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SESQUITERPENE LACTONES OF *ARTEMISIA MEXICANA* VAR. *ANGUSTIFOLIA*

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Key Word Index—*Artemisia mexicana* var. *angustifolia*; Compositae; sesquiterpene lactones; 8α-acetoxyarmexifolin; α-epoxyludalbin; armefolin.

Abstract—Seven eudesmanolides were isolated and characterized from *Artemisia mexicana* var. *angustifolia*. These included arglanin, artemexifolin, ludalbin, santamarine and three new compounds named 8α-acetoxyarmexifolin, α-epoxyludalbin and armefolin. The structure of armexifolin is revised.

INTRODUCTION

In previous phytochemical work with *Artemisia mexicana* L. var. *angustifolia*, we described the isolation and characterization of tulipanolide (**1**), arglanin (**2**) and artemexifolin (**3**). Armexifolin (**10**), was also obtained but its structure was not fully established [1].

Following our studies in search of sesquiterpene lactones, we have analyzed a new collection of the plant, which afforded arglanin (**2**), artemexifolin (**3**), ludalbin (**4**), santamarine (**5**) and three new eudesmanolides **6**, **7** and **8**. Ludalbin (**4**) and santamarine (**5**) have been previously isolated from different species of Compositae, mainly of the tribe Anthemideae [2–6].

RESULTS AND DISCUSSION

Extensive chromatography of a chloroform extract of *Artemisia mexicana* var. *angustifolia* gave seven crystalline eudesmanolides (**2–8**). 8α-Acetoxyarmexifolin (**6**) had the composition $C_{17}H_{20}O_6$, mp 215°. The spectral properties of **6** indicated the presence of an α,β-unsaturated-γ-lactone, an α,β-unsaturated ketone, a hydroxyl and one acetoxy group. The ^{1}H NMR spectrum (Table 1) showed the signal of the C-15 methyl group as a doublet (J = 2 Hz) at δ 2.05 and H-6 appeared as a doublet of quartets (J = 11.3, 2 Hz) at 4.80, thus indicating that 8α-acetoxyarmexifolin (**6**) was an eudesm-4-en-3-one similar to ludovicin C (**9**) [7] and armexifolin (**11**) [1]. The upfield ^{13}C NMR resonance observed (Table 2) for C-15 (δ 11.4) as well as the olefinic singlets at δ 130.2 and 150.2 for C-4 and C-5, respectively, gave further support to this proposition [8]. The *trans*-diaxial disposition of H-6 and H-7 was deduced from the appearance of a doublet of quartets for H-6 (irradiation of the H-7 frequency at δ 3.05 collapsed the signal for H-6 to a quartet, J = 2 Hz). The doublet of doublets at δ 3.87 (J = 10, 6 Hz) which shifted downfield (δ 5.17) after *in situ* formation of the ester upon treatment with trichloroacetyl isocyanate was assigned to H-1. Irradiation of this signal produced change in the δ 2–3 region, where H-2 and H-2′ resonate. The sextet centered at δ 5.25 (J = 11.3, 11.3, 5 Hz) was ascribed to H-8 because decoupling of H-7 collapsed this signal to a quartet (J = 11.3, 5 Hz). Conversely, irradiation of the signal at δ 5.25 simplified the absorption of H-7 at δ 3.05, indicating the β disposition of H-8 since it was axially coupled with H-7. The β disposition at C-1 was established by the observed coupling constants (Table 1), which implied a *trans*-diaxial interaction. The upfield shift of C-14 (δ 18.60) in the ^{13}C NMR spectrum of compound **6** compared to the signal for C-14 in the spectrum dihydro-α-santonine (δ 23.48) [8], confirmed the β-orientation of the hydroxyl group at C-1.

α-Epoxyludalbin (**7**), $C_{17}H_{22}O_6$, mp 185°, contained a hydroxyl group, an α,β-unsaturated-γ-lactone and a *trans*-fused C-6/C-7 lactone according to its UV, IR and ^{1}H NMR spectra. Furthermore, the doublet at δ 60.4 (C-3) and the singlet at δ 58.4 (C-4) observed in the ^{13}C NMR spectrum suggested that **7** contained a C-3/C-4 epoxide ring. The pattern for the C-1 and C-8 protons were similar to those observed for the respective signals of the equivalent protons in ludovicin A and rothin B acetate [9] and are therefore assigned as shown in **7**. The assignment of the hydroxyl group at C-1 as α is supported by the chemical shift of the C-14 methyl (δ 19.3) in the ^{13}C NMR spectrum [10]. Final proof of the structure **7** for epoxyludalbin was provided by correlation with **3** and **4** since epoxidation of **4** afforded the epoxide **7** which, upon oxidation with CrO_3–pyridine, yielded **3**.

