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The mechanism of the spontaneous autoxidation of dihydroartemisinic acid

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Abstract—Dihydroartemisinic acid undergoes slow spontaneous autoxidation to artemisinin and other natural products, which have been reported from the medicinal plant *Artemisia annua*. The mechanism of this complex transformation is shown to involve four steps: (i) initial reaction of the $\Delta^{4,5}$-double bond of dihydroartemisinic acid with molecular oxygen, (ii) Hock cleavage of the resulting tertiary allylic hydroperoxide; (iii) oxygenation of the enol product from Hock cleavage; and (iv) cyclization of the resulting vicinal hydroperoxyl-aldehyde to the 1,2,4-trioxane system of artemisinin.

1. Introduction

It has been known for some time that the 1,2,4-trioxane ring[1,2] in the natural product artemisinin (**1**) from the medicinal plant *Artemisia annua* is responsible for the biological activity of this increasingly important anti-malarial drug. It has been shown both by ourselves[3,4] and by others[5] that the natural product dihydroartemisinic acid (**2**) from this same species can undergo spontaneous autoxidation either as a solid or in organic solution yielding artemisinin (**1**) as well as other natural products which have been reported from *A. annua* (compounds **6**–**8** in Fig. 1). These reactions are slow but seem to proceed in the absence of photosensitizer, which would be required by classical theory for the generation of singlet oxygen ($^1O_2$).

Recently, we have proposed a mechanism[6] for the spontaneous formation of the 1,2,4-trioxane ring of **1** in vitro which begins with the oxygenation of the double bond in **2** to yield the tertiary allylic hydroperoxide **3**. Compound **3** then undergoes Hock cleavage, leading to the enolic intermediate **4**, which reacts with molecular oxygen to form the vicinal hydroperoxyl-aldehyde **5**. The reaction terminates when compound **5** undergoes cyclization to artemisinin (Scheme 1). This proposal was based on previous experimental evidence which had shown that essentially the same mechanism was involved in the formation of peroxofabianane, a very close analog of artemisinin, from the natural product 4-amorphen-11-ol found in *Fabiana imbricata*.[7]

It is interesting to note that, without exception, all chemical syntheses of the 1,2,4-trioxane system in **1** have also involved ring-closure from a hydroperoxyl-aldehyde precursor identical or equivalent in structure to that of compound **5** shown in Scheme 1, which is in turn always derived from oxygenation of an enol identical or equivalent in structure to that of compound **4**.[8–16] In some cases,

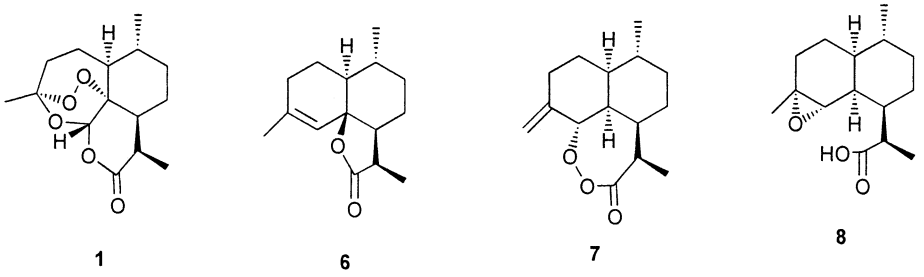

Figure 1. Products of the spontaneous autoxidation of dihydroartemisinic acid (**2**) which have been reported in the literature.

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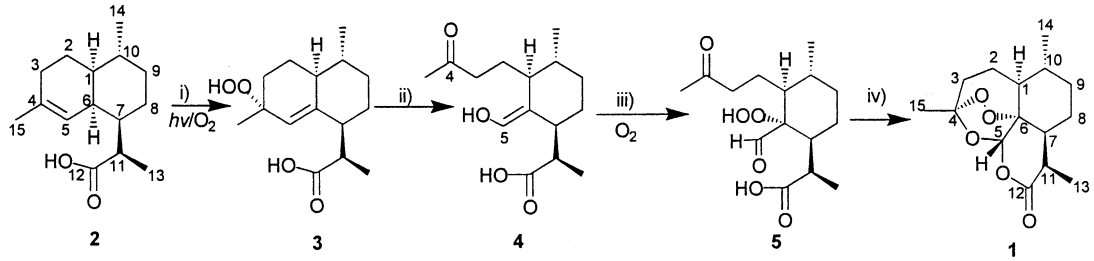


Scheme 1. Proposed mechanism for the spontaneous conversion of dihydroartemisinic acid (**2**) to artemisinin (**1**) via (i) spontaneous autoxidation to the tertiary allylic hydroperoxide **3**; (ii) Hock cleavage of **3** to an enol **4**; (iii) autoxidation of enol **4** to a hydroperoxyl-aldehyde **5**; and (iv) closure of the 1,2,4-trioxane ring.

dihydroartemisinic acid (**2**) itself (or its derivatives) has been converted directly into **1** by photo-oxygenation (presumably via the presumed intermediate **4**).[17–19] Haynes and Vonwiller[20–22] have undertaken studies of the mechanism for the sensitized photo-oxygenation and subsequent Cu^{II}-catalyzed rearrangements of the tertiary allylic hydroperoxide which is derived from arteannuic acid, the 11,13-dehydro analog of **2** (arteannuic acid is also a natural product from *A. annua*). Their results for the chemically induced transformations of arteannuic acid favor the mechanism which we propose for the spontaneous autoxidation of dihydroartemisinic acid (**2**) shown in Scheme 1. However, Roth and Acton[23–25] have also independently studied this same chemically induced transformation of **2**, and although they also found that a tertiary allylic hydroperoxide was involved, they have arrived at a quite different mechanism for carbon–carbon cleavage to that proposed by Haynes and Vonwiller.

In this study we set out to determine whether the mechanism shown in Scheme 1 for the remarkable *spontaneous* transformation of dihydroartemisinic acid (**2**) to artemisinin (**1**) is correct. The answer to this question is important since it may have a bearing on the biogenesis of compound **1** in vivo. In this regard, we note that Pras et al.[26] have recently isolated the tertiary allylic hydroperoxide of dihydroartemisinic acid (compound **3** in Scheme 1) as a natural product from *A. annua*. These authors suggested that this compound may be formed in vivo by oxidation of dihydroartemisinic acid and that it is then transformed into artemisinin, although they presented no direct experimental evidence to support this. The same authors have even suggested that dihydroartemisinic acid might be a natural anti-oxidant in this species.[27]

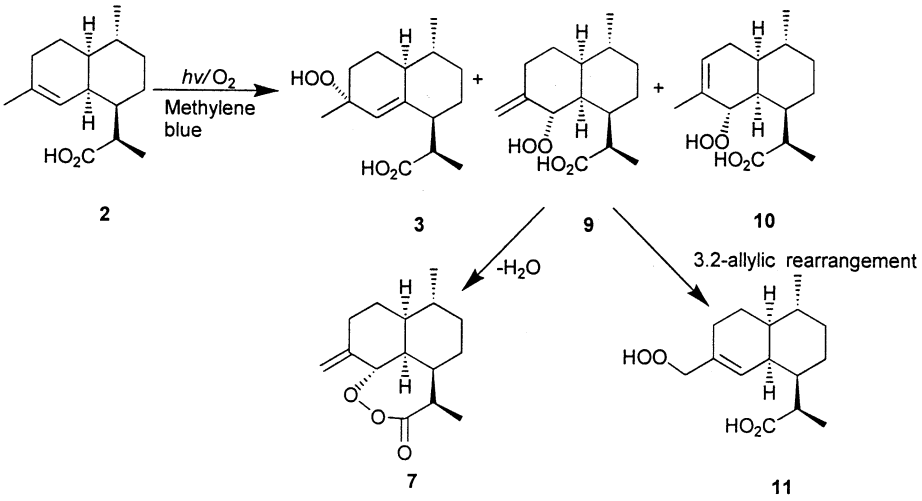
2. Results and discussion

2.1. The first spontaneous autoxidation of dihydroartemisinic acid (2)

A $CDCl_3$ solution of dihydroartemisinic acid (**2**) (isolated from *A. annua*) was kept in an NMR tube under laboratory conditions for several weeks and ^1H NMR spectra were recorded at intervals of every few days. Several new peaks began to appear within a few days in these ^1H NMR spectra and after five weeks the starting material accounted for only around half of the mixture. Although these spectra were complex, some peaks could be identified by comparison with ^1H NMR data reported in the literature, as belonging to the following natural products which have been isolated previously from *A. annua*: artemisinin (**1**),[28] dihydroartemisinic acid tertiary hydroperoxide (**3**),[3,26] dihydro-*epi*-deoxyarteannuin B (**6**)[3,29] and arteannuin H (**7**).[3,6] In particular, it was apparent that peaks belonging to compound **3** appeared early on in the spontaneous transformation of **2** in $CDCl_3$ solution and then declined in intensity, whilst peaks corresponding to compound **1** continued to grow steadily with time. Such behavior is entirely consistent with the role suggested in Scheme 1 for **3** as an intermediate derived from the spontaneous autoxidation of **2** en route to **1**.

