1	100	49	69.2	20.1	100	43.1	49.1	
M/Z	135	122	107	93	81	79	72	67	55
Relative Intensity (%)	50.4	53.3	48.2	42.4	40	50	45.9	44.1	99.9

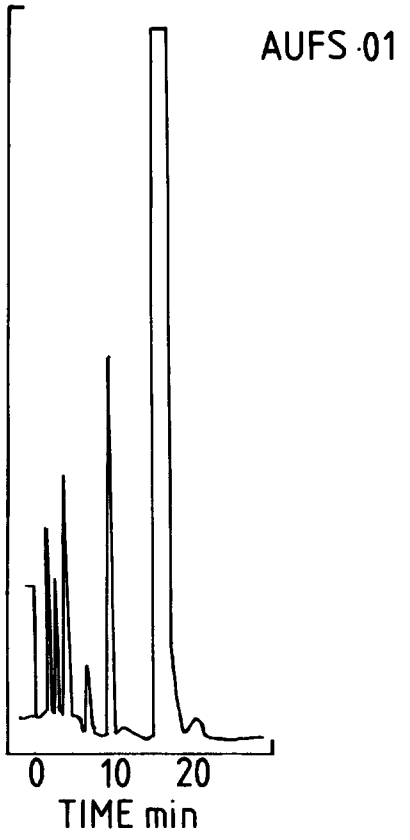


Figure 4 Chromatogram of the reaction mixture obtained from heating arteether with alkaline sodium dithionite at 53°C for 1h.

TABLE 2

Mass spectra of compounds A_3 and A_4

M/Z	Relative intensity (%) Compound A_3	Compound A_4	M/Z	Relative intensity (%) Compound A_3	Compound A_4
295	-	2.9	194	2.8	-
			193	-	12.3
291	24.7		190	20.9	19.7
289	21.2	10.7	167	43	40.7
281	67.2	10.7	167	43	40.7
264	-	12.6	135	17.1	38.6
263	12.9	-	122	15.7	32.1
250	39	29.4	111	19.0	41.6
249	100	75.2	85		93.1
248	12	24.2	83		82
233	8.6	18.1	82		44
221	15	15.2	81		100
208	100	79.7			

TABLE 3

Mass spectrum of compounds A_1 and A_5

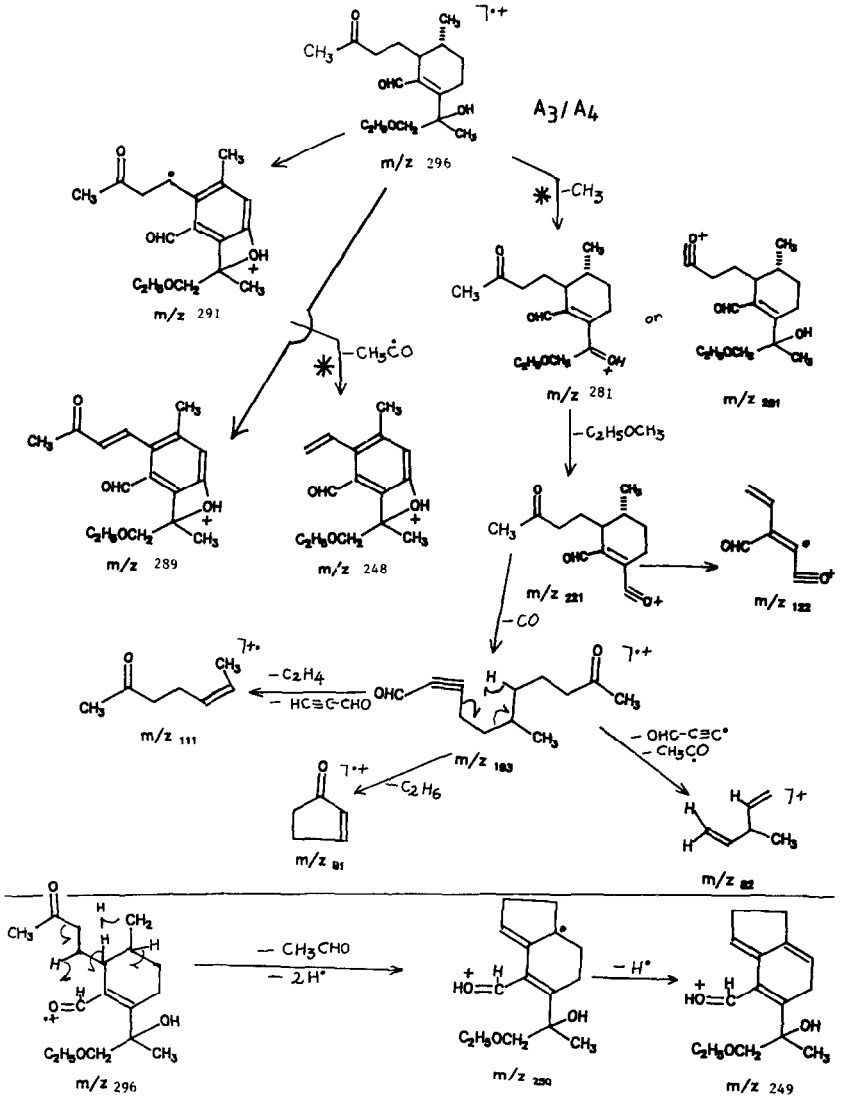
M/Z	Relative intensity (%) Compound A_1	Compound A_5	M/Z	Relative intensity (%) Compound A_1	Compound A_5
296	3.4	-	190	12.9	14.2
295	-	2.9	189	17.5	7.9
291	8.4	13.5	167	34.1	20.6
289	8.8	17.2	149	35.6	21.6
281	22.2	34.5	135	27.2	16.1
277*	3.8	2.8	123	32.3	14.9
276*	3.8	2.1	121	33.2	12.2
275*	5.5	3.4	111	38.5	14.1
250	-	23.7	109	58.3	15.1
249	100	100	86		53.1
238*	4.5	7.3	85		33.6
236*	4.0	1.4	83		30.0
233	25.3	12.6	81		45.4
221	20.0	7.2			
208	38.0	80.9			

