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Phytochemistry 64 (2003) 303–323
PHYTOCHEMISTRY
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Terpenoids from the seeds of *Artemisia annua*

Geoffrey D. Brown[a,b,*], Guang-Yi Liang[c], Lai-King Sy[b]

[a]*School of Chemistry, The University of Reading, Whiteknights Road, Reading, RG6 6AD, UK*
[b]*Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, Area of Excellence Scheme of University Grant Committee (Hong Kong), The University of Hong Kong, Pokfulam Road, Hong Kong*
[c]*The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, 202 Sha-Chong South Road, Guiyang 550002, PR China*

Received 19 February 2003; received in revised form 1 April 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Fourteen sesquiterpenes, three monoterpenes and one diterpene natural product have been isolated from the seeds of *Artemisia annua*. The possible biogenesis of some of these natural products are discussed by reference to recently reported experimental results for the autoxidation of dihydroartemisinic acid and other terpenoids from *Artemisia annua*.

Keywords: *Artemisia annua* L; Compositae; Amorphane sesquiterpene; Caidinane sesquiterpene; Germacrane sesquiterpene; Eudesmane sesquiterpene; Cedrane sesquiterpene; Lavandulane monoterpene; 1,4-Dihydrofuran monoterpene; Phytane diterpene; Autoxidation; Biogenesis; 2D NMR

1. Introduction

Artemisia annua L. (sweet wormwood; Compositae), the source of the potent anti-malarial drug artemisinin (Tu et al., 1982), has been the subject of extensive phytochemical investigations over the past two decades. Some 38 amorphane and cadinane sesquiterpenes are now known from this species (Sy and Brown, 1998; Sy et al., 1998, 2001a), including arteannuic acid (artemisinic acid, qinghao acid) (**1**) (Tu et al., 1982; Misra et al., 1993a; Zheng, 1994), arteannuin B (**2**) (Jeremic et al., 1973; Tu et al., 1982; Zheng, 1994) and artemisinin (**6**) (Tu et al., 1982; Zheng, 1994), which were amongst the first natural products to be reported (Fig. 1) and are generally found to be the most abundant sesquiterpenes in this species. Many of the amorphane and cadinane sesquiterpenes from *A. annua* occur as both their 11,13-dihydro and 11,13-dehydro forms. Thus, the 11,13-dihydro analogues of compounds **1** and **2**, dihydroartemisinic acid (**4**) (Sy et al., 1998) and dihyroarteannuin B (**5**) (Sy et al., 1998) are also present in *A. annua* in significant quantities, while artemisitene (**3**), the 11,13-dehydro analogue of artemisinin (**6**), has been reported on only one occasion and in low abundance (Acton and Klayman, 1985). *A. annua* is also a rich source of numerous other natural products, including monoterpenes (Rucker et al., 1987; Sy and Brown, 2001a), diterpenes (Brown, 1994a; Wong and Brown, 2002a) and flavonoids (Jeremic et al., 1979; Yang et al., 1989, 1995; Sy and Brown, 1998).

All investigations of the phytochemistry of *A. annua* reported to date have employed the aerial parts (leaves, stems and perhaps flowers) as the biological source material. We now report the first study of natural products found in the seeds of *A. annua*,[1] and have found that the seeds of this species are a concentrated source of almost all the secondary metabolites which have been detected in previous studies, as well as of several novel terpenoids.

2. Results and discussion

Extraction of the seeds of *A. annua* by CH_2Cl_2, followed by extensive chromatographic separation by CC

* Corresponding author. Tel.: +44-118-378-7418; fax: +44-118-931-6331.
E-mail address: g.d.brown@reading.ac.uk (G.D. Brown).

[1] Our preliminary investigation of the roots, which have also not been studied previously, indicated a general lack of terpenoids.

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doi:10.1016/S0031-9422(03)00294-2

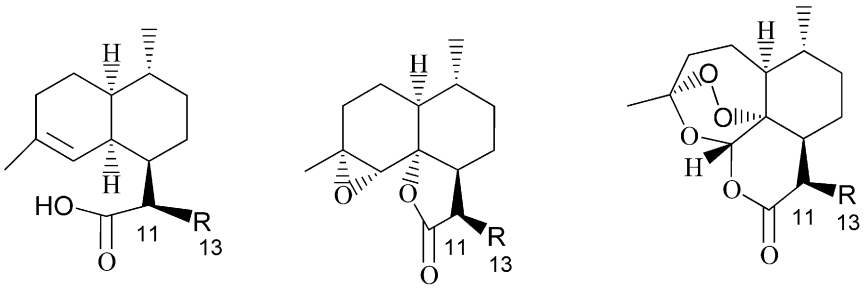


Fig. 1. Common amorphane and cadinane sesquiterpenes reported in the literature from *Artemisia annua*, which are discussed in Section 1.

and HPLC has resulted in the isolation of eighteen new natural products (**7**, **8**, **10**, **15**, **17**, **19**–**22**, **25**–**28**, **32** and **38**–**41**) as shown in Fig. 2. The structures of all of these compounds were elucidated principally from the results of 2D NMR spectroscopic studies (HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY).

HREIMS of compound **7** indicated the molecular formula $C_{15}H_{24}O$, while IR spectroscopy suggested that the oxygen atom in this metabolite was present as a hydroxyl group (a broad absorbance was observed at $\nu = 3435$ cm^{-1}). An inspection of the 1-dimensional (1D-) ^{13}C NMR/DEPT and ^{1}H NMR spectra for **7** revealed resonances which confirmed the presence of this hydroxyl group (δ_C 78.9 ppm (CH) and δ_H 3.45 (*dd*, $J = 11.7$, 4.1 Hz) ppm) and also indicated the presence of a terminal alkene [δ_C 147.9 (C), 109.5 (CH_2) ppm; δ_H 4.81 (*s*), 4.63 (*t*, $J = 2.5$ Hz) ppm] and a tri-substituted alkene [δ_C 144.3 (C), 126.6 (CH) ppm; δ_H 5.57 (s) ppm] in the structure of **7**. 2D NMR spectral analysis (HSQC, HMBC and ^{1}H–^{1}H COSY) proved that these two double bonds were in conjugation with one another and revealed that compound **7** had the skeleton of a eudesmane sesquiterpene (HMBC and ^{1}H–^{1}H COSY correlations used in determining the carbon skeleton of **7** are shown in Fig. 3; full ^{13}C and ^{1}H NMR assignments are given in Tables 1 and 2). The *cis* relationship between the 7-isopropyl and 10-methyl groups in compound **7**, which is the relative stereochemistry normally found for these groups in the eudesmane series of natural products, was confirmed by correlations seen in NOESY spectroscopy. The equatorial orientation of the 1-OH group was deduced both from the results of NOESY and by the coupling constants observed for H–1α ($J_{1\alpha-2\beta} = 11.7$ Hz and $J_{1\alpha-2\alpha} = 4.1$ Hz, representing an axial-axial 3-bond coupling and an axial-equatorial 3-bond coupling, respectively).

Compound **8** was shown to have the same molecular formula as compound **7** by HREIMS and clearly possessed many similar structural features, as shown by its IR and 1D ^{1}H NMR and ^{13}C NMR/DEPT spectra. These included the presence of a hydroxyl group ($\nu = 3440$ cm^{-1}; δ_C 79.5 (CH) ppm; δ_H 3.48 [*dd*, $J = 11.4$, 4.8 Hz) ppm], a terminal alkene double bond [δ_C 148.4 (C), 107.6 (CH_2) ppm; δ_H 4.83 (*s*), 4.65 (*s*) ppm], a tri-substituted double bond [δ_C 141.6 (C), 115.8 (CH) ppm; δ_H 5.33 (*d*, $J = 5.5$ Hz) ppm], an isopropyl group [δ_H 1.02 ([3H] *d*, $J = 6.9$ Hz), δ_H 1.01, ([3H] *d*, $J = 6.6$ Hz) ppm] and a methyl substituent [δ_H 0.66 ([3H] s) ppm] at the 10-position of the eudesmane skeleton. Analysis by 2D NMR spectroscopy (Fig. 3 and Tables 1 and 2) showed conclusively compound **8** to be a regio-isomer of **7**, in which the C-5 double bond has shifted to the C-7 position. Correlations seen by NOESY spectroscopy indicated that compound **8** contains a *trans*-decalin ring system, as is normal for eudesmane sesquiterpenes. The absolute stereochemistry of eudesmanes **7** and **8** was assumed to be as shown in Fig. 2, based on the isolation of the known compound **9** which gave similar NMR spectra[2] and values for its optical rotation to that of 1β,6α-dihydroxy-4(15)-eudesmane, which has been reported as a natural product on several occasions from a variety of species (Guttierez and Herz, 1988; Gonzalez et al., 1989; Nakajima et al., 1994; Ahmed et al., 1995; Hu et al., 1996). A third novel eudesmane, compound **10** (Fig. 2), was identified as the hydroperoxy analogue of the allylic tertiary hydroxide **11**, which has previously been isolated from *A. annua* (Sy and Brown, 1998). The presence of the hydroperoxy group in compound **10** was confirmed by its reduction in the presence of triphenylphosphine, which yielded a compound with identical NMR spectra to those of the natural product **11**. Prior to the present study, compounds **11** and **12** were the only eudesmanes to have been reported from *A. annua*.

Compound **13** is the first germacrane sesquiterpene to have been obtained from *A. annua*. This germacrane allylic hydroxide has been reported as a natural product from various other species on several occasions (Fattorusso et al., 1978; Bohlman et al., 1982; Nagashima et al., 1990) and its isolation from the seeds of *A. annua*

[2] Complete ^{1}H and ^{13}C NMR assignments were made by 2D NMR analysis for compound **9** from *A. annua* and are also included in Tables 1 and 2 to allow for a direct comparison with the NMR assignments of compounds **7** and **8**.

may have some relevance to the biogenesis of all of the eudesmanes **7**–**12**, as is discussed below. After making complete NMR spectroscopic assignments for the germacrane **13** by 2D NMR (presented for the first time in Tables 1 and 2) it was possible to make a detailed interpretation of correlations appearing in the NOESY spectrum of this compound, which in turn have demonstrated that **13** exists in solution predominantly as the conformer shown in Fig. 4 (cf. other recent studies of the conformation of the 10-membered germacrane ring; Barrero et al., 1999; Wong and Brown, 2002b). The cationic cyclization mechanism for germacrane **13** which is shown in Fig. 4, involving protonation of the terminal 10(14)-double bond and addition of the Δ^5

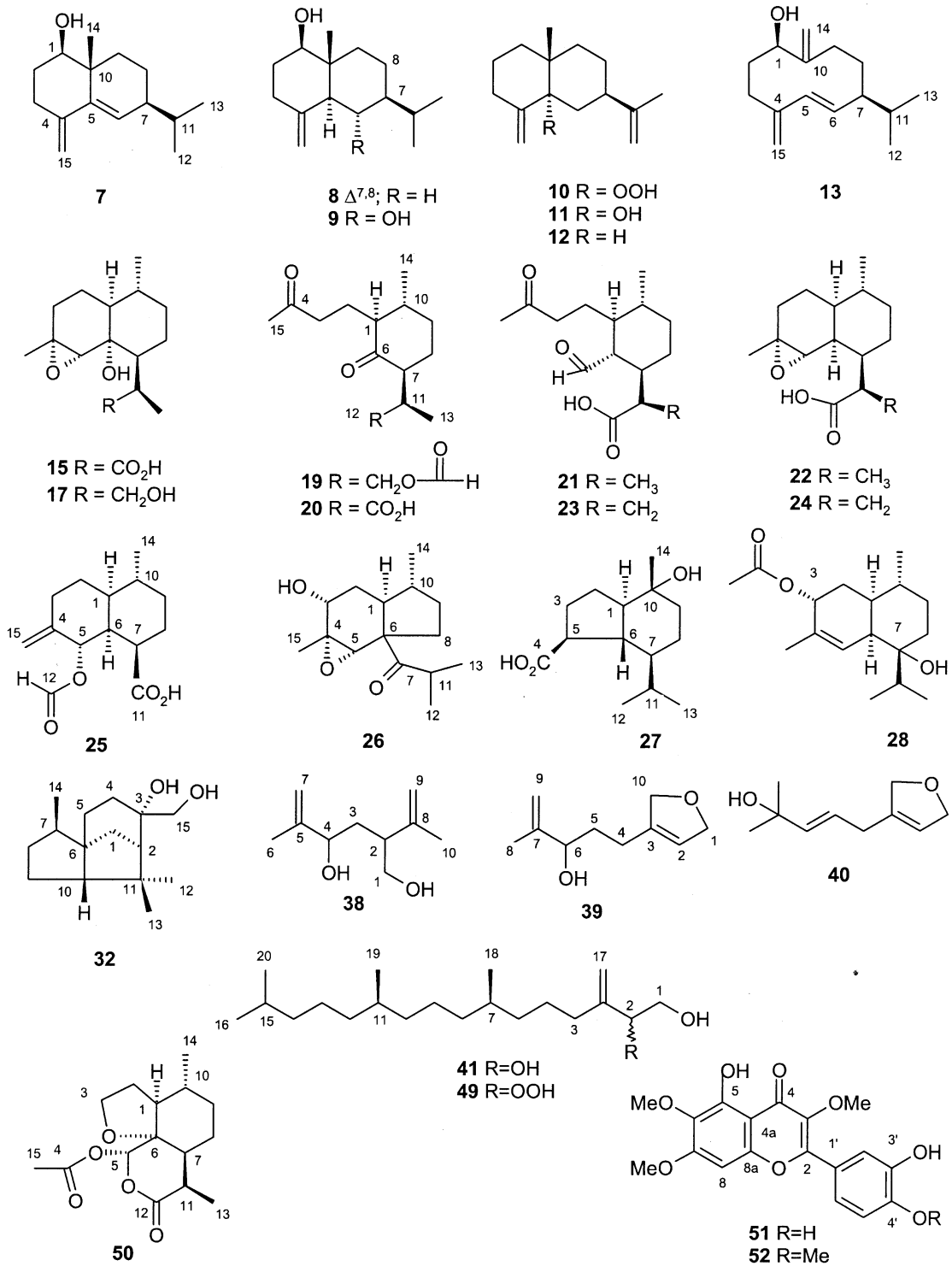


Fig. 2. Natural products isolated from the seeds of *A. annua* which are discussed in Section 2.

double bond, which results in cyclization to the eudesmane cation **14**, has been proposed previously as a general route for the biogenesis of eudesmane sesquiterpenes from germacranes (Bulow and Konig, 2000). As shown in Fig. 4, compounds **7**, **8** and **9** could then arise from the cationic intermediate **14**, respectively, by either proton abstraction at H–5; or by a 1,2-shift of the H-7 proton to the 6-position and subsequent proton abstraction at H-8; or, directly, by quenching of the C-6 carbocation with water. If such a biogenetic mechanism were indeed operative in *A. annua*, then the experimentally determined solution conformation for **13** would naturally lead to the structures proposed for the novel natural products **7** and **8**, thereby providing some additional supporting evidence for the absolute stereochemical assignments which have been suggested for these novel compounds.

