On the Conversion of Dihydroartemisinic Acid into Artemisinin

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Mechanistic possibilities for the title conversion are discussed. By determining the oxygen-18-induced chemical shifts in the ^{13}C NMR spectrum of labeled **3**, some of these mechanisms can be eliminated. Minor products obtained in the oxidative conversion of **2** into **3** have been identified.

Introduction

We recently reported a simple conversion of dihydroartemisinic acid (**1**) into the *Artemisia annua* derived antimalarial drug artemisinin (**3**).[1-4] Dihydroartemisinic acid, obtained by reduction of another *A. annua* constituent, artemisinic acid, was photooxidized at −78 °C in methylene chloride or at 0 °C in acetone, and the photolysate so obtained was then air oxidized in petroleum ether to afford a 17–32% isolated yield of artemisinin (Scheme I). Others reported similar oxidative conversions to produce artemisinin and related compounds.[5-7]

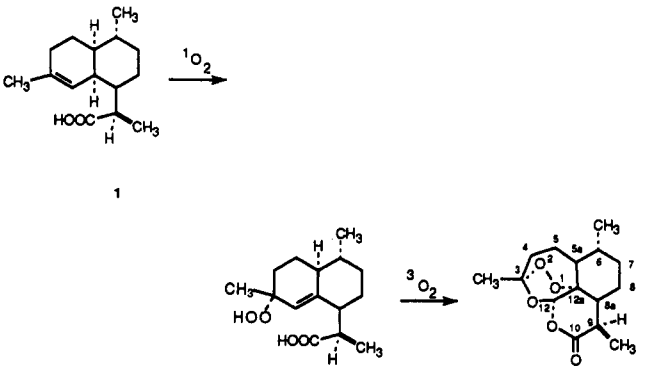
Several mechanistic pathways can be envisaged for the air (triplet oxygen) oxidation of **2** to **3**. Three of these are delineated in Scheme II. Mechanism 1 involves air oxidation of the double bond of **2** with dioxetane formation to give the dioxetane hydroperoxide pictured. This can cleave to give intermediate **A** which in turn can close to artemisinin. This mechanism requires that the oxygens in the endoperoxide bridge of **3** originate in the triplet oxygen oxidation step. Mechanism 2 starts with an allylic rearrangement of hydroperoxide **2**, a reaction with ample precedence.[8] Dioxetane formation should then lead to an intermediate identical (except for possible stereochemical differences) with that formed in mechanism 1. Cleavage of the dioxetane would again lead to intermediate **A**. Mechanism 2 differs from 1 in that the endoperoxide bridge of **3** comes from the singlet oxygen oxidation of **1**. In mechanism 3, the peroxy function in **2** attacks the double bond to give a cyclic radical intermediate which would be subject to autoxidation to give peroxy hydroperoxide.[9] Ring expansion of this intermediate under acid catalysis would give a carbocation which would close to **3**. In this mechanism, the endoperoxide bridge of **3** also comes from the singlet oxygen oxidation step. By using oxygen-18 in the triplet oxygen oxidation and determining the ^{18}O-induced shifts in the ^{13}C NMR spectrum of **3**, we were able to locate the oxygens introduced in this second step of the transformation of **1** to **3**. Using $^{18}O_2$ in the photooxidative conversion of **1** into **2** and then air oxidizing resulted in artemisinin labeled in two of the nonperoxide positions.

During the course of this work, we have isolated other products from this two-step reaction. Establishing the identity of one of these products suggests structural revision of a compound previously reported in the literature.

Results and Discussion

The photooxidation of **1** results in conversion to hydroperoxide **2**, the product expected from the ene reaction with singlet oxygen. In initial experiments, exposure of silica gel purified **2** to air resulted in a very low conversion to artemisinin. This led us to conclude that **2** was not an intermediate in the overall conversion of **1** to **3**.[2] Later experiments have demonstrated, however, that a trace amount of water is required for this transformation, which is acid catalyzed, and that **2** is in fact further oxidized to artemisinin (see Experimental Section).

