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HELIANGOLIDES FROM *LEUCANTHEMOPSIS PULVERULENTA*

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Key Word Index—*Leucanthemopsis pulverulenta*; Compositae; heliangolides.

Abstract—The structure of hispanolide (4,5-*cis*-3-oxogermacranolide) and three other related germacranolides, isolated from *Leucanthemopsis pulverulenta*, have been established by a series of chemical transformations and spectral data.

INTRODUCTION

Leucanthemopsis (Anthemideae, Compositae) is a small genus, with only six species, most of them widespread in Spain and Portugal [1]. They have not previously been studied chemically.

In a previous paper [2], we reported the isolation of two new phloroglucinol derivatives from the roots of *L. pulverulenta*, previously known as *Pyrethrum hispanicum**, and now we report the isolation of six sesquiterpene lactones from the chloroform extract of the aerial parts. Four of these, **1**–**4**, have not been reported previously; the other two lactones are the already known desacetyllaurenobiolide (**5**) and spiformin (**6**) [3].

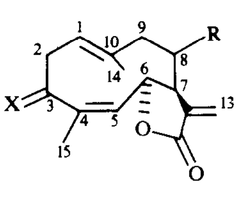
RESULTS AND DISCUSSION

The new lactones are all structurally related heliangolides for which we propose the structure 4,5-*cis*-3-oxogermacranolide (**1**, hispanolide), 4,5-*cis*-3β-acetoxygermacranolide (**2**), 4,5-*cis*-1,10-epoxy-3β-acetoxygermacranolide (**3**) and 4,5-*cis*-1,10-epoxy-3β-hydroxygermacranolide (**4**).

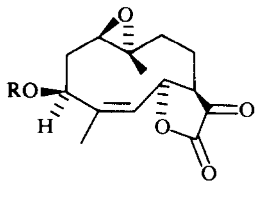
Hispanolide (**1**), mp 130–131°, showed a molecular ion at *m/z* 246, which agreed with the formula $C_{15}H_{18}O_3$. The UV spectrum, with λ_{max}^{EtOH} at 213 (ε = 6.100) and 254 (ε = 3.100) nm, suggested the presence of an α-methylene-γ-lactone ring [4, 5] and a conjugated ketone with a near s-*cis* geometry [6]. This functionality was confirmed by the IR spectrum, which had bands at 1765, 1705 [7, 8], 1670, 1640 and 830 cm^{-1}. The ^{1}H NMR spectrum of **1** (Table 1) was very similar to that exhibited by 3-dehydronobilin (**7**) [9], the only significant difference being the additional ester signal at C-8 of the latter. These facts and the ^{1}H NMR coupling constants $J_{5,6}$, $J_{6,7}$ [10] and $J_{7,13}$ [11] (Table 1), allowed us to assign a heliangolide structure for **1**.

The most probable conformation for hispanolide, deduced from these data and from analysis of the Dreiding models, is shown in **A**.

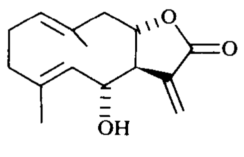
The ^{1}H NMR chemical shift of H-6 (δ4.18) is due to the spatial proximity of the oxygen atom at C-3. That the



1 X=O; R=H
1a X=OH,H; R=H
2 X=OAc,H; R=H
7 X=O; R=Ang



3 R=Ac
4 R=H



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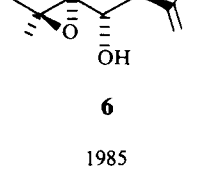

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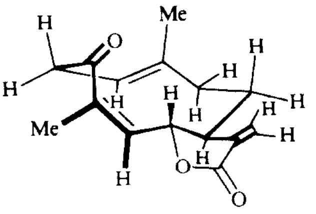
*The plant was identified by Professor B. Casaseca Mena, from the Department of Botany, Salamanca University, where a specimen is held (Herbarium No. 8996).

Table 1. ^{1}H NMR spectra of the lactones from *Leucanthemopsis pulverulenta* (60 MHz, δ, $CDCl_3$, TMS)