From other precedents in the literature,[3,4,6] we propose that compound **6** is formed by S_N2' attack of the carboxylic acid group at the allylic hydroperoxide resulting in elimination of hydrogen peroxide from **3**. The natural product **7** is almost certainly derived from an alternative secondary allylic hydroperoxide product (compound **9** in Scheme 2), which is also produced by spontaneous oxygenation of the $\Delta^{4,5}$-double bond in **2**, but which does not accumulate itself under the conditions of reaction, and is instead trapped by the 12-carboxylic acid group (Scheme 2).[6] Good evidence for this came from the detection of very small peaks in the ^1H NMR spectrum of the mixture corresponding to trace amounts of the primary allylic hydroperoxide **11**, which has been reported previously as a product from the 3,2-allylic rearrangement of **9** that competes with the lactonization reaction involved in the formation of **7** in $CDCl_3$ solution.[6] Several peaks also appeared in the extreme downfield region of the ^1H NMR spectrum (9–12 ppm) but could not be assigned by comparison with the literature. Some of these peaks appeared early on in the experiment, and disappeared towards the end of the experiment, when the conversion of **2** into **1** was complete, and they may represent intermediates in the cyclization of **5** to **1** (see later).

It is interesting to note that the spontaneous formation of all of compounds **1**, **6** and **7** was significantly accelerated in samples of **2** contaminated by stearic acid (which is difficult to separate from dihydroartemisinic acid from the natural source—see Ref. 3). The rates of transformations were in general slower for more concentrated solutions of **2**, which may be due to the rate of dissolution of oxygen in the NMR tube becoming a limiting factor.[30] In support of this, we found that signals due to compound **2** generally disappeared completely from ^1H NMR spectra within a few weeks when



Scheme 2. Products **3**, **9** and **10** from photo-oxygenation of compound **2** and further transformations of the secondary allylic hydroperoxide **9** in $CDCl_3$ which are reported in the literature.[6]

$CDCl_3$ solutions were left in open vessels rather than NMR tubes. No transformations were observed when NMR solutions of **2** were kept in the dark indicating that the initial conversion of **1** into **3** requires light and therefore almost certainly involves singlet oxygen ($^1O_2$).

In confirmation of the suggested intermediacy of **3** in the spontaneous transformations of **2**, it was found that when a pure sample of this tertiary allylic hydroperoxide (obtained by HPLC purification of the crude product from photo-oxygenation of **2**—see Scheme 2 and Ref. 6) was left in a solution of $CDCl_3$ for several days, the formation of compounds **1** and **6** was again noted in ^1H NMR spectra (Fig. 2) and the starting material disappeared within 1–2 weeks. Several other resonances were observed in these spectra in addition to those associated with compounds **1**, **3** and **6**. In particular, a very obvious resonance appeared at δ_H 9.93 (d, J=2.5 Hz) within a few hours of the start of the experiment, reached a maximum after 3 days, and then gradually declined in intensity (Fig. 2). We suspect that this resonance corresponds to the aldehyde proton (H-5) of the vicinal hydroperoxyl-aldehyde intermediate **5** shown in Scheme 1 (an aliphatic resonance at δ_H 2.16 (3H, s) ppm for the H-15 methyl group was also clearly seen and followed the same pattern of change in intensity with time). However, this assignment must remain tentative since we were unable to isolate this compound by chromatography. Additional strongly downfield resonances (δ_H 10.19 (1H, dd, J=1.5, 1.5 Hz), 9.77 (s) and 9.75 (s); not shown in Fig. 2) also reached a maximum intensity after 3–5 days (later than the maximum for the resonance at δ_H 9.93 ppm, which was tentatively assigned to **5**) when the conversion of **3** was ca. 50% complete and then declined. These resonances may correspond to more advanced intermediates in the cyclization of **5** to **1**, although once again this could not be confirmed because none of these components could be

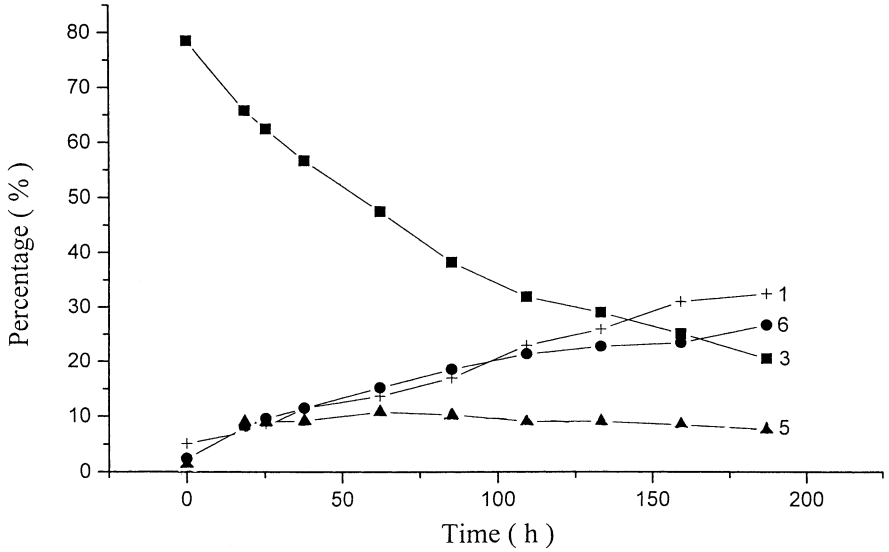
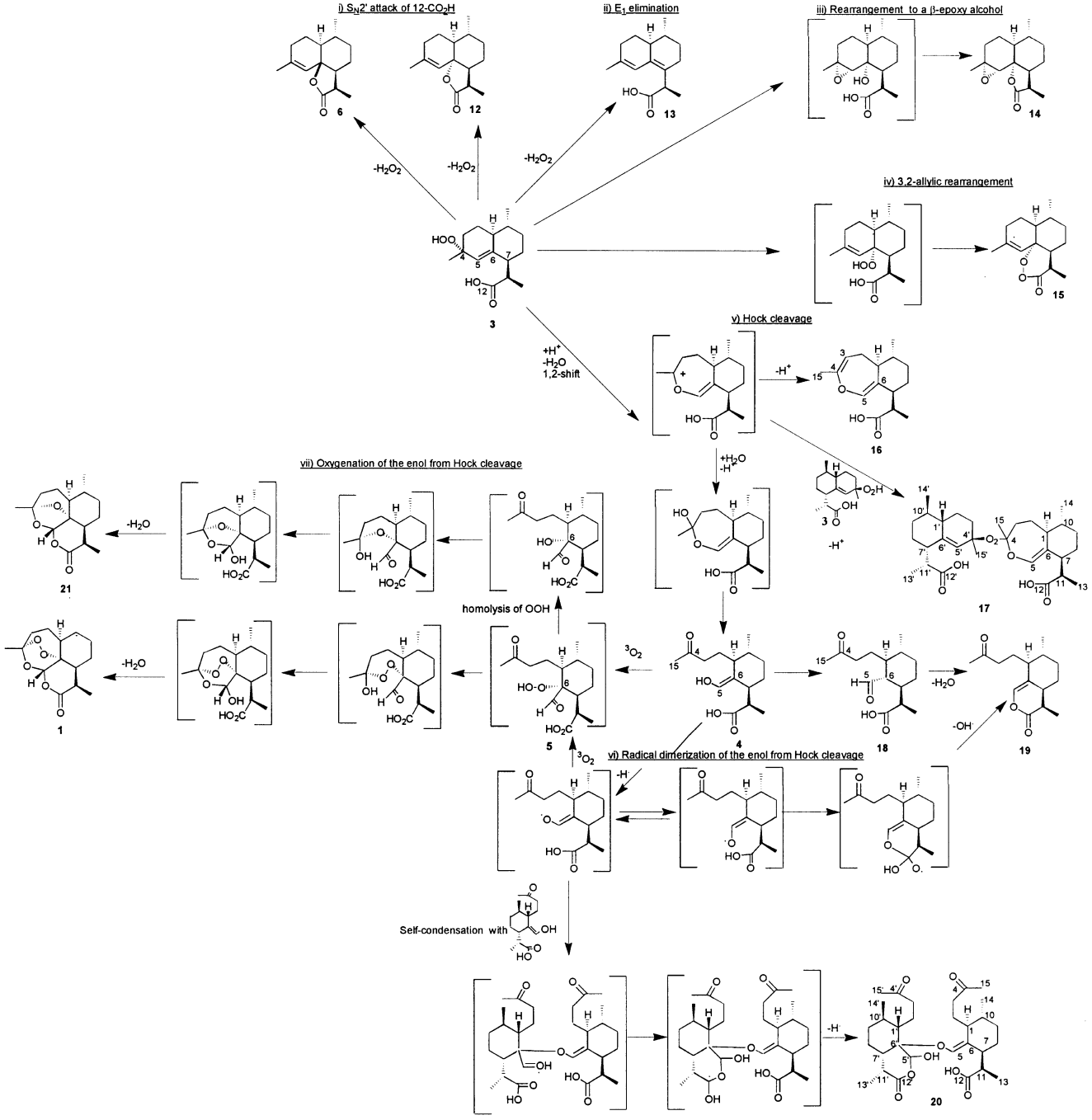


