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The role of the 12-carboxylic acid group in the spontaneous autoxidation of dihydroartemisinic acid

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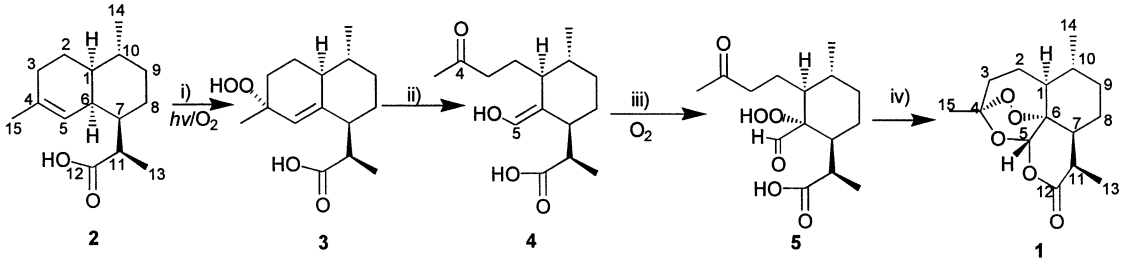
Abstract—Three of the four steps in the slow spontaneous autoxidation of dihydroartemisinic acid to artemisinin ('ene-type' reaction of molecular oxygen with the $\Delta^{4,5}$ double bond, Hock cleavage of the resulting tertiary allylic hydroperoxide, oxygenation of the enol product from Hock cleavage and cyclization of the resulting vicinal hydroperoxyl-aldehyde to the 1,2,4-trioxane system of artemisinin) are shown to be assisted by the proximity of the 12-carboxylic acid functional group in dihydroartemisinic acid to the functional groups participating in these reactions.

1. Introduction

It has previously been shown that the natural product dihydroartemisinic acid[1] (**2**) from *Artemisia annua* L. undergoes spontaneous autoxidation to the important anti-malarial drug, artemisinin (**1**),[2] as well as to other natural products which have been reported from this species. In the preceding paper,[3] we have presented experimental evidence that the mechanism for the slow spontaneous transformation of **2** into **1** involves initial oxygenation of the $\Delta^{4,5}$ double bond in dihydroartemisinic acid (**2**) yielding the tertiary allylic hydroperoxide **3**, which then undergoes Hock cleavage leading to the enolic intermediate **4**. This enol is highly susceptible to autoxidation by a second molecule of oxygen resulting in the presumed vicinal hydroperoxyl-aldehyde intermediate **5**, which finally undergoes cyclization to the 1,2,4-trioxane ring of artemisinin (**1**) (Scheme 1).

In this study we set out to explain why it is that such a



Scheme 1. 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, which was established in the preceding paper.[3]

Keywords: terpenes and terpenoids; autoxidation; mechanism; biomimetic reactions.

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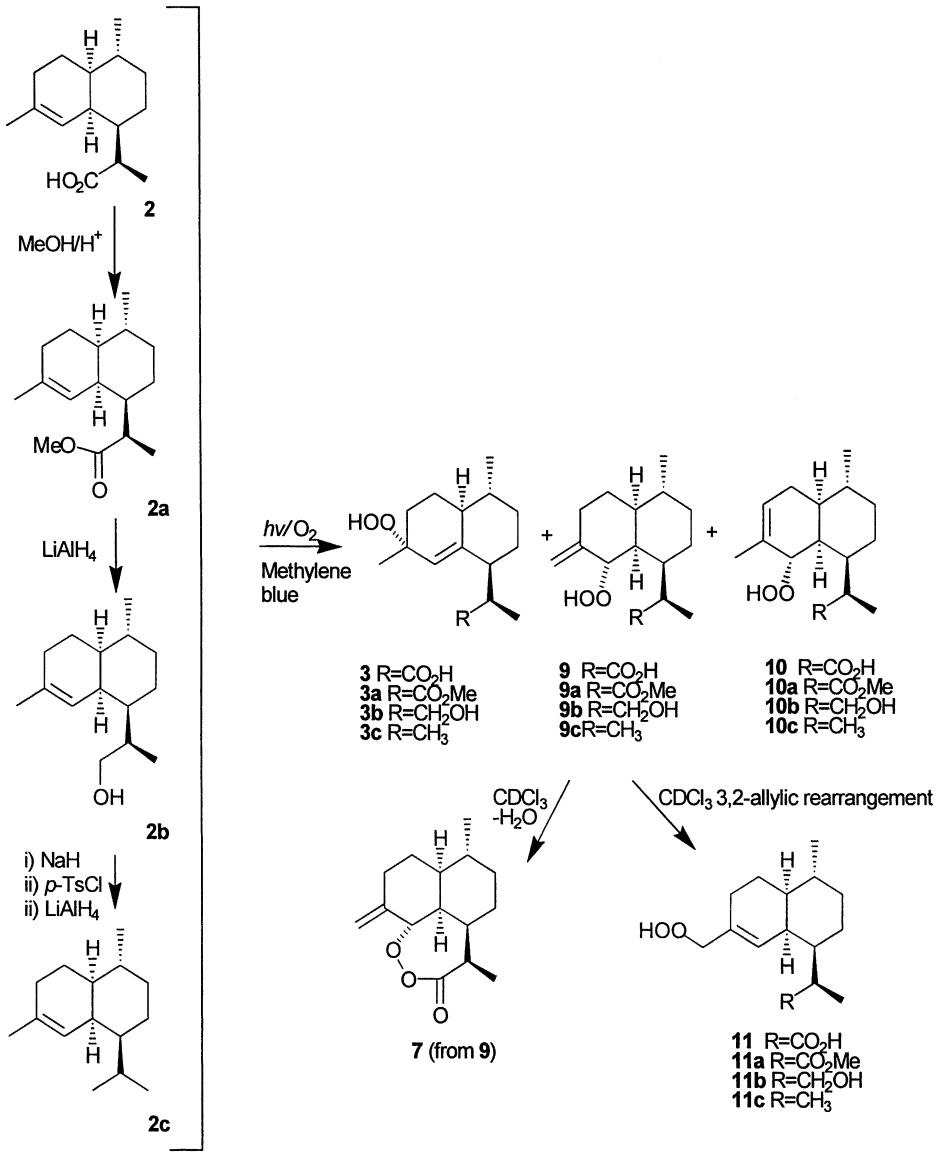


Scheme 2. Modification of the 12-functional group in dihydroartemisinic acid (**2**) yielding derivatives **2a**–**2c**, photo-oxygenation of **2** and **2a**–**2c** and further transformations of secondary allylic hydroperoxides in $CDCl_3$ which have been reported in the literature.[12]

complex series of reactions can apparently proceed so readily in vitro without the need for specific chemical reagents. The answer to this question is, we believe, important for understanding the autoxidation chemistry of tri-substituted double bonds in nature. Tri-substituted double bonds are particularly common in the terpenoid family of natural products, as a result of the way in which their carbon skeletons are constructed by the enzymes involved in terpenoid biosynthesis.[4] We believe that a significant number of highly oxygenated terpenoids which are reported as natural products may, in fact, be products of spontaneous autoxidation reactions of terpenoid precursors containing such double bonds, and we have recently published several examples[5–11] which present circumstantial evidence to illustrate what we believe to be a general principle. The elucidation of the precise mechanism, based on experimental evidence, for the spontaneous transformation of **2** into the important anti-malarial natural product **1** in the preceding paper[3] is thus an important step in substantiating this hypothesis.

2. Results and discussion

From our previous work in the spontaneous autoxidation chemistry of terpenoid natural products[11] we suspected that the 12-carboxylic acid group in dihydroartemisinic acid (**2**), lying in close proximity to the $\Delta^{4,5}$ double bond, might be responsible for assisting some or all of the four reactions which have now been shown to be involved in the spontaneous transformation of **2** into **1**.[3] In order to test this hypothesis, we have modified this functional group in dihydroartemisinic acid. The 12-carboxylic acid group in **2** was converted first to a methyl ester in compound **2a**, then to a primary alcohol in compound **2b** and, finally, it was fully reduced to a methyl substituent in compound **2c**,

as has been described previously[12] (Scheme 2).[†] The influence of the 12-carboxylic acid group on each of the four steps involved in the spontaneous conversion of **2** into **1** which are shown in Scheme 1, i.e. (i) first autoxidation; (ii) Hock cleavage; (iii) second autoxidation; and (iv) 1,2,4-trioxane ring closure, was then investigated by subjecting each of compounds **2a**–**2c** to a similar series of experiments to those which have been described in the preceding paper.[3]

2.1. The first spontaneous autoxidation of the $\Delta^{4,5}$ double bond in 2 requires the presence of oxygen at the 12-position

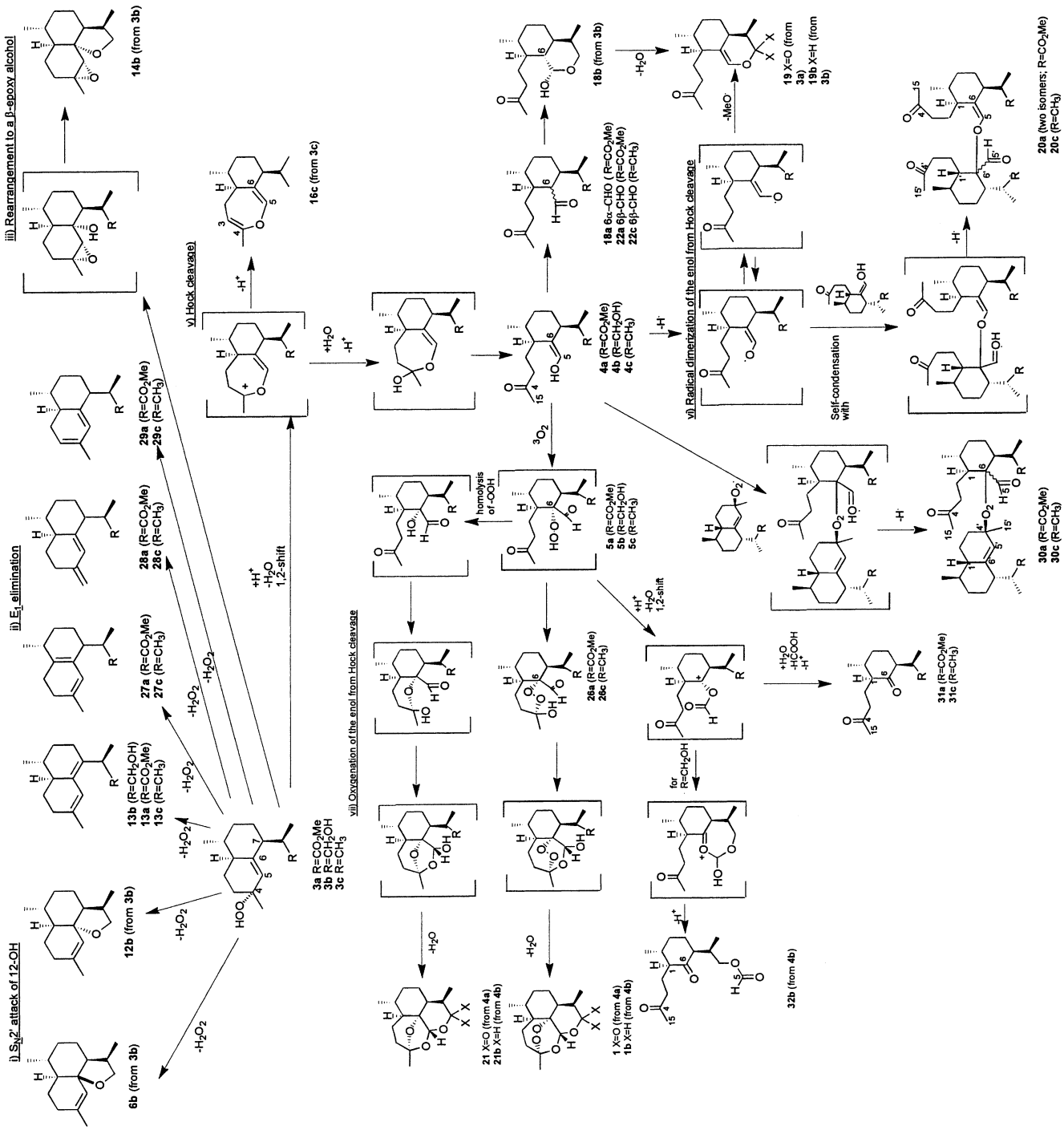
Dilute $CDCl_3$ solutions of each of the derivatives **2a**–**2c** were prepared in NMR tubes and left under laboratory conditions so as to assess their propensity towards spontaneous autoxidation in organic medium. ^{1}H NMR spectra were recorded at intervals of every few days and the spontaneous transformations of each derivative of dihydroartemisinic acid were studied by making a comparison of the chemical shifts and multiplicities of peaks appearing in these spectra with ^{1}H NMR data reported in the literature. It was found that the $\Delta^{4,5}$ double bond in both the methyl ester **2a** and the primary alcohol **2b** was susceptible to spontaneous autoxidation, as has been observed for compound **2** itself in the preceding paper.[3] Peaks corresponding to the tertiary allylic hydroperoxides **3a** and **3b** appeared in the ^{1}H NMR spectra recorded from samples **2a** and **2b** after a few weeks in $CDCl_3$ solution, indicating a rate of spontaneous autoxidation for **2a** and **2b** which was comparable to that observed for **2** itself.[3] Resonances corresponding to small amounts of the alternative secondary allylic hydroperoxide oxidation products, compounds **9a** and **9b** (see Scheme 2 for structures), which are also expected from the 'ene-type' reaction of oxygen with the double bond in **2a** and **2b**, could also be identified by comparison with the literature.[12]

