cis,cis-Germacranolides and Melampolides from *Mikania thapsoides*

César A. N. Catalán,[†,‡] María del R. Cuenca,[‡] Luis R. Hernández,[§] and Pedro Joseph-Nathan*,[⊥]

Instituto de Química Orgánica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, S. M. de Tucumán, 4000, Argentina, Instituto de Ecología, Universidad del Mar, Puerto Ángel, Oaxaca, 70902 México, and Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, D. F., 07000 México

The aerial parts of *Mikania thapsoides* afforded six new *cis,cis*-germacranolide-type sesquiterpene lactones (**1**–**6**), of which three (**1**–**3**) have an unusual 3,4-epoxy function, and also afforded two new melampolides (**7** and **8**). All compounds have a *trans* C-8 lactone ring closure. Their structures were elucidated using 1D and 2D NMR measurements, and the absolute configuration of **1** was determined using the Mosher ester method.
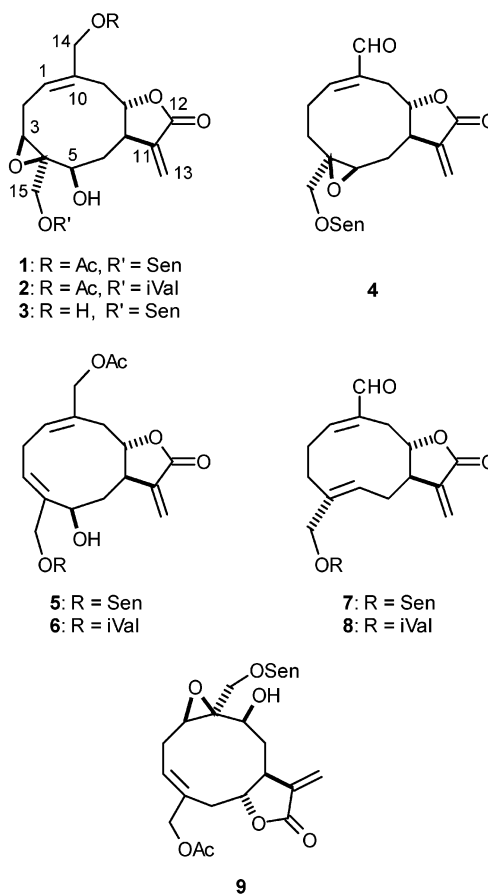
The genus *Mikania* is the largest genus in the tribe Eupatorieae (Asteraceae) with more than 430 mainly American species.[1] Although it is one of the most distinctive and easily recognized genera of the tribe, species delimitation is often difficult due to the very large number of taxa and the existence of highly polymorphic species complexes.[1,2] Some *Mikania* species, particularly those known as "guaco", are used in Central and South American traditional medicine as antiinflammatory agents,[3,4] to treat rheumatism, influenza, and respiratory tract diseases, and also as remedies against snake bite.[5]

Sesquiterpene dilactones of the mikanolide type and germacranolides functionalized at either C-14 or C-15, or at both, are typical secondary metabolites of a significant number of species, while another group of *Mikania* species contains *ent*-kauranes as major terpenoid constituents,[6] although diterpenes rarely co-occur with sesquiterpene lactones in the same plant.

In the present article, we report the isolation of eight new sesquiterpene α-methylene-γ-lactones from *Mikania thapsoides* DC. Six of them (**1**–**6**) are based on a *cis,cis*-germacranolide skeleton, and the remaining two (**7** and **8**) are melampolides. All compounds are *trans* lactones closed at C-8. Considering the plane of symmetry through C-2–C-7 of the *cis,cis*-germacranolide skeleton,[7] the mirror images of structures **1**–**6** can be drawn, as illustrated in structure **9**, which is the enantiomorphic alternative to **1**. To settle this structural ambiguity, and knowing that H-7 is α-oriented in all lactones isolated from higher plants,[8] the absolute configuration of **1** was determined using the Mosher ester method.[9]

Results and Discussion

Compound **1** showed IR bands for hydroxyl, γ-lactone, and ester groups at 3308, 1764, 1738, and 1715 cm^{-1}, respectively. The molecular formula, $C_{22}H_{28}O_8$, followed from its HRMS, which showed a [M]$^+$ at *m/z* 420.1767, accounting for nine degrees of unsaturation, while the LRMS showed peaks at *m/z* 402 [M − H$_2$O]$^+$, 360 [M − AcOH]$^+$, and 83 (100%) [C$_5$H$_7$O]$^+$, indicating the presence