Scheme I



1 **2** **3**

Exposure to oxygen-18 (97.7 atom %) of an argon-purged mixture of **2** in petroleum ether containing trifluoroacetic acid followed by workup and two recrystallizations from ether/hexane afforded a 25% yield of artemisinin, mp 149–151 °C, containing two ^{18}O atoms (**3**-^{18}-O_2). The mass spectrum of **3** is known to be subject to instrumental variations.[10] However, when **3** and **3**-^{18}-O_2 were analyzed by GC/EIMS under identical conditions, **3** showed a small parent peak at 282 and **3**-$^{18}O_2$ at 286. Using CIMS (NH_3), **3** showed a peak at m/e 300 (M + NH_4, 100% relative intensity) as well as peaks at 283 (M + 1, 5%) and at 272 (M + NH_4 − CO, 8%). Under identical conditions, **3**-$^{18}O_2$ has a peak at 304 (M + NH_4, 100%) with negligible peaks at 300 and 302 (<4%). In addition, there are peaks at m/e 287 (M + 1, 8%) and at 276 (M + NH_4 − CO, 5%). The infrared spectrum of **3** contains a band at 883 cm^{-1} which is attributed to the endoperoxide group. The IR spectrum of **3**-$^{18}O_2$ is identical with that of **3** except that this band is shifted to 872 cm^{-1}.

Oxygen-18-induced chemical shifts in ^{13}C NMR spectroscopy have recently been reviewed.[11] Shifts have been

† George Mason University.

(1) A preliminary account of some of the results reported here was presented at the 200th National Meeting of the American Chemical Society, August, 1990, ORGN 287.
(2) Roth, R. J.; Acton, N. *J. Nat. Prod.* **1989**, *52*, 1183–1185.
(3) Roth, R. J.; Acton, N. U.S. Patent 4,992,561, February 12, 1991.
(4) Roth, R. J.; Acton, N. *J. Chem. Ed.* **1991**, *68*, 612–613.
(5) Haynes, R. K.; Vonwiller, S. C. *J. Chem. Soc., Chem. Commun.* **1990**, 451–453.
(6) Bustos, D. A.; Jung, M.; ElSohly, H. N.; McChesney, J. D. *Heterocycles* **1989**, *29*, 2273–2277.
(7) Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. *Tetrrahedron Lett.* **1989**, *30*, 5973–5976.
(8) Dang, H.-S.; Davies, A. G.; Davison, I. G. E.; Schiesser, C. H. *J. Org. Chem.* **1990**, *55*, 1432–1438.
(9) Porter, N. A. *Acc. Chem. Res.* **1986**, *19*, 262–268.
(10) Fales, H. M.; Sokoloski, E. A.; Pannell, L. K.; Quan-long, P.; Klayman, D. L.; Lin, A. J.; Brossi, A.; Kelley, J. A. *Anal. Chem.* **1990**, *62*, 2494–2501.
(11) Risley, J. M.; Van Etten, R. L. *NMR Basic Princ. Prog.* **1990**, *22*, 81–168.

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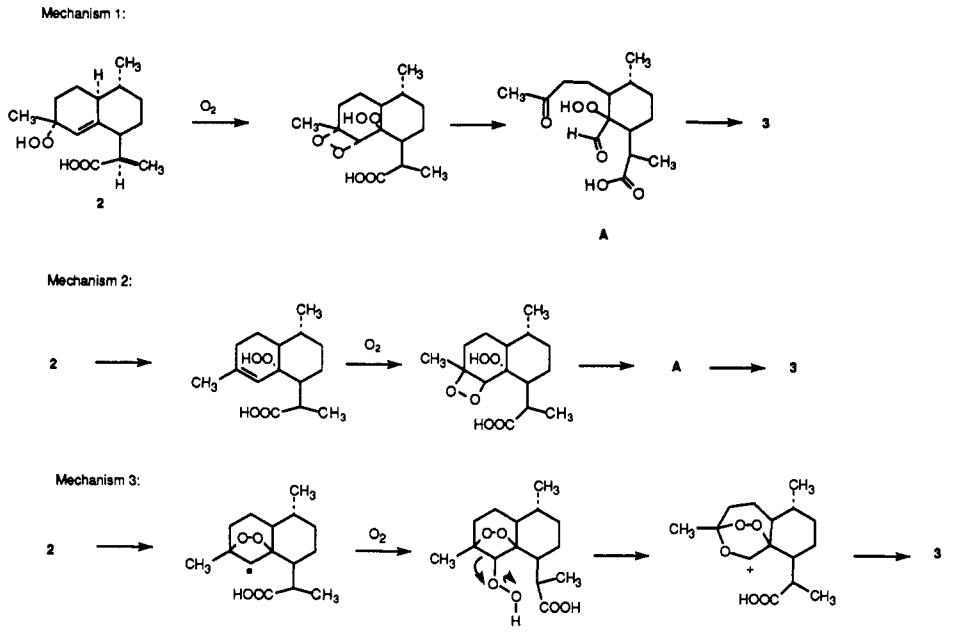
Scheme II