*Peaks which are absent in the spectra of A_3 and A_4.

Formation of the peak at m/Z 277 probably involves thermal dehydration of the molecule before ionisation, a process which would be expected to be facile in the structure A_1 (or A_5) but less favoured in A_3 (or A_4).

Mechanistically speaking, the decomposition of arteether in aqueous acid to give the α,β-unsaturated decalone (compound A_2) may be thought of as being initiated by acid attack on the acetal ring to give the intermediate I_1 as shown in Scheme 2. Intramolecular condensation involving the two carbonyl groups together with acid hydrolysis of the hemiacetal group results in the α,β-unsaturated decalone. However, another possible intermediate that may be formed following the scission of the acetal ring is the α-hydroxyaldehyde, I_{2a} (Scheme 3) which may be the common intermediate of compounds A_1, A_3, A_4 and A_5 as shown in the scheme. The α-hydroxyaldehyde will readily undergo dehydration under the acidic condition of the reaction. The two possible pathways for the dehydration of the α-hydroxyaldehyde lead to two different positions for the olefinic bond as are to be found in the eventual products of the decomposition (A_1; A_5 and α_3; α_4). However, formation of these compounds is interesting and unusual in that it also involves the partial hydrolysis of the acetal ring of arteether to give an ether rather than the expected aldehydic product of complete hydrolysis. Apparently, the secondary carbonium ion involved in the initial steps of the hydrolysis of the hemiacetal function of I_{2a} undergoes a hydride shift by which it is converted to the more favoured tertiary carbonium ion (I_{2b} or I_{2c}). The carbonium ion intermediate I_{2c} may be discharged by proton loss to give a diene derivative or by hydrolysis to give a tertiary alcohol (A_1; A_5) as shown in Scheme 3.

The position of the olefinic bond in the other carbonium ion intermediate (I_{2b}) limits the most favourable pathway to its discharge by hydrolysis to the teritiary alcohol (A_3; or A_4), assuming that the dehydration of the α-hydroxyaldehyde takes place faster than the hydrolysis of the hemiacetal function and the accompanying hydride shift. The formation of the intermediates I_{2b} and I_{2c} will be expected to be equally favoured. Since only A_3 (and its isomeric pair A_4) results from the discharge of I_{2b} while I_{2c} may give a diene derivative in addition to A_1 and A_5, the yields of compounds A_3 (and A_4) will be expected to be higher than the yields of A_1 (and A_5). This is borne out by the sizes of the peaks associated with these