1: R = Ac, R' = Sen
2: R = Ac, R' = iVal
3: R = H, R' = Sen

4

5: R = Sen
6: R = iVal

7: R = Sen
8: R = iVal

9

of hydroxyl, acetate, and an unsaturated five-carbon atom ester, respectively. The ^{1}H NMR spectrum of **1** (Table 1) showed two characteristic α-methylene-γ-lactone doublets corresponding to H-13a and H-13b at δ 6.31 (*J* = 3.6 Hz) and 5.58 (*J* = 3.1 Hz), respectively, whose coupling constant values, larger than 3.0 Hz, are indicative of a *trans* ring lactonization,[10,11] since a *cis* lactone would be evidenced by smaller coupling constant values, typically in the 0–2 Hz range.[12] The nature of the ester residues was evident from the typical acetate signal at δ 2.03 and the senecioate signals at 5.65 (hept., *J* = 1.3 Hz), 2.18 (3H, d, *J* = 1.3 Hz), and 1.91 (3H, d, *J* = 1.3 Hz). Moreover, two AB systems were present, one showing a pair of sharp doublets at δ 4.30 and 3.87 (*J* = 12.6 Hz), assigned to CH$_2$-15, and the other one as a pair of broadened doublets at δ 4.51 and

* To whom correspondence should be addressed. Tel: (52-55) 5747-7112. Fax: (52-55) 5747-7113. E-mail: pjoseph@nathan.chem.cinvestav.mx.
† Research Member of the National Research Council of Argentina (CONICET).
‡ Universidad Nacional de Tucumán.
§ Universidad del Mar.
⊥ Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional.

Table 1. ¹H NMR Data of Compounds **1**–**4**^{a} (300 MHz, CDCl₃, TMS as internal standard)

H	1	1^b	2	3	4
1	5.89 m	5.46 br dd (11.3, 6.0)	5.87	5.88	6.69 ddd (11.6, 5.51, 1.4)
2α	2.65 m	2.16 br ddd (12.7, 6.0, 3.8)	2.65	2.65	2.76–2.86 m
2β	3.10 br q (11.2)	2.82 ddd (12.7, 11.3, 11.2)	3.10	3.10	2.56–2.58 m
3α	3.09	2.60 ddd (11.2, 3.8, 1.0)	3.09	3.09	2.60–2.72 m
3β					1.24–1.42 m
5	4.64 br dd (3.6, 3.4)	4.20 ddd (3.6, 3.4, 1.0)	4.63	4.63	2.76–2.86 m
6α	2.21 ddd (16, 3.4, 1.6)	1.82	2.20	2.20	2.32 ddd (13.2, 3.8, 2.2)
6β	2.10 ddd (16, 10.7, 3.6)	1.55	2.10	2.10	1.24–1.42 m
7	2.99 ddddd (10.7, 7.4, 3.6, 3.1, 1.6)	2.80	2.96	2.98	2.63 m
8	4.54 ddd (7.4, 4.3, 2.4)	4.13	4.53	4.52	4.49 ddd (10.0, 6.6, 2.5)
9α	2.57 dd (15.1, 2.4)	2.33	2.56	2.57	2.94 dd (14.3, 10.0)
9β	3.34 dd (15.1, 4.3)	3.06	3.33	3.30	2.82 dd (14.3, 2.5)
13a	6.31 d (3.6)	6.23	6.34	6.32	6.35 d (3.6)
13b	5.58 d (3.1)	5.20	5.58	5.59	5.64 d (3.1)
14a	4.51 d (12.9)	4.45 br s	4.50	4.06 br s	9.52 br s
14b	4.42 d (12.9)	4.45 br s	4.42	4.06 br s	
15a	4.30 d (12.6)	4.19	4.44	4.32	4.32 d (12.6)
15b	3.87 d (12.6)	3.56	3.76	3.83	4.11 d (12.6)
2′	5.65 hept (1.3)	5.61	2.04–2.26 m	5.65	5.74 hept (1.3)
3′			2.04–2.26 m		
4′	2.18 br d (1.3)	2.06	0.95 (6.5)	2.18	2.21 br d (1.3)
5′	1.91 br d (1.3)	1.43	0.94 (6.5)	1.92	1.95 br d (1.3)
AcO	2.03 s	1.78	2.03		

^{a} Multiplicities and coupling constant values for each compound are equal to those of the previous molecule, excepting the changes shown. ^{b} Measured in benzene-d_6.
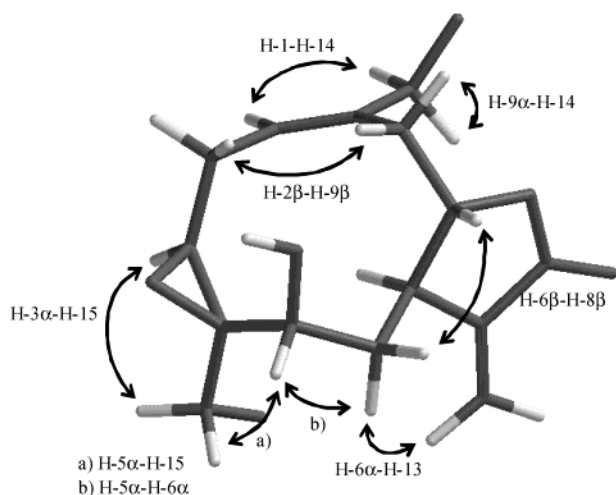
Figure 1. Minimum energy conformation of **1** and some NOESY correlations (ester residues were omitted for clarity).

