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Germacranolides and a New Type of Guaianolide from *Acanthospermum hispidum*

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The aerial parts of an Argentinian collection of *Acanthospermum hispidum* afforded 26 sesquiterpene lactones, including the two guaianolides (**1** and **2**) having a novel oxygen bridge between C-4 and C-14, three new *cis*,*cis*-germacranolides (**4**, **7**, and **8**), and two new melampolides (**25** and **26**). Guaianolides **1** and **2** seem to derive biosynthetically from the germacranolide **27** having the $_1D^{14,15}D_5$ conformation. The structures were elucidated using extensive spectroscopic analysis.

Previous investigations of *Acanthospermum* species[1−4] have led to the isolation of *cis*,*cis*-germacranolides and melampolides,[2] in agreement with the fact that many species of the genera *Acanthospermum*, *Melampodium*, and *Lecocarpus*, belonging to the tribe Heliantheae, subtribe Melampodiinae, contain melampolides.[5] In view of the fact that these compounds show cytotoxic and in vivo anticancer activity,[3] and as a continuation of our work on sesquiterpene lactones of the Argentinian species of Asteraceae,[6−8] we have carried out an exhaustive examination of the minor constituents of *A. hispidum* (Asteraceae), a shrub indigenous to northern Argentina. The aerial parts afforded the new guaianolides hispidunolides A (**1**) and B (**2**) with an unprecedented oxygen bridge between C-4 and C-14; the new *cis*,*cis*-germacranolides **4**, **7**, and **8**; the new melampolides **25** and **26**; and the known sesquiterpene lactones **3**, **5**, **6**, **9**−**18**, previously isolated from American[1,2] and African[3,4] collections of *A. hispidum*; compounds **19**−**23**, previously found in species of *Lecocarpus* from Ecuador;[5,9] compound **24**, previously isolated from an Australian collection of *Siegesbeckia orientalis*;[10] and loliolide.[11]

Results and Discussion

Hispidunolide A (**1**) showed IR bands for alcohol, γ-lactone, and ester groups at 3450, 1760, and 1735 cm^{-1}, respectively. It has the molecular formula $C_{22}H_{28}O_8$ as followed from its mass spectrum, which showed a $[M]^+$ at *m*/*z* 420, accounting for nine degrees of unsaturation. Mass spectral peaks at *m*/*z* 360 $[M - CH_3COOH]^+$, 335 $[M - C_5H_9O]^+$ and 85 $[C_5H_9O]^+$ indicated the presence of an acetate and a saturated five-carbon atom ester. The ^1H NMR spectrum showed the typical signals of a 2-methylbutyrate residue at δ 2.37 (qt, *J* = 7.0, 7.0 Hz), 1.62 (ddq, *J* = 13.5, 7.0, 7.0 Hz), 1.44 (ddq, *J* = 13.5, 7.0, 7.0 Hz), 1.08 (d, *J* = 7.0 Hz), and 0.89 (t, *J* = 7.0 Hz). An α-methylene-γ-lactone moiety was evident by the two doublets at δ 6.28 (*J* = 3.5 Hz) and 5.56 (*J* = 3.0 Hz) in the ^1H NMR spectrum. The ^{13}C NMR spectrum showed 22 signals corresponding to three CH_3, five CH_2, eight CH, and six quaternary carbons, as deduced from a DEPT experiment, in agreement with the molecular formula obtained from the mass spectrum. The ^{13}C NMR spectrum also indicated the presence of a lactone moiety, which showed signals at δ 168.8 (C-12), 134.6 (C-11), and 122.1

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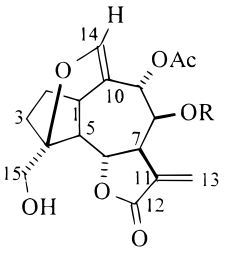
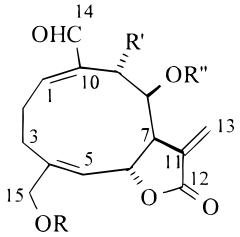
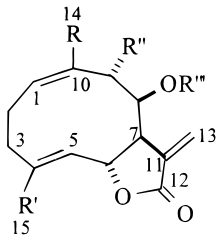


1 R = 2-MeBu
2 R = Ang



	R	R'	R"
3	Ac	H	*i*-Bu
4	Ac	H	Ang
5	Ac	H	2-MeBu
6	Ac	H	*i*-Val
7	Ang	H	Ac
8	H	OAc	2-MeBu



	R	R'	R"	R"'
9	CHO	Me	OAc	2-MeBu
10	CHO	CH_2OH	H	*i*-Bu
11	CHO	CH_2OH	OAc	2-MeBu
12	CHO	CH_2OH	H	2-MeBu
13	CHO	CH_2OH	H	*i*-Val
14	CHO	CH_2OH	OMe	Ang
15	CH_2OH	CH_2OH	OAc	*i*-Bu
16	CH_2OH	CH_2OH	OAc	Ang
17	CH_2OH	CH_2OH	OAc	2-MeBu
18	CHO	CH_2OH	OH	2-MeBu
19	CHO	CH_2OH	OAc	Ang
20	CHO	CH_2OH	H	Ang
21	CHO	CH_2OH	OMe	2-MeBu
22	CHO	CH_2OH	OMe	*i*-Bu
23	CHO	CH_2OH	OH	Ang
24	CHO	CH_2OH	OAc	*i*-Bu
25	CH_2OH	Me	OAc	Ang
26	CH_2OH	Me	OAc	2-MeBu