As mentioned in Section 1, there are some thirty-eight amorphane and cadinane sesquiterpenes which are currently known from *A. annua*, and it would seem that all of the new sesquiterpenes **15**, **17**, **19–22** and **25–28** (Fig. 2), which have been isolated from the seeds of *A. annua* in this study, should also be assigned to these classes. The structures of the amorphane and cadinane natural products **15**, **17** and **19–24** were all suggestive of their derivation from known sesquiterpenes, such as dihydroartemisinic acid (**4**) and arteannuic acid (**1**) (Fig. 1), which were present in the seeds of *A. annua* in

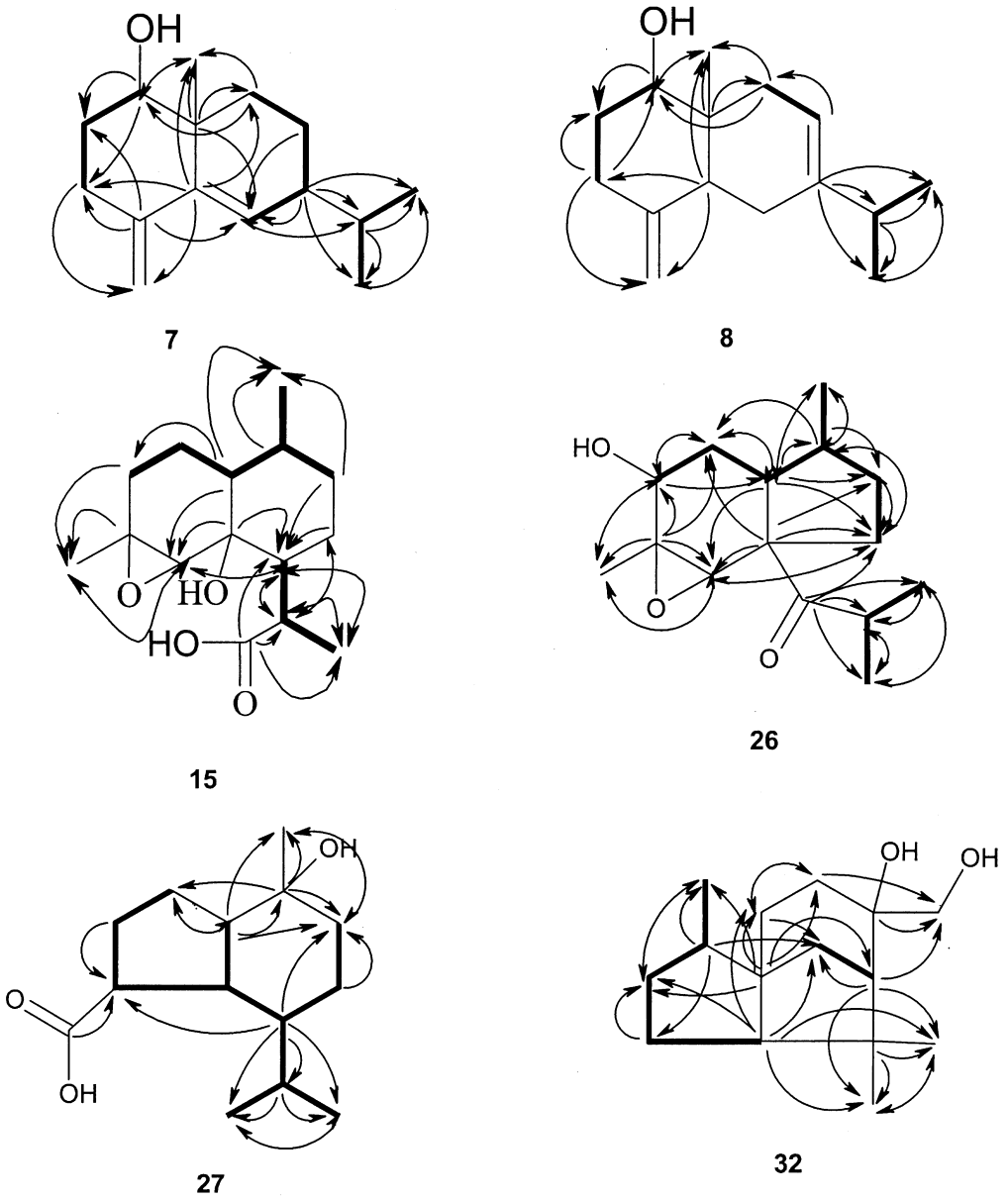


Fig. 3. Two- and three-bond ^{13}C–^{1}H correlations (indicated by arrows from ^{13}C to ^{1}H) and ^{1}H–^{1}H correlations (indicated by bold lines on the structure) which were used in determining the planar structures of compounds **7**, **8**, **15**, **26**, **27** and **32**.

Table 1
^{13}C NMR assignments ($CDCl_3$) determined by 2D NMR for eudesmane, germacrane and amorphane sesquiterpene natural products (and their derivatives) **7**–**10**, **13**, **15**–**18**, **20**, **24**–**28** and **50**, which were isolated from the seeds of *A. annua*

Position (mult.)[a]	**7**	**8**	**9**	**10**	**13**	**15**	**16**	**17**	**18**	**20**	**24**	**25**	**26**	**27**	**28**	**50**
1 (CH)	78.9	79.5	79.1	34.6 (CH)	76.0	48.6	48.6	49.0	48.2	56.6	39.9	45.2	45.1	56.8	39.2	54.8
2 (CH_2)	30.2	31.4	31.9	22.5	36.2	15.2	15.2	15.1	15.0	20.1	22.0	28.8	27.7	25.3	32.0	27.7
3 (CH_2)	32.3	34.3	35.1	32.1	29.9	24.5	24.5	24.5	24.6	41.1	24.8	29.6	67.9 (CH)	29.4	71.0	69.3
4 (C)	147.9	148.4	146.1	148.3	146.8	61.0	60.8	60.3	60.8	209.1	57.7	145.5	60.6	47.3 (CH)	133.9	168.7
5 (C)	144.3	43.0 (CH)	55.9 (CH)	87.3	129.7 (CH)	61.0 (CH)	61.0 (CH)	61.6 (CH)	60.4 (CH)	–	59.3 (CH)	70.7 (CH)	67.3 (CH)	48.7	125.9 (CH)	93.0 (CH)
6 (CH)	126.6	25.4 (CH_2)	67.0	29.1 (CH_2)	138.0	70.7 (C)	70.5 (C)	70.8 (C)	69.3 (C)	213.0 (C)	38.8	45.3	56.9 (C)	179.5	44.2	79.9 (C)
7 (CH)	42.2	141.6 (C)	49.3	39.6	52.5	50.1	50.5	48.4	46.6	53.4	41.3	45.4	214.8 (C)	49.3	74.6 (C)	46.7
8 (CH_2)	20.9	115.8 (CH)	18.1	26.2	36.1	26.2	26.0	26.8	27.2	30.4	27.2	21.9	33.7	23.0	32.0	24.3
9 (CH_2)	35.1	38.3	36.3	34.5	34.5	34.5	34.6	35.0	34.3	34.4	34.9	34.6	33.6	42.0	30.0	34.6
10 (C)	40.4	38.9	41.7	38.8	153.5	30.7 (CH)	30.7 (CH)	30.8 (CH)	30.9 (CH)	40.3 (CH)	29.5 (CH)	28.0 (CH)	37.3 (CH)	73.1	28.4 (CH)	30.9
11 (CH)	32.1	35.0	26.0	150.4 (C)	31.8	39.6	39.3	37.5	143.4 (C)	38.9	141.9 (C)	178.9	36.9	28.8	33.0	35.0
12 (CH_3)	19.5[b]	21.7[b]	21.1[b]	108.5 (CH_2)	20.7[b]	182.0 (C)	176.9 (C)	67.2 (CH_2)	169.4 (C)	179.2 (C)	170.4 (C)	160.2	20.6	15.5[b]	16.1[b]	172.0 (C)
13 (CH_3)	19.0[b]	21.2[b]	16.2[b]	21.1	20.5[b]	18.9	19.0	17.5	122.2 (CH_2)	14.7	126.7 (CH_2)	–	19.6	21.9[b]	15.6[b]	12.5
14 (CH_3)	17.3	10.3	11.6	21.2	110.6 (CH_2)	19.0	19.0	19.1	19.0	20.5	19.1	19.8	17.5	20.3	19.5	20.4
15 (CH_2)	109.5	107.6	107.8	111.8	112.9	23.5 (CH_3)	23.6 (CH_3)	23.6 (CH_3)	23.4 (CH_3)	29.9 (CH_3)	23.5 (CH_3)	105.8	19.3	–	19.7	21.3 (CH_3)
12-OMe	–	–	–	–	–	–	51.3	–	–	–	–	–	–	–	–	–

[a] Multiplicity determined from DEPT.
[b] Interchangeable within column

Table 2
^{1}H NMR assignments ($CDCl_3$) determined by 2D NMR for eudesmane, germacrane and amorphane sesquiterpene natural products (and their derivatives) **7**–**10**, **13**, **15**–**18**, **20**, **24**–**28** and **50**, which were isolated from the seeds of *A. annua*

Position	7	8	9	10	13	15	16	17	18	20	24	25	26	27	28	50
1α	3.45	3.48	3.43	1.03	3.77	1.01	0.99	0.97	1.18	2.10	1.25	1.58	2.08	1.50	1.87	1.47
1β	–	–	–	1.81	–											
2 α	1.82	1.80	1.86	1.66	1.70	1.60	1.61	1.59	1.40	1.85	1.34	1.46	1.45	1.85	2.41	1.58
2β	1.67	1.56	1.55	1.66	2.04	1.41	1.41	1.41	1.40	1.78	1.66	2.00	1.81	1.52	1.48	2.05
3 α	2.17	2.14	2.07	2.48	2.18	1.85	1.84	1.83	1.82	2.53	1.82	2.25	–	2.02	–	3.94
3β	2.35	2.36	2.33	2.20	2.43	1.65	1.67	1.67	1.68	2.36	1.66	2.22	3.91	1.87	5.36	4.21
5	–	1.97	1.75	–	6.00	3.24	3.14	3.17	2.93	–	2.54	5.74	3.15	2.48	5.42	6.64
6 α	5.57	1.98	–	2.11	5.43	–	–	–	–	–	2.17	2.55	–	1.74	2.45	–
6β		1.98	3.72	1.51												
7	2.01	–	1.28	2.46	1.78	1.76	1.74	1.72	3.16	2.61	2.75	2.46	–	1.19	–	1.88
8 α	1.60	5.33	1.53	1.62	2.04	1.79	1.72	1.74	1.70	2.09	1.65	1.75	2.05	1.63	1.69	1.65
8β	1.33		1.21	1.62	1.63	1.26	1.15	1.30	1.42	1.57	1.34	1.04	1.59	1.10	1.13	1.71
9 α	1.34	1.97	1.17	1.18	1.65	1.07	1.06	1.09	1.25	1.54	1.10	1.06	1.10	1.39	1.31	1.08
9β	1.89	2.17	1.92	1.78	2.63	1.68	1.67	1.67	1.75	1.89	1.78	1.90	1.77	1.81	1.44	2.00
10	–	–	–	–	–	1.22	1.21	1.21	1.29	1.61	1.29	1.85	1.58	–	1.41	1.72
11	1.63	2.21	2.24	–	1.49	3.04	3.08	2.10	–	2.81	–	–	3.09	1.81	1.86	3.16
12	0.91 [a]	1.01 [a]	0.95 [a]	4.77[f] 4.74[g]	0.89 [a]	–	–	3.74 3.56	–	–	–	8.00	1.14[a]	0.73[a]	0.93[a]	–
13	0.89 [a]	1.02 [a]	0.87 [a]	1.78	0.81 [a]	1.33	1.30	1.11	5.38[h] 6.08[i]	1.20	5.64[h] 6.48[I]	–	1.15[a]	0.92[a]	0.90[a]	1.21
14	0.91	0.66	0.71	0.94	5.27[e] 5.00[d]	0.87	0.87	0.88	0.90	1.08	0.88	0.91	0.98	1.20	0.97	0.99
15	4.63[b] 4.81[c]	4.83[b] 4.65[c]	5.02[b] 4.74[c]	5.07[b] 4.78[c]	4.92[b] 4.84[c]	1.36	1.36	1.36	1.34	2.12	1.29	4.78 4.62	1.48	–	1.64	2.16
12–OMe	–	–	–	–	–	–	3.66	–	–	–	–	–	–	–	–	–

[a] Interchangeable within column.
[b] Methylene proton *anti* to the 11-isopropyl group.
[c] Methylene proton *syn* with the 11-isopropyl group.
[d] Methylene proton *anti* to the 1-OH group.
[e] Methylene proton *syn* with the 1-OH group.
[f] Methylene proton *anti* to the 13-methyl group.
[g] Methylene proton *syn* with the 13-methyl group.
[h] Proton *anti* to the 12-carbonyl group.
[i] Proton *syn* with the 12-carbonyl group.

substantial amounts, by the kind of spontaneous autoxidation reactions which have been the subject of extensive recent investigations in our laboratories (Ngo and Brown, 1999a, b, 2000; Sy and Brown 1999a, 2002a, b; Sy et al., 1999). Accordingly, the biogenesis of all of compounds **15**, **17** and **19**–**24** might be envisaged as proceeding via tertiary allylic hydroperoxide intermediates, such as compound **29**, as is shown in Fig. 5 (compound **29** is the tertiary allylic hydroperoxide formed by spontaneous autoxidation of dihydroartemisinic acid, which has also recently been reported as a natural product from *A. annua* (Wallaart et al., 1999a, b).

