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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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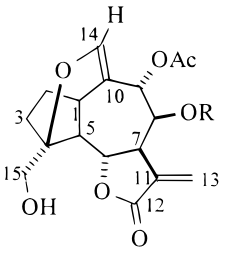
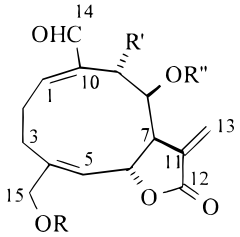
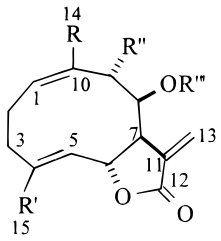


**1** R = 2-MeBu
**2** R = Ang



| | 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 $\delta$ 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 $\delta$ 6.30 (H-14) was long-range coupled with the signals at $\delta$ 5.06 and 2.91, corresponding to H-9 and H-1, respectively. The signals at $\delta$ 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 C4$\beta$−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: H$\beta$C(1)−H$\beta$C(5) = 56° ($J$ = 3.6 Hz), H$\beta$C(5)−H$\beta$C(6) = −49° ($J$ = 5.1 Hz), and H$\beta$C-(6)−HαC(7) = −174° ($J$ = 11.2 Hz); while for the C4$\beta$-O−C14 isomer the values are: HαC(1)−HαC(5) = −48° ($J$ = 5.0 Hz), HαC(5)−H$\beta$C(6) = −142° ($J$ = 6.6 Hz), and H$\beta$C-(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 $\beta$-orientation of the vinyl oxygen at C-4, an NOE experiment irradiating the H-9 signal showed enhancement of the signal at $\delta$ 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)−H$\beta$C(3) = −106° ($J$ = 1.5 Hz), H$\beta$C(2)−HαC-(3) = 134° ($J$ = 6.5 Hz), and H$\beta$C(2)−H$\beta$C(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 $\beta$-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 $\delta$ 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 $\delta$ 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.

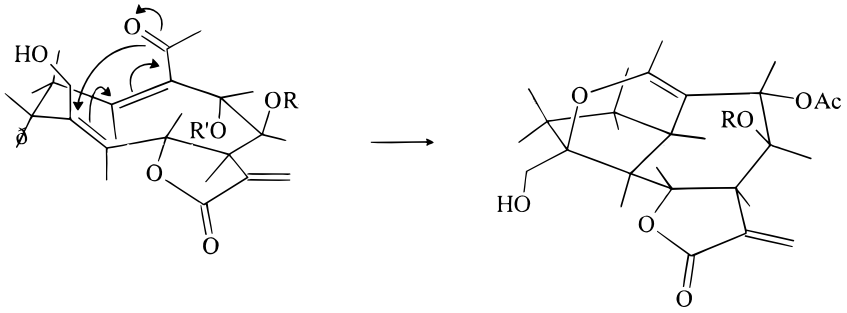
Compound **4** was isolated as a gum. The $^1$H NMR data indicated that we were dealing with a germacranolide-type

**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: $\delta_H$: 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′); $\delta_C$ : 174.9 (C-1′); 41.1 (C-2′); 26.6 (C-3′); 16.8 (C-5′); 11.6 (C-4′). For **2**: Ang: $\delta_H$: 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′); $\delta_C$: 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 ($\delta$, $CDCl_3$, 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 ($\delta$ 6.63, ddd) and H-14 ($\delta$ 9.43,

**Table 3.** $^1$H NMR Data ($\delta$, $CDCl_3$, 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-3$\beta$ | 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-9$\beta$ | 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 ($\delta$), 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 ($\delta$, $CDCl_3$ , 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 ($\delta$ 5.57, br d) and H-6 ($\delta$ 5.48, dd) and the value of $J_{6,7}$ = 4.0 Hz.[2,5] The angelate residue at C-8 is $\beta$-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, $\beta$-oriented ester residues at this position are frequent in germacranolides of the subtribe Melampodiinae. The large coupling constant between H-8 and H-9$\beta$ (10.0 Hz) showed that H-9$\beta$ is trans to H-8. This stereochemistry places H-9$\beta$ and H-14 into a *W* relationship if the aldehyde carbonyl is oriented such that there is maximal overlap between the $\pi$ orbitals of the 1(10)-double bond and the carbonyl group, an arrangement that accounts for the observed long-range coupling between H-9$\beta$ 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 $\delta$ 2.57 and 3.07 were found for the H-9 protons. Instead, a double doublet at $\delta$ 5.80 ($J$ = 9.0, 2.0 Hz) and a singlet at $\delta$ 2.00 revealed the presence of an α-oriented acetate at C-9, as further supported by the signals at $\delta$ 69.6 (C-9), 170.4, and 20.7 (OAc) in the $^{13}$C NMR spectrum. A singlet at $\delta$ 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, $\gamma$-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 ($\delta$ 6.63, ddd) and H-14 ($\delta$ 9.46, d).[1] The *trans*-configuration of the 4,5-double bond was deduced from the typical chemical shifts of H-5 ($\delta$ 5.16, br d) and H-6 ($\delta$ 5.23, t), as well as a large $J_{6,7}$ of 10.5 Hz.[2] The signals at $\delta$ 4.53 (d) and 4.32 (br d) were assigned to H-15a and H-15b, respectively. The signal at $\delta$ 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 $\beta$-oriented. An

allylic coupling between H-1 and H-9α ($J$ = 2.0 Hz) and a *W*-type coupling between H-9$\beta$ and H-14 ($J$ = 1.5 Hz) were observed. The typical signals for an α-methylene-$\gamma$-lactone moiety appeared at $\delta$ 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 $\delta$ 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 ($\delta$ 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) $\lambda_{max}$ (log $\epsilon$) 210 (4.1) nm; IR ($CHCl_3$) $\nu_{max}$ 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) $\lambda_{max}$ (log $\epsilon$) 209 (4.5) nm; IR ($CHCl_3$) $\nu_{max}$ 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-8$\beta$-angeloyloxy-14-oxo-(4*Z*)-acanthospermolide (4):** gum; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 211 (5.3) nm; IR ($CHCl_3$) $\nu_{max}$ 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) $\lambda_{max}$ (log $\epsilon$) 212 (5.1) nm; IR ($CHCl_3$) $\nu_{max}$ 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) $\lambda_{max}$ (log $\epsilon$) 200 (3.6) nm; IR ($CHCl_3$) $\nu_{max}$ 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) $\lambda_{max}$ (log $\epsilon$) 204 (4.1) nm; IR ($CHCl_3$) $\nu_{max}$ 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) $\lambda_{max}$ (log $\epsilon$) 207 (3.8) nm; IR ($CHCl_3$) $\nu_{max}$ 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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