After several weeks, resonances corresponding to primary allylic hydroperoxide **11a** formed from 3,2-allylic rearrangement of **9a**,[12] also appeared in the ^{1}H NMR spectra of compound **2a**, replacing the resonances for **9a** (cf. results for **2** in the preceding paper).[3] In addition, compound **3a** also appeared to undergo slow conversion to **26a**[13] (pathway (vii) in Scheme 3), which is a partially cyclized intermediate en route to the 1,2,4-trioxane ring of artemisinin (**1**) (this contrasts with the much more rapid direct conversion of **3** into **1** described in the preceding study of the autoxidation of dihydroartemisinic acid (**2**)).[3] Products of alternative transformations of **3a**, such as the diene **13a**[13] (pathway ii) in Scheme 3), were also noted in the ^{1}H NMR spectra for **2a**. By contrast, ^{1}H NMR spectra recorded for the spontaneous autoxidation of compound **2b** in $CDCl_3$ solution were considerably simpler than those for **2a**: at the end of the experiment (after two months), the tertiary allylic hydroperoxide autoxidation product **3b** was the dominant species in this experiment, together with small peaks corresponding to compound **11b** (the primary allylic hydroperoxide product from 3,2-allylic rearrangement of the secondary allylic hydroperoxide **9b**). No peaks were seen for products of further reactions of the tertiary allylic hydroperoxide **3b**, indicating that the rates of subsequent transformations for **3b** were slow as compared to its formation.

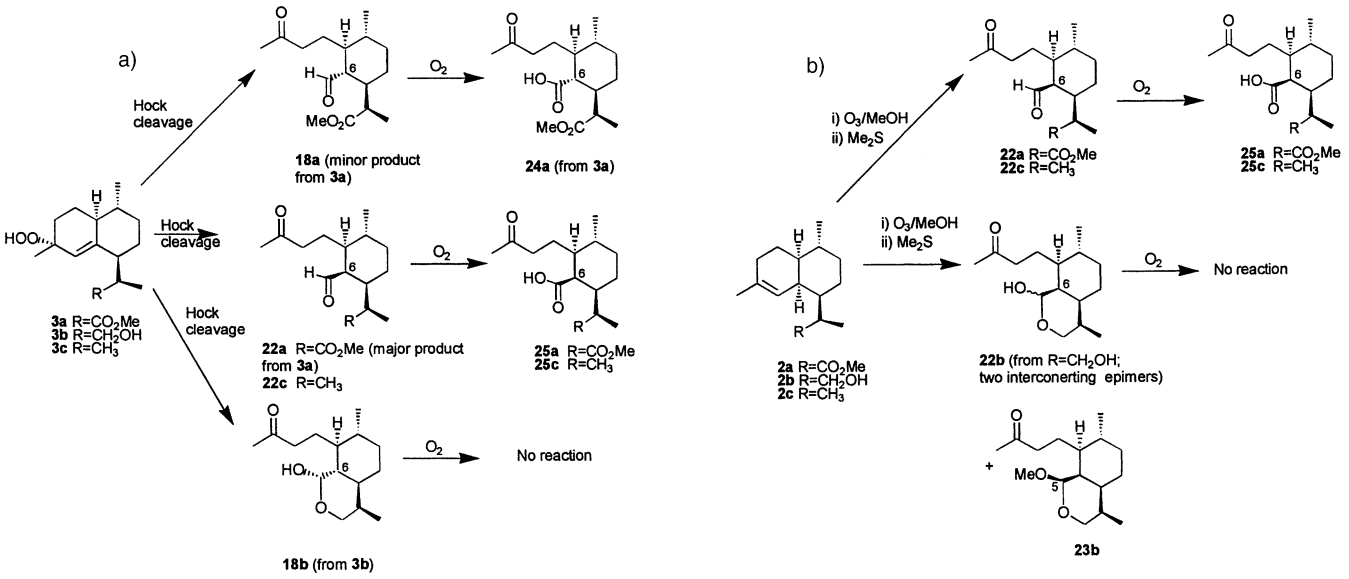
Most interesting of all, compound **2c**, which contains a methyl group in place of the 12-carboxylic acid group in dihydroartemisinic acid (**2**), was found to be almost unchanged on standing in $CDCl_3$ solution after 3 weeks and was clearly undergoing spontaneous autoxidation more slowly than either dihydroartemisinic acid or its derivatives **2a** and **2b**. We have previously shown that oxygen-containing functional groups can assist the autoxidation of tri-substituted double bonds, and in accordance with our previous investigations of such slow non-photosensitized autoxidation reactions,[11] we propose that the very slow rate for the autoxidation of **2c** is due to the absence of an oxygen-containing functional group at the 12-position. (Note that the functional group at the 12-position is held in close proximity to the $\Delta^{4,5}$ double bond and is therefore well placed to assist in its spontaneous oxidation by molecular oxygen, although the mechanism by which this intramolecular assistance occurs is not known).[11]

It also appeared from the above results that, as well as promoting the first autoxidation of dihydroartemisinic acid, the 12-carboxylic acid group in **2** might, in addition, be assisting the subsequent transformations of its autoxidation product **3**, as the further transformations of **3a** and **3b** (containing methyl ester and primary alcohol functional groups, respectively, at the 12-position), which were formed in situ from **2a** and **2b**, were slow when compared with the rate of further transformation of **3** into **1** and other natural products from *A. annua* which are reported in the preceding paper.[3] In order to further investigate the role of the 12-carboxylic acid group in (apparently) catalysing the spontaneous transformation of the tertiary allylic hydroperoxide of dihydroartemisinic acid (**3**) into artemisinin (**1**), we next obtained pure samples of the tertiary allylic hydroperoxide analogues of **3**, compounds **3a**–**3c**, by sensitized photo-oxygenation of the dihydroartemisinic acid derivatives **2a**–**2c** and HPLC separation from smaller quantities of the alternative secondary allylic hydroperoxides **9a**–**9c** and **10a**–**10c**, which are also formed by photo-oxygenation of the $\Delta^{4,5}$ double bond (Scheme 2).[12]

[†] The unconventional numbering system employed for compounds described in this paper is intended both to permit easy comparison with the preceding paper,[3] in which the mechanism for spontaneous oxidation of **2** to **1** was established, and also to immediately indicate which of the three derivatives of compound **2** is being referred to. Thus, the suffix '**a**' indicates compounds containing a 12-CO_2Me group; '**b**' indicates compounds containing (or derived from precursors incorporating) a 12-CH_2OH group; and '**c**' indicates the presence of a 12-CH_3 substituent. Lack of a suffix indicates a compound containing (or derived from a precursor containing) a 12-CO_2H group, and since all such compounds are ultimately derived from dihydroartemisinic acid (**2**) and have already been described in the preceding paper,[3] the numbers given to these compounds are the same as in the preceding paper, so as to aid in immediate cross-referencing. 'Novel analogues' of compounds which have been described in the preceding paper (which incorporate 12-CO_2Me, 12-CH_2OH or 12-CH_3 groups in place of 12-CO_2H), therefore retain the same numbering system (with the appropriate suffix, **a**, **b** or **c**) as in the preceding paper, so as to indicate their relationship. 'Novel structures' described herein, which have no direct analogy in the preceding paper, are then numbered sequentially, starting from number **26** (the last compound number used in the preceding paper was **25**), in order of their appearance in the text.



Scheme 3. Transformations of allylic hydroperoxides **3a**–**3c** in $CDCl_3$.



Scheme 4. Confirmation of the stereochemistry for the 6-aldehyde substituent in compounds **18a**, **18b**, **22a** and **22c**, obtained from (a) Hock cleavage reactions of **3a**–**3c**, by (b) ozonolysis of compounds **2a**–**2c**. All 6-aldehydes undergo slow spontaneous autoxidation by molecular oxygen to carboxylic acids, but the 'trapped' hemi-acetals are stable.

2.2. Spontaneous Hock cleavage of the tertiary allylic hydroperoxide 3 is catalysed by the 12-carboxylic acid

In the preceding paper,[3] we have shown that the tertiary allylic hydroperoxide of dihydroartemisinic acid (**3**) could be converted almost quantitatively into artemisinin (**1**) in organic solution by treatment with trifluoroacetic acid (TFA). Somewhat unexpectedly, hydroperoxide **3c** (in which a methyl group replaces the 12-carboxylic acid substituent in **3**) was converted primarily into the aldehyde **22c**, under these conditions (cf. Ref. 10). Products **1** and **22c** (pathway (v) in Scheme 3) have both clearly undergone carbon–carbon cleavage at C-4/C-5, which is believed to be catalysed by the organic acid in solution. The differing structures of these two product was intriguing, and prompted us to investigate whether such carbon–carbon cleavage would occur spontaneously for $CDCl_3$ solutions of **3a**–**3c** in the absence of exogenous acid.

In the preceding paper we have shown that compound **3**, the tertiary allylic hydroperoxide of dihydroartemisinic acid, was converted predominantly into aldehyde **18** (which is epimeric with **22c** at the 6-position) by spontaneous Hock cleavage at C-4/C-5 when left in $CDCl_3$ solution for a few weeks under an atmosphere of nitrogen.[3] Accordingly, $CDCl_3$ solutions of all three hydroperoxide derivatives **3a**–**3c** were left under laboratory conditions for more prolonged periods of time (the results of Section 2.1 led us to expect that the rates of further reactions of these hydroperoxides would be slow in most cases) under an atmosphere of nitrogen, so as to prevent the occurrence of the second autoxidation step by molecular oxygen following Hock cleavage. These experiments showed that, whereas tertiary allylic hydroperoxide **3**—which contains a 12-carboxylic acid group—was converted into a complex mixture consisting predominantly of the 6α-aldehyde, compound **18** (which has undergone carbon–carbon cleavage at C-4/C-5) over a period of a few weeks, the tertiary allylic hydroperoxides **3a**–**3c** underwent much slower conversions to complex mixtures which consisted predominantly of the products of S_N2' attack and E_1 elimination of hydrogen peroxide (pathways (i) and (ii) in Scheme 3). Thus, the major product from such treatment of compound **3b** was compound **6b** (formed by S_N2' attack of the 12-CH_2OH group at the double bond of the allylic hydroperoxide); while compound **3a** was predominantly converted into compound **13a**; and compound **3c** into compound **13c** (both by E_1 elimination of –OOH with H-7). Alternative products of E_1 elimination (compounds **27a**–**29a** from **3a**; **13b** from **3b**; **27c**–**29c** from **3c**) and of S_N2' attack (compound **12b** from **3b**) were also noted in trace amounts (Scheme 3).

