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SESQUITERPENE LACTONES FROM *ARTEMISIA HISPANICA*

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Key Word Index—*Artemisia hispanica*; Compositae; Anthemideae; sesquiterpenes; sesquiterpene lactones; germacranolides; eudesmanolides; 1-hydroxy-α-bisabololoxide A acetate.

Abstract—Extraction of aerial parts of *Artemisia hispanica* and chromatographic separation yielded the eight known sesquiterpene lactones costunolide, artemorin, anhydroverlotorin, tamaulipin A, reynosin, santamarin, matricarin and deacetylmatricarin, the flavone cirsimaritin, zingerone, 1-hydroxy-α-bisabololoxide A acetate and the new germacranolide 2α-hydroxyartemorin.

INTRODUCTION

We have recently reported on the contents of *A. hispanica* Lam. non Weber ex Stechm. (= *A. lucentica* O. Bolòs, Vallès et Vigo [1]) in flavonoids and other phenolic metabolites [2]. We now wish to communicate our results in the investigation of the terpenoid metabolites. In addition to the known sesquiterpene lactones costunolide (**1**), tamaulipin (**2**), artemorin (**3**), anhydroverlotorin (**4**), santamarin (**6**), reynosin (**7**), deacetylmatricarin (**8**) and matricarin (**9**), the α-bisabololoxide A derivative (**10**) [3], the phenolic ketone zingerone (**11**) [4] and the flavone cirsimaritin (**12**), we have isolated the new germacranolide (**5**), a 2-hydroxyderivative of artemorin.

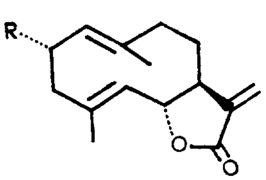
RESULTS AND DISCUSSION

Compound **5** was isolated in a very small amount and crystallized as white needles, mp 220–221°. Strong IR bands at 3380 and 1750 cm^{-1} showed the presence of hydroxyl and lactone functions. The mass spectrum (EI) showed a molecular peak at *m/z* 264, which agreed with a molecular formula $C_{15}H_{20}O_4$. Other significant peaks were visible at *m/z* 249 $[M-Me]^+$, 246 $[M-H_2O]^+$ and 231 $[M-Me-H_2O]^+$. The ^{1}H NMR spectrum at 200 MHz (room temperature) showed the two typical α-methylene lactone doublets at δ6.18 ($J = 3.4$ Hz) and 5.46 ($J = 3.1$ Hz), a broad singlet from an olefinic methyl group at δ1.77 and three broad signals in the range of the oxygenated carbon atoms at δ4.40 (*br t*, $J = ca$ 9.5 Hz), 4.05 (*br m*) and 3.67 (*br d*, $J = ca$ 8 Hz). Further signals were visible in the olefinic range as two broad singlets at δ4.90 and 5.20, the latter overlapping a broadened doublet ($J = ca$ 9 Hz).

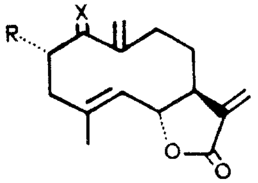
These data suggested a germacranolide structure, as it is known that many lactones with such medium-sized rings exist as mixtures of conformers, which interconvert slowly at room temperature [5, 6]. Further insight into the structure of **5** was gained by recording the NMR spectrum at higher temperature. Apart from experiencing slight shifts, the signals became sharper and could be thus better analysed. Decoupling experiments provided information about the connectivity of the hydrogen atoms. The triplet at δ4.40 collapsed to a doublet after irradiation of the doublet at δ5.20. Furthermore, this irradiation markedly sharpened the olefinic methyl signal at δ1.77. The multiplet at *ca* δ4, which appeared as a double triplet ($J = 9.2$ and 5.6 Hz) after heating, became a double doublet by saturation of the signal at *ca* δ3.6 but was not affected by irradiation of the triplet at δ4.40. In the same way, saturation of the signal at δ4 did not affect the triplet at δ4.40 but transformed the doublet at δ3.6 to a broadened singlet. All these facts can be explained with a germacran-12,6-olide structure bearing two Δ^4 and $\Delta^{10(14)}$-double bonds and two *trans*-vicinal hydroxy groups. As the spectrum displays clear resemblances to that of artemorin (**3**) [7] (Table 1), we propose the structure 2α-hydroxyartemorin (**5**). This structure is biogenetically feasible because **5** could be formed from **2** in the same way as **3** is formed from **1**. Unfortunately, the small amount available of **5** and the intrinsic width of several signals in the NMR spectra of such germacrane derivatives (see comments below) prevented the recording of a ^{13}C NMR spectrum.

