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Structure elucidation of arteannuin O, a novel cadinane diol from *Artemisia annua*, and the synthesis of arteannuins K, L, M and O

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Abstract—The novel cadinane diol, arteannuin O (**1**), has been obtained from *Artemisia annua* and its structure has been established by 2D NMR and X-ray crystallography. A reconstructive synthesis of arteannuin O from artemisinin is described, which also yields the natural products arteannuin K and arteannuin L. Mechanistic considerations have led to the conclusion that the stereochemistry of the 5-hydroxyl group was wrongly assigned when arteannuins K, L and M were first reported as natural products. This was confirmed by derivatization of synthetic arteannuins K, L and M as their Mosher esters.

1. Introduction

Following the discovery of the important anti-malarial amorphane sesquiterpene artemisinin (qinghaosu) (**2**)[1] from *Artemisia annua* in the 1970s, there have been extensive phytochemical investigations of this species, resulting in the description of some 35 amorphane/cadinane sesquiterpenes at the time of writing.[2,3] In 1998, we reported three sesquiterpenes,[2] arteannuins K, L and M from *A. annua*, with structures apparently derived from dihydro-*epi*-deoxyarteannuin B (**3**) (Fig. 1). These natural products are of some interest from both biogenetic and mechanistic perspectives, as it has been proposed that lactone diols such as arteannuin M might undergo Grob fragmentation[4] at C-4/C-5 (the tertiary hydroxyl group behaving as an electron donor and the five-membered lactone behaving as a good leaving group), either in vivo or in vitro, ultimately yielding artemisinin. We herein report a re-investigation of this *A. annua* extract from which we have obtained the novel cadinane diol arteannuin O (**1**), which is a diastereoisomer of arteannuin M. Synthesis of arteannuin O from dihydro-*epi*-deoxyarteannuin B (**3**) has led us to propose a structure revision of the stereochemistry claimed for the 5-OH group in arteannuins K, L and M.

2. Results and discussion

HPLC separation of a polar fraction from column chromatography of the CH_2Cl_2 extract of *A. annua*[2] has resulted in the isolation of a novel cadinane diol, arteannuin O (**1**), in addition to its known diastereoisomer, arteannuin M. The planar structure of **1** was rigorously established by 2D NMR experiments such as HSQC, HMBC and ^1H–^1H COSY

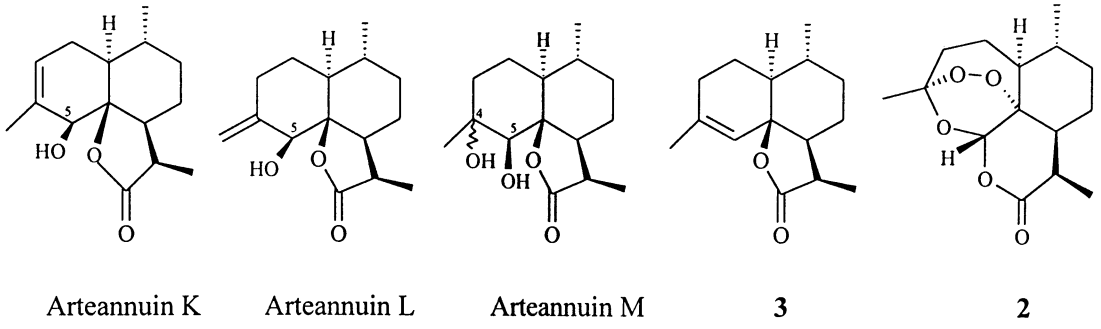

Figure 1. Structures of natural products arteannuins K, L and M as reported in the literature.[2] The stereochemistry of the 5-OH group, which was reported as depicted above, is believed to have been wrongly assigned in all cases. Structures of dihydro-*epi*-deoxyarteannuin B (**3**)[2,5] and artemisinin (**2**)[1] are also shown.

Keywords: arteannuin O; arteannuin K; arteannuin L; arteannuin M; artemisinin; dihydro-*epi*-deoxyarteannuin B; cadinane; *Artemisia annua*; 2D NMR; X-ray crystallography; Mosher ester.

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Table 1. Structure elucidation of the natural product arteannuin O (**1**) by 2D NMR

Position	δ_C[a,b]	δ_H[a]	2 and 3-bond correlations from ^{13}C to ^{1}H in HMBC	Correlations from ^{1}H to ^{1}H in ^{1}H–^{1}H COSY	Correlations from ^{1}H to ^{1}H in NOESY
1	41.7 (CH)	1.48	3.56, 0.93	–	1.06, 0.93
2α	20.2 (CH_2)	1.76	–	1.70, 1.47	1.47, 0.93
2β		1.47		1.76	1.76, 0.93
3α	33.9 (CH_2)	1.70	3.56, 1.23	1.76	1.23[c]
3β		1.70		1.76	1.23[c]
4	72.4 (C)	–	3.56, 1.23	–	–
5	72.7 (CH)	3.56	–	–	3.09, 2.59, 1.23
6	87.7 (C)	–	3.56	–	–
7	38.8 (CH)	2.59	3.09, 1.14	3.09, 1.74, 1.13	3.56, 3.09, 1.74, 1.14
8α	23.9 (CH_2)	1.74	3.09	2.59, 1.13	2.59, 1.63, 1.14, 1.13, 1.06
8β		1.13		2.59, 1.74	1.74, 1.63, 1.37
9α	32.1 (CH_2)	1.06	0.93	1.63	1.74, 1.63, 1.48, 0.93
9β		1.63		1.06	1.74, 1.37, 1.13, 1.06, 0.93
10	30.2 (CH)	1.37	0.93	0.93	1.63, 1.13, 0.93
11	38.9 (CH)	3.09	1.14	2.59, 1.14	3.56, 2.59, 1.14
12	177.9 (C)	–	3.09, 1.14	–	–
13	9.3 (CH_3)	1.14	3.09	3.09	3.09, 2.59, 1.74
14	20.0 (CH_3)	0.93	–	1.37	1.76, 1.63, 1.48, 1.47, 1.37, 1.06
15	26.7 (CH_3)	1.23	–	–	3.56, 1.70

[a] ^{1}H connected to ^{13}C by a single bond determined from correlations observed in HSQC.
[b] Multiplicity in ^{13}C determined by DEPT.
[c] Ambiguity as to which proton is involved in nOe with H-15 as chemical shifts are the same for the 3α- and 3β-positions.

which allowed the assignment of all protons and carbons in the molecule (Table 1). However, the stereochemistry of the vicinal diol remained ambiguous due to the possibility of the A-ring adopting more than one conformation, which in turn allows two alternative diastereoisomeric configurations of the diol at C-4 and C-5 in **1** to be proposed, both of which would be consistent with the observed NOESY correlations for this natural product (Fig. 2). Unlike other chemical shifts around the vicinal diol, the chemical shift of the 4-OH proton (δ_H 3.37) was almost unaffected by the concentration of the sample used in recording NMR spectra, and we propose that this group is therefore axial (4β-OH) and involved in intramolecular hydrogen bonding with the carbonyl group (i.e. the first conformation appearing in Fig. 2). Fortunately, arteannuin O could be crystallized and the relative stereochemistry of the vicinal diol was then unambiguously determined by X-ray crystallography as 4β-OH, 5α-OH (4*S*,5*R*) (Fig. 3).

In order to obtain larger amounts of arteannuin O (**1**) for studies of its reactivity under Grob fragmentation conditions (as part of our on-going investigations into the biogenetic origins of artemisinin) we decided to synthesize **1** from artemisinin (**2**) via dihydro-*epi*-deoxyarteannuin B (**3**).[5]

Artemisinin (**2**) was converted into the *cis*-lactone **6** in two steps. The first step, conversion of **2** being ring-opened to give methyl ester **4**, is best effected by use of strongly acidic conditions (otherwise the 1,2,4-trioxane ring is not completely opened)[6,7] for a short period of time (in order to prevent epimerization at the 1- and 7-positions).[8,9] In the second step, Robinson annulation of **4** in the presence of barium hydroxide octahydrate was accompanied by cleavage of the methyl ester group.[10] If the acidic work-up of this reaction is performed in a controlled way, then it is possible to isolate the decalenone acid **5** in reasonable yield.[8] Work-up under harsher conditions induces intramolecular nucleophilic attack of the carboxylic acid at the α,β-unsaturated ketone functionality, resulting in lactone **6**[8] in good yield (Scheme 1).

Grignard reaction of **6** with methyl iodide then provided the desired intermediate, compound **3**; physical properties of **3** obtained by synthesis were identical to those reported for the natural product dihydro-*epi*-deoxyarteannuin B.[2,5] Presumably **3** is formed in situ by dehydration of the initial products of Grignard reaction at the ketone group of **6**, compounds **7/8** (Fig. 4). One remarkable feature of this reaction is that only the desired Δ^4-dehydration product **3** was obtained, with no detectable formation of the

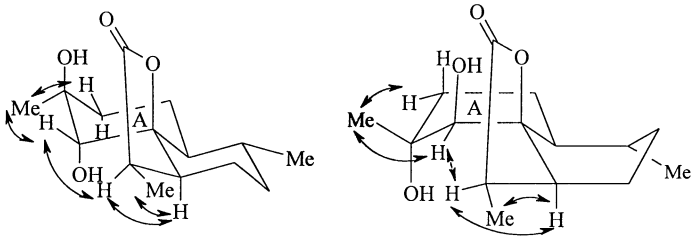


Figure 2. Two possible configurations for the vicinal diol in **1**, which would be consistent with observed nOe data for this compound, depending on the conformation adopted by the A-ring. Critical NOESY correlations are shown by double headed arrows from ^{1}H to ^{1}H.

