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Syntheses of dihydroartemisinic acid and dihydro-*epi*-deoxyarteannuin B incorporating a stable isotope label at the 15-position for studies into the biosynthesis of artemisinin

Lai-King Sy, Nian-Yong Zhu and Geoffrey D. Brown*

Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, People's Republic of China

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Abstract—[15-$^{13}C^{2}H_3$]-Dihydroartemisinic acid (**3a**) and [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteannuin B (**7b**), intended for evaluation in vivo as biosynthetic precursors to artemisinin, have been obtained from a reconstructive synthesis. The decalenone acid **8** from acid degradation of artemisinin (**1**) serves as a common intermediate: following addition of labeled methyl Grignard reagent to **8**, either labeled precursor can be prepared in good yield by varying the work-up conditions employed. It is shown that both compounds are prone to autoxidation on storage and that the products of such oxidation and subsequent rearrangement reactions might be confused with bona fide metabolites when using these labeled precursors in feeding experiments designed to determine the biosynthetic route to artemisinin in *Artemisia annua*.

1. Introduction

Artemisinin (qinghaosu) (**1**), first reported as a constituent of *Artemisia annua* by Chinese scientists in 1972[1] as the result of a screening programme for natural products with anti-malarial activity, is now one of the most promising new drugs for treating malaria and appears to be particularly useful in the treatment of multi-drug resistant forms of *Falciparum* malaria in both Asia and Africa. The 1,2,4-trioxane ring in artemisinin, which is responsible for its anti-malarial activity, has attracted the attention of many natural product chemists and a number of attempts to determine the biosynthetic origins of this unique system have been described. Although most investigators agree that artemisinic acid (**2**)[2] or its 11,13-dihydro analogue, dihydroartemisinic acid (**3**),[3] both of which are natural products present in substantial amounts in *A. annua*,[4] are late precursors in the biogenesis of artemisinin, the precise mechanism by which this remarkable transformation of **2/3** to **1** occurs in vivo has remained elusive. On the one hand, experimental evidence has been presented by Nair[5] for the natural product arteannuin B (**4**) (which is a metabolite of artemisinic acid)[6] and by Jain[7] for its 11,13-dihydro analogue, dihydroarteannuin B (**5**), being converted into **1** by cell free extracts of *A. annua*. On the other hand, Wang et al. found that arteannuin B was *not* transformed into artemisinin,[8] and went on to provide evidence that two alternative oxidation products from compounds **2/3**, *epi*-deoxyarteannuin B (**6**) and its 11,13-dihydro analogue, dihydro-*epi*-deoxyarteannuin B (**7**), are the actual metabolic intermediates derived from **2/3** *en route* to **1** (Fig. 1).[9] The results of Wang et al. were based on leaf homogenate studies, using precursors which were radio-labeled at the 15-position, while Nair et al. reached their conclusions without the benefit of isotopic labeling.

We reasoned that the best way to resolve the apparent controversy, regarding the mechanism for the conversion of **3** into **1** in vivo, would be to synthesize both dihydroartemisinic acid (**3**) and dihydro-*epi*-deoxyarteannuin B (**7**) incorporating stable isotope labels, for use as (postulated) biogenetic precursors which could then be fed to whole plants of *A. annua*. The advantage of using stable-isotope labeled precursors is that their metabolism can be monitored directly by NMR, yielding more extensive and more immediate information on the chemical nature of transformations occurring in vivo than is possible for radio-labeling studies. We herein describe a single synthetic route by which both [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid (**3a**) and [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteannuin B (**7b**) were synthesized from artemisinin (**1**) via a common intermediate **8**. The use of these labeled precursors in feeding experiments with whole plants of *A. annua* will be described in a future communication.

Keywords: *Artemisia annua*; artemisinin; dihydroartemisinic acid; dihydro-*epi*-deoxyarteanuin B; isotopic labeling.

* Corresponding author. Tel.: +852-2859-7915; fax: +852-2857-1586; e-mail: gdbrown@hkucc.hku.hk.

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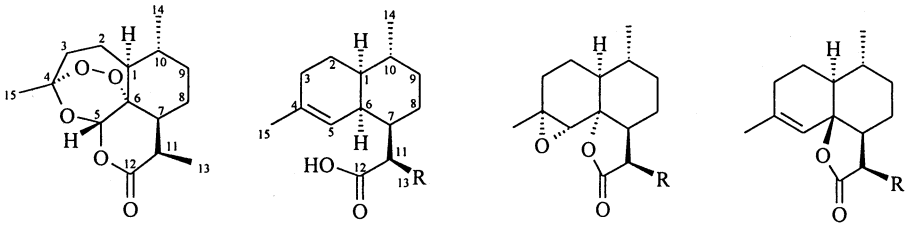


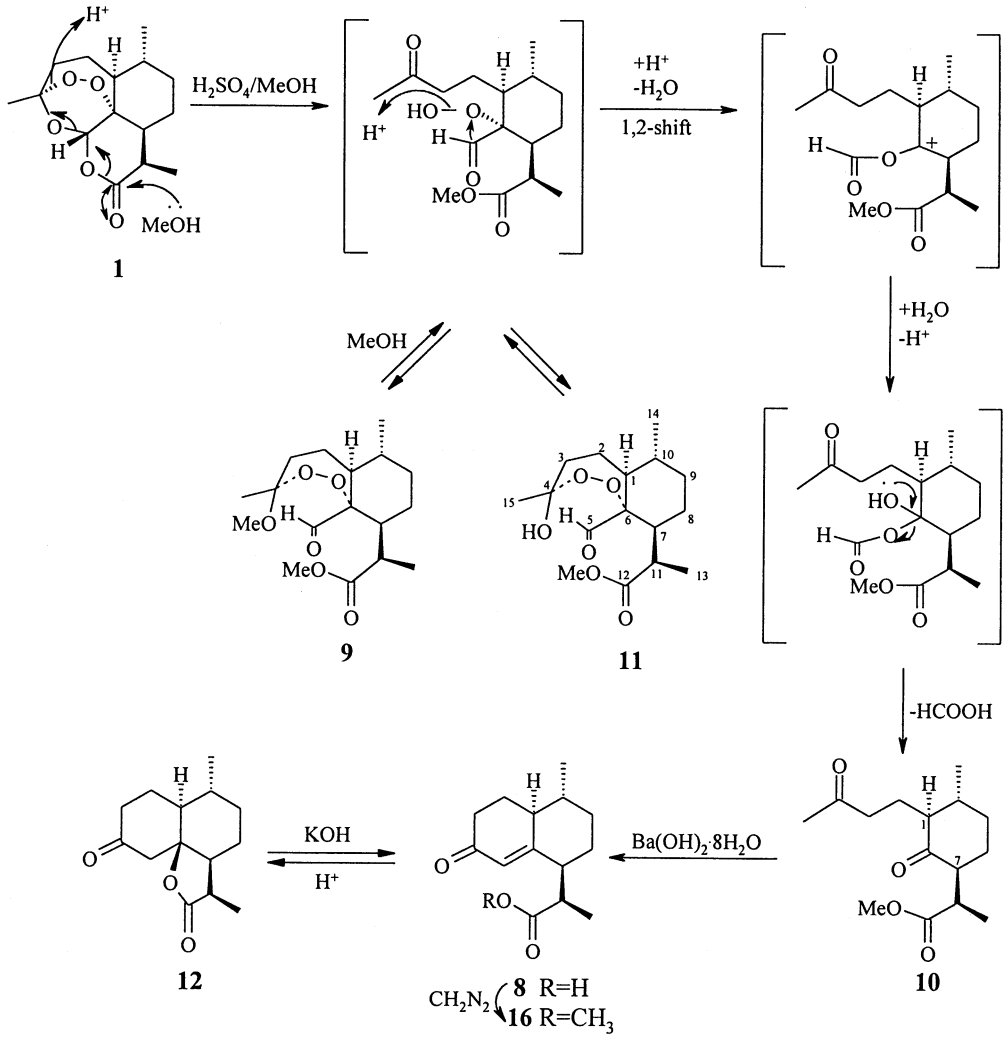
1 **2** R=CH_2 **4** R=CH_2 **6** R=CH_2

3 R=CH_3 **5** R=CH_3 **7** R=CH_3

Figure 1. Natural products from *A. annua* which have been proposed as late biosynthetic precursors of artemisinin (**1**), on the basis of experimental evidence.

2. Results and discussion

The acid degradation of artemisinin (**1**) and its derivatives has been extensively studied and found to yield a wide variety of products such as compounds **9** and **10**, depending upon the precise conditions employed.[10–12] Epimerization at the 1- and 7-positions of the acid degradation products may also become a problem if the reaction conditions are not carefully controlled.[10,12] In the context of this study, we sought to maximize the yield of the *nor*-sesquiterpene **10**[10,11] which can then be converted into the decalenone acid **8** by known reactions.[10,13] Whilst optimizing the acid degradation procedure for converting **1** into **10**, we also occasionally obtained the novel compound **11** as a very minor product (Scheme 1, Tables 1 and 2). Based on both this finding and a detailed recent study of the acid degradation of the closely related compound peroxofabianane,[14] we have proposed a mechanism for the acid degradation of **1** to **10**, involving the 1,2-shift of an aldehyde group to the internal oxygen atom of a tertiary hydroperoxide inter-



Scheme 1. Proposed mechanism for the acid degradation of **1** to **10** in H_2SO_4/MeOH and its application in the reconstructive synthesis of decalenone intermediate **8**, which is common to the syntheses of both dihydroartemisinic acid and dihydro-*epi*-deoxyarteannuin B.

Table 1. ^{13}C NMR data (δ, ppm) for novel compounds described in Schemes 1–4 (rigorous assignments made by the 2D-NMR techniques HSQC, HMBC, ^{1}H–^{1}H COSY in all cases). Splittings observed for labeled compounds (suffix 'a' or 'b') are given in Section 3

Position	**11**	**14a**	**15a**	**17**	**18**	**19**	**20**	**21**	**22**	**23a**	**24a**	**25a**	**26a**
1	59.8	43.2	134.6	44.9	44.7	43.1	45.4	133.7	45.0	42.2	46.8	45.0	40.0
2	22.4	27.8	27.8	23.0	24.3	27.8	26.2	27.9	26.0	27.8	28.5	24.5	28.8
3	41.5	31.2	28.7	34.9	35.6	31.2	27.7	28.9	116.7	119.1	32.3	27.7	30.5
4	106.0	137.9	–[a]	68.6	68.4	137.4	143.7	132.1	130.1	131.8	29.4	30.3	31.1
5	200.4	119.8	123.5	124.4	124.9	119.9	120.4	124.0	118.2	26.3	35.8	123.3	130.1
6	94.2	131.9	127.9	142.9	142.8	131.1	145.1	128.2	136.6	33.4	133.3	140.0	–[a]
7	49.1	127.2	40.1	47.5	46.9	127.8	47.8	40.3	47.5	43.7	128.5	47.4	47.0
8	25.2	25.3	23.3	32.7	31.7	25.4	32.9	23.4	33.7	25.8	24.5	33.4	26.9
9	35.5	31.5	27.8	35.6	35.5	31.5	35.6	27.6	35.5	35.5	30.6	35.9	29.9
10	32.0	34.2	33.2	38.3	39.7	34.2	38.6	33.2	37.7	27.8	35.8	38.3	40.2
11	37.9	40.4	43.1	41.0	41.1	40.5	44.1	43.6	41.1	42.4	41.0	41.1	41.0
12	176.2	180.1	179.5	177.1	176.8	175.5	177.0	176.9	177.0	183.4	179.6	182.1	180.4
13	18.0	15.1	15.5	16.3	15.7	15.3	15.9	15.7	15.9	15.1	15.2	16.1	15.1
14	20.5	20.4	19.0	20.0	20.2	20.3	20.0	19.0	19.9	20.1	20.7	20.0	20.0
15	24.1	24.2[b]	23.1[b]	29.3	29.8	24.1	109.2	23.0	21.6	23.6[b]	17.8[b]	21.9[b]	22.0[b]
12-OMe	51.4	–	–	51.4	51.4	51.6	51.4	51.3	51.4	–	–	–	–

[a] Not resolved.
[b] ^{13}C chemical shift given is for the isotopically-normal compound — see experimental for the effects of substitution of ^{1}H by ^{2}H.