	1	**2**	**3**	**4**
H-1	5.32 *t*	5.20 *m*	2.78 *dd*	2.78 *dd*
	$J_{1,2} = 9$ Hz		$J_{1,2} = 11$, $J_{1,2'} = 4$ Hz	$J_{1,2} = 11$, $J_{1,2'} = 4$ Hz
H-2	3.17 *d*	—	—	—
	$J_{1,2} = 9$ Hz			
H-3	—	5.15 *t*	5.35 *t*	4.45 *t*
		$J_{2,3} = 3$ Hz	$J_{2,3} = 3$ Hz	$J_{2,3} = 3$ Hz
H-5	5.20 *dd*	5.12 *dd*	5.32 *dd*	5.25 *dd*
	$J_{5,6} = 11$, $J_{5,15} = 1.5$ Hz	$J_{5,6} = 11$, $J_{5,15} = 1.5$ Hz	$J_{5,6} = 11$, $J_{5,15} = 2.5$ Hz	$J_{5,6} = 11$, $J_{5,15} = 1.5$ Hz
H-6	4.18 *dd*	5.30 *dd*	5.66 *dd*	6.22 *dd*
	$J_{5,6} = 11$, $J_{6,7} = 2.5$ Hz	$J_{5,6} = 11$, $J_{6,7} = 2.5$ Hz	$J_{5,6} = 11$, $J_{6,7} = 2.5$ Hz	$J_{5,6} = 11$, $J_{6,7} = 2.5$ Hz
H-7	2.70 *m*	2.65 *m*	2.70 *m*	2.70 *m*
H-13	5.70 *d*	5.65 *d*	5.70 *d*	5.65 *d*
	$J_{7,13} = 2.5$ Hz	$J_{7,13} = 2$ Hz	$J_{7,13} = 2.5$ Hz	$J_{7,13} = 2$ Hz
H-13′	6.31 *d*	6.30 *d*	6.38 *d*	6.30 *d*
	$J_{7,13'} = 2.5$ Hz	$J_{7,13'} = 2$ Hz	$J_{7,13'} = 2.5$ Hz	$J_{7,13'} = 2$ Hz
H-14	1.72 *br s*	1.71 *br s*	1.40 *br s*	1.41 *s*
H-15	1.88 *d*	1.75 *d*	1.82 *d*	1.75 *d*
	$J_{5,15} = 1.5$ Hz	$J_{5,15} = 1.5$ Hz	$J_{5,15} = 1.5$ Hz	$J_{5,15} = 1.5$ Hz



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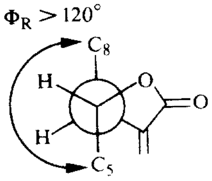

C=O group is not coplanar with the double bond accounts for the partial absence of conjugation (1705 cm^{-1}).

The structure and stereochemistry assigned to hispanolide was confirmed by reduction of **1** to 4,5-*cis*-3β-hydroxygermacranolide (**1a**), of mp 137–138°, whose spectral data were identical with those exhibited by a germacranolide isolated by Bohlmann *et al.* [12] from *Tanacetum tanacetoides*. The ^{1}H NMR spectrum of **1a** was very similar to that of hispanolide, but for the presence of a new allylic β-*O*-proton signal at δ4.46 (*t*, *J* = 3 Hz) and the downfield shift of the H-6 signal (δ5.80, *dd*, *J* = 11 and 2.5 Hz).

Acetylation of **1a**, gave a monoacetate identical with the naturally occurring lactone **2**. The ^{1}H NMR spectral data (Table 1), further support the proposed structure.

4,5-*cis*-1,10-Epoxy-3β-acetoxygermacranolide (**3**) was a gum showing a molecular ion at *m/z* 306, which agreed with the formula $C_{17}H_{22}O_5$. Its IR spectrum suggested the presence of an α-methylene-γ-lactone ring (1770 cm^{-1}), an acetoxyl group (1745 cm^{-1}) and an oxirane ring (1220 and 850 cm^{-1}). The structure was deduced from the characteristic ^{1}H NMR spectrum, which was very similar to that exhibited by **2**, except for the upfield shift of the H-13 resonance from δ1.71 to 1.40 and that of the H-1 resonance from δ5.20 to 2.78. Hence, **3** is the epoxide of **2**. This was confirmed by epoxidation of **2** with *m*-CPB which gave a substance identical in all respects with lactone **3**. 4,5-*cis*-1,10-Epoxy-3β-hydroxygermacranolide (**4**), mp 168–169°, showed a molecular ion at *m/z* 264, which agreed with formula $C_{15}H_{22}O_4$.

The ^{1}H NMR spectrum of **4** was like that of **3**, except for the absence of the upfield shift of the H-3 resonance, which indicated that the C-3 hydroxyl was free. In 4,5-*cis*-1,10-epoxy-3β-hydroxygermacranolide (**4**) the H-6 signal (δ6.22) was more deshielded than in any of the other hispanolide derivatives. This is due to the anisotropy of the three-membered oxirane ring, which has also been observed in other heliangolides [10]. Acetylation of **4**, gave a monoacetate identical with **3**.

The CD curves for hispanolide and its derivatives indicated that their absolute configurations are those depicted in structures **1**–**4** [9, 13–15].

EXPERIMENTAL

Mps are uncorr. and were determined on a Kofler hot stage apparatus.

Collection and isolation of plants. *L. pulverulenta* was collected at the end of May 1980, near Ledesma (Salamanca, Western Spain).

The aerial parts (2.3 kg) were air-dried, finely ground and extracted with hot $CHCl_3$. The extract was concd *in vacuo* and the residual syrup extracted with 50% aq. EtOH. The ethanolic soln was extracted with $CHCl_3$, and the $CHCl_3$ extract was then extracted with aq. 5% $NaHCO_3$, yielding a neutral fraction (6.2 g). The ethanolic soln after acidification with HCl, gave an acidic fraction (4.3 g).

