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THREE SESQUITERPENES FROM *ARTEMISIA ANNUA*

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Key Word Index—*Artemisia annua*; Compositae; sesquiterpenes; cadinane; eudesmane; flavanoids.

Abstract—The dried leaves of *Artemisia annua*, collected from Sichuan Province in Southern China, have yielded an unusual cadinane sesquiterpene oxygenated at the 7-position and a novel eudesmane sesquiterpene, in addition to several known sesquiterpenes and flavanoids.

INTRODUCTION

Artemisia annua L. has been the subject of intensive phytochemical investigation following the discovery of the anti-malarial drug artemisinin. Artemisinin is a cadinane sesquiterpene oxygenated at the 12-position: a further 17 compounds sharing this structural feature have now been described as natural products [1–17] from this species. Recently, two cadinanes which were not oxygenated at the 12-position have also been obtained from *A. annua* [18]. We now report another such cadinane in addition to a new eudesmane sesquiterpene and the methyl ester of arteannuic acid.

RESULTS AND DISCUSSION

Extraction of the dried leaves of *A. annua* followed by CC and HPLC yielded three novel sesquiterpenes. The molecular formula for the cadinane sesquiterpene **1** was determined by HREIMS as $C_{15}H_{26}O_2$ and the location of the allylic 3-hydroxy and 7-hydroxy groups in the cadinane skeleton were rigorously established by 1D (^{1}H, ^{13}C/DEPT) and 2D-NMR techniques (HSQC, HMBC, COSY) (Table 1). The resulting complete NMR assignments for all ^{13}C and ^{1}H resonances in **1** were then particularly useful in establishing the relative stereochemistry from correlations observed in NOESY spectra (Table 1).

Although a large variety of cadinanes have ben reported from *A. annua*, compound **1** is unique in that it is oxygenated at the 7-position rather than the 12-position, as found for most other natural products isolated from this species. Interestingly, an isobutyryl derivative of the 11-hydroxy analogue of **1** has been recently reported from *A. annua* [18]. 7- and 11-Hydroxy cadinanes are extremely rare in nature, although several examples of both types are known from the Chilean plant *Fabiana imbricata* [19], whose phytochemistry closely resembles that of *A. annua* in other aspects, such as the occurrence of 4,5-*seco*-cadinanes [20].

Compound **2** is a eudesmane incorporating an allylic tertiary hydroxide group as shown by the results of HREIMS and 1D/2D-NMR (Table 2). There has only been one previous report of a eudesmane sesquiterpene (*trans*-β-selinene) from *A. annua* which was isolated from a specimen growing in the United Kingdom [15]. Compound **2** is formally the 5-hydroxy derivative of *trans*-β-selinene and the NMR data for **2** gave good agreement with published data for other 5α-hydroxyeudesmanes [21–25].

Compound **3** is the methyl ester of arteannuic acid, which has been obtained previously by synthesis from arteannuic acid [26, 27], but is now recorded for the first time as a natural product. In addition to these three novel sesquiterpenes, the extract yielded a number of known cadinanes. Amongst these was 3α-hydroxydesoxyartemisinin (**4**) which has been reported previously from *A. annua* [6] and recorded as a microbial metabolite of artemisinin and β-arteether [28, 29]. Full ^{1}H and ^{13}C NMR assignments for both **3** and **4**, assigned using the same methodology as for compounds **1** and **2**, are reported in Table 3. A number of other cadinane sesquiterpenes were also isolated including artemisinin, arteannuin B, dihydroarteannuin B, artemisinic acid and dihydroartemisinic acid; rather unusually, the dihydro compounds were present in much greater abundance than their 11, 13-dehydro analogues in this sample.