Table 2. ^{1}H NMR assignments (δ, ppm) of novel compounds reported in Schemes 1–4 (rigorous assignments made by the 2D-NMR techniques HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY in all cases). Splittings for labeled compounds (suffix 'a' or 'b') are given in Section 3

Position	**11**	**14a**	**15a**	**17**	**18**	**19**	**20**	**21**	**22**	**23a**	**24a**	**25a**	**26a**
1	1.15	1.62	–	1.53	1.49	1.61	1.62	–	1.66	1.21	1.52	1.48	1.80
2α	1.80	2.09	2.19	1.89	1.88	2.09	1.82	2.17	2.36	2.15	1.80	1.74	2.05
2β	1.32	1.18	1.96	1.55	1.51	1.19	1.64	1.93	2.16	2.05	1.19	1.61	1.00
3α	1.93	2.03	1.96	1.56	1.47	2.03	2.16	1.94	5.26	5.29	1.50	1.13	0.93
3β	2.20	2.13	2.06	1.64	1.71	2.13	2.27	2.06	–	–	1.65	1.56	2.05
4	–	–	–	–	–	–	–	–	–	–	2.05	2.17	1.70
5α	9.90	6.11	5.55	5.26	5.29	6.11	5.82	5.51	5.37	1.57	2.43	5.11	5.27
5β	–	–	–	–	–	–	–	–	–	1.92	1.87	–	–
6	–	–	–	–	–	–	–	–	–	2.09	–	–	–
7	1.66	–	2.35	2.02	2.04	–	2.15	2.32	2.25	1.69	–	2.02	2.21
8α	1.69	2.10	1.78	1.69	1.68	2.09	–[a]	1.81	1.73	1.70	2.00	1.79	1.86
8β	1.25	2.07	1.50	1.16	1.25	1.89	–[a]	1.42	1.21	1.48	1.94	1.20	1.52
9α	0.95	1.23	1.27	1.18	1.20	1.20	1.24	1.26	1.17	1.00	1.13	1.21	1.20
9β	1.73	1.71	1.88	1.74	1.74	1.70	1.76	1.84	1.74	1.67	1.63	1.76	1.48
10	2.34	1.26	2.22	1.28	1.26	1.25	1.40	2.18	1.47	1.40	1.28	1.37	1.07
11	3.19	3.82	2.68	2.73	2.74	3.77	2.75	2.63	2.74	2.30	3.67	2.70	2.74
13	1.23	1.21	1.18	1.22	1.22	1.20	1.12	1.13	1.24	1.19	1.15	1.26	1.14
14	0.89	0.99	0.98	0.92	0.95	0.99	0.93	0.97	0.87	0.82	0.95	0.90	0.92
15	1.28	1.78[b]	1.78[b]	1.26	1.29	1.78	4.72, 4.69	1.78	1.69	1.63[b]	0.91[b]	0.92[b]	0.85[b]
12-OMe	3.64	–	–	3.67	3.67	3.65	3.66	3.64	3.68	–	–	–	–

[a] Not resolved.
[b] Often absent or seen as low intensity doublet (ca. 126 Hz) in spectra of labeled compound.

mediate, with accompanying loss of the second oxygen atom of the hydroperoxide group as water, which is shown in Scheme 1. In this mechanism, the 5-aldehyde group is ultimately eliminated as formic acid.

Optimal conditions for the preparation of **10** from **1** were found to involve short reaction times and a high concentration of acid. Treatment of **10** with barium hydroxide octahydrate then resulted in the decalenone acid **8**, which has a pronounced tendency to undergo lactonization to compound **12** under the acidic work-up conditions employed. However, subsequent conversion of the lactonized side-product **12** back to the corresponding free acid **8**, by treatment of either purified **12** or a mixture of compounds **8** and **12** with alkali, was found to be a simple and reliable procedure, which could be routinely employed to ensure a high overall yield of **8** from **10**.

Compound **8** then serves as a common intermediate for the synthesis of both of the natural products dihydroartemisinic acid and dihydro-*epi*-deoxyarteannuin B. Treatment of **8** with the Grignard reagent from methyl iodide is expected to form the tertiary allylic alcohol addition product **13** (Fig. 2). Although this putative intermediate was never actually isolated, its formation can be inferred from the differing products of reaction obtained from two alternative work-up procedures (Scheme 2). Thus, at pH 1–2, the predominant product from the Grignard addition of [$^{13}C^{2}H_3$]-methyl iodide with **8** was [15-$^{13}C^{2}H_3$]-6,7-dehydro-11,13-dihydroartemisinic acid (**14a**)[†] (Tables 1 and 2)

[†] The suffix 'a' indicates the replacement of the 15-[CH_3] group by 15-[$^{13}C^{2}H_3$]; the suffix 'b' indicates replacement by 15-[$^{13}CH_3$]; the suffix '*' indicates an unspecified isotopic enrichment at the 15-position.

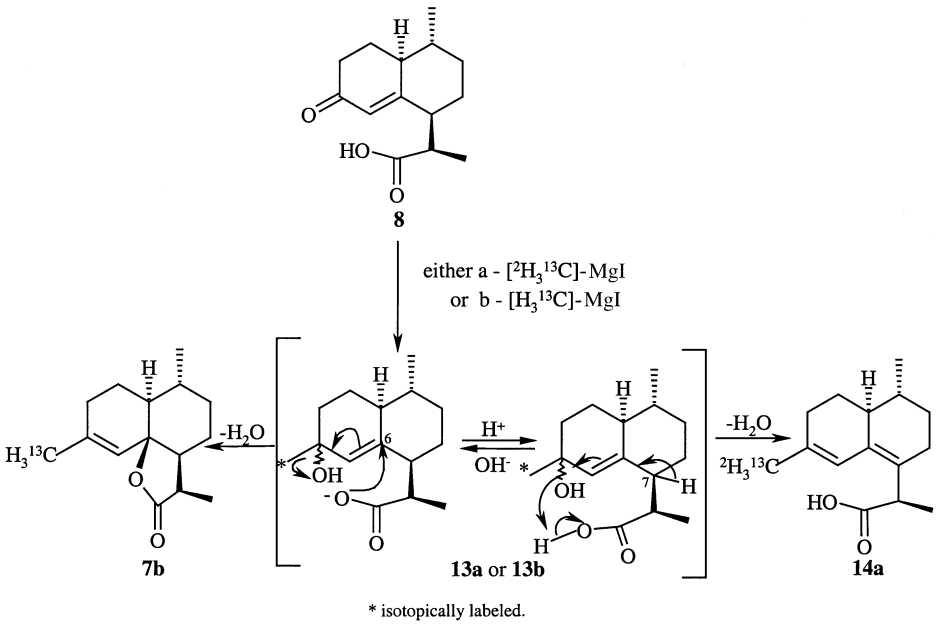
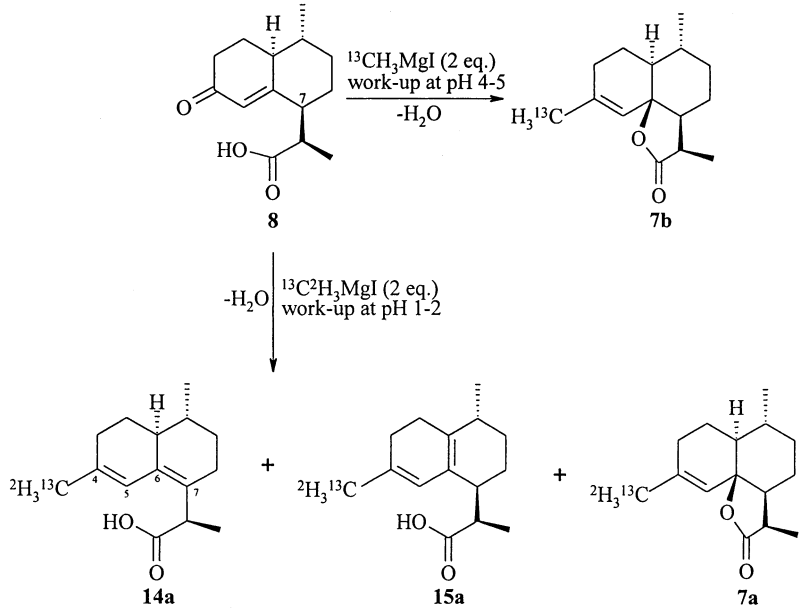


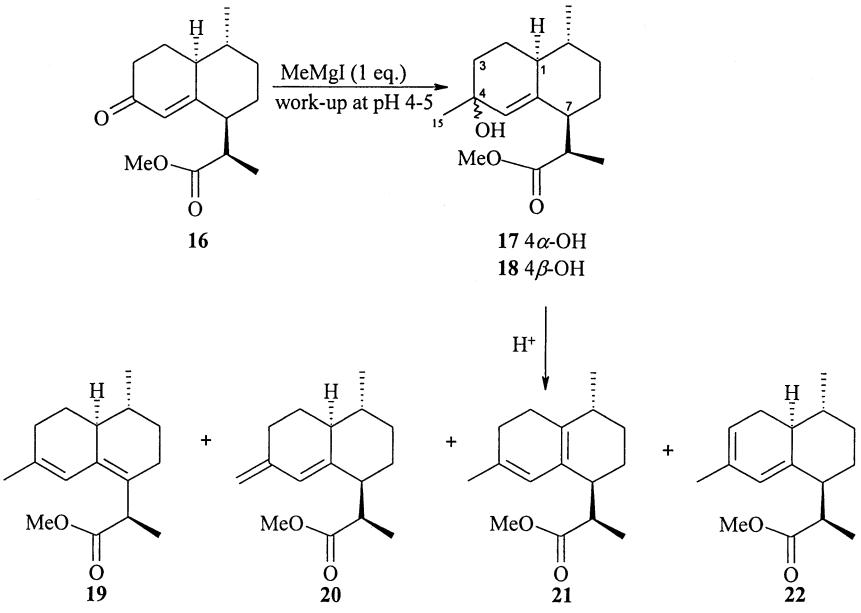
Figure 2. Participation of the 12-carboxylic group in determining the product formed from Grignard addition of methyl iodide to **8**.

which is presumed to be formed by elimination of the H-7 proton and of the allylic hydroxyl group in **13a**. At pH 4–5, by contrast, [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteannuin B (**7b**)[†] was formed as the sole product in good yield from reaction of **8** with [$^{13}CH_3$]-methyl iodide (a better yield than for our recently reported procedure for the preparation of dihydro-*epi*-deoxyarteannuin B by a more direct route).[15] On the basis of these experiments, we propose that when protonated (i.e. under acidic work-up conditions), the carboxylic acid proton in intermediate **13a** undergoes an intramolecular transfer to the 4-hydroxyl group, as shown in Fig. 2, thereby catalyzing the dehydration of **13a** to a diene. Alternatively, at pH 4–5, when the carboxylic acid group exists predominantly in its more nucleophilic conjugate base form, the carboxylate anion instead participates in S_N2' intramolecular addition at the 6-position of the allylic hydroxyl group of intermediate **13b** forming a lactone (Fig. 2).

When the decalenone methyl ester **16** (readily available by treatment of **8** with diazomethane, Scheme 1) is used in place of compound **8** in the Grignard reaction, both of the expected tertiary allylic alcohol intermediates **17** and **18** can be isolated (Tables 1 and 2) following work-up at pH 4–5. Compounds **17** and **18** are the methyl ester derivatives of the addition product **13**, whose existence could only be inferred for the Grignard reaction of the free acid **8**; isolation of these two epimers from the Grignard reaction of methyl ester **16**



Scheme 2. Conversion of key intermediate **8** into either labeled diene **14a** or biosynthetic precursor **7b** by treatment with labeled methyl magnesium iodide followed by different work-up procedures.