Figure 2. Spontaneous transformations of the tertiary allylic hydroperoxide of dihydroartemisinic acid (**3**) into compounds **1** and **6** in $CDCl_3$ solution, via vicinal hydroperoxyl aldehyde **5** (tentative identification only). The percentage of each of these components in the mixture was estimated from integration of clearly resolved characteristic resonances for each compound in the alkene region of ^1H NMR spectra acquired at each time point: compound **1** (δ_H 5.86, s, H-5); compound **3** (δ_H 5.25, s, H-5); compound **6** (δ_H 5.64, s, H-5); compound **5** (δ_H 9.93, d, J=2.5 Hz, H-5)—tentative identification only.



Scheme 3. Proposed transformation pathways (i)–(vii) for the allylic hydroperoxide **3** in $CDCl_3$.

isolated by chromatography. The presence or absence of light had no effect on these further transformations of **3**, and it seems that the second autoxidation reaction required for the spontaneous formation of **1** therefore involves $^3O_2$ rather than $^1O_2$ (see also Section 2.3).

Clearly both the spontaneous autoxidation of **2** to **3** and the subsequent rearrangement reactions of **3** in organic solution (as well as those of the alternative secondary allylic hydroperoxide autoxidation product, compound **9**) are complex processes, producing a variety of products in addition to compounds **1** and **6**. When trifluoroacetic acid (TFA) was added to a petroleum ether solution of **3** (cf. Ref. 7), the transformations of this allylic hydroperoxide were rapid and simplified; after the starting material had disappeared, only two products were isolated, compound **1** (43%) and compound **6** (5%). Such facile conversion of **3** into **1** in the presence of strong acid is also in agreement with the findings of others.[25]

2.2. Spontaneous Hock cleavage of tertiary allylic hydroperoxide (3)

In order to further investigate the mechanism for the carbon–carbon cleavage reaction at C-4/C-5 which is clearly required for the spontaneous transformation of

Table 1. ^{13}C NMR data for novel compounds **4**, **12** and **15**–**25** in $CDCl_3$

Position	**4**[a]	**12**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	Position	**17**	**20**
1	36.8	46.7	50.0	47.3	52.0	42.3	47.3	38.5	44.5	46.1	45.2	43.8	46.1	1′	44.4	47.1
2	28.1	20.1	21.6	26.3	23.8	26.7	20.9	28.0	22.0	24.4	23.0	23.9	24.7	2′	25.9	21.6
3	40.8	26.1	26.7	106.2	33.0	38.1	41.2	41.2	33.9	41.0	41.0	38.6	41.3	3′	32.0	43.5
4	214.6	142.3	123.5	155.9	109.6	208.3	208.3	210.3	109.2	208.6	209.1	210.0	209.0	4′	80.7	208.8
5	140.2	120.5	134.8	136.7	131.2	205.9	131.9	133.3	99.6	206.1	104.3	181.4	184.5	5′	123.0	97.1
6	111.9	86.7	–[b]	124.7	130.1	56.1	124.6	119.4	82.4	49.9	43.1	48.1	45.5	6′	137.0	80.1
7	40.3	49.8	49.7	45.9	43.7	41.0	42.7	41.5	42.3	43.1	40.5	41.4	44.0	7′	43.3	44.4
8	23.1	22.0	21.5	33.2	32.9	23.5	28.6	24.0	23.5	27.3	23.1	31.1	23.7	8′	32.1	23.2
9	22.8	35.3	34.6	34.8	36.1	34.7	35.5	22.9	33.4	35.5	34.9	34.7	35.6	9′	35.2	34.5
10	30.6	30.7	31.1	37.4	39.3	33.4	41.7	30.3	35.3	33.4	32.8	33.5	31.2	10′	39.8	35.1
11	41.9	39.0	38.1	41.1	41.8	41.2	36.6	41.4	32.7	42.9	40.8	43.4	43.5	11′	42.1	32.7
12	184.7	180.6	181.2	179.2	183.0	177.4	171.6	179.0	171.9	181.0	173.8	181.4	181.6	12′	183.4	172.2
13	17.0	13.2	12.7	16.3	9.7	14.4	11.6	17.2	12.6	15.1	13.2	14.5	16.3	13′	9.3	12.7
14	20.0	19.9	18.5	18.8	19.4	19.6	20.0	19.9	18.6	20.2	20.2	19.7	20.3	14′	20.0	21.2
15	30.6	24.1	20.9	20.7	23.9	30.0	30.1	30.6	23.9	29.7	29.4	29.9	30.0	15′	24.6	29.7
5-OMe	–	–	–	–	–	–	–	–	–	–	56.4	–				–

[a] δ values recorded at 233 K in $CDCl_3$/TFA solution.
[b] Not resolved.

tertiary allylic hydroperoxide **3** into compound **1** in organic solution, we attempted to prevent the occurrence of the next step, the second autoxidation reaction shown in Scheme 1 (which forms compound **5**), by preparing a $CDCl_3$ solution of **3** under an atmosphere of nitrogen. Changes observed with time in the ^1H NMR spectra obtained from this experiment bore some parallels with the results summarized in Fig. 2 (e.g. the formation of compound **6** was once again clearly evident), although rather unexpectedly there was now a considerable degree of additional complexity associated with the exclusion of molecular oxygen from the system, even though a single hydroperoxide was employed as the starting material. When all the resonances corresponding to the starting material (**3**) had disappeared from these ^1H NMR spectra, the mixture was separated by HPLC and 12 products could then be characterized by 2D NMR (compounds **1**, **6** and **12**–**21**; Scheme 3 and Tables 1 and 2). These compounds have been grouped together in Scheme 3 according to the mechanisms which, we believe, are involved in their formation. We have identified seven distinct pathways for the transformations of the tertiary allylic hydroperoxide **3** in this Scheme 3: (i) S_N2' attack of the 12-carboxylic acid group, resulting in the elimination of hydrogen peroxide (forming **6** and **12**); (ii) E_1 elimination of the hydroperoxide group and H-7 (forming **13**); (iii) rearrangement to a β-epoxy alcohol (forming **14**); (iv) 3,2-allylic rearrangement (forming **15**); (v) Hock cleavage and tautomerization of the resultant enol (forming **16**–**19**); (vi) Hock cleavage and radical dimerization of the resultant enol (forming **20**); and (vii) oxygenation of the enol from Hock cleavage (forming **1** and **21**).