4.42 (J = 12.9 Hz) assigned to CH₂-14. Other relevant signals were a ddd at δ 4.54 (J = 7.4, 4.3, 2.4 Hz) assigned to H-8 and a broad dd at δ 4.64 (J = 3.6, 3.4 Hz) assigned to H-5. Since in this spectrum, measured in chloroform, the signals at δ 5.89 and 3.09 were not first-order signals, measurement in benzene was also performed, whereby all relevant signals became first order. Thus, the complex signals appearing at δ 5.89 and 3.09 in CDCl₃ appeared at δ 5.46 as a broad dd (J = 11.3, 6.0 Hz) and at 2.60 as a ddd (J = 11.2, 3.8, 1.0 Hz) in benzene-d_6, which could be assigned to H-1 and H-3, respectively. The signal assigned to H-3 clearly showed a long-range *W*-type coupling with that of H-5 at δ 4.20 (ddd, J = 3.6, 3.4, 1.0), indicating the presence of a β-oriented epoxide between C-3 and C-4. In addition, the *E* configuration of the C-1−C-10 double bond and the relative stereochemistry of all chiral centers were confirmed by NOESY experiments in both solvents. It was found that H-1 showed cross-peaks with H-14, H-2β with H-9β, H-3α with H-15, H-5α with H-15 and H-6α, H-6α with H-13b, H-6β with H-8β, and H-9α with H-14 (see Figure 1).

Table 2. ¹³C NMR Data of Compounds **1**, **2**, **4**, and **6** (at 75.4 MHz, CDCl₃, TMS as internal standard)

carbon	1	2	4	6
1	127.3	126.8	157.7	130.0
2	27.2	27.5	26.8	27.1
3	60.5	60.7	32.1	71.2
4	61.8	61.9	59.9	134.7
5	69.4	69.1	60.1	130.9
6	35.5	35.5	31.8	38.7
7	36.7	36.8	39.2	39.0
8	84.3	84.2	79.2	84.3
9	31.0	31.2	28.1	32.5
10	134.6	135.0	139.2	132.7
11	140.2	140.4	137.5	140.3
12	169.6	169.3	168.9	169.6
13	121.4	121.4	123.8	121.2
14	68.4	68.3	194.4	66.5
15	66.0	66.3	62.6	68.7
1′	165.4	172.3	165.8	172.9
2′	114.6	43.0	114.6	43.4
3′	159.3	27.5	159.8	25.7
4′	20.3	22.4	20.5	22.4
5′	27.4	22.4	27.6	22.4
AcO	170.5	170.4		170.7
	20.6	20.8		20.8

Further structural evidence was gained from the ¹³C NMR spectrum, which showed the presence of 22 carbons as shown in Table 2. The DEPT spectrum of **1** exhibited three methyl, six methylene, and six methine signals, while the remaining seven signals corresponded to quaternary carbon atoms. Three carbonyls, six olefinic carbons, and six sp³ carbons bearing oxygen could be readily assigned (Table 2). The chemical shift of the signal assigned to C-8 (δ 84.3) is also indicative of a *trans* lactonization to C-8[11] since in *cis* lactones closed at C-8 the signal appears in the δ 70−79 range.[12] Also, the chemical shift of C-14 at δ 68.4 confirmed the *E* configuration of the C-1−C-10 double bond.[11,13,14] The ester residue positions were deduced from a gHMBC experiment. Complete ¹H and ¹³C NMR signal assignments were achieved with the aid of COSY, DEPT, gHSQC, and gHMBC experiments. The relative stereochemistry of the chiral centers was established by the magnitude of the coupling constants and from a NOESY experiment.

Table 3. Dihedral Angles and Calculated and Observed Coupling Constant Values for **1**

position	θ (deg)	J_{calcd} (Hz)	J_{obs} (Hz)
1,2a	−47	4.5	6.0
1,2b	−160	10.6	11.3
2a,3	58	4.4	3.8
2b,3	171	11.0	11.2
5,6a	54	2.6	3.4
5,6b	−59	4.0	3.6
6a,7	76	1.4	1.6
6b,7	−169	11.9	10.7
7,8	149	8.7	7.4
8,9a	70	2.5	2.4
8,9b	−42	4.4	4.3