Armefolin (**8**) had the composition $C_{15}H_{20}O_4$, mp 175°. The IR spectrum showed a hydroxyl group absorption at 3600 cm^{-1} and bands at 1765 and 1630 cm^{-1} corresponding to an exocyclic methylene conjugated with a γ-lactone. The ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) confirmed these assignments. In particular the spectra demonstrated the *trans*-disposition of the C-6/C-7 lactone (H-6 at δ 4.57, *dq*, J = 11.3, 2 Hz), the presence of two carbinolic carbons (signals at δ 3.85, *t*, J = 8 Hz and at δ 3.97, *t*, J = 3 Hz in the ^{1}H NMR spectrum; resonances at δ 70.6, *d* and at δ 71.7, *d* in the ^{13}C NMR spectrum) and the presence of a C-4/C-5 double

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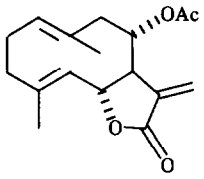

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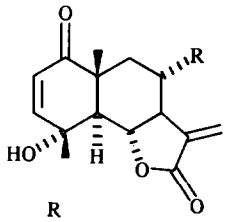

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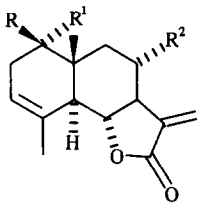
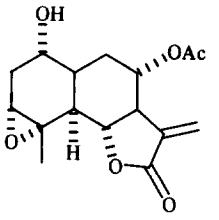
3 R = OAc



4 R = H, R^1 = OH, R^2 = OAc
5 R = OH, R^1 = R^2 = H



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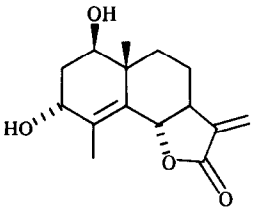

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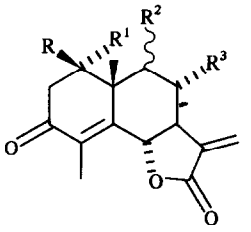

6 R = OH, R^1 = R^2 = H, R^3 = OAc
9 R = R^2 = R^3 = H, R^1 = OH
10 R = R^1 = R^3 = H, R^2 = OH
11 R = OH, R^1 = R^2 = R^3 = H

bond (absence of vinylic protons other than those of the exocyclic methylene group and a doublet at δ 2.02, J = 2 Hz, for the C-15 methyl group in the ^{1}H NMR spectrum; two olefinic singlets at δ 132.9 and 127.3 for C-4 and C-5, respectively, in the ^{13}C NMR spectrum).

The locations of the hydroxyl groups at C-1 and C-3, were established by means of decoupling experiments. Irradiation of either resonances at δ 3.82 or at δ 3.97 changed only the δ 1.87 region, thus indicating that there was a unique vicinal methylene grouping to both carbinolic protons and this was only possible for the C-2 methylene. Additional chemical evidence was obtained by oxidation of **8** with CrO_3–pyridine complex at low temperature. This oxidation afforded ketone **11** whose IR and ^{1}H NMR spectra were identical to those of armexifolin, whose structure was previously proposed as **10** [1].

The ^{1}H NMR spectrum of **11** (Table 1) was very similar to those of 8α-acetoxyarmexifolin (**6**) and ludovicin C (**9**) [7], differing from the former only in the additional signal for the acetoxyl group and from the latter in the signal for the 1-hydroxyl group. H-1 appeared as a doublet of doublets (δ 3.87, J = 11, 7 Hz) in the ^{1}H NMR spectrum; irradiation of this signal caused the collapse of the resonances observed in the δ 2.5–2.7 region, to a well defined singlet at δ 2.60 (H-2). This experiment provided additional evidence of the 1,3 relationship of the hydroxyl group in **8**, since it showed that the selective oxidation of the allylic hydroxyl group caused, as expected, the downfield shift of the C-2 methylene protons from δ 1.87 in **8** to δ 2.60 in **11**. Therefore, the hydroxyl group in armexifolin is at C-1 rather than at C-9 as initially proposed [1]. The stereochemistry of the C-1 hydroxyl group of **11**, and thus in **8**, was determined as β following the same reasoning as for 8α-acetoxyarmexifolin (**6**). In addition, the coupling constant values of H-1 in ludovicin C (**9**), which has the hydroxyl group α-oriented, were different to those of **11**. The stereochemistry at C-3 was established as

