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Synthesis of labelled dihydroartemisinic acid

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Abstract—[15-$^{13}C^{2}H_3$]-Dihydroartemisinic acid (**2a**), [15-$C^{2}H_3$]-dihydroartemisinic acid (**2b**) and [15-$^{13}CH_3$]-dihydroartemisinic acid (**2c**) have been obtained in good yield and high isotopic enrichment by a reconstructive synthesis from artemisinin. These labelled compounds were designed to be used in biosynthetic experiments to determine the origins of artemisinin and other sesquiterpene natural products from *Artemisia annua*.

1. Introduction

The Chinese medicinal plant *Artemisia annua* L has been the subject of intensive phytochemical investigations over the past two decades, following the discovery of the anti-malarial amorphane sesquiterpene artemisinin (qinghaosu) (**1**).[1] Although the biosynthesis of the amorphane carbocycle from which **1** is almost certainly derived seems to proceed by the normal pathways of terpenoid biosynthesis,[2–7] there is considerable uncertainty regarding the latter steps in the biogenesis of artemisinin, which must involve carbon–carbon cleavage at C-4/C-5 in order to produce a compound based on the *seco*-amorphane skeleton which would be the immediate precursor to the 1,2,4-trioxane ring of **1**.[8] All investigators seem to concur that the amorphane sesquiterpene dihydroartemisinic acid (**2**)[9,10] and/or its 11,13-dehydro analogue, artemisinic acid (arteannuic acid),[4,5,10–16] are advanced precursors en route to artemisinin. However, there are several differing, and sometimes directly conflicting, views as to exactly how the transformation of artemisinic acid/dihydroartemisinic acid into **1** occurs in vivo.[8,10,17–20]

We now report a synthetic route to compound **2** which achieves the incorporation of a stable isotopic label at the 15-position with very high isotopic enrichment and in a good overall yield. Three isotopomers were prepared by this procedure: [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid (**2a**); [15-$C^{2}H_3$]-dihydroartemisinic acid (**2b**); and [15-$^{13}CH_3$]-dihydroartemisinic acid (**2c**).[†] These labelled precursors were designed to be used in feeding experiments with *A. annua* in order to determine the biogenesis of artemisinin and other sesquiterpene natural products from this species. The use of stable-isotope labelled precursors such as **2a**–**2c** has the advantage over all previous biosynthetic studies (which have exclusively used radio-isotopically labelled precursors,[2–5,10,12,15,16,19,20] if labelled substrates were used at all[9,17,18]) that one-dimensional (1D) ^{2}H NMR and/or ^{13}C NMR spectroscopy can be used to directly study their transformations in vivo, providing chemical shift information[‡] from the isotopic label at the 15-position by which metabolites can be identified directly in crude plant extracts; since there is no absolute requirement for a prior

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[†] For all compounds in this paper with the suffix '**a**', the isotopically-normal [15-CH_3] group has been replaced by [15-$^{13}C^{2}H_3$]; for compounds labelled with the suffix '**b**', the [15-CH_3] group has been replaced by [15-$C^{2}H_3$]; for compounds labelled with the suffix '**c**', the [15-CH_3] group has been replaced by [15-$^{13}CH_3$]. The suffix '*' indicates an unspecified isotopic enrichment at the 15-position.

[‡] When ^{13}C is isotopically enriched from the natural abundance level of 1.1% to ca. 100%, there is no effect on carbon chemical shift (δ_C). Deuterium chemical shifts (δ_D) are also essentially identical to proton chemical shifts (δ_H) for nuclei in the same chemical environment, but the substitution of ^{1}H by ^{2}H causes an approximately 0.3 ppm upfield shift in δ_C of the directly-bound carbon (and also results in a 1:1:1 triplet splitting). Thus, a CD group appears as a triplet 0.3 ppm upfield of the corresponding CH resonance in ^{13}C NMR spectroscopy; a CD_2 group appears as a 1:2:3:2:1 quintet situated 0.6 ppm upfield of the corresponding CH_2 resonance; and a CD_3 group appears as a 1:3:6:7:6:3:1 septet lying 0.9 ppm upfield of the corresponding CH_3 resonance. Hence, deuterium and carbon chemical shifts can be used to infer the identity of a labelled metabolite, provided that the resonances in the ^{1}H and ^{13}C NMR spectra of that metabolite, in particular the resonances at the 15-position, have been previously assigned.

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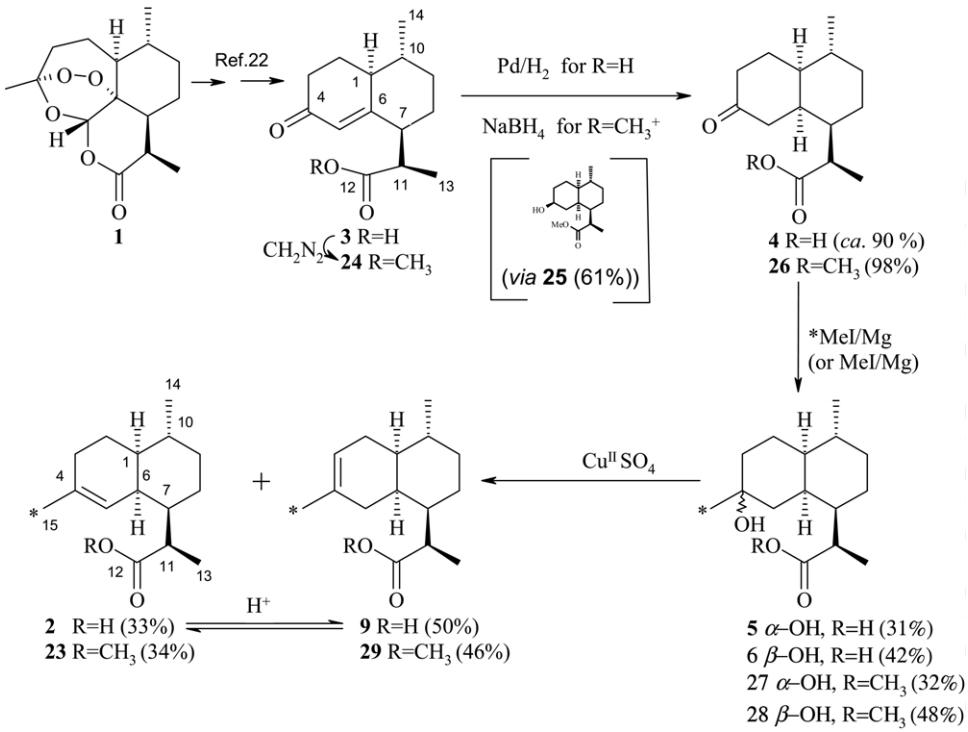


Scheme 1. Synthesis of **2** and its isotopically-labelled forms **2a**–**2c** from **1** via the keto-acid intermediate **4** (∗ =isotopic label at the 15-position). + The methyl ester of **4**, compound **26**, was obtained by Jones oxidation of the immediate reduction product from compound **24**, saturated alcohol **25**.

chromatographic separation of metabolites when using NMR to analyse the metabolism of stable-isotope labelled precursors, the possibility for the introduction of artifacts during extensive sample handling is consequently minimized.[21] The use of stable isotopes also gives more detailed information as to the nature of metabolism than would be possible for studies of the transformations of **2** when conducted using a radio-isotopically labelled precursor, as will be demonstrated in the companion paper, in which experiments involving the feeding of all three labelled precursors **2a**–**2c** to intact plants of *A. annua* are described. In particular, the newly developed two-dimensional (2D) NMR technique of $^{13}C-^{2}H$ correlation spectroscopy ($^{13}C-^{2}H$ COSY)[21] provides a powerful tool by which to analyse the metabolism of the doubly-labelled precursor **2a**.

2. Results and discussion

We have recently reported the preparation of both [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid and [15-$^{13}CH_3$]-dihydro-*epi*-deoxyarteanuin B in moderate yield via a reconstructive synthesis from artemisinin.[22] One disadvantage of this strategy was that there was always an unavoidable and quite extensive depletion of the deuterium label at the 15-position, which made this procedure less than ideal for synthesizing ^{2}H-labelled dihydroartemisinic acid (**2a/2b**) for use in feeding experiments with *A. annua* plants. We have therefore now developed an improved procedure as is shown in Scheme 1, which involves introduction of the isotopic label via Grignard reaction of labelled methyl iodide with the keto-acid **4**, rather than with its synthetic precursor the α,β-unsaturated keto-acid **3** (in our previous synthesis,[22] it was the reduction of the product from Grignard addition with **3** which was responsible for the depletion of the ^{2}H label). The key intermediate, *cis*-decalone **4**, was obtained with quite high stereospecificity from hydrogenation of **3** (less than 10% of the alternative *trans*-decalone stereoisomer was observed in the crude product from this reaction and, in order to keep the overall yield of the synthesis high, the undesired *trans*-decalin isomer was not normally separated at this stage). Although the *trans*-decalones **7** and **8** (Fig. 1) obtained from the Grignard addition of methyl iodide to this crude hydrogenation product were separable from the major reaction products, epimeric *cis*-decalones **5** and **6** (Scheme 1), these minor components were also normally carried through to the next step. Compounds **10** and **11**, the *trans*-decalin analogues of artemisinic acid (**2**) and its Δ^3 regio-isomer (**9**), respectively, were therefore also present as contaminants, following the dehydration of the mixture of tertiary alcohol intermediates **5**–**8** in the last step, and these compounds were most conveniently removed at the very end of the synthesis when the desired product, compound **2**, was separated by HPLC from its regio-isomer **9**.