The structure of compound **15** was determined in the following way. HREIMS indicated the molecular formula $C_{15}H_{24}O_4$. Inspection of the ^{13}C NMR/DEPT spectra of **15** demonstrated 15 carbons with 22 directly attached hydrogen atoms and suggested that four of these carbons [δ_C 61.0 (C), 61.0 (CH), 70.7 (C) and 182.0 (C) ppm] bear oxygen substituents. Two-dimensional NMR spectroscopic analysis of compound **15** indicated the planar structure which is shown in Fig. 3, in which one of the oxygen atoms has been assigned as an epoxide group at C-4 and C-5 by a comparison of the chemical shift values for these carbons (which are overlapped with one another at δ_C 61.0 ppm) with the fully assigned chemical shifts which have been reported previously for dihydroarteannuin B (**5**) (δ_C 58.1 (C-4) and 59.5 (C-5) ppm—see Sy et al., 1998).[3] The nature of the remaining three oxygen atoms in the molecular formula of **15** were revealed by secondary isotope effects observed in the ^{13}C NMR spectrum following a D_2O shake (Christofides and Davies, 1983) which indicated the presence of two exchangeable hydrogen atoms associated with heteroatom substitutents at C-6 (δ_C 70.7 ppm; upfield chemical shift of $\Delta\delta_C$ −0.11 ppm, following the D_2O shake) and C-12 (δ_C 182.0 ppm, $\Delta\delta_C$ −0.55

[3] The chemical shift values at C-4 and C-5 in both compounds **5** and **15** were unusually upfield for carbons which are substituted by oxygen, and this is to be expected as the result of the strain associated with the epoxide ring.

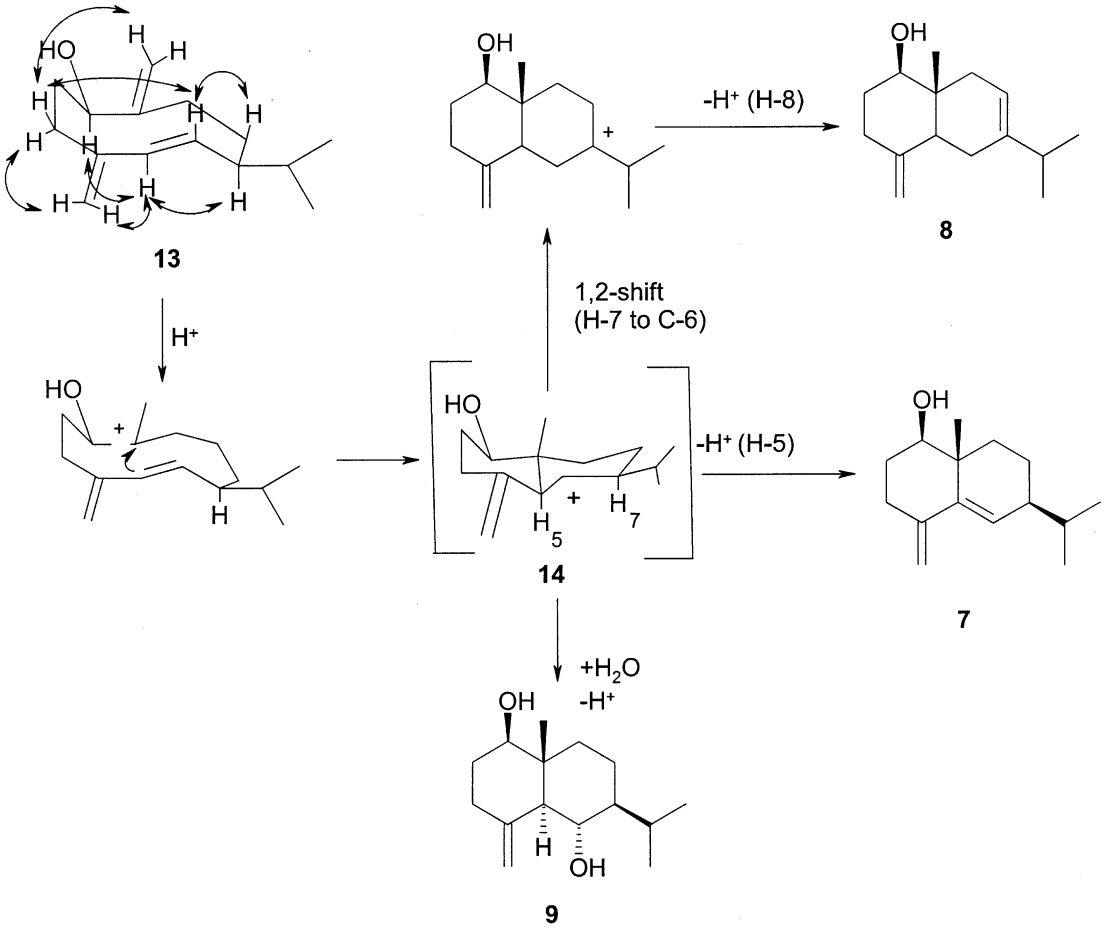


Fig. 4. A proposed biogenesis of eudesmanes **7**–**9** from germacrene **13**, which would be consistent with the observed solution conformation of **13**, as determined by NOESY spectroscopy (critical NOESY correlations for **13** are indicated on the structure by double-headed arrows from ^{1}H to ^{1}H).

ppm). Inspection of the IR spectrum of **15** confirmed that these two exchangeable protons were associated, respectively, with OH ($\nu = 3537$ cm^{-1}) and CO_2H ($\nu = 3400$–2600 and 1703 cm^{-1}) functional groups and the presence of a free carboxylic acid functional group in **15** was further supported by treatment of this compound with diazomethane, which resulted in a clean conversion into compound **16**, the 12-methyl ester of the natural product (Fig. 6, Tables 1 and 2).

Although most of the ^{13}C and ^{1}H chemical shifts assigned to compound **15** by 2D NMR spectral analysis (Tables 1 and 2) were very similar to those reported for dihydroarteannuin B (**5**) (Sy et al., 1998), the ^{13}C resonances determined for the C-6, C-12 and C-13 positions all showed some significant differences (δ_C 70.7, 182.0 and 18.9 ppm, respectively, for **15**, as compared with δ_C 83.0, 179.6 and 12.6 ppm, for **5**). These differences were consistent with ring-opening of the lactone group in dihydroarteannuin B to a tertiary hydroxyl group at C-6 and a carboxylic acid group at C-12 in compound **15** and were also in agreement with the upfield shifts which were observed for the ^{13}C for resonances at these two positions following a D_2O shake, as has been discussed above. The decalin ring in **15** was shown to be *cis*-fused, as is the case for dihydroarteannuin B (**5**), by correlations which were observed in its NOESY spectrum. Based on these results and the autoxidation scheme shown in Fig. 5, compound **15** can therefore be regarded as the lactone-ring opened analogue of dihydroarteannuin B (**5**). Although **15** is a new natural product, it has recently been proposed as an intermediate in the formation of dihydroarteannuin B (**5**) from experiments performed in vitro with dihydroartemisinic acid (**4**) in which compound **4** had first been converted into the tertiary allylic hydroperoxide of dihydroartemisinic acid (**29**) (Sy and Brown, 2002a). The 11-epimer of compound **15** has been reported previously from treatment of the 11-epimer of dihydroarteannuin B with potassium hydroxide in methanol (Ge et al., 1983).

The fully assigned NMR spectral data for the new natural product **17** from the seeds of *A. annua* was clearly very similar to that of both compounds **15** and **16** (Tables 1 and 2) and was fully consistent with the structure proposed for this compound, which is the 12-deoxy analogue of **15**. Stefanovic et al. (1977) have reported previously that compound **17** was obtained as

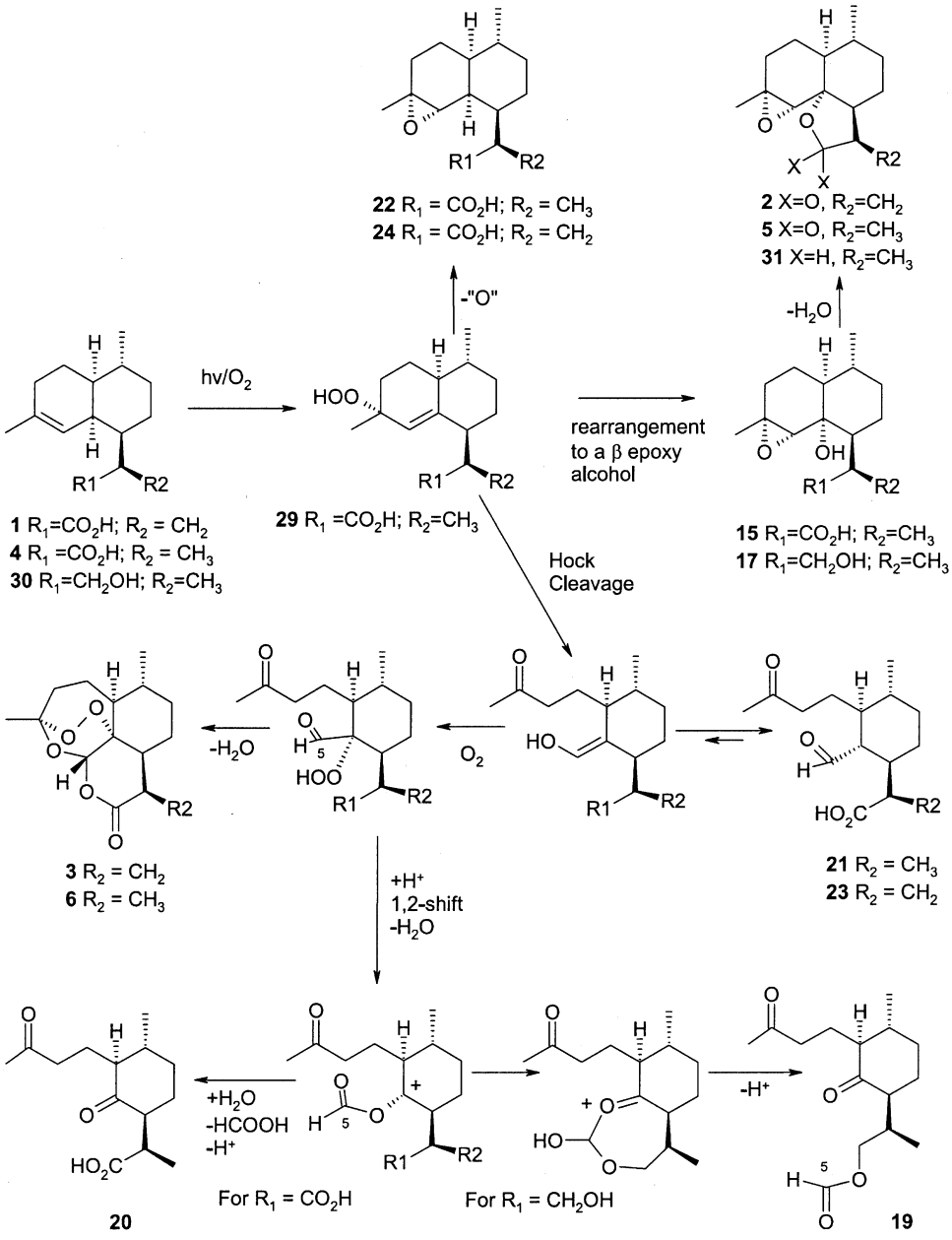


Fig. 5. Proposed biogenesis of the novel amorphane and cadinane sesquiterpene natural products **15**, **17** and **19**–**24** by autoxidation reactions of either artemisinic acid (**1**), dihydroartemisinic acid (**4**) or the 12-deoxy analogue of dihydroartemisinic acid (**30**); the suggested pathways are based on recent in vitro studies with dihydroartemisinic acid (**4**) and its analogues which indicated that a tertary allylic hydroperoxide (such as compound **29**) is likely to be a common mechanistic intermediate (Sy et al., 1999; Sy and Brown, 2002a,b).

the sole product from the complete reduction of the α-methylene-γ-butyrolactone group in aretannuin B (**2**) by sodium borohydride. However Ge et al. (1983) have claimed that the same reagent only reduced the terminal double bond in **2**, resulting in the 11-epimer of dihydroarteannuin B; these workers then subsequently employed the more powerful reducing agent, lithium aluminium hydride, to reductively open the lactone ring, thereby obtaining the 11-epimer of compound **17**. As mentioned in Section 1, arteannuin B (**2**) is one of the most abundant natural products in *A. annua* and, as expected, substantial amounts of this compound were also isolated from the seeds of this species in the present study. In our hands, sodium borohydride did not appear to effect reduction of the $\Delta^{11,13}$-double bond in arteannuin B (**2**) at all:[4] rather the lactone ring-opened

[4] The difficulties which we encountered in attempting to reduce the α-methylene group in **2** may be associated with steric hindrance in this molecule. Thus, the terminal double bond in the closely related natural product, *epi*-deoxyarteannuin B (see Sy et al., 2001a), was readily reduced under the same conditions, yielding its 11,13-dihydro derivative, dihyro-*epi*-deoxyarteannuin B (see Brown, 1992).