Six known flavanoids were also isolated from the CH_2Cl_2 extract (artemetin (**5**), chrysoplenetin (**6**), 5-hydroxy-3,4′,6,7-tetramethoxyflavone (**7**), penduletin (**8**), retusin (**9**) and pachypodol (**10**)). Previous

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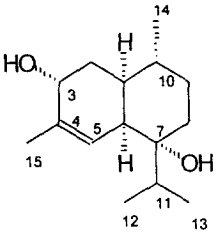

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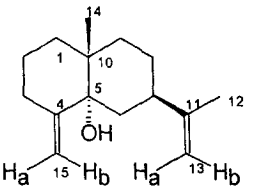

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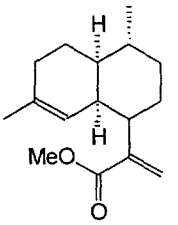

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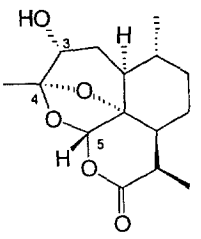

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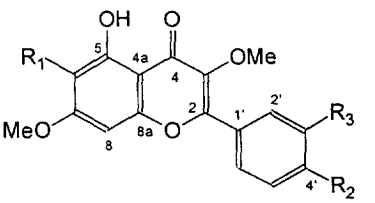

5 R_1 = OMe; R_2 = OMe; R_3= OMe
6 R_1 = OMe; R_2 = OH; R_3= OMe
7 R_1 = OMe; R_2 = OMe; R_3= H
8 R_1 = OMe; R_2 = OH; R_3= H
9 R_1 = H; R_2 = OMe; R_3= OMe
10 R_1 = H; R_2 = OH; R_3= OMe

Table 1. NMR assignments for compound **1**

Atom	δ_C	δ_H	HMBC	^1H–^1H COSY	NOESY
1	39.4	1.84	5.31, 0.94	—	2.04, 0.94
2α	36.3	2.41	—	4.08, 1.40	4.08, 1.40, 0.94
2β		1.40		4.08, 2.41	4.08, 2.41
3	68.2	4.08	5.31, 1.76	2.41, 1.40	2.41, 1.76, 1.40
4	137.2	—	1.76	—	—
5	123.8	5.31	1.76	2.40, 1.76	2.40, 1.86, 1.76, 0.89
6	44.4	2.40	—	5.31	5.31, 1.84, 0.89
7	74.7	—	0.89, 0.93	—	—
8α	31.9	1.69	—	1.42, 1.11	1.11, 0.93
8β		1.11		1.69, 1.42	1.69
9	30.1	1.42	0.94	1.69, 1.11	—
		1.30		—	—
10	28.3	1.36	0.94	0.94	0.94
11	33.1	1.86	0.93, 0.89	—	5.31, 0.93, 0.89
12*	16.1	0.93	0.89	1.86	1.86, 1.69
13*	15.6	0.89	0.93	1.86	5.31, 2.40, 1.86
14	19.5	0.94	—	1.36	2.41, 1.84, 1.36
15	19.8	1.76	5.31	5.31	5.31, 4.08

* Assignments interchangeable.

Table 2. NMR assignments for compound **2**

Atom	δ_C	δ_H	HMBC	^1H–^1H COSY	NOESY
1α	35.0	1.06	—	1.87, 1.66	1.87, 1.61
1β		1.87		1.06	1.66, 1.61, 1.06
2α	22.2	1.61	—	2.61, 2.12	2.61, 2.12, 1.87, 1.06
2β		1.66		2.61, 2.12, 1.06	2.12, 1.87
3α	31.7	2.61	4.82, 4.69	2.12, 1.66, 1.61	2.12, 1.61
3β		2.12		2.61, 1.66, 1.61	4.82, 2.61, 1.66, 1.61
4	151.9	—	—	—	—
5	75.8	—	4.82, 4.69, 0.88	—	—
6α	35.4	1.77		2.53	4.74, 4.69, 2.53
6β		1.55		2.53	4.74, 4.69
7	40.0	2.53	4.74, 4.72, 1.76	1.77, 1.55	4.74, 1.77, 1.58
8α	26.0	1.58	—		2.53
8β		1.52		1.20	1.76
9α	34.3	1.81	0.88	1.20	1.20
9β		1.20		1.81, 1.52	1.81
10	37.7	—	0.88	—	—
11	150.5	—	1.76	—	—
12	21.1	1.76	4.74, 4.72	—	4.72, 1.52, 0.88
13a	108.5	4.74	1.76	—	2.53, 1.77, 1.55
13b		4.72			1.76
14	19.9	0.88	—	—	4.69, 1.76
15a	107.6	4.82	—	—	4.69, 2.12
15b		4.69			4.82, 1.77, 1.55, 0.88