The synthesis of both of the labelled precursors **2a** and **2b** by this method was superior to our previous procedure[22] in that the retention of the deuterium label in the 15-[$^{13}C^{2}H_3$] and 15-[$C^{2}H_3$] groups of **2a** and **2b** was close to 100%, as shown by the NMR spectra of these compounds (Figs. 2 and 3).[§] We have also been able to enhance the overall yield for this synthesis of **2**, as compared with previously published

[§] However, note that it is important to carefully control the conditions for the Cu(II)-catalysed dehydration step, as use of excess Cu(II) can also lead to an almost complete depletion of ^{2}H label from the 15-position (we were unable to achieve this dehydration by the use of acid catalysis, although this procedure has been reported by others).[23,24]

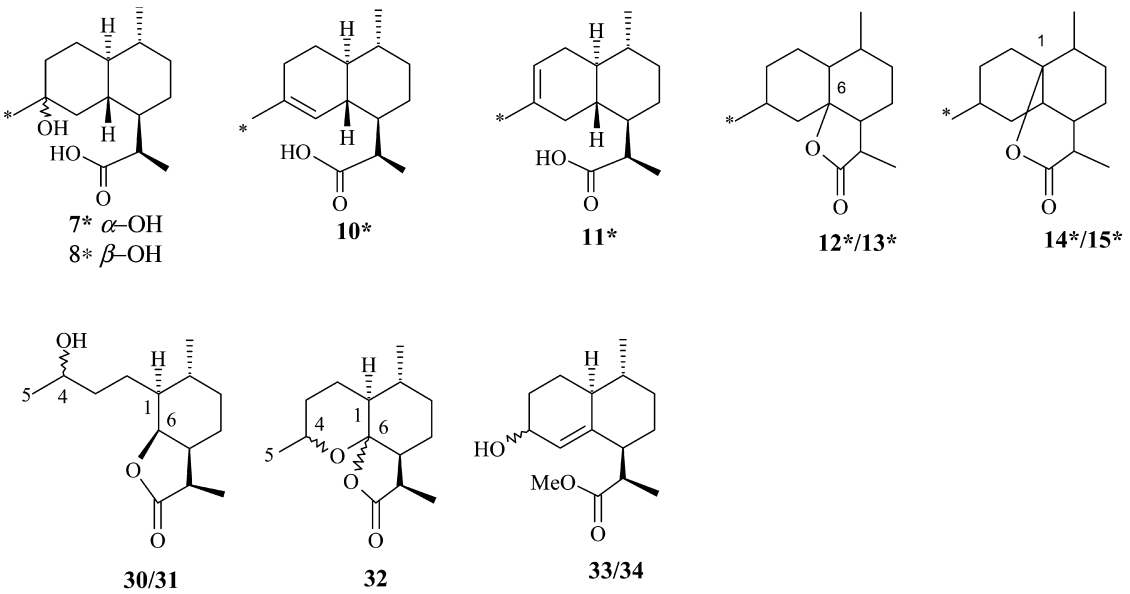


Figure 1. Minor products from the syntheses described in Scheme 1 (* =unspecified isotopic enrichment).

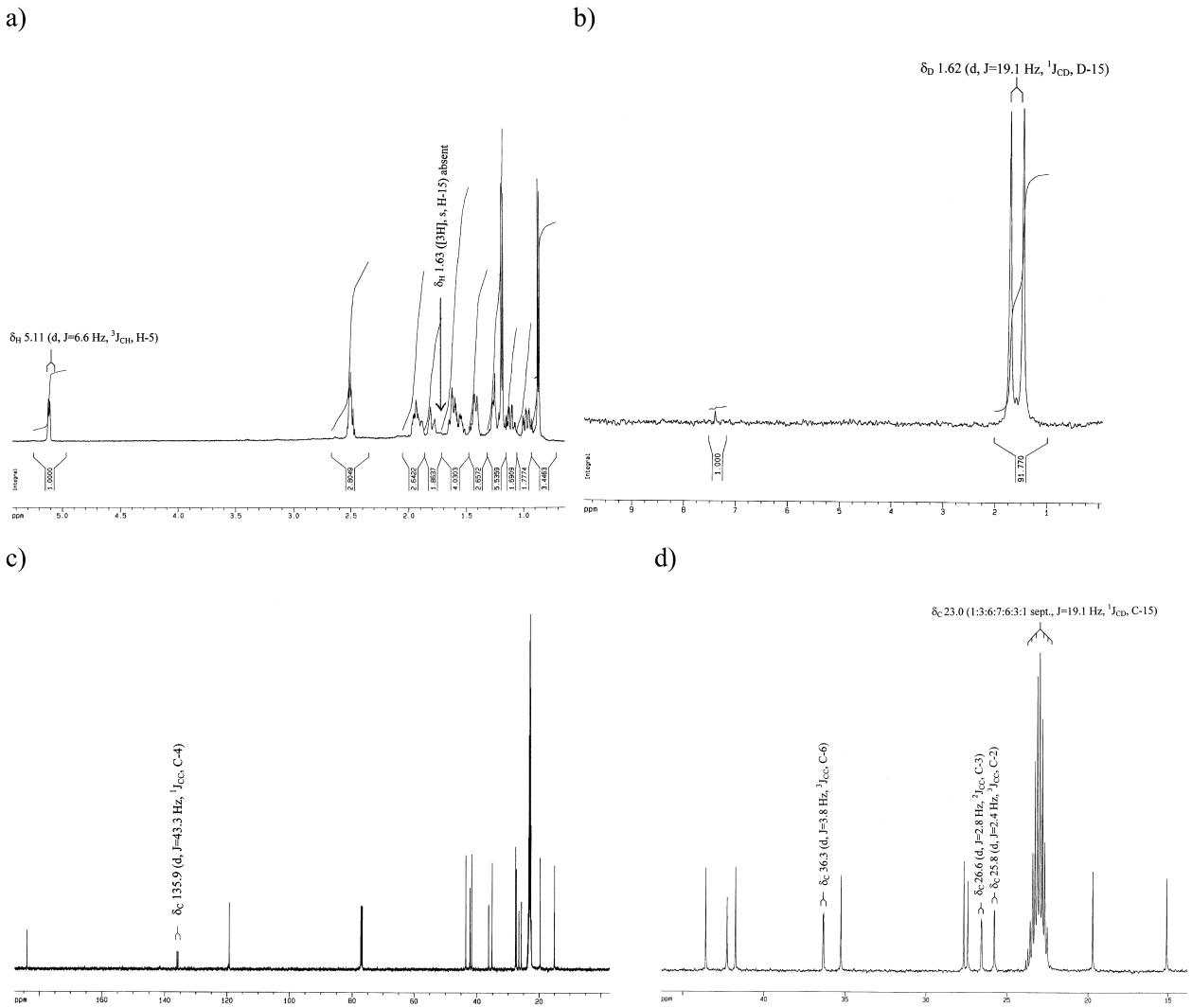


Figure 2. (a) ^{1}H NMR, (b) ^{2}H NMR and (c) ^{13}C NMR (and expansion d) spectra of [15-$^{13}C^{2}H_3$]-dihydroartemisinic acid (**2a**) prepared from artemisinin (**1**), following introduction of the isotopic label to intermediate **4**, showing ca. 100% labelling of both ^{13}C and ^{2}H at the 15-position.

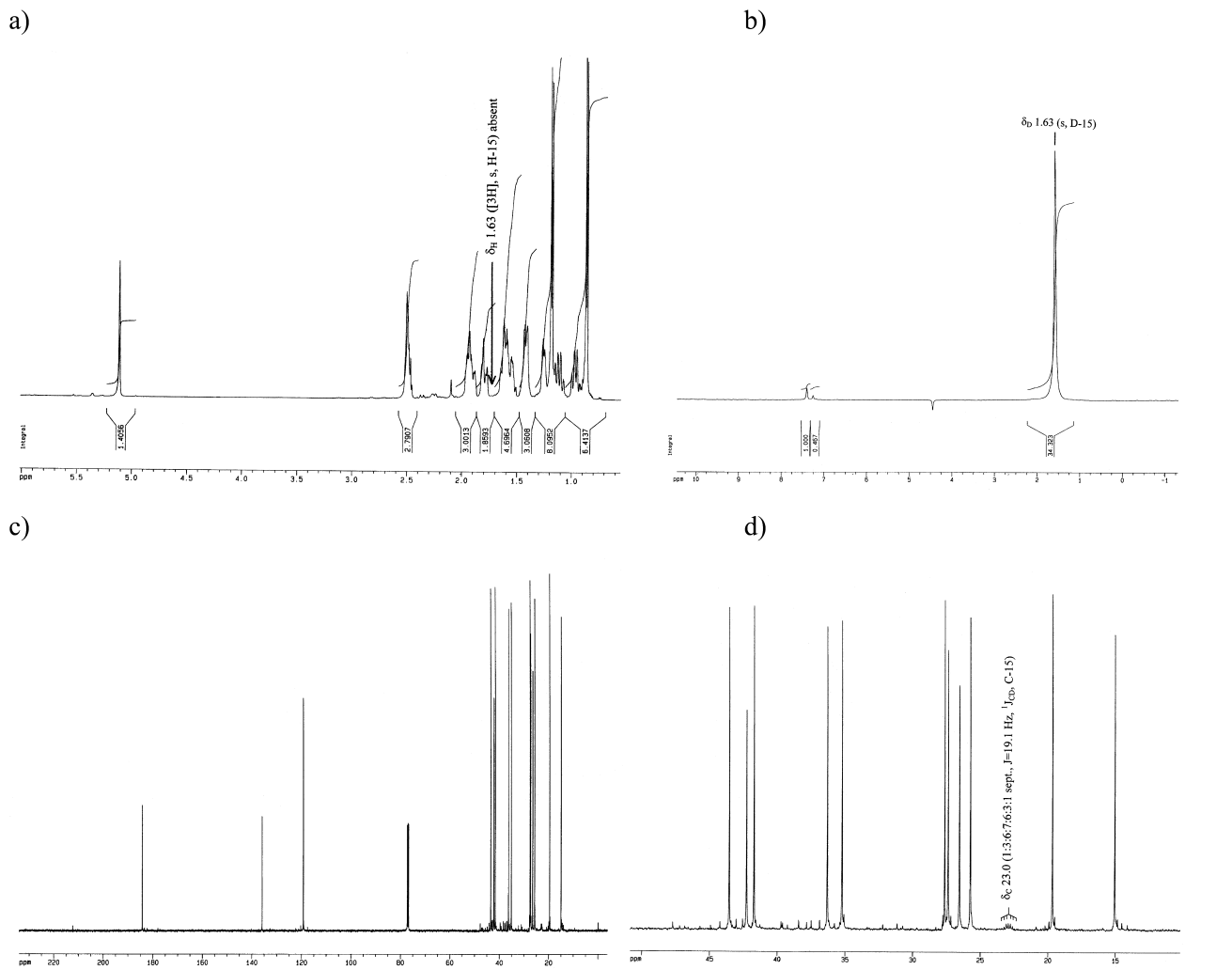
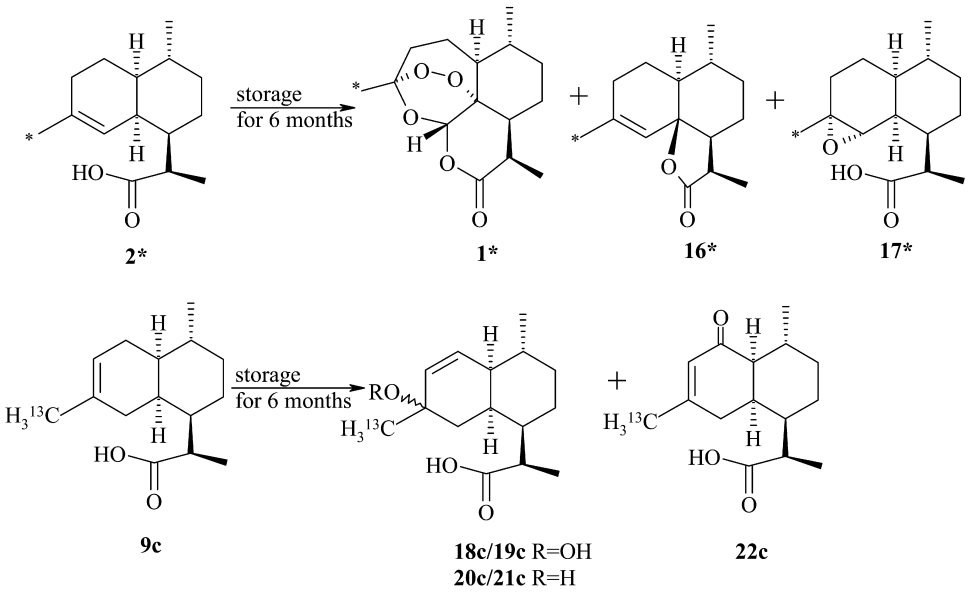