Table 4. Selected ^{1}H NMR Data for (*R*)- and (*S*)-MTPA Esters of **1**

H	*R*-MTPA	*S*-MTPA	$\Delta\delta_{R-S}$
1	5.79	5.68	0.11
2α	2.60	1.72	0.88
3	2.99	2.79	0.20
6α	2.27	2.41	−0.14
7	2.81	3.02	−0.21
8	3.06	4.40	−1.34
9α	1.71	2.53	−0.82
9β	2.30	3.12	−0.82
13a	6.28	6.34	−0.06
13b	5.56	5.63	−0.07
14a	4.35	4.44	−0.09
14b	4.24	4.35	−0.11

The relative stereochemistry of all chiral centers on the germacranolide skeleton was confirmed by molecular modeling. The minimum energy conformation of **1** (Figure 1) was obtained using the PCMODEL program[15] and is in agreement with the correlations observed in the NOESY experiments. Dihedral angles, along with calculated and observed coupling constants, are listed in Table 3.

Owing to the C-2−C-7 symmetry plane of the germacranolide skeleton,[7] an alternative structure having a *trans* lactone closed at C-6 can be drawn. Such an alternative structure to **1** would have a 1(10)-epoxy group, a double bond between C-3 and C-4, and a hydroxyl group at C-9, as depicted in **9**. Since lactone **1** contains the very unusual 3β,4β-epoxy group (or 1β,10β-epoxy in the eventuality of **9**), it was necessary to settle the ambiguity between the two possible alternatives, i.e., **1** or **9**. This is possible since H-7 is α-oriented for both alternative structures, as is the case for all sesquiterpene lactones isolated from higher plants.[8] Thus, if the new natural product has structure **1**, the hydroxyl group-bearing carbon (C-5) is *R*, while if the structure is **9**, the corresponding carbon (C-9) should be *S*, and therefore the ambiguity can be solved using the Mosher ester method.[9] The (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters were prepared to determine the absolute configuration of the carbon bearing the hydroxyl group. Selected hydrogen chemical shifts of the respective MTPA esters are listed in Table 4. From the obtained values, the *R* configuration was deduced, and consequently the natural product has structure **1**. It is interesting to note the high shielding undergone by H-8 (1.34 ppm) of the *R*-MTPA ester as compared to the *S*-MTPA ester, which indicated that H-8 is strongly affected by the phenyl group of the MTPA moiety. On the other hand, the H-7 shielding (0.21 ppm) was smaller than that of H-8, which is in agreement with the *trans* lactone ring closure.

Compound **2** showed in the HRMS the [M]$^+$ at *m/z* 422.1925, in agreement with the molecular formula, C$_{22}$H$_{30}$O$_8$. Peaks at *m/z* 362 and 85 in the LRMS indicated

the presence of [M − C$_2$H$_4$O$_2$]$^+$ and [C$_5$H$_9$O]$^+$ ions, respectively, which correspond to acetyloxy and saturated five-carbon ester substituents. The ^{1}H NMR spectrum of **2** was almost identical to that of **1** (see Table 1) except for the signals of the senecioyl residue, which were replaced by those corresponding to an isovalerate residue. This was confirmed by the ^{13}C NMR spectrum, which displayed the typical signals of an isovalerate ester (Table 2).

The ^{1}H NMR spectrum of **3** was also very similar to that of **1** (see Table 1). Particularly evident were the lack of the acetate signal and the upfield shift of the CH$_2$-14 protons, which appeared as a broad singlet at δ 4.06, exhibiting the presence of a hydroxyl group at CH$_2$-14.

Compound **4** showed in the HRDCIMS the [M + NH$_4$]$^+$ at *m/z* 378.1929, in agreement with the molecular composition, C$_{20}$H$_{24}$O$_6$, and in the LRMS the base peak at *m/z* 83 corresponded to the [C$_5$H$_7$O]$^+$ ion, which is indicative of an unsaturated five-carbon atom ester. The ^{1}H NMR spectrum showed characteristic doublets corresponding to H-13a and H-13b of a *trans*-α-methylene-γ-lactone at δ 6.35 (*J* = 3.6 Hz) and 5.64 (*J* = 3.1 Hz). The presence of a 1(10)-*cis*-double bond with an aldehyde group at C-10 followed from the chemical shift of H-1 (δ 6.69, ddd) and H-14 (δ 9.52, br s).[16] Other absorptions were typical for a methylene group (AB system at δ 4.32 and 4.11, *J* = 12.6 Hz) bearing a senecioate ester assigned to CH$_2$-15 and for the lactone H-8 signal, which appeared at δ 4.49 as a ddd (*J* = 10, 6.6, and 2.5 Hz). The signal for the epoxide proton appeared overlapped with those of H-2α and H-9β in the δ 2.76−2.86 region. The epoxide group was assigned at C-4−C-5, as deduced from COSY, gHMBC, and gHSQC experiments. Its β-orientation was deduced from the minimum energy conformational calculations performed using the PCMODEL program for the two isomers having the epoxide either α- or β-oriented. Thus, in the isomer with the epoxide α-oriented, the dihedral angles H-5−C-5−C-6−H-6α and H-5−C-5−C-6−H-6β were 152.8° and 39.1°, respectively, corresponding to the calculated coupling constant values, $J_{5,6\alpha}$ = 8.6 Hz and $J_{5,6\beta}$ = 7.3 Hz, while the minimum energy conformation of the isomer having the epoxide in the β-position showed H-5−C-5−C-6−H-6α and H-5−C-5−C-6−H-6β dihedral angles of 57.1° and −52.4°, respectively, having 2.2 and 5.0 Hz as the calculated coupling constant values, respectively. Although the H-6β signal was superimposed on another signal, the β-orientation of the epoxide was easily deduced from the observed H-6α coupling constants, whose values were 13.2, 3.8, and 2.2 Hz. The ^{13}C NMR spectrum showed 20 signals, as shown in Table 2. The DEPT spectrum of **4** exhibited two methyls, six methylenes, six methines, and six quaternary carbons. The signals at δ 60.1 and 59.9 corresponded to carbons bearing an epoxide function, and the assignment of the remaining signals was straightforward. The complete assignments of both ^{1}H and ^{13}C NMR signals were made by the combined use[17] of COSY, DEPT, gHSQC, and gHMBC experiments.