The neutral fraction was dry chromatographed, using C_6H_6–Et_2O (7:3), to afford five fractions. Each of them was rechromatographed on Si gel or Si gel impregnated with 20% $AgNO_3$ and purified by crystallization or TLC. Fraction 1 (0.60 g) afforded **2** (200 mg). Fraction 2 (1.25 g) afforded **1** (82 mg) and **5** (340 mg). Fraction 3 (1.53 g) afforded **3** (240 mg) and **6** (50 mg). Fraction 4 (1.42 g) afforded **4** (535 mg). Fraction 5 (1.25 g) was a complex mixture.

Hispanolide (**1**). Compound **1** was eluted with $CHCl_3$–Et_2O (95:5); mp 130–131° ($CHCl_3$–Et_2O) and $[\alpha]_D = +148°$ (*c* 0.47, $CHCl_3$); UV λ_{max}^{EtOH} nm: 213 (ε = 6.100) and 254 (ε = 6.100) nm; IR ν_{max}^{KBr} cm^{-1}: 1765, 1705, 1670, 1640, 1340, 1280, 1150, 1030, 980, 940, 890, 860, 830; MS *m/z* (rel. int.): 246 $[M]^+$ (5), 231 (7), 203 (16), 175 (28), 161 (100), 147 (72), 135 (33), 122 (31), 105 (94), 69 (43), 43 (58); CD: $\Delta\varepsilon_{215} = -1.18$; $\Delta\varepsilon_{305} = +1.12$.

Reduction of **1**. To a soln of **1** (23 mg) in MeOH (3 ml), $NaBH_4$ (16 mg) in MeOH (2 ml) was added. The reaction mixture was kept at room temp. for 1 hr. The MeOH was evaporated, H_2O and two drops of HOAc were added and the organic phase was extracted with $CHCl_3$. Evaporation of the solvent gave 22 mg **1a**, mp 137–138° (hexane–Et_2O), $[\alpha]_D = -18.1°$ (*c* 0.43, $CHCl_3$).

Acetylation of **1a** (14 mg) gave a monoacetate (13 mg) identical with **2**.

4,5-cis-3*β*-*Acetoxygermacranolide* (**2**). Compound **2** was eluted with C_6H_6 as a gum, $[\alpha]_D = -21°$ (*c* 0.45, $CHCl_3$); IR ν_{max} cm^{-1}: 1770, 1740, 1670, 1460, 1380, 1250, 1190, 1140, 1050, 970, 890, 820; MS *m/z* (rel. int.): 290 $[M]^+$ (5), 248 $[M-42]^+$ (4), 230 $[M-60]^+$ (10), 149 (19), 121 (26), 107 (30), 93 (32), 67 (32), 43 (100); CD: $\Delta\varepsilon_{212} = -3.48$; $\Delta\varepsilon_{233} = +0.56$.

4,5-cis-1,10-*Epoxy*-3*β*-*acetoxygermacranolide* (**3**). This was eluted with $CHCl_3$–Et_2O (9:1), $[\alpha]_D = -47°$ (*c* 1.70, $CHCl_3$); IR ν_{max} cm^{-1}: 1770, 1750, 1660, 1450, 1380, 1250, 1140, 1090, 990, 890, 825, 805; MS *m/z* (rel. int.): 306 $[M]^+$ (3), 264 $[M-42]^+$ (17), 246 $[M-60]^+$ (58), 218 (39), 203 (52), 175 (62), 159 (44), 147 (56), 135 (95), 123 (100), 122 (91), 95 (91), 43 (86).

Epoxidation of **2**. To a stirred soln of **2** (17 mg) in CH_2Cl_2 (2 ml) at 0°, *m*-CPBA (8.6 mg) in CH_2Cl_2 (2 ml) was added and the mixture stirred for 30 min at 25°. A 10% aq. soln of Na_2SO_3 was added dropwise and the organic layer was washed × 3 with a 5% aq. soln of Na_2CO_3, dried over Na_2SO_4 and evaporated to give 16 mg of **3**.

4,5-cis-1,10-*Epoxy*-3*β*-*hydroxygermacranolide* (**4**). Compound **4** was eluted with $CHCl_3$–Et_2O (8:2), mp 168–170° (Et_2O), $[\alpha]_D = -23.5°$ (*c* 1.8, $CHCl_3$); IR ν_{max} cm^{-1}: 3600–3100, 1765, 1670, 1450, 1380, 1280, 1140, 970, 880, 860, 820; MS *m/z* (rel. int.): 264 $[M]^+$ (3), 246 $[M-18]^+$ (9), 231 (3), 203 (7), 175 (14), 161 (14), 147 (19), 135 (15), 123 (24), 95 (100), 91 (62), 69 (55), 55 (45); CD: $\Delta\varepsilon_{211} = -6.85$; $\Delta\varepsilon_{235} = +1.68$.

Acetylation of **4** (22 mg) gave a monoacetate (20 mg) identical with **3**.

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