Figure 3. (a) ^{1}H NMR, (b) ^{2}H NMR and (c) ^{13}C NMR (and expansion d) of spectra of [15-$C^{2}H_{3}$]-dihydroartemisinic acid (**2b**) prepared from artemisinin (**1**), following the introduction of isotopic label to intermediate **4**, showing ca. 100% enrichment of ^{2}H label at the 15-position.

procedures which have involved a similar dehydration of a tertiary alcohol to Δ^{3}/Δ^{4} amorphene products,[23–25] by introducing an additional step after the dehydration, in which the (unwanted) Δ^{3} regio-isomer, compound **9**, is equilibrated with its Δ^{4}-isomer, compound **2**. Thus, following the HPLC separation of the two double bond regio-isomers, dihydroartemisinic acid (**2**) and compound **9** (produced in an approximately 5:7 ratio by the dehydration of **5/6**), the purified Δ^{3}-isomer (**9**) was converted back into this same equilibrating mixture with the desired Δ^{4}-isomer, compound **2**, by treatment with acid; and the two isomers were then separated again by HPLC. By applying two such cycles of equilibration and purification, the yield of labelled dihydroartemisinic acid (**2**) was raised from 33% (as had been the case in the (now) penultimate step of the reaction for dehydration of epimeric alcohols **5/6**) to almost 60%, with no associated loss of label from the 15-position. This procedure required careful optimization in order to avoid contamination by other products of double bond rearrangement (such as the five-membered lactones **12** and **13**; and the six-membered lactones **14** and **15** (Fig. 1), which were presumably formed by more extensive acid-catalysed migrations of the Δ^{3} double bond, allowing for the formation of carbocations at C-6 and then C-1, which became ‘trapped’ by the carboxylic acid group).

As reported in our previous communication,[22] dihydroartemisinic acid which had been labelled with a stable isotope at the 15-position was found to be susceptible to autoxidation after prolonged storage for several months, even when kept in the dark at −20 °C in the freezer. This was a significant observation in connection with the proposed use of this compound as a labelled precursor for feeding studies which were designed to establish biosynthetic routes in *A. annua*, because all three of the compounds which were obtained following such storage[22] (compounds **1**, **16** and **17** in Scheme 2), have also been reported as natural products from this species.[26,27] Hence, failure to pay attention to the susceptibility of **2** towards spontaneous autoxidation during storage could lead to an erroneous interpretation when analyzing the results of feeding experiments. Interestingly, the Δ^{3}-isomer of dihydroartemisinic acid (**9**) was also found to be susceptible to spontaneous autoxidation on storage, although, in this case, the isolable oxidation products were simply either allylic hydroperoxides (**18** and **19**), which are expected from the ‘ene-type’ reaction of $^{1}O_{2}$ with the Δ^{3} double bond in **9**, or hydroxides (**20** and **21**) which are probably formed by the homolysis of such hydroperoxides (see the companion paper for a discussion of these processes). The unusual α,β-unsaturated ketone **22**, which was a very minor product of



Scheme 2. Autoxidation of both the desired biosynthetic precursor, labelled dihydroartemisinic acid (**2***) (see Ref. 22), and of its Δ^3-isomer, compound **9c**, after storage for several months (* =unspecified isotopic enrichment).

autoxidation, may be formed by 3,2-allylic rearrangement of either one of the tertiary allylic hydroperoxides **18** or **19** and a subsequent dehydration reaction (cf. similar reactions of closely related compounds which are proposed in Refs. 22,26). There was no sign of any alternative products from the further reactions of such hydroperoxides; and, in particular, the complex rearrangement reactions, which are responsible for the appearance of **1** and **17** during the autoxidation of dihydroartemisinic acid (**2**) on storage, appear not to be occurring in the case of its Δ^3 isomer, compound **9**.

In view of the instability of dihydroartemisinic acid towards prolonged storage, it was clearly preferable that the synthesis of each of the labelled precursors **2a**–**2c** should be performed immediately prior to their use as biogenetic precursors in feeding experiments, so as to avoid the possibility of confusing the products of spontaneous in vitro autoxidation with any products arising from the metabolism of dihydroartemisinic acid in vivo. However, on some occasions this was found to be impractical, and an alternative procedure was therefore developed which parallels the approach already described in Scheme 1. The rationale for this alternative strategy was that the methyl ester of dihydroartemisinic acid, compound **23**, is known to be more stable towards autoxidation than dihydroartemisinic acid (**2**) itself,[28,29] and that it can readily be converted back to **2**, by hydrolysis of the ester group, as and when required for use in feeding experiments.

The synthesis of this alternative product, the methyl ester of dihydroartemisinic acid (**23**), was a straightforward procedure. It is reported that the unsaturated keto-acid **3** from the acid degradation of artemisinin (**1**) can be readily converted into its methyl ester,[30] compound **24**, by treatment with diazomethane,[22] and it was found that **24** could then be subjected to a similar series of transformations as for **3** (Scheme 1). The reduction of the $\Delta^{5,6}$ double bond in compound **24** by sodium borohydride was significantly more stereoselective[¶] than had been the case for the reduction of this same double bond in its free acid analogue compound **3** by hydrogen over a palladium catalyst such that, under optimized conditions, only the *cis*-decalin methyl ester, compound **25**, was observed in the crude reaction product (and no *trans*-decalin isomer was 'carried through' to the key intermediate, compound **26**, as a result); and this is one reason why it was found preferable to esterify the carboxylic acid group early on in the synthesis of **23**, rather than in the final step. In addition, only 1 equiv. of labelled Grignard reagent was then required in the preparation of epimeric tertiary allylic alcohols **27/28** from **26**:[31] by contrast, 2 equiv. had been required for the formation of the free acid analogues of these products, compounds **5/6** in the direct synthesis of dihydroartemisinic acid (**2**) (in this case, 1 equiv. of labelled Grignard reagent was lost in deprotonating the carboxylic acid group). The final step in this alternative synthesis, the dehydration of the mixture of epimeric alcohols **27/28**, yielded the methyl ester of dihydroartemisinic acid (**23**) as a mixture with its Δ^3 regio-isomer (**29**), as expected.

The full ^{13}C and ^{1}H NMR assignments which are reported for the synthetic intermediates and products **4**–**15**, **23** and **25**–**34** in Tables 1–4 were made by the 2D NMR techniques HSQC, HMBC, and ^{1}H–^{1}H COSY; these unambiguous assignments were necessary in order to make an independent verification of issues of stereo- and regio-isomerism at each step of the synthesis described in Scheme 1 based on the analysis of the NOESY spectrum of

[¶] However, note that several alternative reduction products, such as compounds **30**–**34**, which are shown in Figure 1, were obtained from the treatment of **24** with $NaBH_4$ under non-optimized reaction conditions (compounds **30**–**32** probably arise from an initial retro-aldol reaction of the αβ-unsaturated ketone group in **24** under the basic conditions of this reaction).

Table 1. ^{13}C NMR data for isotopically-normal compounds described in Scheme 1 (see Section 4 for the effects of isotopic enrichment on the appearance of the NMR spectra of labelled compounds with suffix '**a**', '**b**' and '**c**')

Position[a]	**4**	**5**	**6**	**9**	**23**	**25**	**26**	**27**	**28**	**29**
1 (CH)	42.6	43.2	43.4	42.2	41.7	42.9	42.6	43.1	43.4	42.2
2 (CH_2)	27.8	23.6	25.7	27.8	25.8	26.3	27.8	23.6	25.8	27.9
3 (CH_2)	36.9	33.6	35.0	119.1 (CH)	26.6	30.4	36.9	33.5	35.2	119.2 (CH)
4 (C)	212.6	70.0	72.1	131.8	135.9	71.8 (CH)	212.2	69.9	71.9	131.8
5 (CH_2)	37.5	33.3	34.9	26.3	119.5 (CH)	30.1	37.5	33.2	34.9	26.3
6 (CH)	42.2	32.2	35.1	33.4	36.4	36.0	38.5	32.2	35.1	33.5
7 (CH)	43.1	43.5	43.2	43.7	44.0	43.7	43.4	43.7	43.6	44.1
8 (CH_2)	25.7	26.4	26.3	25.8	27.5	26.5	25.7	26.4	26.3	25.9
9 (CH_2)	35.1	35.6	35.6	35.6	35.3	35.6	35.1	35.6	35.5	35.6
10 (CH)	27.2	26.5	26.7	27.8	27.7	27.4	27.2	26.5	26.7	27.9
11 (CH)	42.2	42.0	42.3	42.5	42.2	42.3	42.1	42.2	42.2	42.4
12 (C)	182.6	183.0	182.6	183.9	178.0	177.7	177.2	177.8	177.8	177.8
13 (CH_3)	14.9	15.0	14.8	15.1	15.1	15.0	14.9	15.0	14.8	15.2
14 (CH_3)	19.5	19.7	19.6	20.1	19.7	19.6	19.5	19.8	19.6	20.1
15 (CH_3)	—	32.1	26.1	23.6	23.8	—	—	32.1	26.2	23.6
12-OMe	—	—	—	—	51.4	51.4	51.5	51.4	51.4	51.3

[a] Multiplicity determined from DEPT.

each of these compounds. Complete isotopic labelling by three ^2H atoms at the 15-position (as in the case of all of the compounds with the suffix '**a**' and '**b**') generally caused an upfield shift in the ^{13}C NMR spectrum of ca. 0.9 ppm at C-15 and resulted in the splitting of this resonance into a 1:3:6:7:6:3:1 septet[22] due to the 19–20 Hz single-bond carbon–deuterium coupling constant ($^1J_{CD}$), as well as resulting in an absence of the H-15 resonance from the ^1H NMR spectrum. The ca. 100% isotopic enrichment of ^{13}C at the 15-position (as in all of the compounds with the suffix '**a**' and '**c**') resulted in the appearance of doublet splittings in the ^{13}C NMR spectrum due to single-bond ($^1J_{CC}$) and long-range carbon–carbon couplings ($^2J_{CC}$ and $^3J_{CC}$) at some or all of C-3, C-4, C-5 and C-6; as well as a doublet splitting for H-5 in the ^1H NMR spectrum, due to a long-range carbon–proton coupling ($^3J_{CH}$). These effects are described in greater detail in Section 4.