(C-13). The guaianolide skeleton was easily deduced from the ^{1}H NMR spectrum and sequential spin decoupling involving H-1, H-5, H-6, and H-7. The vinyl proton signal at C-14 appeared at δ 6.30 in agreement with the chemical-shift range for protons attached to the α-carbon in enol-ethers.[12] The COSY experiment showed that the signal at δ 6.30 (H-14) was long-range coupled with the signals at δ 5.06 and 2.91, corresponding to H-9 and H-1, respectively. The signals at δ 114.2 and 140.5 were assigned to the enol-ether carbons C-10 and C-14, respectively. To establish the relative configuration of the fragment C-1−C-5−C-6−C-7 and that of C-4, the minimum energy conformations of **1**, having either a C4β−O−C14 or a C4α−O−C14 bridge, was calculated using the PCMODEL program.[13] These calculations showed that the dihedral angles and coupling constant values for the fragment CH(1)−CH(5)−CH(6)−CH(7) in the C4α-O−C14 isomer are: HβC(1)−HβC(5) = 56° (J = 3.6 Hz), HβC(5)−HβC(6) = −49° (J = 5.1 Hz), and HβC-(6)−HαC(7) = −174° (J = 11.2 Hz); while for the C4β-O−C14 isomer the values are: HαC(1)−HαC(5) = −48° (J = 5.0 Hz), HαC(5)−HβC(6) = −142° (J = 6.6 Hz), and HβC-(6)−HαC(7) = −165° (J = 10.8 Hz). The latter set of values was in good agreement with the observed coupling constants, as can be seen in Table 1. To confirm the β-orientation of the vinyl oxygen at C-4, an NOE experiment irradiating the H-9 signal showed enhancement of the signal at δ 6.30 (6%) corresponding to H-14. The minimum energy conformation of **1** is shown in Scheme 1. The individual assignment of the protons attached to C-3 was deduced from the minimum energy conformation of hispidunolide A (**1**), in which the dihedral angles and calculated coupling constants are: HαC(2)−HαC(3) = 14° (J = 11.5 Hz), HαC(2)−HβC(3) = −106° (J = 1.5 Hz), HβC(2)−HαC-(3) = 134° (J = 6.5 Hz), and HβC(2)−HβC(3) = 13° (J = 11.5 Hz), in good agreement with the experimental coupling constants given in Table 1. The small coupling constant between H-7 and H-8 indicated that the ester residue at C-8 is β-oriented. Therefore, with the small coupling constant between H-8 and H-9 also taken into account, the acetate group at C-9 is α-oriented, as occurs in many known sesquiterpene lactones isolated from this species[1,2] and also found in the present investigation.

The mass spectrum of hispidunolide B (**2**) showed $[M]^+$ at m/z 418, corresponding to the molecular formula $C_{22}H_{26}O_8$ in agreement with the ^{13}C NMR spectrum and DEPT experiment, which showed 22 signals, three of them corresponding to CH_3, four to CH_2, eight to CH, and seven to quaternary carbons. Relevant mass peaks at m/z 358 $[M - CH_3COOH]^+$, 335 $[M - C_5H_7O]^+$, and 83 $[C_5H_7O]^+$ were indicative of an acetate and an unsaturated five-carbon atom ester. Both the ^{1}H and ^{13}C NMR signals of hispidunolide B (**2**) indicated the presence of the same skeleton as in hispidunolide A (**1**), with the only difference being the ester moiety at C-8, since for **2** the ^{1}H NMR spectrum shows signals at δ 1.96 (dq, J = 7.0, 1.5 Hz), 1.81 (dq, J = 1.5, 1.5 Hz), and 6.15 (qq, J = 7.0, 1.5 Hz), which indicated the presence of an angelate residue. The ^{13}C NMR spectrum further confirmed the angelate moiety due to the signals at δ 165.6, 141.1, 126.4, 20.5, and 16.0.[14] It is interesting to note that, from the biosynthetic point of view, hispidunolides A (**1**) and B (**2**) might be biosynthesized through a hetero Diels−Alder transformation[15] from germacranolide **27**, which has the $_1$D^{14}, ^{15}D$_5$ conformation,[16] as shown in Scheme 1.

Compound **3** was previously reported by Kraus et al.,[4] but no spectral data were provided to support the structure. Therefore, the ^{1}H and ^{13}C NMR data of **3** are included in Tables 2 and 4, respectively.