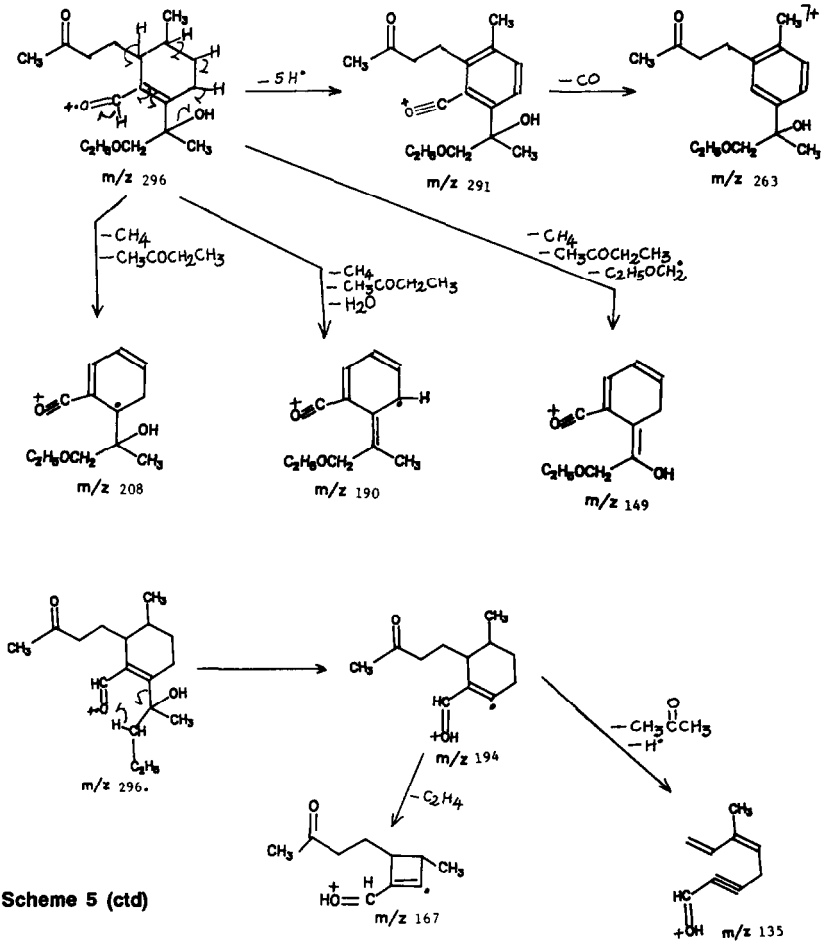


Scheme 5

* metastable transition

compounds in the chromatogram shown in Figure 2. Since the molecular masses of the compounds are the same, the chromophores can be considered identical and the retention times are fairly close, the larger peaks associated with compounds A_3 and A_4 shows that they were present in the reaction mixture in greater concentrations than compounds A_1 and A_5 as would be expected from the mechanism presented in Scheme 3.

Although there has been no previous report on the reaction of a peroxide with sodium dithionite, a disulphide, which may be considered to be closely related to a peroxide has been reduced to a thiol by this reagent[4].



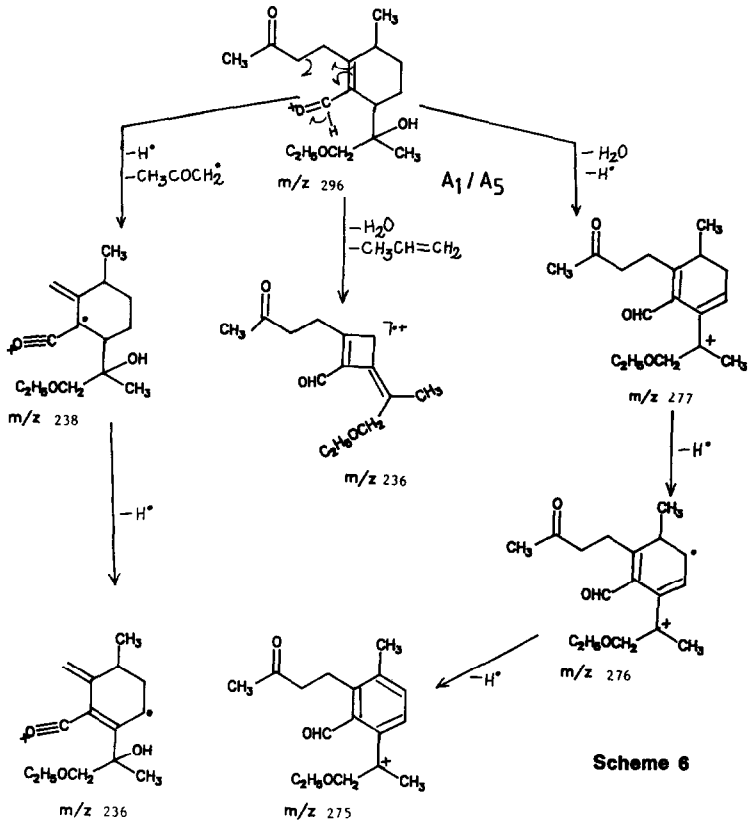
Scheme 5 (ctd)