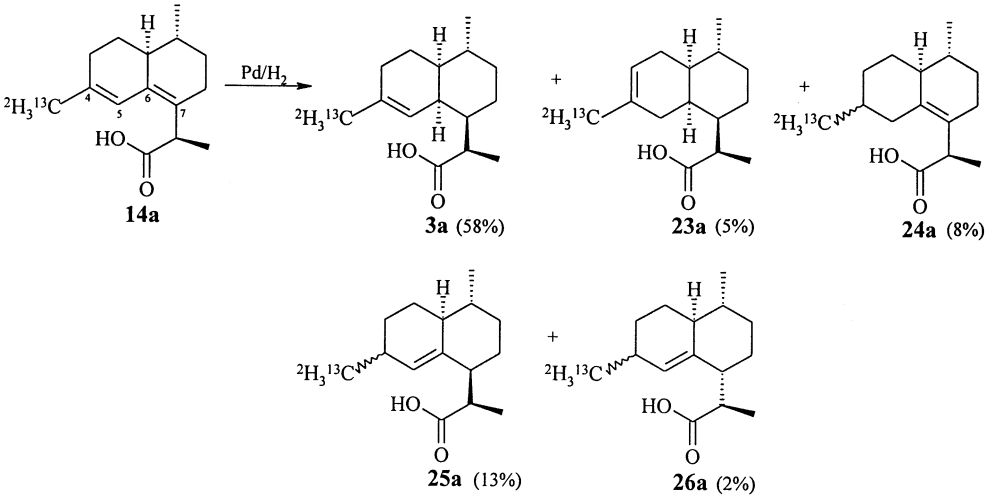


Scheme 3. Products from treatment of **16** with isotopically-normal methyl magnesium iodide.

provides support for the mechanism shown in Fig. 2. When **17/18** were treated with acid or when acidic conditions (pH 1–2) were used in the work-up of the Grignard reaction of **16**, compound **19**, which is the methyl ester derivative of **14**, was the predominant dehydration product. Three alternative dehydration products **20**–**22** were also isolated as very minor components (Scheme 3): all four products presumably arise from the E_1 elimination of the tertiary hydroxyl group in **17/18** followed by elimination of either the H-7/H-1 allylic proton (forming **19/21**) or the H-15/H-3 proton (forming **20/22**) from the resulting carbocation. (It is also possible that these diene isomers may be able to interconvert with one another under the conditions of the reaction.)[16]

Hydrogenation of the $\Delta^{4,5}\Delta^{6,7}$-diene in compound **14a**, proceeded both regiospecifically, at the more highly substituted $\Delta^{6,7}$-double bond, and stereospecifically, from the α-face, to give the desired $\Delta^{4,5}$-alkene, [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid **3a**, as the predominant product. Alternative hydrogenation products **23a**–**26a** were also formed in small amounts (Scheme 4) and could be separated by HPLC. A previous report of the synthesis of dihydroartemisinic acid labeled at the 15-position[13] employed dehydration of an intermediate, which is the 5,6-dihydro analogue of **13a/b** (see Fig. 2), resulting in both labeled dihydroartemisinic acid (**3***; Δ^4) and its unwanted Δ^3-isomer, compound **23***, as a roughly 1:1 mixture, which was difficult to separate. The current procedure, in which the desired Δ^4-isomer, compound **3a**, is obtained by hydrogenation of diene **14a** in 58% yield after chromatography, is an improvement on the previously reported procedure.

It should be noted that one disadvantage of our strategy is that there is some unavoidable loss of ^2H label from the 15-postion of **14a** during the hydrogenation step. Thus, while compound **14a** was formed largely as the 15-[$^{13}C^{2}H_3$]-isotopomer from Grignard reaction with labeled



Scheme 4. Conversion of diene intermediate **14a** into [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid **3a** (and smaller quantities of other alkenes) by hydrogenation.

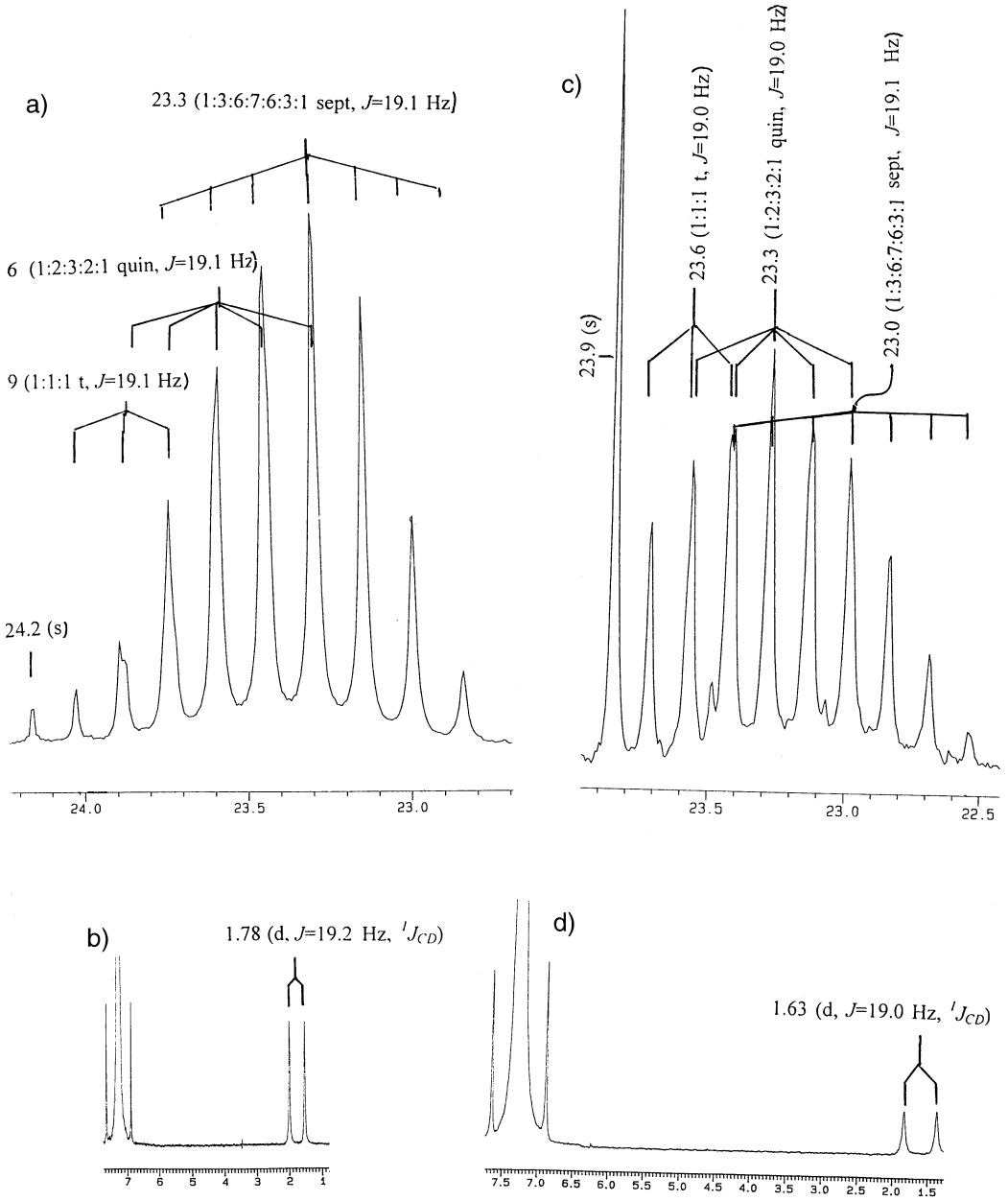


Figure 3. ^{13}C NMR and ^{2}H NMR spectra of compounds **3a** and **14a** showing ca. 32% overall depletion of ^{2}H label (but not ^{13}C) from the 15-position during the hydrogenation step. (a) Expansion of ^{13}C NMR spectrum of **14a**; (b) ^{2}H NMR spectrum of **14a**; (c) expansion of ^{13}C NMR spectrum of **3a**; (d) ^{2}H NMR spectrum of **3a**.

methyl iodide, compound **3a** from hydrogenation of **14a** was shown to be a mixture of all four possible isotopomers at the 15-position by ^{13}C NMR (Fig. 3). Interpretation of the ^{13}C NMR spectra depicted in Fig. 3 rests on two assumptions. Firstly, the carbon resonance for the 15-position is expected to be shifted upfield by approximately 0.3 ppm for each ^{2}H atom directly attached at C-15. Secondly, a single replacement of ^{1}H by ^{2}H at C-15 is predicted to produce a 1:1:1 triplet ($^{1}J_{CD}\sim19$ Hz) in the proton-decoupled ^{13}C NMR spectrum; two deuterium replacements result in a 1:2:3:2:1 quintet; and complete deuteriation at the 15-position produces a 1:3:6:7:6:3:1 septet. Such a septet pattern dominates in the expansion of the ^{13}C spectrum of **14a** at δ_C 23.3 ppm (which is the expected chemical shift for the [15-$^{13}C^{2}H_3$] isotopomer of **14** since δ_C for isotopically-normal **14** is 24.2 ppm) shown in Fig. 3, which is consistent with approximately 88% deuteriation at the 15-position (77% [15-$^{13}C_1{}^{2}H_3$]-**14**; 13% 15-[$^{13}C_1{}^{2}H_2H$]-**14**; 8% 15-[$^{13}C_1{}^{2}HH_2$]-**14**; 2% 15-[$^{13}CH_3$]-**14**). However, all three possible splitting patterns are seen superimposed on one another for the 15-position of **3a** in Fig. 3; we estimate that **3a** is approximately 56% deuteriated at the 15-position from this ^{13}C NMR expansion (35% [15-$^{13}C^{2}H_3$]-**3**, 21% [15-$^{13}C^{2}H_2H$]-**3**, 21% [15-$^{13}C^{2}HH_2$]-**3** and 24% [15-$^{13}CH_3$]-**3**). The hydrogenation step has thus resulted in the loss of approximately one deuterium atom from the 15-position. However, these NMR analyses must be considered as semi-quantitative only, because no account is taken of the poorer ability of ^{2}H to induce a nuclear Overhauser enhancement at ^{13}C as compared with ^{1}H, which may in

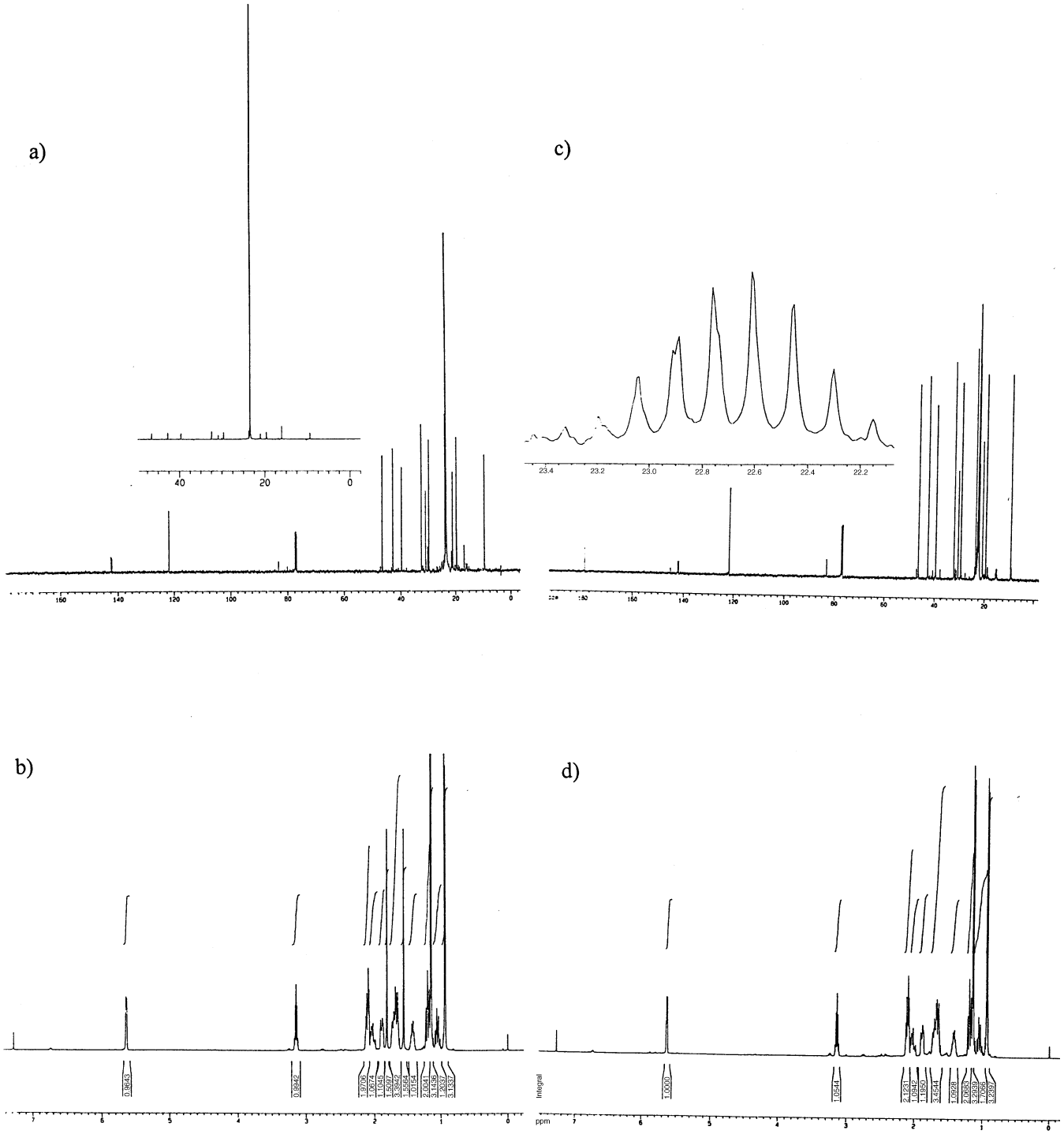


Figure 4. (a) ^{13}C NMR and (b) ^{1}H NMR spectra of **7b** showing essentially complete ^{13}C labeling at the 15-position. (c) ^{13}C NMR and (d) ^{1}H NMR spectra of **7a** showing ca. 100% retention of both ^{13}C and ^{2}H labels at the 15-position.

turn lead to an over-estimation of the extent of deuterium depletion.