3. Conclusion

Two methods for the preparation of dihydroartemisinic acid, which is labelled at the 15-position by either ^{13}C or $^2H_3$ (or both), have been developed and optimized such that the appropriate labelled precursor can be synthesized in good yield and with close to 100% isotopic enrichment. The first method involved the direct preparation of labelled dihydroartemisinic acid (**2**) from artemisinin (**1**). In view of the tendency of this compound to undergo spontaneous autoxidation on prolonged storage, such labelled versions of dihydroartemisinic acid should be used immediately in feeding experiments with *A. annua* plants. The second synthesis resulted in the methyl ester of dihydroartemisinic acid (**23**), which can be prepared in advance of any biological experiments, as it is more stable to storage than dihydroartemisinic acid itself; it is then a simple

Table 2. ^1H NMR data for isotopically-normal compounds described in Scheme 1 (see Section 4 for the effects of isotopic enrichment in the NMR spectra of labelled compounds with suffix '**a**', '**b**' and '**c**')

Position	**4**	**5**	**6**	**9**	**23**	**25**	**26**	**27**	**28**	**29**
1	1.40	1.20	1.17	1.23	1.25	1.14	1.38	1.19	1.17	1.21
2α	1.69	1.68	1.40	2.05	1.55	1.31	1.70	1.67	1.39	2.04
2β	2.23	1.76	1.88	2.15	1.94	1.95	2.21	1.75	1.89	2.15
3α	2.25	1.43[a]	1.53[a]	5.29	1.91	1.72	2.22	1.67	1.53[a]	5.28
3β	2.35	1.40[a]	1.46[a]	—	1.80	1.34	2.32	1.45	1.45[a]	—
4	—	—	—	—	—	3.59	—	—	—	—
5α	2.07	1.45	1.57	1.92	5.12	1.53	2.05	1.40	1.53	1.88
5β	2.38	1.19	1.24	1.57	—	1.30	2.35	1.19	1.24	1.57
6	2.18	2.17	1.86	2.09	2.50	1.82	2.24	2.16	1.84	2.07
7	1.81	1.68	1.68	1.70	1.62	1.68	1.80	1.66	1.68	1.69
8α	1.56	1.45	1.68	1.49[a]	1.25	1.30	1.36	1.27	1.71	1.38[a]
8β	1.34	1.25	1.44	1.42[a]	1.08	1.24	1.30	1.20	1.23	1.31[a]
9α	1.11	1.00	0.99	1.00	0.94	0.99	1.10	0.98	0.97	0.97
9β	1.82	1.69	1.70	1.69	1.59	1.68	1.79	1.66	1.68	1.65
10	1.82	1.59	1.71	1.39	1.41	1.66	1.80	1.57	1.71	1.37
11	2.18	2.29	2.28	2.29	2.50	2.30	2.19	2.26	2.29	2.31
13	1.15	1.17	1.13	1.18	1.13	1.10	1.10	1.12	1.09	1.13
14	0.96	0.84	0.83	0.82	0.86	0.83	0.96	0.83	0.83	0.81
15	—	1.23	1.28	1.63	1.63	—	—	1.22	1.27	1.63
12-OMe	—	—	—	—	3.68	3.66	3.67	3.66	3.66	3.66

[a] Assignments as α and β interchangeable.

Table 3. ^{13}C NMR data for compounds reported in Figure 1 (see Section 4 for the effects of isotopic enrichment in the NMR spectra of labelled compounds with suffix '**a**', '**b**' and '**c**')

Position[a]	7	8	10	11	12c	13c	14c	15c	30	31	32	33/34
1 (CH)	48.7	48.5	46.8	44.3	49.3	44.4	84.0 (C)	84.6 (C)	45.1	45.1	47.5	45.10/44.93
2 (CH_2)	28.3	25.8	26.6	31.2	24.9	22.8	28.1	34.5	24.9	25.4	22.0	22.92/22.94
3 (CH_2)	39.8	38.1	30.8	120.4 (CH)	34.7	28.3	26.6	30.9	36.2	36.3	33.5	30.11/29.09
4 (C)	71.6	70.4	135.1	132.8	28.0 (CH)	28.6 (CH)	26.4 (CH)	32.6 (CH)	68.2 (CH)	68.6 (CH)	68.3 (CH)	66.53/66.47 (CH)
5 (CH_2)	44.0	42.5	122.4 (CH)	35.5	43.9	42.2	31.6	36.0	23.4 (CH_3)	23.7 (CH_3)	21.0 (CH_3)	120.63/121.69 (CH)
6 (CH)	41.8	39.3	43.4	39.8	85.2 (C)	87.7 (C)	36.4	44.9	79.2	79.1	106.9 (C)	144.76/144.29 (C)
7 (CH)	45.8	46.4	45.7	47.7	43.1	43.7	37.1	37.3	40.4	40.4	44.1	47.31/47.19
8 (CH_2)	29.0	27.6	28.2	27.3	24.3	21.1	27.5	20.9	22.7	22.7	25.1	32.59/32.40
9 (CH_2)	35.5	35.6	35.8	35.3	32.8	31.7	26.5	25.9	33.1	33.0	33.0	35.47
10 (CH)	36.9	36.8	36.2	37.9	30.7	28.4	41.8	30.7	31.3	31.4	31.6	39.59/39.52
11 (CH)	40.3	39.3	39.4	39.3	39.3	38.7	33.6	41.1	42.2	42.2	39.2	41.01/41.05
12 (C)	181.2	179.8	183.4	180.4	179.7	180.1	180.4	176.2	179.7	179.8	179.8	176.86
13 (CH_3)	13.0	14.4	14.3	14.6	9.4	13.1	13.6	14.0	9.1	9.1	8.7	16.19
14 (CH_3)	20.1	20.0	19.8	19.9	19.9	19.5	14.4	14.2	20.0	20.0	18.8	20.19/20.24
15 (CH_3)	25.7	31.5	23.8	23.5	22.3	22.4	16.7	22.0	—	—	—	—
12-OMe	—	—	—	—	—	—	—	—	—	—	—	51.4

[a] Multiplicity determined from DEPT.

procedure to convert this derivative back to labelled dihydroartemisinic acid, just prior to performing a feeding experiment.

4. Experimental

4.1. General

All ^1H and ^{13}C NMR experiments were recorded on either a Bruker DRX 500 or an AV 600 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 ^1H NMR spectra without recourse to 2D NMR analysis (see Tables 1–4 in the main text for full ^{13}C and ^1H NMR assignments, which were made by 2D NMR in all cases). ^2H NMR spectra were recorded at 76.7 MHz in $CHCl_3$ solution containing C_6D_6 (10 μl/100 ml), as an internal reference (δ_D 7.43 ppm). HSQC, HMBC, ^1H–^1H COSY and NOESY spectra were recorded with 1024 data points in F_2 and 256 data points in F_1. High-resolution MS were recorded in EI mode at 70 eV on a Finnigan-MAT 95 MS spectrometer. IR spectra were recorded in $CHCl_3$ on a Shimadzu 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.

4.2. Acid degradation of artemisinin (1) to 2-(4-methyl-7-oxo-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7-octahydro-naphthalen-1-yl)-propionic acid (3)

See Ref. 22 for the procedure for converting **1** into **3** (see Ref. 30 for other physical properties of **3**).

4.3. Hydrogenation of decalenone 3

To a solution of decalenone **3** (2 g) in EtOAc (100 ml) was added a catalytic amount of Pd/charcoal. The reaction mixture was connected to an atmospheric pressure hydrogenation apparatus and was left stirring overnight. The mixture was filtered and the solvent was removed under reduced pressure to yield a crude product (1.98 g, 98%) consisting predominantly of compound **4**, together with a little of its *trans*-decalone isomer (less than 10% by ^1H NMR spectroscopic analysis of the crude product), which could not be separated chromatographically.

4.3.1. 2-(4-Methyl-7-oxo-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid (4). Solid. Mp 160–162 °C; $[\alpha]_D$ −4.7 (*c* 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 3024, 2961, 2928, 2878, 1717, 1705, 1458 cm^{-1}; ^1H NMR (δ, $CDCl_3$) ppm: 1.15 (3H, d, *J*=6.8 Hz), 0.96 (3H, d, *J*=6.1 Hz)—see

Table 4. ^{1}H NMR data for compounds described in Figure 1 (see Section 4 for the effects of isotopic enrichment observed in the NMR spectra of labelled compounds with suffix '**a**', '**b**' and '**c**')

Position	7	8	10	11	12c	13c	14c	15c	30	31	32	33/34
1	0.63	0.55	0.76	0.86	0.99	1.35	—	—	1.17	1.19	1.26	1.50
2α	1.94	1.78	1.98	2.27	1.81[a]	1.76[a]	1.92[a]	1.45[a]	1.65	1.82	1.83[a]	1.70, 1.70[a]
2β	0.93	1.25	1.78	1.56	1.43[a]	0.91[a]	1.38[a]	2.17[a]	1.55	1.42	1.55[a]	1.70/1.70[a]
3α	1.39	1.29	1.98[a]	5.36	0.98[a]	0.91[a]	1.78[a]	1.66[a]	1.64	1.64	1.76[a]	1.92/1.70[a]
3β	1.73	1.71	1.94[a]	—	1.78[a]	1.46[a]	1.38[a]	1.03[a]	1.41	1.42	1.35[a]	1.35/1.55[a]
4	—	—	—	—	1.82	1.80	2.08	1.61	3.81	3.78	3.98	4.24/4.14
5α	1.05	0.94	5.54	1.57	0.96[a]	1.64[a]	1.78[a]	1.57[a]	1.21	1.20	1.11	5.38/5.41
5β	1.96	2.06	—	2.27	2.02[a]	1.45[a]	1.21[a]	1.44[a]	—	—	—	—
6	1.13	1.43	1.80	1.38	—	—	1.83	1.88	4.40	4.42	—	—
7	1.50	1.27	1.37	1.33	2.02	2.16	1.74	1.78	2.25	2.27	2.16	2.04
8α	1.72	1.77	1.72	1.79	1.66[a]	1.63[a]	1.52	1.67	1.62	1.64	1.78	1.69
8β	1.26	1.25	1.31	1.25	1.13[a]	1.23[a]	1.08	1.03	1.06	1.06	1.04	1.22
9α	1.06	1.01	1.12	1.05	1.13[a]	1.20[a]	1.50	1.06	0.97	1.01	1.07	1.73
9β	1.72	1.71	1.65	1.72	1.63[a]	1.67[a]	1.91	1.50	1.66	1.67	1.66	1.20
10	1.06	1.10	1.31	1.07	1.31	1.57	1.54	2.11	1.35	1.36	1.29	1.19
11	2.69	2.77	2.95	2.83	3.11	2.80	2.74	2.65	2.78	2.78	3.28	2.76
13	1.11	1.12	1.16	1.16	1.12	1.27	1.25	1.27	1.14	1.14	1.09	1.24
14	0.88	0.87	0.89	0.88	0.89	0.90	0.92	0.89	0.95	0.95	0.88	0.92
15	1.22	1.23	1.65	1.63	0.87	0.87	1.02	0.95	—	—	—	—
12-OMe	—	—	—	—	—	—		—	—	—	—	3.67

[a] Assignments as α and β interchangeable.

Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 238.1572 [M^+, $C_{14}H_{22}O_3$ requires 238.1569, Δ=−0.3 mmu] (2), 220 (4), 192 (4), 165 (37), 164 (100).

4.4. Grignard reaction of keto-acid 4 with methyl iodide

To small Mg chips (0.40 g) in anhyd. Et_2O (200 ml) was added a solution of isotopically-normal MeI (1.2 ml) in anhyd. Et_2O (50 ml) and the mixture was refluxed for 2 h. A solution of **4** (1.58 g, containing a small amount of its *trans* isomer—see above) in anhyd. Et_2O (200 ml) was added to the Grignard reagent and the reaction was allowed to reflux for a further 3.5 h until completion, as determined by TLC. The 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 (3×200 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, 96%), consisting predominantly of the *cis*-decalin 4-hydroxy epimers **5** and **6** (in an approximately 3:4 ratio) which could be separated by HPLC (40% EtOAc/*n*-hexane/5% AcOH) for individual characterization, although a crude mixture of the two epimers was generally used in the final step of this synthesis (Section 4.6). Much smaller amounts of the corresponding *trans*-decalin isomers **7** and **8** (Fig. 1) were also isolated by HPLC.

4.4.1. 2-(7α-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid (5). Oil (490 mg, 31%, R_t 13.6 min). $[\alpha]_D$ −66.2 (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2937, 2858, 1709, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$)[‖] ppm: 2.29 (1H, dq, *J*=7.0, 6.8 Hz), 2.17 (1H, dd, *J*=13.5, 3.7 Hz), 1.23 (3H, s), 1.17 (3H, d, *J*=6.8 Hz), 0.84 (3H, d, *J*=6.4 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 254.1879 [M^+, $C_{15}H_{26}O_3$ requires 254.1882, Δ=0.3 mmu] (4), 236 (10), 221 (6), 208 (7), 193 (19), 163 (45), 162 (100).

4.4.2. 2-(7β-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid (6). Oil (664 mg, 42%, R_t 16.8 min). $[\alpha]_D$ +9.8 (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3599, 3480 (br), 3400–2600, 2930, 2870, 1705, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 5.60 (2H, br s, –OH), 2.28 (1H, dq, *J*=7.0, 6.6 Hz), 1.28 (3H, s), 1.13 (3H, d, *J*=6.6 Hz), 0.83 (3H, d, *J*=6.1 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 254.1880 [M^+, $C_{15}H_{26}O_3$ requires 254.1882, Δ=0.2 mmu] (9), 236 (24), 221 (10), 208 (22), 193 (44), 163 (90), 162 (100).

4.4.3. 2-(7α-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aβ-*H*)-decahydro-naphthalen-1-yl)-propionic acid (7). Oil (25 mg, 2%, R_t 18.8 min). $[\alpha]_D$ −56.7 (*c* 2.5, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3429 (br), 3400–2600, 2974, 2928, 2858, 1703, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 5.34 (2H, br s, –OH), 2.69 (1H, dq, *J*=2.5, 7.1 Hz), 1.22 (3H, s), 1.11 (3H, d, *J*=7.1 Hz), 0.88 (3H, d, *J*=5.6 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3; HREIMS *m/z* (rel. int.): 254.1880 [M^+, $C_{15}H_{26}O_3$ requires 254.1882, Δ=0.2 mmu] (2), 236 (20), 221 (18), 208 (28), 193 (35), 163 (100), 162 (98).

4.4.4. 2-(7β-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aβ-*H*)-decahydro-naphthalen-1-yl)-propionic acid (8). Oil (30 mg, 2%, R_t 15.1 min). $[\alpha]_D$ −90.3 (*c* 3.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3418 (br), 3400–2600 (br), 2970, 2926, 2860, 1705, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) ppm: 6.26 (2H, br s, –OH), 2.77 (1H, dq, *J*=2.5, 7.0 Hz), 2.06 (1H, d, *J*=10.6 Hz), 1.23 (3H, s), 1.12 (3H, d, *J*=7.0 Hz), 0.87 (3H, d, *J*=6.4 Hz), 0.55 (1H, dddd, *J*=10.5, 10.5, 10.5, 3.4 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3; HREIMS *m/z* (rel. int.): 254.1882 [M^+, $C_{15}H_{26}O_3$ requires 254.1882, Δ=0.0 mmu] (6), 236 (23), 221 (12), 208 (30), 193 (32), 163 (60), 162 (100).

[‖] The sample was poorly soluble in this solvent.

4.5. Preparation of isotopically-labelled hydroxy-acids 5 and 6 from 4

4.5.1. [15-$^{13}C^{2}H_3$]-Labelled 5a and 6a. When $^{13}C^{2}H_3I$ (Cambridge Isotope Laboratories, Inc.) was used in place of isotopically-normal MeI, labelled compounds **5a** and **6a** were isolated from Grignard reaction with **4**. Physical properties for **5a** were as for **5** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.23 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.23 (d, J=19.2 Hz, $^{1}J_{CD}$, D-15); ^{13}C NMR (ppm): 31.2 (1:3:6:7:6:3:1 septet, J=19.2 Hz, $^{1}J_{CD}$, C-15). Physical properties for **6a** were as for **6** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.28 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.28 (d, J=19.2 Hz, $^{1}J_{CD}$, D-15); ^{13}C NMR (ppm): 25.2 (1:3:6:7:6:3:1 septet, J=19.2 Hz, $^{1}J_{CD}$, C-15). HREIMS of the mixture of **5a** and **6a** *m/z* (rel. int.): 258.2103 [M^+, $C_{14}{}^{13}C_1H_{23}{}^{2}H_3O_3$ requires 258.2103, Δ=0.0 mmu] (3), 240 (10), 221 (5), 212 (7), 193 (12), 167 (38), 166 (100), 165 (21).

4.5.2. [15-$C^{2}H_3$]-Labelled 5b and 6b. When $C^{2}H_3I$ (Cambridge Isotope Laboratories, Inc.) was used in place of isotopically-normal MeI, labelled compounds **5b** and **6b** were isolated from Grignard reaction with **4**. Physical properties for **5b** were as for **5** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.23 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.23 (s, D-15); ^{13}C NMR (ppm): 31.2 (C-15) not seen, presumably due to splitting into a 1:3:6:7:6:3:1 septet by the three deuterium atoms, and the reduced NOE effect from deuterium as compared to hydrogen. Physical properties for **6b** were as for **6** with the following differences: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.28 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.28 (s, D-15); ^{13}C NMR (ppm): 25.2 (C-15) not seen, presumably due to splitting into a 1:3:6:7:6:3:1 septet by the three deuterium atoms, and the reduced NOE effect from deuterium as compared to hydrogen. HREIMS of the mixture of **5b** and **6b** *m/z* (rel. int.): 257.2077 [M^+, $C_{15}H_{23}{}^{2}H_3O_3$ requires 257.2070, Δ=−0.7 mmu] (8), 239 (11), 221 (4), 211 (7), 193 (9), 166 (45), 165 (100), 164 (22).

4.5.3. [15-$^{13}CH_3$]-Labelled 5c and 6c. When $^{13}CH_3I$ (Cambridge Isotope Laboratories, Inc.) was used in place of isotopically-normal MeI, labelled compounds **5c** and **6c** were isolated from Grignard reaction with **4**. Physical properties for **5c** were as for **5** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.23 (3H, d, J=126.3 Hz, $^{1}J_{CH}$, H-15); ^{13}C NMR (ppm): 32.1 (ca. 90× normal intensity, C-15). Physical properties for **6c** were as for **6** with the following differences: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.28 (3H, d, J=126.3 Hz, $^{1}J_{CH}$, H-15); ^{13}C NMR (ppm): 26.1 (ca. 90× normal intensity, C-15).

4.6. Dehydration of the mixture of epimeric alcohols 5 and 6

To a mixture of the epimeric alcohols **5** and **6** (1.5 g, containing a little of the *trans*-decalin isomers **7** and **8**) in C_2Cl_4 (50 ml) was added $CuSO_4$ absorbed onto silica gel[32] (6.35 g; prepared by dissolving 1 equiv. by weight of $CuSO_4{\cdot}5H_2O$ (1.6 g) in a minimum volume of water and then adding 3 equiv. by weight of silica gel (4.8 g), stirring the mixture under vacuum until homogeneous, and then drying in an oven).** The reaction mixture was refluxed overnight and completion was determined by TLC, then the mixture was filtered and the silica gel was washed several times by $CHCl_3$. The combined organic solvents were removed under reduced pressure to yield a crude product (1.46 g, 97%) consisting predominantly of compounds **2** and **9**, in an approximately 5:7 ratio, which could be separated by HPLC (2.5% EtOAc/*n*-hexane/0.7% AcOH). Minor amounts of the *trans*-decalin analogues of **2** and **9**, compounds **10** and **11** respectively, were also isolated by using alternative HPLC conditions, but it was generally the crude mixture of the four compounds that was used in this last step (see also Section 4.7).

4.6.1. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid [dihydroartemisinic acid] (2). 490 mg, 33%, R_t 18.7 min—see Refs. 26,29 for physical properties.