Although, the seventh pathway is clearly the dominant one for the transformation of compound **3** in the presence of oxygen (resulting predominantly in conversion to **1**, as discussed in Section 2.1) it is much less significant in this experiment—in which compound **18**, believed to be formed by Hock cleavage and tautomerization of the resultant enol **4** to an aldehyde in the absence of molecular oxygen (pathway v) in Scheme 3), was found as the predominant product. Given that great care was taken to exclude atmospheric oxygen from the $CDCl_3$ solution it was a little surprising

Table 2. ^1H NMR data for novel compounds **4**, **12** and **15**–**25** in $CDCl_3$

Position	**4**[a]	**12**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	Position	**17**	**20**
1	2.21	1.53	1.75	1.79	1.58	1.46	1.68	2.34	1.28	1.25	1.17	1.47	1.15	1′	1.57	1.56
2α	1.61	2.03	1.92	2.57[b]	1.74[b]	1.56	2.06	1.78	1.92	2.40	1.97	1.82	2.03	2α′	1.17	1.82
2β	1.61	1.80	1.72	2.04[b]	1.48[b]	1.22	1.49	1.56	1.28	2.02	1.21	1.58	1.32	2β′	2.05	2.10
3α	2.57	2.03	1.81	4.91	1.64[b]	2.32	2.45	2.60	1.63	2.66	2.75	2.46	2.73	3α′	1.53	2.52
3β	2.68	1.93	2.35	–	1.72[b]	2.45	2.63	2.48	1.78	2.66	2.33	2.33	2.38	3β′	1.24	2.79
5	6.31	5.53	5.79	6.07	5.88	9.58	6.08	6.19	5.70	9.97	5.02	–	–	5′	5.47	5.69
6	–	–	–	–	–	2.36	–	–	–	2.45	2.16	2.21	2.88	6′	–	–
7	2.15	2.19	2.12	2.04	2.64	1.93	2.16	2.23	2.00	1.81	1.73	2.42	1.69	7′	2.74	1.92
8α	1.77[b]	1.68	1.80	1.85	1.63	1.87	1.83	1.79	1.92	1.67	1.67	1.66	1.97	8α′	1.41	1.25
8β	1.34[b]	1.44	1.61	1.22	1.44	1.47	1.10	1.41	1.01	1.30	1.03	1.38	1.54	8β′	1.59	1.89
9α	1.28[b]	1.16	1.19	1.17	1.19	1.11	1.19	1.24	1.09	1.19	1.03	1.19	0.97	9α′	1.75	1.88
9β	1.83[b]	1.82	1.90	1.77	1.78	1.81	1.86	1.91	1.81	1.90	1.78	1.74	1.78	9β′	1.24	1.13
10	1.74	1.55	2.05	1.57	1.45	1.27	1.24	1.73	1.28	1.65	1.34	1.19	1.80	10′	1.05	1.64
11	2.62	2.74	2.63	2.58	2.67	2.48	2.95	2.75	3.18	2.41	2.61	2.51	2.49	11′	2.58	2.74
13	1.12	1.35	1.32	1.26	1.19	1.18	1.22	1.15	1.20	1.23	1.21	1.19	1.29	13′	1.23	1.12
14	0.93	0.96	0.94	0.87	0.89	0.94	0.99	0.86	0.94	0.98	0.97	0.92	0.90	14′	0.92	1.07
15	2.32	1.70	1.60	1.77	1.43	2.14	2.16	2.18	1.53	2.15	2.14	2.14	2.16	15′	1.30	2.07
5-OMe	–	–	–	–	–	–	–	–	–	–	3.46	–	–			

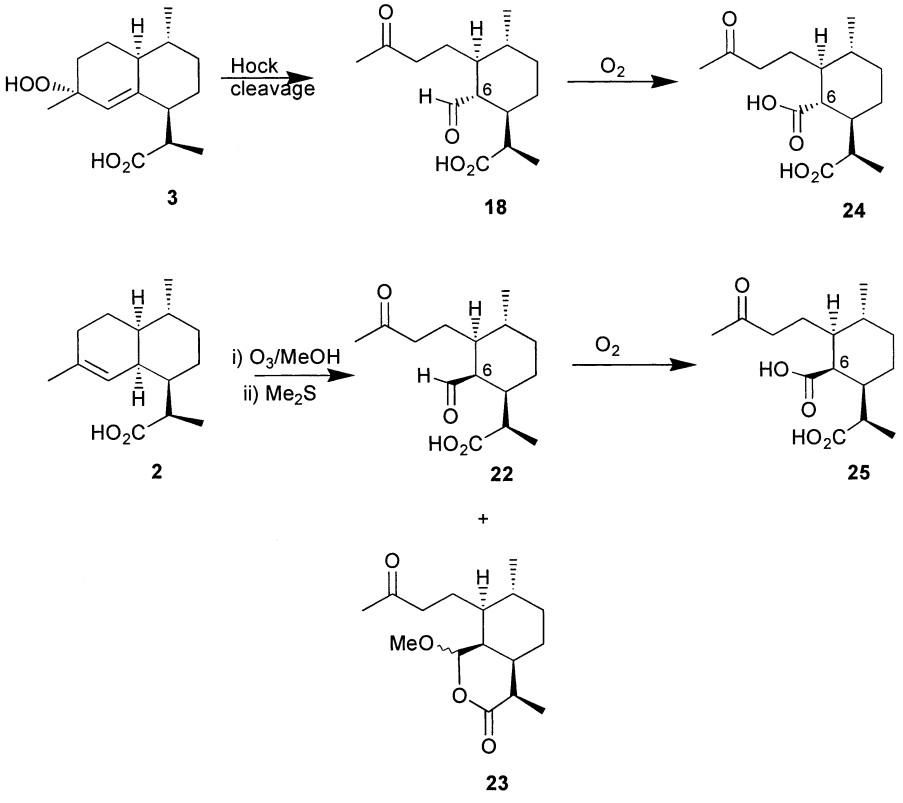
[a] δ values recorded at 233 K in $CDCl_3$/TFA solution.
[b] α and β assignments uncertain (due to extensive chemical exchange in NOESY spectra, which are dominated by EXSY-type peaks in the case of **4**).

that compounds such as **1** and **21** (from pathway vii) in Scheme 3) were isolated at all: we believe that the small amounts of these compounds obtained are the result of the occurrence of oxidation by hydrogen peroxide, which is formed in solution as a by-product from the S_N2' and E_1 pathways, rather than by molecular oxygen.

Although the complex nature of the varying transformations undergone by **3** in this experiment were unexpected (and undesired), they are of considerable interest because several of the reaction products have themselves been reported as natural products from *A. annua* (note that **3** is also itself claimed as a natural product).[26,27] These include the lactones dihydro-*epi*-deoxyarteannuin B (**6**)[3,29] and dihydro-deoxyarteannuin B (**12**).[31] 6,7-Dehydro-dihydroartemisinic acid (**13**) is the 11,13-dihydro analog of the natural product 6,7-dehydroartemisinic acid from this species.[32,33] We have recently provided experimental evidence from the 4-hydroxyl analog of compound **3**[4] for both of the mechanisms proposed in the formation of these natural product/natural product analogs—i.e. S_N2' attack of the 12-carboxylic acid group at the 6-position of the allylic hydroperoxide which forms both **6** and **12**; and E_1 elimination of the allylic hydroperoxyl group with H-7 which is responsible for forming diene **13**. Similar results have been obtained from photo-oxygenation of arteannuic acid, the 11,13-dehydro analog of **2**.[34]

Dihydroarteannuin B (**14**) has also been described as a natural product from *A. annua*[3,35]—the mechanism proposed for its formation from **3**, by rearrangement of the tertiary allylic hydroperoxide group to a β-epoxy alcohol, has been well documented in the oxidation chemistry of fatty acids,[36] and we have also recently isolated the β-epoxy alcohol, which is now proposed to be an intermediate in pathway (iii) of Scheme 3, as a natural product from this species (unpublished results). In further support of this mechanism, we note that a more abundant natural product from *A. annua*, arteannuin B (the 11,13-dehydro analog of compound **14**), has previously been obtained, together with *epi*-deoxyarteannuin B (the 11,13-dehydro analog of **6**), from photo-oxygenation of arteannuic acid (the 11,13-dehydro analog of **2**).[37]