The structure of the α-bisabololoxide A derivative **10** could not be ascertained at 200 MHz because of extensive overlapping of signals in the range 1–2 ppm. At 400 MHz, however, the resolution of individual signals and the results of decoupling experiments permitted a structural assignment. Further information was gained from comparison of the spectral data of **10** with those of its oxidation product **10a**. Although no compound with the proposed structure had been described in the literature, we then learned that compound **10** had been isolated several months ago by Jakupovic and coworkers from *Artemisia abrotanum* [3]. A comparison of our and Dr Jakupovic's compound revealed the identity of both substances.

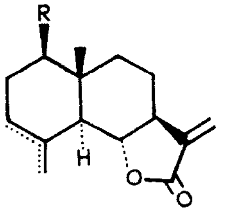
Lactone **13** was found in some fractions from the column chromatography of the plant extract. However, we noticed that the product appeared, merely by standing some time, in solutions of fractions which did not initially contain it. Since these fractions contained tamaulipin A (**2**), we think that **13** is not a true natural product but rather an artifact derived from **2** by oxy-Cope rearrangement [8, 9]. This has probably happened by some kind of



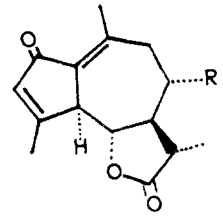
1 R= H
2 R= OH
2a R= OAc



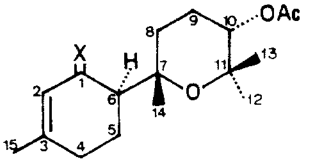
3 X= H, βOH R= H
4 X= O R= H
5 X= H, βOH R= OH



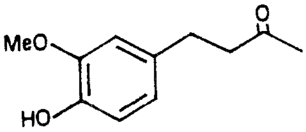
6 R= OH; Δ^3
6a R= OAc; Δ^3
7 R= OH; $\Delta^{4(15)}$



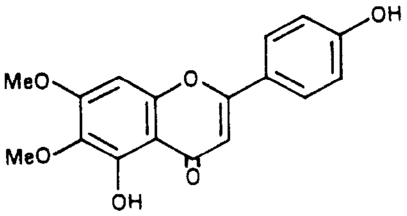
8 R= OH
9 R= OAc



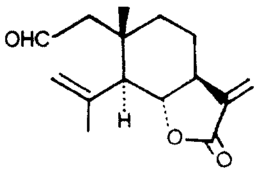
10 X= H, αOH
10a X= O



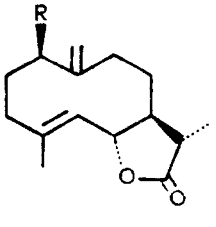
11



12



13



14a R= OH
14b R= OAc

catalytic induction, because no heating of plant material above 50° ever took place.

All other compounds could be identified by direct comparison with authentic samples or by comparison of their spectral data with those of the literature (see Experimental). Acetylation of **3** gave a product which was shown to be identical with artemorin acetate. On the other hand, reduction of **3** with sodium borohydride gave a compound which proved identical with gallicin **14a** [10]. The ^{13}C NMR spectra of **3**, **14a** and **14b** (Table 2) had to be recorded at high temperature in order to observe all carbon signals. At room temperature, the peaks of some carbon atoms were very low and broad, so that they almost did not emerge from the background. In the same way, the ^1H NMR spectra of these three compounds had also to be recorded at high temperature, as in the case of **5**, for many signals were broad and unresolved at ambient temperature. This fact was not specifically mentioned in the corresponding literature citations [7, 10]. It should be emphasized that not only germacran-12,8-olides [5, 11] but also many germacran-12,6-olides, especially those bearing a $\Delta^{10(14)}$-double bond, undergo

Table 1. ^1H NMR data of compounds **3**, **4**, **5**, **10**, **10a** and **13***