Analysis of the molecular ions and daughter ions formed by fragmentation of the 7-substituent ($M^{+}-C_3H_6O_2$), which constitute the base peak in the mass spectrum of **3a**, should be a more straightforward procedure for establishing the extent of deuterium depletion relative to **14a** since each isotopomer is expected to give only a single peak differing by one mass unit. All possible isotopomers for the molecular ion then span the range 240–237 and 166–163 daltons for the base peak. However, there are believed to also be inaccuracies inherent in such mass spectral analysis because additional peaks caused by hydrogen/deuterium loss in fragmentation reactions may also occur superimposed in these same mass ranges, and these fragments are then of sufficiently similar mass in high resolution mass spectroscopy as to be confused with the isotopomer peaks under study. Mass spectroscopic analysis indicated a lesser degree of deuterium depletion for **3a** as compared to ^{13}C analysis

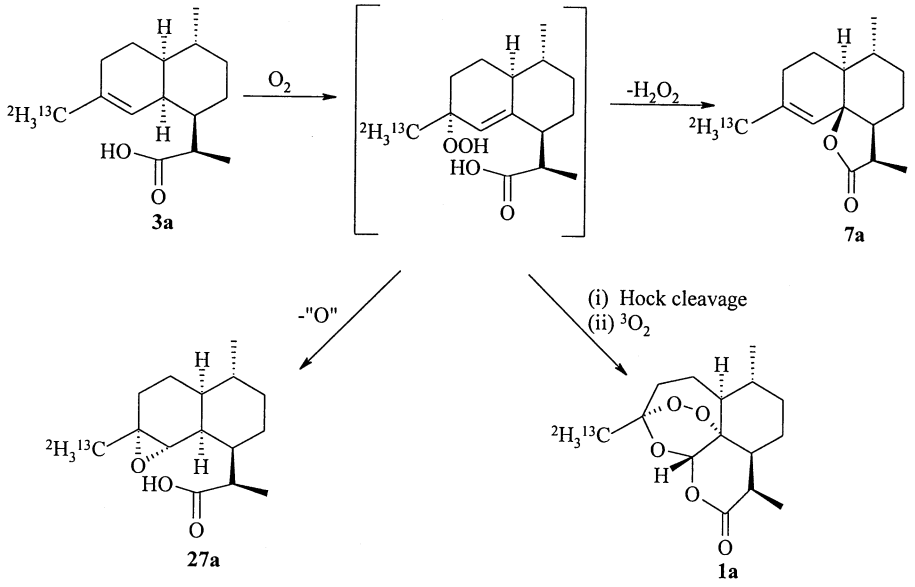
and no depletion at all in the ^{13}C label. This was confirmed by ^{2}H NMR spectroscopy (Fig. 3) in which signal for the 15-position appears entirely as a doublet ($^{1}J_{CD}$ ca. 19 Hz) for both **3a** and **14a**—any replacement of ^{13}C by ^{12}C would be expected to lead to the appearance of a singlet peak in ^{2}H NMR.

^{13}C, ^{2}H and mass spectral analyses of the other minor products **23a**–**26a** from hydrogenation of **14a** indicated varying degrees of depletion in the ^{2}H label at the 15-position, and we believe that this effect might therefore be ascribed to the exchange of the allylic ^{2}H atoms at the 15-position of **14a** with H radicals adsorbed on the Pd catalyst surface. The effect is particularly marked in compound **23a** which has also clearly undergone double bond isomerization (again probably by a radical process).

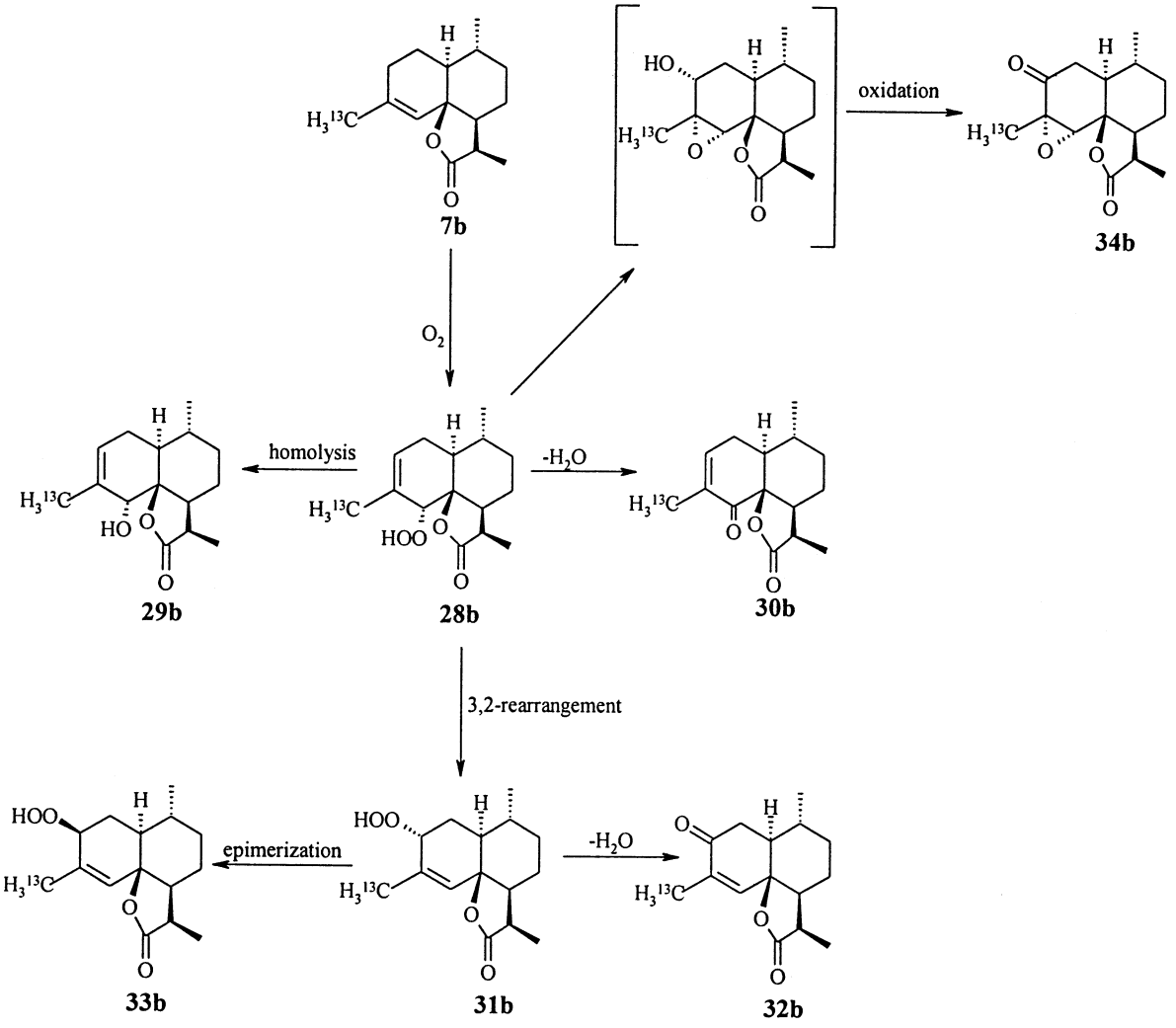
The ^{1}H and ^{13}C NMR spectra of [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteannuin B (**7b**) indicated ca. 100% incorporation of ^{13}C label at the 15-position as expected (Fig. 4: the corresponding spectra for [15-$^{13}C^{2}H_3$]-dihydro-*epi*-deoxyarteannuin B (**7a**), showing close to 100% incorporation of both ^{13}C and ^{2}H labels at the 15-position is also shown for comparison). We judged that the extent of stable isotope-labeling determined for both compounds **3a** and **7b** was sufficiently high that both labeled precursors might be used in feeding experiments with *A. annua* in order to delineate the biosynthesis of artemisinin (**1**) by studying the ^{13}C and/or ^{2}H NMR spectra of extracts and purified compounds which would be isolated from the fed plants. The labeled precursors **3a** and **7b** were stored in the freezer at −20°C whilst awaiting the growth of *A. annua* plants to be used in such feeding experiments. Surprisingly, it was found that both samples of **3a** and **7b** had undergone some changes after storage for six months (Schemes 5 and 6).

Re-chromatography of sample **3a** after storage yielded significant amounts of [15-$^{13}C^{2}H_3$]-artemisinin (**1a**), [15-$^{13}C^{2}H_3$]-dihydro-*epi*-deoxyarteannuin B (**7a**) and [15-$^{13}C^{2}H_3$]-α-epoxy-dihydroartemisinic acid (**27a**) in addition to much larger quantities of the labeled precursor itself. The structures of **1a** and **7a** were confirmed by their 1D ^{1}H NMR spectra, while the planar structure of novel compound **27a** was determined by the 2D-NMR experiments HSQC, HMBC and ^{1}H–^{1}H COSY as for all other novel compounds reported herein (Table 3). Unfortunately, the relative stereochemistry of the newly formed epoxide group in **27a** was not clearly shown by NOESY experiments. However, we were able to synthesize much larger amounts of an isotopically normal compound with NMR spectra exactly matching those of **27a** (after making allowances for the effects of isotopic enrichment) by treating dihydroartemisinic acid (**3**) with *m*-chloroperoxybenzoic acid. The X-ray crystallographic structure of **27** from synthesis confirmed that the epoxy group had been introduced to the α-face of dihydroartemisinic acid (Fig. 5) and the oxidation product **27a** formed from **3a** on storage has been assigned as α-epoxy-dihydroartemisinic acid accordingly. We have recently isolated a natural product from *A. annua* with NMR spectra identical to those of isotopically normal α-epoxy-dihydroartemisinic acid (**27**) (unpublished results); compound **27** is the 11,13-dihydro analogue of the natural product α-epoxy-artemisinic acid, which has been described previously from this species.[17]

We propose that all three products **1a**, **7a** and **27a** arise by the initial reaction of oxygen with the $\Delta^{4,5}$-double bond of the labeled precursor **3a**. This results in an allylic tertiary hydroperoxide, which then undergoes spontaneous conversion to these three isolated products under the conditions of storage. There are several precedents in the literature for the transformations of **3** suggested in Scheme 6.[3,18–21] All these references state or imply that it is singlet oxygen ($^{1}O_2$), which reacts with the $\Delta^{4,5}$-double bond in dihydroartemisinic acid in order to form the initial tertiary allylic hydroperoxide. Since our sample of **3a** was stored in the dark there is no obvious source of singlet oxygen which would be required to initiate the formation of **1a**, **7a** and **27a**, according to our understanding of current theory (which states that both a photosensitizer and a light source



Scheme 5. Proposed autoxidation and subsequent rearrangement reactions of **3a** occurring during storage at −20°C for six months.



Scheme 6. Proposed autoxidation and subsequent rearrangement reactions of **7b** occurring during storage at −20°C for six months.