Several products of alternative reaction pathways available to allylic hydroperoxides, all of which have analogies in the preceding paper,[3] were also isolated as very minor constituents from these mixtures. Thus, rearrangement of the tertiary allylic hydroperoxide to a β-hydroxy epoxide (pathway (iii) in Scheme 3) must still be occurring to a limited extent, in order to explain the formation of a small amount of the five-membered cyclic ether **14b** (from hydroperoxide **3b**, in which there is a primary alcohol group at the 12-position). The products of spontaneous carbon–carbon cleavage reactions (pathway (v) in Scheme 3) were of relatively low abundance in the mixtures formed from the slow transformations of **3a**–**3c** in $CDCl_3$ under an atmosphere of nitrogen. This contrasts markedly with results described in the preceding paper[3] in which Hock cleavage was the dominant pathway for transformation of the tertiary allylic hydroperoxide of dihydroartemisinic acid (**3**) in $CDCl_3$ solution under an atmosphere of nitrogen. One such minor product, compound **16c**, is the analogue of compound **16** described in the preceding paper[3] and its isolation can be considered as providing good supporting evidence for the first step of a Hock cleavage mechanism in which the $\Delta^{5,6}$ double bond of **3c** participates in a 1,2-shift to the internal oxygen atom of the hydroperoxide, which accompanies the loss of the external oxygen atom as water, thereby generating

Table 1. ^{13}C NMR assignments for novel products isolated from spontaneous autoxidation reactions of **2a**–**2c**, with direct analogues from the spontaneous transformations of **2** in the preceding paper[3]

Position	**1b**	**4a**[a]	**4b**[a]	**4c**[a]	**6b**	**12b**	**13b**	**14b**	**18a**	**18b**	**19b**	**20a**[b]	**20a**[c]	**20c**	**21b**	**22a**	**22c**	Position	**20a**[b]	**20a**[c]	**20c**
1	52.2	36.8	37.3	37.3	47.5	47.7	43.4	46.2	41.8	42.5	47.1	39.4	45.6	46.3	45.9	46.2	46.5	1′	49.6	50.9	50.7
2	24.8	28.2	28.5	28.6	21.3	20.5	28.5	16.6	27.1[d]	21.0	20.7	29.2	21.4	21.0	22.1	24.7	24.8	2′	22.8	23.3	22.3
3	36.3	40.9	41.2	41.0	31.2	26.4	31.1	24.6	38.2	38.6	41.4	42.8	42.4	42.6	34.5	41.0	41.0	3′	43.9	43.5	44.2
4	104.2	214.8	214.0	214.3	137.3	139.0	136.9	58.4	208.5	209.4	209.1	208.9	208.6	209.1	107.5	208.4	208.7	4′	207.3	207.6	208.4
5	92.2	139.9	139.1	138.4	127.3	122.3	120.2	60.1	205.5	91.6	135.5	134.9	134.8	133.5	96.2	206.3	207.5	5′	204.4	204.8	206.4
6	81.0	112.3	113.4	114.8	81.7	82.0	132.9	79.5	56.2	39.6	118.6	117.6	117.3	118.9	–[e]	50.1	51.6	6′	87.0	87.2	88.1
7	44.9	40.6	39.4	45.2	44.2	51.3	129.7	51.6	42.1[d]	35.8	38.7	42.0	36.4	40.9	40.1	43.5	47.8	7′	49.5	47.6	49.5
8	20.8	22.8	21.7	21.8	22.8	22.7	23.7	22.2	23.4[d]	30.4	28.5	24.7	23.5	24.7	23.9	27.3	26.4	8′	21.4	29.9	22.5
9	34.1	23.2	23.5	23.3	34.6	36.3	31.5	35.7	34.8	35.1	35.9	23.9	23.9	30.2	34.5	35.7	36.3	9′	35.1	35.0	35.2
10	37.4	30.7	31.6	31.6	30.4	30.4	34.6	30.4	33.5	34.8	39.3	32.9	33.2	34.3	35.3	33.6	33.4	10′	33.4	33.1	34.0
11	28.0	41.9	35.3	28.6	35.2	34.2	36.5	33.7	41.2[d]	32.9	30.5	42.4	43.3	30.5	26.4	43.2	30.9	11′	36.8	37.7	24.6
12	66.2	179.0	66.0	22.3[d]	70.5	73.3	65.9	74.5	175.1	65.5	67.7	177.6	177.1	22.1[d]	64.5	176.6	20.8[d]	12′	174.7	174.8	23.2[d]
13	13.1	16.9	16.6	21.6[d]	11.9	16.7	15.1	16.3	14.3	11.9	12.9	17.2	16.7	20.7[d]	16.6	15.3	20.6[d]	13′	17.3	17.4	18.7[d]
14	20.3	20.0	20.2	20.3	19.8	19.8	20.5	18.5	19.6	20.0	20.3	19.9	19.8	21.9	18.9	20.5	21.2	14′	20.6	20.4	20.2
15	26.1	30.7	30.6	30.6	23.6	24.3	24.1	23.6	29.7	30.0	30.0	29.9	29.9	29.7	24.0	30.0	29.9	15′	29.7	29.7	30.0
12-OMe	–	52.3	–	–	–	–	–	–	51.6	–	–	51.6	51.6	–	–	51.6	–	12′-OMe	51.2	51.1	–

[a] Assignments made at 233 K in TFA/$CDCl_3$ solution.
[b] Isomer 1 of compound **20a**.
[c] Isomer 2 of compound **20a**.
[d] Interchangeable within column.
[e] Not assigned.

Table 2. [1]H NMR assignments for novel products isolated from spontaneous autoxidation reactions of **2a–2c**, with direct analogues from the spontaneous transformations of **2** in the preceding paper[3]

Position	**1b**	**4a**[a]	**4b**[a]	**4c**[a]	**6b**	**12b**	**13b**	**14b**	**18a**	**18b**	**19b**	**20a**[b]	**20a**[c]	**20c**	**21b**	**22a**	**22c**	Position	**20a**[b]	**20a**[c]	**20c**
1	1.24	2.20	2.22	2.19	1.12	1.27	1.63	1.11	[d]	1.20	1.49	2.70	1.67	1.58	1.17	1.23	1.09	1′	1.75	1.30	1.22
2α	1.87	1.61	1.68	1.74	1.84	2.00	2.13	1.70	1.85	1.77	1.98	1.81	1.71	1.76	1.65	2.02	1.99	2α′	1.45	1.60	1.67
2β	1.49	1.61	1.57	1.56	1.57	1.77	2.13	1.45	1.45	1.73	1.57	1.66	1.60	1.55	1.25	1.28	1.24	2β′	1.78	1.71	1.79
3α	2.01	2.57	2.54	2.52	2.06	1.88	2.15	1.88	2.28	2.36	2.43	2.59	2.43	2.51	1.72	2.34	2.33	3α′	2.41	2.32	2.36
3β	2.36	2.68	2.63	2.66	1.96	1.88	2.02	1.65	2.46	2.40	2.62	2.49	2.43	2.38	1.57	2.63	2.64	3β′	2.41	2.43	2.49
5	5.21	6.31	6.23	6.21	5.59	5.65	6.22	2.92	9.54	5.17	6.04	6.24	6.17	6.05	5.25	9.95	9.97	5′	9.69	9.77	9.81
6	–	–	–	–	–	–	–	–	2.27	1.45	–	–	–	–	–	2.66	2.69	6′	–	–	–
7	1.53	2.15	1.76	1.48	1.61	1.63	–	1.76	–[d]	1.83	1.84	2.23	3.20	2.50	1.88	1.79	1.18	7′	1.41	1.98	1.80
8α	1.66	1.82	1.74[e]	1.61	1.52	1.65	2.10	1.77	–[d]	1.43	1.71	1.77	1.64	1.79	1.64	1.55	1.86	8α′	0.94	1.29	1.02
8β	1.44	1.24	1.20[e]	1.61	1.22	1.22	1.96	1.34	–[d]	1.21	1.15	1.26	1.25	1.07	1.20	1.55	1.48	8β′	1.74	1.67	1.57
9α	1.08	1.15	1.28[e]	1.56	0.99	1.10	1.22	1.15	–[d]	1.08	1.16	1.21	1.18	1.72	1.00	1.17	1.11	9α′	1.79	1.80	1.81
9β	1.70	1.75	1.67[e]	1.72	1.67	1.76	1.74	1.81	–[d]	1.70	1.82	1.88	1.88	1.80	1.70	1.87	1.91	9β′	1.00	1.03	1.02
10	1.35	1.72	1.68	1.68	1.42	1.43	1.22	1.32	1.21	1.20	1.18	1.81	1.79	1.74	1.22	1.65	1.64	10′	1.97	1.98	2.04
11	2.65	2.64	1.75	1.65	2.83	2.46	3.14	2.51	2.44	1.58	2.18	2.76	2.74	1.75	2.28	2.36	1.45	11′	2.84	2.87	2.16
12	3.74, 3.46	–	3.78, 3.52	0.79[f]	3.37, 3.90	3.52, 4.18	3.48, 3.48	4.29, 3.60	–	4.16, 3.33	3.69, 3.48	–	–	0.95[f]	3.94, 3.29	–	0.91[f]	12′	–	–	0.93[f]
13	0.78	1.06	0.88	0.88[f]	0.96	1.10	0.97	1.11	1.14	0.99	0.87	1.05	1.20	0.94[f]	0.92	1.17	0.93[f]	13′	1.09	1.19	0.65[f]
14	0.96	0.92	0.93	0.93	0.90	0.93	1.01	0.91	0.92	0.88	0.94	0.93	0.94	0.95	0.89	0.96	0.94	14′	0.92	0.89	0.90
15	1.43	2.33	2.28	2.29	1.66	1.68	1.76	1.35	2.15	2.15	2.13	2.15	2.14	2.12	1.53	2.15	2.14	15′	2.05	2.08	2.07
12-OMe	–	3.73	–	–	–	–	–	–	3.67	–	–	3.70	3.70	–	–	3.67	–	12′-OMe	3.56	3.56	–

[a] Assignments made at 233 K in TFA/$CDCl_3$ solution.
[b] Isomer 1 of compound **20a**.
[c] Isomer 2 of compound **20a**.
[d] Not resolved.
[e] α and β assignments uncertain due to extensive chemical exchange in NOESY spectra, which are dominated by EXSY-type peaks.
[f] Interchangeable within column.