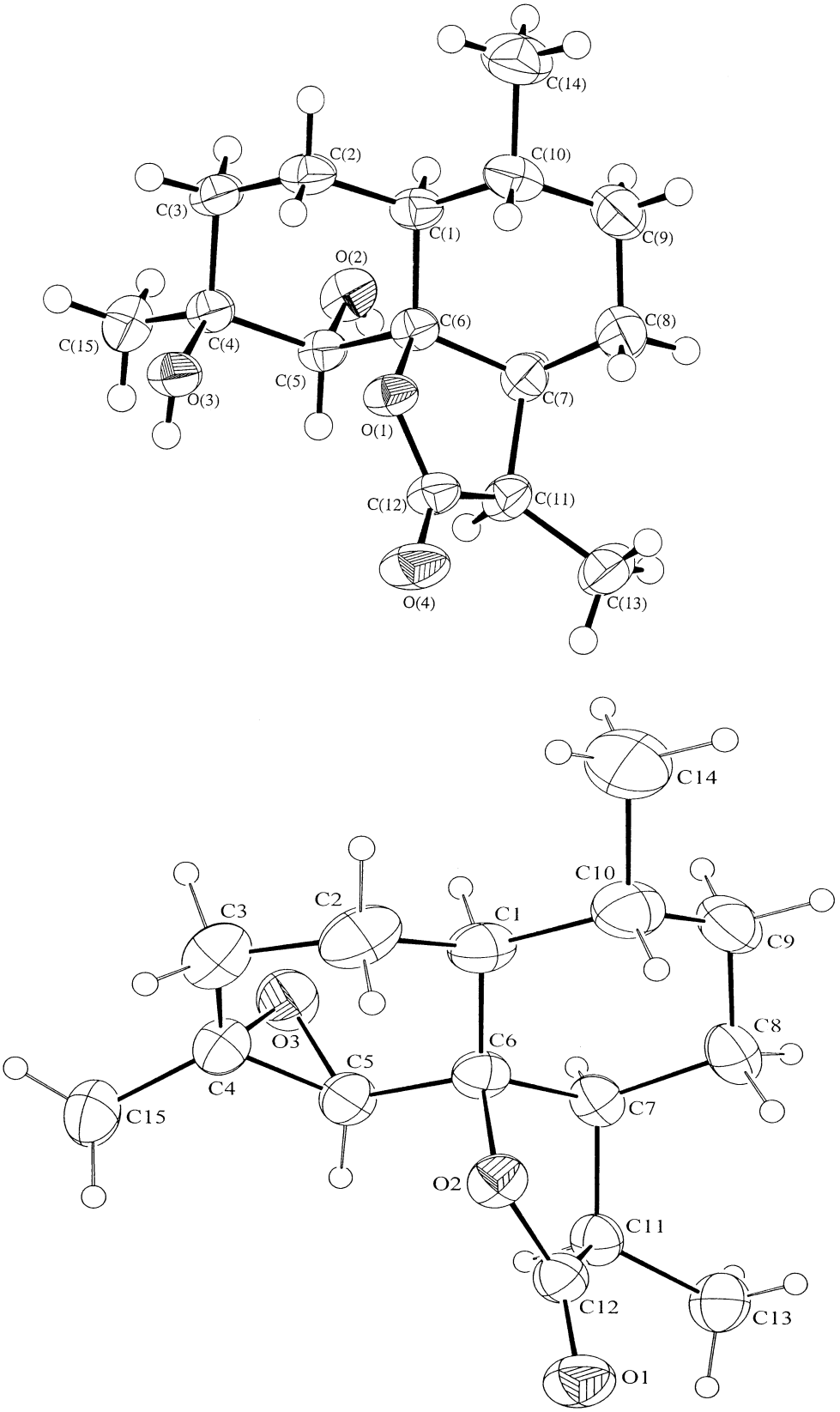
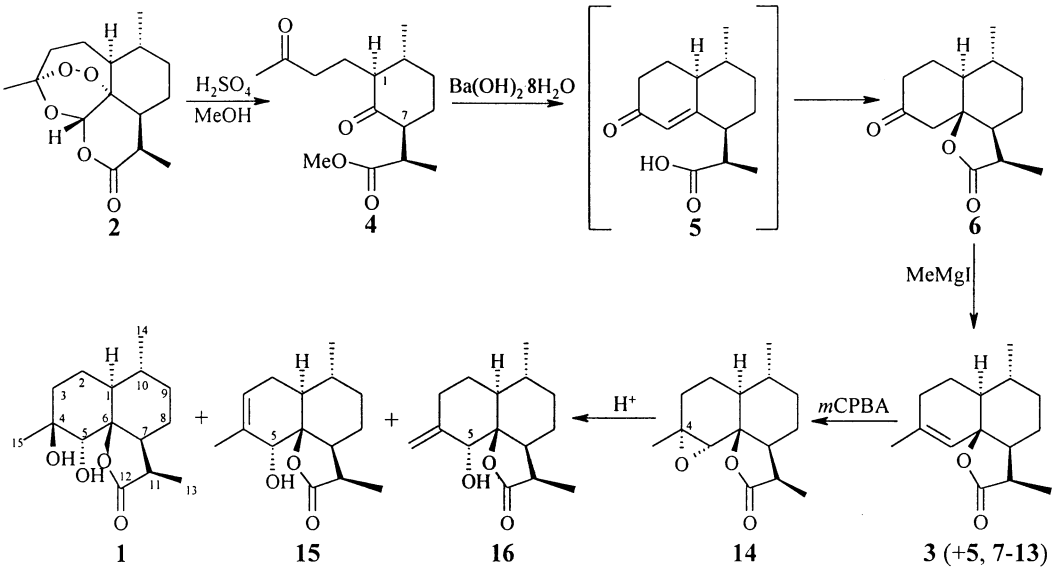


Figure 3. ORTEP diagrams of the natural product arteannuin O (**1**) and the synthetic product dihydro-*epi*-arteannuin B (**14**).

alternative Δ^3 regio-isomer. This was contrary to our expectation, based on recent precedents in the literature[11,12] for very closely related compounds (amorphanes and cadinanes containing a tertiary hydroxyl group at the 4-position) which are reported to undergo dehydration to give nearly equal amounts of both double bond regio-isomers. One possible explanation for this unexpected selectivity is the participation of the lactone group, which may be able to undergo



Scheme 1. Synthesis of arteannuins O, K and L (**1**, **15** and **16**, respectively) from artemisinin (**2**) via dihydro-*epi*-deoxyarteannuin B (**3**). The correct stereochemistry is shown at the 5-position for arteannuins K (**15**) and L (**16**), as determined by the results of Mosher ester studies.

reversible ring-opening under the conditions of the reaction as shown in Fig. 4, thereby assisting formation of the Δ^4-isomer. Such selectivity is clearly desirable in the context of this synthesis, and it acts to offset the main difficulty associated with this reaction, which is that the optimized yield of **3** from **6** was quite low (26%). The reasons for the low yield are believed to be the tendency for the lactone ring to open (as in the formation of **5**) or to participate in Grignard reaction itself, as in the formation of compounds **9**–**12** and **13** (Fig. 5, Tables 1 and 2), which have incorporated 2 and 3 equiv. of the methyl Grignard reagent respectively. (N.B. The use of fewer equivalents of Grignard reagent resulted only in the recovery of starting material and lactone ring-opened product **5**).

Treatment of **3** with *m*-chloroperoxybenzoic acid[13,14] gave a single epoxide product, compound **14** (Scheme 1). Oxygen was delivered to the less hindered α-face of the double bond in **3** as shown by X-ray crystallography of **14** (Fig. 3). We have recently isolated a novel natural product as a minor constituent of *A. annua* (unpublished results) with NMR spectra identical to those of synthetic dihydro-*epi*-arteannuin B (**14**) reported in Tables 2 and 3. Hydrolysis of epoxide **14** under acidic conditions resulted predominantly in the desired *trans* diol **1**, possessing identical physical properties to the natural product arteannuin O. Also separable from the crude reaction product, were small quantities of the allylic alcohol **15**, together with smaller amounts of its regio-isomer **16** (Scheme 1) and trace quantities of the chlorinated product **17** (Fig. 5; Tables 2 and 3). Although no molecular ion was seen for **17** in HREIMS, the presence of chlorine and the relative stereochemistry at the 4- and 5-positions could be confirmed by X-ray crystallography (structure not shown). Formation of all four products probably involves opening of the epoxide ring to yield a tertiary carbocation at C-4, which is then either quenched by addition of water/chloride (as in compounds **1**/**17**) or which eliminates a proton from H-3/H-15 (as in compounds **15**/**16**).

The NMR spectra of synthetic products **15** and **16** matched those previously published for the natural products arteannuins K and L, respectively.[2] This was somewhat surprising as direct opening of the α-epoxide group in **14** would be expected to yield allylic alcohol products containing a 5α-hydroxyl group, rather than the 5β-hydroxyl functionality which has been claimed for these natural products (see Fig. 1). (N.B. none of the products **1**, **15**, **16** or **17** underwent interconversion with one another under the

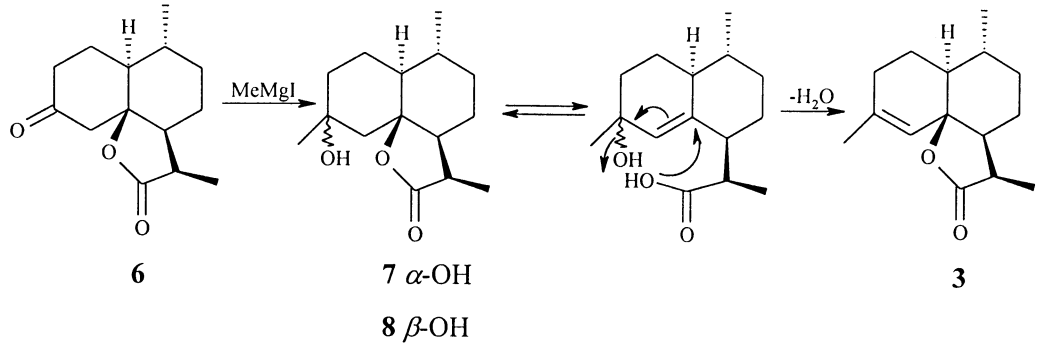


Figure 4. A possible explanation for the unexpected regio-selectivity observed in the conversion of **6** to only the Δ^4-isomer **3** via dehydration of Grignard addition products **7**/**8**.

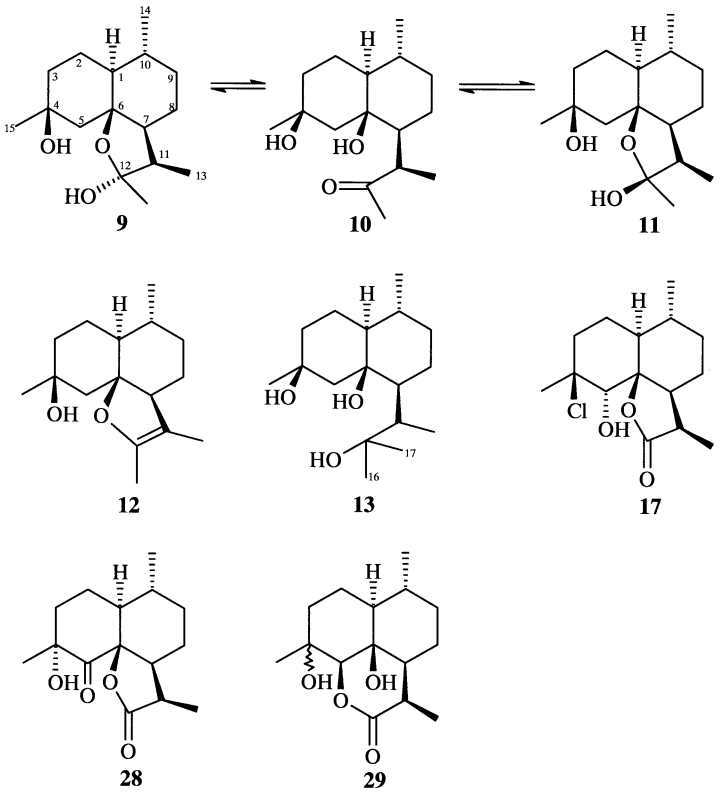


Figure 5. Side-products from reactions shown in Schemes 1 and 2.