Table 3. NMR assignments for compounds **3** and **4**

	δ_C		δ_H	
Atom	**3**	**4**	**3**	**4**
1	41.4	40.6	1.43	1.55
2	25.6	30.3	1.93, 1.53	1.99, 1.53
3	26.6	69.1	1.87, 1.76	3.63
4	134.9	108.8	—	—
5	120.3	98.9	4.99	5.64
6	37.9	82.7	2.54	—
7	42.3	42.1	2.72	2.07
8	25.9	23.5	1.40, 1.31	1.95, 1.00
9	35.2	33.4	1.71, 1.07	1.82, 1.10
10	27.6	35.2	1.44	1.29
11	143.5	32.7	—	3.19
12	167.9	171.5	—	—
13	124.2	12.6	6.28, 5.43	1.20
14	19.8	18.4	0.90	0.93
15	23.7	20.5	1.59	1.58
-OMe	51.8		3.74	

phytochemical investigations of *A. annua* have resulted in the characterization of some 33 flavanoids of which we are aware [30–41]. Of the six flavanoids isolated in this study, retusin and pachypodal are reported for the first time from this species. Fully assigned NMR data (using 2D-NMR methodology) for all six flavanoids is given in Table 4 (partially assigned ^{13}C NMR data has been provided previously for pachypodol [42, 43] and artemetin [44, 45], and partially assigned ^1H NMR data has been reported for penduletin [46] and chrysoplenetin [47]).

EXPERIMENTAL

Methods

NMR: Bruker DRX 500 instrument, chemical shifts expressed in ppm (δ) relative to TMS as int. standard. Two dimensional spectra were recorded with 1024 data points in F_2 and 256 data points in F_1; HREIMS: 70 eV on a Finnigan-MAT 95 MS spectrometer; IR: $CHCl_3$. TLC plates developed using *p*-anisaldehyde; CC: silica gel 60–200 μm (Merck); HPLC: PREP-SIL 20 mm × 25 cm column, flow rate 8 ml/min.

Plant material

Leaves of *A. annua* were collected from the mountains in You Yang county in Sichuan Province, Southern China in July 1996 and dried in the sun. A voucher specimen is deposited in the University of Hong Kong herbarium (GDB97/3).

Extraction and isolation

The dried leaves (1 kg) were pulverized to a fine powder under liq. N_2, repetitively extracted with CH_2Cl_2, dried and solvent removed under red. pres. to yield a dark green gum (97 g). A portion of the extract (25 g) was subjected to gradient CC (developing solvents 100% hexane to 100% EtOAc) and crude

Table 4. NMR assignments for flavanoids **5–10** (in $CDCl_3$)