Table 3. NMR assignments (δ, ppm) for labeled compounds formed from the slow autoxidation of **3a** and **7b** on storage (Schemes 5 and 6) (rigorous assignments made by the 2D-NMR techniques HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY in all cases). Splittings for labeled compounds (suffix 'a' or 'b') are given in Section 3

	δ_C								δ_H							
Position	**27a**	**28b**	**29b**	**30b**	**31b**	**32b**	**33b**	**34b**	**27a**	**28b**	**29b**	**30b**	**31b**	**32b**	**33b**	**34b**
1	40.2	39.9	38.8	47.3	41.4	47.7	46.4	38.6	1.07	1.49	1.44	1.64	1.58	1.85	1.33	2.04
2α	22.3	27.2	27.2	27.9	25.9	37.5	26.1	37.4	1.31	2.27	2.27	2.50	2.43	2.70	2.05	2.60
2β									1.65	1.93	2.00	2.40	1.85	2.61	2.30	2.19
3α	24.8	129.6	126.5	145.1	81.3	194.0	83.5	203.2	1.84	5.78	5.67	6.73	4.35	–	4.43	–
3β									1.67							
4	57.8	127.0	131.2	132.6	137.0	139.4	140.7	61.3	–	–	–	–	–	–	–	–
5	58.7	84.4	70.1	195.7	128.5	141.0	126.9	61.9	2.63	4.17	3.70	–	5.88	6.76	5.82	3.46
6	38.1	84.9	85.2	84.1	82.1	81.1	82.2	83.3	2.04	–	–	–	–	–	–	–
7	42.8	38.8	38.0	37.9	42.0	42.5	42.6	41.0	1.74	2.93	2.72	2.75	2.13	2.25	2.09	2.54
8α	28.7	24.4	24.4	24.1	23.7	24.0	23.6	24.1	1.64	1.80	1.76	1.85	1.75	1.84	1.72	1.85
8β									1.10	1.15	1.13	1.16	1.20	1.23	1.20	1.21
9α	34.8	32.0	32.1	31.9	32.2	32.2	32.3	31.8	1.05	1.13	1.09	1.02	1.09	1.11	1.07	1.10
9β									1.69	1.67	1.67	1.70	1.69	1.75	1.71	1.69
10	29.5	31.9	31.7	31.5	29.2	30.3	29.5	30.7	1.27	1.36	1.37	1.56	1.47	1.55	1.51	1.37
11	42.0	39.5	39.0	40.8	39.4	39.8	39.6	39.2	2.60	3.21	3.11	3.25	3.12	3.16	3.13	3.24
12	181.2	179.7	180.4	179.3	179.1	178.1	178.8	177.9	–	–	–	–	–	–	–	–
13	15.5	9.4	9.3	9.2	9.4	9.3	9.4	9.3	1.29	1.17	1.14	1.12	1.15	1.19	1.15	1.21
14	19.0	19.8	19.9	19.0	19.4	18.8	20.8	19.2	0.86	0.93	0.94	0.96	0.99	0.94	0.96	0.90
15	23.6	20.8	21.2	15.3	21.0	15.8	19.5	14.6	1.32[a]	1.79	1.79	1.77	1.84	1.81	1.82	1.42

[a] Low intensity and split into a doublet (125.9 Hz) in spectrum of isotopically-labeled **27a**.

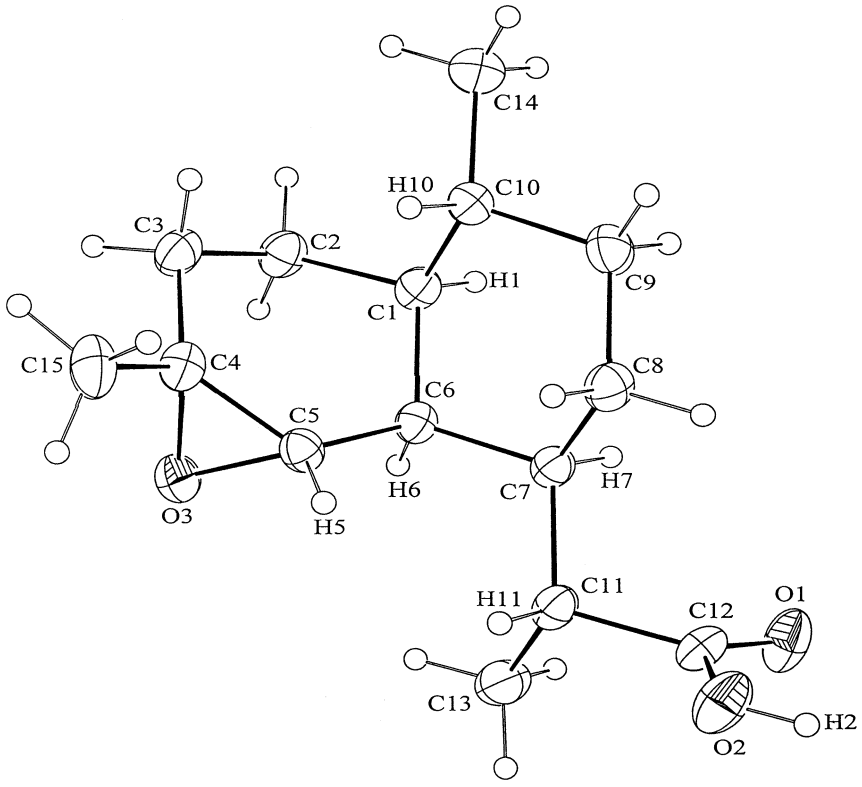


Figure 5. ORTEP diagram for compound **27**, which was obtained by treatment of isotopically-normal **3** with *m*CPBA.

are required in order to produce the singlet state of molecular oxygen ($^1O_2$)). Based on the foregoing results, we suggest that there may be something lacking in current theories regarding the generation of singlet oxygen and/or the absolute requirement for singlet oxygen in reacting with tri-substituted double bonds, at least when it comes to the slow spontaneous autoxidation of unsaturated organic compounds, which, although a matter of common experience is a phenomenon which has not often been studied or reported. In addition to the current unexpected finding, we would draw the reader's attention to previous reports both by ourselves[18] and others[3] regarding the slow spontaneous autoxidation of dihydroartemisinic in the absence of photosensitizers and a recent report of a much more rapid and apparently un-photosensitized spontaneous autoxidation of a natural product.[22]

Re-chromatography of sample **7b** after a period of six months storage at −20°C resulted in the isolation of seven compounds **28b**–**34b** (Table 3), of which six are novel, in addition to much larger amounts of the labeled precursor starting material. The secondary allylic hydroperoxide **28b** is once again clearly formed from 'ene-type' reaction of oxygen with the $\Delta^{4,5}$-double bond in **7b** (note that because there is no H-6 proton in **7b**, formation of a tertiary allylic hydroperoxide is not possible). Compound **29b** is the [15-$^{13}CH_3$]-isotopomer of the natural product arteannuin K,[18] which might then be formed by homolysis of the hydroperoxide group in **28b**.[23] (N.B. The stereochemistry at the 5-position of this natural product, originally reported as 5β-OH,[18] is believed to have been wrongly assigned and we have recently submitted a manuscript revising the stereochemistry of the 5-OH group in arteannuin K to that shown in Scheme 6. The formation of **29b** from α-hydroperoxide **28b** is thus consistent with this structure revision for arteannuin K).[15] The formation of the α,β-unsaturated ketone **30b** can be explained in an analogous fashion by dehydration of **28b**[23] (cf. the proposed biogenesis of arteannuin N in *A. annua* from dihydroartemisinic acid).[18] The secondary allylic hydroperoxide **31b** is almost certainly the result of 3,2-rearrangement (Schenck reaction) of allylic hydroperoxide **28b**[23] (several analogous rearrangement reactions of the secondary allylic hydroperoxide from photo-oxidation of dihydroartemisinic acid have been reported recently),[19] and **32b** may be formed by dehydration of this rearranged hydroperoxide. The well-known Smith epimerization reaction of allylic hydroperoxides[23] can then be invoked to account for the formation of **33b** from compound **31b**. Compound **34b** was shown to have the rather unusual structure of an epoxy-ketone by 2D-NMR analysis (summarized in Table 3). A tentative suggestion is made for the derivation of **34b** from **28b** in Scheme 6, based on the reported rearrangements of allylic hydroperoxides of fatty acids to β-hydroxy epoxides;[24] further oxidation of the alcohol group of such a hydroxy-epoxide intermediate would then yield **33b**, possibly by a radical process.[23]

We have obtained evidence for some of the transformations proposed in Scheme 6 for allylic hydroperoxide **28b** by allowing a pure sample of this compound to stand in $CDCl_3$ for several weeks. By recording the ^1H NMR spectra of this sample at regular intervals it was possible to observe the appearance of peaks characteristic of all of the products **29b**–**32b** within a few days. After 45 days, peaks of the starting material had almost disappeared, leaving a complex

mixture containing ca. 10% **29b**, 10% **30b**, 15% **31b** and 10% **32b** by ^{1}H NMR. Although no peaks due to **33b** or **34b** were seen in ^{1}H NMR under these conditions, there were a large number of unidentified signals in these spectra, suggestive of alternative transformations occurring in $CDCl_3$, which are not observed in the solid state. Once again, conventional wisdom would require a source of singlet oxygen, in order to produce the allylic hydroperoxide **28b** (from which all of **29b**–**34b** are proposed to be derived) from **7b**, although there is no obvious source for this species under the conditions of storage.

The formation of the labeled natural products artemisinin (**1a**), dihydro-*epi*-deoxyarteannuin B (**7a**) and α-epoxy-dihydroartemisinic acid (**27a**) from the slow spontaneous autoxidation of [15-$^{13}C^2H_3$]-dihydroartemisinic acid (**3a**), as well as the appearance of the labeled natural product arteannuin K (**29b**) from prolonged storage of [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteannuin B (**7b**), is of some concern from the point of view of feeding experiments in which the labeled precursors **3a** and **7b** are intended to be tested as biosynthetic precursors en route to natural products from *A. annua*. Clearly, great care must be taken to ensure that allylic hydroperoxides and their rearrangement products, which can readily be formed from autoxidation of the double bond in these compounds in vitro even in the absence of light and photosensitizers, are not confused with bona fide metabolites which might then be re-isolated in vivo. It is thus important to prepare these labeled precursors freshly or to re-purify them after storage and prior to their use in feeding experiments. Finally, we make the suggestion that the unexpected ease by which both dihydroartemisinic acid and dihydro-*epi*-deoxyarteannuin B undergo spontaneous oxidation (by non-enzymatic processes) may have some bearing on the rather confusing results concerning the biogenesis of artemisinin which have been reported in the literature.

3. Experimental

3.1. General

All ^{1}H and ^{13}C NMR experiments were recorded on a Bruker DRX 500 instrument. Chemical shifts are expressed in ppm (δ) relative to TMS as internal standard. Proton chemical shifts, multiplicities, coupling constants and integrals reported in this section are those which were clearly resolved in 1D-^{1}H NMR without recourse to 2D-NMR analysis (see tables in the main text for full assignments made by 2D-NMR). ^{2}H NMR spectra were recorded in $CDCl_3$ at 42.4 MHz and $CDCl_3$ was referenced to 7.29 ppm. 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 FT-IR-8201 PC instrument. Column chromatography (CC) 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 either a normal phase Intersil PREP-SIL or a YMC diol 20 mm×25 cm column, flow rate 8 ml/min. Melting points were recorded by a Perkin–Elmer differential scanning calorimeter 7 (DSC 7). 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 solvent.

δ_C values for the 15-position of compounds labeled by ^{2}H at the 15-position (suffix 'a') generally appeared as 4 sets of superimposed signals: a singlet at the isotopically-normal value [15-$^{13}CH_3$]; a 1:1:1 triplet which is 0.3 ppm upfield of this [15-$^{13}CH_2{}^2H$]; a 1:2:3:2:1 quintet 0.6 ppm upfield [15-$^{13}CH^2H_2$]; and a 1:3:6:7:6:3:1 septet 0.9 ppm upfield [15-$^{13}C^2H_3$]. $^1J_{CD}$ couplings for these multiplets were in the range 19.0–19.3 Hz for all compounds reported. For brevity, only the four chemical shifts at the labeled position are quoted, together with an estimated percentage for each isotopomer. Four sets of molecular ions and four sets of base peaks were generally observed in MS for $^{13}C/^2H$-labeled compounds (suffix 'a') corresponding, in order of decreasing mass, to [15-$^{13}C^2H_3$], [15-$^{13}CH^2H_2$], [15-$^{13}CH_2{}^2H$] and [15-$^{13}CH_3$] isotopomers, respectively. For brevity, experimental and calculated high-resolution mass data is given only for the [15-$^{13}C^2H_3$] isotopomer. High-resolution data for the other isotopomers are not explicitly stated, but were universally found to be within the same range of tolerance as that for the [15-$^{13}C^2H_3$] isotopomer.