Table 2. ^{13}C NMR data (δ, ppm) for novel compounds reported in Schemes 1 and 2 and Fig. 4

Position	7	8	9[a]	10[a]	11[a]	12	13	14	17	27	28	29
1	49.0	49.1	50.5	51.5	50.4	44.0	52.1	39.6	42.3	44.7	52.4	50.0
2	23.3	21.1	21.0	21.3	21.1	23.0	21.4	20.1	20.9	22.0	21.5	19.4
3	40.5	39.1	39.8	39.0	39.6	39.3	38.7	27.2	36.6	37.6	42.7	36.7
4	71.1	69.9	70.8	70.3	71.2	70.3	70.9	63.1	67.6	73.3	77.6	72.8
5	48.4	46.0	46.6	46.3	46.0	47.7	45.7	60.6	74.0	79.7	210.7	84.3
6	84.9	86.1	85.8	83.9	83.4	86.6	73.4	84.6	85.7	88.0	88.4	70.5
7	43.7	43.3	45.2	53.2	46.6	51.9	56.6	40.7	39.5	41.1	38.6	42.7
8	23.9	23.8	23.3	21.0	23.5	20.8	22.5	23.4	23.9	20.1	23.5	23.3
9	32.5	32.4	34.5	35.9	34.2	26.5	37.3	31.7	32.4	27.2	32.3	35.0
10	30.0	30.3	30.5	31.5	30.9	28.9	31.8	29.5	29.9	28.9	30.6	30.6
11	39.8	39.8	44.1	44.6	45.9	106.1	40.5	39.1	39.0	38.3	40.7	37.2
12	179.0	178.0	105.1	216.0	106.7	144.1	74.3	179.3	178.6	180.4	177.8	175.9
13	9.4	9.3	9.3	17.8	10.5	10.2	19.5	9.4	9.4	10.5	9.1	15.2
14	19.9	19.9	19.8	19.9	19.8	21.1	20.1	20.0	20.0	21.0	19.9	19.6
15	28.8	30.2	30.4	30.9	30.4	30.1	31.0	24.2	31.4	26.4	25.6	27.2
16	–	–	29.2	30.5	26.7	11.3	33.5[b]	–	–	–	–	–
17	–	–	–	–	–	–	25.9[b]	–	–	–	–	–

Assigned by the same 2D NMR techniques shown in Table 1.
[a] Assigned as a mixture.
[b] Interchangeable.

conditions of the reaction, and **15** and **16** are thus presumed to be formed directly from the epoxide). Unfortunately, neither synthetic compound **15** nor **16** could be obtained as crystals for X-ray crystallographic determination of the true stereochemistry at the 5-position of arteannuins K and L. However, the secondary alcohol group in both compounds did react cleanly with the *R*-(−) and *S*-(+) forms of α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPCl)[15,16] yielding either the *S*- or *R*-α-methoxy-α-(trifluoromethyl)phenylacetate esters (OMTP) **18/19** (from **15**) and **20/21** (from **16**).[†] The complete ^{1}H (and ^{13}C) NMR chemical shift assignments for all four derivatives were then made by 2D NMR (Table 4), allowing the configuration of the secondary hydroxyl group to be reliably determined as 5*R* (5α-OH) for both **15** and **16** (by analysis of ^{1}H chemical shift differences between the diastereoisomeric ester pairs made by subtracting the ^{1}H chemical shifts for the *R*-OMTP

[†] The *R*/*S* nomenclature is reversed when the acid chloride derivatizing agent is converted into the Mosher ester product.

Table 3. ^{1}H NMR data (δ, ppm) for novel compounds reported in Schemes 1 and 2 and Fig. 4

Position	**7**	**8**	**9**[a]	**10**[a]	**11**[a]	**12**	**13**	**14**	**17**	**27**	**28**	**29**
1	1.06	1.01	0.93	0.74	0.87	1.02	0.77	1.40	1.39	1.11	1.19	0.85
2α	1.89	1.84	1.75	1.66	–[b]	1.72	1.67	1.59	1.70	1.76	1.92	1.64
2β	1.44	1.58	1.55	1.55	–[b]	1.59	1.49	1.45	1.82	1.51	1.79	1.48
3α	1.52	1.46	1.38	1.31	1.33	1.38	1.32	1.91	1.85	1.52	1.62	1.41
3β	1.87	1.87	1.78	1.74	1.77	1.78	1.75	1.87	1.98	1.95	2.27	1.96
5α	1.52	1.41	1.15	1.15	1.21	1.12	1.14	–	–	–	–	3.78
5β	2.14	2.17	1.97	1.99	2.38	2.20	2.27	3.04	3.83	3.26	–	–
7	2.04	1.99	1.65	1.28	1.70	2.20	1.32	2.43	2.62	2.55	2.89	1.83
8α	1.69	1.69	1.90	–[b]	1.65	1.50	1.80	1.75	1.72	1.58	1.82	1.74
8β	1.13	1.15	1.50	–[b]	1.28	1.25	1.62	1.20	1.17	1.43	1.12	1.42
9α	1.02	1.03	0.98	0.98	0.94	0.99	0.92	1.04	1.04	1.13	1.02	1.01
9β	1.65	1.66	1.65	1.74	1.62	1.61	1.78	1.61	1.64	1.48	1.69	1.77
10	1.35	1.36	1.37	1.56	1.31	1.46	1.60	1.33	1.42	1.51	1.58	1.64
11	3.08	3.11	2.56	3.02	2.80	–	2.27	3.25	3.02	3.60	2.80	2.79
13	1.13	1.14	1.01	1.25	0.98	1.53	0.95	1.18	1.14	1.15	1.12	1.37
14	0.92	0.94	0.90	0.85	0.88	0.94	0.86	0.88	0.94	0.98	0.98	0.88
15	1.36	1.19	1.14	1.19	1.17	1.14	1.21	1.33	1.63	1.23	1.54	1.34
16	–	–	1.55	2.21	1.40	1.71	1.27[c]	–	–	–	–	–
17	–	–	–	–	–	–	1.18[c]	–	–	–	–	–

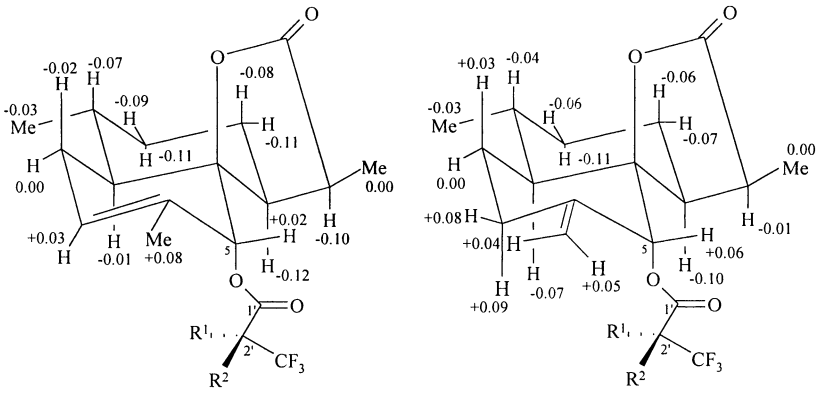
Assigned by the same 2D NMR techniques shown in Table 1.
[a] Assigned as a mixture.
[b] Not resolved.
[c] Interchangeable.

ester **19/21** from those for the corresponding ester *S*-OMTP ester **18/20**). Inspection of Fig. 6 shows that all differences to the left of the newly formed Mosher ester groups are positive, while all values to the right are negative: such a clear division in upfield and downfield shifts about the OMTP functionality is generally considered as good evidence that the Mosher ester is adopting the expected conformation (shown in Fig. 6), which can then be used in reliably assigning the absolute stereochemistry for a secondary hydroxyl group.[17]

Table 4. ^{1}H and ^{13}C NMR data (δ, ppm) for the *S*-OMTP esters of compounds **15**, **16** and **22** (**18**, **20** and **23/25**, respectively) and the *R*-OMTP esters of compounds **15**, **16** and **22** (**19**, **21** and **24/26**, respectively) fully assigned by 2D NMR

	δ_H								δ_C					
Position	**18**	**19**	**20**	**21**	**23**	**24**	**25**	**26**	**18**	**19**	**20**	**21**	**23**	**24**
1	1.38	1.39	1.36	1.43	1.20	1.22	1.57	1.57	39.5	39.8	43.0	43.2	43.0	43.4
2α	2.31	2.31	1.83	1.83	1.78	1.82	1.83	1.82	27.3	27.3	24.7	24.8	22.1	22.2
2β	1.98	2.00	1.46	1.43	1.32	1.40	1.40	1.38						
3α	5.81	5.78	2.15	2.06	1.49	1.51	1.94	1.91	129.9	129.5	29.8	29.5	35.2	35.7
3β	–	–	2.33	2.25	1.66	1.70	2.43	2.47						
4	–	–	–	–	–	–	–	–	126.8	126.9	140.8	140.6	71.7	71.5
5	5.33	5.31	5.55	5.49	5.04	5.05	3.93	3.94	72.1	72.8	75.3	75.9	76.6	77.2
6	–	–	–	–	–	–	–	–	83.5	83.4	84.3	84.4	84.7	84.6
7	1.75	1.87	1.92	2.02	1.73	1.88	2.63	2.61	38.2	38.6	38.7	39.0	39.4	39.8
8α	1.53	1.64	1.60	1.67	1.50	1.59	1.71	1.71	24.2	24.2	24.2	24.2	23.9	23.8
8β	0.99	1.07	1.06	1.12	1.00	1.06	1.12	1.11						
9α	0.70	0.81	0.77	0.88	0.74	0.81	1.05	1.05	31.6	31.8	32.1	32.3	32.1	32.3
9β	1.53	1.62	1.55	1.61	1.51	1.59	1.62	1.62						
10	1.25	1.32	1.28	1.32	1.28	1.33	1.35	1.36	31.7	31.7	30.6	30.7	29.9	29.9
11	3.16	3.26	3.17	3.18	3.11	3.20	3.06	3.06	38.9	38.9	38.7	38.7	38.8	38.7
12	–	–	–	–	–	–	–	–	178.8	178.8	178.4	178.3	178.1	178.0
13	1.09	1.09	1.13	1.13	1.08	1.09	1.12	1.12	9.2	9.2	9.3	9.3	9.3	9.3
14	0.88	0.91	0.87	0.90	0.88	0.90	0.92	0.92	19.6	19.7	19.3	20.0	19.9	20.0
15	1.66	1.58	5.15, 5.12	5.10, 5.08	1.43	1.40	1.74	1.75	21.2	20.8	118.0	118.1	26.4	25.6
1′	–	–	–	–	–	–	–	–	166.5	166.5	165.7	165.4	166.6	166.0
2′	–	–	–	–	–	–	–	–	84.5	84.7	84.5	84.8	84.7	84.8
3′	–	–	–	–	–	–	–	–	131.8	131.4	131.9	131.6	131.8	131.5
4′/8′	7.60	7.60	7.49	7.52	7.67	7.61	7.51	7.52	127.2	127.5	127.3	127.5	127.6	127.5
5′/7′	7.43	7.44	7.43	7.44	7.44	7.47	7.44	7.43	128.6	128.7	128.6	128.6	128.7	128.7
6′	7.43	7.44	7.43	7.44	7.44	7.47	7.44	7.43	130.1	129.9	129.9	129.8	130.0	130.0
2′-OMe	3.57	3.53	3.52	3.51	3.59	3.55	3.52	3.54	55.6	55.4	55.3	55.4	55.7	55.6