reported for many types of compounds and many oxygen-containing functional groups, but the only report of ^{13}C NMR shifts for an ^{18}O-labeled peroxide is that of Vleggaar et al., who labeled verruculogen, a peroxide metabolite of *Penicillium verruculosum*, with ^{18}O.[12,13] The complete assignment of ^{13}C NMR peaks of artemisinin has been reported and confirmed.[14,15] The peak at 105.3 ppm is that of carbon-3, and C-12a is at 79.4 ppm. A 2:1 mixture of **3** and **3**-$^{18}O_2$ shows upfield shifts for these two peaks of 32 ppb and 47 ppb, respectively. Since no other carbons in the molecule show shifts of sufficient magnitude to be one-bond shifts, this demonstrates that the labeled oxygen is exclusively in the endoperoxide bridge (Figure 1, A and B). The magnitudes of the one-bond shifts observed here are close to those reported for verruculogen (26 and 42 ppb[12]). This result rules out mechanisms 2 and 3 and any others in which the endoperoxide bridge of **3** comes from the singlet oxygen reaction. Additional two-, three-, and four-bond ^{18}O-induced shifts can also be detected. Thus two-bond upfield shifts of 13 ppb can be seen for C-4 at 35.8 ppm and 5 ppb for the 3-CH_3 at 25.2 ppm as can a three-bond shift of 4 ppb for C-6 at 37.4 ppm. Most interestingly, a four-bond shift is observed for the carbonyl carbon at 172 ppm. This last shift of 3 ppb is downfield and is apparently only the second reported instance of a downfield oxygen-18-induced chemical shift in ^{13}C-NMR spectroscopy. The first such downfield shift was recently reported and is a three-bond shift.[16]

We next turned to the more difficult experiment of performing the photooxidative conversion of **1** into **2** using labeled oxygen. Although this reaction is rapid and complete when done using an oxygen purge,[2-4] it is slow under a static oxygen blanket, and the dye bleaches before the reaction is complete. The labeled experiment was carried out by adding at intervals additional dye dissolved in a small amount of argon-purged solvent. The hydroperoxide was not separated from residual **1**; the mixture was exposed to air to give, after flash chromatography and recrystallization, a 12% yield of artemisinin (**3**-$^{18}O_x$). The CIMS (NH_3) of this material showed peaks at m/e 300 (53%), 302 (100%), and 304 (43%), corresponding respectively to unlabeled, monolabeled, and dilabeled compound. The infrared spectrum of this labeled mixture has a split carbonyl absorption at 1730 and 1741 cm^{-1}.

There are three peaks of interest in the ^{13}C spectrum of this material: C-10, C-3, and C-12 (Figure 1, C, D, and E). The carbonyl carbon, C-10, at 172 ppm appears as a split peak with an upfield shift of 11 ppb for the labeled material. Spiking the sample with additional unlabeled material increases the downfield peak, thus confirming the expected upfield shift in the labeled material. The size of the shift implies that the label is in position 11 rather than in the carbonyl oxygen.[11] The peak at 105 ppm (C-3) appears as two peaks, the larger at 105.280 and the smaller at 105.254 in a ratio of approximately 3:1 for a chemical shift difference of 26 ppb. Spiking with unlabeled material increases the downfield peak. This, in combination with the mass spectra ratios, indicates that the larger peak at 105.280 goes with both unlabeled and monolabeled compound, and the smaller peak at 105.254 ppm corresponds to dilabeled compound. Since C-3 is shifted only by dilabeling, this indicates that in monolabeled material ^{18}O is largely in position 11, the lactone oxygen. Dilabeled compound contains ^{18}O in positions 11 and 13. This is confirmed by the pattern displayed by C-12 which appears as three peaks: 93.617 is the unlabeled material (confirmed by the spiking experiment); 93.590 is monolabeled, and 93.572 is dilabeled.[17] This corresponds to an upfield shift of 28 ppb for the monolabel and 45 ppb for the dilabel. The NMR intensity ratio of these peaks is 65:100:48, which is not so very different from the mass spectra ratio of 53:100:43.

Of the three mechanisms in Scheme II, we are left with mechanism 1. This mechanism does have precedence in its favor. In a communication published shortly after our initial report of the conversion of **1** into **3**, Haynes and Vonwiller reported the same conversion, but they used

(12) Horak, R. M.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1987**, 1568–1570.
(13) We thank a referee for bringing this reference to our attention.
(14) Zhongshan, W.; Nakashima, T. T.; Kopecky, K. R.; Molina, J. *Can. J. Chem.* **1985**, *63*, 3070–3074.
(15) Blasko, G.; Cordell, G. A. *J. Nat. Prod.* **1988**, *51*, 1273–1276.
(16) Arias, W.; Risley, J. M. *J. Org. Chem.* **1991**, *56*, 3741–3744.
(17) A referee has pointed out that the shoulder observed for C-12 (Figure 1E) at 93.598 ppm may be from material monolabeled at position 13. This is probably no more than about 5%.