3.2. Acid degradation of artemisinin (1) to 10

To a cooled solution of conc. H_2SO_4 in MeOH (120 ml; 180 ml) in an ice bath was added artemisinin (**1**) (12.0 g, 42.6 mmol). The mixture was stirred for 10 min after which iced water (250 ml) was added. The mixture was filtered and extracted with $CHCl_3$ (5×100 ml) and the combined organic layers were washed with brine (3×50 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a crude product (9.05 g; 75% w/w), consisting predominantly of **10** (90% by ^{1}H NMR).

Compound **10**: $[\alpha]_D=-34.4$ (c 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3028, 2928, 1728, 1712 cm^{-1}; other physical properties as for Ref. 10. Compound **11** was sometimes isolated as a very minor product by CC.

Compound **11**: ^{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), 1.23 (3H, d, J=7.2 Hz), 0.89 (3H, d, J=6.5 Hz)—see Table 2; ^{13}C NMR: see Table 1.

3.3. Robinson annulation of 10 to 8

$BaOH_2{\cdot}8H_2O$ (8 g) in MeOH (150 ml) was added to the crude product consisting mostly of **10** (9.05 g, 33.8 mmol, 90% purity by ^{1}H NMR) and the reaction mixture was stirred at room temperature for 3 h, then filtered and neutralized to pH 7 by dropwise addition of CH_3COOH under ice bath cooling. The volume of the solvent was reduced in vacuo (to ca. 30 ml) and the mixture was gradually acidified by addition of HCl (5%) until no more solid precipitated. (N.B. addition of excess HCl causes lactonization of product **8**). The precipitate was filtered and dried by suction to yield a crude product (7.08 g, 78% w/w) from which compound **8** (4.98 g, 21.1 mmol, 62%) could be obtained by recrystallization from EtOAc. See Ref. 10 for physical properties of

8. Compound **8** sometimes underwent a substantial degree of lactonization to **12** during the acidic work-up, see Ref. 10 for physical properties of **12**. The free acid **8** could be recovered by treatment of **12** with alkali as described below.

3.3.1. Hydrolysis of the lactone side-product 12 back to decalenone acid 8. Compound **12** (2.5 g, 10.6 mmol) was stirred with KOH solution (15%, 30 ml) at room temperature for 15 min. The mixture was cooled in an ice bath and HCl (10%) was added to pH 2, until no more precipitate was formed. The precipitate was filtered, washed with H_2O (5 ml) and dried by suction to yield compound **8** (2.3 g, 9.8 mmol, 93%) without need for further purification.

3.4. Synthesis of either diene 14a or lactone 7b from keto acid 8 by Grignard reaction with labeled methyl iodide

To small Mg chips (0.436 g, 17.9 mmol) in anhyd. Et_2O (300 ml) was added a solution of labeled MeI (either [$^{13}C^2H_3$] or [$^{13}CH_3$]) (1.32 ml; 21.0 mmol) in anhyd. Et_2O (100 ml) and the reaction mixture was refluxed for 1.5 h. A solution of **8** (1.69 g, 7.16 mmol) in anhyd. Et_2O (200 ml) was added and the reaction was allowed to reflux for a further 2.5 h until completion, as determined by TLC.

Work-up at pH 1–2 for [$^{13}C^2H_3$]-methyl iodide: the mixture was cooled in an ice bath and HCl (10%) was added until pH 1–2. Then the reaction mixture was extracted with Et_2O (3×300 ml) and the combined organic layers were washed with brine (3×50 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield a crude product (1.52 g, 90% w/w) consisting mostly of **14a** with compounds **7a** and **15a** as side-products. The mixture was separated either by CC (15% EtOAc/*n*-hexane) or by HPLC (10% EtOAc/*n*-hexane/1% CH_3COOH).

Compound **7a**: Oil (200 mg, 0.85 mmol, 12%, R_t 13.1 min). Physical properties as for isotopically-normal **7** in Ref. 18, with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) ppm: 5.63 (^{1}H, d, J=6.2 Hz, $^3J_{CH}$, H-5), 1.69 (H-15) is not clearly seen; ^{2}H NMR (δ, $CDCl_3$) ppm: 1.69 (d, J=19.2 Hz, $^1J_{CD}$); ^{13}C NMR: 142.2 (d, J=42.9 Hz, $^1J_{CC}$, C-4), 83.2 (d, J=4.0 Hz, $^3J_{CC}$, C-6), 30.8 (d, J=3.0 Hz, $^2J_{CC}$, C-3), 23.5/23.2/22.9/22.6 (1:6:10:83), 21.0 (d, J=2.8 Hz, $^3J_{CC}$, C-2); HREIMS *m/z* (rel. int.) 238.1826 [M^+, $C_{14}{}^{13}C_1H_{19}{}^2H_3O_2$ requires 238.1842] (15), 237 (1.4), 236 (0.16), 235 (0.01), 219 (10), 194 (90), 165.1543 [$M^+-C_3H_5O_2$; $C_{11}{}^{13}C_1H_{14}{}^2H_3$ requires 165.1552] (100), 164 (19), 163 (6), 162 (1).

Compound **14a**: solid (1.18 g, 4.96 mmol, 69%, R_t 20.8 min), Mp 119–120°C $[\alpha]_D=-25.3$ (*c* 4.8, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2400, 2912, 2874, 2829, 1707, 1458 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 6.11 (1H, d, J=6.3 Hz, $^3J_{CH}$), 3.82 (1H, q, J=7.0 Hz), 1.78 (H-15) is not clearly seen in the spectrum of the isotopically enriched sample, 1.21 (3H, d, J=7.0 Hz), 0.99 (3H, d, J=6.1 Hz)—see Table 2; ^{2}H NMR (δ, $CDCl_3$) ppm: 1.78 (d, J=19.2 Hz, $^1J_{CD}$); ^{13}C NMR: see Table 1—137.9 (d, J=43.4 Hz, $^1J_{CC}$), 131.9 (d, J=4.9 Hz, $^3J_{CC}$), 31.2 (d, J=2.9 Hz, $^2J_{CC}$), 27.8 (d, J=2.7 Hz, $^3J_{CC}$), 24.2/23.9/23.6/23.3 (2:8:13:77); HREIMS *m/z* (rel. int.) 238.1835 [M^+, $C_{14}{}^{13}C_1H_{19}{}^2H_3O_2$ requires 238.1842] (4.7), 237 (0.18), 236 (0.03), 235 (0.04), 193 (30), 165.1549 [$M^+-C_3H_5O_2$; $C_{11}{}^{13}C_1H_{14}{}^2H_3$ requires 165.1552] (100), 164 (15), 163 (10), 162 (1).

Compound **15a**: oil (96 mg, 0.41 mmol, 6%, R_t 21.0 min); IR ν_{max} ($CHCl_3$): 3400–2400 (br), 3026, 2940, 1715, 1603, 1458 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 5.55 (1H, dd, J=6.0 Hz, $^3J_{CH}$, 2.1 Hz), 2.68 (1H, dq, J=6.9, 7.1 Hz), 2.35 (1H, m), 1.78 (H-15) is not clearly seen in the spectrum of the isotopically enriched sample, 1.18 (3H, d, J=7.1 Hz), 0.98 (3H, d, J=7.0 Hz)—see Table 2; ^{2}H NMR (δ, $CDCl_3$) ppm: 1.78 (d, J=19.3 Hz, $^1J_{CD}$); ^{13}C NMR: see Table 1—127.9 (d, J=4.5 Hz, $^3J_{CC}$), 28.7 (d, J=4.0 Hz, $^2J_{CC}$), 23.1/22.8/22.5/22.2 (2:10:20:68); HREIMS *m/z* (rel. int.) 238.1806 [M^+, $C_{14}{}^{13}C_1H_{19}{}^2H_3O_2$ requires 238.1842] (1.0), 237 (1.2), 236.1673 [M^+-H_2; $C_{14}{}^{13}C_1H_{17}{}^2H_3O_2$ requires 236.1686] (7.5), 192 (18), 163.1392 [$M^+-H_2-C_3H_5O_2$; $C_{11}{}^{13}C_1H_{12}{}^2H_3$ requires 163.1396] (100), 162 (40), 161 (17), 160 (13).

Work-up at pH 4–5 for [$^{13}CH_3$]-methyl iodide: the reaction mixture was cooled in an ice bath and HCl (10%) was added dropwise to pH 4–5. Then the reaction mixture was extracted with Et_2O (3×300 ml) and the combined organic layers were washed by brine (3×50 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield lactone **7b** without need for further purification.

Compound **7b**: oil (1.60 g; 6.81 mmol, 95%). See Ref. 18 for physical properties—with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) ppm: 5.64 (1H, d, J=6.0 Hz, $^3J_{CH}$, H-5), 1.69 (3H, d, J=126.0 Hz, $^1J_{CH}$, H-15); ^{13}C NMR: 142.2 (d, J=42.9 Hz, $^1J_{CC}$, C-4), 121.7 (d, J=2.0 Hz, $^2J_{CC}$), 83.2 (d, J=4.0 Hz, $^3J_{CC}$, C-6), 30.8 (d, J=3.0 Hz, $^2J_{CC}$, C-3), 23.5 (ca. 70 times intensity of other ^{13}C peaks, C-15); HREIMS *m/z* (rel. int.) 235.1643 [M^+, $C_{14}{}^{13}C_1H_{22}O_2$ requires 235.1654] (28), 191 (100), 162 (100).

3.5. Preparation of methyl ester 16 from decalenone 8

Diazomethane was freshly prepared from Diazald® (*N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide) (1.88 g, 88 mmol) in Et_2O (30 ml) cooled in an ice bath, by adding a solution of KOH (0.436 g) in EtOH; (96%, 10 ml) with stirring for 5–10 min. The ethereal diazomethane solution was distilled from a water bath and added dropwise to a stirred solution of decalenone **8** (2.05 g, 8.69 mmol) in Et_2O (70 ml) until no more bubbles were observed. Then CH_3COOH (10%, 1 ml) was added to destroy the excess diazomethane and the reaction mixture was washed with brine (3×10 ml), dried ($MgSO_4$) and concentrated to yield the methyl ester **16** (2.0 g, 8 mmol, 98% w/w) without need for further purification—see Ref. 10 for physical properties.

3.5.1. Reaction of methyl ester 16 with the Grignard reagent from methyl iodide. To small Mg chips (89 mg, 3.66 mmol) in anhyd. Et_2O (20 ml) was added a solution of MeI (0.30 ml, 4.80 mmol) in anhyd. Et_2O (5 ml) and the reaction mixture was refluxed for 1.5 h. A solution of **16** (375 mg, 1.5 mmol) in anhyd. Et_2O (10 ml) was added and the reaction was allowed to reflux for a further 2.5 h until completion, as determined by TLC.

Work-up at pH 4–5: the reaction mixture was cooled in an

ice bath and H_2O (5 ml) was added to pH 4–5. Then, the reaction mixture was extracted with Et_2O (3×20 ml) and the combined organic layers were washed with brine (3×10 ml), dried ($MgSO_4$) and solvent removed under reduced pressure to yield a crude product (330 mg, 88% w/w) consisting of epimers **17** and **18** which was separated by HPLC (27% EtOAc/*n*-hexane).

Compound **17**: oil (87 mg, 0.33 mmol, 22%, R_t 27.3 min), $[\alpha]_D=-24.8$ (*c* 0.33, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3590, 3450 (br), 3005, 2928, 2856, 1728, 1456, 1437 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 5.26 (1H, s), 3.67 (3H, s), 2.73 (1H, dq, *J*=6.9, 6.8 Hz), 1.26 (3H, s), 1.22 (3H, d, *J*=6.8 Hz), 0.92 (3H, d, *J*=6.3 Hz)—see Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 248.1780 [M^+-H_2O, $C_{16}H_{24}O_2$ requires 248.1776] (89), 214 (12), 189 (30), 161 (100).