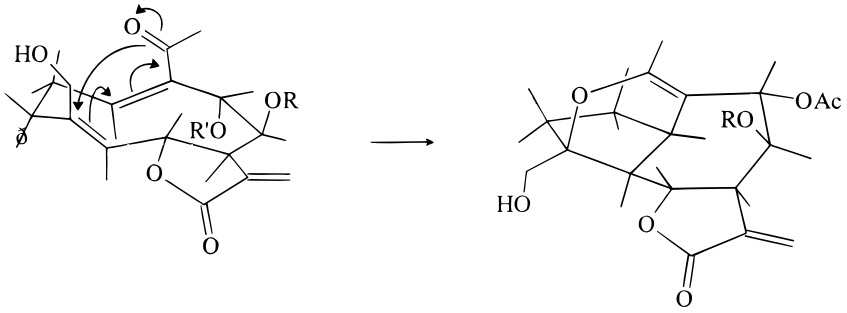
Compound **4** was isolated as a gum. The ^{1}H NMR data indicated that we were dealing with a germacranolide-type

Table 1. ^{1}H[a] and ^{13}C NMR[b] Data ($CDCl_3$, TMS) for Hispidunolide A (**1**) and B (**2**)[c]

	1		**2**	
	$^\delta$H	$^\delta$C	$^\delta$H	$^\delta$C
1	2.91 br t (5.5)	32.8	2.94 br t (5.5)	32.8
2a	1.99[d]	29.3	1.99[d]	29.4
2b	1.99[d]		1.99[d]	
3α	1.77 ddd (10.5, 10.0, 3.5)	36.9	1.78 ddd (10.5, 10.0, 3.5)	36.9
3*β*	2.23 br t (10.5)		2.23 br t (10.5)	
4		88.6		88.6
5	2.52 t (5.5)	45.8	2.53 t (5.5)	45.9
6	4.46 dd (9.5, 5.5)	75.6	4.48 dd (9.5, 5.5)	75.8
7	3.48 dddd (9.5, 3.5, 3.0, 1.5)	46.0	3.51 dddd (9.5, 3.5, 3.0, 1.5)	46.0
8	5.48 t (1.5)	71.9	5.56 t (1.5)	72.1
9	5.06 d (1.5)	73.7	5.15 d (1.5)	73.6
10		114.2		114.3
11		134.6		134.6
12		168.8[e]		168.8[e]
13a	6.28 d (3.5)	122.1	6.29 d (3.5)	122.3
13b	5.56 d (3.0)		5.60 d (3.0)	
14	6.30 br s	140.5	6.31 br s	140.6
15a	3.91 br s	64.3	3.96 d (17.0)	64.3
15b	3.91 br s		3.90 d (17.0)	
OAc	2.15 s	168.9,[e] 21.0	2.16 s	169.0[e], 21.0

[a] 300 MHz. *J* values are given in Hz in parentheses. [b] 75.4 MHz. [c] Other signals for **1**: 2-MeBu: δ_H: 2.37 (qt, 7.0, 7.0, H-2′); 1.62 (ddq, 13.5, 7.0, 7.0, H-3′a); 1.44 (ddq, 13.5, 7.0, 7.0, H-3′b); 1.08 (d, 7.0, H-5′); 0.89 (t, 7.0, H-4′); δ_C : 174.9 (C-1′); 41.1 (C-2′); 26.6 (C-3′); 16.8 (C-5′); 11.6 (C-4′). For **2**: Ang: δ_H: 6.15 (qq, 7.0, 1.5, H-3′); 1.96 (dq, 7.0, 1.5, H-4′); 1.81 (dq, 1.5, 1.5, H-5′); δ_C: 165.6 (C-1′); 141.1 (C-3′); 126.4 (C-2′); 20.5 (C-5′); 16.0 (C-4′). [d] Overlapping signals. [e]Interchangeable.

Scheme 1. Proposed Biosynthetic Path and Minimum Energy Conformation of **1** and **27**



27 R = 2-MeBu; R' = OAc **1**

Table 2. ^{1}H NMR Data (δ, $CDCl_3$, TMS) for Germacranolides **3**, **4**, **7**, and **8**[a]