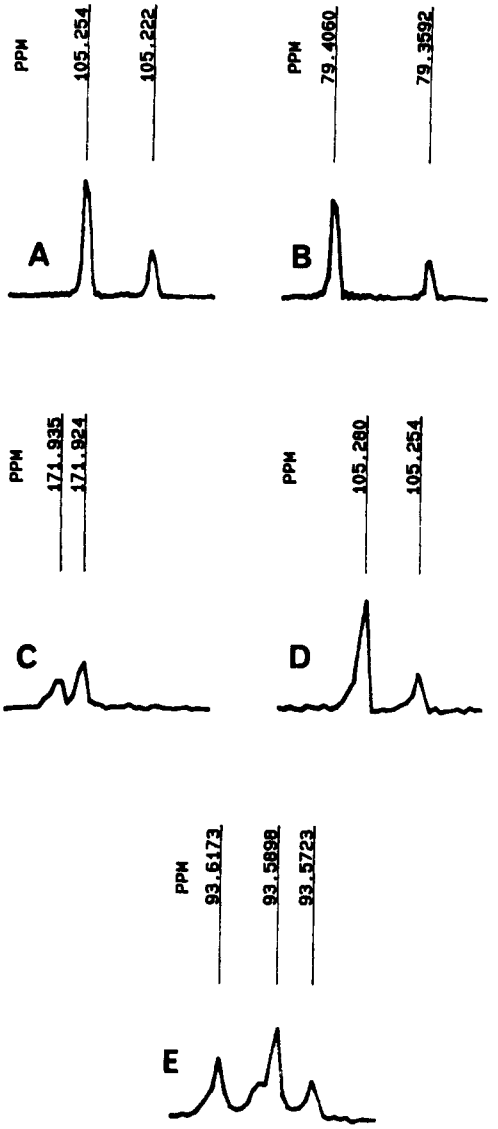
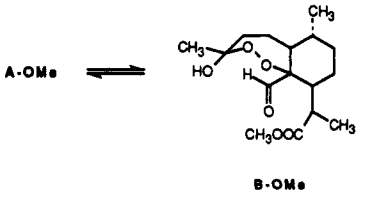


Figure 1. ^{13}C NMR signals (A) for C-3 and (B) for C-12a in a 2:1 mixture of **3** and **3**-$^{18}O_2$ and (C) for C-10, (D) for C-3, and (E) for C-12 in **3**-$^{18}O_x$.

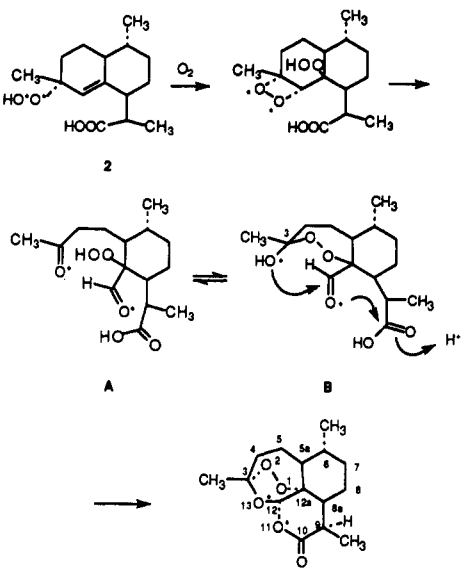
oxygen and a copper catalyst rather than acid to effect the oxidation of **2** to **3**.[5] When the methyl ester of **1** was used as starting material, an equilibrating mixture of the methyl ester of **A** (**A-OMe**) and **B-OMe** was identified by NMR.



Treatment of this mixture with *p*-toluenesulfonic acid resulted in a 28% yield of **3**. In fact, our conditions for the conversion (mixing the substrate with hydrocarbon solvent containing a trace of trifluoroacetic acid and allowing to stand in air for several days) also converts the methyl ester of **2** into **3** (23% overall yield from **1**).

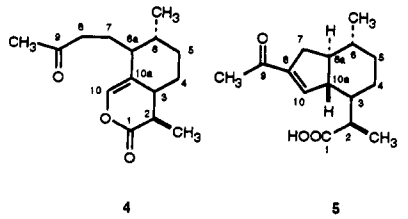
The label distribution in **3**-$^{18}O_x$ tells something about the equilibria involved in the conversion of intermediate **A** to **3** (Scheme III). The ratio of unlabeled:monolabeled:dilabeled compound obtained must depend to some extent on the amount of water present. However, the fact that little monolabeled material is found containing a label in position 13 must mean that the equilibration of oxygen

Scheme III



in the keto position of **A** is faster than equilibration at the aldehyde position. Protonation of the OH of intermediate **B** followed by loss of water results in a carbonium ion at C-3. Stabilization of this carbonium ion by the adjacent oxygen atom may be the reason for this rate difference. Equilibration with unlabeled water gives artemisinin labeled in position 11. Further equilibration of the aldehyde oxygen of **B** leads to unlabeled **3**.