The *S*-OMTP esters **18**, **20** and **23/25** are formed from reaction of *R*-MTPCl with compounds **15**, **16** and **22**, respectively; the *R*-OMTP esters **19**, **21** and **24/26** are formed from reaction of *S*-MTPCl with compounds **15**, **16** and **22**, respectively; the CF_3 group in the Mosher ester was generally not observed due to ^{19}F-^{13}C coupling.



18 R^1= Ph, R^2= OMe
19 R^1= OMe, R^2= Ph

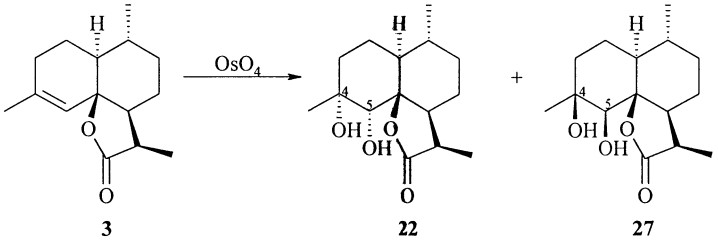
20 R^1 = Ph, R^2= OMe
21 R^1= OMe, R^2= Ph

Figure 6. $\Delta\delta$ ^{1}H NMR values (*S*-OMTP–*R*-OMTP; see Table 4) for Mosher ester derivatives **18** and **19**, which were used in determining the absolute stereochemistry at the 5-position of compound **15**; and $\Delta\delta$ ^{1}H NMR values (*S*-OMTP–*R*-OMTP; see Table 4) for Mosher ester derivatives **20** and **21**, which were used in determining the absolute stereochemistry at the 5-position for compound **16**. The analysis of these results assume the conformation of the Mosher ester which is depicted.

Hence, based on both mechanistic considerations and the results of Mosher ester studies, we propose that the stereochemistry at the 5-position for natural products arteannuins K and L, which was originally assigned as 5*S* (5β-OH) should be revised to 5*R* (5α-OH). Given that the 5-hydroxyl stereochemistry of arteannuins K and L appears to have been wrongly assigned, we next turned our attention to the natural product arteannuin M, which was also isolated from *A. annua* in the same study.[2] Treatment of synthetic dihydro-*epi*-deoxyarteannuin B (**3**) with osmium tetroxide[18] resulted predominantly in the cadinane diol **22** (Scheme 2), with NMR spectra matching those reported for arteannuin M.

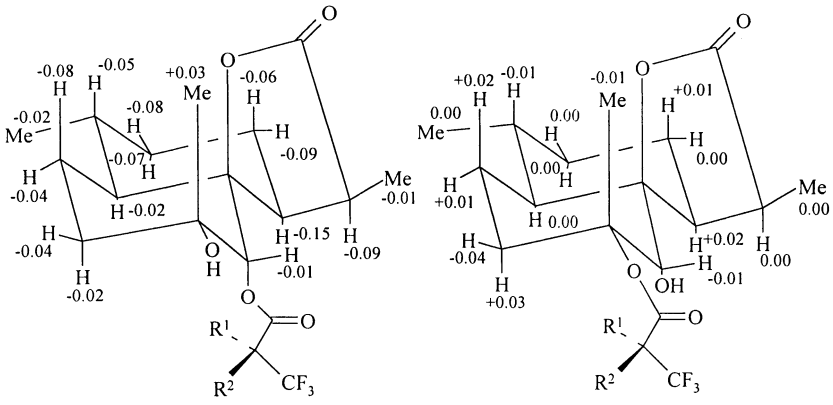
Because of the mechanism of the osmylation reaction, the vicinal diol in **22** ought to have *cis* stereochemistry. Steric considerations would suggest that osmium tetroxide is more likely to attack the double bond in **3** from the less hindered α-face (cf. α-epoxidation of **3** to **14** in Scheme 1), which in turn leads to the conclusion that the true stereochemistry of the vicinal diol in the natural product arteannuin M is 4*R*, 5*R* (4α-OH, 5α-OH): in the original report, the stereochemistry at the 4-position of arteannuin M was undefined, but the 5-position was assigned as 5*S* (5β-OH).

The need for structure revision of arteannuin M, as well as arteannuins K and L, was confirmed by the reacion of *R*- and *S*-MTPCl with the secondary hydroxyl group in **22**, which yielded the *S*- and *R*-OMTP esters **23** and **24**, respectively. ^{1}H chemical shifts differences between the two Mosher ester derivatives did indeed suggest that the stereochemistry which was originally proposed at the 5-position for arteannuin M as 5*S* (β-OH) should also be revised to 5*R* (5α-OH) (Table 4, Fig. 7). However, the Mosher ester results for compound **22** are slightly less convincing than those for compounds **15** and **16**, with the assignment of absolute stereochemistry at the secondary hydroxyl group resting on only a single positive chemical shift difference at the 15-position (all other differences were negative). This less satisfactory result may be due to hydrogen bonding between the oxygen substituents at the 4- and 5-positions in **23** and **24**, which distorts the normally preferred conformation of the Mosher ester, and perturbs the expected pattern of negative/positive chemical shift differences. Evidence for hydrogen bonding of the secondary hydroxyl group to the oxygen at C-4 was seen in the ^{1}H NMR spectrum of **22**, in which the ^{1}H chemical shift for the 5-OH group underwent comparatively smaller concentration-dependent changes than was the case for the 4-OH group. The yields of both Mosher esters from **22** were extremely low (see Section 3), and this might also be ascribed to a reduced reactivity of the secondary hydroxyl group as a result of hydrogen bonding with the tertiary hydroxyl group.



3 **22** **27**

Scheme 2. Synthesis of arteannuin M (**22**) from dihydro-*epi*-deoxyarteannuin B (**3**). The correct stereochemistry is shown for the 5-position of arteannuin M, as determined by the results of Mosher ester studies with **22**. The stereochemistry at the 4-position is more tentatively assigned based on the expected *cis* mechanism for the osmylation reaction. Cadinane diol **27** was obtained as a minor product.



23 R^1=Ph, R^2=OMe
24 R^1=OMe, R^2=Ph

25 R^1=Ph, R^2=OMe
26 R^1=OMe, R^2=Ph

Figure 7. $\Delta\delta$ ^{1}H NMR values (*S*-OMTP–*R*-OMTP: see Table 4) for Mosher ester derivatives **23** and **24** used in tentatively assigning the absolute stereochemistry at the 5-position of compound **22** assuming the conformation of the Mosher ester is as depicted; and $\Delta\delta$ ^{1}H NMR values (*S*-OMTP–*R*-OMTP: see Table 4) for Mosher ester derivatives **25** and **26** used in a failed attempt to determine the stereochemistry at the 4-position for compound **22**—the ester probably adopts no single well-defined conformation in this case.

Rather unexpectedly, we were also able to obtain the *S*- and *R*-OMTP esters of the tertiary hydroxyl group in **22**, compounds **25** and **26**, respectively. Analysis of ^{1}H chemical shift differences between **25** and **26** (Table 4, Fig. 7) was inconclusive in assigning the absolute stereochemistry at the 4-position—chemical shift differences were small and there is no clear pattern of positive and negative values. Most probably, the Mosher esters of the tertiary hydroxyl group in **22** adopt no single well-defined conformation — indeed we are unaware of any previous attempts to study the Mosher esters of tertiary alcohols. In summary, the results of Mosher ester studies with **22**, although lending some support to the revision of the stereochemistry at the 5-position of arteannuin M to 5*R* (5α-OH), do not shed any light on the stereochemistry of the tertiary hydroxyl group at C-4, and this is assumed to be 4*R* on mechanistic grounds only. Further synthetic studies in order to establish more rigorously the stereochemistry of the vicinal diol in arteannuin M, particularly at the 4-position, would be most welcome.

A second cadinane diol, compound **27**, was also obtained as a minor product from the osmylation reaction. The NMR spectra of **27** are different from those of both **1** and **22**, and this compound has been assigned as 4*S*, 5*S* (4β-OH, 5β-OH), on the basis of correlations observed in NOESY and mechanistic considerations, which require a *cis*-diol. Compound **27** is the third of four diastereoisomers which are possible from di-hydroxylation of the double bond in dihydro-*epi*-deoxyarteannuin B; because **27** was only isolated in small amounts, presumably as a consequence of steric hindrance to osmylation of the β-face of **3**, we have been unable to obtain further evidence to corroborate the proposed stereochemistry. The structures of two further minor products of the osmylation reaction, compounds **28** and **29**, are shown in Fig. 5 (see also Tables 2 and 3).

[15-^{13}C]-Isotopomers of all of the natural products, arteannuin O (**1a**), arteannuin K (**15a**) and arteannuin L (**16a**)[‡] (as well as the chloro-compound **17a**) have been synthesised by substituting [15-^{13}C]-dihydro-*epi*-deoxyarteannuin B (**3a**), obtained by use of $^{13}CH_3MgI$ in the Grignard reaction with **6**, for isotopically normal **3** in the epoxidation and acid hydrolysis reactions shown in Scheme 1. These labeled compounds will be used in future feeding experiments with *A. annua* in order to establish the status of arteannuins K, L and O as potential precursors in the biogenesis of artemisinin (**2**) in vivo.