Arteether was, therefore, treated with sodium dithionite in an attempt to convert it to a diol. Further, sodium dithionite was chosen as the reducing agent because it could be used under alkaline conditions in which arteether is otherwise stable. Chromatography of a reaction mixture obtained from treating arteether with sodium dithionite in an alkaline medium at 53°C for 1h shows the presence of two UV-detectable compounds (Figure 4).

The ultraviolet spectrum of an extract of the reaction mixture shows a maximum at about 220 nm. Mass spectometry of the eluate fractions corresponding to the two peaks B1 and B2 shows that these compounds have the structures depicted in Scheme 4.

The mass spectra are shown in Tables 4 and 5. The proposed fragmentation patterns are shown in schemes 7 and 8.

The optimum conditions for the formation of the predominant compound (B2) were a reaction temperature of 70°C and a time of 2.5h. Under these conditions only the compound B2 was formed. The compound B1 is apparently the first product in the reduction of arteether by alkaline sodium dithionite. Further reduction of the carbonyl and vic-diol groups of B1 leads to B2. The reduction of vic-diols with sodium eithionite has not been described before while the reduction of ketones and aldehydes to alcohols with this reagent has been reported[5].



Scheme 6

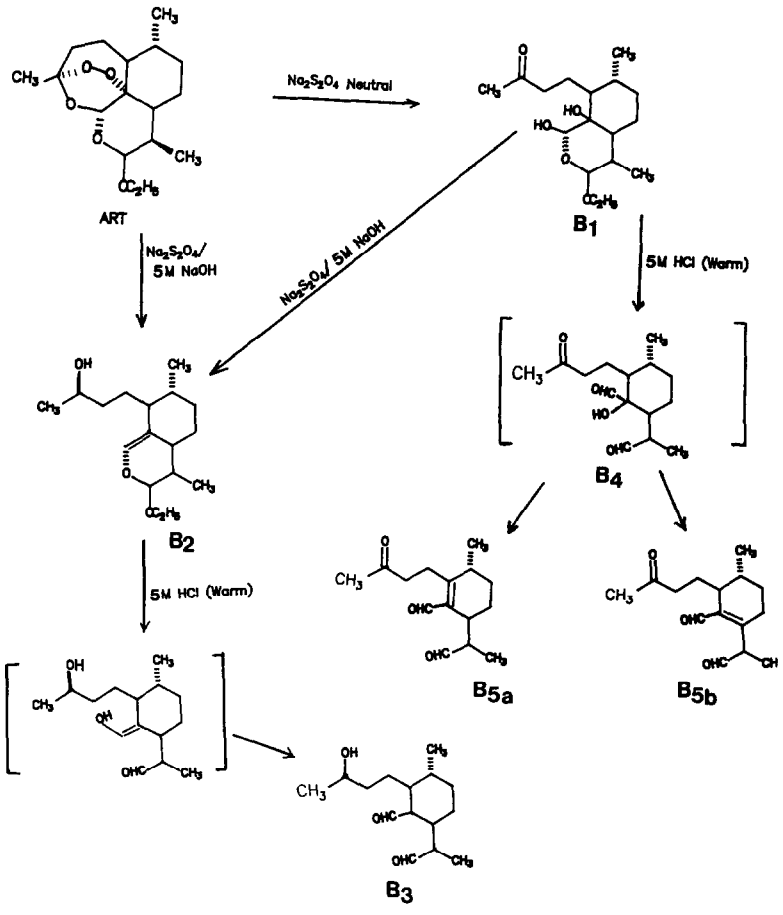
Since the reduction potential of sodium dithionite is known to depend on the concentration of base in the medium[6], the reaction of arteether with sodium dithionite in absence of alkai was examined. In the neutral medium only compound B1 was formed. However, if after heating arteether with sodium dithionite at neutral pH for 2h, the reaction mixture was treated with 5M sodium hydroxide solution and then heated for another 1h, only compound B2 was detected upon chromatography of the reaction mixture.

