# Preparation, Stereochemistry, and Cytotoxic Activity of the Melampolides from *Mikania minima*

Alejandro F. Barrero,*,† J. Enrique Oltra,† Ignacio Rodríguez-García,‡ Armando Barragán,† and Míriam Álvarez†

*Departamento de Química Orgánica, Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain, and Departamento de Química Orgánica, Facultad de Ciencias Experimentales, Universidad de Almería, 04120 Almería, Spain*

*Received September 3, 1999*

The semisyntheses of melampolides **1** and **2**, previously found in *Mikania minima*, were carried out in order to confirm their chemical structures and to establish their absolute configurations. Their conformational analyses, performed using molecular mechanics, experimental $^1$H NMR coupling constants, and NOE experiments, showed a preferred DU conformer in solution at room temperature. The cytotoxic activities of **1** and **2** against three tumor cell lines were also determined. Conjugated aldehyde **2** showed higher activity than alcohol **1**.

Among the four configurational types of germacranolides, germacrolides (*trans*,*trans*-germacra-1(10),4-dienolides) are the best-known compounds. On the other hand, numerous chemical and biological aspects of melampolides, heliangolides, and *cis*,*cis*-germacranolides are still poorly documented. Although a rigorous conformational analysis of germacrolides was carried out 13 years ago,[1] there is not much information about the conformational behavior of the other germacranolide types. Recently, small amounts of melampolides **1** and **2** have been isolated from *Mikania minima*, and their chemical structures have been established by means of $^1$H NMR spectroscopy.[2] Because we are interested in the chemistry and biological activity of natural sesquiterpene lactones,[3−5] as well as in the stereochemistry of medium-size ring sesquiterpenoids,[6] we have synthesized melampolides **1** and **2** from (+)-salonitenolide (**3**). Subsequently, the conformational analysis of **1** and **2** has been carried out. The cytotoxic activities of both melampolides have also been tested against three tumor cell lines.

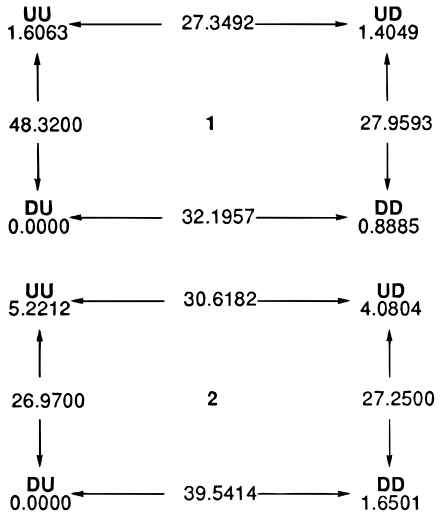
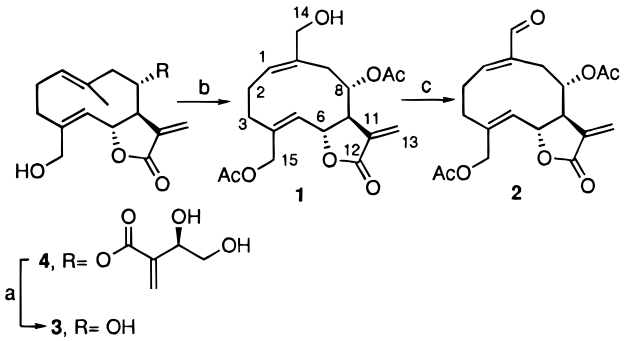



**Figure 1.** Relative energies (kcal/mol) of the four conformers of **1** and **2** and calculated energy barriers (kcal/mol) between them (MM2).

## Results and Discussion

(+)-Salonitenolide (**3**) may be isolated from *Centaurea calcitrapa*.[5] However, for this study, **3** was obtained through side-chain saponification of (+)-cnicin (**4**) isolated from *C. malacitana*, to establish a chemical correlation between **4** and the melampolides from *M. minima*. Regioselective allylic oxidation of the diacetyl derivative of **3** took place with concomitant isomerization of the $\Delta^{1(10)}$ double bond, leading to **1** (70% yield from **3**) (Scheme 1). The $^1$H NMR spectrum of semisynthetic **1** matched that of the natural product.[2] The $^{13}$C NMR chemical shift of C-14 supported the *E* stereochemistry of the $\Delta^{1(10)}$ double bond, which was confirmed by the NOE observed between H-1 and H-14. Mechanistic justification for the endocyclic double-bond isomerization in germacrolides, under treatment with $SeO_2$ and *tert*-butyl hydroperoxide, has been previously discussed.[7] Mild oxidation of the allylic alcohol **1** gave the conjugated aldehyde **2** (73% yield), the $^1$H NMR spectrum of which matched that of the aldehyde from *M. minima*.[2] In the $^{13}$C NMR spectrum of **2**, the chemical shift

**Scheme 1**




a) $K_2CO_3$. b) i: $Ac_2O$/Py; ii: $SeO_2$, *t*-BuOOH. c) PDC

of C-14 indicated that the *E* stereochemistry of the $\Delta^{1(10)}$ double bond remained unchanged after oxidation. This was further supported by the observed NOE between H-1 and the aldehydic hydrogen.