H	**3**	**3**†	**4**	**5**	**5**‡	**10**§	**10a**§	**13**
1	3.95 *m*	3.65 *t*	—	3.67 *br d*	3.64 *br d*	4.41 *br d*	—	2.39 *dd*
								2.51 *dd*
2α			2.65–2.25 *m*	—	—			
						5.28 *br s*	5.76 *br s*	9.82 *t*
2β	2.70–1.80 *m*	2.30–1.50 *m*	3.10 *m*	4.05 *m*	4.01 *ddd*			
3			2.65–2.25 *m*	3.00–1.50 *m*				5.08 *dq*
								4.76 *br s*
5	5.19 *br d*	4.93 *br d*	5.06 *br d*	5.22 *br d*	5.20 *br d*			2.56 *br d*
6	4.37 *t*	4.00 *t*	4.31 *t*	4.40 *br t*	4.36 *t*			4.03 *dd*
7	2.80 *m*	2.35 *br dd*	2.65–2.25 *m*					2.50 *m*
8α		2.30–1.50 *m*	2.22 *dddd*					2.03 *dddd*
8β		1.10 *m*	1.40 *m*	3.00–1.50 *m*				1.58 *m*
	2.70–1.80 *m*							
9α		2.18 *br dd*	2.65–2.25 *m*					
								1.90–1.70 *m*
9β		2.30–1.50 *m*	2.65–2.25 *m*					
10	—	—	—	—	—	4.69 *dd*	4.68 *dd*	—
12	—	—	—	—	—	1.12 *s*	1.11 *s*[a]	—
13	6.13 *d*	6.10 *d*	6.19 *d*	6.18 *d*	6.18 *d*	1.30 *s*	1.17 *s*[a]	6.05 *d*
	5.41 *d*	4.96 *d*	5.46 *d*	5.46 *d*	5.44 *d*			5.39 *d*
14	5.16 *br s*	4.86 *br s*	5.80 *br s*	5.20 *br s*	5.19 *br s*	1.30 *s*	1.22 *s*[a]	1.11 *s*
	4.83 *br s*	4.48 *br s*	5.63 *br s*	4.90 *br s*	4.94 *br s*			
15	1.69 *br s*	1.37 *d*	1.74 *d*	1.77 *br s*	1.78 *d*	1.62 *br s*	1.90 *br s*	1.80 *d*
OAc	—	—	—	—	—	2.04 *s*	2.05 *s*	—

*At 200.13 MHz in $CDCl_3$ (27°) unless otherwise stated.

†In C_6D_6 at 62°.

‡In $CDCl_3$ at 55°.

§The non indicated protons gave an unresolved multiplet in the range 1–2 ppm for **10** or two multiplets of three (δ2.50–2.20) and six protons (δ2.10–1.70) for **10a**. Compound **10** gave a broad singlet at δ4.90 from the hydroxylic proton. For high-resolution data (400 MHz), see ref. [3].

[a] Signals may be interchanged.

Coupling constants in Hz: **3** $J_{1,2(2')}=6.3$; $J_{5,6}=J_{6,7}=9.8$; $J_{5,15}=1.3$; $J_{7,8\beta}=10$; $J_{8\beta,9\alpha}=10$; $J_{9\alpha,9\beta}=15$; $J_{7,13'}=3.5$; $J_{7,13}=3.1$. **4** $J_{5,6}=J_{6,7}=9.8$; $J_{5,15}=1.4$; $J_{7,8\alpha}=3.3$ or 6.4; $J_{8\alpha,8\beta}=15$; $J_{8\alpha,9\alpha}=6.4$ or 3.3; $J_{8\alpha,9\beta}=2$; $J_{7,13'}=3.5$; $J_{7,13}=3.2$. **5**‡ $J_{1,2}=J_{2,3\alpha}=9.5$; $J_{2,3\beta}=5.6$; $J_{5,6}=J_{6,7}=9.7$; $J_{5,15}=1.3$; $J_{7,13'}=3.4$; $J_{7,13}=3.2$. **10** $J_{1,2}=$ *ca.* 0; $J_{1,6}=8$; $J_{9,10}=3.5$; $J_{9',10}=2$. **10a** $J_{9,10}=6.2$; $J_{9',10}=2.8$. **13** $J_{1,1'}=16$; $J_{1(1'),2}=2.4$; $J_{3,5}=J_{3,15}=1.5$; $J_{5,6}=11.7$; $J_{6,7}=10.7$; $J_{7,8\alpha}=J_{8\alpha,9\alpha}=J_{8\alpha,9\beta}=3.5$; $J_{8\alpha,8\beta}=12.5$; $J_{7,13'}=3.2$; $J_{7,13}=3.0$

slow conformational equilibria at room temperature [12, 13] and thus display broad signals in their NMR spectra.

The isolation of germacranolides, eudesmanolides and guaianolides provides an additional support for the inclusion of *A. hispanica* (= *A. lucentica*) into the subgenus *Artemisia*, sect. *Abrotanum*, rather than into the sect. *Seriphidium*, in which guaianolides are rare [14]. The chemical data thus confirm the conclusions derived from the botanical and morphological features [1].