4.6.2. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid [Δ^3-isomer of dihydroartemisinic acid] (9). Solid. Mp 113–116 °C. (730 mg, 50%, R_t 20.3 min). $[\alpha]_D$ +32.8 (*c* 4.6, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2968, 2928, 2853, 1705, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.29 (1H, br), 2.29 (1H, dq, J=11.2, 6.8 Hz), 1.63 (3H, s), 1.18 (3H, d, J=6.8 Hz), 0.82 (3H, d, J=6.4 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 236.1774 [M^+, $C_{15}H_{24}O_2$ requires 236.1776, Δ=0.2 mmu] (3), 218 (1), 180 (1), 163 (28), 162 (100).

4.6.3. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,(8aβ-*H*)-octahydro-naphthalen-1-yl)-propionic acid (10). ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.54 (1H, s), 2.95 (1H, dq, J=2.9, 6.8 Hz), 1.65 (3H, s), 1.16 (3H, d, J=6.8 Hz), 0.89 (3H, d, J=6.7 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3. HREIMS *m/z* (rel. int.): 236.1776 [M^+, $C_{15}H_{24}O_2$ requires 236.1776, Δ=0.0 mmu] (15), 218 (12), 163 (51), 162 (100).

4.6.4. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aβ-*H*)-octahydro-naphthalen-1-yl)-propionic acid (11). Oil (41 mg, 3%, R_t 24.5 min, 1.5% EtOAc/*n*-hexane/0.25% AcOH). $[\alpha]_D$ −195.5 (*c* 0.6, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600, 3034, 2926, 2872, 1705, 1460 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.36 (1H, s), 2.83 (1H, dq, J=6.8, 7.1 Hz), 1.63 (3H, s), 1.16 (3H, d, J=7.1 Hz), 0.88 (3H, d, J=5.9 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3; HREIMS *m/z* (rel. int.): 236.1779 [M^+, $C_{15}H_{24}O_2$ requires 236.1776, Δ=−0.2 mmu] (9), 218 (6), 163 (57), 162 (100).

** N.B. It is important not to use more than 1 equiv. of $CuSO_4$ per mole of starting material, otherwise there is appreciable depletion of the ^{2}H-label at the 15-position.

4.7. Preparation of isotopically-labelled dihydroartemisinic acid (2) and its Δ^3 isomer (9) from the dehydration of isotopically-labelled compounds 5 and 6

4.7.1. [15-$^{13}C^2H_3$]-Labelled 2a and 9a. When a mixture of **5a/6a** was used in place of the isotopically-normal starting material, compounds **2a** and **9a** were isolated. Physical properties for **2a** were as for **2** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.11 (1H, d, J=6.4 Hz, $^3J_{CH}$, H-5), 1.63 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.63 (d, J=19.1 Hz, $^1J_{CD}$, D-15); ^{13}C NMR (ppm): 135.9 (d, J=43.1 Hz, $^1J_{CC}$, C-4), 36.3 (d, J=3.9 Hz, $^3J_{CC}$, C-6), 26.6 (d, J=3.1 Hz, $^2J_{CC}$, C-3), 25.8 (d, J=2.4 Hz, $^3J_{CC}$, C-2), 23.9 (s, 1% of 15-$^{13}CH_3$), 23.6 (1:1:1 t, J=19.1 Hz, $^1J_{CD}$, 4% of 15-$^{13}CH_2{}^2H$), 23.3 (1:2:3:2:1 quin, J=19.1 Hz, $^1J_{CD}$, 14% of 15-$^{13}CH_2{}^2H$), 23.0 (1:3:6:7:6:3:1 septet, J=19.1 Hz, $^1J_{CD}$, 81% of 15-$^{13}C^2H_3$); HREIMS: *m/z* (rel. int.) 240.1996 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998, Δ=0.2 mmu] (3), 211 (2), 193 (2), 167 (30), 166 (100), 165 (16). Physical properties for **9a** were as for **9** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.29 (1H, dd, J=5.3 Hz, $^3J_{CH}$, 5.3 Hz, H-3), 1.63 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.63 (d, J=18.9 Hz, $^1J_{CD}$, D-15); ^{13}C NMR (ppm): 131.7 (d, J=43.4 Hz, $^1J_{CC}$, C-4), 33.4 (d, J=2.4 Hz, $^3J_{CC}$, C-6), 26.3 (d, J=2.0 Hz, $^2J_{CC}$, C-5), 22.8 (1:3:6:7:6:3:1 septet, J=18.9 Hz, $^1J_{CD}$, C-15); HREIMS *m/z* (rel. int.): 240.1995 [M^+, $C_{14}{}^{13}C_1H_{21}{}^2H_3O_2$ requires 240.1998, Δ=0.3 mmu] (3), 194 (2), 167 (32), 166 (100), 165 (15).

4.7.2. [15-C^2H_3]-Labelled 2b and 9b. Compounds **2b** and **9b** were isolated when a mixture of **5b/6b** was used in place of the isotopically-normal starting material. Physical properties for **2b** were as for **2** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.63 (H-15) absent from spectrum; ^{2}H NMR (ppm): 1.63 (s, D-15); ^{13}C NMR (ppm): 23.0 (1:3:6:7:6:3:1 septet, J=19.1 Hz, $^1J_{CD}$, C-15) ca. 1% of the height of the other ^{13}C peaks, presumably the very low intensity is due to the effects of both splitting and the reduced NOE effect from deuterium as compared to hydrogen; HREIMS: *m/z* (rel. int.): 239.1959 [M^+, $C_{15}H_{21}{}^2H_3O_2$ requires 239.1964, Δ=0.6 mmu] (4), 221 (1), 193 (1), 166 (30), 165 (100), 164 (8). Physical properties for **9b** were as for **9** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.63 (H-15) absent from spectrum; ^{2}H NMR: 1.63 (s, D-15); ^{13}C NMR (ppm): 22.8 (C-15) not seen, presumably due to splitting into a 1:3:6:7:6:3:1 septet by the three deuterium atoms, and the reduced NOE effect from deuterium as compared to hydrogen; HREIMS *m/z* (rel. int.): 239.1959 [M^+, $C_{15}H_{21}{}^2H_3O_2$ requires 239.1964, Δ=0.5 mmu] (4), 193 (1), 166 (30), 165 (100), 164 (30).

4.7.3. [15-$^{13}CH_3$]-Labelled 2c and 9c. When a mixture of **5c/6c** was used in place of the isotopically-normal starting material, compounds **2c** and **9c** were isolated. Physical properties for **2c** were as for **2** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.11 (1H, d, J=6.4 Hz, $^3J_{CH}$, H-5), 1.63 (3H, d, J=125.1 Hz, $^1J_{CH}$, H-15); ^{13}C NMR (ppm): 135.9 (d, J=43.3 Hz, $^1J_{CC}$, C-4), 36.3 (d, J=3.8 Hz, $^3J_{CC}$, C-6), 26.6 (d, J=3.1 Hz, $^2J_{CC}$, C-3), 25.7 (d, J=2.6 Hz, $^3J_{CC}$, C-2), 23.8 (ca. 85× the intensity of the peak in the isotopically-normal spectrum, C-15). HREIMS *m/z* (rel. int.): 237.1799 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=1.1 mmu] (3), 179 (3), 164 (78), 163 (100). Physical properties for **9c** were as for **9** with the following differences due to isotopic enrichment: ^{1}H NMR (δ, $CDCl_3$) (ppm): 1.63 (3H, d, J=125.2 Hz, $^1J_{CH}$, H-15); ^{13}C NMR (ppm): 131.8 (d, J=43.6 Hz, $^1J_{CC}$, C-4), 33.4 (d, J=2.5 Hz, $^3J_{CC}$, C-6), 27.8 (br, $^3J_{CC}$, C-2), 26.3 (br, $^2J_{CC}$, C-5) 23.6 (ca. 75× the intensity of the corresponding peak in the isotopically-normal spectrum); HREIMS *m/z* (rel. int.): 237.1794 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=1.6 mmu] (3), 180 (2), 164 (80), 163 (100).

4.8. Conversion of purified 2-(4,[7-$^{13}CH_3$]-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aα-*H*)-octahydronaphthalen-1-yl)-propionic acid [the Δ^3-isomer of dihydroartemisinic acid] (9c) to an equilibrating mixture of 9c and dihydroartemisinic acid (2c)

To a solution of **9c** (46 mg) in $CHCl_3$ (50 ml) was added H_2SO_4 (70%, 4.6 ml). The reaction was stirred at room temperature for 2 h, then extracted by $CHCl_3$ (3×100 ml), washed with brine (3×30 ml) and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a crude product (42 mg, 91%) consisting predominantly of **2c** and **9c** in an approximately 5:7 ratio, which could be separated by HPLC as described in Section 4.6. These conditions were found to be optimal in that they allowed the mixture of **2c** and **9c** to attain their equilibrium ratio, while minimizing the extent of further double bond isomerization, resulting in unwanted products. Thus, the use of longer reaction times led to the formation of significant amounts of the lactones **12c**–**15c** in addition to the desired compound **2c**. Compounds **12c**–**15c** were separated by HPLC (8% EtOAc/*n*-hexane/0.5% AcOH).

4.8.1. 3,6,[9-$^{13}CH_3$]-Trimethyl-decahydro-1-oxa-cyclopentane[*d*]naphthalene-2-one (12c). Oil (major 'unwanted' rearrangement product—ca. 80% of the total of compounds **12c**–**15c**, R_t 17.1 min). $[\alpha]_D$ +50.6 (*c* 3.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3026, 2943, 2866, 2851, 1755, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 3.11 (1H, dq, J=6.9, 7.3 Hz), 1.12 (3H, d, J=7.3 Hz), 0.89 (3H, d, J=6.4 Hz), 0.87 (3H, dd, J=124.5 Hz, $^1J_{CH}$, 6.4 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3—splittings observed for isotopically-labelled compound: 85.2 (d, J=4.2 Hz, $^3J_{CC}$, C-6), 28.0 (d, J=35.5 Hz, $^1J_{CC}$, C-4), 24.9 (d, J=4.8 Hz, $^3J_{CC}$, C-2), 22.3 (C-15, ca. 90× the intensity of other ^{13}C peaks, C-15); HREIMS *m/z* (rel. int.): 237.1807 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=0.3 mmu] (28), 222 (8), 193 (20), 179 (52), 165 (100), 164 (60), 151 (15), 136 (20), 125 (29).