Table 3. ^{13}C NMR data for novel products obtained from the spontaneous autoxidation and ozonolysis of **2a**–**2c**, which have no direct analogy with the spontaneous transformations of **2**

Position	**22b**[a]	**22b**[b]	**23b**	**24a**	**25a**	**25c**	**26a**	**26c**	**30a**	**32b**	Position	**30a**
1	45.9	47.9	45.9	44.9	45.8	46.0	59.7	59.8	49.4	57.0	1′	45.0
2	22.9	24.9	22.8	23.8	24.8	24.9	22.4	22.4	22.5	20.2	2′	22.2
3	41.8	42.5	41.8	38.4	41.5	41.4	41.3	41.6	44.2	41.3	3′	29.5
4	209.1	210.8	209.1	209.2	208.9	209.0	106.0	105.9	208.3	209.1	4′	79.5
5	92.8	94.7	99.6	–[c]	176.5	176.5	200.4	201.3	202.6	161.2	5′	120.1
6	40.2	43.3	40.4	51.0	43.7	44.9	94.2	95.5	90.1	212.4	6′	146.4
7	39.3	41.7	39.5	44.1	43.3	47.6	48.9	51.5	48.2	53.3	7′	47.8
8	21.6	20.4	21.5	26.4	25.8	24.6	25.2	22.7	22.7	30.3	8′	32.7
9	35.7	36.2	35.7	34.8	35.3	35.9	35.5	35.6	35.2	34.7	9′	35.5
10	31.8	32.3	31.7	33.7	31.0	30.9	32.0	32.2	34.0	40.5	10′	38.3
11	34.3	34.4	34.2	41.8	43.8	31.1	37.7	23.7	36.9	31.7	11′	41.0
12	61.4	67.1	61.3	175.3	176.8	21.3[d]	175.3	24.2[d]	175.2	66.5	12′	176.8
13	14.2	13.7	14.2	14.9	15.6	21.0[d]	18.0	19.2[d]	17.3	15.6	13′	16.3
14	21.1	20.7	21.1	19.6	20.3	20.4	20.5	20.6	20.6	20.6	14′	20.0
15	29.9	29.9	29.9	29.9	30.0	29.9	24.1	23.9	29.8	29.9	15′	25.6
12-OMe	–	–	–	51.6	51.6	–	51.5	–	50.9	–	12′-OMe	51.4
5-OMe	–	–	54.7	–	–	–	–	–	–	–	–	–

[a] Major 5β-OH isomer of **22b**.
[b] Minor 5α-OH isomer of **22b**; the two isomers were inseparable and are believed to be interconverting in $CDCl_3$ solution.
[c] Not resolved.
[d] Interchangeable within column.

Table 4. ^{1}H NMR data for novel products obtained from the spontaneous autoxidation and ozonolysis of **2a**–**2c**, which have no direct analogy with the spontaneous transformations of **2**

Position	**22b**[a]	**22b**[b]	**23b**	**24a**	**25a**	**25c**	**26a**	**26c**	**30a**	**32b**	Position	**30a**
1	0.95	1.03	0.92	1.43	1.13	1.10	1.13	1.13	1.59	2.04	1′	1.58
2α	1.96	1.83	1.95	1.84	2.04	2.02	1.80	1.80	1.93	1.80	2α′	1.58
2β	1.51	1.78	1.33	1.59	1.32	1.28	1.32	1.31	1.55	1.76	2β′	1.95
3α	2.56	2.84	2.56	2.53	2.38	2.39	1.90	2.20	2.46	2.55	3α′	2.02
3β	2.38	2.32	2.34	2.36	2.74	2.74	2.20	1.92	2.39	2.38	3β′	1.53
5	5.17	5.09	4.54	–	–	–	9.90	9.91	9.56	8.07	5′	5.25
6	1.92	1.76	1.94	2.37	2.93	2.98	–	–	–	–	6′	–
7	1.50	1.69	1.48	1.83	1.74	1.14	1.63	1.43	2.05	2.34	7′	2.05
8α	1.73	1.49	1.62	1.83	1.74	1.64	1.68	1.65	1.75	2.07	8α′	1.20
8β	1.41	1.49	1.36	1.09	1.32	1.64	1.25	1.18	1.50	1.41	8β′	1.72
9α	1.03	1.05	0.88	1.09	1.00	0.95	0.95	0.92	0.98	1.50	9α′	1.75
9β	1.79	1.82	1.74	1.77	1.74	1.78	1.75	1.78	1.75	1.90	9β′	1.20
10	1.91	1.48	1.80	1.22	1.78	1.79	2.34	2.34	1.92	1.56	10′	1.31
11	1.97	2.00	1.98	2.55	2.36	1.47	3.19	2.58	3.20	2.24	11′	2.73
12	3.73, 3.30	3.64, 3.43	3.47, 3.25	–	–	0.99[c]	–	0.93[c]	–	4.20, 4.07	12′	–
13	0.81	0.78	0.79	1.16	1.24	0.91[c]	1.22	0.75[c]	1.16	1.00	13′	1.21
14	0.87	0.90	0.84	0.90	0.89	0.89	0.89	0.89	0.88	1.08	14′	0.93
15	2.14	2.13	2.14	2.15	2.16	2.15	1.28	1.28	2.09	2.13	15′	1.36
12-OMe	–	–	–	3.69	3.67	–	3.64	–	3.54	–	12′-OMe	3.68
5-OMe	–	–	3.29	–	–	–	–	–	–	–	–	–

[a] Major 5β-OH isomer of **22b**.
[b] Minor 5α-OH isomer of **22b**; the two isomers were inseparable and are believed to be interconverting in $CDCl_3$ solution.
[c] Interchangeable within column.

a transient tertiary carbocation at C-4, which is converted into **16c** by elimination of H^+ from the 3-position (see pathway (v) in Scheme 3). Re-addition of water to this carbocation would instead generate an enol and although none of the enols **4a**–**4c** were observed directly in this experiment, there was some evidence for their formation from the isolation of small amounts of compound **18b** in which the 5-aldehyde group formed by tautomerization of the enol **4b** has been 'trapped' by the 12-hydroxyl group. The stereochemistry of the 6-substituent in this compound was shown to be α by correlations observed in its NOESY spectrum. This is the same stereochemical outcome as was noted for the spontaneous Hock cleavage reaction of **3** in Scheme 3 of the preceding paper,[3] from which the aldehyde tautomer **18** of enol **4** was isolated. It was confirmed in the same way, by ozonolysis of the corresponding derivative of dihydroartemisinic acid, compound **2b** (Scheme 4). Hemi-acetal **22b** obtained from ozonolysis of **2b** is expected to be formed from 'trapping' of the epimeric 6β-aldehyde, and its spectral properties were clearly different from those of **18b** (Tables 1–4).[‡]

[‡] Compound **23b** was also isolated as a minor product from this reaction (cf. the results for the ozonolysis of dihydroartemisinic acid in the preceding paper).[3] Previous reports of the ozonolysis of **2b** in the presence of acid have described the enol ether **19b**,[14,15] which might be formed by dehydration/elimination of MeOH from either or both of compounds **22b** and **23b**.

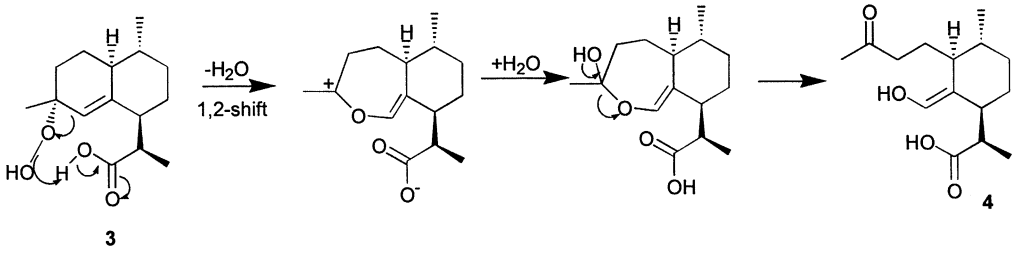


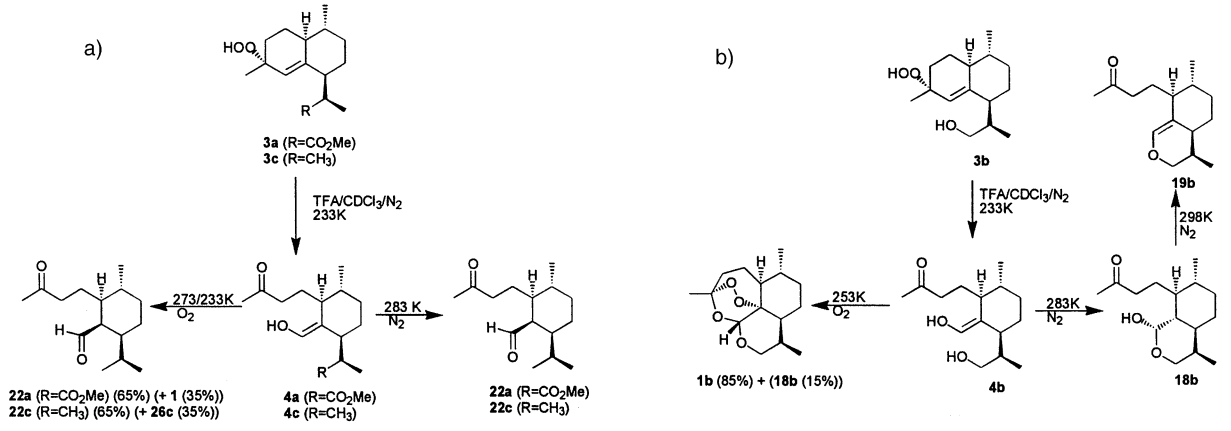
Figure 1. Proposed mechanism by which the 12-carboxylic acid group catalyses the spontaneous Hock cleavage reaction of **3**.

The isolation of small amounts of dimers **20a** (two isomers)/**20c** and **30a/30c** (pathway (vi) in Scheme 3) all attest to the possibility of radical reactions of the enol product from Hock cleavage, as was proposed for the formation of **20** from **3**, in the preceding paper.[3] As previously, it was difficult to confirm the molecular weights of some of these dimeric products by mass spectrometry and, when problems were encountered, we resorted to a rough determination of molecular weight by DOSY-NMR. As before, several products of oxygenation of these assumed enol intermediates, i.e. compounds **1**, **21**, **26a** and **31a** from **4a**; compounds **21b** and **32b** from **4b**; and compounds **26c** and **31c** from **3c**, were also isolated from these mixtures in small quantities even though great care had been taken to exclude molecular oxygen from $CDCl_3$ solution. We believe that these products arise from reactions with hydrogen peroxide, which is formed as a by-product of the more dominant pathways (i) S_N2' and (ii) E_1 elimination for the transformation of hydroperoxides **3a**–**3c**.

From the foregoing results, we believe that the Hock cleavage[16] reactions observed for compounds **3a**–**3c**, which occur to only a very limited extent in $CDCl_3$ solution, are being catalysed by the traces of acid naturally present in deuteriated chloroform. By contrast, we attribute the much greater tendency of the tertiary allylic hydroperoxide **3** to undergo Hock cleavage under the same conditions (see the preceding paper)[3] to the presence of the acidic proton in the 12-carboxylic acid group which can participate in an intramolecular transfer to the terminal oxygen atom of the allylic hydroperoxide, as shown in Fig. 1, thereby initiating carbon–carbon cleavage at C-4/C-5 (see also our previous observations concerning the involvement of the 12-carboxylic acid group in the facile dehydration of the 4-hydroxyl analogue of compound **3**).[13] The increased rate of the spontaneous formation of **1** from $CDCl_3$ solutions of **2** containing stearic acid impurities, noted in the preceding paper,[3] might also be explained by such acid catalysis.

2.3. The second spontaneous autoxidation of the enolic intermediate is favoured when an OH group is present at the 12-position

In order to study the effect of the 12-carboxylic acid substituent on the second autoxidation reaction shown in Scheme 1, it was first necessary to obtain the enolic derivatives **4a**–**4c** from Hock cleavage of the corresponding hydroperoxides **3a**–**3c** (incorporating CO_2Me, CH_2OH and CH_3 groups, respectively, at the 12-position). As in the preceding paper, when performed at 233 K under an atmosphere of nitrogen, the addition of TFA to $CDCl_3$ solutions of all of these hydroperoxides led to rapid near quantitative conversion into the desired enols **4a**–**4c** (as before, there was also a low level of contamination by expectable side-products of alternative reactions, such as **6b** and **13a/13c**, which are formed by the elimination of hydrogen peroxide as shown in pathways i and ii in Scheme 3). Enols **4a**–**4c** were all stable over a period of several hours at low temperature in the bore of the magnet of the NMR spectrometer and could be characterized by 2D-NMR. In the case of compounds **4a** and **4c**, for which CO_2Me and CH_3 groups, respectively, were present at the 12-position, it was possible to unambiguously determine the *E*-geometry of the enolic double bond from correlations (negative cross-peaks) observed in NOESY spectroscopy between H-5 and H-13/H-7. Thus, the enol geometry which would be predicted by



Scheme 5. (a) Conversion of enols **4a** and **4c** predominantly into aldehydes **22a** and **22c** under either aerobic or anaerobic conditions; (b) the differing reactions of enol **4b** in the presence and absence of molecular oxygen.

the mechanism shown for Hock cleavage of **3a** and **3c** in Schemes 3 and 5 could be confirmed experimentally for these two examples. However the NOESY spectrum of compound **4b**, for which a CH_2OH group is present at the 12-position, was dominated by positive 'EXSY-type' cross-peaks, indicating rapid chemical exchange and making stereochemical assignment impossible (cf. the similar NOESY results in the preceding paper for enol **4** which incorporates a CO_2H group at the 12-position).