The ^{1}H and ^{13}C NMR spectral patterns of lactones **5** and **6** (Table 5) resembled those of lactones **1** and **2**, respectively, suggesting the same substitution pattern and stereochemistry of the chiral centers, with the only difference being the replacement of the 3,4-epoxy group in **1** and **2** by the C-3−C-4 double bond. As deduced from the mass spectral (see Experimental Section) and ^{1}H and ^{13}C NMR data, their molecular formulas are C$_{22}$H$_{28}$O$_7$ and C$_{22}$H$_{30}$O$_7$, respectively, i.e., one oxygen atom less than in lactones **1** and **2**, respectively. Comparison of the corresponding ^{1}H NMR data showed that the only relevant difference between **1** and **5**, as well as between **2** and **6**, is the downfield

Table 5. ^{1}H NMR Data of Compounds **5−8**[a] (300 MHz, CDCl$_3$, TMS as internal standard)

H	5	6	7	8
1	5.84 dd (9.5, 8.0)	5.84	6.60 br t (7.5)	6.66
2α	2.72 ddd (13.5, 8.0, 7.0)	2.73	2.4−2.8 m	2.4−2.8
2β	3.66 ddd (13.5, 9.5, 9.5)	3.67	2.4−2.8 m	2.4−2.8
3α	5.84 dd (9.5, 7.0)	5.84	2.4−2.8 m	2.4−2.8
3β			2.4−2.8 m	2.4−2.8
5	4.82 br dd (6.3, 3.0)	4.81	5.31 dd (11.0, 5.8)	5.35
6α	2.20 ddd (14.8, 3.0, 3.0)	2.20	2.70 m	2.70
6β	1.98 ddd (14.8, 10.2, 6.3)	1.98	2.15 (obsc.)	2.16 dt (13.0, 11.0)
7	2.94 ddddd (10.2, 5.6, 3.6, 3.2, 3.0)	2.94	2.70 m	2.70
8	4.47 ddd (5.6, 5.2, 2.0)	4.47	3.99 m	3.99
9α	3.28 dd (15.4, 5.2)	3.25	2.90 br d (15)	2.93
9β	2.57 dd (15.4, 2.0)	2.58	2.50−2.60 (obsc.)	2.50−260
13a	6.26 d (3.6)	6.27	6.23 d (3.3)	6.28
13b	5.52 d (3.2)	5.53	5.53 d (2.9)	5.58
14a	4.50 br s	4.52 d (12.4)	9.48 br s	9.53
14b	4.50 br s	4.41 d (12.4)		
15a	4.56 d (12.1)	4.55	4.59 br s	4.58
15b	4.42 d (12.1)	4.45	4.59 br s	4.58
2′	5.65 hept. (1.3)	2.19 m	5.66 hept (1.3)	2.04−2.26 m
3′		2.10 m		2.04−2.26 m
4′	2.17 d (1.3)	0.95 d (6.5)	2.18 br d (1.3)	1.01 d (6.5)
5′	1.91 d (1.3)	0.95 d (6.5)	1.92 br d (1.3)	1.01 d (6.5)
AcO	2.03 s	2.04		

[a] Multiplicities and coupling constant values for each compound are equal to those of the previous molecule, excepting the changes shown.

shift of H-3 from δ 3.09 to δ 5.84, indicating that lactones **5** and **6** have a double bond instead of the 3,4-epoxy group found in **1** and **2**. The ^{13}C NMR spectrum of **6** (Table 2) confirmed the presence of a C-3−C-4 double bond in addition to the C-1−C-10 and C-11−C-13 double bonds.