	3	**4**	**7**	**8**
H-1	6.62 ddd (8.0, 7.0, 1.5)	6.63 ddd (8.5, 6.5, 1.5)	6.67 br t (6.5)	6.78 dd (9.0, 6.0)
H-2α	2.70 m[b]	2.70 m[b]	2.70 m[b]	3.25 dddd (15.0, 8.0, 6.0, 2.0)
H-2*β*	2.85 dddd (15.0, 7.0, 4.0, 1.0)	2.85 dddd (15.0, 6.5, 4.0, 1.0)	2.85 dddd (15.0, 6.5, 4.0, 1.0)	2.80 m[b]
H-3α	2.69 m[b]	2.69 m[b]	2.69 m[b]	2.58 ddd (14.0, 8.0, 2.0)
H-3*β*	2.40 ddd (14.5, 7.5, 4.0)	2.40 ddd (14.5, 7.5, 4.0)	2.40 ddd (14.5, 7.5, 4.0)	2.99 ddd (14.0, 11.0, 8.0)
H-5	5.55 br d (9.5)	5.57 br d (9.5)	5.55 br d (9.5)	5.58 br d (9.5)
H-6	5.46 dd (9.5, 4.0)	5.48 dd (9.5, 4.0)	5.41 dd (9.5, 4.0)	5.43 dd (9.5, 4.5)
H-7	2.64 dddd (4.0, 3.0, 2.5, 2.5)	2.67 dddd (4.0, 3.0, 2.5, 2.5)	2.75 dq (4.0, 3.0, 2.5, 1.5)	2.75 m[b]
H-8	5.93 ddd (10.0, 7.0, 2.5)	5.98 ddd (10.0, 7.0, 2.5)	6.13 ddd (10.0, 7.0, 1.5)	6.53 dd (9.0, 2.5)
H-9α	3.07 br ddd (14.0, 7.0, 1.5)	3.07 br ddd (14.0, 7.0, 1.5)	3.07 br dd (14.0, 7.0)	
H-9*β*	2.40 ddd (14.0, 10.0, 1.5)	2.57 ddd (14.0, 10.0, 1.5)	2.57 ddd (14.0, 10.0, 2.0)	5.80 dd (9.0, 2.0)
H-13a	6.35 d (3.0)	6.36 d (3.0)	6.40 d (3.0)	6.41 d (3.0)
H-13b	5.71 d (2.5)	5.72 d (2.5)	5.79 d (2.5)	5.86 d (2.5)
H-14	9.41 d (1.5)	9.43 d (1.5)	9.45 d (2.0)	9.40 d (2.0)
H-15a	4.49 s	4.53 dd (13.5, 1.5)	4.49 s	4.08 s
H-15b	4.49 s	4.46 dd (13.5, 1.0)	4.49 s	4.08 s
OAc	2.12 s	2.12 s	2.12 s	2.00 s
H-2′	2.49 sept (7.0)			2.31 sext (7.0)
H-3′a	1.12 d (7.0)	6.09 qq (7.0, 1.5)	6.15[b]	1.60 m[b]
H-3′b				1.39 m
H-4′	1.09 d (7.0)	1.96 dq (7.0, 1.5)	1.96 dq (7.0, 1.5)	0.85 t (7.0)
H-5′		1.81 dq (1.5, 1.5)	1.85 dq (1.5, 1.5)	1.05 d (7.0)

[a] 300 MHz. *J* values are given in Hz in parentheses. [b] Overlapping signals.

sesquiterpene lactone containing acetate and angelate esters. These data are similar to those of related *cis,cis*-germacranolides with 2-methylbutyrate or isovalerate ester residues attached to C-8.[2,4] The presence of a 1,10-*cis*-double bond with an aldehyde group at C-10 followed from the chemical shifts of H-1 (δ 6.63, ddd) and H-14 (δ 9.43,

Table 3. ^{1}H NMR Data (δ, $CDCl_3$, TMS) for Melampolides **10**, **14**, **16**, **25**, and **26**[a,b]

	10	14	16	25	26
H-1	6.63 ddd (9.5, 7.0, 2.0)	6.82 dd (10.0, 7.5)	5.80 dd (9.0, 8.0)	5.77 dd (9, 7.5)	5.75 br dd (8.5, 7.5)
H-2a	2.47[c]	2.74 m	2.42 m[c]	2.44 m[c]	2.44 m[c]
H-2b	2.42[c]	2.62[c]	2.34 m[c]	2.3[c]	2.3[c]
H-3α	2.04 ddd (12.5, 12.5, 2.0)	2.04 ddd (12.0, 12.0, 2.0)	1.90 ddd(12.5, 12.5, 2.5)	2.0[c]	2.0[c]
H-3β	2.83 ddd (12.5, 6.0, 2.5)	2.85 ddd (12.0, 5.5, 2.5)	2.69 ddd (12.5, 5.5, 2.5)	2.3[c]	2.3[c]
H-5	5.16 br d (10.5)	5.03 br d (10.0)	5.15 br d (10.0)	5.03 br d (10.5)	5.01 br d (10.5)
H-6	5.23 t (10.5)	5.20 t (10.0)	5.35 t (10.0)	5.10 dd (10.5, 9)	5.10 dd (10.5, 9)
H-7	2.48[c]	2.63[c]	3.35 dddd (10.0, 3.5, 3.0, 2.0)	3.31dddd (9.0, 3.5, 3.0, 2.0)	3.29 dddd (9.0, 3.5, 3.0, 2.0)
H-8	6.36 ddd (10.0, 8.0, 2.0)	6.65 dd (8.5, 1.5)	6.16 dd (9.5, 2.0)	6.16 dd (9.5, 2.0)	6.06 dd (9.5, 2.0)
H-9α	2.75 ddd (14.0, 8.0, 2.0)				
H-9β	2.06 ddd (14.0, 10.0, 1.5)	3.88 dd (8.5, 2.0)	5.53 d (9.5)	5.42 d (9.5)	5.35 d (9.5)
H-13a	6.24 d (3.5)	6.30 d (3.5)	6.24 d (3.5)	6.24 d (3.5)	6.24 d (3.5)
H-13b	5.58 d (3.0)	5.87 d (3.0)	5.68 d (3.0)	5.68 d (3.0)	5.63 d (3.0)
H-14a	9.46 d (1.5)	9.52 d (2.0)	4.40 br d (12.5)	4.38 d (12.5)	4.37 br d (12.5)
H-14b			4.23 br d (12.5)	4.21 br d (12.5)	4.19 d (12.5)
H-15a	4.53 d (12.5)	4.47 br d (13.0)	4.49 br s	1.97 br s	1.98 br s
H-15b	4.32 br d (12.5)	4.37 br d (13.0)	4.49 br s		