From these observations, we make the tentative suggestion that it is the presence of a labile hydrogen atom in the functional group at the 12-position (as found in **4** and **4b**, but not in **4a** and **4c**) which is responsible for this rapid chemical exchange process. It is possible that enols which contain such a labile hydrogen have a greater tendency to exist as radicals and that extensive scrambling of hydrogen atoms throughout the molecule would then account for the all-pervasive EXSY-type peaks seen in the NOESY spectra of such radicals from **4** and **4b**. This proposal is supported by our observations of the differing behaviour of the three enols **4a**–**4c** on warming under atmospheres which either contain or exclude oxygen. Thus, in the presence of oxygen, enols **4a** and **4c**, containing CO_2Me and CH_3 groups, respectively, at the 12-position, preferred to undergo tautomerization to aldehydes (compounds **22a**[§] and **22c**) rather than oxygenation (yielding a 1,2,4-trioxane ring as in compound **1** from **4a**, or an endoperoxide, as in its precursor compound **26c** from **4c**—see Scheme 5). By contrast, enol **4b**, for which a CH_2OH group is present at the 12-position, was preferentially transformed into the 12-deoxy analogue of artemisinin, compound **1b**, accompanied by only a little of the enol tautomer **18b** (Scheme 5; cf. exclusive conversion of **4** into **1** in the preceding paper).[3] We propose that the increased reactivity of both of the enols **4** and **4b** towards $^3O_2$ is the result of their greater tendency to exist as an enol radical which is then intercepted by molecular oxygen, yielding a presumed hydroperoxyl-aldehyde intermediate (**5** and **5b**—see pathway (vii) in Scheme 3) from which cyclization to a 1,2,4-trioxane ring can occur.

Under an atmosphere of nitrogen, all three enols were converted primarily to products of tautomerization, as shown in Scheme 5. This is not surprising given our results for compound **4** in the preceding paper;[3] however, it is interesting to note that enols **4a** and **4c**, incorporating CO_2Me and CH_3, respectively, at the 12-position were converted largely into the 6β-aldehydes **22a** and **22c**, with physical properties identical to the products obtained from ozonolysis of the corresponding derivatives of dihydroartemisinic acid, compounds **2a** and **2c** in Scheme 4b (Tables 1 and 2). By contrast, enol **4b** containing a 12-CH_2OH group underwent tautomerization only to product **18b** which is derived from a 6α-aldehyde (see Section 2.2 for a discussion of the stereochemistry of this compound). We propose that it is the labile proton in the 12-OH functional group, which is transferred to the 6-position of this enol when it undergoes tautomerization, and that this intramolecular process is then constrained to occur from the β-face of the molecule by the geometry of the 7-substituent in compound **4b**. The first step in the tautomerization of enols **4a** and **4c** to the aldehydes **22a** and **22c**, which do not contain any labile hydrogen atom in the functional group at the 12-position, is an intermolecular transfer of a proton from the organic acid in the $CDCl_3$ medium to the 6-position, which occurs preferentially from the less sterically hindered α-face of the molecule.

None of the products isolated from the tautomerization reaction of enols **4a**–**4c** under an atmosphere of nitrogen showed any sign of undergoing further oxidation to 1,2,4-trioxane ring-containing compounds in the presence of molecular oxygen. Instead, all of the aldehydes **18a**, **22a** and **22c** underwent slow spontaneous autoxidation to the carboxylic acids **24a**, **25a** and **25c**, respectively, as shown in Scheme 4. This confirms our deduction in the preceding paper[3] that it is the enol which is formed directly by Hock cleavage, and not the aldehyde to which it may subsequently tautomerize, which is the reactive species in the formation of the 1,2,4-trioxane ring of artemisinin.

2.4. Closure of the 1,2,4-trioxane ring occurs from a vicinal hydroperoxyl aldehyde

The 1,2,4-trioxane ring is partially formed in the minor endoperoxide products **26a**[¶] and **26c** which were obtained from experiments involving precursors containing 12-CO_2Me and 12-CH_3 groups, respectively. This might suggest that the final steps in the formation of the 1,2,4-trioxane ring occurs from vicinal hydroperoxyl-aldehydes such as **5a** and **5c** by the mechanism shown in pathway (vii) of Scheme 3, in which it is the 6-hydroperoxyl group which initiates the ring closure, although further experiments would need to be devised in order to prove this mode of cyclization, since neither **5a** nor **5c** were ever isolated. In support of this, *nor*-sesquiterpenes **31a** and **31c** are believed to have lost one carbon atom as formic acid and we have recently proposed[10,13] that the mechanism of formation of such compounds involves Hock cleavage of vicinal hydroperoxyl-aldehydes, such as **5a** and **5c**, before they can cyclize to endoperoxides such as **26a** and **26c**, as is shown in pathway (vii) of Scheme 3. The mechanism for this reaction is believed to involve protonation of the terminal oxygen atom of such a hydroperoxide (which is then lost as water), and accompanying 1,2-shift of the vicinal aldehyde functional group to the internal oxygen atom of the hydroperoxide, which would generate a transient carbocation at C-6 adjacent to a formate ester. The isolation of compound **32b** from reactions of **3b** provides further supporting evidence for this proposal, as this formate group has clearly been trapped by the primary hydroxyl group at the 12-position. Thus, we believe that there is now considerable circumstantial evidence to support the hypothesis that vicinal hydroperoxyl-aldehydes of general structure **5** are the immediate products of oxygenation of enols of general structure **4**, and that the formation of the 1,2,4-trioxane ring of artemisinin proceeds from cyclization of such vicinal hydroperoxyl-aldehydes, as in Scheme 1.

[§] Obtained previously by synthesis.[17]

[¶] Compound **26a** has been reported previously from synthesis.[13,18]

3. Conclusion

In summary, we find that the presence of the 12-carboxylic acid group, in close proximity to the $\Delta^{4,5}$ double bond in dihydroartemisinic acid, appears to be a pre-requisite for the spontaneity of the complex series of transformations involved in the conversion of **2** to **1**, assisting (at least) three of the four steps which have been identified previously.[3] If the functional group at the 12-position lacks an oxygen atom (as for derivative **2c**), then the rate of the first spontaneous autoxidation of the double bond is significantly reduced in organic solution. Only when a carboxylic acid is present at the 12-position does spontaneous Hock cleavage become the dominant pathway for further reaction of the resulting tertiary allylic hydroperoxide, as was observed for compound **3**, but not for any of compounds **3a**–**3c**. Only when the functional group at the 12-position contains a labile hydrogen atom does the enol product from Hock cleavage preferentially react with molecular oxygen, as found for **4** and **4b**, but not for **4a** and **4c**. The vicinal hydroperoxyl-aldehydes **5/5b** are assumed to be the immediate products from this reaction, based on the isolation of compounds **26a/26c**, in which the 1,2,4-troxane ring is partially formed, and of compounds **31a**, **31c** and **32b**, which are very likely to be formed by Hock cleavage of this intermediate. Thus, the spontaneous formation of the 1,2,4-trioxane ring of artemisinin (**1**) from the $\Delta^{4,5}$ double bond of dihydroartemisinic acid (**2**) appears to be a consequence of the presence and the proximity of the 12-carboxylic acid group in dihydroartemisinic acid to the functional groups participating in the four steps of the remarkable spontaneous transformation of **2** into **1**.

4. Experimental

4.1. General

All compounds reported were fully characterized by the 2D NMR experiments HSQC, HMBC, ^{1}H–^{1}H 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 ^{1}H 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 tubes equipped with a teflon valve which can be used to isolate the contents of the NMR tube from the atmosphere (J. Young, 528-VL-7). HSQC, HMBC ^{1}H–^{1}H 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.

4.1.1. Preparation of dihydroartemisinic acid derivatives 2a–2c and their spontaneous transformations in $CDCl_3$ solution. Samples of dihydroartemisinic acid (**2**) were obtained from *A. annua* plants as has been described previously,[1] and derivatized to **2a**–**2c** as described in Ref. 12. Solutions of **2a**–**2c** (1 mg) in $CDCl_3$ (0.6 ml) were left in NMR tubes under laboratory conditions and ^{1}H NMR spectra (δ_H, ppm) were acquired every 3–7 days over a period of 2 months. Disappearance of starting materials and appearance of oxidation products were monitored by observing characteristic resonances in these spectra. Compound **2a**:[12] 5.12 (s, H-5); **2b**:[12] 5.22 (s, H-5); **2c**:[12] 5.23 (s, H-5); **3a**:[12] 5.22 (s, H-5); **3b**:[12] 5.33 (s, H-5); **3c**:[12] 5.26 (s, H-5); **9a**:[12] 5.06 (s, H-15a/b), 4.92 (s, H-15a/b), 4.72 (d, J=11.7 Hz, H-5); **9b**:[12] 5.05 (s, H-15a/b), 4.94 (s, H-15a/b), 4.71 (d, J=10.0 Hz, H-5); **9c**:[12] 5.04 (s, H-15a/b), 4.93 (s, H-15a/b), 4.67 (d, J=9.5 Hz, H-5); **11a**:[12] 5.54 (s, H-5), 4.37 (m, H-15), 4.34 (m, H-15); **11b**:[12] 5.65 (s, H-5), 4.38 (d, J=11.5 Hz, H-15a/b), 4.35 (d, J=11.5 Hz, H-15a/b); **11c**:[12] 5.66 (s, H-5), 4.37 (m, H-15a/b), 4.35 (m, H-15a/b); **13a**:[13] 6.11 (s, H-5); **1**:[19] 5.86 (s, H-5).