The ^{1}H NMR data of lactones **7** and **8** (Table 5) closely matched those reported for a number of melampolides isolated from *Mikania cordifolia*,[11] with the only difference being the signals corresponding to the ester residues. Lactones **7** and **8** showed the typical signals of a senecioate and an isovalerate ester residue, respectively, in addition to the absorptions corresponding to the melampolide framework.

So far, germacranolides having the very rare 3,4-epoxy function exhibited by lactones **1−3** of *M. thapsoides* have not been reported from any other member of the genus *Mikania*. However, lactones closely related to **5** and **6**, which most likely are the biogenetic precursors of **1−3**, were isolated from a Costa Rican collection of *Mikania holwayana*.[18]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were determined on a Perkin-Elmer Lambda 12 UV/vis spectrophotometer. IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrophotometer. NMR measurements were recorded on two Varian Associates Mercury spectrometers operating at 300 MHz for hydrogen and at 75 MHz for carbon, using 5 mm sample tubes in deuteriochloroform solution containing TMS as the internal standard. LREIMS were recorded at 20 eV on a Hewlett-Packard 5989A spectrometer or at 70 eV on a Varian Saturn 2000 spectrometer, while HREIMS were measured at the UCR Mass Spectrometry Facility, University of California, Riverside, CA, on a VG 7070 high-resolution mass spectrometer. For separation of mixtures, HPLC with a differential refractometer detector was used. The columns employed were a Phenomenex Luna phenylhexyl (5 μm, 10 × 250 mm) (column A) and a Phenomenex Maxsil 10 C$_8$ (10 μm, 10 × 250 mm) (column B). Retention times (t_R) were measured from the solvent peak. For column chromatography, Si gel Merck 70−230 or 230−400 mesh ASTM was used.

Plant Material. Aerial parts of *M. thapsoides* were collected in April 1997 at Centinela, Santo Tomé, Corrientes Province, on Route 14 close to the boundary with Misiones Province, Argentina. A voucher specimen (Leg. Schinini y Dematheis No. 675, LIL 602,421) is on deposit in the herbarium of the Instituto Miguel Lillo, Tucumán, Argentina.

Extraction and Isolation. Flowers and leaves (297 g) were extracted with CHCl$_3$ (2 × 2.5 L) at room temperature for 5 days. The extract was evaporated at reduced pressure (40 °C) to give 12.5 g of crude residue, which was suspended in EtOH (110 mL) at 60 °C, diluted with H$_2$O (85 mL), and extracted successively with hexane (3 × 150 mL) and CHCl$_3$ (3 × 150 mL). Evaporation of the hexane fraction at reduced pressure gave 5.74 g of residue. Evaporation of the CHCl$_3$ extract gave 4.43 g of residue, which was column chromatographed over Si gel (220 g) using CHCl$_3$ with increasing amounts of EtOAc (0−100%), with 80 fractions being collected. These were grouped according to their TLC profiles and monitored by IR spectroscopy. Fractions showing γ-lactone absorption were processed by RP-HPLC.

Fractions 24−27 (125 mg) were combined, and a portion (85 mg) was processed by HPLC using column A (MeOH−H$_2$O, 4:1, 1.5 mL min^{-1}) to give 58 mg of **1**, t_R 6.0 min, and several partially resolved peaks, which were rechromatographed on the same column (MeOH−H$_2$O, 7:3, 2.2 mL min^{-1}) to give an additional 2.9 mg of **1**, t_R 11.8 min, and 6 mg of **4**, t_R 13.2 min.

Fractions 28−33 (264 mg) were combined, and a portion (50 mg) was processed by RP-HPLC using column B (MeOH−H$_2$O, 3:2, 2.2 mL min^{-1}) to give 16 mg of **1**, t_R 15.5 min; 4.8 mg of **2**, t_R 18.0 min; 2.2 mg of **5**, t_R 22.5 min; 3.4 mg of **7**, t_R 24.5 min; and 5.2 mg of **8**, t_R 28.5 min.

Fractions 34−36 (476 mg) were combined, and a portion (120 mg) was chromatographed using column B (MeOH−H$_2$O, 3:2, 2.0 mL min^{-1}) to give 40 mg of **1**, t_R 17.4 min; 7.9 mg of **2**, t_R 20.7 min; 3.7 mg of **5**, t_R 28.5 min; and 6.1 mg of **6**, t_R 33.7 min.

Fractions 62−85 (601 mg) were combined, and a portion thereof (150 mg) was chromatographed using column B (MeOH−H$_2$O, 1:1, 2.5 mL min^{-1}); the poorly resolved peaks obtained were rechromatographed on column A (MeOH−H$_2$O, 4:3, 2.2 mL min^{-1}) to give 2.6 mg of **3**, t_R 15.0 min, and 69 mg of a inseparable complex mixture of lactones.