[a] 300 MHz. *J* values are given in Hz in parentheses. [b] Other signals (δ), for **10**: *i*-Bu: 2.53 (sept, 7.0, H-2′); 1.14 (d, 7.0, H-4′); 1.12 (d, 7.0, H-3′a). For **14**: Ang: 6.05 (qq, 7.0, 1.5, H-3′); 1.96 (dq, 7.0, 1.5, H-4′); 1.88 (dq, 1.5, 1.5, H-5′); OMe: 3.10 s. For **16**: Ang: 6.11 (qq, 7.0, 1.5, H-3′); 1.95 (dq, 7.0, 1.5, H-4′); 1.82 (dq, 1.5, 1.5, H-5′); Ac: 1.95 s. For **25**: Ang: 6.10 (qq, 6.0, 1.5, H-3′); 1.95 (overlapped, H-4′); 1.82 (dq, 1.5, 1.5, H-5′); Ac: 1.94 s. For **26**: 2-MeBu: 2.30 (m, H-2′); 1.60 (m, H-3′a); 1.39 (m, H-3′b); 1.06 (d, 7.0, H-5′); 0.86 (t, 7.5, H-4′). Ac: 1.97 s. [c]Overlapping signals.

Table 4. ^{13}C NMR Data (δ, $CDCl_3$, TMS) for Compounds **3**, **8**, **13**, **16**, **17**, **24**, and **26**[a]

	3	8	13[b]	16	17	24	26
1	153.2	158.9	153.8	134.5[d]	134.5[d]	158.5	134.3[d]
2	25.0	24.9	27.0	26.3	26.5[e]	27.6	26.5
3	26.1	27.3[c]	32.6	33.1	33.1	32.4	37.7
4	135.0[d]	139.3[c]	140.4[d]	139.9	141.8	140.9[d]	139.1
5	129.2	127.0	128.4	127.9	128.0	128.5	125.9
6	73.2	73.5	73.8	72.3	72.3	73.4	72.8
7	46.8	46.4	49.4	50.9	51.0	51.1	50.8
8	71.9	72.2	65.6	68.8	68.8	69.8	68.9
9	28.6	69.6	28.8	74.2	74.0	67.9	75.6
10	142.0	140.3[c]	142.7[d]	136.0	136.0	141.2[d]	136.2
11	134.4[d]	133.4	134.8	134.1[d]	134.0[d]	133.7	134.8[d]
12	169.3	169.0	169.3	169.4	169.3	169.0	164.6
13	124.7	126.2	121.2	121.3	121.3	122.3	121.1
14	194.8	193.3	195.5	64.0	64.0	193.8	64.0
15	66.6	65.8	60.5	60.9	60.9	60.6	16.7
OAc	170.4	170.4		170.0	169.9	170.5	
	20.9	20.7		20.8	21.0	20.8	
1′	175.7	175.0	171.7	166.5	175.4	175.4	175.4
2′	34.0	41.3	43.3	142.0	41.4	34.1	41.4
3′	19.1	26.5[c]	25.8	126.8	26.3[e]	19.0	25.4
4′	18.6	11.6	22.4	15.8	11.6	19.0	11.7
5′		17.1	22.3	20.4	16.9		16.7

[a] 75.4 MHz. [b] Distinction of C-2 and C-3′ followed from APT measurements. [c] Distinction of C-4 from C-10 and of C-3 from C-3′ followed from HMBC measurements. [d,e] Interchangeable signals.

d).[1] The *cis*-configuration of the 4,5-double bond was deduced from the typical chemical shifts of H-5 (δ 5.57, br d) and H-6 (δ 5.48, dd) and the value of $J_{6,7}$ = 4.0 Hz.[2,5] The angelate residue at C-8 is β-oriented because $J_{7,8}$ = 2.5 Hz, in agreement with the well-known *syn*-periplanar orientation of H-8 and H-7.[5] In addition, β-oriented ester residues at this position are frequent in germacranolides of the subtribe Melampodiinae. The large coupling constant between H-8 and H-9β (10.0 Hz) showed that H-9β is trans to H-8. This stereochemistry places H-9β and H-14 into a *W* relationship if the aldehyde carbonyl is oriented such that there is maximal overlap between the π orbitals of the 1(10)-double bond and the carbonyl group, an arrangement that accounts for the observed long-range coupling between H-9β and H-14.[1] An allylic coupling between H-1 and H-9α was also observed.

The ^{1}H NMR spectrum of **7** (Table 2) was very similar to that of **4**. It only differed in the H-8 and H-15 chemical shifts, and therefore in **7** the angelate ester is located at C-15, while the acetate group is located at C-8.