4.1.2. Spontaneous transformation of tertiary allylic hydroperoxide 3a–3c in $CDCl_3$ solution under an atmosphere of nitrogen. Tertiary allylic hydroperoxides **3a**–**3c** were prepared by photo-oxygenation of **2a**–**2c**—see Ref. 12 for details. Dissolved oxygen was removed from solutions of **3a**–**3c** (30 mg) in $CDCl_3$ (1 ml), in NMR tubes fitted with a valve, by the freeze–thaw method. The samples were left under nitrogen under laboratory conditions for three and a half months and the resulting mixtures were then separated by HPLC. Compound **1**:[19] (1 mg, 3%); **13a**:[13] Oil. (12 mg, 40%; R_t 9.5 min, 15% EtOAc/*n*-hexane). $[\alpha]_D$=−13.6 (*c* 0.07, $CHCl_3$); ^{1}H NMR (δ, $CDCl_3$) ppm: 6.11 (1H, s, H-5), 3.77 (1H, q, J=7.0 Hz, H-11), 3.65 (3H, s, –OMe), 1.78 (3H, s, H-15), 1.20 (3H, d, J=7.0 Hz, H-13), 0.98 (3H, d, J=6.0 Hz, H-14); ^{13}C NMR—175.5 (C, C-12), 137.6 (C, C-4), 131.1 (C, C-6), 127.8 (C, C-7), 119.8 (CH, C-5), 51.7 (CH_3, –OMe), 43.1 (CH, C-1), 40.6 (CH, C-11), 34.3 (CH, C-10), 31.6 (CH_2, C-9), 31.2 (CH_2, C-3), 27.8 (CH_2,C-2), 25.4 (CH_2, C-8), 24.2 (CH_3, C-15), 20.4 (CH_3, C-14), 15.4 (CH_3, C-13); **20a** (isomer 1): Oil. (1.5 mg, 4%; R_t 37.6 min, 30% EtOAc/*n*-hexane). $[\alpha]_D$=−22.9 (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2928, 2855, 1734, 1717, 1458 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.69 (1H, s), 6.24 (1H, s), 3.70 (3H, s), 3.56 (3H, s), 2.84 (1H, dq, J=2.9, 7.2 Hz), 2.76 (1H, dq, J=7.0, 6.9 Hz), 2.70 (1H, m), 2.59 (1H, ddd, J=17.0, 9.5, 6.4 Hz), 2.49 (1H, ddd, J=17.0, 9.7, 5.7 Hz), 2.41 (2H, m), 2.15 (3H, s), 2.05 (3H, s), 1.09 (3H, d, J=7.2 Hz), 1.05 (3H, d, J=6.9 Hz), 0.93 (3H, d, J=6.8 Hz), 0.92 (3H, d, J=6.8 Hz)—see Table 2; ^{13}C NMR—see Table 1; CIMS: 563 [$M^+(C_{32}H_{50}O_8)$+1] (4), 504 [M^+−CO_2Me] (1), 281 (100); EIMS *m*/*z* (rel. int.) 281 (80), 249 (80), 231 (68), 195 (100); **20a** (isomer 2): Oil. (1 mg, 3%; R_t 34.5 min, 30% EtOAc/*n*-hexane). $[\alpha]_D$=−9.1 (*c* 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2930, 2855, 1734, 1717, 1458 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.77 (1H, s), 6.17 (1H, s), 3.70 (3H, s), 3.56 (3H, s), 3.20 (1H, dd, J=11.3, 6.0 Hz), 2.87 (1H, dq, J=3.3, 7.2 Hz), 2.74 (1H, dq, J=11.3, 7.0 Hz), 2.14 (3H, s), 2.08 (3H, s), 1.20 (3H, d, J=7.0 Hz), 1.19 (3H, d, J=7.2 Hz), 0.94 (3H, d, J=6.9 Hz), 0.89 (3H, d, J=6.5 Hz)—see Table 2; ^{13}C NMR—see Table 1; CIMS: 563 [$M^+(C_{32}H_{50}O_8)$+1] (4), 504 [M^+−CO_2Me] (1), 281

(100); EIMS *m/z* (rel. int.) 281 (70), 249 (91), 231 (60), 204 (100), 195 (100); **21**:[20] (1 mg, 3%, R_t 12.4 min, 30% EtOAc/*n*-hexane); **26a**: ^{1}H NMR δ ($CDCl_3$) ppm: 9.90 (1H, d, *J*=2.6 Hz), 3.64 (3H, s), 3.19 (1H, m), 1.28 (3H, s), 1.22 (3H, d, *J*=7.2 Hz), 0.89 (3H, d, *J*=6.5 Hz); **27a**, **28a** and **29a**[13]—isolated as a mixture with **13a** and identified by characteristic NMR resonances in the mixture (ca. 2% each from ^{1}H NMR of crude mixture): δ_H 5.51 (H-5) ppm; δ_H 5.82 (H-5), 4.72 (H-15a/b), 4.69 (H-15a/b) ppm; δ_H 5.37 (H-5), 5.26 (H-3) ppm, respectively; **30a**: Oil. (1.5 mg, 4%; R_t 13.0 min, 30% EtOAc/*n*-hexane). The sample was unstable under laboratory conditions after purification and decomposed into compounds **13a**, **21**, **26a** and **31a**. ^{1}H NMR δ ($CDCl_3$) ppm: 9.56 (1H, s), 5.25 (1H, s), 3.68 (3H, s), 3.54 (3H, s), 3.20 (1H, dq, *J*=1.8, 7.1 Hz), 2.73 (1H, dq, *J*=7.1, 6.8 Hz), 2.46 (1H, m), 2.39 (1H, m), 2.09 (3H, s), 1.36 (3H, s), 1.21 (3H, d, *J*=6.8 Hz), 1.16 (3H, d, *J*=7.1 Hz), 0.93 (3H, d, *J*=6.4 Hz), 0.88 (3H, d, *J*=6.3 Hz)—see Table 4; ^{13}C NMR—see Table 3; CIMS: 281 (3), 269 (10), 251 (60), 249 (100), 237 (25), 209 (35); **31a**:[21] (1 mg, 3%; R_t 20.8 min, 30% EtOAc/*n*-hexane).

Compound **6b**: Oil. (12 mg, 40%; R_t 7.6 min, 50% EtOAc/*n*-hexane). $[\alpha]_D$=+42.4 (*c* 0.18, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2930, 2872, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 5.59 (1H, s), 3.90 (1H, dd, *J*=8.4, 8.4 Hz), 3.37 (1H, dd, *J*=9.6, 8.4 Hz), 2.83 (1H, m), 2.06 (1H, dd, *J*=18.4, 6.8 Hz), 1.66 (3H, s), 0.96 (3H, d, *J*=6.9 Hz), 0.90 (3H, d, *J*=6.6 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.) 220.1822 [M^+, $C_{15}H_{24}O$ requires 220.1827] (70), 205 (55), 177 (42), 165 (100); **12b**: Oil. (4.5 mg, 15%; R_t 8.4 min, 50% EtOAc/*n*-hexane). ^{1}H NMR δ ($CDCl_3$) ppm: 5.65 (1H, s), 4.18 (1H, dd, *J*=9.1, 9.0 Hz), 3.52 (1H, dd, *J*=9.1, 5.6 Hz), 2.46 (1H, m), 1.68 (3H, s), 1.10 (3H, d, *J*=7.4 Hz), 0.93 (3H, d, *J*=6.5 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.) 220.1820 [M^+, $C_{15}H_{24}O$ requires 220.1827] (40), 205 (42), 165 (100); **13b**: Oil. (2.5 mg, 9%; R_t 9.9 min, 50% EtOAc/*n*-hexane). $[\alpha]_D$=+100.3 (*c* 0.38, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3538 (br), 3009, 2965, 2928, 2874, 2830, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 6.22 (1H, s), 3.48 (2H, m), 3.14 (1H, sext, *J*=7.1 Hz), 1.76 (3H, s), 1.01 (3H, d, *J*=5.9 Hz), 0.97 (3H, d, *J*=7.1 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.) 220.1821 [M^+, $C_{15}H_{24}O$ requires 220.1827] (27), 189 (100), 159 (33), 133 (26), 119 (40); **14b**: Oil. (1.5 mg, 6%; R_t 13.7 min, 50% EtOAc/*n*-hexane). ^{1}H NMR δ ($CDCl_3$) ppm: 4.29 (1H, dd, *J*=9.2, 8.9 Hz), 3.60 (1H, dd, *J*=9.2, 6.0 Hz), 2.92 (1H, s), 2.51 (1H, m), 1.35 (3H, s), 1.11 (3H, d, *J*=7.0 Hz), 0.91 (3H, d, *J*=6.6 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.) 236.1774 [M^+, $C_{15}H_{24}O_2$ requires 236.1776] (10), 221 (12), 178 (50), 165 (100); **18b**: Oil. (1 mg, 3%; R_t 21.3 min, 50% EtOAc/*n*-hexane). $[\alpha]_D$=+71.4 (*c* 0.98, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3603, 3400 (br), 3011, 2963, 2924, 2858, 1711, 1460 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 5.17 (1H, dd, *J*=1.8, 1.8 Hz), 4.16 (1H, dd, *J*=11.1, 2.8 Hz), 3.33 (1H, dd, *J*=11.1, 1.1 Hz), 2.40 (1H, m), 2.36 (1H, m), 2.25 (1H, br s, –OH), 2.15 (3H, s), 0.99 (3H, d, *J*=7.1 Hz), 0.87 (3H, d, *J*=6.4 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS *m/z* (rel. int.) 254.1884 [M^+, $C_{15}H_{26}O_3$ requires 254.1882] (2), 236 (56), 181 (45), 178 (70), 150 (100); **21b**: (ca. 5% by ^{1}H NMR of the crude mixture; isolated as a mixture with **12b**). NMR assignments made by 2D-NMR of the mixture. ^{1}H NMR δ ($CDCl_3$) ppm: 5.25 (1H, s), 3.94 (1H, dd, *J*=11.6, 6.7 Hz), 3.29 (1H, dd, *J*=11.6, 4.5 Hz), 1.53 (3H, s), 0.92 (3H, d, *J*=7.3 Hz), 0.89 (3H, d, *J*=6.1 Hz)—see Table 2; ^{13}C NMR—see Table 1; **32b**: Oil. (2.5 mg, 8%; R_t 11.8 min, 50% EtOAc/*n*-hexane). $[\alpha]_D$=−175 (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3015, 2932, 2876, 1713, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 8.07 (1H, s), 4.20 (1H, dd, *J*=10.9, 4.7 Hz), 4.07 (1H, dd, *J*=10.9, 6.3 Hz), 2.55 (1H, ddd, *J*=17.1, 8.7, 5.9 Hz), 2.36 (2H, m), 2.24 (1H, m), 2.13 (3H, s), 1.08 (3H, d, *J*=7.0 Hz), 1.00 (3H, d, *J*=6.7 Hz)—see Table 4; ^{13}C NMR—see Table 3; HREIMS *m/z* (rel. int.) 268.1671 [M^+, $C_{15}H_{24}O_4$ requires 268.1675] (10), 250 (36), 222 (21), 207 (50), 182 (53), 165 (100), 124 (100).

Compound **13c**:[22] Oil. (11 mg, 35%; R_t 10.2 min, *n*-hexane). $[\alpha]_D$=+40.8 (*c* 0.13, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3020, 2958, 2937, 2853 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 6.22 (1H, s, H-5), 3.03 (1H, sept, *J*=6.9 Hz, H-11), 1.77 (3H, s, H-15), 0.99 (3H, d, *J*=5.9 Hz, H-14), 0.96 (3H, d, *J*=6.9 Hz, H-12/13), 0.95 (3H, d, *J*=6.9 Hz, H-12/13); ^{13}C NMR—135.3 (C, C-4), 135.0 (C, C-7), 127.9 (C, C-6), 120.4 (CH, C-5), 43.2 (CH, C-1), 34.6 (CH, C-10), 31.8 (CH_2, C-9), 31.2 (CH_2, C-3), 28.2 (CH_2, C-2), 28.1 (CH, C-11), 24.1 (CH_3, C-15), 23.9 (CH_2, C-8), 21.0 (CH_3, C-12/13), 20.5 (CH_3, C-14), 20.4 (CH_3, C-12/13); **16c**: Oil. (4.5 mg, 15%; R_t 12.0 min, *n*-hexane). The sample was unstable under laboratory conditions following purification and was converted into **22c** and **26c** within 24 h. Tentative identification by ^{1}H NMR only (however, cf. data for compound **16** in the preceding paper).[3] ^{1}H NMR δ ($CDCl_3$) ppm: 6.10 (1H, s, H-5), 4.93 (1H, dd, *J*=6.4, 6.4 Hz, H-3), 2.53 (1H, m, H-2), 2.07 (1H, m, H-7), 1.77 (3H, s, H-15), 0.94 (3H, d, *J*=6.6 Hz, H-12/13), 0.88 (3H, d, *J*=6.6 Hz, H-12/13), 0.85 (3H, d, *J*=6.4 Hz, H-14); **20c**: Oil. (2.5 mg, 8%; R_t 31.3 min, 10% EtOAc/*n*-hexane). $[\alpha]_D$=−11.0 (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2959, 2928, 1734, 1717, 1458 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.81 (1H, dd, *J*=1.6, 1.6 Hz), 6.05 (1H, s), 2.12 (3H, s), 2.07 (3H, s), 0.95 (6H, d, *J*=6.7 Hz), 0.94 (3H, d, *J*=6.7 Hz), 0.93 (3H, d, *J*=7.1 Hz), 0.90 (3H, d, *J*=6.5 Hz), 0.65 (3H, d, *J*=6.8 Hz)—see Table 2; ^{13}C NMR—see Table 1; CIMS: 474 [M^+] (7), 431 (2), 281 (6), 237 (100), 219 (14); HREIMS *m/z* (rel. int.) 474.3704 [M^+, $C_{30}H_{50}O_4$ requires 474.3709] (3), 432 (3), 416 (3), 237 (100), 219 (36), 195 (100); **26c**: Oil. (1 mg, 3%; R_t 50.8 min, 13% EtOAc/*n*-hexane and 47.3 min in 10% EtOAc/*n*-hexane). $[\alpha]_D$=+78.4 (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3580, 3393 (br), 3028, 2963, 2936, 2872, 1732, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.91 (1H, d, *J*=2.6 Hz), 2.58 (1H, d sept, *J*=6.9, 6.7 Hz), 1.28 (3H, s), 0.93 (3H, d, *J*=6.7 Hz), 0.89 (3H, d, *J*=6.7 Hz), 0.75 (3H, d, *J*=6.7 Hz)—see Table 4; ^{13}C NMR—see Table 3; CIMS: 253 [M+1−H_2O] (10), 237 (41), 235 (60), 225 (100); HREIMS *m/z* (rel. int.) 238.1928 [M^+-O_2, $C_{15}H_{26}O_2$ requires 238.1933] (5), 224 (16), 209 (30), 195 (100); **27c**, **28c** and **29c**—isolated as a mixture with **13c** and identified by characteristic resonances in ^{1}H NMR (ca. 2% each from ^{1}H NMR of the crude mixture):[22] δ_H 5.61 (H-5) ppm; δ_H 5.90 (H-5), 4.72 (H-15a/b), 4.66 (H-15a/b) ppm; δ_H 5.48 (H-5), 5.30 (H-3) ppm, respectively; **30c**: Oil. (1.5 mg, 5%; R_t 11.7 min, 10% EtOAc/*n*-hexane). Sample was unstable under laboratory conditions after isolation and decomposed into **13c**, **22c** and **26c** within 24 h. Tentative identification by ^{1}H NMR