The structures of compounds B1 and B2 were further confirmed by treating arteether with sodium dithionite under either alkaline or neutral conditions followed by treatment of extracts of the reaction mixtures with 5M hydrochloric acid solution. In both cases the ultraviolet spectra of the reaction mixtures were measured after the reduction reaction and then after treating the reduction products with aqueous acid. The results are shown in Figure 5. Thus, after the reduction of arteether to compound B2 under alkaline conditions, subsequent treatment with acid leads to hydrolysis of the acetal ring and the destruction of the olefinic bond of B2 to give the dialdehyde B3 which has no UV spectrum in the accessible region and is also not detectable following chromatography of the final reaction mixture.

In contrast however, when arteether was treated with sodium dithionite at neutral pH, to give only compound B1, subsequent treatment of an extract of the reaction mixture with acid leads to a bathochromic as well as hyperchromic shift in the absorbance of the reaction mixture (C→D, figure 5). As shown in Scheme 4, this is accounted for by the acid hydrolysis of compound B1 to the hydroxyaldehyde B4 which is then dehydrated to the α,β-unsaturated aldehydes B5a

and B5b. These two compounds and their epimers are readily detected on chromatography of the final reaction mixture obtained after reduction of arteether at neutral pH followed by acid treatment (see Figure 6).

These results are in contrast to earlier work on the action of reducing agents on QHS and its derivatives which have shown that the peroxy group in these compounds is resistant to sodium borohydride while catalytic hydrogenation in the presence of $Pd/CaCO_3$ catalyst causes the loss of only one of the peroxide oxygen atoms to give the corresponding epoxide derivatives[1,2].



Scheme 4

EXPERIMENTAL

Materials

Arteether was provided by SAPEC S.A. (Switzerland) through the World Health Organisation. Sodium dithionite (reagent grade, > 85%) and dichloromethane (Hipersolve^R; HPLC grade) were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Methanol, acetonitrile (both of HPLC grade) and concentrated hydrochloric acid (specific gravity 1.16, A.R. grade) were obtained from Fisons Laboratory Supplies, Loughborough, U.K.

Chromatographic conditions

The chromatographic system used consisted of a Spectra-Physics model SP 8700 solvent delivery system, a SP 8750 organiser module fitted with a Rheodyne Model 7125 injector with a 100μl sample loop; a Beckman Ultrasphere-ODS C_{18} reverse phase column (5μ particle diameter; 15 cm x 4.6 mm) and a SP 8300 fixed wavelength (254 nm) ultraviolet detector. The mobile phase was acetonitrile-water (60:40 v/v) and the flow rate was 0.50 ml/min.