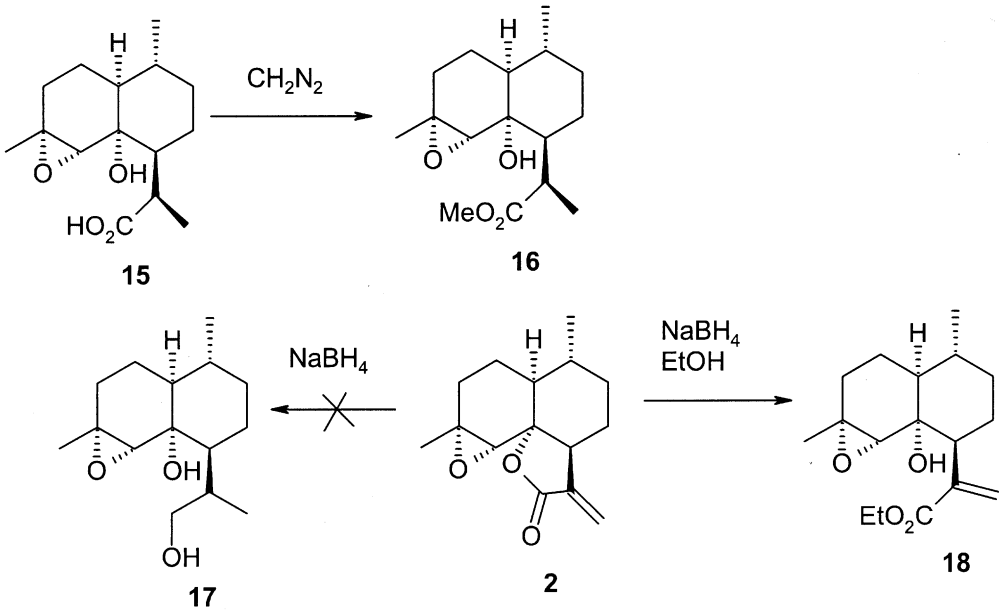


Fig. 6. Derivatization reactions for compounds **2** and **15** which were used in confirming the structures of the novel natural products **15** and **17** from the seeds of *A. annua*.

product **18** was the only component we were able to isolate from this reaction (Fig. 6). The formation of compound **18** must involve nucleophilic attack by sodium ethoxide (formed in situ from $NaBH_4$ and EtOH) at C-12, and although not the expected product, the complete 2D NMR spectral assignments for the ring-opened compound **18**, which are reported in Tables 1 and 2, were of some value, as they compared favorably with those for the natural products **15** and **17**. The new natural product **17** is the 12-deoxy analogue of **15**, and it is worth noting that this compound has been previously suggested to be an intermediate in the in vitro autoxidation of compound **30**, the 12-deoxy analogue of dihydroartemisinic acid, to the 12-deoxy analogue of dihydroarteannuin B (**31**) (Sy and Brown, 2002b), as is shown in Fig. 5.

Although the *seco*-cadinane **19** (Fig. 2) is new as a natural product, it has also been obtained recently from the in vitro autoxidation of compound **30**, the 12-deoxy analogue of dihydroartemisinic acid (Sy and Brown, 2002b), and compound **19** from the seeds of *A. annua* was readily identified by comparison with an authentic standard of this compound. Similarly, the new *nor-seco*-cadinane natural product **20** is the free acid analogue of a methyl ester which has been reported previously from the acid degradation of artemisinin in a methanol/sulfuric acid medium (Hui et al., 1997) and the NMR assignments for **20** which are shown in Tables 1 and 2 gave good agreement with those which have been reported for this degradation product. The new *seco*-cadinane natural product **21** (Fig. 2) which was isolated in this study from the seeds of *A. annua* has also been obtained recently from the in vitro autoxidation of dihydroartemisinic acid (**4**) in organic solution (Sy and Brown, 2002a) and was suggested to have been formed by the mechanism which is shown in Fig. 5. The new natural product amorphane epoxide, compound **22**, was also obtained previously as a minor component from the much slower autoxidation of dihydroartemisinic acid as a solid (Sy et al., 2001a) which was presumed to arise in vitro by a mechanism involving the tertiary allylic hydroperoxide **29**, as is shown in Fig. 5. The $\Delta^{11,13}$-dehydro analogues of the *seco*-cadinane **21** and the epoxide **22**, compound **23** (Brown, 1994b) and α-epoxy-arteannuic acid[5] (**24**) (Wu and Wang, 1984) respectively, were both also obtained in this study and have been reported previously as natural products from *A. annua*.

The *nor*-amorphane **25** from the seeds of *A. annua*, is the formyl ester of a bis-*nor*-amorphane which has been identified previously from this species and was referred to by Misra as norannuic acid (Misra et al., 1993b; Ahmad and Misra, 1994). The formyl ester group in compound **25** (Fig. 2) might have been derived from the C-12 carbon of an amorphane precursor which became "trapped" by the 5-hydroxyl group during extensive autoxidation and further rearrangement reactions of the C-11–C-13 substituent, in a similar manner to that which has been proposed for the biogenesis of the formyl group in compound **19** in Fig. 5 (in this case it is the

[5] Very little spectroscopic evidence was provided to support the structure of α-epoxy-arteannuic acid (**24**) in the first and only report of its isolation from *A. annua* (Wu and Wang, 1984). Complete ^{13}C and ^{1}H NMR assignments for **24** which were made by 2D NMR, as for all other compounds reported in Tables 1 and 2, are now given for the first time.

Table 3
^{13}C and ^{1}H NMR spectral assignments for cedrene sesquiterpene derivatives **32–37** which provided supporting evidence for the structure of the natural product **32**

Position (mult.)[a]	δ_C						δ_H					
	32	**33**	**34**	**35**	**36**	**37**	**32**	**33**	**34**	**35**	**36**	**37**
1 (CH_2)	39.6	45.1	40.7	40.0	42.0	41.4	1.90 1.57	1.78 1.20	1.66 1.40	1.90 1.52	1.65 1.36	1.93 1.59
2 (CH)	56.7	60.7	55.0	61.6	61.1	67.1	1.73	2.19	1.75	1.56	1.58	2.33
3 (C)	75.4	151.8	140.6	73.3	75.1	214.5	–	–	–	–	–	–
4 (CH_2)	29.7	29.7	119.3 (CH)	34.4	35.4	36.7	1.69 1.57	2.32 2.32	5.22	1.66 1.59	1.85 1.70	2.49 2.32
5 (CH_2)	30.2	33.7	38.8	30.6	31.6	32.0	1.61 1.40	1.48 1.38	1.78 2.17	1.59 1.34	1.44 1.38	1.74 1.65
6 (C)	54.2	55.4	53.9	53.5	54.1	54.4	–	–	–	–	–	–
7 (CH)	41.9	42.1	41.6	41.8	41.5	41.6	1.70	1.69	1.74	1.69	1.69	1.86
8 (CH_2)	36.9	37.0	36.2	37.0	37.0	36.8	1.89 1.29	1.87 1.30	1.87 1.37	1.88 1.27	1.88 1.29	1.94 1.43
9 (CH_2)	25.4	25.7	24.9	25.4	25.4	25.5	1.54 1.41	1.55 1.43	1.58 1.39	1.53 1.41	1.54 1.40	1.66 1.52
10 (CH)	56.4	56.5	59.0	56.3	56.6	56.9	1.75	1.82	1.71	1.74	1.80	1.99
11 (C)	42.0	42.3	48.2	41.9	43.4	42.8	–	–	–	–	–	–
12 (CH_3)	28.8	26.6	25.6	29.1	28.9[b]	26.2[b]	1.01	0.97	0.95	1.00	1.00[b]	1.01[b]
13 (CH_3)	28.0	25.9	27.7	28.2	27.7[b]	25.9[b]	1.13	0.94	1.02	1.13	1.32[b]	1.02[b]
14 (CH_3)	15.5	15.4	15.5	15.5	15.6	15.5	0.86	0.85	0.84	0.84	0.84	0.90
15 (CH_2)	70.0	107.6	24.8 (CH_3)	30.7 (CH_3)	30.2 (CH_3)	–	3.67 3.59	4.58 4.51	1.68	1.31	1.26	–

[a] Multiplicity determined by DEPT;
[b] Interchangeable within column

C-5 carbon which was "trapped" by the 12-hydroxyl group).

The unusual structures of both of compounds **26** and **27** were determined from the results of extensive 2D NMR spectral analyses (Fig. 3) and these natural products are proposed to be derived from amorphanes which have undergone rearrangement of the decalin ring. The novel skeleton of compound **26** (Fig. 2) might be derived from the amorphane skeleton by migration of C-8 from C-7 to C-6 [i.e. compound **26** is an 8(7→6) *abeo* amorphane] resulting in a contraction of the B ring from six atoms to five. Similarly, the unusual carbon skeleton of **27** might also arise from an amorphane precursor, in which the A ring has been contracted down from six to five atoms, by carbon–carbon bond migration of C-3 from C-4 to C-5. If this were the case, then compound **27** could be thought of as a member of the oplopane class of natural products—i.e. the 15-*nor* derivative of oplopanone (Bohlmann and Zdero, 1978; Piers and Gavai, 1990)—oplopanes have previously been proposed to be derived from amorphanes by this kind of mechanism. Compound **28**, the last amorphane to be described from the seeds of *A. annua*, was identified as the 3-acetate derivative of a dihydroxy-amorphane, which has been obtained previously from this species (Sy and Brown, 1998), by 2D NMR. The presence of a hydroxyl group at the 7-position of the amorphane skeleton is unusual for natural products from *A. annua* and this is only the second such metabolite to be isolated, although a sesquiterpene synthase has been reported recently from *A. annua*, with apparent enzymatic activity for forming this class of natural product (Mercke et al., 2000).

The new compound **32** (Fig. 2) is the first cedrane sesquiterpene to be described from *A. annua* and was characterized as 3α,15-dihydroxycedrane by 2D NMR spectroscopy (Fig. 3, Table 3). A survey of the literature revealed that this same compound had apparently been described previously from *Juniperus chinensis* L. by Kuo and Chen (1992) (compound **32** was referred to as 8α,12-dihydroxycerdane by these authors). However, the NMR spectral data reported for the natural product from *J. chinensis* was radically different from that determined by ourselves from 2D NMR analysis of compound **32** from the seeds of *A. annua,* and this prompted us to undertake a more detailed investigation in order to confirm our deductions. Full 2D NMR spectroscopic assignments for the commercially available cedrenes, (+)-β-cedrene (**33**), (−)-α-cedrene (**34**), (−)-*epi*-cedrol (**35**) and (+)-cedrol (**36**) (Fig. 7), which are reported for the first time in Table 3, all tended support our NMR assignments for the cedrane skeleton of **32** from *A. annua* and suggested that the structural assignment made by Kuo and Chen was incorrect. The fully-assigned NMR data for the 3α-OH cedrane derivative (−)-*epi*-cedrol (**35**) (Table 3), in particular, were very similar to those of compound **32** (with the obvious exception of the ^{13}C and ^{1}H resonances at the

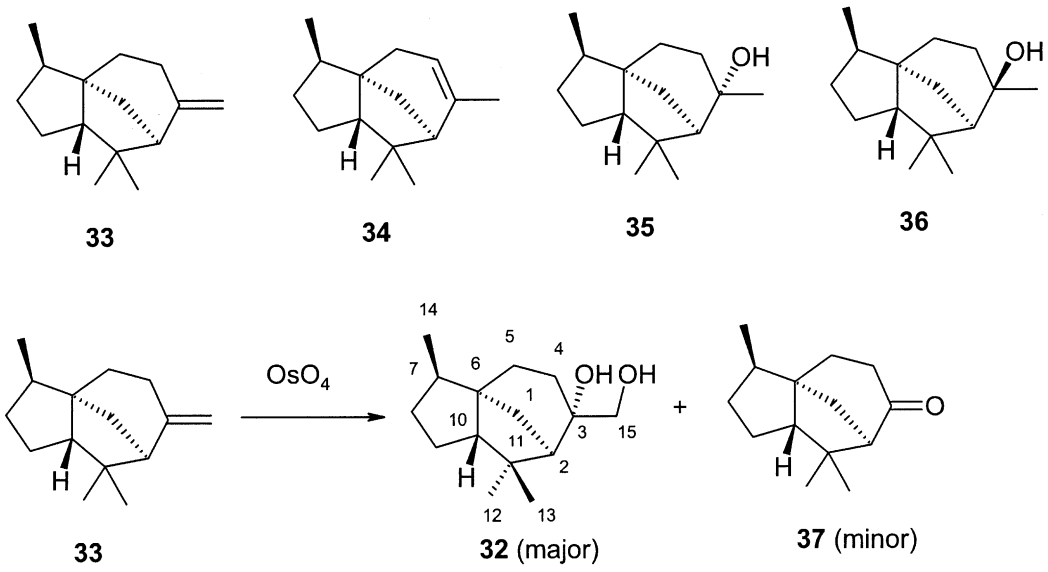


Fig. 7. Commercially available cedranes **33**–**36** which were fully assigned by 2D NMR for the first time in Table 3, and the chemical transformation of **33** to **32** (and **37**) which was used in confirming the structure of the natural product 3α,15-dihydroxy cedrane from *A. annua*.