4.8.2. 3,6,[9-$^{13}CH_3$]-Trimethyl-decahydro-1-oxa-cyclopentane[d]naphthalene-2-one (13c). Oil (2nd major 'unwanted' rearrangement product—ca. 15% of the total of compounds **12c**–**15c**, R_t 27.4 min). $[\alpha]_D$ +71.0 (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3015, 2943, 2866, 1751, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 2.80 (1H, dq, J=10.4, 7.8 Hz), 2.16 (1H, ddd, J=10.4, 5.8, 5.8 Hz), 1.27 (3H, d, J=7.8 Hz), 0.90 (3H, d, J=6.5 Hz), 0.87 (3H, dd,

J=124.6 Hz, $^1J_{CH}$, 6.6 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3—splittings observed for isotopically-labelled compound: 87.7 (d, J=4.0 Hz, $^3J_{CC}$, C-6), 28.6 (d, J=35.7 Hz, $^1J_{CC}$, C-4), 22.8 (d, J=4.2 Hz, $^3J_{CC}$, C-2), 22.4 (C-15, ca. 90× the intensity of other ^{13}C peaks, C-15); HREIMS m/z (rel. int.): 237.1809 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=0.1 mmu] (31), 222 (12), 193 (54), 179 (57), 165 (100), 164 (79), 140 (33), 125 (97).

4.8.3. [4-^{13}CH$_3$],8,11-Trimethyl-10-oxa-tricyclo-[5.3.3.0*1,6*]tridecan-9-one (14c). Oil (very minor rearrangement product—ca. 1% of the total of compounds **12c**–**15c**, R_t 26.1 min). $[\alpha]_D$ −32 (c 0.05, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2926, 2864, 1705, 1460 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 2.74 (1H, dq, J=6.6, 7.2 Hz), 2.08 (1H, m), 1.25 (3H, d, J=7.2 Hz), 1.02 (3H, dd, J=124.3 Hz, $^1J_{CH}$=7.3 Hz), 0.92 (3H, d, J=6.5 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3—splittings observed for labelled compound: 26.4 (d, J=32.5 Hz, $^1J_{CC}$, C-4), 16.7 (C-15, ca. 90× the intensity of other ^{13}C peaks, C-15); HREIMS m/z (rel. int.): 237.1805 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=0.5 mmu] (56), 193 (6), 180 (100), 152 (79), 125 (72), 124 (43).

4.8.4. [4-^{13}CH$_3$],8,11-Trimethyl-10-oxa-tricyclo-[5.3.3.0*1,6*]tridecan-9-one (15c). Oil (minor rearrangement product—ca. 4% of the total of compounds **12c**–**15c**, R_t 35.1 min). $[\alpha]_D$ −14.3 (c 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3018, 2939, 2866, 1705, 1456 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 2.65 (1H, dq, J=5.7, 7.2 Hz), 2.17 (1H, ddd, J=12.7, 3.0, 3.0 Hz), 2.11 (1H, m), 1.88 (1H, ddd, J=12.7, 2.7, 2.7 Hz), 1.27 (3H, d, J=7.2 Hz), 0.95 (3H, dd, J=124.7 Hz, $^1J_{CH}$=6.2 Hz), 0.89 (3H, d, J=6.7 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3—splittings observed for labelled compound: 44.9 (d, J=4.2 Hz, $^3J_{CC}$, C-6), 34.5 (d, J=4.2 Hz, $^3J_{CC}$, C-2), 32.6 (d, J=35.5 Hz, $^1J_{CC}$, C-4), 22.0 (C-15, ca. 90× intensity of other ^{13}C peaks, C-15); HREIMS m/z (rel. int.): 237.1806 [M^+, $C_{14}{}^{13}C_1H_{24}O_2$ requires 237.1810, Δ=0.4 mmu] (1), 193 (15), 164 (15), 125 (100), 124 (49), 113 (25).

4.9. Autoxidation of labelled dihydroartemisinic acid (2a/2b) on storage

Conditions and results were similar to those described in Ref. 22.

4.10. Autoxidation of 2-(4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid [Δ^3-isomer of dihydroartemisinic acid] (9c) on storage

After storage at −20 °C for 6 months, compound **9c** was found to have undergone autoxidation to a complex mixture of products, which were difficult to separate chromatographically. Compounds **18c**–**22c** were isolated from the mixture in varying degrees of purity by HPLC (30% EtOAc/*n*-hexane/0.7% AcOH).

4.10.1. 2-(7ξ-Hydroperoxy-4,[7-^{13}CH$_3$]-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),7,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid (18c). Oil (major component: ca. 30% of compounds **18c**–**22c**, R_t 25.2 min); IR ν_{max} ($CHCl_3$): 3400–2600, 3020, 2930, 1705, 1653, 1456 cm^{-1}; ^{1}H NMR (characterized by 2D NMR as a mixture with **22c**) (δ, $CDCl_3$) (ppm): 7.30 (1H, br s, −OOH), 6.23 (1H, dd, J=9.8, 5.4 Hz, H-2), 5.54 (1H, d, J=9.8 Hz, H-3), 2.45 (1H, dq, J=10.1, 6.9 Hz, H-11), 1.35 (3H, d, J=127.6 Hz, $^1J_{CH}$, H-15), 1.24 (3H, d, J=6.9 Hz, H-13), 0.95 (3H, d, J=6.5 Hz, H-14); HREIMS m/z (rel. int.): 251.1603 [M^+-H_2O, $C_{14}{}^{13}C_1H_{22}O_3$ requires 251.1602, Δ=−0.1 mmu] (25), 236 (32), 178 (38), 162 (100), 161 (78).

4.10.2. 2-(7ξ-Hydroperoxy-4,[7-^{13}CH$_3$]-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),7,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid (19c). Oil (major component: ca. 40% of compounds **18c**–**22c**, R_t 27.5 min). IR ν_{max} ($CHCl_3$): 3400–2600 (br), 2928, 2853, 1707, 1458 cm^{-1}; ^{1}H NMR (characterized by 2D NMR as a mixture with **21c**) (δ, $CDCl_3$) (ppm): 7.33 (1H, br s, −OOH), 6.12 (1H, dd, J=10.1, 5.0 Hz, H-2), 5.55 (1H, d, J=10.1 Hz, H-3), 2.41 (1H, dq, J=7.1, 6.7 Hz, H-11), 1.30 (3H, d, J=128.5 Hz, $^1J_{CH}$, H-15), 1.18 (3H, d, J=6.7 Hz, H-13), 0.93 (3H, d, J=6.1 Hz, H-14); HREIMS m/z (rel. int.): 235.1648 [$M^+-H_2O_2$, $C_{14}{}^{13}C_1H_{22}O_2$ requires 251.1653, Δ=0.5 mmu] (3), 162 (100), 161 (70).

4.10.3. 2-(7ξ-Hydroxy-4,[7-^{13}CH$_3$]-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),7,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid (20c). Oil (minor component: ca. 10% of compounds **18c**–**22c**). R_t 32.7 min). $[\alpha]_D$ −5.9 (c 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400–2600, 2937, 2855, 1717, 1603, 1458 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) (ppm): 5.91 (1H, dd, J=10.1, 5.2 Hz, H-2), 5.53 (1H, d, J=10.1 Hz, H-3), 2.40 (1H, dq, J=11.2, 7.0 Hz, H-11), 1.32 (3H, d, J=126.1 Hz, $^1J_{CH}$, H-15), 1.17 (3H, d, J=6.9 Hz, H-13), 0.94 (3H, d, J=6.3 Hz, H-14); HREIMS m/z (rel. int.): 235.1646 [M^+-H_2O, $C_{14}{}^{13}C_1H_{22}O_2$ requires 235.1653, Δ=0.8 mmu] (20), 217 (20), 191 (41), 163 (70), 162 (100).

4.10.4. 2-(7ξ-Hydroxy-4,[7-^{13}CH$_3$]-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),7,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid (21c). Oil (minor component: ca. 15% of compounds **18c**–**22c**). ^{1}H NMR (characterized by 2D NMR as a mixture with **19c**) (δ, $CDCl_3$) (ppm): 6.02 (1H, dd, J=9.9, 5.3 Hz, H-2), 5.61 (1H, d, J=9.9 Hz, H-3), 2.40 (1H, dq, J=6.6, 6.9 Hz, H-11), 1.30 (3H, d, J=128.5 Hz, $^1J_{CH}$, H-15), 1.21 (3H, d, J=6.9 Hz, H-13), 0.94 (3H, d, J=6.1 Hz, H-14).

4.10.5. 2-(4,[7-^{13}CH$_3$]-dimethyl-5-oxo-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid (22c). Oil (very minor component: ca. 5% of compounds **18c**–**22c**). ^{1}H NMR (characterized by 2D NMR as a mixture with **18c**) (δ, $CDCl_3$) (ppm): 5.82 (1H, d, J=5.5 Hz, $^3J_{CH}$, H-3,), 1.95 (3H, d, J=126.9 Hz, $^1J_{CH}$, H-15), 1.20 (3H, d, J=6.8 Hz, H-13), 0.84 (3H, d, J=6.5 Hz, H-14).

4.11. Preparation of 2-(4-methyl-7-oxo-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7-octahydro-naphthalen-1-yl)-propionic acid methyl ester (24) from decalenone keto-acid (3)

See Ref. 22 for the procedure used for converting **3** into **24** in the presence of diazomethane and Ref. 30 for the physical properties of **24**.

4.12. Reduction of α,β-unsaturated ketone 24 to secondary alcohol 25 by $NaBH_4$ in pyridine

To a solution of the methyl ester **24** (902 mg) in pyridine (5 ml) was added a solution of $NaBH_4$ (650 mg) in pyridine (4 ml). The reaction mixture was stirred for 3 h at room temperature then diluted with Et_2O (50 ml). HCl (10%) was added to neutralize the solution while cooling in an ice bath. The mixture was extracted by Et_2O (3×100 ml), washed with brine (3×50 ml) and dried ($MgSO_4$). Solvent was removed by a rotary evaporator to yield a crude product (686 mg, 76%) which was purified by CC (50% EtOAc/*n*-hexane) to obtain the alcohol **25**.

4.12.1. 2-(7β-Hydroxy-4-methyl-(1α-H),2,3,(4β-H),(4aα-H),5,6, 7,8,(8aα-H)-decahydro-naphthalen-1-yl)-propionic acid (25). Oil (605 mg, 61%, R_f 0.48). $[\alpha]_D$ −10.4 (*c* 1.5, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3421 (br), 3028, 2936, 2868, 1728, 1448, 1437 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 3.66 (3H, s), 3.59 (1H, dddd, *J*=11.7, 11.7, 4.9, 4.9 Hz), 2.30 (1H, dq, *J*=11.1, 6.9 Hz), 1.10 (3H, d, *J*=6.9 Hz), 0.87 (3H, d, *J*=6.2 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 254.1874 [M^+, $C_{15}H_{26}O_3$ requires 254.1882, Δ=0.8 mmu] (1), 236 (3), 205 (2), 149 (100).