	δ_C						δ_H					
Atom	**5**	**6**	**7**	**8**	**9**	**10**	**5**	**6**	**7**	**8**	**9**	**10**
2	155.9	156.0	156.0	155.9	155.9	156.0	—	—	—	—	—	—
3	138.9	138.7	138.8	138.8	139.1	138.6	—	—	—	—	—	—
4	178.9	178.9	178.8	179.0	178.8	178.8	—	—	—	—	—	—
4a	106.7	106.6	107.6	107.7	107.8	107.8	—	—	—	—	—	—
5	152.9	152.8	152.8	152.8	162.1	162.1	—	—	—	—	—	—
6	132.4	132.4	132.4	132.4	97.9	97.9	—	—	—	—	6.36[e]	6.35[e]
7	158.8	158.8	158.8	158.8	165.5	165.5	—	—	—	—	—	—
8	90.4	90.4	90.4	90.4	92.3	92.2	6.51	6.51	6.51	6.51	6.45[e]	6.44[e]
8a	152.4	152.3	152.4	152.4	156.8	156.8	—	—	—	—	—	—
1′	123.0	122.5	122.9	123.1	123.0	122.5	—	—	—	—	—	—
2′	111.5	111.0	130.2	130.4	111.4	111.0	7.69[b]	7.70[b]	8.07[d]	8.03[d]	7.69[b]	7.70[b]
3′	148.9	146.4	114.1	115.7	148.9	146.4	—	—	7.03[d]	6.97[d]	—	—
4′	151.5	148.4	161.7	158.1	151.5	148.4	—	—	—	—	—	—
5′	111.0	114.6	114.1	115.7	111.0	114.6	6.99[c]	7.05[c]	7.03[d]	6.97[d]	6.99[c]	7.04[c]
6′	122.2	122.6	130.2	130.4	122.2	122.7	7.73[a]	7.66[a]	8.07[d]	8.03[d]	7.74[a]	7.67[a]
3-OMe	60.2	60.2	60.2	60.2	60.2	60.2	3.86	3.86	3.87	3.86	3.87	3.86
6-OMe	60.9	60.9	60.9	60.9	—	—	3.93	3.93	3.93	3.93	—	—
7-OMe	56.4	56.3	56.3	56.3	55.8	55.8	3.97	3.97	3.96	3.96	3.89	3.88
3′-OMe	56.0	56.1	—	—	56.1	56.2	3.98	3.99	—	—	3.97	3.98
4′-OMe	56.2	—	55.5	—	56.0	—	3.98	—	3.90	—	3.98	—

a–dd, J = 8.5, 2.0 Hz; *b–d*, J = 2.0 Hz; *c–d*, J = 8.5 Hz; *d–d*, J = 8.1 Hz; *e–d*, J = 2.2 Hz

fractions further purified by HPLC to yield **1** (11.1 mg), **2** (3.5 mg) and **3** (5 mg).

3α,7α-Dihydroxy-cadin-4-ene (**1**). Oil. $[\alpha]_D$ +71.5° (*c* 0.07, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1} 3612, 3443 (br), 3013, 2970, 2873, 1705; ^1H NMR ($CDCl_3$): δ 5.31 (1H, *s*), 4.08 (1H, *br m*), 2.40 (2H, *br m*), 1.76 (3H, *s*), 0.94 (3H, *d*, J = 6.7 Hz), 0.93 (3H, *d*, J = 7.4 Hz), 0.89 (3H, *d*, J = 7.4 Hz); HREIMS *m/z* (rel. int.): 238.1926 [M^+, Δ = 0.7 mmu for $C_{15}H_{26}O_2$] (20), 220 (30), 204 (11), 202 (7), 195 (21), 177 (21), 159 (17), 132 (100).

5α-Hydroxy-eudesma-4(15),11-diene (**2**). Oil $[\alpha]_D$ +80.0° (*c* 0.3, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1} 3607, 3428 (br), 3011, 2930, 2856, 1643; ^1H NMR ($CDCl_3$): δ 4.82 (1H, *s*), 4.74 (1H, *s*), 4.72 (1H, *s*), 4.69 (1H, *s*), 2.61 (1H, *ddd*, J = 13.3, 13.3, 6.2 Hz), 2.53 (1H, *m*), 1.76 (3H, *s*), 0.88 (3H, *s*); HREIMS *m/z* (rel. int.): 220.1821 [M^+, Δ = 0.6 mmu for $C_{15}H_{24}O$] (16), 205 (38), 202 (57), 187 (100), 177 (18), 162 (12), 137 (24), 108 (21), 95 (27).

Artemisinic acid methyl ester (**3**). Oil, $[\alpha]_D$ +6.2° (*c* 1.45, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1} 3009, 2928, 2856, 1717; ^1H NMR ($CDCl_3$): δ 6.28 (1H, *s*), 5.43 (1H, *s*), 4.99 (1H, *s*), 3.74 (3H, *s*), 2.72 (1H, *m*), 2.54 (1H, *br s*), 1.59 (3H, *s*), 0.90 (3H, *d*, J = 6.6 Hz); HREIMS *m/z* (rel. int.): 248.1774 [M^+, Δ = 0.2 mmu for $C_{16}H_{24}O_2$] (57), 216 (29), 188 (31), 162 (17), 136 (31), 121 (100), 119 (60), 105 (23), 93 (41).

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