15-position) which prompted us to attempt to prepare an authentic sample of **32** from one of these commercially available precursors. Gratifyingly, treatment of (+)-β-cedrene (**33**) with osmium tetroxide (Fig. 7) did indeed yield a product with identical physical properties to those of the natural product **32**,[6] thereby conclusively confirming the structure assignment of this compound as 3α,15-dihydroxycedrane, and casting doubt on the previous report of this compound as a natural product. The isolation of this class of sesquiterpene, which was previously unknown from *A. annua*, is particularly significant because a sesquiterpene cyclase which catalyses the formation of all of (+)-β-cedrene (**33**), (−)-α-cedrene (**34**), (−)-*epi*-cedrol (**35**) and (+)-cedrol (**36**) (as well as several other unidentified products) has recently been isolated from this species (Mercke et al., 1999) and the authors of this paper have been unsure how to explain the significance of their findings in the absence of any reports of cedrane natural products (P. Brodelius, personal communication) .

In addition to the fourteen new sesquiterpene natural products described above, the seeds of *A. annua* have also yielded three new monoterpenes (**38**, **39** and **40**) and one diterpene (**41**) which is a new natural product (Fig. 2). All four secondary metabolites might be derived by autoxidation reactions of unsaturated terpenoid precursors (see Fig. 8), in a similar manner to that proposed for the sesquiterpenes **15**, **17** and **19**–**24** in Fig. 5. The diol **38** was characterized as a derivative of the irregular monoterpene, lavandulol, by 2D NMR spectroscopy, as previously described (Fig. 9 and Table 4). Lavandulane **38** contained a secondary allylic alcohol group that was isomeric with the tertiary allylic alcohol group in the natural product **42**, which has been reported recently from this species (Sy and Brown, 2001a) and which was, at the time, suggested to have been formed by autoxidation reactions of lavandulol (**43**) (see Fig. 8 for a suggested mechanism). Given the similarity in structure between the two natural products **38** and **42**, it seemed highly likely that the novel compound **38** might also be derived by oxygenation of lavandulol. This was confimed by performing a biomimetic synthesis, in which lavandulol (**43**) was subjected to photo-oxygenation, resulting in all three allylic hydroxides (**44**–**46**) (see Fig. 8 and Sy and Brown, 2001a), followed by reduction of one of these secondary allylic hydroperoxides (**44**) in the presence of triphenylphosphine, to yield a product with identical physical properties to those of compound **38** from *A. annua*.

Compounds **39** and **40** were identified by 1D and 2D NMR spectroscopy (Fig. 9 and Table 4) as being derivatives of the regular acyclic monoterpene, myrcene, which incorporated oxygen substituents at C-1, C-6/C-7 and C-10.[7] The results of a D_2O shake (Christofides and Davies, 1983) indicated a significant upfield chemical shift for the carbons at C-6/C-7 ($\Delta\delta_C$ −0.12 and −0.13 ppm, respectively), which must therefore be associated with hydroxyl groups in both compounds **39** and **40**. No significant secondary isotope effects were observed at any other position, which led to the conclusion that the remaining two oxygen-substitued carbons at C-1 and C-10 must therefore be present as part of a 1,4-dihydrofuran

[6] The 15-*nor*-cedrane, compound **37**, was also isolated as a minor side-product from this reaction.

[7] Both α- and β-myrcene hydroperoxides, which are closely related to the intermediates proposed in the biogenesis of compounds **39** and **40** in Fig. 9, were also isolated in significant quantities from the seeds of *A. annua*, in agreement with previous reports concerning the phytochemistry of this species (Rucker et al., 1987).

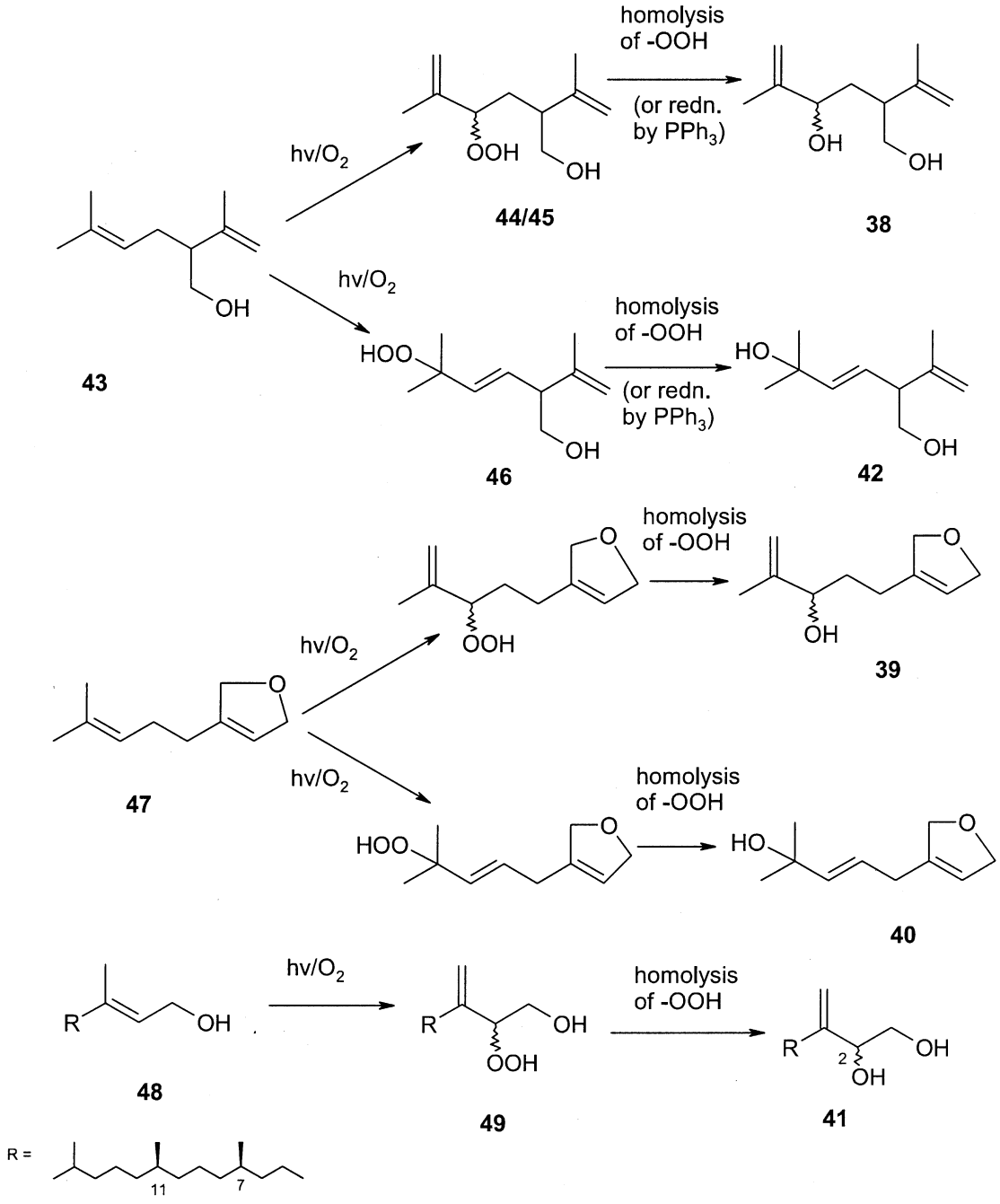


Fig. 8. Proposed biogenesis of the natural products **38**–**41** by autoxidation of mono- and diterpene precursors containing a tri-substituted double bond. The biomimetic synthesis of compound **38** which was used in confirming its structure, followed the same route as for the synthesis of compound **42**, which has been reported previously (Sy and Brown, 2001a).

ring in both **39** and **40**. (*Z*)-6-Hydroxymethyl-2-methylocta-1,6-diene-3,8-diol from *Chondrococcus hornemanii* (Coll and Wright, 1989) has the closest structure of any natural product, of which we are aware, to the novel monoterpenes **39** and **40**. It incorporates two primary hydroxyl groups in place of the 1,4-dihydrofuran functional group proposed for **39** and **40** and, as expected, this compound has slightly different physical properties to those of compound **39**. The 1,4-dihydrofuran functional group suggested for both of compounds **39** and **40** is, to the best of our knowledge, unique amongst natural products. We propose that the biogenesis of the allylic hydroxide functionality in both compounds (Fig. 8) might be explained by autoxidation reactions of a putative monoterpene precursor (**47**) which also incorporates the 1,4-dihydrofuran functional group, which proceed by a similar pathway to that proposed for the lavandulane natural products **38** and **42**.

Table 4
^{13}C and ^{1}H NMR spectral assignments for compounds **38**–**41** and **48** from the seeds of *Artemisia annua*

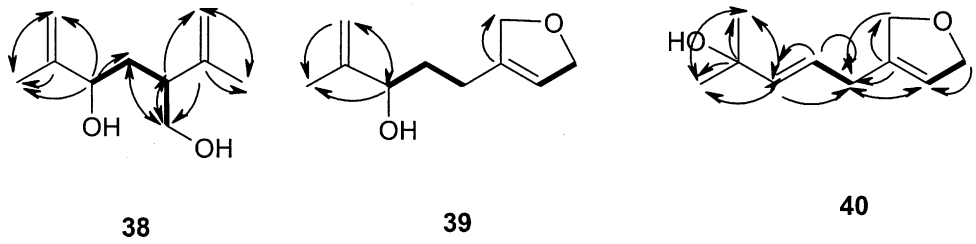
Position (mult.)[a]	δ_C					δ_H				
	38	**39**	**40**	**41**	**48**	**38**	**39**	**40**	**41**	**48**
1 (CH_2)	64.9	70.0	70.0	65.66	59.45	3.59, 3.58	4.57, 4.57	4.56, 4.56	3.71, 3.54	4.16, 4.16
2 (CH)	47.1	117.4	118.4	75.00	123.11	2.53	5.68	5.68	4.20	5.41
3 (CH_2)	36.1	134.4 (C)	134.3 (C)	148.20 (C)	140.33 (C)	1.65, 1.60	–	–	–	–
4 (CH)	74.2	28.3 (CH_2)	35.2 (CH_2)	33.01 (CH_2)	39.88 (CH_2)	4.07	2.13, 2.05	2.75, 2.75	2.04, 1.97	1.99, 1.99
5 (C)	148.0	32.3 (CH_2)	122.9 (CH)	25.53 (CH_2)	25.15 (CH_2)	–	1.71, 1.71	5.60	1.46, 1.46	1.41, 1.41
6 (CH_3)	17.9	75.3 (CH)	140.8 (CH)	36.86/36.82 (CH_2)	36.68 (CH_2)	1.74	4.10	5.69	1.32, 1.13	1.40, 1.32
7 (CH_2)	110.6	147.3 (C)	70.7 (C)	32.71 (CH)	32.70 (CH)	4.97, 4.83	–	–	1.39	1.39
8 (C)	145.4	17.6 (CH_3)	29.9 (CH_3)	37.45[b] (CH_2)	37.44[b] (CH_2)	–	1.74	1.32	1.25, 1.08	1.25, 1.08
9 (CH_2)	113.5	111.4 (CH_2)	29.9 (CH_3)	24.49	24.48 (CH_2)	4.94, 4.87	4.96, 4.87	1.32	1.27, 1.27	1.28, 1.28
10 (CH_3)	19.6	72.5 (CH_2)	72.2 (CH_2)	37.41/37.39[b](CH_2)	37.37[b] (CH_2)	1.73	4.48, 4.48	4.44, 4.44	1.25, 1.08	1.25, 1.08
11 (CH)	–	–	–	32.82	32.80	–	–	–	1.39	1.39
12 (CH_2)	–	–	–	37.31[b]	37.30[b]	–	–	–	1.25, 1.08	1.25, 1.08
13 (CH_2)	–	–	–	24.81	24.80	–	–	–	1.27, 1.27	1.28, 1.28
14 (CH_2)	–	–	–	39.39	39.38	–	–	–	1.14, 1.14	1.14, 1.14
15 (CH)	–	–	–	28.00	27.98	–	–	–	1.52	1.52
16 (CH_3)	–	–	–	22.63	22.62	–	–	–	0.87	0.87
17 (CH_2)	–	–	–	110.57	16.18 (CH_3)	–	–	–	5.13, 4.98	1.67
18 (CH_3)	–	–	–	19.70/19.71	19.72[c]	–	–	–	0.86	0.85[b]
19 (CH_3)	–	–	–	19.77	19.75[c]	–	–	–	0.84	0.84[b]
20 (CH_3)	–	–	–	22.73	22.71	–	–	–	0.87	0.87

[a] Multiplicity determined from DEPT.
[b] Interchangeable within column.
[c] Interchangeable within column.

The diterpene secondary metabolite, phytene-1,2-diol (**41**) (Fig. 2), has previously been obtained in completely racemic form and as a minor product, together with its 2-hydroperoxy analogue, compound **49**,[8] from the in vitro photo-oxidation of commercially available phytol (**48**) (Wong and Brown, 2002a). Phytol (**48**) was also obtained from the CH_2Cl_2 extract of the seeds of *A. annua*, and is expected to have the 7*R*,11*R* absolute configuration (Connolly and Hill, 1991) which is shown in Fig. 8. The expectation that natural phytol (**48**) from this source should be biosynthesized as a single enantiomer was supported by the observation of only a single resonance for each of C-1 through C-20 in the fully assigned ^{13}C NMR spectrum of **48** (Table 4). By contrast, the ^{13}C NMR spectral data recorded for compound **41** from the seeds of *A. annua* (Table 4) suggested that this natural product was a 1:1 mixture of diastereoisomers, because two distinct resonances were resolved for the carbons at the C-6, C-10 and C-18 positions (note that the ^{13}C NMR data for the completely racemic form of **41**, which had been obtained from photo-oxidation of **48**, had indicated the presence of at least four species which could be distinguished by NMR, which were accordingly assigned to the eight possible diastereoisomers associated with complete racemization at each of the three chiral centers (C-2, C-7 and C-11) in **41**—see Wong and Brown, 2002a). In order to account for the foregoing observations concerning the ^{13}C NMR spectra of these two compounds, we propose that phytene-1,2-diol (**41**) in the seeds of *A. annua* may be derived from 7*R*,11*R*-phytol (**48**), which is present naturally in the same source, by autoxidation processes which are similar to those that have been suggested for the monoterpenes **38**–**40** in Fig. 8, and that natural **41** is therefore a mixture of epimers only at the 2-position.