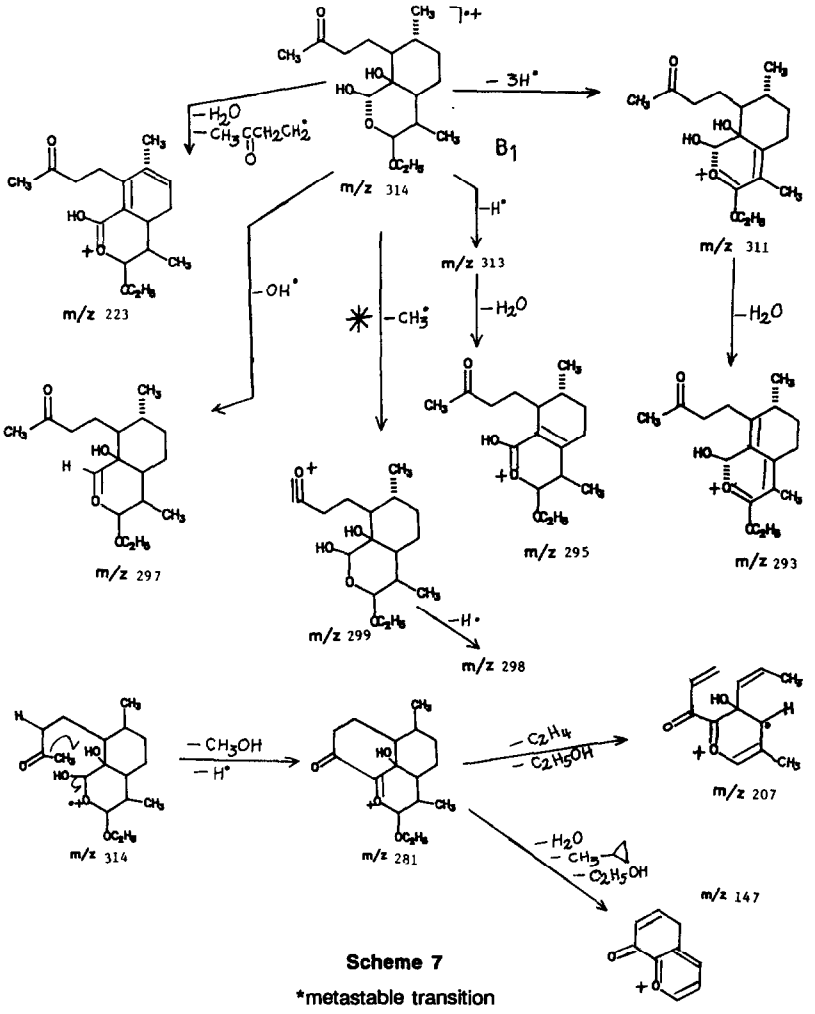
Spectrometers

The mass spectra were determined using a VG Tritech TS 250 spectrometer integrated with a VG 11-250J data system and operated in the EI mode (for compound A_2) or in the CI mode using isobutane as reagent gas. Analysis was by direct insertion (source temperature, 180°C) and the accelerating voltage was 4.12 KV. The ultraviolet spectra were obtained using a Cecil Instruments CE 505 double beam UV-visible spectrophotometer.

Method

Decomposition of Arteether in aqueous acid solution

A solution of arteether in methanol (100 μg.ml) was prepared. A 10.0 ml portion of this solution was taken and the volume reduced to about 1.0 ml by heating in a warm water bath (40°C) under a stream of nitrogen. The solution was then treated with 1.0 ml of 5M hydrochloric acid solution and the mixture heated in a water bath at 53°C for 15 min. After cooling to room temperature, the mixture was extracted with dichloromethane (3.0 ml) by shaking on a vortex mixer.



Scheme 7

*metastable transition

After removing the alkaline aqueous layer, the extract was further shaken with distilled water (1.0 ml) for 10-15 sec. The aqueous layer was discarded and a little of anhydrous sodium sulphate added to the extract to dry it. The extract was transferred to a tappered glass centrifuge tube and the solvent evaporated at 40°C under a stream of nitrogen. The residue was redissolved in 0.25 ml of acetonitrile and the solution chromatographed in ten 25 μl portions.