only (but cf. results for **30a**). ^{1}H NMR δ ($CDCl_3$) ppm: 9.75 (1H, s, H-5), 5.33 (1H, s, H-5′), 2.55 (1H, ddd, J=16.2, 12.0, 4.8 Hz, H-3), 2.46 (1H, d sept, J=6.6, 6.6 Hz, H-11′), 2.38 (1H, ddd, J=16.8, 12.4, 4.8 Hz, H-3), 2.07 (3H, s, H-15), 1.37 (3H, s, H-15), 0.94 (3H, d, J=6.7 Hz), 0.93 (3H, d, J=6.6 Hz), 0.92 (3H, d, J=6.6 Hz), 0.90 (3H, d, J=6.6 Hz), 0.87 (3H, d, J=6.6 Hz), 0.64 (3H, d, J=6.9 Hz); **31c**:[10,22] Oil. (3 mg, 10%; R_t 20.3 min, 10% EtOAc/*n*-hexane). $[\alpha]_D$=−41.5 (*c* 0.05, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2928, 2855, 1707, 1458 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 2.55 (1H, ddd, J=17.1, 8.8, 5.9 Hz, H-3), 2.34 (1H, ddd, J=17.1, 9.1, 6.4 Hz, H-3), 2.13 (3H, s, H-15), 1.07 (3H, d, J=6.3 Hz, H-14), 0.89 (3H, d, J=6.3 Hz, H-12/13), 0.85 (3H, d, J=6.4 Hz, H-12/13).

4.1.3. Ozonolysis of 2b. A cooled (−78°) solution of **2b** (20 mg) in CH_2Cl_2/CH_3OH (1:1, 40 ml) was subjected to ozonolysis and the resulting oil (18 mg, 90%, w/w) was separated by HPLC (32% EtOAc/*n*-hexane). Compound **22b** (13 mg, 65%) was isolated as a broad peak (R_t 48.3 min) which was found to consist of two interconverting acetals which could not be separated chromatographically and were assigned as a mixture by 2D-NMR. *Major product* (5β-OH): ^{1}H NMR δ ($CDCl_3$) ppm: 5.17 (1H, dd, J=3.2, 3.2 Hz), 3.73 (1H, dd, J=11.5, 11.5 Hz), 3.30 (1H, dd, J=11.5, 4.4 Hz), 2.56 (1H, ddd, J=16.0, 10.0, 4.6 Hz), 2.38 (1H, m), 2.14 (3H, s), 0.87 (3H, d, J=6.5 Hz), 0.81 (3H, d, J=7.0 Hz)—see Table 4; ^{13}C NMR: see Table 3; *Minor product* (5α-OH): ^{1}H NMR δ ($CDCl_3$): 5.09 (1H, dd, J=8.9, 4.8 Hz), 3.64 (1H, dd, J=11.5, 4.9 Hz), 3.43 (1H, dd, J=11.5, 11.5 Hz), 3.06 (1H, d, J=4.8 Hz, –OH), 2.84 (1H, ddd, J=16.5, 8.7, 6.2 Hz), 2.38 (1H, m), 2.13 (3H, s), 0.90 (3H, d, J=6.5 Hz), 0.78 (3H, d, J=7.1 Hz)—see Table 4; ^{13}C NMR—see Table 3. Very many positive 'EXSY-type' peaks were seen in NOESY corresponding to interconversion of the following resonances between the two isomers: 3.73→3.43 (H-12a), 3.30→3.64 (H-12b), 2.56→2.84 (H-3), 1.96→1.83 (H-2a), 1.92→1.76 (H-6), 1.91→1.48 (H-10), 1.78→1.51 (H-2b), 1.73→1.49 (H-8α), 1.50→1.69 (H-7), 0.95→1.03 (H-1) indicating that the two 5-OH epimers are interconverting in $CDCl_3$ solution; HREIMS *m*/*z* (rel. int.) 236.1768 [M^+-H_2O, $C_{15}H_{24}O_2$ requires 236.1776] (15), 190 (1), 178 (100), 165 (26). Compound **23b**: Oil. (2 mg, 10%, R_t 12.4 min). $[\alpha]_D$=−93.1 (*c* 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3015, 2957, 2932, 2878, 1713, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 4.54 (1H, d, J=2.4 Hz), 3.47 (1H, dd, J=11.5, 11.5 Hz), 3.29 (3H, s), 3.25 (1H, dd, J=11.5, 4.5 Hz), 2.56 (1H, ddd, J=16.1, 10.5, 5.0 Hz), 2.34 (1H, m), 2.14 (3H, s), 0.84 (3H, d, J=6.3 Hz), 0.79 (3H, d, J=7.1 Hz)—see Table 4; ^{13}C NMR—see Table 3; HREIMS *m*/*z* (rel. int.) 268.2036 [M^+, $C_{16}H_{28}O_3$ requires 268.2038] (4), 237 (3), 219 (3), 195 (100), 178 (11), 150 (27), 108 (88).

4.1.4. Characterization of enols 4a–4c from treatment of 3a–3c with TFA at 233 K under an atmosphere of nitrogen. Dissolved oxygen was removed from solutions of **3a**–**3c** in $CDCl_3$ (33 mg; 1 ml) in NMR tubes as above and TFA (2 μl) was added to just-thawed solutions. The NMR tubes were immediately transferred to the NMR spectrometer (probehead pre-cooled to 233 K). Formation of compounds **4a**–**4c** was completed within 30 min and the disappearance of starting material and appearance of enols and other minor products was monitored by observing characteristic peaks in ^{1}H NMR. Some ^{13}C values of minor components were assigned from 2D NMR spectra of the crude mixtures (all δ_C and δ_H values are in $CDCl_3$/TFA at 233 K (ppm)). **3a**: 5.28 (s, H-5); **3b**: 5.39 (s, H-5); **3c**: 5.20 (s, H-5); **4a**: 6.31 (1H, s), 3.73 (3H, s), 2.72–2.53 (3H, m), 2.33 (3H, s), 1.06 (3H, d, J=6.3 Hz), 0.92 (3H, d, J=4.2 Hz)—see also Table 2; NOESY correlations were observed from δ_H 6.31 (H-5) ppm to δ_H 1.06 (H-13) ppm and δ_H 2.15 (H-7) ppm, thus proving the E stereochemistry for the enolic double bond; ^{13}C NMR—see Table 1; **4b**: 6.23 (1H, s), 3.78 (1H, dd, J=11.4, 10.3 Hz), 3.52 (1H, dd, J=11.4, 5.6 Hz), 2.28 (3H, s), 0.93 (3H, d, J=6.4 Hz), 0.88 (3H, d, J=6.6 Hz)—see Table 2; almost all peaks seen in the NOESY spectrum were positive 'EXSY-type' peaks[23] (e.g. δ_H 6.23 (H-5) correlated with δ_H 1.75 (H-11) and δ_H 0.88 (H-13) ppm) making stereochemical assignment impossible; ^{13}C NMR—see Table 1; **4c**: 6.21 (1H, s), 2.29 (3H, s), 0.93 (3H, d, J=6.4 Hz), 0.88 (3H, d, J=6.0 Hz), 0.79 (3H, d, J=5.8 Hz)—see Table 2; ^{13}C NMR—see Table 1; NOESY correlations were seen from δ_H 6.21 (H-5) ppm to δ_H 0.79 (H-12/13) ppm and δ_H 1.48 (H-7) ppm, thereby proving the *E*-stereochemistry for the enolic double bond; **6b**: 5.59 (1H, s, H-5), 4.01 (1H, dd, J=8.8, 8.5 Hz, H-12), 3.44 (1H, dd, J=9.1, 8.8 Hz, H-12), 1.66 (3H, s, H-15), 0.97 (3H, coupling obscured, H-13), 0.90 (3H, coupling obscured, H-14); ^{13}C NMR—138.6 (C, C-4), 125.6 (CH, C-5), 83.1 (C, C-6), 69.7 (CH_2, C-12), 46.7 (CH, C-1), 43.6 (CH, C-7), 34.1 (CH_2, C-9), 30.5 (CH_2, C-3), 30.1 (CH, C-10), 23.8 (CH_3, C-15); **13a**: 6.11 (1H, s, H-5), 3.86 (1H, q, J=7.3 Hz, H-11), 3.60 (3H, s, 12-OMe); ^{13}C NMR—177.2 (C, C-12), 138.8 (C, C-4), 130.8 (C, C-6), 127.3 (C, C-7), 118.9 (CH, C-5), 30.7 (CH_2, C-3), 24.9 (CH_2, C-8), 14.9 (CH_3, C-13); **13c**:[22] 6.25 (1H, s, H-5), 3.03 (1H, coupling obscured, H-11), 1.79 (3H, s, H-15); ^{13}C NMR—136.2 (C, C-4), 135.2 (C, C-7), 131.8 (C, C-6), 119.6 (CH, C-5), 42.1 (CH, C-1), 30.8 (CH_2, C-3), 24.2 (CH_2, C-15), 22.6 (CH_3, C-12/13), 20.7 (CH_3, C-12/13); **18b**: 5.22 (1H, s, H-5), 4.23 (1H, d, J=10.4 Hz, H-12), 3.39 (1H, d, J=10.4, H-12), 2.21 (3H, s, H-15), 0.98 (3H, coupling obscured, H-13), 0.88 (3H, coupling obscured, H-14); ^{13}C NMR—91.2 (CH, C-5), 65.4 (CH_2, C-12), 11.8 (CH_3, C-13); **22a**: 9.96 (1H, d, J= 5.1 Hz, H-5), 3.73 (3H, s, 12-OMe), 2.27 (3H, s, H-15); ^{13}C NMR—212.6 (C, C-4), 206.2 (C, C-5), 177.2 (C, C-12), 49.9 (CH, C-6); **22c**: 9.96 (1H, d, J=6.2 Hz, H-5), 2.86 (1H, m, H-3), 2.36 (1H, m, H-3); ^{13}C NMR—211.3 (C, C-4), 209.4 (CH, C-5), 51.4 (CH, C-6).