In addition to artemisinin, several other products have been isolated from this air oxidation, among them the enol lactone **4**. This compound appears in the literature in a frequently cited paper that reports its synthesis via ozonolysis of **1** as part of an unsuccessful attempt to effect the conversion of **1** into artemisinin.[18] Treatment of the purported **4** with singlet oxygen did not produce artemisinin. The authors suggested that this failure might be caused by decreased electron density of the double bond. Others suggested hydroperoxidation as the reaction pathway or inappropriate experimental conditions.[19] Since the enol lactone which we isolated and identified as **4** had spectral properties which were not identical with those reported for **4** in the literature, we have repeated this literature preparation. The material which we isolated has IR and proton NMR peaks identical with those reported by Jung et al.,[18] but on the basis of more complete spectral data we believe what we obtained to be the isomeric carboxylic acid **5** resulting from a simple aldol condensation of the keto–aldehyde ozonolysis product.



Thus, acid **1** was treated with ozone followed by dimethyl sulfide and then with perchloric acid in THF/water as described.[18] The major compound isolated can be extracted into aqueous sodium carbonate. The IR spectrum of **5** determined in KBr (rather than in the $CHCl_3$ used

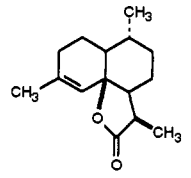
(18) Jung, M.; ElSohly, H. N.; Croom, E. M.; McPhail, A. T.; McPhail, D. R. *J. Org. Chem.* **1986**, *51*, 5417–5419.
(19) Jefford, C. W.; Wang, Y.; Bernardinelli, G. *Helv. Chim. Acta* **1988**, *71*, 2042–2052.

in ref 18) shows a carboxylic acid absorption. The DEPT NMR determination of carbon multiplicities indicates that the compound has three quaternary, six tertiary, three secondary, and three primary carbons as required for structure 5 but not 4. Standard COSY and HETCORR NMR experiments provided the assignments listed in the Experimental Section. The trans ring junction is deduced from the absence of splitting of H-10 in the ^{1}H-NMR spectrum of 5 (6.67 ppm, s, $W_{1/2}$ = 5 Hz). A Dreiding model of this compound and molecular modeling using the Alchemy II program show a dihedral angle close to 90° for H-10–H-10a. The mass spectrum of 5 has a parent ion at m/e 250 and a base peak at m/e 177 corresponding to loss of the acid side chain. This fragmentation is expected for 5 but not for 4.

The enol lactone 4 can be isolated in trace amounts on treatment of the photooxidation products of 1 with trifluoroacetic acid in petroleum ether and air. The mass spectrum of this compound shows a parent ion at m/e 250 and a base peak at m/e 192 corresponding to loss of acetone from the side chain. The IR spectrum has a split lactone carbonyl absorption at 1755 and 1748 cm^{-1}, a ketone carbonyl absorption at 1706, and a double-bond absorption at 1676 cm^{-1}. The DEPT NMR experiment gives appropriate ^{13}C multiplicities for the enol lactone structure.

Although enol lactone 4 is readily photooxidized to an as yet unidentified material, there is no evidence that this procedure produces a significant amount of artemisinin. Moreover, a trifluoroacetic acid-containing petroleum ether solution of 4 is stable in air for at least several days. When 4 is subjected to photooxidation, and the photolysate allowed to stand in petroleum ether solution with trifluoroacetic acid, no more than 1–2% of artemisinin can be detected by liquid chromatography using an electrochemical detector.[20]

In addition to 4, the chromatography of the mother liquors afforded trace amounts of three other sesquiterpene lactones: the known dihydro-*epi*-deoxyarteannuin B[21,22] (6) and two others, assigned structures 7 (isolated on only one occasion) and 8. The structures of 7 and 8 were



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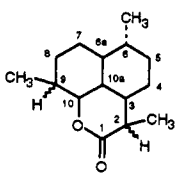

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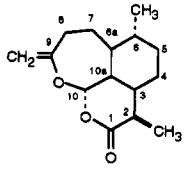

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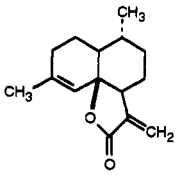