3. Experimental

3.1. General

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 are clearly resolved in 1D ^{1}H NMR without recourse to 2D NMR analysis (see Tables 1–4 for full assignments by 2D NMR). All NMR experiments were run on a Bruker DRX 500 instrument. HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY spectra were recorded with 1024 data points in F_2 and 256 data points in F_1. High-resolution MS were recorded in EI mode at 70 eV on a Finnigan-MAT 95 MS spectrometer. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8201 PC instrument. Column chromatography (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 a YMC diol 20 mm×25 cm column, flow rate 8 ml min^{-1}. Melting points were recorded by a Perkin–Elmer differential scanning calorimeter 7 (DSC7). Optical rotations were measured by a Perkin–Elmer 343 Polarimeter with polarized light (Na

[‡] The suffix 'a' is used to indicate that the isotopically normal [15-CH_3] group has been replaced by [15-$^{13}CH_3$].

589 nm). $[\alpha]_D$ Values are given in 10^{-1} deg cm^2 g^{-1} and $CHCl_3$ was used as a solvent.

3.2. Isolation of the natural products, arteannuins M and O

Leaves of *A. annua* were collected from the mountains in You Yang Country in Sichuan Province, Southern China, in July and dried in the sun for two weeks before being sent to Hong Kong for extraction. The dried leaves (1 kg) were pulverized to a fine powder under liquid N_2, repetitively extracted with CH_2Cl_2 (AR grade), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a dark green gum (97 g; 9.7% w/w). A portion of the extract (25 g) was subjected to gradient column chromatography (developing solvent: 100% *n*-hexane to 100% EtOAc) and crude fractions eluted by 40–50% EtOAc/*n*-hexane were further purified by CC and HPLC (38% EtOAc/*n*-hexane). *Arteannuin M* (158 mg, R_t 25.4 min)—see Ref. 2 for physical properties. *Arteannuin O* (1): Solid. (182 mg, R_t 21.4 min). $[\alpha]_D$=−86.9 (*c* 0.64, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3574, 2937, 1773, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.56 (1H, s), 3.37 (1H, br s, 4-OH), 3.09 (1H, dq, *J*=6.8, 7.1 Hz), 2.59 (1H, ddd, *J*=11.0, 6.8, 5.4 Hz), 1.23 (3H, s), 1.14 (3H, d, *J*=7.1 Hz), 0.93 (3H, d, *J*=6.4 Hz) — see Table 1 for full assignments; ^{13}C NMR ($CDCl_3$): see Table 1; HREIMS *m/z* (rel. int.) 268.1675 [M^+, $C_{15}H_{24}O_4$ requires 268.1675] (1), 250 (5), 232 (8), 195 (100), 192 (28), 179 (45), 177 (35).

3.3. Conversion of artemisinin (2) to lactone 6 by acid degradation and Robinson annulation

To a cooled solution of conc. H_2SO_4 (120 ml) in MeOH (180 ml) in an ice bath was added artemisinin (**2**) (12.0 g, 42.6 mmol). The mixture was stirred for 10 min (a colour change to pale yellow was noted) after which iced water (250 ml) was added to quench the reaction. The mixture was filtered and extracted with $CHCl_3$ (5×100 ml) and the combined organic layers were washed with brine (3×50 ml) and dried ($MgSO_4$), then solvent was removed under reduced pressure to yield a crude product (9.05 g; 75% w/w) consisting predominantly of methyl ester **4**—see Ref. 8 for physical properties. $BaOH_2{\cdot}8H_2O$ in MeOH (8 g; 150 ml) was added to the crude product (9.05 g) 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 then gradually acidified by dropwise addition of HCl (3 M) to pH 2, while stirring was maintained for 15 min in an ice bath. The mixture was extracted by $CHCl_3$ (3×100 ml), washed with brine (3×20 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield a crude product (8.0 g; 88% w/w) consisting mostly of lactone **6** (6.8 g, 28.8 mmol; 68%) which was purified by recrystallization from EtOAc (to remove impurities of uncyclized compound **5**). Compound **6**—see Ref. 8 for physical properties: Solid. Mp 171–172°C; $[\alpha]_D$=−50.9 (*c* 3.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3024, 2934, 2881, 1771, 1719, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.02 (1H, dq *J*=6.9, 7.1 Hz), 2.79 (1H, dd, *J*=14.9, 2.0 Hz), 2.54 (1H, ddd, *J*=15.4, 2.5, 2.5 Hz), 2.35 (1H, d, *J*=14.9 Hz), 2.33 (1H, m), 1.13 (3H, d, *J*=7.1 Hz), 1.00 (3H, d, *J*=6.5 Hz); ^{13}C NMR ($CDCl_3$): 207.0 (C-4), 178.0 (C-12), 85.9 (C-6), 50.7 (C-5), 48.1 (C-1), 43.3 (C-7), 40.3 (C-3), 39.9 (C-11), 32.5 (C-9), 31.2 (C-10), 24.4 (C-8), 24.0 (C-2), 19.9 (C-14), 9.2 (C-13); HREIMS *m/z* (rel. int.) 236.1412 [M^+, $C_{14}H_{20}O_3$ requires 236.1415] (93), 208 (100), 180 (75), 179 (72), 163 (67), 134 (35).

3.4. Preparation of 3 by reaction of 6 with a methyl Grignard reagent

To small Mg chips (1.33 g, 54.7 mmol) in anhyd. Et_2O (250 ml) was added a solution of MeI (4.16 ml, 66.8 mmol) in anhyd. Et_2O (100 ml) and the reaction mixture was refluxed for 1.5 h. A solution of lactone **6** (6.5 g, 27.5 mmol) in anhyd. Et_2O (250 ml) was added dropwise and the reaction was allowed to reflux for a further 2.5 h. Completion of the reaction was determined by TLC and the reaction mixture was cooled in an ice bath and H_2O (250 ml) was added to destroy the excess Grignard reagent. The mixture was extracted with Et_2O (5×100 ml) and the combined organic layers for this 'neutral extract' were washed by brine (3×50 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a crude product (4.2 g; 65% w/w) consisting of compounds **7**–**13** which were separated by HPLC (30% EtOAc/*n*-hexane). The aqueous layer left behind after the 'neutral extraction' was acidified by dropwise addition of HCl (3 M) to pH 2 and then extracted again by Et_2O (3×100 ml). The combined organic layers from this 'acidic extract' were washed with brine (3×50 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield a crude mixture (2.1 g; 32% w/w) consisting mostly of dihydro-*epi*-deoxyarteannuin B (**3**) together with a little of decalenone acid **5**, which could be separated by CC (10% EtOAc/*n*-hexane). Compound **3** (1.65 g, 7.05 mmol; 26%)—see also Refs. 2 and 5 for physical properties: Oil. $[\alpha]_D$=+104.1 (*c* 0.32, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3020, 2939, 2876, 1751, 1668, 1454 cm^{-1}; ^{1}H NMR ($CDCl_3$): 5.64 (1H, d, *J*=1.4 Hz), 3.14 (1H, dq, *J*=7.0, 7.2 Hz), 1.69 (3H, s), 1.14 (3H, d, *J*=7.2 Hz), 0.94 (3H, d, *J*=6.6 Hz); ^{13}C NMR ($CDCl_3$): 179.4 (C-12), 142.2 (C-4), 121.9 (C-5), 83.2 (C-6), 46.6 (C-1), 42.9 (C-7), 39.6 (C-11), 32.5 (C-9), 30.9 (C-3), 29.7 (C-10), 23.7 (C-15), 23.4 (C-8), 21.1 (C-2), 19.6 (C-14), 9.4 (C-13); HREIMS *m/z* (rel. int.) 234.1616 [M^+, $C_{15}H_{22}O_2$ requires 234.1620] (10), 219 (5), 190 (100), 175 (20), 161 (85). Compound **5** (0.20 g, 0.85 mmol; 3%)—see Ref. 8 for physical properties. Compound **7**: Oil. (R_t 34.6 min, 1.00 g, 3.97 mmol; 15%); $[\alpha]_D$=−68.5 (*c* 0.78, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3587, 3009, 2974, 2934, 2880, 1765, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.08 (1H, dq, *J*=6.9, 7.1 Hz), 2.14 (1H, dd, *J*=13.9, 2.1 Hz), 2.04 (1H, ddd, *J*=11.7, 6.9, 5.3 Hz), 1.52 (1H, d, *J*=13.5 Hz), 1.44 (1H, dddd, *J*=11.9, 11.9, 11.9, 2.3 Hz), 1.36 (3H, s), 1.13 (3H, d, *J*=7.1 Hz), 0.92 (3H, d, *J*=6.6 Hz) - see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS *m/z* (rel. int.) 252.1722 [M^+, $C_{15}H_{24}O_3$ requires 252.1725] (6), 234 (38), 206 (17), 179 (36), 178 (60), 161 (100), 160 (68), 151 (54). Compound **8**: Oil. (R_t 15.6 min, 0.79 g, 3.13 mmol; 11%); $[\alpha]_D$=−88.6 (*c* 0.61, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3568, 3007, 2976, 2936, 2880, 1767, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.31 (1H, br s, OH), 3.11 (1H, dq, *J*=6.8, 7.1 Hz), 2.17 (1H, dd, *J*=14.5, 2.3 Hz), 1.99 (1H, ddd, *J*=11.0, 6.8, 6.3 Hz), 1.58 (1H, dddd, *J*=12.5, 12.5, 12.5, 3.3 Hz), 1.46 (1H, dd, *J*=13.4,