4.1.5. Further conversions of 4a–4c in the presence of O_2 (at 273, 253 and 233 K, respectively). The valve of the NMR tube was opened and the temperature of the probehead was raised. Conversion of **4a** was completed in 100 min, when 6β-aldehyde **22a** (δ_H 9.95 d, J=5.0 Hz (H-5) ppm) accounted for 65% of the mixture; endoperoxide **26a** (δ_H 9.93 d, J=2.6 Hz (H-5) ppm) reached a maximum after 20 min and then declined, being completely replaced by a peak for H-5 of the 1,2,4-trioxane ring of **1** (consistent with the role for **26a** as an intermediate en route to **1** from **4a**) which accounted for ca. 35% of the mixture by the end of the experiment. HPLC yielded: **1**[19] (8 mg, 25%); and **22a**: Oil. (17 mg, 51%, R_t 62.2 min, 22% EtOAc/*n*-hexane). $[\alpha]_D$=−59.2 (*c* 0.33, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2932, 2853, 1715, 1461 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.95

(1H, d, J=5.0 Hz), 3.67 (3H, s), 2.15 (3H, s), 1.17 (3H, d, J=6.9 Hz), 0.96 (3H, d, J=6.4 Hz)—see Table 2; ^{13}C NMR—see Table 1. HREIMS: m/z (rel. int.) 282.1776 (M^+, $C_{15}H_{26}O_4$) (1), 267 (10), 241 (60), 223 (75), 193 (100).

Conversion of **4b** was completed in 100 min, and **1b** accounted for 85% of the mixture by the end of the reaction: ^{1}H NMR: 5.30 (1H, s), 3.83 (1H, dd, J=11.6, 4.0 Hz), 3.51 (1H, dd, J=11.8, 11.6 Hz), 1.42 (3H, s), 0.96 (3H, d, J=6.2 Hz), 0.80 (3H, d, J=7.2 Hz). HPLC yielded: **1b**: Oil. (16 mg, 48%). $[\alpha]_D$=+18.3 (c 0.14, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3013, 2928, 2855, 1456, 1379 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 5.21 (1H, s), 3.74 (1H, dd, J=11.2, 3.7 Hz), 3.46 (1H, dd, J=11.8, 11.2 Hz), 2.65 (1H, m), 1.43 (3H, s), 0.96 (3H, d, J=6.3 Hz), 0.78 (3H, d, J=7.2 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS m/z (rel. int.) 268.1675 [M^+, $C_{15}H_{24}O_4$ requires 268.1675] (0.5), 252.1732 [M^+-O, $C_{15}H_{24}O_3$ requires 252.1725] (3), 236.1775 [M^+-O_2, $C_{15}H_{24}O_2$ requires 236.1776] (18), 178 (100), 165 (81); **6b**: Oil. (1 mg, 2%; R_t 10.9 min, 7.5% EtOAc/n-hexane); and **18b**: Oil. (3 mg, 9%).

Conversion of **4c** was completed in 3 h and **22c** (δ_H 9.95, J=6.5 Hz (H-5) ppm) accounted for about 65% of the mixture; compound **26c** (δ_H 9.89, d, J=2.5 Hz (H-5) ppm) accounted for ca. 35%. HPLC (30% EtOAc/n-hexane) yielded **13c**:[22] (1.5 mg, 5%; R_t 9.1 min); **22c**: Oil. (18 mg, 55%; R_t 10.7 min). $[\alpha]_D$=−34.5 (c 0.68, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3018, 2959, 2872, 1709, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 9.97 (1H, d, J=6.5 Hz), 2.69–2.64 (2H, m), 2.33 (1H, ddd, J=16.9, 10.0, 6.0 Hz), 2.14 (3H, s), 0.94 (3H, d, J=6.6 Hz), 0.93 (3H, d, J=6.6 Hz), 0.91 (3H, d, J=6.6 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS m/z (rel. int.) 238.1929 [M^+, $C_{15}H_{26}O_2$ requires 238.1933] (18), 220 (20), 195 (100), 177 (55); and **26c** (8 mg, 25%; R_t 34.6 min).

4.1.6. Further conversions of 4a–4c under an atmosphere of N_2 at 283 K. The valve of the NMR tube was kept closed as the temperature of the probehead was raised, and the reaction was completed after 50–100 min. The crude product from **4a** was subjected to HPLC yielding: **1**[19] (2 mg, 6%, R_t 30.3 min, 15% EtOAc/n-hexane); **13a**:[13] (1 mg, 4%, R_t 8.7 min, 15% EtOAc/n-hexane); **18a**: Oil. (1 mg, 4%, R_t 21.8 min, 15% EtOAc/n-hexane). ^{1}H NMR δ ($CDCl_3$) ppm: 9.54 (1H, d, J=5.2 Hz), 3.67 (3H, s), 2.15 (3H, s), 1.14 (3H, d, J=7.2 Hz), 0.92 (3H, d, J=6.4 Hz)—see Table 2; ^{13}C NMR—see Table 1; **19**:[3] (0.5 mg, 1%). ^{1}H NMR δ ($CDCl_3$) ppm: 6.08 (1H, s, H-5), 2.95 (1H, dq, J=7.0, 7.2 Hz, H-11), 2.64 (1H, ddd, J=17.6, 9.2, 5.0 Hz, H-3), 2.45 (1H, ddd, J=17.6, 8.7, 7.0 Hz, H-3), 2.17 (3H, s, H-15), 1.23 (3H, d, J=7.2 Hz, H-13), 0.99 (3H, d, J=6.2 Hz, H-14); **22a** (21 mg, 66%); and **26a**:[13] Oil. (0.5 mg, 1%); ^{1}H NMR δ ($CDCl_3$) ppm: 9.90 (1H, d, J=1.7 Hz), 3.64 (3H, s), 3.19 (1H, m), 1.28 (3H, s), 1.22 (3H, d, J=7.2 Hz), 0.89 (3H, d, J=6.5 Hz)—see Table 4; ^{13}C NMR—see Table 3. On HPLC separation, there was some conversion of **26a** into **1**.

The crude mixture from **4b** (δ_H and δ_C values in $CDCl_3$/TFA at 283 K) consisted mostly of **18b**: δ_H 5.22 (1H, s, H-5), 4.21 (1H, d, J=10.7 Hz, H-12), 3.37 (1H, d, J=10.7 Hz, H-12), 2.18 (3H, s, H-15), 0.99 (3H, d, J=7.5 Hz, H-13), 0.88 (3H, d, J=5.3 Hz, H-14); δ_C 210.6 (C-4), 91.6 (CH, C-5), 65.5 (CH_2, C-12), 42.3 (CH, C-1), 39.3 (CH, C-6), 38.4 (CH_2, C-3), 35.6 (CH, C-7), 34.9 (CH_2, C-9), 34.6 (CH, C-10), 32.7 (CH, C-11), 30.2 (CH_2, C-8), 30.1 (CH_3, C-15), 20.7 (CH_2, C-2), 19.9 (CH_3, C-14), 11.8 (CH_3, C-13); minor peaks due to **6b** were also identified by 2D-NMR of the mixture: δ_H 5.57 (1H, s, H-5), 3.99 (1H, dd, J=8.4, 8.4 Hz, H-12), 3.41 (1H, dd, J=8.9, 8.9 Hz, H-12), 1.65 (3H, s, H-15), 0.95 (3H, d, J=6.8 Hz, H-13), 0.90 (3H, d, J=7.0 Hz, H-14); δ_C 138.2 (C-4), 126.1 (CH, C-5), 70.1 (CH_2, C-12), 47.1 (CH, C-1), 44.1 (CH, C-7), 34.4 (CH_2, C-9), 30.8 (CH_2, C-3), 30.3 (CH, C-10). HPLC yielded **6b**: (2 mg, 5%, R_t 10.9 min, 7.5% EtOAc/n-hexane); **18b**: (21 mg, 64%; R_t 38.8 min, 30% EtOAc/n-hexane); and **19b**: Oil. (3 mg, 9%; R_t 34.7 min, 7.5% EtOAc/n-hexane). $[\alpha]_D$=−104.5 (c 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2964, 2928, 2872, 1713, 1661, 1462 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 6.04 (1H, s), 3.69 (1H, dd, J=10.3, 4.3 Hz), 3.48 (1H, dd, J=10.3, 10.3 Hz), 2.62 (1H, ddd, J=17.8, 10.0, 4.8 Hz), 2.43 (1H, ddd, J=17.8, 9.0, 6.1 Hz), 2.13 (3H, s), 0.94 (3H, d, J=6.2 Hz), 0.87 (3H, d, J=7.1 Hz)—see Table 2; ^{13}C NMR—see Table 1; HREIMS m/z (rel. int.) 236.1773 [M^+, $C_{15}H_{24}O_2$ requires 236.1776] (8), 218 (10), 178 (100), 165 (24).

HPLC (30% EtOAc/n-hexane) of the crude product from **4c** yielded: **13c** (1.5 mg, 4%); **22c** (6.8 mg, 20%); and **26c** (2 mg, 6%).

4.2. Ozonolysis of 2a and 2c

The same reaction procedures were followed as for the ozonolysis of **2b**, yielding compounds **22a** and **22c**—see previous sections for physical properties.

4.2.1. Compounds 24a, 25a and 25c from spontaneous autoxidation of aldehydes 18a, 22a and 22c. Compound **24a**: Oil. $[\alpha]_D$=−23.1 (c 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2928, 2854, 1734, 1717, 1458 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 3.69 (3H, s), 2.15 (3H, s), 1.16 (3H, d, J=7.1 Hz), 0.90 (3H, d, J=6.4 Hz)—see Table 4; ^{13}C NMR—see Table 3; HREIMS m/z (rel. int.) 280.1669 [M^+-H_2O, $C_{16}H_{24}O_4$ requires 280.1675] (2), 268 (5), 248 (8), 241 (30), 223 (100), 193 (96). Compound **25a**: Oil. $[\alpha]_D$=−38.8 (c 0.33, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2930, 2855, 1730, 1717, 1456 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 3.67 (3H, s), 2.95 (1H, br), 2.74 (1H, ddd, J=16.1, 10.1, 5.3 Hz), 2.38 (1H, ddd, J=16.1, 10.0, 6.4 Hz), 2.36 (1H, m), 2.16 (3H, s), 1.24 (3H, d, J=7.0 Hz), 0.89 (3H, d, J=6.3 Hz)—see Table 4; ^{13}C NMR—see Table 3; HREIMS m/z (rel. int.) 280.1678 [M^+-H_2O, $C_{16}H_{24}O_4$ requires 280.1675] (5), 267 (18), 241 (50), 223 (65), 193 (100). Compound **25c**: Oil. (R_t 50.8 min, 13% EtOAc/n-hexane). $[\alpha]_D$=−13.3 (c 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2961, 2932, 1715, 1462 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) ppm: 2.98 (1H, dd, J=4.4, 4.4 Hz), 2.74 (1H, ddd, J=16.5, 10.1, 4.5 Hz), 2.39 (1H, ddd, J=16.5, 8.9, 5.2 Hz), 2.15 (3H, s), 0.99 (3H, d, J=6.6 Hz), 0.91 (3H, d, J=6.8 Hz), 0.89 (3H, d, J=6.7 Hz)—see Table 4; ^{13}C NMR—see Table 3; HREIMS m/z (rel. int.) 254.1886 [M^+, $C_{15}H_{26}O_3$ requires 254.1882] (0.5), 236 (50), 208 (44), 193 (100), 151 (77).

Compounds **18b** and **22b** did not undergo autoxidation in $CDCl_3$ solution.

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