Table 1. ^{1}H NMR spectra of compounds **6–8**, **11** and **6** plus trichloroacetyl isocyanate (TAI) (100 MHz, $CDCl_3$, TMS as internal standard)*

	6	6+TAI	7	8	11
H-1	3.87 *dd* (10, 6)	5.17 *dd* (10, 6)	3.20 *t* (3)	3.82 *t* (8)	3.87 *dd* (11, 7)
H-2	—	—	—	1.87 *m*	2.60*m*
H-3	—	—	3.00 *t* (1.5)	3.98 *t* (3)	—
H-5	—	—	2.42 *d* (11.2)	—	—
H-6	4.80 *dq* (11.3, 2)	4.80 *dq* (11.3, 2)	4.00 *t* (11.3)	4.55 *dq* (11.3, 2.0)	4.27 *dq* (11, 2.0)
H-7	3.05 *tt* (11, 2.0)	3.05 *tt* (11, 2.0)	2.77 *tt* (11.3, 2.0)	2.63 *m*	2.75 *m*
H-8	5.25 *ddd* (11.3, 11.3, 5)	5.25 *ddd* (11.3, 11.3, 5)	5.20 *m* (11.3, 8, 2.6)	—	—
H-13a	6.27 *d* (3.0)	6.27 *d* (3.0)	6.11 *d* (3.0)	6.3 *d* (3.0)	6.22 *d* (3.0)
H-13b	5.67 *d* (3.0)	5.67 *d* (3.0)	5.50 *d* (2.8)	5.46 *d* (2.8)	5.5 *d* (2.8)
H-15	2.05 *d*† (1.8)	2.05 *d*† (1.8)	1.50 *s*†	2.02 *d*† (2.0)	2.06 *d*† (1.8)
H-14	1.35 *s*†	1.47 *s*†	0.90 *s*†	1.07 *s*†	1.30 *s*†
H-16	2.15 *s*†	2.15 *s*†	2.10 *s*†	—	—
OH‡	3.87	—	3.25	3.75	3.76

*Figures in parentheses are coupling constants in Hz.
†Intensity three protons.
‡Disappeared after equilibration with D_2O.

Table 2. ^{13}C NMR spectra of compounds **2–4**, **6–8** and **11** (20.0 MHz, $CDCl_3$, TMS as internal standard)

C	2	3	4	6	7	8	11
1	201.4 *s*	200.3 *s*	72.2 *d*	74.2 *d*	72.2 *d*	70.6 *d*	74.4 *d*
2	125.4 *d*	124.1 *d*	32.1 *t*	42.3 *t**	29.10 *t*	38.0 *t**	42.6 *t*
3	152.0 *d*	153.4 *d*	119.6 *d*	196.6 *s*	60.4 *d*	71.8 *d*	196.7 *s*
4	70.0 *s*	69.4 *s*	132.7 *s*	130.2 *s*	58.4 *s*	132.9 *s*	129.6 *s*
5	55.1 *d*	53.9 *d*	43.8 *d*	150.2 *s*	45.6 *d*	127.4 *s*	152.2 *s*
6	79.7 *d*	76.9 *d*	79.6 *d*	78.5 *d*	78.5 *d*	82.7 *d*	82.0 *d*
7	49.7 *d*	52.2 *d*	53.6 *d*	50.8 *d*	53.5 *d*	49.4 *d*	48.8 *d*
8	21.17 *t*	68.7 *d*	69.6 *d*	69.9 *d*	69.1 *d*	22.9 *t*	23.2 *t*
9	34.0 *t*	40.4 *t*	40.0 *t*	44.1 *t**	39.8 *t*	36.3 *t**	38.0 *t*
10	46.4 *s*	45.6 *s*	40.4 *s*	43.2 *s*	39.9 *s*	42.5 *s*	43.9 *s*
11	169.5 *s*	169.8 *s*	170.2 *s*	170.0 *s*	170.1 *s*	169.8 *s*	169.0 *s*
12	137.9 *s*	135.9 *s*	136.8 *s*	135.3 *s*	136.2 *s*	139.1 *s*	137.9 *s*
13	118.2 *t*	120.0 *t*	118.9 *t*	122.3 *t*	119.4 *t*	118.2 *t*	119.6 *t*
14	23.7 *q*	23.0 *q*	18.4 *q*	18.6 *q*	19.3 *q*	17.5 *q**	17.6 *q*
15	19.7 *q*	20.4 *q*	23.5 *q*	11.0 *q*	22.1 *q*	17.3 *q**	11.0 *q*
16	—	20.9 *q*	20.9 *q*	20.9 *q*	20.9 *q*	—	—
17	—	169.03 *s*	169.9 *s*	168.2 *s*	169.2 *s*	—	—

*Assignments may be interchanged.