In addition to the above natural products, three well known secondary metabolites were also isolated from the seeds of *A. annua* which have not been reported previously from this species: these included the sesquiterpene caryophyllene oxide (Tsui and Brown, 1996), and the shikimate derivatives phenylpropanoic acid and benzoic acid. Almost all of the natural products which have been previously reported from the aerial parts of *A. annua* were also isolated from this investigation of the seeds (and were identified from their 1D ^{1}H and ^{13}C NMR spectra). These included the monoterpenes artemisia ketone (Woerdenbag et al., 1993) and α-myrcene hydroperoxide/β-myrcene hydroperoxide (Rucker et al., 1987), as well as the sesquiterpenes, arteannuic acid (**1**) (Misra et al., 1993a), arteannuin B (**2**) (Zheng, 1994), dihydroartemisinic acid (**4**) (Sy et al., 1998), dihydroarteannuin B (**5**) (Sy et al., 1998), artemisinin (**6**) (Zheng, 1994), the *seco*-cadinane **23** (Brown, 1994b), α-epoxy-arteannuic acid (**24**) (Wu and Wang, 1984),

[8] The 2-hydroperoxy analogue of **41**, compound **49**, is, in fact, also a natural product from *A. annua*, which had previously been erroneously identified as phytene-1,2-ol (Brown, 1994a).



38 39 40

Fig. 9. Characterization of novel monoterpenes **38**, **39** and **40** by 2D NMR (two and three-bond correlations from ^{13}C to ^{1}H are indicated by arrows; ^{1}H–^{1}H correlations are indicated by bold lines on the structure).

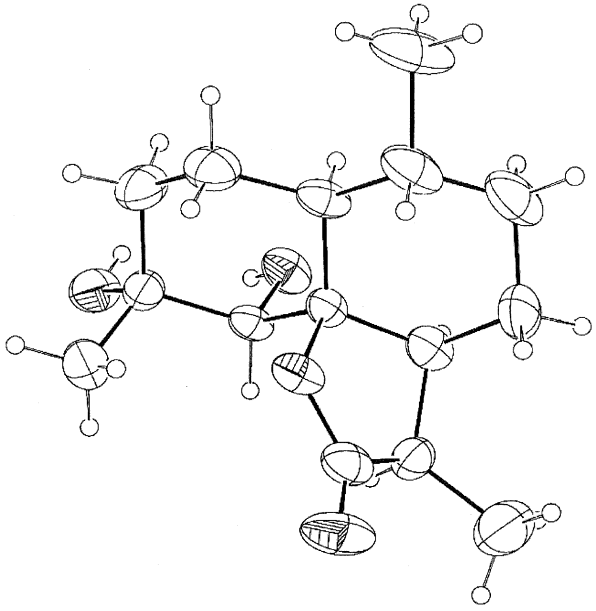


Fig. 10. ORTEP diagram showing the correct stereochemistry of the geminal diol group in the natural product arteannuin M, as established by X-ray crystallography.

dihydro-*epi*-deoxyarteannuin B (Sy et al., 1998), deoxyartemisinin (Sy and Brown, 2001b), 3α-hydroxyartemisinin (Tu et al., 1982), *epi*-deoxyarteannuin B (El-Feraly et al., 1989), artemisinin G (**50**) (Wei et al., 1992), arteannuins H, I, J, K, L, M and N (Sy et al., 1998) and arteannuin O (Sy et al., 2001b). The full ^{1}H and ^{13}C NMR spectroscopic assignments for artemisinin G, which were determined by 2D NMR techniques, as for all the novel compounds reported above, are given in Tables 1 and 2 for the first time. Following two recent syntheses of both natural (−)-arteannuin M (Sy et al., 2001b) and of its (+)-enantiomer (Barriault and Deon, 2001) it has become apparent that the stereochemistry reported for the geminal diol group had been incorrectly assigned when this compound was first described as a natural product from *A. annua* (Sy et al., 1998). An X-ray crystallographic analysis of arteannuin M obtained from the seeds of *A. annua* has now confirmed that the geminal hydroxyl groups in this compound are *cis* to one another, and by implication, that the absolute stereochemistry of arteannuin M must be as shown in the ORTEP diagram in Fig. 10 (X-ray crystallographic data deposited with the Cambridge Crystallographic Data Centre; deposit number 208262).

The well known steroids stigmasterol and sitosterol (Hai et al., 2000) were also isolated from the extracts of the seeds together with the known flavonoids: chrysosplenol D (**51**) (Chen Liu et al., 1992), casticin (**52**) (Baeva et al., 1988; Chen Liu et al., 1992), chrysosplenetin (Baeva et al., 1988; Chen Liu et al., 1992), artemetin (Baeva et al., 1988; Chen Liu et al., 1992), penduletin (Sy and Brown, 1998) and 5-hydroxy-3,6,7,4′-tetramethoxyflavone (Sy and Brown, 1998); full ^{13}C and ^{1}H NMR assignments were made by 2D NMR for compounds **51** and **52** and are reported for the first time in the Experimental section. Coumarin (Brown, 1992) and its derivatives, scopoletin and isofraxidin (Saitibaeva and Sidyakin, 1970; Brown, 1994c; Sy and Brown, 1999b) were also obtained from the CH_2Cl_2 extract of the seeds of *A. annua*, together with 2,4-dihydroxy-6-methoxyacetophenone (Brown, 1992; Singh et al., 1997) and 5-nonadecylresorcinol 3-*O*-methyl ether (Brown, 1992), which have all been reported previously from *A. annua*. The polyacetylenes, pontica epoxide and annuadiepoxide which were isolated from the seeds in this study, have also both been described previously from the aerial parts of *A. annua* (Manns and Hartmann, 1992).

3. Experimental

3.1. General

Chemical shifts are expressed in ppm (δ) relative to TMS as an internal standard. Proton chemical shifts, multiplicities, coupling constants and integrals reported in this section are those which were clearly resolved in one-dimensional ^{1}H NMR without recourse to 2D NMR analysis (see tables in the main text for full assignments which were made by 2D NMR). All NMR experiments were run either on a Bruker DRX 500 or an Avance 600 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. 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 silica or a YMC diol column (20 mm × 25 cm), flow rate 8 ml/minute. Optical rotations were measured by a Perkin-Elmer 343 polarimeter with polarized light (Na 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. Plant material

Seeds of *A. annua* were collected around Guiyang in Guizhou Province, P. R. China and extracted immediately after collection. Taxonomic identification was made in the field by one of us (G.-Y. L.) of the Key Laboratory of Chemistry for Natural Products of Guizhou Province. A voucher specimen (voucher no. HKU 2285) of plant material grown from these seeds has also been deposited in the herbarium at The University of Hong Kong.

3.3. Extraction and isolation

The seeds of *A. annua* (2 kg) were frozen in liquid N_2 and converted into a powder by grinding with a pestle and mortar. The powder was repetitively extracted with CH_2Cl_2, dried ($MgSO_4$) and solvent removed under reduced pressure to yield an aromatic green gum (103 g, 5.2% w/w) which was subjected to gradient CC yielding 32 crude fractions: fraction A (5.19 g) and fraction B (5.13 g) from 5% EtOAc/*n*-hexane; fraction C (5.95 g) and fraction D (2.41 g) from 10% EtOAc/*n*-hexane; fraction E (1.84 g), fraction F (2.05 g), fraction G (3.00 g) and fraction H (7.96 g) from 15% EtOAc/*n*-hexane; fraction I (3.59 g) and fraction J (2.79 g) from 20% EtOAc/*n*-hexane; fraction K (6.67 g), fraction L (2.94 g) and fraction M (7.59 g) from 25% EtOAc/*n*-hexane; fraction N (2.08 g) and fraction P (8.32 g) from 30% EtOAc/*n*-hexane; fraction Q (3.40 g) and fraction R (1.25 g) from 35% EtOAc/*n*-hexane; fraction S (1.29 g) and fraction T (3.24 g) from 40% EtOAc/*n*-hexane; fraction U (2.47 g) and fraction V (3.23 g) from 45% EtOAc/*n*-hexane; fraction W (2.16 g), fraction X (2.22 g) and fraction Y (1.07 g) from 50% EtOAc/*n*-hexane; fraction Z (0.94 g) and fraction K-1 (2.02 g) from 60% EtOAc/*n*-hexane; fraction K-2 (1.33 g) and fraction K-3 (1.95 g) from 70% EtOAc/*n*-hexane; fraction K-4 (1.12 g) from 80% EtOAc/*n*-hexane; and fraction K-5 (1.05 g) and fraction K-6 (0.69 g) from 100% EtOAc.

The crude fractions from CC were further purified by repeated prep HPLC, using *n*-hexane/EtOAc/HOAc in varying proportions, according to the polarity of the crude fraction which was under investigation. Only the HPLC solvent used in the final purification of the novel compounds obtained from the seeds of *A. annua* is given below: **7** (6 mg) R_t 23.3 min in 8% EtOAc/*n*-hexane (from fraction N); **8** (1.6 mg) R_t 68.0 min in 8% EtOAc/*n*-hexane (from fraction N); **15** (70.5 mg) R_t 17.0 min in 35% EtOAc/*n*-hexane/1% HOAc (from fraction X)/R_t 14.0 min in 40% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-1)/R_t 12.2 min in 45% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-3); **17** (1.2 mg) R_t 55.5 min in 30% EtOAc/*n*-hexane (from fraction V); **19** (56.1 mg) R_t 38.8 min in 22% EtOAc/*n*-hexane (from fraction S)/R_t 22.9 min in 30% EtOAc/*n*-hexane (from fraction V); **20** (21.6 mg) R_t 24.0 min in 35% EtOAc/*n*-hexane/1% HOAc (from fraction X)/R_t 20.5 min in 45% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-3)/; **21** (5.4 mg) R_t 23.0 min in 45% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-3); **22** (24.4 mg) R_t 20.3 min in 30% EtOAc/*n*-hexane (from fraction V)/R_t 14.1 min in 35% EtOAc/*n*-hexane/1% HOAc (from fraction X); **23** (21.4 mg) R_t 21.5 min in 45% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-3); **24** (6.5 mg) R_t 13.4 min in 35% EtOAc/*n*-hexane/1% HOAc (from fraction X)/R_t 11.2 min in 40% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-1); **25** (8.4 mg) R_t 24.6 min in 30% EtOAc/*n*-hexane (from fraction V); **26** (17.6 mg) R_t 42.2 min in 25% EtOAc/*n*-hexane (from fraction U)/R_t 31.3 min in 30% EtOAc/*n*-hexane (from fraction V); **27** (5.4 mg) R_t 28.5 min in 45% EtOAc/*n*-hexane/0.5% HOAc (from fraction K-3); **28** (2.2 mg) R_t 31.1 min in 8% EtOAc/*n*-hexane (from fraction N); **32** (6.4 mg) R_t 61.5 min in 30% EtOAc/*n*-hexane (from fraction V); **38** (5.8 mg) R_t 81.0 min in 35% EtOAc/*n*-hexane/1% HOAc (from fraction X); **39** (59.8 mg) R_t 36.7 min in 25% EtOAc/*n*-hexane (from fraction U)/R_t 27.5 min in 30% EtOAc/*n*-hexane (from fraction V); **40** (66 mg) R_t 48.0 min in 25% EtOAc/*n*-hexane (from fraction U); and **41** (12 mg) R_t 59.5 min in 25% EtOAc/*n*-hexane/(from fraction U).

3.4. 1β-Hydroxy-4(15),5-eudesmadiene (7)

Colorless oil. $[\alpha]_D$ +6.9 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3435 (*br*), 2966, 2922, 2878, 1460 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 5.57 (1H, *s*), 4.81 (1H, *s*), 4.63 (1H, *t*, $J=2.5$ Hz), 3.45 (1H, *dd*, $J=11.7$, 4.1 Hz), 2.35 (1H, *d*, $J=13.7$ Hz), 0.91 (3H, *s*), 0.91 (3H, *d*, $J=7$ Hz), 0.89 (3H, *d*, $J=6.6$ Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 220.1807 (19) [M^+, calc. for 220.1827, $C_{15}H_{24}O$], 202 (25), 177 (20), 159 (100), 153 (62).

3.5. 1β-Hydroxy-4(15),7-eudesmadiene (8)

Colorless oil. IR ($CHCl_3$) ν_{max}: 3440 (*br*), 2951, 2862, 1458 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 5.33 (1H, *d*, $J=5.5$ Hz), 4.83 (1H, *s*), 4.65 (1H, *s*), 3.48 (1H, *dd*, $J=11.4$, 4.8 Hz), 2.36 (1H, *d*, $J=14.2$ Hz), 1.02 (3H, *d*, $J=6.9$ Hz), 1.01 (3H, *d*, $J=6.6$ Hz), 0.66 (3H, s)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z*

(rel. int.): 220.1825 (85) [M^+, calc. for 220.1827, $C_{15}H_{24}O$], 202 (27), 187 (19), 177 (28), 159 (100), 153 (34).

3.6. 4(15)-Eudesmene-1β,6α-diol (9)

Colorless oil. $[\alpha]_D$ +4.5 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} : 3420 (*br*), 3011, 2932, 2854, 1454 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 5.02 (1H, *s*), 4.74 (1H, *s*), 3.72 (1H, *dd*, *J* = 9.8, 9.8 Hz), 3.43 (1H, *dd*, *J* = 11.7, 4.6 Hz), 0.95 (3H, *d*, *J* = 6.9 Hz), 0.87 (3H, *d*, *J* = 7.1 Hz), 0.71 (3H, *s*)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 220.1828 (38) [M^+–H_2O, calc. for 220.1827, $C_{15}H_{24}O$], 202 (25), 189 (21), 177 (45), 159 (60), 121 (100).