Compound **18**: oil (144 mg, 0.54 mmol, 36%, R_t 24.3 min), $[\alpha]_D=-70.7$ (*c* 1.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3599, 3447 (br), 3005, 2972, 2930, 2872, 1728, 1458, 1437 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 5.29 (1H, s), 3.67 (3H, s), 2.74 (1H, dq, *J*=7.1, 6.8 Hz), 1.29 (3H, s), 1.22 (3H, d, *J*=6.8 Hz), 0.95 (3H, d, *J*=6.1 Hz)—see Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 248.1774 [M^+-H_2O, $C_{16}H_{24}O_2$ requires 248.1776] (100), 216 (14), 189 (40), 161 (76).

Work-up at pH 1–2: the reaction mixture was cooled in an ice bath and HCl (10%) was added to pH 1–2. Then, the reaction mixture was extracted with Et_2O (3×50 ml) and the combined organic layers were washed with brine (3×10 ml), dried ($MgSO_4$) and solvent removed under reduced pressure to yield a crude product (328 mg, 88% w/w) consisting predominantly of **19** with very small amounts of compounds **20**–**22**.

Compound **19**: oil. $[\alpha]_D=-13.6$ (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2930, 1719, 1603 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 6.11 (1H, s), 3.77 (1H, q, *J*=7.0 Hz), 3.65 (3H, s), 1.78 (3H, s), 1.20 (3H, d, *J*=7.0 Hz), 0.99 (3H, d, *J*=6.1 Hz)—see Table 2; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.) 248.1776 [M^+, $C_{16}H_{24}O_2$ requires 248.1776] (10), 211 (10), 189 (20), 149 (30), 99 (100).

Compound **20**: oil. ^1H NMR (δ, $CDCl_3$) ppm: 5.82 (1H, s), 4.72 (1H, s), 4.69 (1H, s), 3.66 (3H, s), 2.75 (1H, dq, *J*=7.1, 7.1 Hz), 1.12 (3H, d, *J*=7.1 Hz), 0.93 (3H, d, *J*=6.4 Hz)—see Table 2; ^{13}C NMR: see Table 1.

Compound **21**: oil. ^1H NMR (δ, $CDCl_3$) ppm: 5.51 (1H, s), 3.64 (3H, s), 2.63 (1H, dq, *J*=7.1, 7.1 Hz), 1.78 (3H, s), 1.13 (3H, d, *J*=7.1 Hz), 0.97 (3H, d, *J*=7.0 Hz)—see Table 2; ^{13}C NMR: see Table 1.

Compound **22**: oil. ^1H NMR (δ, $CDCl_3$) ppm: 5.37 (1H, s), 5.26 (1H, br s), 3.68 (3H, s), 2.74 (1H, dq, *J*=8.8, 6.9 Hz), 1.69 (3H, d, *J*=2.3 Hz), 1.24 (3H, d, *J*=6.9 Hz), 0.87 (3H, d, *J*=6.4 Hz)—see Table 2; ^{13}C NMR: see Table 1.

3.5.2. Conversion of 17/18 to 19 by treatment with concentrated sulphuric acid. To a solution of the crude mixture of compounds **17** and **18** (20 mg, 0.075 mmol) in Et_2O (5 ml) was added 70% H_2SO_4 (1 ml) and the reaction mixture was stirred at room temperature for 5 h until completion, as shown by TLC. H_2O (5 ml) was added and the reaction mixture was extracted by Et_2O (3×10 ml), washed with brine (3×5 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a crude product (16 mg, 80% w/w), consisting mostly of compound **19** (95% by ^1H NMR).

3.6. Hydrogenation of diene acid 14a

To diene acid **14a** (430 mg, 1.81 mmol) in EtOAc (200 ml) was added a catalytic amount of palladium activated charcoal and the solution was left in a hydrogenation apparatus overnight. The reaction mixture was filtered and solvent was removed under reduced pressure to yield a crude product (414 mg, 96% w/w) which was separated by HPLC (4% EtOAC/*n*-hexane/1% CH_3COOH). Compound **3a**: oil (248 mg, 1.03 mmol, 58%, R_t 15.3 min). Physical properties as for Ref. 18—with the following differences due to isotopic enrichment: ^1H NMR (δ, $CDCl_3$) ppm: 5.11 (1H, d, *J*=6.4 Hz, $^3J_{CH}$, H-5), 1.63 (H-15) is not clearly seen; ^2H NMR (δ, $CDCl_3$) ppm: 1.63 (d, *J*=19.0 Hz, $^1J_{CD}$); ^{13}C NMR—136.0 (d, *J*=43.2 Hz, $^1J_{CC}$, C-4), 36.4 (d, *J*=3.4 Hz, $^3J_{CC}$, C-6), 26.6 (d, *J*=3.5 Hz, $^2J_{CC}$, C-3), 25.8 (d, *J*=3.0 Hz, $^3J_{CC}$, C-2), 23.9/23.6/23.3/23.0 (24:21:21:35); HREIMS *m/z* (rel. int.) 240.1989 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998] (2), 239 (0.4), 238 (0.2), 237 (0.08), 192 (5), 166.1626 [$M^+-C_3H_6O_2$; $C_{11}{}^{13}C_1H_{15}{}^2H_3$ requires 166.1630] (100), 165 (30), 164 (14), 163 (7).

Compound **23a**: oil (20 mg, 0.083 mmol, 5%, R_t 19.3 min). $[\alpha]_D=+44.7$ (*c* 1.34, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 3040, 2928, 2903, 2847, 1705, 1458, 1445 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 5.29 (1H, br), 2.30 (1H, dq, *J*=10.7, 6.9 Hz), 2.15 (1H, dd, *J*=17.6, 5.4 Hz), 1.63 (d, *J*=125.9 Hz, $^1J_{CH}$)—ca. 30% intensity expected for 3H in the isotopically-normal spectrum, 1.19 (3H, d, *J*=6.9 Hz), 0.82 (3H, d, *J*=6.4 Hz)—see Table 2; ^2H NMR (δ, $CDCl_3$) ppm: 1.63 (d, *J*=18.9 Hz, $^1J_{CD}$); ^{13}C NMR: see Table 1—131.8 (d, *J*=48.3 Hz, $^1J_{CC}$), 33.4 (d, *J*=2.5 Hz, $^3J_{CC}$), 27.8 (br, $^3J_{CC}$), 26.3 (br, $^2J_{CC}$), 23.6/23.3/23.0/22.7 (52:26:15:7); HREIMS *m/z* (rel. int.) 240.1986 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998] (2.0), 239 (1.2), 238 (1.2), 237 (0.8), 166.1624 [$M^+-C_3H_6O_2$; $C_{11}{}^{13}C_1H_{15}{}^2H_3$ requires 166.1630] (100), 165 (63), 164 (57), 163 (40).

Compound **24a**: oil (34 mg, 0.148 mmol, 8%, R_t 13.7 min). $[\alpha]_D=-80.9$ (*c* 3.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600, 3030, 2924, 2872, 1705, 1458 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 3.67 (1H, q, *J*=7.1 Hz), 2.43 (1H, d, *J*=13.6 Hz), 1.15 (3H, d, *J*=7.1 Hz), 0.95 (3H, d, *J*=6.4 Hz), 0.92 is not clearly seen—see Table 2; ^2H NMR (δ, $CDCl_3$) ppm: 0.92 (d, *J*=19.3 Hz, $^1J_{CD}$), ^{13}C NMR: see Table 1—29.4 (d, *J*=35.4 Hz, $^1J_{CC}$), 17.8/17.5/17.2/16.9 (13:17:19:50); HREIMS *m/z* (rel. int.) 240.1995 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998] (2.9), 239 (0.4), 238 (0.1), 237 (0.02), 166.1625 [$M^+-C_3H_6O_2$; $C_{11}{}^{13}C_1H_{15}{}^2H_3$ requires 166.1630] (100), 165 (24), 164 (8), 163 (3).

Compound **25a**: oil (54 mg, 0.23 mmol, 13%, R_t 14.6 min). $[\alpha]_D=-45.2$ (*c* 7.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600, 3032, 2924, 2864, 1705, 1458, 1447 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 5.11 (1H, s), 2.70 (1H, dq, *J*=8.8, 6.8 Hz), 2.17 (1H, m), 2.02 (1H, dd, *J*=9.5, 8.8 Hz), 1.26 (3H, d,

J=6.8 Hz), 0.93 is not clearly seen, 0.90 (3H, d, J=6.4 Hz)—see Table 2; ^{2}H NMR (δ, $CDCl_3$) ppm: 0.93 (d, J=19.1 Hz, $^1J_{CD}$); ^{13}C NMR: see Table 1—30.3 (d, J=35.4 Hz, $^1J_{CC}$), 24.5 (d, J=3.9 Hz, $^3J_{CC}$), 21.9/21.6/21.3/21.0 (25:24:29:23); HREIMS m/z (rel. int.) 240.1984 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998] (0.08), 239 (0.03), 166.1631 [$M^+-C_3H_6O_2$; $C_{11}{}^{13}C_1H_{15}{}^2H_3$ requires 166.1630] (100), 165 (35), 164 (14), 163 (1).

Compound **26a**: oil (10 mg, 0.042 mmol, 2%, R_t 9.9 min). $[\alpha]_D$=−52.3 (c 0.7, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600, 3032, 2926, 2855, 1703, 1458, 1445 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 5.27 (1H, s), 2.74 (1H, dd, J=11.3, 6.7 Hz), 2.21 (1H, dd, J=11.3, 4.2 Hz), 1.14 (3H, d, J=6.7 Hz), 0.92 (3H, d, J=6.3 Hz), 0.85 (3H, dd, J=125.4 Hz, $^1J_{CH}$, 7.0 Hz)—ca. 10% of peak intensity expected for 3H in the isotopically normal spectrum—see Table 2; ^{2}H NMR (δ, $CDCl_3$) ppm: 0.85 (d, J=18.7 Hz, $^1J_{CD}$); ^{13}C NMR: see Table 1—31.1 (d, J=34.5 Hz, $^1J_{CC}$), 28.8 (d, J=3.9 Hz, $^3J_{CC}$), 22.0/21.7/21.4/21.1(48:29:16:7); HREIMS m/z (rel. int.) 240.1989 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998] (0.24), 239 (0.12), 238 (0.10), 237 (0.05), 167.1706 [$M^+-C_3H_5O_2$; $C_{11}{}^{13}C_1H_{16}{}^2H_3$ requires 167.1709] (100), 166 (95), 165 (85), 164 (60).

3.7. Separation of compounds 1a, 7a and 27a formed by autoxidation of 3a on storage

Compound **3a** (29 mg, 0.12 mmol) stored at −20°C, was found to have undergone some changes by ^{1}H NMR spectroscopy after six months. The mixture was separated by HPLC (8% EtOAc/*n*-hexane/0.5% CH_3COOH).

Compound **1a**: oil (6 mg, 0.021 mmol, 17%, R_t 28.6 min). ^{1}H NMR spectrum as for artemisinin—see Ref. 25—with the following differences due to isotopic enrichment: δ_H 1.44 (d, J=129.3 Hz, $^1J_{CH}$, H-15)—ca. 10% of peak intensity expected for 3H in the isotopically normal spectrum.

Compound **7a**: oil (2 mg, 0.008 mmol, 7% R_t 14.9 min). ^{1}H NMR spectrum as for dihydro-*epi*-deoxyarteannuin B—see Ref. 18—with the following differences due to isotopic enrichment: δ_H 5.63 (1H, d, J=5.9 Hz, $^3J_{CH}$, H-5), 1.69 (3H, J=125.4 Hz, $^1J_{CH}$, H-15)—ca. 10% of peak intensity expected for 3H in the isotopically normal spectrum.