1(10)E-(3S,4R,5R,7S,8S)-14-Acetyloxy-3,4-epoxy-5-hydroxy-15-senecioyloxygermacra-1(10),11(13)-dien-8,12-olide (1): gum; $[\alpha]_{589}^{26}$ +27° (c 1.98, CHCl$_3$); UV (EtOH) λ_{max} (log ϵ) 209 (3.6) nm; IR (CHCl$_3$) ν_{max} 3308, 1764, 1738, 1715,

1650 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 420 [M]$^+$ (7), 402 [M − H$_2$O]$^+$ (0.2), 360 [M − C$_2$H$_4$O$_2$]$^+$ (2), 214 (4), 83 [C$_5$H$_7$O]$^+$ (100), 55 (22); HREIMS *m/z* 420.1767 [M]$^+$ (calcd for C$_{22}$H$_{28}$O$_8$, 420.1784).

1(10)*E*-(3*S*,4*R*,5*R*,7*S*,8*S*)-14-Acetyloxy-3,4-epoxy-5-hydroxy-15-isovaleroyloxygermacra-1(10),11(13)-dien-8,12-olide (2): gum; [α]$_{589}^{26}$ +52° (*c* 0.52, CHCl$_3$); UV (EtOH) λ_{max} (log ϵ) 210 (3.5) nm; IR (CHCl$_3$) ν_{max} 3300, 1765, 1739, 1720, 1680 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 422 [M]$^+$ (8), 362 [M − C$_2$H$_4$O$_2$]$^+$ (2), 214 (18), 260 (18), 109 (19), 85 [C$_5$H$_9$O]$^+$ (67), 57 (100), 43 (56); HREIMS *m/z* 422.1925 [M]$^+$ (calcd for C$_{22}$H$_{30}$O$_8$, 422.1941).

1(10)*E*-(3*S*,4*R*,5*R*,7*S*,8*S*)-3,4-Epoxy-5,14-dihydroxy-15-senecioyloxygermacra-1(10),11(13)-dien-8,12-olide (3): gum; UV (EtOH) λ_{max} (log ϵ) 210 (3.5) nm; IR (CHCl$_3$) ν_{max} 3350, 1763, 1715, 1685 cm^{-1}; ^{1}H NMR, see Table 1; HREIMS *m/z* 378.1660 [M]$^+$ (calcd for C$_{20}$H$_{26}$O$_7$, 378.1678).

1(10)*E*-(4*R*,5*R*,7*S*,8*S*)-4,5-Epoxy-14-oxo-15-senecioyloxygermacra-1(10),11(13)-dien-8,12-olide (4): gum; [α]$_{589}^{26}$ +54° (*c* 0.54, CHCl$_3$); UV (EtOH) λ_{max} (log ϵ) 212 (3.9) nm; IR (CHCl$_3$) ν_{max} 2730, 1762, 1724, 1684, 1628 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* 360 [M]$^+$ (1), 231 (7), 215 (4), 121 (4), 105 (3), 83 [C$_5$H$_7$O]$^+$ (100), 82 (36), 55 (28), 53 (11); HRDCIMS (NH$_3$) *m/z* 378.1929 [M + NH$_4$]$^+$ (calcd for C$_{20}$H$_{24}$O$_6$ + NH$_4^+$ 378.1917).

1(10)*E*-3*Z*-(5*R*,7*S*,8*S*)-14-Acetyloxy-5-hydroxy-15-senecioyloxygermacra-1(10),3,11(13)-trien-8,12-olide (5): gum; UV (EtOH) λ_{max} (log ϵ) 211 (3.7) nm; IR (CHCl$_3$) ν_{max} 3315, 1763, 1737, 1718, 1683 cm^{-1}; ^{1}H NMR, see Table 5; EIMS *m/z* 404 [M]$^+$ (2), 345 (6), 244 (15), 216 (12), 198 (12), 171 (7), 131 (9), 91 (15), 83 [C$_5$H$_7$O]$^+$ (100), 55 (22); HREIMS *m/z* 404.1821 [M]$^+$ (calcd for C$_{22}$H$_{28}$O$_7$, 404.1835).

1(10)*E*-3*Z*-(5*R*,7*S*,8*S*)-14-Acetyloxy-5-hydroxy-15-isovaleroyloxygermacra-1(10),3,11(13)-trien-8,12-olide (6): gum; [α]$_{589}^{26}$ +58° (*c* 0.24, CHCl$_3$); UV (EtOH) λ_{max} (log ϵ) 209 (3.8) nm; IR (CHCl$_3$) ν_{max} 3310, 1765, 1739, 1720, 1680 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 5 and 2, respectively; EIMS *m/z* 406 [M]$^+$ (5), 244 (73), 226 (54), 216 (51), 198 (54), 171 (32), 131 (42), 105 (38), 91 (61), 85 [C$_5$H$_9$O]$^+$ (50), 57 (100), 43 (86); HREIMS *m/z* 406.2008 [M]$^+$ (calcd for C$_{22}$H$_{30}$O$_7$, 406.1991).