The remaining novel compounds **15**–**20**, which were isolated from transformations of **3** in organic solution in the absence of molecular oxygen have not, as yet, been reported as natural products from *A. annua*. Evidence for the mechanism proposed for the formation of the lactone endoperoxide **15** by 3,2-allylic rearrangement and subsequent 'trapping' of the rearranged allylic hydroperoxide by the 12-carboxylic acid group comes from close analogies with our recently reported biomimetic synthesis of arteannuin H (**7**) from secondary allylic hydroperoxide **9** (Scheme 2).[6] There are several precedents in the literature[20–22] to suggest that the C-4/C-5 carbon–carbon bond scission which has occurred in all of the products **16**–**20** is the result of Hock cleavage of the tertiary allylic hydroperoxide **3**, as shown in pathway (v) of Scheme 3. The structure of compound **16** is particularly revealing as it is only easily accounted for by the elimination of H^+ from the tertiary carbocation which is expected to be formed from the 1,2-shift of the $\Delta^{5,6}$-double bond of **3** to the internal oxygen atom of the hydroperoxide (with the accompanying loss of



Scheme 4. Confirmation of the stereochemistry for the 6-aldehyde substituent in compound **18** produced from Hock cleavage of **3**, by ozonolysis of compound **2** which yielded the epimeric aldehyde **22**. Both 6-aldehydes undergo slow spontaneous autoxidation to carboxylic acids, not 1,2,4-trioxanes.

the external oxygen atom as water) which is the initial step in Hock cleavage. Nucleophilic attack at this cation by a second molecule of **3**, instead of the elimination of H^+, would then explain the formation of the dimer **17**. Alternatively, re-addition of water to this same carbocation would result in the enol **4**, which is the starting point for the further transformations shown in pathways (v)–(vii) of Scheme 3. Although not isolated in this experiment, the existence of compound **4** as the immediate product of Hock cleavage might be inferred from the formation of all of the products **18**–**20**. Thus, the enol functional group in proposed intermediate **4** has clearly undergone tautomerization to an aldehyde in compound **18** which was the major reaction product isolated from this experiment. The stereochemistry of the aldehyde group in **18** was proven to be 6α-by correlations observed in NOESY sepctroscopy (following assignment of all ^1H and ^{13}C resonances by the 2D NMR experiments HSQC, HMBC and ^1H–^1H COSY, as for all other novel compounds reported herein). Ozonolysis of dihydroartemisinic acid (**2**) (Scheme 4) yielded the epimeric 6β-aldehyde, compound **22**, which had substantially different NMR spectra to those of **18** (Tables 1 and 2), particularly in the vicinity of the 6-aldehyde substituent, thereby tending to confirm this assignment.[†] Enol lactone **19** may be formed either by attack of the 12-carboxylic acid at the aldehyde group in **18** with ensuing loss of water from a hemi-acetal intermediate, or it may be the result of geometrical isomerism of the *E*-enolic double bond in compound **4** to the *Z*-form, perhaps occurring by a radical process as shown in pathway (vi), prior to lactonization.

Dimer **20** may also be derived from such a radical, which attacks the enolic double bond of a second molecule of **4**, as shown in the self-condensation pathway (vi) in Scheme 3. Obtaining confirmation of the molecular weight of dimers **17** and **20** by mass spectroscopy was difficult, as they fragmented very easily under all ionization techniques which were available (in addition, compound **17** was also unstable when purified and decomposed to deoxyartemisinin (**21**) in a matter of days under normal laboratory conditions). However, the presence of a correlation between the two halves of the molecule in the HMBC spectrum of **20** (from δ_C 80.1 (C-6′) ppm to δ_H 6.19 (H-5) ppm) clearly showed that it was a dimer. In addition, we were able to use diffusion ordered NMR spectroscopy (DOSY) to confirm that the molecular weight for **20** was approximately twice that of the starting material **3**.[38,39]

With the benefit of hindsight, resonances corresponding to all of the compounds **1**, **6** and **12**–**21** now isolated by HPLC from the various possible reactions of **3** when left to stand in $CDCl_3$ solution under an atmosphere of nitrogen, could now be identified in the ^1H NMR spectra which had been acquired over the three-week period of the reaction (e.g. δ_H 9.58 (br) and 2.14 (3H, s) for the 5-aldehyde and 15-methyl groups of compound **18**, which accounted for more than 50% of the final crude reaction product by ^1H NMR). With the exception of dimers **17** and **20**, all these characteristic resonances were seen to grow steadily in intensity with time, confirming that all of compounds **1**, **6**, **12**–**16**, **18**, **19** and **21** probably represent end-points in the varied transformations of **3**, and that only **17** and **20** are intermediates. Having thus obtained good evidence that Hock cleavage is the mechanism by which spontaneous cleavage occurs at C-4/C-5 in **3** (from the isolation of compounds **16** and **17**) and some indications for the transient formation of the enol intermediate **4** which would be expected from such a Hock cleavage mechanism (by the isolation of end products **18** and **19**), we next set out to obtain more direct evidence for the intermediacy of compound **4**, which is proposed to undergo a second autoxidation reaction in the transformation of **3** into **1** in Scheme 1.

2.3. The second spontaneous autoxidation of enolic intermediate 4

When TFA was added to a $CDCl_3$ solution of **3** maintained under an atmosphere of nitrogen at room temperature there was an almost immediate conversion into a single product, the enol lactone **19**, which was proposed to be derived via Hock cleavage of intermediate **4** in pathways (v)/(vi) of Scheme 3. Based on this finding, we reasoned that it might be possible to actually characterize the presumed enol intermediate **4**, before it became trapped by the 12-carboxylic acid group as a lactone in compound **19**, and without interference from the plethora of other products which can be formed by alternative elimination, rearrangement, dimerization and oxygenation reactions of the hydroperoxide **3** (i.e. pathways (i)–(iv) and (vii) in Scheme 3), by using this same treatment under more controlled conditions. There were some precedents in the literature for this expectation from the work of Haynes and Vonwiller who had previously obtained evidence from 1D ^1H NMR spectra to support the existence of the 11,13-dehydro 12-methyl ester analog of **4**, which was reported to be unexpectedly stable at low temperature.[20,22]

Gratifyingly, when performed under an atmosphere of nitrogen at 233 K, treatment of a $CDCl_3$ solution of tertiary allylic hydroperoxide **3** with TFA did indeed result in conversion into a single novel compound (contaminated by small amounts of the expectable side-products **1**, **6** and **19**) in less than 30 min as observed by ^1H NMR (Fig. 3). When kept in the bore of the magnet of the NMR spectrometer at low temperature under nitrogen, the $CDCl_3$ solution of this compound was stable for several hours, permitting its characterization by the 2D NMR experiments HSQC, HMBC and ^1H–^1H COSY. This data unambiguously indicated the planar structure of enol **4**, although the stereochemistry of this compound, in particular the geometry of the enolic double bond, could not be determined from nuclear Overhauser enhancements, as the NOESY spectrum of **4** was dominated by positive exchange-type (EXSY) cross-peaks,[40] making observation of through-space connections from negative nOe correlation cross-peaks impossible. The *E*-geometry depicted for **4** in Schemes 1, 3 and 5 is therefore only an assumption based on the expected mechanism for the Hock cleavage reaction.

When the temperature of the solution was raised to 298 K, whilst maintaining an atmosphere of nitrogen, compound **4** was converted predominantly into the enol lactone **19**, as

[†] Compound **23** was also isolated as a minor product from ozonolysis.