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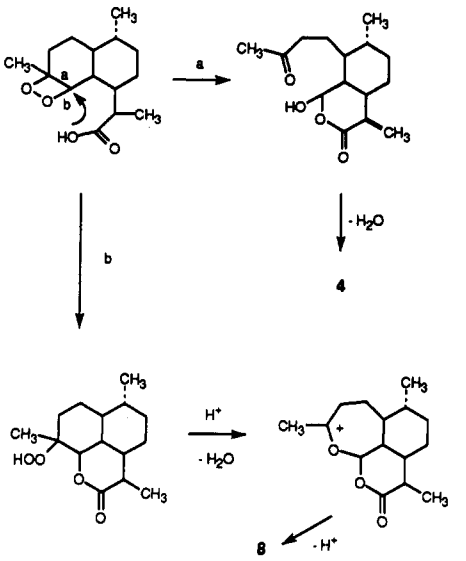
deduced from their ^{1}H- and ^{13}C-NMR spectra including standard DEPT, COSY, and HETCORR experiments, as well as from their IR and mass spectra (see Experimental Section).

Scheme IV



A conceivable route to 4 might be via the keto–aldehyde–acid obtained by ozonolysis of 1.[18] However, allowing this compound to stand in TFA-containing petroleum ether did not produce any lactone 4 (HPLC analysis). A possible alternative is outlined in Scheme IV, wherein a dioxetane is intramolecularly attacked and bond a is cleaved. Lactone 6 must arise from decomposition of hydroperoxide 2 in a manner analogous to that in which *epi*-deoxyarteannuin B (9) is formed from the analogous hydroperoxide derived from artemisinic acid.[23–26] Lactone 7 could come from (anti-Markovnikov) acid-catalyzed lactonization of dihydroartemisinic acid. Cleavage of bond b in the dioxetane shown in Scheme IV leads to a hydroperoxide lactone. Protonation of this lactone hydroperoxide followed by loss of water and ring expansion would give a resonance-stabilized carbocation which on deprotonation leads to the lactone 8.

Conclusions

Although a variety of reaction pathways for the two-step oxidation of dihydroartemisinic acid (1) to artemisinin (3) can be imagined, we have been able to demonstrate unambiguously that the peroxide bridge oxygens are introduced during the triplet oxygen (air) oxidation step. Further, the oxygen atoms of the hydroperoxide 2 are not completely incorporated in the artemisinin formed, nor are they randomly distributed among the non-peroxide positions of this molecule. Since a series of complex equilibria must be established before intermediate A closes to 3, it is not clear how reaction conditions might be manipulated to increase the yield of 3. Enol lactone 4 has been isolated and characterized; it is not a precursor of artemisinin even though its deoxo analogue is a precursor of deoxoartemisinin.[27] Several other minor oxidation byproducts have been isolated, characterized, and identified.

Experimental Section

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured in $CDCl_3$ on a Bruker AC-F 300 spectrometer. GC/MS (EI) were

(20) Acton, N.; Klayman, D. L.; Rollman, I. J. *Planta Med.* **1985**, 445–446.
(21) Xu, X.-X.; Xue, T.-H.; Zhou, W.-S. *Acta Chim. Sin.* **1985**, *43*, 1056–1059.
(22) We have isolated traces of 6 from *A. annua* extracts: Acton, N.; unpublished results.
(23) Acton, N.; Roth, R. J. *Phytochem.* **1989**, *28*, 3530–3531.
(24) Jung, M.; Yoo, Y.; ElSohly, H. N.; McChesney, J. D. *J. Nat. Prod.* **1987**, *50*, 972–973.
(25) Xu, X.; Zhu, J.; Zhou, W. *Acta Chim. Sin.* **1985**, *43*, 48–52.
(26) El-Feraly, F. S.; Al-Meshal, I. A.; Al-Yahya, M. A.; Hifnawy, M. S. *Phytochem.* **1986**, *25*, 2777–2778.
(27) Ye, B.; Wu, Y.-L. *J. Chem. Soc., Chem. Commun.* **1990**, 726–727.

obtained from a Hewlett Packard 5890 GC with a 5970 mass selective detector. CI spectra were obtained from a Nermag R 10-10C spectrometer. Melting points are uncorrected. Oxygen-18 was purchased from MSD Isotopes.