α on the basis of the coupling pattern observed for H-3 (Table 1). Arglanin (**2**), artemexifolin (**3**), ludalbin (**4**) and santamarine (**5**) were characterized by direct comparison.

The absence of tulipanolide (**1**) and armexifolin (**11**) in the different fractions of the chloroform extract may be attributed to a seasonal variation of the sesquiterpene lactones of the plant. The co-occurrence of **3**, **4** and **7** is suggestive of their biogenetic inter-relationship. Also the co-existence of **5**, **6** and **8**, all of them bearing a hydroxyl group at C-1, might be indicative of a common biosynthetic pathway. It is of interest to note the presence of 1α- and 1β-hydroxy substituted compounds, which has no precedent in the *Artemisia* genus.

EXPERIMENTAL

Plant material. A. mexicana L. var. *angustifolia* was collected *ca* 4 km SSW of Pachuca, Hidalgo, Hwy 45, in December 1981. Reference specimens are deposited in the National Herbarium, voucher No. MEXU 294323.

Extraction and preliminary fractionation. Dried and shredded aerial parts of the plant (7.1 kg) were exhaustively extracted with $CHCl_3$ at room temp. The residue remaining after evaporation of the solvent (224.3 g) was adsorbed to tonsil (900 g) and then eluted with hexane–$CHCl_3$ (95:5), $CHCl_3$–EtOAc (9:1) and EtOAc, yielding three fractions respectively: a low 'A' (122.6 g), a medium 'B' (74.2 g) and a high 'C' (21.3 g) polarity fractions. Fraction 'A' containing waxes and fats was discarded.

Resolution of fraction 'B'. The fraction 'B' was treated with activated charcoal and the residue (64.2 g) obtained was resolved by CC over silica gel (1.8 kg) using a hexane–EtOAc gradient elution system, 500 ml fractions being collected.

Isolation of artemexifolin (**3**). From fractions 20–36 eluted with hexane–EtOAc (1:1), a crystalline powder was separated and recrystallized from *iso*-Pr_2O–Me_2CO to yield 1.9 g of artemexifolin, mp and mmp 260°, identical in all respects with an authentic sample.

Isolation of arglanin (**2**), *ludalbin* (**4**) *and* 8α-*acetoxyarmexifolin* (**6**). The mother liquors obtained after isolation of **3** were combined with fractions 15–23. The resulting residue (16 g) was then rechromatographed on silica gel (500 g) using as eluent $CHCl_3$ with increasing amounts of Me_2CO. Fractions 34–41 eluted with $CHCl_3$–Me_2CO (95:5), afforded 230 mg of ludalbin (**4**), mp 155° (lit. [2] mp 169°). An additional 120 mg of ludalbin were obtained by further CC of the combined mother liquors and fraction 33 (2 g) on silica gel using a C_6H_6–EtOAc gradient elution system. The total yield of **4** was 350 mg (0.0048% of the dry wt). IR ν_{max}^{KBr} cm^{-1}: 3480, 1760, 1650, 1240; EIMS, *m/z* (rel. int.): 306 $[M]^+$ (1.7), 228 (35.4), 213 (37), 43 (100).

Fractions 52–56, eluted also with $CHCl_3$–Me_2CO (95:5), upon treatment with $CHCl_3$–*iso*-Pr_2O yielded 200 mg (0.003% of the dry wt) of arglanin (**2**), mp and mmp 185–187°. This compound was identified by comparison with an authentic sample. Fractions 60–73 when triturated with *iso*-Pr_2O–Me_2CO gave an additional 125 mg of **3**. The total yield of **3** was 2.025 g (0.030% of the dry wt). Finally, fractions 74–100 eluted with $CHCl_3$–Me_2CO (9:1), and fractions 101–111 eluted with $CHCl_3$–Me_2CO (4:1) were combined (3.5 g) and rechromatographed over silica gel (120 g). Development was made starting with C_6H_6–EtOAc (4:1) followed by using increasing amounts of EtOAc. Fractions 61–66, eluted with C_6H_6–EtOAc (7:3), crystallized spontaneously to yield 37.5 mg of **6**, mp 215°. An additional crop of compound **2** (30 mg) was obtained from fractions 61–72, which had been shown to be present by TLC (red orange colour). The total yield of **6** was 67.5 mg (0.001% of the dry wt); $[\alpha]_D$ +147° (*c* 1.33, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 209 (3.99), 237 (4.13); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 1782, 1745, 1680, 1220; (calc. for $C_{17}H_{20}O_6$: MW 320.1258. Found MW (MS) 320.1267). Other significant peaks in the low resolution MS were at *m/z* (rel. int.): 260 (26.0), 234 (35.4), 216 (23.7), 188 (23.9), 43 (100).