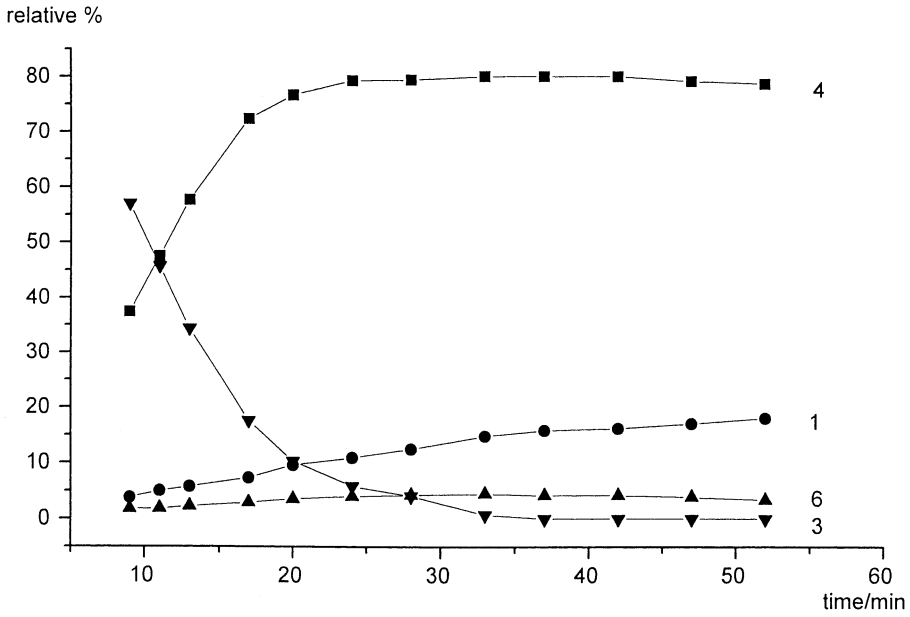
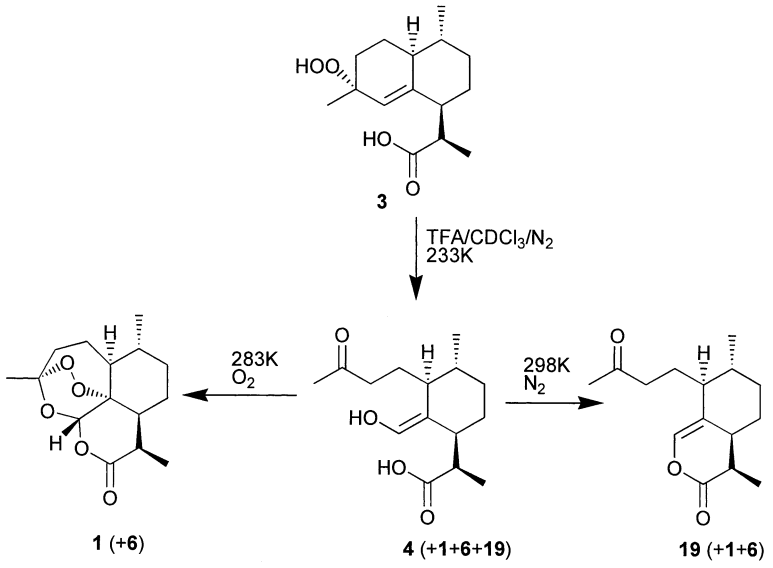


Figure 3. Percentage of compounds **1**, **3**, **4** and **6** (estimated by integration of characteristic alkene resonances in ^{1}H NMR) as a function of time following treatment of a $CDCl_3$ solution of **3** with TFA at 233 K under an atmosphere of nitrogen.

expected (smaller quantities of compounds **1** and **6** were also isolated by HPLC). The reaction was completed within 1 h as shown by ^{1}H NMR spectra of the $CDCl_3$/TFA solution, which were acquired at intervals of every few minutes. By contrast, when the temperature was raised to 283 K, whilst at the same time allowing oxygen in the atmosphere access to the solution, there was instead an almost quantitative conversion of enol **4** into artemisinin (**1**) (Scheme 5) within a few hours. This conversion of **4** into **1** is presumably a radical process involving $^3O_2$ (pathway (vii) in Scheme 3) since the reaction could be studied in the darkened environment of the bore of the magnet of an NMR spectrometer (cf. comments in the first section regarding the spontaneous transformation of **3** into **1** in $CDCl_3$ solution which was unaffected by light).

It is worth pointing out that although the Hock cleavage product **4** has thus been shown to be extremely susceptible to spontaneous autoxidation by molecular oxygen, yielding essentially only a single product, artemisinin (**1**), its aldehyde tautomer, compound **18**, was much less reactive towards oxygen and was not converted to a 1,2,4-trioxane ring system in organic solution. Instead, a solution of this 6α-aldehyde in $CDCl_3$ underwent spontaneous autoxidation to the corresponding 6α-carboxylic acid, compound **24** only after several weeks at room temperature and with no detectable formation of artemisinin. The same was true of the 6β-aldehyde **22**, obtained from ozonolysis of dihydroartemisinic acid, which underwent slow oxidation to the corresponding 6β-carboxylic acid, compound **25** (Scheme 4).



Scheme 5. Differing transformations of enol **4**, obtained from Hock cleavage of **3**, under atmospheres which either contain or exclude oxygen.

3. Conclusion

Based on the preceding experimental results: (i) dihydroartemisinic acid (**2**) undergoes spontaneous autoxidation to the tertiary allylic hydroperoxide **3** in $CDCl_3$ solution; (ii) this allylic hydroperoxide in turn undergoes transformations into a wide variety of products, several of which appear to be derived from the transient enol intermediate **4**, which is expected from Hock Cleavage; (iii) it was actually possible to characterize compound **4** by 2D NMR at low temperature and in the absence of molecular oxygen, following treatment of **3** with acid; and (iv) compound **4** was converted rapidly and exclusively into artemisinin (**1**) in the presence of molecular oxygen; we believe that we now have good evidence to support the mechanism for the spontaneous autoxidation of **2** to **1** which has been suggested in Scheme 1. Based on our experience with the spontaneous autoxidation of other natural products,[30] we suspected that the proximity of the 12-carboxylic acid group to the $\Delta^{4,5}$-double bond in **2** might be responsible for the apparent ease of this complex series of transformations. This hypothesis is explored in the companion paper.

4. Experimental

4.1. General

All novel compounds reported were fully characterized by the 2D NMR experiments HSQC, HMBC, ^1H–^1H COSY and NOESY. Chemical shifts are expressed in ppm (δ) relative to TMS as internal standard. Proton chemical shifts, multiplicities, coupling constants and integral reported in this section are those which are clearly resolved in 1D ^1H NMR without recourse to 2D NMR analysis (see Tables in the main text for full assignments by 2D NMR). All NMR experiments were run on a Bruker DRX 500 instrument. NMR experiments under an atmosphere of nitrogen were performed using 5 mm NMR tubes equipped with a teflon valve which isolates the NMR tube from the atmosphere (J. Young, 528-VL-7). HSQC, HMBC ^1H–^1H COSY and NOESY spectra were recorded with 1024 data points in F_2 and 256 data points in F_1. High-resolution MS were recorded in EI mode at 70 eV on a Finnigan—MAT 95 MS spectrometer. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8201 PC instrument. Column chromatography was performed using silica gel 60–200 μm (Merck). HPLC separations were performed using a Varian chromatograph equipped with RI star 9040 and UV 9050 detectors and a Prep-Sil 20 mm×25 cm column, flow rate 8 ml/min. Optical rotations were measured by a Perkin–Elmer 343 polarimeter (Na 589 nm). $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} and $CHCl_3$ was used as a solvent.

4.2. Spontaneous transformations of dihydroartemisinic acid 2 in $CDCl_3$ solution

Samples of dihydroartemisinic acid were obtained from *A. annua* plants as described previously.[3] A solution of dihydroartemisinic acid (**2**) (1 mg) in $CDCl_3$ (0.6 ml) was left in an NMR tube under laboratory conditions and ^1H NMR spectra were acquired every 3–7 days over a period of 2 months. The disappearance of **2** was monitored by measuring the decrease in the integral for the H-5 resonance (δ_H 5.11 ppm).[3] Formation of natural products **1**, **3**, **6** and **7** was detected by identification of characteristic peaks associated with these products in the ^1H NMR spectra of the mixture.

Compound **1**:[28] δ_H 5.86 (1H, s, H-5), 3.40 (1H, dq, J=12.6, 7.2 Hz, H-11) ppm.

Compound **3**:[3,26] δ_H 5.25 (1H, s, H-5), 2.73 (1H, dq, J=7.0, 7.0 Hz, H-11) ppm.