The spectral features of **8** were similar to those described for *cis*,*cis*-germacranolides **4** and **7**, but no signals at δ 2.57 and 3.07 were found for the H-9 protons. Instead, a double doublet at δ 5.80 (J = 9.0, 2.0 Hz) and a singlet at δ 2.00 revealed the presence of an α-oriented acetate at C-9, as further supported by the signals at δ 69.6 (C-9), 170.4, and 20.7 (OAc) in the ^{13}C NMR spectrum. A singlet at δ 4.08 (2H) indicated that a hydroxyl group was bonded to a methylene group, and it was assigned to the protons at C-15. The 500-MHz HMBC contour plot showed, among others, correlations between C-5 and H-3, H-6, and H-6; between C-6 and H-5 and H-8; between C-7 and H-6, H-8, and the two hydrogens at C-13, between C-8, and H-6 and H-9, between C-9 and H-1, H-8, and H-14; between the acetate carbonyl and H-9, and between the 2-methylbutyrate carbonyl and H-8, the two H-3′ signals, and the H-5′ methyl, which further supported the structure of **8**. The experiment also allowed distinction of the C-4 and C-10 signals; the former had correlations with one H-2 and one H-3, H-5, and H-15, while the latter had correlations with one H-2, H-9, and H-14. There was also distinction of the C-3 and C-3′ signals, was much as the former had correlations with H-5 and H-15, while the latter correlated with H-2′, H-4′, and H-5′.

The IR spectrum of **10** showed strong absorptions at 3450, 1760, 1735, and 1685 cm^{-1}, indicating the presence of a hydroxyl, γ-lactone, ester, and conjugated carbonyl with a double bond, respectively. The ^{1}H NMR spectrum exhibited signals ascribable to a germacranolide-type compound bearing an isobutyrate ester, because it was similar to the spectra of related melampolides.[1,2,5,9] The presence of a 1,10-*cis*-double bond with an aldehyde group at C-10 followed from the chemical shifts of H-1 (δ 6.63, ddd) and H-14 (δ 9.46, d).[1] The *trans*-configuration of the 4,5-double bond was deduced from the typical chemical shifts of H-5 (δ 5.16, br d) and H-6 (δ 5.23, t), as well as a large $J_{6,7}$ of 10.5 Hz.[2] The signals at δ 4.53 (d) and 4.32 (br d) were assigned to H-15a and H-15b, respectively. The signal at δ 6.36 (ddd) is typical for a proton attached to a carbon supporting an ester group and was assigned to H-8. The small coupling constant between H-7 and H-8 (2.0 Hz) indicated that the ester residue at C-8 is β-oriented. An

allylic coupling between H-1 and H-9α (J = 2.0 Hz) and a *W*-type coupling between H-9β and H-14 (J = 1.5 Hz) were observed. The typical signals for an α-methylene-γ-lactone moiety appeared at δ 6.24 (d, J = 3.5 Hz, H-13a) and 5.58 (d, J = 3.0 Hz, H-13b). A previous report on this compound by Kraus et al.[4] provided no spectral data to support the structure. The ^{1}H NMR data are given in Table 3.

Compounds **25** and **26** were melampolides having an acetate and an angelate in the case of **25**, and an acetate and a 2-methylbutyrate in the case of **26**. They differed in the ester group attached to C-8, as can be seen from the ^{1}H NMR data given in Table 3. Neither the IR nor NMR spectra showed bands or signals for an aldehyde group. However, in the ^{1}H NMR spectrum of **25** an AB system at δ 4.38 (d, J = 12.5 Hz) and 4.21 (br d, J = 12.5 Hz), assigned to the H-14 protons, was present. The chemical shift of these protons and those of H-1 (δ 5.77, dd) were similar to those observed for related melampolides bearing a CH_2OH at C-10.[1] Similar signals were observed for **26**, as can be seen in Table 3. Noteworthy for melampolides with carbonyl groups attached to C-10 is the chemical shift of H-1, found 1 ppm downfield. The total signal assignment was achieved by comparison with a melampolide obtained by Herz and Kalyanaraman[1] by reduction of a precursor with a carbonyl attached to C-10.

The ^{1}H NMR spectrum of melampolide **14** differed from that of lecocarpinolide J (**21**), previously isolated from *Lecocarpus lecocarpoides*,[5] only in the signals corresponding to the ester residue at C-8. Because **14** has an allylic methoxyl group and we used methanol for the HPLC separation, one could suspect it to be an artifact. However, lecocarpinolide J and lecocarpinolide M, which also contain an allylic methoxyl group at C-9, were found in the genus *Lecocarpus*,[5] belonging to the same subtribe of *Acanthospermum*, for which no methanol was used during the isolation procedures. Compound **16** displayed similar spectral data to those of **25**. However, the ^{1}H and ^{13}C NMR spectra of **16** accounted for an additional CH_2OH group at C-4. Compounds **14** and **16** were previously reported by Kraus et al.,[4] but no spectral data were provided to support the structures. Herein we report the ^{1}H NMR data of **14** and **16** in Table 3 and the ^{13}C NMR data of **13**[2], **16**,[4] **17**,[1] and **24**,[10] which were not published previously, in Table 4.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrophotometer. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on Varian XL-300GS or Unity-500 spectrometers. The EIMS were obtained on a Hewlett-Packard 5989-A spectrometer at 20 eV. For separation of mixtures, a Gilson HPLC instrument with a 305 pump and a 112-differential refractometer detector was used. Columns: A (Beckman ultrasphere C_{18}, 10 × 250 mm) and B (Beckman ultrasphere C_8, 10 × 250 mm) were employed. Retention times (t_R) were measured from the solvent peak. For column chromatography, Si gel Merck 70−230 or 230−400 mesh ASTM were used.