Artemisinin-$^{18}O_2$ (3-$^{18}O_2$). A solution of 1 (500 mg) in acetone (80 mL) containing 2–3 mg of methylene blue was photooxidized at 0 °C as previously described.[3,4] After removal of solvent and most of the dye, the photolysate was mixed with 80 mL of argon-purged petroleum ether. Trifluoroacetic acid (4 drops) was added, the mixture was cooled in dry ice–acetone, and the flask was evacuated and flushed with argon 3 times. After warming to rt, the mixture was exposed to oxygen-18 (97.7 atom %, 1 L) for 5 days. Workup as previously described for the unlabeled material, followed by two recrystallizations from ether/hexane, afforded 150 mg (25%) of 3-$^{18}O_2$, mp 149–151 °C, $[\alpha]^{21}_D$ +64° (*c* 1.2, $CDCl_3$). The IR, ^1H NMR, and ^{13}C NMR spectra were identical with those of the unlabeled material with the exceptions discussed in the text.

Artemisinin-$^{18}O_x$ (3-$^{18}O_x$). A solution of 1 (505 mg) and ca. 5 mg of methylene blue in acetone (70 mL) was cooled in dry ice–acetone on a vacuum manifold. The solution was evacuated for 10 min and the manifold then closed. The dry ice bath was replaced with an ice bath, and the solution was then exposed to a 1-L container of 97.7% $^{18}O_2$. After irradiation for 2 h,[3,4] the dye had bleached. HPLC analysis showed a large amount of residual starting material. Additional dye dissolved in a small amount of argon-purged acetone was added and irradiation continued. This procedure was repeated once more. After a total irradiation time of 4 h, the solvent was evaporated and replaced with ether. After filtering to remove most of the dye, the solvent was removed. The residue was mixed with 75 mL of hexane containing 3 drops of TFA and allowed to stand in air at room temperature for 3 days. At the end of this time, ECLC analysis[20] of an aliquot showed a 14% yield of artemisinin. Removal of solvent followed by two flash chromatographies (silica gel, 1:1 hexane/ether) and then recrystallization from ether/hexane afforded 71 mg (12%) of artemisinin, mp 151–153 °C.

Artemisinin (3) and Enol Lactone 4. A solution of 500 mg of 1 in acetone was photooxidized in acetone at 0 °C, and the photolysate was then air oxidized as previously described.[2-4] After isolation of 3 by recrystallization, flash chromatography of the mother liquor (silica gel, 1:1 ether/petroleum ether) afforded small additional amounts of artemisinin and 10–20 mg each of 4, 6, 7, and 8.

Enol Lactone 4. Flocculent needles, mp 98–101 °C, after recrystallization from ether/petroleum ether. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.07; H, 9.07. IR max (KBr): 1755, 1748, 1706, 1676, 1160, 1127, 1084 cm^{-1}. ^1H NMR: δ 1.00 (3 H, d, J = 6 Hz, 6-CH_3), 1.15 (1 H, m, H-4 or -5), 1.22 (3 H, d, J = 7 Hz, 2-CH_3) superimposed on 1.22 (2 H, m's, H-6 and 1 × H-4 or 1 × H-5), 1.50 (1 H, m, H-7 or -7′), 1.67 (1 H, m, H-6a), 1.85 (2 H, m, 1 × H-4 and 1 × H-5), 2.05 (2 H, m, H-7′ or -7 and H-3), 2.15 (3 H, s, 9-CH_3), 2.45 and 2.60 (2 H, AB m, H-8 and -8′), 2.95 (1 H, apparent pentuplet, H-2), 6.08 (1 H, d, J = 1 Hz, H-10). ^{13}C NMR: δ 11.5 (q, 2-CH_3), 20.0 (q, 6-CH_3). 20.9 (t, C-7), 28.5 (t, C-4 or -5), 30.0 (q, 9-CH_3), 35.4 (t, C-5 or -4), 36.6 (d, C-2), 41.2 (t, C-8), 41.7 (d, C-6), 42.7 (d, C-3), 47.2 (d, C-6a), 124.4 (s, C-10a), 131.9 (d, C-10), 171.6 (s, C-1), 208.4 (s, C-9).

Dihydro-*epi*-deoxyarteannuin B (6). This was identified by comparison of its ^1H NMR with that of material prepared by sodium borohydride reduction of *epi*-deoxyarteannuin B[23] according to the literature procedure.[21]

Sesquiterpene Lactone 7. Oil, EIMS: *m/e* 236 (0.5, M^+), 192 (18, M – CO_2), 163 (100). IR: max (neat) 2924, 2870, 1734, 1445, 1376, 1197, 1176, 1152, 1100, 1040 cm^{-1}. ^1H NMR: δ 0.86 (3 H, d, J = 6 Hz, 6- or 9-CH_3), 1.07 (3 H, d, J = 6 Hz, 9- or 6-CH_3), 1.20 (3 H, d, J = 7 Hz, 2-CH_3), 0.9–1.9 (m's), 2.69 (1 H, apparent pent, J ca. 7 Hz, H-2), 4.12 (1 H, t, J = 10.5 Hz, H-10). ^{13}C NMR: δ 13.4 (q, 2-CH_3), 18.2 (q, 6- or 9-CH_3), 19.9 (q, 9- or 6-CH_3), 23.0 (t), 27.0 (t), 28.3 (d), 28.8 (t), 35.1 (t), 38.7 (d), 39.8 (d), 40.4 (d, C-2), 43.3 (d), 43.9 (d), 81.2 (d, C-10), 175.0 (s, C-1).