4.2 Hz), 1.41 (1H, d, J=14.5 Hz), 1.36 (1H, m), 1.19 (3H, s), 1.14 (3H, d, J=7.1 Hz), 0.94 (3H, d, J=6.6 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS m/z (rel. int.) 252.1730 [M^+, $C_{15}H_{24}O_3$ requires 252.1725] (20), 234 (30), 219 (5), 191 (20), 179 (65), 178 (100), 161 (75). Compounds **9**–**11** were isolated as an inseparable mixture by HPLC (R_t 17.5 min, 0.98 g, 3.66 mmol; 13%): Oil. IR ν_{max} ($CHCl_3$): 3429 (br), 2932, 2874, 2849, 1688, 1454 cm^{-1}; HREIMS m/z (rel. int.) 268.2040 [M^+, $C_{16}H_{28}O_3$ requires 268.2038] (3), 250 (100), 232 (20), 217 (17), 178 (30), 160 (55). The planar structures of all three compounds were determined, as a mixture, by correlations observed in the 2D NMR experiments HSQC, HMBC and ^{1}H–^{1}H COSY (Tables 2 and 3). Positive cross-peaks observed in NOESY (most obviously 2.56/3.02/2.80 for H-11; 1.97/1.99/2.38 for H-5β; 1.55/2.21/1.40 for H-16; 1.01/1.25/0.98 for H-13 in **9**, **10** and **11** respectively) indicated that all three compounds were involved in chemical exchange with one another (see Fig. 5)—hence the observation that they cannot be separated chromatographically. The stereochemistry of each of compounds **9**, **10** and **11** has been tentatively assigned from negative correlations observed in the same NOESY spectrum (the exchange process causes some ambiguity in interpreting correlations due to dipole–dipole interactions). Compound **9** (ca. 50% of the mixture by NMR): ^{1}H NMR ($CDCl_3$): 4.93 (1H, br s, OH), 4.54 (1H, br s, OH), 2.56 (1H, dq, J=6.9, 7.1 Hz), 1.97 (1H, dd, J=13.8, 2.5 Hz), 1.55 (3H, s), 1.14 (3H, s), 1.01 (3H, d, J=7.1 Hz), 0.90 (3H, d, J=6.6 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2. Compound **10** (ca. 30% of the mixture by NMR): ^{1}H NMR ($CDCl_3$): 4.73 (1H, br s, OH), 3.02 (1H, dq, J=2.4, 7.3 Hz), 2.28 (1H, br s, OH), 2.21 (3H, s), 1.99 (1H, dd, J=13.6, 2.5 Hz), 1.19 (3H, s), 1.25 (3H, d, J=7.3 Hz), 0.85 (3H, d, J=6.6 Hz), 0.74 (1H, ddd, J=12.0, 12.0, 3.4 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2. Compound **11** (ca. 20% of the mixture by NMR): ^{1}H NMR ($CDCl_3$): 4.84 (1H, br s, OH), 4.64 (1H, br s, OH), 2.80 (1H, dq, J=6.9, 7.3 Hz), 2.38 (1H, dd, J=14.0, 2.5 Hz), 1.40 (3H, s), 1.17 (3H, s), 0.98 (3H, d, J=7.3 Hz), 0.88 (3H, d, J=6.7 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2. Compound **12** (R_t 9.2 min, 0.42 g, 1.68 mmol; 6%): Oil. $[\alpha]_D$=−4.9 (c 0.32, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3471, 2930, 2872, 2856, 1717, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.59 (1H, br s, OH), 2.20 (1H, m), 2.00 (1H, dd, J=14.2, 2.8 Hz), 1.71 (3H, s), 1.53 (3H, d, J=0.9 Hz), 1.14 (3H, s), 0.94 (3H, d, J=6.8 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS m/z (rel. int.) 250.1935 [M^+, $C_{16}H_{26}O_2$ requires 250.1933] (100), 232 (12), 217 (24), 189 (20), 161 (36), 160 (46). Compound **13** (R_t 18.5 min, 0.27 g, 0.95 mmol; 4%): Oil. $[\alpha]_D$=−10.9 (c 0.22, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3321 (br), 2970, 2930, 2872, 2849, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.68 (1H, br s, OH), 2.27 (1H, dq, J=1.8, 7.3 Hz), 2.27 (1H, d, J=14.4, 2.5 Hz), 1.27 (3H, s), 1.21 (3H, s), 1.18 (3H, s), 0.95 (3H, d, J=7.3 Hz), 0.92 (3H, dddd, J=12.5, 12.5, 12.5, 3.9 Hz), 0.86 (3H, d, J=6.6 Hz), 0.77 (1H, ddd, J=14.0, 10.7, 3.7 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS m/z (rel. int.) 284.2350 [M^+, $C_{17}H_{32}O_3$ requires 284.2351] (6), 266 (17), 248 (60), 233 (42), 230 (80), 215 (50), 193 (42), 190 (47), 180 (75), 161 (100).

3.4.1. Reaction of 6 with 1 equiv. of Grignard reagent. When the same procedures described in Section 3.4 were repeated with 1 equiv. of Grignard reagent being added to the lactone **6** (50 mg, 0.22 mmol), the starting material was recovered from the 'neutral fraction' (30 mg, 0.13 mmol; 60%) while the 'acidic fraction' contained only compound **5** (15 mg, 0.06 mmol; 30%).

3.5. Epoxidation of dihydro-*epi*-deoxyarteannuin B (3) to dihydro-*epi*-arteannuin B (14)

To a solution of compound **3** (1.6 g, 6.83 mmol) in $CHCl_3$ (100 ml) was added *m*-chloroperoxybenzoic acid (*m*CPBA, 1.77 g, 50–55%). The reaction mixture was stirred overnight at room temperature and when completion had been determined by TLC, H_2O (100 ml) was added and the reaction mixture was extracted with $CHCl_3$ (3×100 ml). The combined organic extracts were washed successively with $NaHCO_3$ (30%; 3×50 ml) and brine (3×50 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield a crude product (1.59 g; 99% w/w) which was purified by column chromatography (15% EtOAc/*n*-hexane) to afford epoxide **14** which was further purified by recrystallization from *n*-hexane. Compound **14** (1.52 g, 6.08 mmol; 89%): Solid. Mp 133–135°C; $[\alpha]_D$=−12.0 (c 6.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3024, 3013, 2932, 2878, 1767, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.25 (1H, dq, J=6.9, 7.1 Hz), 3.04 (1H, s), 2.43 (1H, ddd, J=10.4, 6.9, 6.5 Hz), 1.33 (3H, s), 1.18 (3H, d, J=7.1 Hz), 0.88 (3H, d, J=6.5 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS m/z (rel. int.) 250.1557 [M^+, $C_{15}H_{22}O_3$ requires 250.1568] (2), 232 (1), 222 (12), 193 (15), 180 (37), 179 (100).

3.6. Preparation of compounds 1, 15, 16 and 17 from the treatment of dihydro-*epi*-arteannuin B (14) with acid

To a solution of epoxide **14** (1.45 g, 5.80 mmol) in Et_2O (100 ml) was added HCl (3 M, 12 ml) and the reaction mixture was stirred overnight at room temperature. When completion had been determined by TLC, H_2O (50 ml) was added and the mixture was extracted with Et_2O (3×100 ml). The combined organic layers were washed with brine (3×30 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield a crude product (1.43 g; 99% w/w), which was separated by HPLC (20% EtOAc/*n*-hexane/1% CH_3COOH) to afford compound **1** (R_t 51.7 min) as the predominant product (1.23 g, 4.59 mmol; 79%). Compound **1** was further purified by recrystallization from EtOAc and its structure was confirmed to be identical with the natural product arteannuin O by X-ray crystallography. Compound **1**: physical properties of **1** were similar to arteannuin O. NMR spectra in $CDCl_3$ solution for natural and synthetic arteannuin O were identical within the resolution of the instrument (±0.01 ppm for ^{1}H and ±0.1 ppm for ^{13}C) when recorded at the same concentration (7.2 mg/0.6 ml $CDCl_3$). Chemical shifts for some resonances in the vicinity of the oxygen-containing functional groups showed concentration-dependent changes (e.g. C-6 and C-12 ($\Delta\delta_C$ 0.2 and 0.3 ppm, respectively) and H-5, 4-OH and 5-OH ($\Delta\delta_H$ 0.02, 0.03 and 0.46 ppm, respectively)) when more concentrated solutions were studied. Molecular modeling showed that the 4-OH group is involved in intramolecular

hydrogen-bonding with the lactone functional group, whilst the 5-OH group does not participate in intramolecular hydrogen bonding, and these changes in chemical shift are thus consistent with the expected changes in the extent of hydrogen bonding of the 4-OH and 5-OH groups as the concentration is changed. Solid. Mp 197–198°C; $[\alpha]_D$= −106.6 (*c* 0.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3568, 3410 (br), 3024, 2932, 2862, 1773, 1456 cm^{-1}; ^1H NMR (7.2 mg/0.6 ml $CDCl_3$): 3.55 (1H, s), 3.38 (1H, br s, 4-OH), 3.09 (1H, dq, *J*=6.9, 7.1 Hz), 2.59 (1H, ddd, *J*=10.9, 6.9, 5.0 Hz), 2.29 (1H, br s, 5-OH), 1.23 (3H, s), 1.14 (3H, d, *J*=7.1 Hz), 0.94 (3H, d, *J*=6.4 Hz); ^{13}C NMR ($CDCl_3$): 178.0 (C-12), 87.7 (C-6), 72.7 (C-5), 72.5 (C-4), 41.7 (C-1), 38.9 (C-7), 38.8 (C-11), 33.9 (C-3), 32.1 (C-9), 30.2 (C-10), 26.7 (C-15), 23.9 (C-8), 20.2 (C-2), 20.0 (C-14), 9.2 (C-13); HREIMS *m/z* (rel. int.) 250.1563 [M^+-H_2O, $C_{15}H_{22}O_3$ requires 250.1569] (10), 232 (15), 222 (10), 207 (12), 195 (100), 192 (35), 179 (55), 177 (60). Compound **15** (R_t 28.0 min, 80 mg, 0.32 mmol; 6%): physical properties as for arteannuin K—see Ref. 2. NMR spectra in $CDCl_3$ solution for natural and synthetic arteannuin K were identical within the resolution of the instrument (±0.01 ppm for ^1H and ±0.1 ppm for ^{13}C) when recorded at the same concentration (5.7 mg/0.6 ml $CDCl_3$). The appearance of the H-5 resonance (δ_H 3.70 ppm) varied with concentration, appearing as either a singlet, a broad singlet or a doublet (*J*=7.5 Hz). Molecular modeling showed that the 5-OH group does not participate in intramolecular hydrogen bonding, and the observed changes in multiplicity for the H-5 proton may be related to the rate of exchange of the 5-OH proton to which it is coupled, as the concentration is changed. Oil. $[\alpha]_D$=−148.7 (*c* 0.62, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3591, 3447 (br), 3028, 2928, 1759, 1456 cm^{-1}; ^1H NMR (5.7 mg/0.6 ml; $CDCl_3$): 5.66 (1H, q, *J*=1.7 Hz), 3.70 (1H, d, *J*=7.5 Hz (coupling to OH)), 3.11 (1H, dq, *J*=6.9, 7.2 Hz), 2.72 (1H, ddd, *J*=12.1, 6.9, 4.7 Hz), 2.27 (1H, m), 1.79 (3H, dd, *J*=2.4, 1.7 Hz), 1.14 (3H, d, *J*=7.2 Hz), 0.94 (3H, d, *J*=6.4 Hz); ^{13}C NMR ($CDCl_3$): 179.5 (C-12), 131.2 (C-4), 126.4 (C-3), 85.2 (C-6), 70.1 (C-5), 39.0 (C-11), 38.8 (C-1), 38.0 (C-7), 32.1 (C-9), 31.7 (C-10), 27.2 (C-2), 24.4 (C-8), 21.2 (C-15), 19.8 (C-14), 9.3 (C-13); HREIMS *m/z* (rel. int.) 250.1564 [M^+, $C_{15}H_{22}O_3$ requires 250.1569] (3), 167 (100), 151 (20). Compound **16** (R_t 26.9 min, 35 mg, 0.14 mmol; 2%): physical properties as for arteannuin L—see Ref. 2. NMR spectra in $CDCl_3$ solution for natural and synthetic arteannuin L were identical within the resolution of the instrument (±0.01 ppm for ^1H and ±0.1 ppm for ^{13}C) when recorded at the same concentration (7.0 mg/0.6 ml $CDCl_3$). No significant changes were noted in the NMR spectra when the concentration was changed. Oil. $[\alpha]_D$=−48.8 (*c* 0.77, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3400 (br), 3026, 2931, 2873, 1763, 1460 cm^{-1}; ^1H NMR ($CDCl_3$): 4.93 (1H, dd, *J*=1.5, 1.5 Hz), 4.91 (1H, dd, *J*=1.5, 1.5 Hz), 4.10 (1H, s), 3.11 (1H, dq, *J*=6.9, 7.2 Hz), 2.60 (1H, ddd, *J*=11.3, 6.9, 5.5 Hz), 1.14 (3H, d, *J*=7.2 Hz), 0.94 (3H, d, *J*=6.5 Hz); ^{13}C NMR ($CDCl_3$): 179.2 (C-12), 146.2 (C-4), 114.5 (C-15), 86.2 (C-6), 73.5 (C-5), 41.8 (C-1), 38.8 (C-11), 38.4 (C-7), 32.4 (C-9), 30.7 (C-10), 29.2 (C-3), 25.2 (C-2), 24.3 (C-8), 20.1 (C-14), 9.3 (C-13); HREIMS *m/z* (rel. int.) 250.1565 [M^+, $C_{15}H_{22}O_3$ requires 250.1569] (5), 232 (10), 222 (6), 204 (6), 179 (95), 177 (100). Compound **17**: (R_t 34.9 min, 50 mg, 0.17 mmol; 3%) Oil. $[\alpha]_D$=−74.2 (*c* 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3422 (br), 3013, 2934, 2876, 1763, 1452 cm^{-1}; ^1H NMR ($CDCl_3$): 3.83 (1H, d, *J*=5.8 Hz), 3.02 (1H, dq, *J*=6.8, 7.1 Hz), 2.62 (1H, ddd, *J*=11.4, 6.8, 5.0 Hz), 2.29 (1H, d, *J*=5.8 Hz, OH), 1.63 (3H, s), 1.14 (3H, d, *J*=7.1 Hz), 0.94 (3H, d, *J*=6.3 Hz) - see Table 3 for full assignments; ^{13}C NMR ($CDCl_3$): see Table 2; HREIMS *m/z* (rel. int.) 250.1574 [M^+−HCl, $C_{15}H_{22}O_3$ requires 250.1569] (20), 232 (10), 222 (35), 207 (20), 192 (18), 180 (48), 179 (100), 167 (65).