Isolation of santamarine (**5**) *and* α-*epoxyludalbin* (**7**). Fractions 40–61 from the original column (3 g) were rechromatographed on silica gel (120 g). The elution was accomplished with $CHCl_3$ and increasing amounts of Me_2CO. Fractions 7–10 eluted with $CHCl_3$ gave 85 mg (0.0012% of the dry wt) of **5**, mp 124°. Successive washing with hexane raised the mp to 135°. The solid was identified as santamarine by standard procedures.

Evaporation of the mother liquor of santamarine left a residue which crystallized when triturated with *iso*-Pr_2O–Me_2CO. The compound, α-epoxyludalbin **7**, formed colourless needles, mp 185°. Fraction 11 afforded an additional 50 mg to give a total yield of 62 mg (0.0004% of the dry wt); $[\alpha]_D$ +146.6° (*c* 1.5 $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 205 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3470, 1775, 1745, 1635, 1260; (Calc. for $C_{17}H_{22}O_6$: MW 322.1414. Found MW (MS): 322.1422). Other important peaks in the low resolution MS were at *m/z* (rel. int.): 247 (55), 205 (100), 117 (86), 43 (84).

Resolution of fraction 'C'. The polar fraction (21 g) was chromatographed on silica gel (690 g), starting elution with $CHCl_3$–Me_2CO (4:1) and then with increasing amounts of Me_2CO.

Isolation of armefolin (**8**). Fractions 73–157 (3.5 g) were rechromatographed over silica gel (144 g) with elution with EtOAc with increasing amounts of MeOH. Fractions 33–41 and 42–60 eluted with EtOAc yielded upon treatment with *iso*-Pr_2O–Me_2CO 110 mg of **8** (0.0016 of the dry wt), mp 175°; $[\alpha]_D$ +105.5° (*c* 2, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 205 (4.25); IR ν_{max}^{KBr} cm^{-1}: 3600, 1765, 1630; EIMS *m/z* (rel. int.): 264, (41.4), 246 (100), 231 (64.8), 107 (57.6), 91 (50).

Epoxidation of ludalbin (**4**) *to* α-*epoxyludalbin* (**7**). To a soln of 100 mg of **4** in $CHCl_3$ (40 ml) was added *m*-chloroperbenzoic acid (100 mg). The mixture was left overnight, then washed successively with 10% aq. $NaHCO_3$ and H_2O, dried and evaporated. The crystalline residual material, once recrystallized from *iso*-Pr_2O–Me_2CO, was found identical with the epoxide **7** isolated from the natural source.

Oxidation of epoxyludalbin (**7**) *to artemexifolin* (**3**). A soln of 60 mg of epoxyludalbin (**7**) in 1 ml pyridine was oxidized with 60 mg CrO_3 at room temp. The soln was diluted and extracted with EtOAc. The residue obtained after evaporation of the solvent was chromatographed over silica gel. Elution with hexane–EtOAc (1:1) afforded 35 mg of a crystalline product, mp 260°. The identity of the compound with **3** was established by direct comparison (mmp, TLC, IR).

Oxidation of armefolin (**8**) *to ketone* **11**. A soln of 42 mg of **8** in 1 ml pyridine was oxidized with 62 mg CrO_3 on an ice bath, during 2 hr. The mixture was then diluted with H_2O, extracted with EtOAc, dried and concd. The residual material was chromatographed on silica gel (10 g). Elution with EtOAc yielded 32 mg of **11**, mp 198°; UV λ_{max}^{MeOH} nm (log ε): 210 (4.00), 241 (4.08); IR ν_{max}^{KBr} cm^{-1}: 3480, 1755, 1660, 1615; EIMS *m/z* (rel. int.): 262 (20.9), 191 (60), 79 (66.8), 53 (100), 43 (76.3), 41 (67.6).

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