Compound **27a**: solid (1 mg, 0.004 mmol, 4%, R_t 30.9 min). Physical properties as for isotopically normal **27**—see below—with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) ppm: 1.32 (3H, d, J=125.9 Hz, $^1J_{CH}$)—ca. 10% of peak intensity expected for 3H in the isotopically normal spectrum; ^{13}C NMR: 57.8 (d, J=46.8 Hz, $^1J_{CC}$), 23.6/23.3/23.0/22.7 (25:22:21:32); HREIMS m/z (rel. int.) 238.1836 [M^+ ($C_{14}{}^{13}C_1H_{21}{}^2H_3O_3$)–$H_2O$; $C_{14}{}^{13}C_1H_{19}{}^2H_3O_2$ requires 238.1841] (40), 237 (27), 236 (4), 235 (4), 183.1652 [$M^+-C_3H_5O_2$; $C_{11}{}^{13}C_1H_{16}{}^2H_3O$ requires 183.1658] (100), 182 (35), 181 (16), 180 (12), 165.1563 [M^+-H_2O-$C_3H_5O_2$; $C_{11}{}^{13}C_1H_{14}{}^2H_3$ requires 165.1552] (62), 164 (44), 163 (15), 162 (5).

3.7.1. Preparation of 27 by treatment of isotopically normal dihydroartemisinic (3) with *m*CPBA. To a solution of dihydroartemisinic acid **3** (400 mg, 1.66 mmol) in $CHCl_3$ (20 ml) was added *m*-chloroperoxybenzoic acid (*m*CPBA; 440 mg, 2.55 mmol) and the mixture was stirred overnight at room temperature. H_2O (20 ml) was added and the mixture was extracted by $CHCl_3$ (3×100 ml), and washed with $NaHCO_3$ (10%, 3×50 ml). The combined organic layers were washed with brine (3×20 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to obtain a crude product from which the excess *m*CPBA was removed by HPLC (12% EtOAc/*n*-hexane/0.5% CH_3COOH). Crystals of epoxide **27** were obtained by recrystallization from *n*-hexane. Compound **27**: solid (350 mg, 1.37 mmol, 82%, R_t 29.0 min). Mp 153–154°C. $[\alpha]_D$=−3.4 (c 1.5, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3028, 2928, 2878, 2853, 1705, 1458 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 2.65 (1H, s), 2.60 (1H, dq, J=10.7, 6.9 Hz), 2.04 (1H, br m), 1.84 (1H, dd, J=15.3, 6.6 Hz), 1.32 (3H, s), 1.29 (3H, d, J=6.9 Hz), 0.86 (3H, d, J=6.4 Hz)—see Table 3; ^{13}C NMR: see Table 3; HREIMS m/z (rel. int.) 252.1725 [M^+, $C_{15}H_{24}O_3$ requires 252.1725] (2), 234 (60), 206 (27), 179 (100), 161 (45).

3.8. Separation of compounds 28b–34b formed by autoxidation of 7b on storage

Compound **7b** (61 mg, 0.26 mmol) was stored at −20°C in the freezer and found to have undergone some changes by ^{1}H NMR after six months. The mixture was separated by HPLC (12% EtOAc/*n*-hexane). Compound **7b** (45 mg, 0.19 mmol, 74%, R_t 12.8 min).

Compound **28b**: oil (1.8 mg, 0.067 mmol, 3%, R_t 39.3 min). $[\alpha]_D$=−45.4 (c 0.18, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3300, 2930, 1763, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 8.44 (1H, br s, OOH), 5.78 (1H, ddd, J=5.9, 5.9, 2.1 Hz), 4.16 (1H, s), 3.21 (1H, dq, J=6.6, 7.3 Hz), 2.93 (1H, ddd, J=11.9, 6.6, 6.4 Hz), 2.27 (1H, ddd, J=17.8, 5.9, 5.3 Hz), 1.93 (1H, ddd, J=17.8, 12.1, 5.9 Hz), 1.49 (1H, ddd, J=12.1, 11.4, 5.3 Hz), 1.79 (d, J=126.0 Hz, $^1J_{CH}$), 1.17 (3H, d, J=7.3 Hz), 0.93 (3H, d, J=6.4 Hz)—see Table 3; ^{13}C NMR: see Table 3—129.6 (d, J=2.9 Hz, $^2J_{CC}$), 127.0 (d, J=52.9 Hz, $^1J_{CC}$), 21.5 (ca. 70 times intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 249.1433 [M^+-H_2O, $C_{14}{}^{13}C_1H_{20}O_3$ requires 249.1446] (18), 225 (15), 197 (25), 167 (65), 139 (55), 125 (85), 111 (100).

Compound **29b**: oil (1 mg, 0.004 mmol, 2%, R_t 44.3 min). Physical properties as for arteannuin K—see Ref. 18; ^{1}H NMR (δ, $CDCl_3$) ppm: 5.67 (1H, dd, J=5.3, $^3J_{CH}$, 5.3 Hz), 3.70 (1H, s), 3.11 (1H, dq, J=7.3, 7.1 Hz), 2.72 (1H, ddd, J=12.0, 6.4, 6.4 Hz), 2.27 (1H, m), 2.00 (1H, m), 1.79 (3H, d, J=126.2 Hz, $^1J_{CH}$), 1.44 (1H, ddd, J=11.0, 11.0, 5.3 Hz), 1.14 (3H, d, J=7.1 Hz), 0.94 (3H, d, J=6.3 Hz)—see Table 3; ^{13}C NMR: see Table 3—27.2 (d, J=5.0 Hz, $^3J_{CC}$), 21.2 (ca. 75 times intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 251.1601 [M^+, $C_{14}{}^{13}C_1H_{22}O_3$ requires 251.1603] (3), 167.1048 [$M^+-C_4{}^{13}C_1H_7O$ for a retro-Diels Alder fragmentation requires 167.1072] (100).

Compound **30b**: oil (6.3 mg, 0.025 mmol, 10%, R_t 14.8 min) $[\alpha]_D$=−89.3 (c 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3028, 2932, 2856, 1769, 1678, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 6.73 (1H, ddd, J=5.5, $^3J_{CH}$, 5.5, 2.6 Hz),

3.25 (1H, dq, J=10.4, 6.8 Hz), 2.75 (1H, ddd, J=10.4, 6.5, 6.5 Hz), 2.50 (1H, ddd, J=17.5, 4.9, 4.9 Hz), 2.40 (1H, dd, J=17.5, 9.5 Hz), 1.77 (3H, d, J=124.6 Hz, $^1J_{CH}$), 1.12 (3H, d, J=6.8 Hz), 0.96 (3H, d, J=6.0 Hz)—see Table 3; ^{13}C NMR: see Table 3—132.6 (d, J=45.9 Hz, $^1J_{CC}$), 16.2 (ca. 70 times intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 249.1444 [M^+, $C_{14}{}^{13}C_1H_{20}O_3$ requires 249.1446] (100), 221 (30), 176 (28).

Compound **31b**: oil (4.0 mg, 0.015 mmol, 6%, R_t 53.6 min). $[\alpha]_D$=+62.6 (c 0.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3531, 3309 (br), 2934, 1759, 1454 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 7.89 (1H, s, OOH), 5.88 (1H, d, J=6.0 Hz, $^3J_{CH}$), 4.35 (1H, br s), 3.12 (1H, dq, J=6.4, 7.1 Hz), 2.43 (1H, d, J=14.6 Hz), 2.13 (1H, ddd, J=10.2, 6.4, 6.1 Hz), 1.84 (3H, d, J=127.1 Hz, $^1J_{CH}$), 1.15 (3H, d, J=7.1 Hz), 0.99 (3H, d, J=6.5 Hz)—see Table 3; ^{13}C NMR: see Table 3—137.0 (d, J=43.9 Hz, $^1J_{CC}$), 21.0 (ca. 95 times the intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 249.1440 [M^+-H_2O, $C_{14}{}^{13}C_1H_{20}O_3$ requires 249.1446] (100), 234 (30), 221 (45), 205 (50), 179 (35), 178 (95), 160 (80).

Compound **32b**: oil (6.9 mg, 0.028 mmol, 11%, R_t 22.4 min). $[\alpha]_D$=+65.6 (c 0.7, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3020, 2932, 1767, 1682, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 6.76 (1H, d, J=5.7 Hz, $^3J_{CH}$), 3.16 (1H, dq, J=5.9, 7.1 Hz), 2.70 (1H, dd, J=17.3, 4.3 Hz), 2.61 (1H, dd, J=17.3, 12.3 Hz), 2.25 (1H, ddd, J=11.5, 5.9, 5.9 Hz), 1.81 (3H, dd, J=128.4, $^1J_{CH}$, 1.5 Hz), 1.19 (3H, d, J=7.1 Hz), 0.94 (3H, d, J=6.6 Hz)—see Table 3; ^{13}C NMR: see Table 3—139.4 (d, J=44.9 Hz, $^1J_{CC}$), 15.8 (ca. 70 times the intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 249.1442 [M^+, $C_{14}{}^{13}C_1H_{20}O_3$ requires 249.1446] (100), 221 (25), 205 (35), 176 (40).

Compound **33b**: oil (1.4 mg, 0.005 mmol, 2%, R_t 24.4 min). $[\alpha]_D$=+43.5 (c 0.14, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3408 (br), 2930, 1759, 1460 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 7.73 (1H, br s, OOH), 5.82, (1H, dd, J=6.3 Hz, $^3J_{CH}$=1.6 Hz), 4.43 (1H, dd, J=10.1, 6.5 Hz), 3.13 (1H, dq, J=6.5, 7.2 Hz), 2.30 (1H, ddd, J=12.5, 6.5, 2.6 Hz), 2.05 (1H, dd, J=12.5, 10.1 Hz), 1.82 (3H, ddd, J=127.4 Hz, $^1J_{CH}$, 1.0, 1.0 Hz), 1.15 (3H, d, J=7.2 Hz), 0.96 (3H, d, J=6.5 Hz)—see Table 3; ^{13}C NMR: see Table 3—19.5 (ca. 95 times the intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 249.1444 [M^+-H_2O, $C_{14}{}^{13}C_1H_{20}O_3$ requires 249.1446] (40), 225 (28), 183 (30), 169 (100).

Compound **34b**: oil (0.6 mg, 0.002 mmol, 1%, R_t 19.5 min); $[\alpha]_D$=−16.7 (c 0.06, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3024, 2932, 2860, 1774, 1713, 1458 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 3.46 (1H, d, J=1.9 Hz), 3.24 (1H, dq, J=6.9, 7.1 Hz), 2.60 (1H, dd, J=18.9, 5.9 Hz), 2.54 (1H, ddd, J=11.4, 6.9, 5.7 Hz), 2.19 (1H, dd, J=18.9, 12.3 Hz), 2.04 (1H, ddd, J=12.3, 5.9, 5.3 Hz), 1.42 (3H, d, J=124.6 Hz, $^1J_{CH}$), 1.21 (3H, d, J=7.1 Hz), 0.90 (3H, d, J=6.6 Hz)—see Table 3; ^{13}C NMR: see Table 3—15.4 (ca. 90 times intensity of other ^{13}C peaks); HREIMS m/z (rel. int.) 265.1409 [M^+, $C_{14}{}^{13}C_1H_{20}O_4$ requires 265.1409] (2), 177 (85), 176 (70), 162 (100).

3.8.1. ^{1}H NMR investigation of the transformation of 28b in $CDCl_3$ solution. Compound **28b** (1.8 mg) was left in $CDCl_3$ (0.6 ml) for forty five days and ^{1}H NMR spectra were acquired approximately every 5 days. Over this time characteristic peaks for compounds **29b**–**32b** were observed to grow in intensity, until the starting material had almost entirely disappeared leaving a complex mixture consisting of ca. 10% **29b**, 10% **30b**, 15% **31b** and 10% **32b** together with a large number of unidentified peaks. Characteristic peaks used in identifying **29b** in the mixture: 5.67 (1H, m), 3.71 (1H, d, J=2.7 Hz), 2.72 (1H, ddd, J=12.0, 6.4, 6.4 Hz). Characteristic peaks used in identifying **30b** in the mixture: 6.73 (1H, ddd, J=5.5, 5.5, 2.6 Hz), 3.25 (1H, dq, J=10.4, 6.8 Hz), 2.75 (1H, ddd, J=10.4, 6.5, 6.5 Hz). Characteristic peaks used in identifying **31b** in the mixture: 5.88 (1H, d, J=6.0 Hz), 4.35 (1H, br s), 0.99 (3H, d, J=6.5 Hz). Characteristic peaks used in identifying **32b** in the mixture: 6.76 (1H, d, J=5.6 Hz), 2.61 (1H, dd, J=17.2, 12.5 Hz), 1.20 (3H, d, J=7.0 Hz).

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