Plant Material. The aerial parts (flowers and leaves) of *A. hispidum* DC. were collected in Vipos, Tucumán Province, Argentina, in April 1995. A voucher specimen (LIL 604458) is on deposit at the Herbarium of Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and Isolation. The plant material (330 g) was extracted with $CHCl_3$ (2 × 3 L) at room temperature for 14 days to give 15.9 g (yield 4.8%) of a crude extract, which was suspended in EtOH (130 mL) at 55 °C, diluted with H_2O (100 mL), and extracted successively with hexane (3 × 150 mL) and $CHCl_3$ (3 × 150 mL). The chloroform extract on evaporation at reduced pressure furnished a residue (3.38 g), which was column chromatographed over Si gel using $CHCl_3$ with increasing amounts of EtOAc (0−100%) and finally MeOH, to give nine fractions. Fractions containing sesquiterpene lactones, as evidenced by IR, were further processed.

A portion (200 mg) of fraction 2 (463 mg) was chromatographed by HPLC (Column A, MeOH−H_2O, 3:2, 1.5 mL min^{-1}) to give 5 mg of **3**, t_R 27 min, 4 mg of **1**, t_R 32 min; 2.3 mg of **4**, t_R 43 min; 33.3 mg of **6**, t_R 52 min; 2 mg of **9**, t_R 84 min; and mixtures further purified by HPLC (Column B, MeOH−H_2O, 3:2, 1.3 mL min^{-1}) to give 1.5 mg of **2**, t_R 30 min, and 0.9 mg of **5**, t_R 45 min.

Fraction 3 (168 mg) was chromatographed by HPLC (Column A, MeOH−H_2O, 4:3, 1.5 mL min^{-1}) to give 2.1 mg of loliolide,[11] t_R 6 min; 5.5 mg of **2**, t_R 23 min; 2.4 mg of **1**, t_R 25 min; 1.3 mg of **25**, t_R 76 min; 6.4 mg of **26**, t_R 88 min; and mixtures further purified by HPLC (Column B, MeOH−H_2O, 1:1, 1.3 mL min^{-1}) to give 1 mg of **10**, t_R 24 min; 0.7 mg of **19**, t_R 35 min; 7.4 mg of **11**, t_R 28 min; 4.4 mg of **20**, t_R 40 min; 8.7 mg of **12**, t_R 44 min; and 11.4 mg of **13**, t_R 48 min.

A portion (110 mg) of fraction 4 (193 mg) was processed by HPLC (Column A, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 1.7 mg of **24**, t_R 14 min; 0.6 mg of **7**, t_R 19 min; 5.8 mg of **13**, t_R 31 min; and mixtures further purified by HPLC (Column B, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 1 mg of **14**, t_R 12 min; 1.9 mg of **10**, t_R 14 min; 3.3 mg of **21**, t_R 16 min; 9.6 mg of **19**, t_R 20 min; 30.8 mg of **11**, t_R 25 min; and 1.6 mg of **12**, t_R 16 min.

A portion (200 mg) of fraction 5 (423 mg) was processed by HPLC (Column B, MeOH−H_2O, 6:5, 2 mL min^{-1}) to give 96 mg of **11**, t_R 45 min; 4.5 mg of a mixture of **13** and **24**, t_R 54 min; and mixtures further purified by HPLC (Column A, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 0.5 mg of **22**, t_R 13 min; 3.1 mg of **24**, t_R 19 min; and 4.7 mg, of **19**, t_R 30 min.

A portion (200 mg) of fraction 6 (487 mg) was chromatographed by HPLC (Column A, MeOH−H_2O, 1:1, 1.8 mL min^{-1}) to give 10.3 mg of **24**, t_R 15 min, and 35.8 mg of **11**, t_R 28 min.

A portion (200 mg) of fraction 7 (549 mg) was processed by HPLC (Column A, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 1.1 mg of **15**, t_R 18 min; 18.8 mg of **17**, t_R 30 min; 8.5 mg of **8**, t_R 61 min; and mixtures further purified by HPLC (Column B, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 1.5 mg of **15**, t_R 21 min; 4.7 mg of **16**, t_R 27 min; and 0.7 mg of **17**, t_R 32 min.

Fraction 8 (60 mg) was chromatographed by HPLC (Column B, MeOH−H_2O, 1:1, 2 mL min^{-1}) to give 2.3 mg of **23**, t_R 9 min, and 2.8 mg of **18**, t_R 10 min.

9-Acetyloxy-15-hydroxy-8-(2-methylbutanoyloxy)-10(14),11(13)-guaiadien-6,12-olide-4,14-oxide (hispidunolide A) (1): gum; $[\alpha]^{25}_{589}$ −46°, $[\alpha]^{25}_{578}$ −48°, $[\alpha]^{25}_{546}$ −56°, $[\alpha]^{25}_{436}$ −102°, $[\alpha]^{25}_{365}$ −174° (*c* 5.0, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 210 (4.1) nm; IR ($CHCl_3$) ν_{max} 3450, 1760, 1735, 1635 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (direct inlet) *m/z* 420 $[M]^+$ (3), 402 (9) $[M - H_2O]^+$, 360 $[M - CH_3COOH]^+$ (1), 335 $[M - CH_3CH_2CH(CH_3)CO]^+$ (11), 318 $[M - CH_3CH_2CH(CH_3)COOH]^+$ (1), 317 $[M - H_2O - CH_3CH_2CH(CH_3)CO]^+$ (4), 293 $[M - CH_2CO - CH_3CH_2CH(CH_3)CO]^+$ (66), 275 $[M - CH_3COOH - CH_3CH_2CH(CH_3)CO]^+$ (74), 258 $[M - CH_3COOH - CH_3CH_2CH(CH_3)COOH]^+$ (16), 240 $[M - H_2O - CH_3COOH - CH_3CH_2CH(CH_3)COOH]^+$ (34), 85 $[CH_3CH_2CH(CH_3)CO]^+$ (39), 57 $[C_4H_9]^+$ (100).