Compound **6**:[3,29] δ_H 5.64 (1H, s, H-5), 3.15 (1H, dq, J=6.7, 6.7 Hz, H-11) ppm.

Compound **7**:[3,6] δ_H 5.02 (1H, d, J=11.0 Hz, H-5), 4.91 (1H, s, H-15a/b), 4.84 ppm (1H, s, H-15a/b).

Peaks at δ_H 4.38 (m), 4.35 (m) and 5.56 (s) ppm were identified as belonging, respectively, to H-15a/H-15b and H-5 of the primary allylic hydroperoxide **11** formed by 3,2-allylic rearrangement of secondary allylic hydroperoxide **9** (not detected—but see Ref. 6).

4.3. Spontaneous transformations of dihydroartemisinic acid tertiary hydroperoxide 3 in $CDCl_3$ solution

Dihydroartemisinic acid tertiary allylic hydroperoxide (**3**) was prepared by photo-oxygenation of dihydroartemisinic acid (**2**)—see Ref. 6 for experimental details and purification. A solution of **3** (1 mg) in $CDCl_3$ (0.6 ml) was left in an NMR tube under laboratory conditions and ^1H NMR spectra were acquired once or twice a day over a period of two weeks. Formation of known compounds **1** and **6** was confirmed by identification of characteristic peaks associated with these products in the ^1H NMR spectra of the mixture as above. A minor peak at δ_H 6.12 ppm seen in some experiments was assigned to the H-5 proton of compound **13b**,[4] and a more significant peak at δ_H 8.02 ppm was assigned to formaldehyde (possibly formed as a by-product of Hock cleavage reactions of **5**).

4.4. Spontaneous transformations of the tertiary allylic hydroperoxide 3 in $CDCl_3$ solution under an atmosphere of nitrogen

Dissolved oxygen was removed from a solution of **3** (30 mg) in $CDCl_3$ (1 ml) in a NMR tube equipped with a valve by the freeze–thaw method. The sample was left under an atmosphere of N_2 under laboratory conditions and ^1H NMR spectra were acquired every one or two days over a period of three weeks. Formation of compounds **1** and **6** was confirmed by identification of characteristic peaks associated with these products in the ^1H NMR spectra of the mixture, as earlier. The mixture was separated by repeated HPLC after 3 weeks.

Compound **1**:[28] (1.6 mg, 5%; R_t 41.7 min, 15% EtOAc/*n*-hexane).

Compound **6**:[3,29] oil. (1.2 mg, 4%; R_t 29.8 min, 15% EtOAc/*n*-hexane).

Compound **12**:[31] oil. (2.1 mg, 6%; R_t 10.2 min, 50% EtOAc/*n*-hexane; R_t 35.1 min, 15% EtOAc/*n*-hexane). IR ν_{max} ($CHCl_3$): 3026, 2934, 2874, 1751, 1456 cm^{-1}; ^1H NMR δ ($CDCl_3$) ppm: 5.53 (1H, s), 2.74 (1H, dq, *J*=9.0, 8.0 Hz), 2.19 (1H, ddd, *J*=12.5, 9.0, 2.5 Hz), 1.70 (3H, s), 1.35 (3H, d, *J*=8.0 Hz), 0.96 (3H, d, *J*=6.2 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 234.1613 [M^+, $C_{15}H_{22}O_2$ requires 234.1620] (19), 219 (4), 190 (91), 161 (100).

Compound **13**:[4] oil. (0.9 mg, 3%; R_t 11.5 min, 50% EtOAc/*n*-hexane; R_t 21.0 min, 15% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 6.13 (1H, s, H-5), 3.84 (1H, q, *J*=6.9 Hz, H-11), 1.79 (3H, s, H-15), 1.23 (3H, d, *J*=6.9 Hz, H-13), 0.99 (3H, d, *J*=6.0 Hz, H-14); HREIMS *m/z* (rel. int.) 234.1614 [M^+, $C_{15}H_{22}O_2$ requires 234.1620] (44), 189 (30), 161 (100).

Compound **14**:[3,35] oil. (2.4 mg, 8%; R_t 18.0 min, 50% EtOAc/*n*-hexane; R_t 28.8 min, 15% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 3.02 (1H, s, H-5), 2.77 (1H, dq, *J*=7.5, 7.8 Hz, H-11), 2.25 (1H, ddd, *J*=13.4, 7.5, 2.9 Hz, H-7), 1.38 (3H, d, *J*=7.8 Hz, H-13), 1.36 (3H, s, H-15), 0.96 (3H, d, *J*=6.6 Hz, H-14); ^{13}C NMR: 179.5 (C, C-12), 82.9 (C, C-6), 59.5 (CH, C-5), 58.1 (C, C-4), 50.2 (CH, C-7), 45.6 (CH, C-1), 38.6 (CH, C-11), 34.8 (CH_2, C-9), 30.5 (CH, C-10), 24.1 (CH_2, C-3), 23.1 (CH_3, C-15), 21.6 (CH_2, C-8), 18.6 (CH_3, C-14), 16.3 (CH_2, C-2), 12.6 (CH_3, C-13).

Compound **15**: oil. (0.9 mg, 3%; R_t 9.2 min, 50% EtOAc/*n*-hexane; R_t 13.1 min, 15% EtOAc/*n*-hexane). IR ν_{max} ($CHCl_3$) 3013, 2927, 2854, 1759 cm^{-1}; ^1H NMR δ ($CDCl_3$) ppm: 5.79 (1H, s), 2.63 (1H, dq, *J*=8.4, 7.7 Hz), 2.35 (1H, m), 2.12 (1H, ddd, *J*=12.8, 8.4, 3.4 Hz), 2.05 (1H, m), 1.60 (3H, s), 1.32 (3H, d, *J*=7.7 Hz), 0.94 (3H, d, *J*=6.6 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 250.1571 [M^+, $C_{15}H_{22}O_3$ requires 250.1569] (11), 222 (8), 192 (2), 179 (100), 151 (26); CIMS: 251 [M+1] (100), 233 (34), 223 (11), 205 (35).

Compound **16**: oil. (1.8 mg, 6%; R_t 14.2 min, 50% EtOAc/*n*-hexane; R_t 22.0 min, 15% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 6.07 (1H, s), 4.91 (1H, dd, *J*=6.8, 6.8 Hz), 2.58 (2H, m), 1.77 (3H, s), 1.26 (3H, d, *J*=6.8 Hz), 0.87 (3H, d, *J*=6.4 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 250.1569 [M^+, $C_{15}H_{22}O_3$ requires 250.1569] (35), 232 (4), 177 (100), 159 (58), 133 (70).

Compound **17**: oil. (1.0 mg, 3%; R_t 11.1 min, 50% EtOAc/*n*-hexane; R_t 17.3 min, 15% EtOAc/*n*-hexane). The sample decomposed to **21** after purification under laboratory conditions within a few days. ^1H NMR δ ($CDCl_3$) ppm: 5.88 (1H, s), 5.47 (1H, s), 2.74 (1H, m), 2.67–2.64 (2H, m), 2.58 (1H, dq, *J*=4.4, 7.1 Hz), 1.43 (3H, s), 1.30 (3H, s), 1.23 (3H, d, *J*=7.1 Hz), 1.19 (3H, d, *J*=7.0 Hz), 0.92 (3H, d, *J*=6.4 Hz), 0.89 (3H, d, *J*=7.0 Hz)—see also Table 2; ^{13}C NMR: see Table 1; CIMS: 477 (100), 281 (52).

Compound **18**: oil. (9.1 mg, 30%; R_t 30.8 min, 50% EtOAc/*n*-hexane; R_t 68.0 min, 30% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 9.58 (1H, d, *J*=4.9 Hz), 2.14 (3H, s), 1.18 (3H, d, *J*=6.9 Hz), 0.94 (3H, d, *J*=6.4 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 250.1570 [M^+-H_2O, $C_{15}H_{22}O_3$ requires 250.1569] (22), 232 (23), 222 (7), 192 (16), 167 (36), 149 (100); CIMS: 269 [M+1] (35), 251 (28), 233 (100), 205 (52).