1(10)*E*-4*Z*-(7*S*,8*S*)-14-Oxo-15-senecioyloxygermacra-1(10),4,11(13)-trien-8,12-olide (7): gum; UV (EtOH) λ_{max} (log ϵ) 208 (3.7) nm; IR (CHCl$_3$) ν_{max} 2727, 1758, 1725, 1680, 1630 cm^{-1}; ^{1}H NMR, see Table 5; EIMS *m/z* 344 [M]$^+$ (1), 244 (18), 215 (8), 188 (10), 121 (4), 105 (8), 91 (13), 83 [C$_5$H$_7$O]$^+$ (100), 82 (14), 55 (26), 53 (9); HREIMS *m/z* 344.1613 [M]$^+$ (calcd for C$_{20}$H$_{24}$O$_5$, 344.1624).

1(10)*E*-4*Z*-(7*S*,8*S*)-14-Oxo-15-isovaleroyloxygermacra-1(10),4,11(13)-trien-8,12-olide (8): gum; UV (EtOH) λ_{max} (log ϵ) 208 (3.6) nm; IR (CHCl$_3$) ν_{max} 2728, 1760, 1725, 1682, 1632 cm^{-1}; ^{1}H NMR, see Table 5; EIMS *m/z* 346 [M]$^+$ (1), 244 (64), 215 (32), 188 (42), 169 (24), 148 (24), 133 (25), 120 (33), 105 (39), 91 (61), 77 (32), 85 [C$_5$H$_9$O]$^+$ (46), 57 (100); HREIMS *m/z* 346.1768 [M]$^+$ (calcd for C$_{20}$H$_{26}$O$_5$, 346.1780).

References and Notes

(1) King, R. M.; Robinson, H. *The Genera of the Eupatorieae (Asteraceae)*; Monographs in Systematic Botany; Missouri Botanical Garden: St. Louis, MO, 1987; p 419.
(2) Holmes, W. C. A Revision of *Mikania scandens* and Relatives. Ph.D. Thesis, Mississippi State University, Starkville, MS, 1975.
(3) Morton, J. F. In *Atlas of Medicinal Plants of Middle America, Bahamas to Yucatan*; Thomas, C. C., Ed.; Springfield, IL, 1981; pp 947−948.
(4) Pereira, A. M. S.; Camara, F. L. A.; Celeghini, R. M. S.; Vilegas, J. H. Y.; Lanças, F. M.; França S. C. *J. Herbs Spices Med. Plants* **2000**, *7*, 1−10.
(5) Gupta, M. P. In *270 Plantas Medicinales Iberoamericanas*; Gupta, M. P., Ed.; Convenio Andrés Bello, CYTED-SECAB: Bogotá, 1995; p 124.
(6) Herz, W. *J. Indian Chem. Soc.* **1998**, *75*, 559−564.
(7) Rogers, D.; Moss, G. P.; Neidle, S. *J. Chem. Soc., Chem. Commun.* **1972**, 142−143.
(8) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer; Vienna, 1979; Vol. 38, pp 47−390.
(9) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(10) Díaz, J. G.; Goedken, V. L.; Herz, W. *Phytochemistry* **1992**, *31*, 597−608.
(11) Gutiérrez, A. B.; Oberti, J. C.; Sosa, V. E.; Herz, W. *Phytochemistry* **1987**, *26*, 2315−2320.
(12) Delgado, G.; Tejeda, V.; Salas, A.; Chávez, M. I.; Guzmán, S.; Bolaños, A.; Aguilar, M. I.; Navarro, V.; Villarreal, M. L. *J. Nat. Prod.* **1998**, *61*, 1082−1085.
(13) Rüngeler, P.; Brecht, V.; Tamayo-Castillo, G.; Merfort, I. *Phytochemistry* **2001**, *56*, 475−489.
(14) Cartagena, E.; Bardón, A.; Catalán, C. A. N.; de Hernández, Z. N. J.; Hernández, L. R.; Joseph-Nathan, P. *J. Nat. Prod.* **2000**, *63*, 1323−1328.
(15) Burket, U.; Allinger N. L. *Molecular Mechanics*; ACS Monograph 177; American Chemical Society: Washington, DC, 1982.
(16) Herz, W.; Kalyanaraman, P. S. *J. Org. Chem.* **1975**, *40*, 3486−3491.
(17) Morales-Ríos, M. S.; Santos-Sánchez, N. F.; Suárez-Castillo, O. R.; Joseph-Nathan, P. *Magn. Reson. Chem.* **2002**, *40*, 677−682.
(18) Castro, V.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1989**, *28*, 527−530.

NP030055P