3.7. 5α-Hydroperoxy-eudesma-4(15),11-diene (10)

Colorless oil. IR ($CHCl_3$) ν_{max}: 3524 (*br*), 2937, 2862, 1643, 1464 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 6.80 (1H, *s*,–OOH), 5.07 (1H, *s*), 4.78 (1H, *s*), 4.77 (1H, *s*), 4.74 (1H, *s*), 2.20 (1H, *br d*, *J* = 13.2 Hz), 2.11 (1H, *br d*, *J* = 13.8 Hz), 1.78 (3H, *s*), 1.51 (1H, *dd*, *J* = 13.8, 12.6 Hz), 0.94 (3H, s)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 218.1676 (41) [M^+–H_2O, calc. for 218.1671, $C_{15}H_{22}O$], 203 (100), 187 (63), 175 (25), 161 (30).

3.8. Reduction of 10 to 11 by triphenylphosphine

To a solution of **10** (1.8 mg) in MeOH (2 ml) was added triphenylphosphine (2.5 mg) and the mixture was stirred for 2 h, until completion of the reaction, as determined by TLC. The organic solution was evaporated to dryness and the crude residue (4.0 mg) was purified by HPLC (10% EtOAc/hexane) to yield compound **11** (1.1 mg) with identical NMR spectral properties to those reported in the literature for 5α-hydroxy eudesma-4(15),11-diene (Sy and Brown, 1998). ^1H ($CDCl_3$) δ (ppm): 4.82 (1H, *s*), 4.74 (1H, *s*), 4.72 (1H, *s*), 4.69 (1H, *s*), 1.76 (3H, *s*), 0.88 (3H, *s*); ^{13}C ($CDCl_3$) δ (ppm): 151.9, 150.5, 108.5, 107.6, 75.8, 40.0, 35.5, 35.0, 34.3, 31.7, 26.0, 22.2, 21.1, 19.9.

3.9. 1β-Hydroxy-4(15),5E,10(14)-germacratriene (13)

Colorless oil. $[\alpha]_D$ −106 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} : 3406 (*br*), 3018, 2930, 2856, 1454, 1209 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 6.00 (1H, *d*, *J* = 15.0 Hz), 5.43 (1H, *dd*, *J* = 15.5, 10.1 Hz), 5.27 (1H, *s*), 5.00 (1H, *s*), 4.92 (1H, *s*), 4.84 (1H, *s*), 3.77 (1H, *dd*, *J* = 11.9, 3.9 Hz), 2.43 (1H, *ddd*, *J* = 12.3, 12.3, 4.8 Hz), 0.89 (3H, *d*, *J* = 6.6 Hz), 0.81 (3H, *d*, *J* = 6.6 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 220.1827 (3) [M^+, calc. for 220.1827, $C_{15}H_{24}O$], 202 [M^+–H_2O] (31), 202 (28), 187 (9), 175 (17), 159 (100).

3.10. 4α,5α-Epoxy-6α-hydroxy amorphan-12-oic acid (15)

Colorless oil. $[\alpha]_D$−62.5 (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3537 (*br*), 3400–2600 (*br*), 2955, 2928, 2855, 1703, 1456 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 3.24 (1H, *s*), 3.04 (1H, *dq*, *J* = 4.1, 7.1 Hz), 1.36 (3H, *s*), 1.33 (3H, *d*, *J* = 6.9 Hz), 1.07 (1H, *dddd*, *J* = 13.2, 13.2, 13.2, 2.5 Hz), 0.87 (3H, *d*, *J* = 6.4 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 268.1676 (1) [M^+, calc. for $C_{15}H_{24}O_4$, 268.1675], 250.1576 (18) [M^+–H_2O, calc. for $C_{15}H_{22}O_3$, 250.1569], 232 (69), 222 (38), 207 (15), 195 (48), 192 (54), 179 (100), 177 (55), 167 (60).

3.11. Conversion of the carboxylic acid group in 15 to a methyl ester in 16

To a solution of compound **15** (5.6 mg, 0.0209 mmol) in Et_2O (3 ml) was added an ethereal solution of diazomethane (1 ml) [freshly prepared by dissolving Diazald® (0.645 g) in Et_2O (10ml), adding KOH (0.145 g) in EtOH (3 ml; 95%), stirring at 0 °C for 10 min, and distilling the diazomethane solution from a water bath] and the mixture was stirred at room temperature for 5 min. HOAc (10%, 1 ml) was added to the mixture to remove the excess diazomethane and the organic layer was washed with brine (2×2 ml), dried ($MgSO_4$) and solvent was removed under reduced pressure to yield compound **16** (4 mg; 0.0142 mmol, 68%), without the need for further purification.

3.12. 4α,5α-Epoxy-6α-hydroxy amorphan-12-oic acid methyl ester (16)

Colorless oil. $[\alpha]_D$−45.4 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3537, 2955, 2928, 2876, 1703, 1456 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 3.66 (3H, *s*), 3.14 (1H, *s*), 3.08 (1H, *dq*, *J* = 11.4, 7.1 Hz), 1.36 (3H, *s*), 1.30 (3H, *d*, *J* = 7.1 Hz), 0.87 (3H, *d*, *J* = 6.6 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 264.1732 (4) [M^+–H_2O, calc. for $C_{16}H_{24}O_3$, 264.1725], 252 (4), 250 (10), 246 (13), 232 (32), 222 (10), 204 (15), 179 (28), 177 (33), 159 (100).

3.13. 4α,5α-Epoxy-6α-hydroxy amorphan-12-ol (17)

Colorless oil. IR ($CHCl_3$) ν_{max}: 3602, 3400 (*br*), 3011, 2963, 2924, 2858, 1460 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 3.72 (1H, *dd*, *J* = 11.2, 6.9 Hz), 3.56 (1H, *dd*, *J* = 11.2, 5.9 Hz), 3.17 (1H, br *s*), 1.36 (3H, *s*), 1.11 (3H, *d*, *J* = 7.1 Hz), 0.88 (3H, *d*, *J* = 5.7 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 254.1879 (1) [M^+, calc. for 254.1882, $C_{15}H_{26}O_3$], 236 (60), 193 (45), 181 (54), 178 (40), 165 (66), 150 (100).

3.14. Conversion of arteannuin B (2) to 18 by $NaBH_4$ in EtOH

To a solution of arteannuin B (**2**) (10 mg) in EtOH (5 ml) was added a solution of $NaBH_4$ in EtOH (6.4 mg/5 ml) over 15 min with stirring. The mixture was stirred for a further 8 h, then taken up in H_2O (40 ml) and extracted with Et_2O (3 × 20 ml). The combined organic layers were dried ($MgSO_4$) and the solvent removed to yield a crude product, containing compound **18**, without the need for further purification.

3.15. 4α,5α-Epoxy-6α-hydroxy amorph-11-en-12-oic acid ethyl ester (18)

Colorless oil. $[\alpha]_D$−15.9 (*c* 0.06, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3018, 2923, 1713, 1624, 1460, 1209 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 6.08 (1H, *s*), 5.38 (1H, *s*), 4.21 (2H, m, –OC$\underline{H}_2$CH$_3$), 3.16 (1H, *dd*, *J* = 12.9, 1.8 Hz), 2.99 (1H, *br s*, 6-OH), 1.82 (1H, *dd*, *J* = 14.7, 4.2 Hz), 1.34 (3H, *s*), 1.30 (3H, *t*, *J* = 7.1 Hz, –OCH$_2$C$\underline{H}_3$), 0.90 (3H, *d*, *J* = 5.9 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 294.1828 (9) [M^+, calc. for $C_{17}H_{26}O_4$, 294.1831], 276 (9), 265 (21), 258 (25), 248 (19), 230 (71), 219 (100), 177 (70).

3.16. 1-Oxo-2β-[3-butanone]-3α-methyl-6β-[2-propanol formyl ester]-cyclohexane (19)

See Sy and Brown (2002b) for physical properties.

3.17. 1-Oxo-2β-[3-butanone]-3α-methyl-6β-[2-propanoic acid]-cyclohexane (20)

Colorless oil. $[\alpha]_D$ −36.7 (*c* 1.2, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3400–2600 (*br*), 3009, 2932, 2874, 1745 (*sh*), 1709, 1456, 1209 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 2.81 (1H, *dq*, *J* = 6.6, 7.0 Hz), 2.61 (1H, *m*), 2.53 (1H, *ddd*, *J* = 17.2, 8.8, 5.3 Hz), 2.36 (1H, *ddd*, *J* = 17.2, 9.1, 6.3 Hz), 2.12 (3H, *s*), 1.20 (3H, *d*, *J* = 7.0 Hz), 1.08 (3H, *d*, *J* = 6.2 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 254.1517 (5) [M^+, calc. for 254.1518, $C_{14}H_{22}O_4$], 236 (100), 221 (10), 208 (58), 193 (31), 180 (74), 166 (88), 151 (73).

3.18. 1α-Aldehyde-2β-[3-butanone]-3α-methyl-6β-[2-propanoic acid]-cyclohexane (21)

Colorless oil. $[\alpha]_D$ −21.8 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3400–2600 (*br*), 2930, 2854, 1745 (*sh*), 1715, 1454 cm^{-1}; HREIMS *m*/*z* (rel. int.): 250.1564 (35) [M^+–H_2O, calc. for 250.1569, $C_{15}H_{22}O_3$], 232 (31), 222 (17), 207 (18), 193 (20), 177 (72), 167 (40), 149 (100). See Sy and Brown, 2002a for ^1H and ^{13}C NMR data.

3.19. α-Epoxy-dihydroartemisinic acid (22)

See Sy et al. (2001a) for physical properties.

3.20. 1α-Aldehyde-2β-[3-butanone]-3α-methyl-6β-[2-propenoic acid]-cyclohexane (23)

See Brown (1994b) for physical properties.

3.21. α-Epoxy-arteannuic acid (24)

Colorless oil. $[\alpha]_D$ −15.5 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3400–2600, 2928, 2862, 1705, 1450 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 6.48 (1H, *s*), 5.64 (1H, *s*), 2.75 (1H, *d*, *J* = 13.2 Hz), 2.54 (1H, *s*), 2.17 (1H, br *s*), 1.29 (3H, *s*), 0.88 (3H, *d*, *J* = 5.9 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 250.1566 (41) [M^+, calc. for 250.1569, $C_{15}H_{22}O_3$], 232 (100), 217 (36), 204 (55), 187 (40), 177 (27), 165 (40).

3.22. Norannuic acid formyl ester (25)

Colorless oil. $[\alpha]_D$ −14.1 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3400–2600 (*br*), 3018, 2930, 1728, 1450 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 8.00 (1H, *s*), 5.74 (1H, *d*, *J* = 11.5 Hz), 4.78 (1H, *s*), 4.62 (1H, *s*), 2.55 (1H, *ddd*, *J* = 11.5, 3.9, 3.6 Hz), 2.46 (1H, *ddd*, *J* = 12.5, 3.6, 3.6 Hz), 0.91 (3H, *d*, *J* = 6.3 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 206.1323 (77) [M^+–HCOOH, calc. for 206.1307, $C_{13}H_{18}O_2$], 191 (18), 177 (28), 167 (40), 161 (46), 119 (100).

3.23. 3α-Hydroxy-4α,5α-epoxy-7-oxo-(8[7→6]-abeo-amorphane (26)

Colorless oil. $[\alpha]_D$ −9.3 (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3391 (*br*), 2966, 2930, 2856, 1707, 1458, 1383 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 3.91 (1H, *dd*, *J* = 11.2, 5.7 Hz), 3.15 (1H, *s*), 3.09 (1H, *sept*, *J* = 6.6 Hz), 1.48 (3H, *s*), 1.15 (3H, *d*, *J* = 5.9 Hz), 1.14 (3H, *d*, *J* = 6.6 Hz), 0.98 (3H, *d*, *J* = 6.6 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 252.1720 (1) [M^+, calc. for 252.1725, $C_{15}H_{24}O_3$], 234 (6), 218 (8), 208 (9), 193 (11), 164 (68), 138 (100).

3.24. 15-nor-10-hydroxy-oplopan-4-oic acid (27)

Colorless oil. IR ($CHCl_3$) ν_{max}: 3500–2800 (*br*), 3444, 2930, 2854, 1707, 1460, 1375, 1269 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 2.48 (1H, *ddd*, *J* = 10.3, 9.7, 5.2 Hz), 1.20 (3H, *s*), 0.92 (3H, *d*, *J* = 7.1 Hz), 0.73 (3H, *d*, *J* = 6.8 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 224.1751 (2) [M^+, calc. for 224.1776, $C_{14}H_{24}O_2$], 206.1678 (4) [M^+–H_2O, calc. for 206.1671, $C_{14}H_{22}O$], 179 (12), 155 (100).

3.25. 3α,7α-Dihydroxy amorph-4-ene 3-acetate (28)

Colorless oil. $[\alpha]_D$ −9.4 (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3011, 2922, 2862, 1720, 1454 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 5.42 (1H, *quin*, *J* = 1.6 Hz), 5.36 (1H, *m*), 2.45 (1H, *br s*) 2.41 (1H, *ddd*, *J* = 13.3, 6.6, 4.1 Hz), 2.07 (3H, *s*), 0.97 (3H, *d*, *J* = 6.2 Hz), 0.93 (3H, *d*, *J* = 6.9 Hz), 0.90 (3H, *d*, *J* = 6.9 Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1; HREIMS *m*/*z* (rel. int.): 220.1824 (12) [M^+–CH_3COOH, calc. for 220.1827, $C_{15}H_{24}O$], 202 (45), 187 (10), 177 (16), 159 (52), 132 (100).