3.6.1. Derivitization of 15 and 16 as Mosher esters. To a solution of **15** (21.8 mg, 0.087 mmol) in pyridine (0.25 ml) was added *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.05 ml, 0.29 mmol) and the solution was allowed to stand overnight at room temperature. *N*,*N*-diisopropylethylamine (0.03 ml, 0.17 mmol) was added, and after 10 min the solvent was evaporated to yield a crude residue (40 mg) which was purified by HPLC (13% EtOAc/*n*-hexane) to afford the *S*-OMTP ester, compound **18** (R_t 15.6 min, 12.1 mg, 0.026 mmol; 30%): ^1H NMR ($CDCl_3$): 7.60 (2H, m), 7.43 (3H, m), 5.81 (1H, br), 5.33 (1H, s), 3.57 (3H, s), 3.16 (1H, dq, *J*=6.9, 7.1 Hz), 2.31 (1H, m), 1.98 (1H, m), 1.75 (1H, ddd, *J*=12.1, 6.9, 5.5 Hz), 1.66 (3H, s), 1.38 (1H, ddd, *J*=11.0, 11.0, 5.3 Hz), 1.09 (3H, d, *J*=7.1 Hz), 0.88 (3H, d, *J*=6.6 Hz), 0.70 (1H, ddd, *J*=12.1, 12.1, 12.1 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments. The same procedure was applied to compound **15** with *S*-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride to yield a crude residue (42 mg) which was purified by HPLC (13% EtOAc/*n*-hexane) to afford the *R*-OMTP ester, compound **19** (R_t 16.9 min, 13.2 mg, 0.028 mmol; 32%): ^1H NMR ($CDCl_3$): 7.60 (2H, m), 7.44 (3H, m), 5.78 (1H, br), 5.31 (1H, s), 3.53 (3H, s), 3.26 (1H, dq, *J*=6.9, 7.3 Hz), 2.31 (1H, m), 1.87 (1H, ddd, *J*=11.9, 6.9, 5.5 Hz), 1.58 (3H, d, *J*=1.8 Hz), 1.39 (1H, ddd, *J*=11.0, 11.0, 5.2 Hz), 1.09 (3H, d, *J*=7.2 Hz), 0.91 (3H, d, *J*=6.4 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments.

To a solution of **16** (22 mg, 0.087 mmol) in pyridine (0.25 ml) was added *R*-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (0.05 ml, 0.29 mmol) and the solution was allowed to stand overnight at room temperature. *N*,*N*-diisopropylethylamine (0.03 ml, 0.17 mmol) was added, and after 10 min the solvent was evaporated to yield a crude residue (42 mg) which was purified by HPLC (13% EtOAc/*n*-hexane) to yield the *S*-OMTP ester, compound **20** (R_t 15.6 min, 13 mg, 0.028 mmol; 32%): ^1H NMR ($CDCl_3$): 7.49 (2H, m), 7.43 (3H, m), 5.55 (1H, s), 5.15 (1H, s), 5.12 (1H, s), 3.52 (3H, s), 3.17 (1H, dq, *J*=6.6, 7.1 Hz), 1.92 (1H, ddd, *J*=12.1, 6.6, 5.4 Hz), 1.36 (1H, ddd, *J*=10.7, 10.7, 3.2 Hz), 1.13 (3H, d, *J*=7.1 Hz), 0.87 (3H, d, *J*=6.4 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments. The same procedure was applied to compound **16** with *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to yield a crude residue (39 mg) which was purified by HPLC (13% EtOAc/*n*-hexane) to afford the *R*-OMTP ester, compound **21** (R_t 16.9 min, 11 mg, 0.024 mmol; 27%): ^1H NMR ($CDCl_3$): 7.52 (2H, m), 7.44 (3H, m), 5.49 (1H, s), 5.10 (1H, s), 5.08 (1H, s), 3.51 (3H, s), 3.18 (1H, dq, *J*=6.6, 7.1 Hz), 1.13 (3H, d, *J*=7.1 Hz), 0.90 (3H, d, *J*=6.6 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments.

3.7. *cis*-Hydroxylation of dihydro-*epi*-deoxyarteannuin B (3) by OsO_4

To a solution of compound **3** (47 mg, 0.20 mmol) in *t*-BuOH/H_2O (1.5 ml/1.5 ml) was added $K_3Fe(CN)_6$ (0.198 g, 0.60 mmol), K_2CO_3 (0.083 g, 0.60 mmol) and a small portion (0.05 ml) of a solution of OsO_4 in *t*-BuOH, (prepared from 1 g, 3.9 mmol, OsO_4 in 80 ml *t*-BuOH, incorporating several drops of *t*-BuOOH (70%)). The reaction mixture was stirred overnight at room temperature and completion was determined by TLC. Na_2SO_3 (0.05 g, 0.40 mmol) was added and stirring continued for a further 2 h before the solution was concentrated to dryness under reduced pressure and extracted with Et_2O (3×20 ml). The combined organic layers were washed with brine (3×5 ml), dried ($MgSO_4$) and solvent removed under reduced pressure to yield a crude product (45 mg; 96% w/w) which was separated by HPLC (40% EtOAc/*n*-hexane). Compound **22** (R_t 23.0 min, 31 mg, 0.12 mmol; 58%): physical properties as for arteannuin M—see Ref. 2. NMR spectra in $CDCl_3$ solution for natural and synthetic arteannuin M were identical within the resolution of the instrument (±0.01 ppm for ^1H and ±0.1 ppm for ^{13}C) when recorded at the same concentration (7.1 mg/0.6 ml $CDCl_3$). Chemical shifts for some resonances in the vicinity of the oxygen-containing functional groups showed concentration-dependent changes (e.g. C-4, C-5, C-6, C-12 and C-15 ($\Delta\delta_C$ 0.3, 0.2, 0.3, 0.3 and 0.3 ppm, respectively) and H-15, 4-OH and 5-OH ($\Delta\delta_H$ 0.03, 0.73 and 0.38 ppm, respectively)) when more concentrated solutions were studied. Molecular modeling showed that the 5-OH group is involved in an intramolecular hydrogen-bond to the oxygen of the 4-OH group, and these changes in chemical shift are thus consistent with the expected changes in the extent of hydrogen bonding of the 4-OH and 5-OH groups as the concentration is changed. Oil. $[\alpha]_D$=−53.0 (*c* 0.88, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3510, 3421 (br), 3024, 2941, 2876, 1763, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): 3.45 (1H, s), 3.18 (1H, br s, 5-OH), 3.09 (1H, dq, *J*=6.9, 7.1 Hz), 2.65 (1H, ddd, *J*=10.8, 6.9, 5.6 Hz), 1.52 (1H, ddd, *J*=12.5, 11.1, 3.3 Hz), 1.39 (3H, s), 1.34 (1H, dddd, *J*=12.7, 12.7, 12.7, 3.7 Hz), 1.13 (3H, d, *J*=7.1 Hz), 0.92 (3H, d, *J*=6.4 Hz); ^{13}C NMR ($CDCl_3$): 179.2 (C-12), 86.2 (C-6), 74.2 (C-5), 72.7 (C-4), 41.7 (C-1), 39.1 (C-7), 38.8 (C-11), 34.2 (C-3), 32.3 (C-9), 29.9 (C-10), 26.6 (C-15), 23.9 (C-8), 22.1 (C-2), 20.1 (C-14), 9.4 (C-13); HREIMS *m/z* (rel. int.) 268.1676 [M^+, $C_{15}H_{24}O_4$ requires 268.1675] (1), 250 (8), 222 (18), 195 (84), 179 (100). Compound **27** (R_t 18.4 min, 2.2 mg, 0.008 mmol; 4%): Oil. $[\alpha]_D$=−24.5 (*c* 0.22, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3553, 3026, 2936, 2878, 1763, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): 3.60 (1H, dq, *J*=6.9, 7.5 Hz), 3.26 (1H, d, *J*=9.4 Hz), 2.69 (1H, d, *J*=9.4 Hz, 5-OH), 2.65 (1H, br s, 4-OH), 2.55 (1H, ddd, *J*=10.5, 6.9, 4.8 Hz), 1.23 (3H, s), 1.15 (3H, d, *J*=7.5 Hz), 0.98 (3H, d, *J*=6.6 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS *m/z* (rel. int.) 250.1570 [M^+-H_2O, $C_{15}H_{22}O_3$ requires 250.1569] (7), 232 (15), 222 (5), 195 (100), 192 (48), 179 (38), 177 (38), 159 (35). Compound **28** (R_t 11.7 min, 3.0 mg, 0.011 mmol; 6%): Oil. $[\alpha]_D$=−69.0 (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3510, 3026, 2934, 2862, 1778, 1713, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): 3.77 (1H, s, OH), 2.89 (1H, ddd, *J*=11.7, 6.9, 5.4 Hz), 2.80 (1H, dq, *J*=6.9, 7.1 Hz), 2.27 (1H, ddd, *J*=13.0, 3.2, 3.2 Hz), 1.92 (1H, dddd, *J*=14.2, 3.7, 3.4, 3.2 Hz), 1.54 (3H, s), 1.19 (1H, ddd, *J*=11.4, 10.7, 3.4 Hz), 1.12 (3H, d, *J*=7.1 Hz), 0.98 (3H, d, *J*=6.6 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS *m/z* (rel. int.) 266.1522 [M^+, $C_{15}H_{22}O_4$ requires 266.1518] (13), 248 (6), 238 (90), 220 (26), 210 (42), 205 (18), 164 (34), 152 (100). Compound **29** (R_t 16.3 min, 1.4 mg, 0.005 mmol; 3%): Oil. $[\alpha]_D$=−22.8 (*c* 0.14, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3545, 2955, 2930, 2874, 1717, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): 4.34 (1H, s, 6-OH), 3.78 (1H, s), 2.79 (1H, dq, *J*=7.2, 7.5 Hz), 2.53 (1H, br s, 4-OH), 1.37 (3H, d, *J*=7.5 Hz), 1.34 (3H, s), 0.88 (3H, d, *J*=6.6 Hz)—see Table 3 for full assignments; ^{13}C NMR: see Table 2; HREIMS *m/z* (rel. int.) 250.1571 [M^+-H_2O, $C_{15}H_{22}O_3$ requires 250.1569] (21), 232 (30), 195 (60), 192 (100), 179 (32), 177 (37).