Sesquiterpene Lactone 8. Oil, MS: This compound showed considerable decomposition under GC/MS conditions. However, the major GC peak showed the following EI peaks: *m/e* 204 (11, M – HCO_2H), 109 (100), CI(NH_3) *m/e* 268 (100, M + NH_4^+). IR (neat): 2924, 2873, 1766, 1651, 1446, 1382, 1258, 1154, 1136, 1116, 1086, 1023, 993, 900 cm^{-1}. ^1H NMR: δ 0.87 (3 H, d, J = 6 Hz, 6-CH_3), 1.21 (3 H, d, J = 7 Hz, 2-CH_3), 0.7–2.1 (m's), 3.53 (1 H, q, J = 7 Hz, H-2), 4.83 and 4.90 (2 H, s's, 9=CH_2), 5.03 (1 H, d, J = 11.8 Hz, H-10). ^{13}C NMR: δ 15.6 (q, 2-CH_3), 19.7 (q, 6-CH_3), 22.7 (t, C-4 or -5), 28.7 (d and t superimposed), 30.7 (t, C-8), 35.5 (t, C-5 or -4), 40.3 (d, C-2), 43.3 (d, C-3 or 6a), 45.2 (d, C-6a or 3), 50.9 (d, C-10a), 84.8 (d, C-10), 106.6 (t, 9=CH_2), 145.4 (s, C-9), 180.1 (s, C-1).

Effect of Water on the Conversion of 2 to 3. An ether solution of the hydroperoxide 2 was dried for 1 h over 3-Å molecular sieves. Two aliquots, each containing ca. 20 mg of 2, were withdrawn, and the ether was removed from each in a stream of argon. Cyclohexane-d_{12} (0.5 mL) and trifluoroacetic acid (2 drops) were added to each, and 20 μL of D_2O was added to one. After purging with oxygen, the solutions were allowed to stand in air for 3 days. The cyclohexane-d_{12} was replaced with $CDCl_3$. ^1H NMR of the D_2O-containing mixture showed the clear presence of artemisinin, while the mixture without D_2O contained no artemisinin by ^1H NMR.

Keto–Acid 5. This compound was prepared as described for the "enol lactone" in ref 18. Flash chromatography (8:1 $CHCl_3$/MeOH, silica gel) resulted in a 35% yield of an oil which could be crystallized from cold ether/petroleum ether as off-white crystals, mp 152–155 °C. Found: C, 71.80; H, 8.79. IR: max ($CHCl_3$) 1709, 1659 cm^{-1} [lit.[18] 1705, 1650 cm^{-1}]. IR: max (KBr) ca. 3500–3000 (COOH), 1728, 1639, 1167 cm^{-1}. ^1H NMR: δ 0.88 (3 H, d, J = 6-CH_3), ca. 1.0 (1 H, hidden, H-6), 0.98, 1.04 (2 H, apparent AB, J = 12 Hz, H-4,5 β or H-4,5 α), 1.26 (3 H, d, J = 7 Hz, 2-CH_3), 1.63 (2 H, m, H-4,5 α or H-4,5 β), 1.84 (2 H, m, H-3, H-6a), 2.33 (3 H, s, 9-CH_3), 2.55–2.50 (3 H, m, H-2, H-7), 3.10 (1 H, br s, H-10a), 6.67 (1 H, br s, $W_{1/2}$ = 5 Hz, H-10), 10.15 (1 H, br, COOH); lit. for "4"[18] 2.4 (3 H, s), 3.19 (1 H, m), 6.72 (1 H, br s). ^{13}C NMR: δ 15.4 (q, 2-CH_3), 20.6 (q, 6-CH_3), 26.4 (q, 9-CH_3), 28.3 (t, C-4 or -5), 32.0 (d, C-6), 33.4 (t, C-5 or -4), 35.5 (t, C-7), 40.2 (d, C-3 or -6a), 44.3 (d, C-2), 46.7 (d, C-6a or -3), 47.7 (d, C-10a), 143.0 (d, C-10), 147.0 (s, C-8), 182.7 (s, C-1), 197.2 (s, C-9). UV (95% ethanol): λ_{max} 246 nm (ϵ 1.08 × 10^4), 223 nm (ϵ 5.8 × 10^3).

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