9-Acetyloxy-15-hydroxy-8-angeloyloxy-10(14),11(13)-guaiadien-6,12-olide-4,14-oxide (hispidunolide B) (2): gum; $[\alpha]^{25}_{589}$ −40°, $[\alpha]^{25}_{578}$ −42°, $[\alpha]^{25}_{546}$ −50°, $[\alpha]^{25}_{436}$ −87°, $[\alpha]^{25}_{365}$ −147° (*c* 6.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209 (4.5) nm; IR ($CHCl_3$) ν_{max} 3500, 1760, 1730, 1640 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (direct inlet) *m/z* 418 $[M]^+$ (1), 400 $[M - H_2O]^+$ (4), 358 $[M - CH_3COOH]^+$ (0.2), 335 $[M - CH_3CHC(CH_3)CO]^+$ (12), 317 (2), 293 $[M - CH_2CO - CH_3CHC(CH_3)CO]^+$ (35), 275 $[M - CH_3COOH - CH_3CHC(CH_3)CO]^+$ (41), 258 $[M - CH_3COOH - CH_3CHC(CH_3)COOH]^+$ (5), 240 $[M - CH_3COOH - CH_3CHC(CH_3)COOH - H_2O]^+$ (12), 83 $[CH_3CHC(CH_3)CO]^+$ (100), 55 $[C_4H_7]^+$ (28).

15-Acetyloxy-8β-angeloyloxy-14-oxo-(4*Z*)-acanthospermolide (4): gum; UV (EtOH) λ_{max} (log ϵ) 211 (5.3) nm; IR ($CHCl_3$) ν_{max} 2720, 1760, 1735, 1630 cm^{-1}; ^{1}H NMR, see Table

2; EIMS (direct inlet) *m*/*z* 402 $[M]^+$ (1), 342 (4), 302 (15), 242 (20), 213 (11), 82 (44), 54 (100).

8*β*-Acetyloxy-15-angeloyloxy-14-oxo-(4*Z*)-acanthospermolide (7): gum; UV (EtOH) λ_{max} (log ϵ) 212 (5.1) nm; IR ($CHCl_3$) ν_{max} 2720, 1760, 1735, 1630 cm^{-1}; ^1H NMR, see Table 2; EIMS (direct inlet) *m*/*z* 402 $[M]^+$ (2), 342 (4), 302 (10), 242 (19), 213 (11), 82 (50), 54 (100).

9α-Acetyloxy-8*β*-(2-methylbutanoyloxy)-14-oxo-(4*Z*)-acanthospermolide (8): gum; $[\alpha]^{25}_{589}$ −73°, $[\alpha]^{25}_{578}$ −78°, $[\alpha]^{25}_{546}$ −90°, $[\alpha]^{25}_{436}$ −168°, $[\alpha]^{25}_{365}$ −278° (*c* 6.3, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 200 (3.6) nm; IR ($CHCl_3$) ν_{max} 2725, 1765, 1730, 1640 cm^{-1}; ^1H and ^{13}C NMR, see Table 2 and Table 4, respectively; EIMS (direct inlet) *m*/*z* 420 $[M]^+$ (1), 402 (15), 342 (20), 240 (26), 212 (10), 85 (32), 57 (100).

9α-Acetyloxy-8*β*-angeloyloxy-14-hydroxyacanthospermolide (25): gum; UV (EtOH) λ_{max} (log ϵ) 204 (4.1) nm; IR ($CHCl_3$) ν_{max} 3455, 1760, 1735, 1630 cm^{-1}; ^1H NMR, see Table 3; EIMS (direct inlet) *m*/*z* 404 $[M]^+$ (0.2), 386 (9), 326 (15), 226 (22), 83 (100).

9α-Acetyloxy-14-hydroxy-8*β*-(2-methylbutanoyloxy)-acanthospermolide (26): gum; $[\alpha]^{25}_{589}$ −20°, $[\alpha]^{25}_{578}$ −22°, $[\alpha]^{25}_{546}$ −24°, $[\alpha]^{25}_{436}$ −38°, $[\alpha]^{25}_{365}$ −67° (*c* 4.5, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 207 (3.8) nm; IR ($CHCl_3$) ν_{max} 3450, 1760, 1735, 1635 cm^{-1}; ^1H and ^{13}C NMR, see Table 3 and Table 4, respectively; EIMS (direct inlet) *m*/*z* 406 $[M]^+$ (1), 388 (9), 328 (17), 226 (11), 85 (32), 57 (100), 43 (56).

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