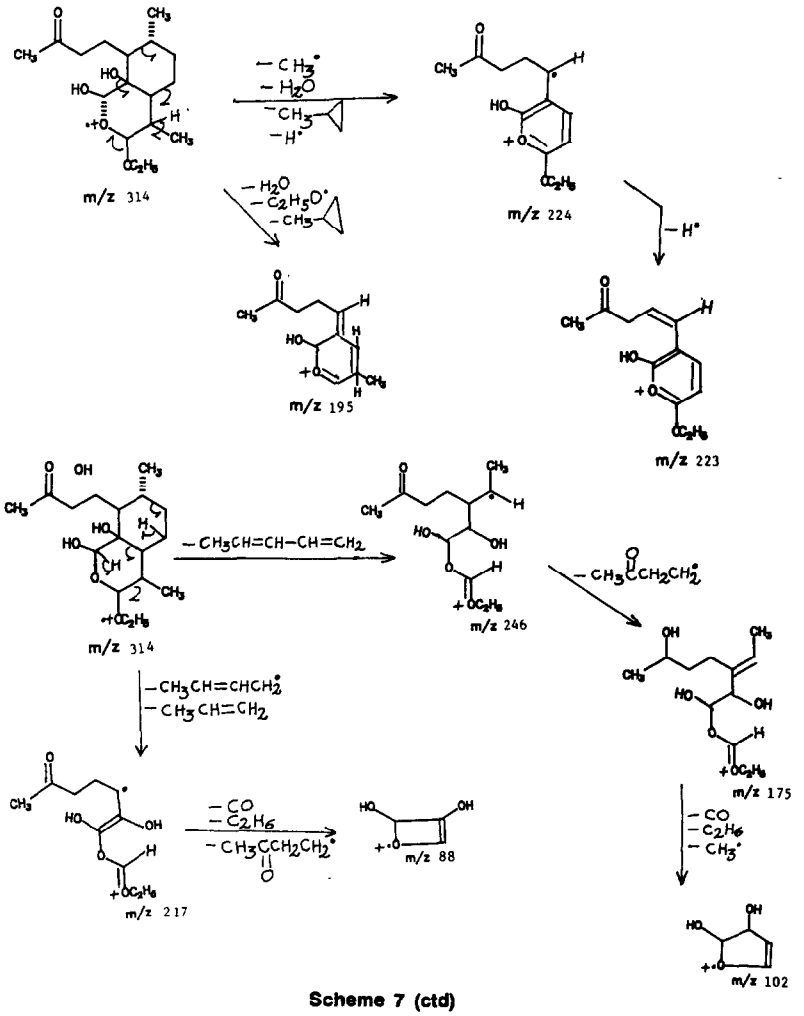
TABLE 4

Mass Spectrum of Compound B1

M/Z:	314	313	312	311	299	298	297				
Relative Intensity (%)	4.0	12.0	12.5	3.0	35.4	44.0	100				
M/Z:	295	281	223	207	195	163	149	147	133	102	88
Relative Intensity (%)	26.0	100	36.0	8.0	7.5	16.0	57.0	82.5	18.5	22.5	44.5

During each chromatographic run the eluate fraction corresponding to each peak was collected as it emerged from the detector. The fractions collected for a particular peak were pooled and extracted with dichloromethane (approximately 1.5 ml or extractant to 5 ml of eluate) for 1 min on a vortex mixer. The extract was dried over anhydrous sodium sulphate, transferred to a tappered glass tube and the solvent evaporated at 40°C under nitrogen. The residue was redissolved in 100 μl of methanol and the solution examined by mass spectrometry. A 20 μl portion of the solution was re-chromatographed to establish the purity of the separated compounds.

The overall procedure is equivalent to reacting 1.0 mg of arteether with aqueous acid, separating the five main products formed (represented by peaks A_1 to A_5 in the chromatogram of Figure 2) and identifying these by mass spectrometry.



Scheme 7 (ctd)

For spectrophotometry, a 2.0 ml aliquot of the solution of arteether was reduced in volume and reacted with hydrochloric acid as described above. After extracting the reaction mixture with dichloromethane, drying the extract and evaporating to dryness as described, the residue was redissolved in 4.0 ml of methanol and the UV-visible spectrum of the solution measured using methanol as reference.