4.13. 'Non-optimized' procedures for the reduction of 24 yielding the alternative products 30–32. Reduction by alkaline sodium borohydride

To a solution of **24** (1.0 g) in MeOH (10 ml) cooled in an ice bath, was added dropwise a solution of $NaBH_4$ (0.76 g) in NaOH solution (1 ml, 30%) over a period of 10 min. The mixture was acidified with HCl (2 ml, 3 M), diluted with water (30 ml), extracted with Et_2O (2×30 ml) and the combined organic layers were washed with brine (2×5 ml), dried ($MgSO_4$) and solvent removed under reduced pressure to yield a crude product (611 mg, 61%) consisting of compounds **30**, **31** and **32** which were separated by CC (50% EtOAc/*n*-hexane).

4.13.1. 7-(3ξ-Hydroxy-butyl)-3,6,dimethyl-hexahydro-benzofuran-2-one (30). Oil (R_f 0.26). IR ν_{max} ($CHCl_3$): 2934, 1763, 1462, 1383 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 4.40 (1H, dd, *J*=3.4, 3.4 Hz), 3.81 (1H, quin, *J*=6.2 Hz), 2.78 (1H, dq, *J*= 7.2, 7.2 Hz), 2.25 (1H, m), 1.21 (3H, d, *J*=6.2 Hz), 1.14 (3H, d, *J*=7.2 Hz), 0.95 (3H, d, *J*=6.4 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3; HREIMS *m/z* (rel. int.): 222.1620 [M^+-H_2O, $C_{14}H_{22}O_2$ requires 222.1620, Δ= 0.0 mmu] (4), 207 (18), 196 (21), 165 (15), 122 (80), 94 (100).

4.13.2. 7-(3ξ-Hydroxy-butyl)-3,6,dimethyl-hexahydro-benzofuran-2-one (31). Oil (R_f 0.28). IR ν_{max} ($CHCl_3$): 2934, 1763, 1462, 1383 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 4.42 (1H, dd, *J*=3.4, 3.4 Hz), 3.78 (1H, quin, *J*=6.2 Hz), 2.78 (1H, dq, *J*=7.2, 7.2 Hz), 2.27 (1H, m), 1.20 (3H, d, *J*=6.2 Hz), 1.14 (3H, d, *J*=7.2 Hz), 0.95 (3H, d, *J*=6.4 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3; HREIMS *m/z* (rel. int.): 222.1620 [M^+-H_2O, $C_{14}H_{22}O_2$ requires 222.1620, Δ=0.0 mmu] (13), 207 (21), 196 (29), 165 (19), 122 (92), 94 (100).

4.13.3. 3,6,9-Trimethyl-octahydro-1,10-dioxa-cyclopenta[d]napthalen-2-one (32). Oil (R_f 0.74). ^1H NMR (δ, $CDCl_3$) (ppm): 3.98 (1H, m), 3.28 (1H, dq, *J*=6.3, 7.3 Hz), 1.11 (3H, d, *J*=6.4 Hz), 1.09 (3H, d, *J*=7.3 Hz), 0.88 (3H, d, *J*=6.3 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3.

4.14. Non-optimized procedures for the reduction of 24 yielding the alternative products 33 and 34. Reduction by methanolic sodium borohydride

To a solution of **24** (1.23 g) in MeOH (10 ml) cooled in an ice bath was added $NaBH_4$ (0.92 g) in portions, over a period of 5 min. After a further 10 min, HCl (5 ml, 3 M) was added to pH 4 and the mixture was diluted with water (50 ml) and extracted with Et_2O (2×50 ml). The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to yield a colourless oil (0.61 g, 50%) consisting of a mixture of the two epimeric alcohols **33** and **34**, which could not be separated chromatographically.

4.14.1. 2-(7ξ-Hydroxy-4-methyl-(1α-H),2,3,(4β-H),(4aα-H),5,6,7octahydro-naphthalen-1-yl)-propionic acid methyl ester (33/34). Oil. ^1H NMR (δ, $CDCl_3$) (ppm): 5.41/5.38 (1H, d, *J*=1.6 Hz), 4.24/4.14 (1H, br s), 3.67 (3H, s), 2.76 (1H, m), 1.24 (3H, d, *J*=6.9 Hz), 0.92 (3H, d, *J*=6.6 Hz)—see Table 4 for full assignments; ^{13}C NMR: see Table 3.

4.15. Jones oxidation of alcohol 25

To a solution of **25** (600 mg) in acetone (5 ml), cooled in an ice bath, was added Jones reagent (freshly prepared by mixing CrO_3 (180 mg), H_2O (0.4 ml) and conc. H_2SO_4 (0.16 ml) and washing the resulting precipitate with water). The reaction mixture was stirred for 30 min, until the starting material had disappeared, as judged by TLC. Then MeOH (10 ml) was added and the mixture was taken up in H_2O (50 ml) and extracted by Et_2O (2×50 ml). The combined organic layers were washed with H_2O (10 ml) and brine (10 ml), dried ($MgSO_4$) and solvent removed under reduced pressure to yield compound **26** (588 mg, 98%) without the need for further purification.

4.15.1. 2-(4-Methyl-7-oxo-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid methyl ester (26). Solid. Mp 158–161 °C. $[\alpha]_D$ −18.1 (*c* 2.6, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3013, 2955, 2928, 1713, 1456 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 3.67 (3H, s), 1.10 (3H, d, *J*=6.9 Hz), 0.96 (3H, d, *J*=6.2 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 252.1732 [M^+, $C_{15}H_{24}O_3$ requires 252.1725, Δ=−0.7 mmu] (2), 221 (1), 175 (5), 165 (38), 164 (100).

4.16. Grignard reaction of methyl ester 26 with methyl iodide

To a Grignard reagent freshly prepared from Mg (13 mg) and MeI (88 mg) was added a solution of compound **26** (124 mg) in Et_2O (30 ml). The reaction mixture was refluxed for 3 h, then cooled to 0 °C and H_2O (50 ml) was added. The mixture was extracted by Et_2O (2×50 ml), and the combined organic extracts washed with H_2O (10 ml) and

brine (50 ml), dried ($MgSO_4$) and solvent removed on a rotary evaporator to yield a crude product (116 mg, 94%) consisting of a mixture of the 4-hydroxy epimers **27** and **28**, which could be separated by HPLC (30% EtOAc/*n*-hexane) for purposes of characterization, but which were normally used as a mixture for the last step in the synthesis (Section 4.17).

4.16.1. 2-(7α-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid methyl ester (27). Oil (41 mg, 32%, R_t 22.1 min). $[\alpha]_D$ −11.1 (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3546, 2928, 2856, 1717, 1458 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 3.66 (3H, s), 2.26 (1H, dq, *J*=11.1, 6.8 Hz), 2.16 (1H, ddd, *J*=16.9, 3.8, 3.8 Hz), 1.22 (3H, s), 1.12 (3H, d, *J*=6.8 Hz), 0.83 (3H, d, *J*=6.4 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1. HREIMS: *m*/*z* (rel. int.): 250.1935 [M^+-H_2O, $C_{16}H_{26}O_2$ requires 250.1933, Δ=−0.2 mmu] (3), 236 (1), 218 (2), 201 (1), 191 (4), 175 (4), 163 (81), 162 (100).

4.16.2. 2-(7β-Hydroxy-4,7-dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,7,8,(8aα-*H*)-decahydro-naphthalen-1-yl)-propionic acid methyl ester (28). Oil (60 mg, 48%, R_t 24.2 min). $[\alpha]_D$ +2.3 (*c* 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3599, 3447 (br), 3007, 2932, 2870, 1728, 1456 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 3.66 (3H, s), 2.29 (1H, dq, *J*=11.1, 6.9 Hz), 1.27 (3H, s), 1.09 (3H, d, *J*=6.9 Hz), 0.83 (3H, d, *J*=6.2 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m*/*z* (rel. int.): 268.2039 [M^+, $C_{16}H_{28}O_3$ requires 268.2038, Δ=−0.1 mmu] (2), 250 (3), 236 (2), 218 (3), 191 (4), 175 (4), 163 (75), 162 (100).

4.17. Dehydration of tertiary alcohols 27/28

The dehydration of the mixture of epimeric alcohols **27/28** (50 mg) was effected in the same way as for the alcohols **5/6** (see Section 4.6) resulting in a crude mixture (45 mg, 90%) containing the double bond regio-isomers, compounds **23** and **29**, which were separated by HPLC (2.5% EtOAc/*n*-hexane/0.7% AcOH).

4.17.1. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,6,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid methyl ester [dihydroartemisinic acid methyl ester] (23). Oil (16 mg, 34%, R_t 13.7 min). $[\alpha]_D$ −9.6 (*c* 2.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2924, 2872, 2851, 1728, 1456, 1437 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 5.12 (1H, s), 3.68 (3H, s), 2.50 (2H, m), 1.63 (3H, d, *J*=0.7 Hz), 1.13 (3H, d, *J*=6.9 Hz), 0.86 (3H, d, *J*=6.5 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m*/*z* (rel. int.): 250.1939 [M^+, $C_{16}H_{26}O_2$ requires 250.1933, Δ=−0.6 mmu] (4), 219 (3), 201 (3), 163 (55), 162 (100).

4.17.2. 2-(4,7-Dimethyl-(1α-*H*),2,3,(4β-*H*),(4aα-*H*),5,8,(8aα-*H*)-octahydro-naphthalen-1-yl)-propionic acid methyl ester [Δ^3-isomer of dihydroartemisinic acid methyl ester] (29). Oil (21 mg; 46%, R_t 15.0 min). $[\alpha]_D$ +34.0 (*c* 10.6, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2970, 2930, 2849, 1728, 1456 cm^{-1}; ^1H NMR (δ, $CDCl_3$) (ppm): 5.28 (1H, d, *J*=3.6 Hz), 3.66 (3H, s), 2.31 (1H, dq, *J*=11.1, 6.8 Hz), 1.63 (3H, s), 1.13 (3H, d, *J*=6.8 Hz), 0.81 (3H, d, *J*=6.4 Hz)—see Table 2 for full assignments; ^{13}C NMR: see Table 1; HREIMS *m*/*z* (rel. int.): 250.1937 [M^+, $C_{16}H_{26}O_2$ requires 250.1933, Δ=−0.4 mmu] (2), 219 (4), 191 (5), 163 (65), 162 (100).

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