EXPERIMENTAL

Spectrometer features have been described in our former publications [2]. CC was made on silica gel Merck. HPLC was performed in the reverse phase mode (Knauer chromatograph, detection by refractive index).

Plant material. Aerial parts of *A. hispanica* (= *A. lucentica*) were collected in the vicinity of Callosa de Segura (Alicante, Spain) in October 1987 and authenticated by Dr A. Aguilella (Department of Botany at the Faculty of Biology, University of Valencia, Spain). A voucher specimen has been deposited in the herbarium of this Department.

Extraction and chromatography. The air-dried plant material (600 g) was extracted at room temp. with hexane–Et_2O–MeOH (1:1:1) (10 l, 5 days) [15]. The extract (30 g) was defatted by ppt. from MeOH and prefractionated by CC. Fractions A–G were collected, corresponding to elution with, resp., hexane, 10%, 25%, 50%, 75% Et_2O in hexane, Et_2O and 20% MeOH in Et_2O.

Fractions A and B (*ca* 3 g overall) contained mainly waxes and essential oils and were discarded. Fraction C (4.3 g) was subjected to CC (hexane–CH_2Cl_2–Et_2O, 10:5:1). This enabled the separation of costunolide (**1**, 465 mg) from the accompanying waxes and other aliphatic compounds.

Fraction D (7.7 g) consisted mainly of sterols. Repeated CC (hexane–Et_2O and $CHCl_3$–Et_2O mixtures) gave two sterol-free fractions. One of them contained **11** (5 mg). The other one was further purified by HPLC (LiChrosorb RP-8, 8 × 250 mm column, elution with MeOH–H_2O 3:1, *ca* 140 bar). This gave pure **10** (R_t = 6.7 min) (100 mg).

CC (CH_2Cl_2–Et_2O 15:1 to Et_2O) of fraction E (6.6 g) gave 7 main fractions (E-1 to E-7) after inspection by TLC. Fraction E-1 was rechromatographed (elution with $CHCl_3$–Et_2O 20:1) to give anhydroverlotorin (**4**) (12 mg). Column chromatography of

Table 2. ^{13}C NMR data of compounds **3**, **4**, **10**, **10a**, **13**, **14a** and **14b***

C	**3**	**3**†	**4**	**10**	**10a**	**13**	**14a**‡	**14b**‡
1	78.24	77.74	204.42	67.91	200.14	55.14	78.16	78.97
2	32.32§[a]	33.31[a]	35.57	124.72	127.82	201.90	33.02[a]	31.21[a]
3	*ca* 30‖[a]	29.95§[a]	28.08[a]	135.75	160.90	117.61	31.16§[a]	32.61§[a]
4	*ca* 145‖	144.62§	142.89	30.55	31.39	139.17	144.49	144.43
5	122.88§	123.96	124.90	23.12	30.50	55.75	123.09	123.34
6	80.52	80.16	80.92	52.30	55.81	80.75	80.43	80.43
7	*ca* 49‖	48.02§	49.50§	79.21	73.04[a]	49.18	52.54§	53.17§
8	25.65§	25.77	29.00	22.56	23.57	21.78	27.30	27.40
9	36.10§[a]	35.86[a]	38.29[a]	20.52	21.17	38.32	36.25[a]	36.53[a]
10	150.94§	151.72	150.81	71.07	73.26	39.78	151.84	147.97
11	140.11	141.48	139.53	74.08	74.44[a]	140.43	42.06	42.01
12	170.37	169.71	170.10	27.48	28.62	170.23	178.43	178.33
13	118.35	116.94	119.14	25.56	25.87	117.38	12.75	12.77
14	110.85§	109.90	123.36	27.41	22.86	21.37	110.32	112.51
15	17.88	17.98	17.11	22.86	23.76	22.30	17.86	17.83
OAc				170.35	170.49			170.02
				20.96	21.17			21.18

*At 50.32 MHz in $CDCl_3$ (27°) unless otherwise stated.

†In C_6D_6 at 62°.

‡In $CDCl_3$ at 55°.

§Broadened.

‖Very low and broad.

[a] The signals may be interchanged.

fraction E-3 (hexane–Et_2O 2:3) gave three fractions; two of them contained, resp., reynosin (**7**, 195 mg) and the elemanolide **13** (150 mg). The other one was a mixture of **2**, **6** and **9** (NMR) and could be resolved into individual components only after acetylation (hexane–Et_2O 1:1). This enabled the separation of tamaulipin A acetate (**2a**, 16 mg), santamarin acetate (**6a**, 12 mg), and matricarin (30 mg). Fractions E-2, E-4, E-5, E-6 and E-7 did not contain defined products and were discarded.