3.26. 3α,15-Dihydroxy cedrane (32)

Colorless oil. $[\alpha]_D$ −7.4 (*c* 1.4, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3568 (*br*), 3420 (*br*), 2947, 2872, 1460 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 3.67 (1H, *d*, *J* = 10.8 Hz), 3.59 (1H, *d*, *J* = 10.8 Hz), 1.13 (3H, *s*), 1.01 (3H, *s*), 0.86 (3H, *d*, *J* = 7.3 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3; HREIMS *m*/*z* (rel. int.): 238.1930 (3) [M^+, calc. for 238.1933, $C_{15}H_{26}O_2$], 220 (11), 207 (100), 189 (21), 177 (10), 150 (25), 135 (14).

3.27. (+)-β-Cedrene (33) (ex Fluka, Cat. No. 22134)

^1H ($CDCl_3$) δ (ppm): 4.58 (1H, *t*, *J* = 2.2 Hz), 4.51 (1H, *t*, *J* = 2.2 Hz), 2.19 (1H, *d*, *J* = 4.4 Hz), 0.97 (3H, *s*), 0.94 (3H, *s*), 0.85 (3H, *d*, *J* = 6.9 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3.

3.28. (−)-α-Cedrene (34) (ex Fluka, Cat. No. 22133)

^1H ($CDCl_3$) δ (ppm): 5.22 (1H, *s*), 2.17 (1H, *d quin*, *J* = 16.4, 2.5 Hz), 1.02 (3H, *s*), 0.95 (3H, *s*), 0.84 (3H, *d*, *J* = 7.2 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3.

3.29. (−)-epi-Cedrol (35) (ex Fluka, Cat. No. 45310)

^1H ($CDCl_3$) δ (ppm): 1.90 (1H, *d*, *J* = 11.3 Hz), 1.31 (3H, *s*), 1.13 (3H, *s*), 1.00 (3H, *s*), 0.84 (3H, *d*, *J* = 7.5 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3.

3.30. (+)-Cedrol (36) (ex Fluka, Cat. No. 22135)

^1H ($CDCl_3$) δ (ppm): 1.32 (3H, *s*), 1.26 (3H, *s*), 1.00 (3H, *s*), 0.84 (3H, *d*, *J* = 7.2 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3.

3.31. Conversion of (+)-β-cedrene (33) to 3α,15-Dihydroxy cedrane (32)

To a solution of (+)-β-cedrene (**33**) (47 mg, 0.23 mmol) in $^tBuOH/H_2O$ (1.5 ml/1.5 ml) was added succesively, K_3FeCN_6 (0.228 g/0.69 mmol), K_2CO_3 (0.095 g; 0.69 mmol) and OsO_4 in $^tBuOH/H_2O$ (0.06 ml from a a stock solution prepared from OsO_4 (1g; 3.9 mmol) in ^tBuOH (80 ml) containing several drops of ^tBuOOH). The reaction was stirred overnight, then Na_2SO_3 (58 mg) was added, and stirring continued for a further 2 h, before concentrating to dryness under reduced pressure. The residue was extracted by $CHCl_3$ (3 × 20 ml) and the combined organic layers washed with brine (3 × 15 ml), dried ($MgSO_4$) and solvent removed on a rotary evaporator to yield a crude product which was separated by HPLC (30% EtOAc/*n*-hexane) into compounds **32** (14 mg, R_t 64.0 min) and **37** (2 mg, R_t 19.3 min). The physical properties for **32** obtained from synthesis were identical with those of the natural product which was isolated from the seeds of *A. annua*.

3.32. 15-Nor-3-oxo cedrane (37)

Colorless oil. $[\alpha]_D$ +72.5 (*c* 4.2, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3011, 2959, 2872, 1693, 1458 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 2.49 (1H, *dd*, *J* = 18.3, 7.9 Hz), 2.33 (2H, *m*), 1.02 (3H, *s*), 1.01 (3H, *s*), 0.90 (3H, *d*, *J* = 6.8 Hz)—see also Table 3; ^{13}C ($CDCl_3$) δ (ppm)—see Table 3; HREIMS *m*/*z* (rel. int.): 206.1675 (100) [M^+, calc. for 206.1671, $C_{14}H_{22}O$], 191 (14), 188 (35), 177 (4), 173 (11), 163 (35), 147 (26), 136 (6).

3.33. 4-Hydroxy-2-isopropenyl-5-methylene-hexan-1-ol (38)

Colorless oil. $[\alpha]_D$ −9.3 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3406 (*br*), 3011, 2937, 2854, 1454 cm^{-1}; ^1H ($CDCl_3$) δ (ppm): 4.97 (1H, *s*), 4.94 (1H, *t*, *J* = 1.6 Hz), 4.87 (1H, *s*), 4.83 (1H, *t*, *J* = 1.1 Hz), 4.07 (1H, *dd*, *J* = 9.6, 3.2 Hz), 3.59 (1H, *dd*, *J* = 10.5, 6.9 Hz), 3.58 (1H, *dd*, *J* = 10.5, 6.8 Hz), 2.53 (1H, *dddd*, *J* = 7.3, 7.3, 6.9, 6.8 Hz), 1.74 (3H, *s*), 1.73 (3H, *s*)—see also Table 4; ^{13}C ($CDCl_3$) δ (ppm)—see Table 4; EIMS *m*/*z* (rel. int.): 152 (100) [M^+–H_2O], 139 (18), 127 (20), 115 (14).

3.34. Preparation of 38 by reduction of a lavandulane secondary allylic hydroperoxide in the presence of triphenylphosphine

The secondary allylic hydroperoxide derivatives of lavandulol (**43**), compounds **44** and **45**, were prepared by photo-autoxidation [together with the tertiary allylic hydroperoxide (**46**)] and separated from one another as described in Sy and Brown (2001a). To a solution of the more polar of the two secondary hydroperoxides, compound **44** (25.8 mg—identical with compound **15** in Sy and Brown, 2001a), in MeOH (5 ml), was added triphenylphosphine (36.4 mg). The reaction mixture was stirred at room temperature until reduction was complete, as indicated by TLC, and the solution was then evaporated to dryness under reduced pressure and the

crude product (62.2 mg) was subjected to HPLC (50% EtOAc/*n*-hexane/1% HOAc), yielding a single compound (R_t 32.2 min) with physical properties identical to those of the natural product **38** from *A. annua*.

Reduction of the less polar hydroperoxide, compound **45** (identical with compound **14** in Sy and Brown, 2001a), by the same procedures resulted in a single compound (R_t 31.6 min) with slightly different NMR spectra to those of compound **38**, which was believed to be diastereoisomeric with **38** at the 4-position. ^{1}H ($CDCl_3$) δ (ppm): 4.96 (1H, *s*), 4.93 (1H, *t*, $J=1.4$ Hz), 4.87 (1H, *s*), 4.84 (1H, *s*), 4.15 (1H, *t*, $J=6.5$ Hz), 3.57 (2H, *d*, $J=6.6$ Hz), 2.35 (1H, *m*), 1.73 (6H, *s*); ^{13}C ($CDCl_3$) δ (ppm): 146.9 (C), 145.6 (C), 113.4 (CH_2), 111.9 (CH_2), 74.2 (CH), 64.2 (CH_2), 46.0 (CH), 34.8 (CH_2), 19.8 (CH_3), 17.4 (CH_3).

3.35. 1,10-Oxy-α-myrcene hydroxide (39)

Colorless oil. $[\alpha]_D$ −4.7 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3427 (*br*), 3003, 2922, 2862, 1591, 1454 cm^{-1}; ^{1}H ($CDCl_3$) δ (ppm): 5.68 (1H, *s*), 4.96 (1H, *s*), 4.87 (1H, *s*), 4.57 (2H, *s*), 4.48 (2H, *s*), 4.10 (1H, *t*, $J=7.0$ Hz), 1.74 (3H, *s*)—see also Table 4; ^{13}C ($CDCl_3$) δ (ppm)—see Table 4; HREIMS *m*/*z* (rel. int.): 167.1073 (30) [M^+–H, calc. for 167.1702, $C_{10}H_{15}O_2$], 151 (100).

3.36. 1,10-Oxy-β-myrcene hydroxide (40)

Colorless oil. $[\alpha]_D$ −22.2 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3435 (*br*), 2997, 2928, 2856, 1456 cm^{-1}; ^{1}H ($CDCl_3$) δ (ppm): 5.69 (1H, *d*, $J=16.0$ Hz), 5.68 (1H, *m*), 5.60 (1H, *m*), 4.56 (2H, *s*), 4.44 (2H, *s*), 2.75 (2H, *d*, $J=6.9$ Hz), 1.32 (6H, *s*)—see also Table 4; ^{13}C ($CDCl_3$) δ (ppm)—see Table 4; HREIMS *m*/*z* (rel. int.): 167.1071 (100) [M^+–H, calc. for 167.1072, $C_{10}H_{15}O_2$], 151 (11).

3.37. Phytene-1,2-diol (41)

Colorless oil. IR ($CHCl_3$) ν_{max}: 3400 (*br*), 3018, 2930, 1450 cm^{-1}; ^{1}H ($CDCl_3$) δ (ppm): 5.13 (1H, *s*), 4.98 (1H, *s*), 4.20 (1H, *dd*, $J=6.8$, 2.7 Hz) 3.71 (1H, *d*, $J=11.4$ Hz), 3.54 (1H, *dd*, $J=11.4$, 6.8 Hz), 0.87 (6H, *d*, $J=6.9$ Hz), 0.86 (3H, *d*, $J=6.9$ Hz), 0.84 (3H, *d*, $J=6.6$ Hz)—see also Table 4; ^{13}C ($CDCl_3$) δ (ppm)—see Table 4; HREIMS *m*/*z* (rel. int.): 312.3035 (10) [M^+, calc. for 312.3028, $C_{20}H_{40}O_2$], 294 (5), 281 (18), 263 (14), 222 (10), 199 (13), 179 (16), 165 (30), 109 (100).

3.38. Artemisinin G (50)

^{1}H ($CDCl_3$) δ (ppm): 6.64 (1H, *s*), 4.21 (1H, *dd*, $J=8.0$, 8.0 Hz), 3.94 (1H, *ddd*, $J=8.0$, 8.0, 8.0 Hz), 3.16 (1H, *dq*, $J=12.3$, 7.3 Hz), 2.16 (3H, *s*), 1.21 (3H, *d*, $J=7.3$ Hz), 0.99 (3H, *d*, $J=6.6$ Hz)—see also Table 2; ^{13}C ($CDCl_3$) δ (ppm)—see Table 1. See Wei et al., 1992, for other physical properties.

3.39. Chrysosplenol D (51)

Colourless oil. IR ($CHCl_3$) ν_{max}: 3523 (*br*), 3018, 2934, 2856, 1653, 1597, 1559, 1458 cm^{-1}; ^{1}H ($CDCl_3$) δ (ppm): 12.52 (1H, s, 5-OH), 7.77 (1H, *s*, H-2′), 7.58 (1H, *d*, $J=8.8$ Hz, H-6′), 7.01 (1H, *d*, $J=8.8$ Hz, H-5′), 6.50 (1H, *s*, H-8), 3.94 (3H, *s*, 7-OMe), 3.91 (3H, *s*, 6-OMe), 3.82 (3H, *s*, 3-OMe); ^{13}C ($CDCl_3$) δ (ppm): 178.9 (C) [C-4], 158.8 (C) [C-7], 156.4 (C) [C-2], 152.6 (C) [C-5], 152.4 (C) [C-8a], 147.5 (C) [C-4′], 144.1 (C) [C-3′], 138.6 (C) [C-3], 132.7 (C) [C-6], 121.8 (CH) [C-6′], 115.6 (CH) [C-2′], 115.4 (CH) [C-5′], 106.5 (C) [C-4a], 90.4 (CH) [C-8], 60.9 (CH_3) [6-OMe], 60.1 (CH_3) [3-OMe], 56.3 (CH_3) [7-OMe].

3.40. Casticin (52)

Colourless oil. IR ($CHCl_3$) ν_{max}: 3544 (*br*), 3018, 2972, 2939, 1652, 1597, 1558, 1458 cm^{-1}; ^{1}H ($CDCl_3$) δ (ppm): 12.59 (1H, *s*, 5-OH), 7.73 (1H, *dd*, $J=8.7$, 2.3 Hz, H-6′), 7.68 (1H, *d*, $J=2.3$ Hz, H-2′), 6.97 (1H, *d*, $J=8.7$ Hz, H-5′), 6.51 (1H, *s*, H-8), 3.99 (3H, *s*, 4′-OMe), 3.96 (3H, *s*, 7-OMe), 3.93 (3H, *s*, 6-OMe), 3.87 (3H, *s*, 3-OMe); ^{13}C ($CDCl_3$) δ (ppm): 179.0 (C) [C-4], 158.8 (C) [C-7], 155.7 (C) [C-2], 152.7 (C) [C-5], 152.4 (C) [C-8a], 148.8 (C) [C-4′], 145.6 (C) [C-3′], 139.0 (C) [C-3], 132.3 (C) [C-6], 123.6 (C) [C-1′], 121.6 (CH) [C-6′], 114.4 (CH) [C-2′], 110.4 (CH) [C-5′], 106.6 (C) [C-4a], 90.4 (CH) [C-8], 60.9 (CH_3) [6-OMe], 60.2 (CH_3) [3-OMe], 56.3 (CH_3) [7-OMe], 56.1 (CH_3) [4′-OMe]. HREIMS *m*/*z* (rel. int.): 374.1010 (100) [M^+, calc. for $C_{19}H_{18}O_8$, 374.1002], 373 (33), 359 (25).

Acknowledgements

We thank the Generic Drug Research Programme of the Chemistry Department of The University of Hong Kong for providing a postdoctoral fellowship to Dr. L.-K. Sy. This project was funded by a grant from the Committee on Conference and Research Grants (CRCG). Professor G.-W. Qin of the Shanghai Institute of Materia Medica helped to organize this collaboration between Prof. Liang (who collected the seeds of *A. annua*) and Dr. Brown. Dr. Nian-Yong Zhu of the Chemistry Department of the University of Hong Kong solved the X-ray structure of arteanuin M.

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