3.7.1. Derivitization of 22 as Mosher esters 23–26. To a solution of **22** in pyridine (13.5 mg, 0.050 mmol; 0.25 ml) was added *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.2 ml, 1.15 mmol) and the solution was allowed to stand overnight at room temperature. *N*,*N*-diisopropylethylamine (0.2 ml, 1.15 mmol) was added, and after 10 min the solvent was evaporated to yield a crude residue (25 mg) which was purified by HPLC (20% EtOAc/*n*-hexane) to afford the *S*-OMTP ester of the secondary hydroxyl group, compound **23** (R_t 20.8 min, 0.8 mg, 0.0017 mmol; 3%): Oil. ^1H NMR ($CDCl_3$): 7.67 (2H, m), 7.44 (3H, m), 5.04 (1H, s), 3.59 (3H, s), 3.11 (1H, dq, *J*=6.9, 6.6 Hz), 1.78 (1H, dddd, *J*=13.9, 3.7, 3.7, 3.7 Hz), 1.73 (1H, ddd, *J*=11.1, 6.9, 4.5 Hz), 1.43 (3H, s), 1.08 (3H, d, *J*=6.6 Hz), 0.88 (3H, d, *J*=6.4 Hz), 0.74 (1H, dddd, *J*=12.8, 12.8, 12.8, 2.5 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments. The same procedure was applied to compound **22** with *S*-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride to yield a crude residue (28 mg) which was purified by HPLC (20% EtOAc/*n*-hexane) to afford the *R*-OMTP ester of the secondary hydroxyl group, compound **24** (R_t 20.4 min, 0.5 mg, 0.0011 mmol; 2%): Oil. ^1H NMR ($CDCl_3$): 7.61 (2H, m), 7.47 (3H, m), 5.05 (1H, s), 3.55 (3H, s), 3.20 (1H, dq, *J*=6.8, 7.1 Hz), 1.88 (1H, ddd, *J*=11.7, 6.8, 4.5 Hz), 1.82 (1H, dddd, *J*=13.5, 3.4, 3.4, 3.4 Hz), 1.40 (3H, s), 1.09 (3H, d, *J*=7.1 Hz), 0.90 (3H, d, *J*=6.4 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments. Also isolated were the *S*-OMTP and *R*-OMTP esters of the tertiary hydroxyl group in **22**, compounds **25** and **26**, respectively. Compound **25** (R_t 17.1 min, 0.7 mg, 0.0015 mmol; 3%): Oil. ^1H NMR ($CDCl_3$): 7.51 (2H, m), 7.44 (3H, m), 3.93 (1H, s), 3.52 (3H, s), 3.06 (1H, dq, *J*=6.9, 7.3 Hz), 2.63 (1H, ddd, *J*=10.9, 6.9, 5.6 Hz), 2.43 (1H, m), 2.33 (1H, br s, OH), 1.94 (1H, ddd, *J*=13.3, 13.3, 4.8 Hz), 1.74 (3H, s), 1.12 (3H, d, *J*=7.3 Hz), 0.92 (3H, d, *J*=6.4 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments. Compound **26** (R_t 17.2 min, 0.6 mg, 0.0013 mmol; 3%): Oil. ^1H NMR (δ $CDCl_3$) ppm: 7.52 (2H, m), 7.43 (3H, m), 3.94 (1H, s), 3.54 (3H, s), 3.06 (1H, dq, *J*=6.9, 7.1 Hz), 2.61 (1H, ddd, *J*=10.7, 6.9, 5.1 Hz), 2.47 (1H, d, *J*=12.4 Hz), 2.34 (1H, br s, OH),1.91 (1H, ddd, *J*=12.9, 12.9, 3.7 Hz), 1.75 (3H, s), 1.12 (3H, d, *J*=7.1 Hz), 0.92 (3H, d, *J*=6.2 Hz)—see Table 4 for full ^1H and ^{13}C NMR assignments.

3.8. Synthesis of labelled compounds 1a, 15a, 16a and 17a

^{13}C-Labelled methyl iodide ($^{13}CH_3I$) (Aldrich 27,718-5, 99

atom %) was used in place of isotopically normal CH_3I in the Grignard reaction with **6** which forms **3a**. Subsequent epoxidation of **3a** to **14a** and acid hydrolysis of **14a** yielding **1a**, **15a**, **16a** and **17a** was performed as described for the isotopically-normal compounds in the preceding sections. Compound **3a**: physical properties as for **3**, with the following differences: ^{1}H NMR ($CDCl_3$): 5.64 (1H, dd, J=5.6 ($^3J_{CH}$), 1.4 Hz), 1.69 (3H, d, J=126 Hz ($^1J_{CH}$)); ^{13}C NMR ($CDCl_3$): 142.2 (d, J=43 Hz ($^1J_{CC}$)), 121.7 (d, J=2 Hz ($^2J_{CC}$)), 83.3 (d, J=4 Hz ($^3J_{CC}$)), 30.8 (d, J=3 Hz ($^2J_{CC}$)), 23.4 (ca. 90 times normal intensity); HREIMS *m/z* (rel. int.) 235.1643 [M^+, $C_{14}{}^{13}C_1H_{22}O_2$ requires 235.1653] (10), 191 (95), 162 (100). Compound **14a**: physical properties as for **14**, with the following differences: ^{1}H NMR ($CDCl_3$): 1.32 (3H, d, J=127 Hz ($^1J_{CH}$)); ^{13}C NMR ($CDCl_3$): 63.1 (d, J=45 Hz ($^1J_{CC}$)), 27.3 (d, J=4 Hz ($^2J_{CC}$)), 24.4 (ca. 95 times normal intensity); HREIMS *m/z* (rel. int.) 251.1602 [M^+, $C_{14}{}^{13}C_1H_{22}O_3$ requires 251.1602] (1), 223 (10), 179 (100), 167 (35). Compound **1a**: physical properties as for **1**, with the following differences: ^{1}H NMR ($CDCl_3$): 3.53 (1H, d, J=4 Hz ($^3J_{CH}$)), 3.37 (1H, d, J=6.8 Hz, 4-OH ($^3J_{CH}$)), 1.22 (3H, d, J=126 Hz ($^1J_{CH}$)); ^{13}C NMR ($CDCl_3$): 72.2 (d, J=42 Hz ($^1J_{CC}$)), 26.7 (ca. 90 times normal intensity); 20.2 (d, J=3 Hz ($^2J_{CC}$)). Compound **15a**: physical properties as for **15**, with the following differences: ^{1}H NMR ($CDCl_3$): 5.67 (1H, dq, J=5.7 ($^3J_{CH}$), 1.7 Hz), 1.78 (3H, dd, J=126 ($^1J_{CH}$), 1.7 Hz); ^{13}C NMR ($CDCl_3$): 131.2 (d, J=45 Hz ($^1J_{CC}$)), 126.4 (d, J=2 Hz ($^2J_{CC}$)), 70.1 (d, J=3 Hz ($^2J_{CC}$)), 27.2 (d, J=4 Hz ($^3J_{CC}$)), 21.2 (ca. 75 times normal intensity); HREIMS *m/z* (rel. int.) 251.1606 [M^+, $C_{14}{}^{13}C_1H_{22}O_3$ requires 251.1602] (2), 167 (100). Compound **16a**: physical properties as for **16**, with the following differences: ^{1}H NMR ($CDCl_3$): 4.93 (1H, dd, J=157 ($^1J_{CH}$), 1.8 Hz), 4.90 (1H, dd, J=156 ($^1J_{CH}$), 1.5 Hz); ^{13}C NMR ($CDCl_3$): 114.6 (ca. 90 times normal intensity). Compound **17a**: physical properties as for **17**, with the following differences: ^{1}H NMR ($CDCl_3$): 1.62 (3H, d, J=128 Hz ($^1J_{CH}$)); ^{13}C NMR ($CDCl_3$): 31.4 (ca. 95 times normal intensity).

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