Fraction F was submitted to CC (CH_2Cl_2–Et_2O, 9:1), which gave four main fractions (F-1 to F-4). Fraction F-1 contained mainly polymethylated flavonoids [2]. Fraction F-2 was crystallized from EtOAc. The yellow crystals (425 mg) were shown by NMR to be essentially a mixture of the flavones cirsilineol [2] and cirsimaritin (**12**). The mother liquors were purified by CC (hexane–EtOAc 2:3), allowing the separation of artemorin (**3**, 285 mg). Fraction F-3 was also subjected to CC (CH_2Cl_2–Et_2O 1:1), affording deacetylmatricarin (**8**, *ca* 1 g). Finally, fraction G was purified by CC (toluene–Et_2O 1:9 to Et_2O). This gave compound **5** (7 mg).

Compounds **1**, **4**, **6**–**9**, **10** and **12** were compared with authentic samples. Lactone **3** was unequivocally identified via the acetylated and reduced derivatives, for which authentic samples were available. Compounds **2** and **13** were identified by comparison of their spectra with literature data [8]. Zingerone (**11**) was compared with a synthetic sample, prepared from vanillin by aldol condensation with acetone and subsequent hydrogenation [4, 16].

1β,2α-Dihydroxygermacra- 4,10(14), 11*-trien-6β,7α*H-12,6*-olide* (**5**). White needles, mp 220–221° (EtOAc). IR ν_{max}^{KBr} cm^{-1}: 3480, 3380 (OH), 1750 (C=O), 1269, 1260, 1148, 1044, 1028, 950. EIMS (probe) *m/z* (rel. int.): 264 $[M]^+$ (6), 249 $[M-Me]^+$ (3), 246 $[M-H_2O]^+$ (5), 231 $[M-Me-H_2O]^+$ (4), 228 $[M-2H_2O]^+$ (5), 220 (6), 218 (5), 217 (5), 203 (6), 180 (23), 163 (20), 91 (50), 41 (100). For ^1H NMR data, see Table 1.

1*-Hydroxy-α-bisabololoxide A acetate* (**10**). Colourless oil, $[\alpha]_D^{23°}$ +2.5° ($CHCl_3$; *c* 2.5); $[\alpha]_{365}^{23°}$ −2.5°. IR ν_{max}^{Film} cm^{-1}: 3480 (OH), 1740 (acetate C=O), 1260, 1120, 1020. EIMS (probe) *m/z* (rel. int.): 296 $[M]^+$ (2.5), 278 $[M-H_2O]^+$ (1.5), 263 $[M-H_2O-Me]^+$ (1.5), 236 $[M-HOAc]^+$ (1.5), 218 $[M-H_2O-HOAc]^+$ (4), 203 $[M-H_2O-HOAc-Me]^+$ (4), 185 (82), 143 (83), 125 (100). An aliquot of **10** (40 mg) was oxidized with a modified Collins reagent [17], affording **10a** (20 mg) as a colourless oil: IR ν_{max}^{Film} cm^{-1}: 1738 (acetate C=O), 1673 (ketone C=O), 1255; UV λ_{max}^{EtOH} nm: 234 (log ε_{max} 4.07); EIMS (probe) *m/z* (rel. int.): 294 $[M]^+$ (1), 279 $[M-Me]^+$ (2), 234 $[M-HOAc]^+$ (2), 219 $[M-Me-HOAc]^+$ (5), 185 (100), 143 (83), 125 (98). For NMR data of **10** and **10a**, see Tables 1 and 2.

Zingerone (**11**). Viscous oil, lit. [4, 16] solid, mp 40–41°. ^1H NMR, ($CDCl_3$): δ6.82 (*d*, $J = 8$ Hz), 6.71 (*d*, $J = 1.8$ Hz), 6.64 (*dd*, $J = 8$ and 1.8 Hz), 5.5 (*br s*, OH), 3.80 (*s*, 3H; OMe), 2.85–2.65 (*m*, A_2B_2 system; CH_2CH_2), 2.11 (*s*, 3H: MeCO). ^{13}C NMR, ($CDCl_3$): δ208.22 (*s*), 146.22 (*s*), 143.51 (*s*), 132.25 (*s*), 120.09 (*d*), 114.08 (*d*), 110.85 (*d*), 55.22 (*q*), 44.72 (*t*), 29.27 (*q*), 28.83 (*t*).

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