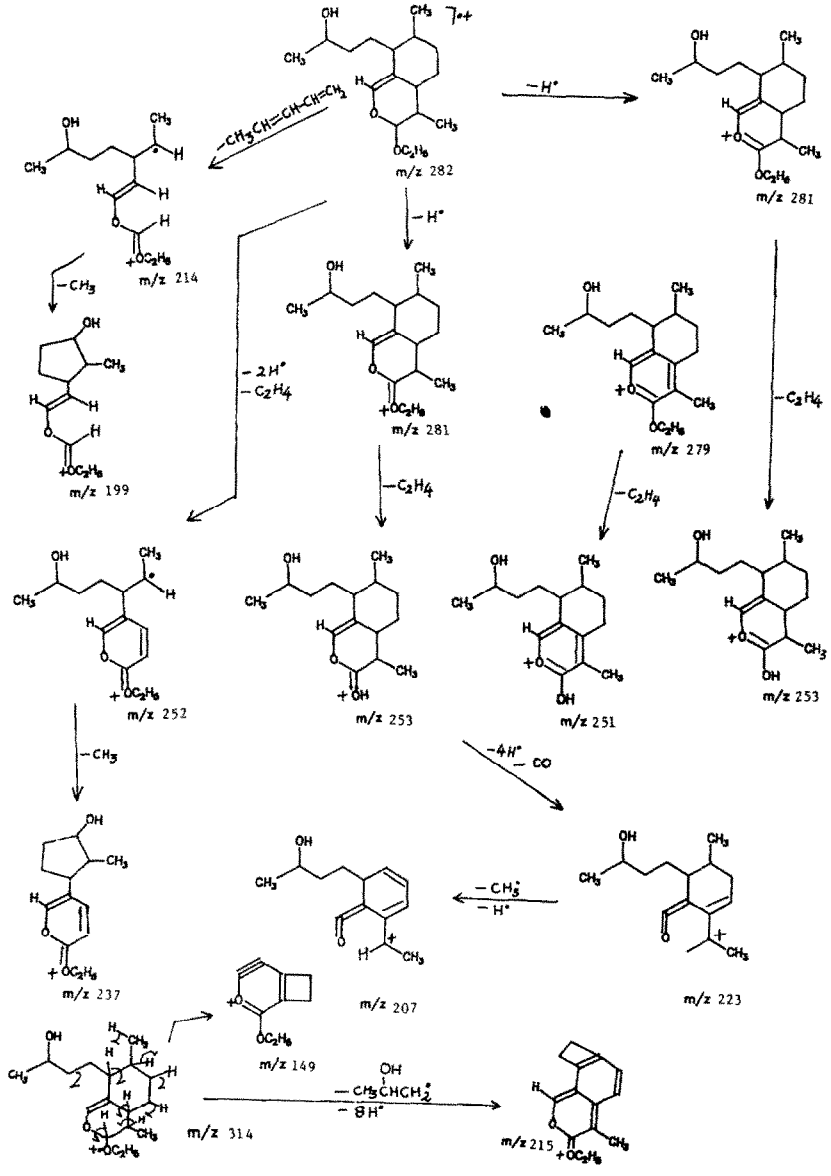
TABLE 5

Mass Spectrum of Compound B2

M/Z	282	281	279	253	251	237	215	214	207
Relative Intensity (%)	4.4	14.9	4.0	3.7	12.3	30.8	100	78.7	14.6
M/Z	199	163	149	123	111	109			
Relative Intensity (%)	28.0	18.8	24.4	27.4	27.8	36.7			

Reduction of arteether with sodium dithionite

An aliquot of arteether solution in methanol (0.5 ml: 100 μg/ml) was treated with sodium hydroxide solution (1.0 ml; 5M) and a solution of dithionite (1.0 ml; 5% m/v). The reaction mixture was heated in a water bath at 53°C for 1h. After cooling to room temperature the reaction mixture was extracted with dichloromethane (3.0 ml) by shaking on a vortex mixer for 1 min. The aqueous layer was removed and the extract dried with anhydrous sodium sulphate. The extract was transferred to a tappered glass centrifuge tube and then evaporated to dryness by heading in water bath (40°C) under a stream of nitrogen. The residue was redissolved in 50 μl of acetonitrile and the solution chromatographed in two 25 μl portions. During each chromatographic run the eluate fractions corresponding to peaks B1 and B2 (Figure 3) were collected. The pooled fractions were extracted and the extracts analysed by mass spectrometry as described for the decomposition of arteether in aqueous acid.



Scheme 8

For spectrophotometry, 200 μg of arteether (2.0 ml of 100 μg/ml solution in methanol) was treated with sodium dithionite solution (1.0 ml; 5% m.v) and 1.0 ml of 5M sodium hydroxide solution. The mixture was heated at 70°C for 2h. After extracting with dichloromethane, drying the extract and evaporating under nitrogen as described above the residue was redissolved in 3.0 ml of methanol. The UV-visible spectrum was measured using methanol as reference.

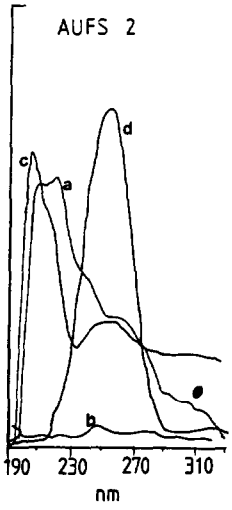


Figure 5: UV spectra of reaction mixtures obtained by reduction of ART after
a: Alkaline medium; b: A+ acid treatment;
c: Neutral medium; d: C+ acid treatment.

Figure 6: Chromatogram of reaction mixture obtained by the reduction of ART in neutral medium followed by acid treatment.

To assess the effect of alkali on the reduction of arteether with sodium dithionite, two aliquots of arteether solution (2.0 ml; 100 μg/ml) were reacted with sodium dithionite (1.0 ml; 5% w/v) in the absence of alkali. After cooling to room temperature, one reaction mixture was extracted and the UV-visible spectrum measured. The other was extracted and the extract dried and evaporated as described earlier. The residue was redissolved in methanol (0.5 ml) and the solution treated with HCl (1.0 ml: 5M). The mixture was heated in a water bath at 70°C for 15 min. After cooling to room temperature the mixture was extracted with dichloromethane (3.0 ml), and the extract dried and evaporated. The residue was redissolved in methanol (3.0 ml) and the UV-visible spectrum measured. This procedure involving reduction in neutral media with or without subsequent acid treatment was repeated, but the final residues were redissolved in 50 μl of acetonitrile and 25 μl portions of the solutions chromatographed as described previously. The reduction of arteether in alkaline media with or without subsequent acid treatment, followed by spectrophotometric or chomatographic analysis of extracts of the reaction mixtures was carried out as above.

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