Compound **19**: oil. (2.3 mg, 8%; R_t 16.6 min, 50% EtOAc/*n*-hexane; R_t 60.0 min, 22% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 6.08 (1H, s), 2.95 (1H, dq, *J*=7.2, 7.0 Hz), 2.63 (1H, ddd, *J*=17.8, 9.2, 4.8 Hz), 2.45 (1H, ddd, *J*=17.8, 8.5, 8.5 Hz), 2.16 (3H, s), 1.22 (3H, d, *J*=7.0 Hz), 0.99 (3H, d, *J*=6.1 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 250.1575 [M^+, $C_{15}H_{22}O_3$ requires 250.1569] (3), 232 (3), 211 (1), 192 (100).

Compound **20**: oil. (1.0 mg, 3%; R_t 42.3 min, 50% EtOAc/*n*-hexane; R_t 42.5 min, 15% EtOAc/*n*-hexane). IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2930, 2856, 1740, 1715 cm^{-1}; ^1H NMR δ ($CDCl_3$) ppm: 6.19 (1H, s), 5.92 (1H, d, *J*=13.2 Hz, 5′-OH), 5.69 (1H, d, *J*=13.2 Hz), 2.18 (3H, s), 2.07 (3H, s), 1.15 (3H, d, *J*=6.9 Hz), 1.12 (3H, d, *J*=7.2 Hz), 1.07 (3H, d, *J*=6.3 Hz), 0.86 (3H, d, *J*=7.1 Hz)—see also Table 2; ^{13}C NMR: see Table 1; CIMS: *m/z* (rel. int.) 515 [M+1] (62), 281 (100).

Compound **21**:[28,41] oil. (1.4 mg, 4%; R_t 10.8 min, 50% EtOAc/*n*-hexane; R_t 12.3 min, 30% EtOAc/*n*-hexane); ^1H NMR δ ($CDCl_3$) ppm: 5.70 (1H, s), 3.18 (1H, dq, *J*=12.1, 7.2 Hz), 2.00 (1H, ddd, *J*=12.1, 4.4, 4.4 Hz), 1.53 (3H, s), 1.20 (3H, d, *J*=7.2 Hz), 0.94 (3H, d, *J*=5.6 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 266.1517 [M^+, $C_{15}H_{22}O_4$ requires 266.1518] (7), 224 (28), 195 (18), 165 (100), 151 (60).

4.5. Ozonolysis of dihydroartemisinic acid 2

A cooled (−78°) solution of dihydroartemisinic acid (**2**) (57 mg, 0.24 mmol) in CH_2Cl_2/CH_3OH (1:1, 40 ml) was subjected to ozonolysis to give an oil (52 mg, 90% w/w), which was separated by HPLC (35% EtOAc/*n*-hexane).

Compound **22**: oil. (20 mg, 0.07 mmol, 31%; R_t 31.0 min). $[\alpha]_D$=−46.7 (*c* 0.57, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2936, 2855, 1706 cm^{-1}; ^1H NMR δ ($CDCl_3$) ppm: 9.97 (1H, br s), 2.67 (2H, br s), 2.15 (3H, s), 1.23 (3H, d, *J*=7.0 Hz), 0.98 (3H, d, *J*=6.3 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 250.1574 [M^+-H_2O, $C_{15}H_{22}O_3$ requires 250.1569] (3), 227 (31), 209 (100), 181 (100).

Compound **23**: oil. (5 mg, 0.02 mmol, 7%; R_t 23.5 min); ^1H NMR δ ($CDCl_3$) ppm: 5.02 (1H, d, *J*=6.4 Hz), 3.46 (3H, s), 2.75 (1H, ddd, *J*=16.9, 9.6, 3.8 Hz), 2.61 (1H, dq, *J*=3.8, 7.1 Hz), 2.33 (1H, ddd, *J*=16.9, 8.0, 5.2 Hz), 2.14 (3H, s), 1.21 (3H, d, *J*=7.1 Hz), 0.97 (3H, d, *J*=6.4 Hz)—see also Table 2; ^{13}C NMR: see Table 1.

4.6. Conversion of a $CDCl_3$ solution of 3 into enol lactone 19 by treatment with TFA under an atmosphere of nitrogen

Dissolved oxygen was removed from a solution of **3** (10 mg) in $CDCl_3$ (0.6 ml) as earlier and TFA (2 μl) was

added at room temperature. Analysis by ^{1}H NMR indicated the complete transformation of **3** into **19** in less than 5 min (the time than it took to lock, shim and acquire a ^{1}H NMR spectrum).

4.7. Characterization of enol intermediate 4 from treatment of 3 with TFA under an atmosphere of nitrogen

Dissolved oxygen was removed from a solution of **3** (25 mg, 0.09 mmol) in $CDCl_3$ (1 ml) as earlier and TFA (ca. 1 μl) was added to a just-thawed solution. The NMR tube was immediately transferred to the NMR spectrometer (probe-head pre-cooled to 233 K) and formation of compound **4** was complete within 30 min as determined by ^{1}H NMR (disappearance of δ_H 5.21 ppm (H-5) for the starting material). Some ^{13}C resonances of products were assigned by 2D NMR (all δ_C and δ_H values are in $CDCl_3$/TFA at 233 K (ppm)).

Compound **4**: δ_H 6.31 (1H, s), 2.68–2.57 (3H, m), 2.32 (3H, s), 1.12 (3H, d, J=5.9 Hz), 0.93 (3H, d, J=6.9 Hz)—see also Table 2; almost all ^{1}H resonances showed positive EXSY-type correlations in NOESY spectra: e.g. δ_H 6.31 (H-5) correlated with 2.15 (H-7), 1.12 (H-13) ppm etc.; ^{13}C NMR: see Table 1.

Compound **1**: δ_H 5.95 (1H, s, H-5), 3.46 (1H, dq, J=7.0, 7.0 Hz, H-11), 1.49 (3H, s, H-15); ^{13}C NMR 105.7 (C, C-4), 94.1 (CH, C-5), 79.5 (C, C-6), 32.8 (C-11), 12.6 (CH_3, C-13).

Compound **6**: δ_H 5.67 (1H, s, H-5), 3.29 (1H, dq, J=7.0, 6.9 Hz, H-11), 1.72 (3H, s, H-15), 1.18 (3H, d, J=6.9 Hz, H-13); ^{13}C NMR 182.0 (C, C-12), 143.4 (C, C-4), 120.9 (CH, C-5), 42.5 (C-7), 40.2 (C-11), 30.4 (C-3), 23.8 (C-8), 9.5 (CH_3, C-13).

Compound **19**: δ_H 6.08 (1H, s, H-5), 3.02 (1H, dq, J=7.0, 7.0 Hz, H-11), 2.17 (3H, s, H-15), 1.25 (3H, d, J=7.0 Hz, H-13), 1.00 (3H, d, J=6.8 Hz, H-14); ^{13}C NMR 212.4 (C, C-4), 173.4 (C, C-12), 131.4 (CH, C-5), 124.9 (C, C-6), 46.8 (CH, C-1), 36.3 (CH, C-11), 28.5 (C-8), 11.7 (CH_3, C-13).

*Further conversion of **4** in the presence of O_2 at 283 K or under an atmosphere of nitrogen at 298 K*: the valve of the NMR tube was opened to admit O_2 or kept closed to maintain an atmosphere of N_2 as the temperature of the probe-head was raised.

4.8. Compounds 24 and 25 from spontaneous autoxidation of aldehydes 18 and 22 in $CDCl_3$ solution

Compound **24**: Oil. (R_t 30.9 min, 50% EtOAc/*n*-hexane); ^{1}H NMR δ ($CDCl_3$) ppm: 2.14 (3H, s), 1.19 (3H, d, J=7.2 Hz), 0.92 (3H, d, J=5.7 Hz)—see also Table 2; ^{13}C NMR: see Table 1; CIMS: 285 [M+1] (40), 267 (100), 221 (46).

Compound **25**: Oil. $[\alpha]_D$=−36.5 (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2930, 1709, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 2.88 (1H, br), 2.73 (1H, m), 2.16 (3H, s), 1.29 (3H, d, J=8.4 Hz), 0.90 (3H, d, J=6.2 Hz)—see also Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 266.1510 [M^+-H_2O, $C_{15}H_{22}O_4$ requires 266.1518] (4), 248 (19), 227 (23), 209 (60), 181 (100).

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