Thus, the semisynthesis of **1** and **2** from **3** confirmed the structures previously proposed on the basis of $^1$H NMR spectral features.[2] Inasmuch as the absolute stereochemistry of (+)-cnicin (**4**) was previously established by X-ray

* To whom correspondence should be addressed. Tel. 34 958 24 33 18. Fax: 34 958 24 84 37. E-mail:afbarre@goliat.ugr.es.
† Universidad de Granada, Spain.
‡ Universidad de Almería, Spain.

10.1021/np990436w CCC: $19.00 
Published on Web 01/28/2000

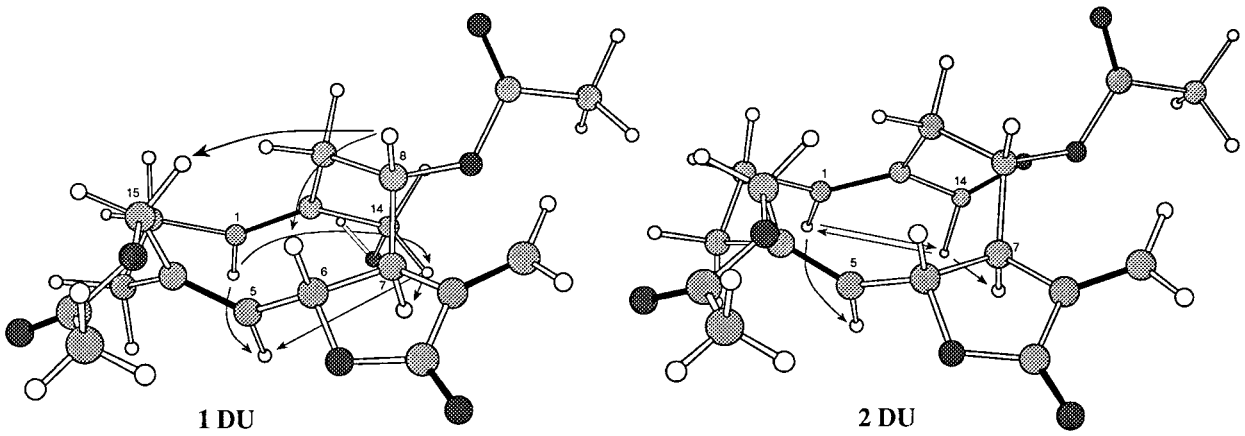



**Figure 2.** Minimum energy conformers of compounds **1** and **2**. Arrows show the observed NOEs and start at the irradiated hydrogens.

**Table 1.** Selected Coupling Constants of Compounds **1** and **2** (in Hz)

| | **1** | | **2** | |
|---|---|---|---|---|
| | $J_{theor}$[a] | $J_{exp}$[b] | $J_{theor}$[a] | $J_{exp}$[b] |
| $J_{1,2\alpha}$ | 9.4 | 7.4 | 9.4 | 9.1 |
| $J_{1,2\beta}$ | 5.2 | 7.4 | 5.2 | 7.0 |
| $J_{5,6}$ | 11.6 | 10.5 | 11.6 | 10.6 |
| $J_{6,7}$ | 11.0 | 9.7 | 10.9 | 9.7 |
| $J_{7,8}$ | 10.6 | 10.9 | 10.6 | 10.6 |
| $J_{8,9\alpha}$ | 3.1 | 3.2 | 2.8 | 2.7 |
| $J_{8,9\beta}$ | 3.7 | 3.2 | 3.9 | 4.3 |

[a] $J_{theor}$ = theoretical values obtained using MM2 calculations. [b] $J_{exp}$ = experimental values measured on the $^1$H NMR spectra.

analysis,[8] the chemical correlation between **4** and the (+)-enantiomers of **1** and **2** indicated that the absolute configuration of these compounds is as shown. Unfortunately, optical rotation of the melampolides from *M. minima* was not previously reported.

Once the chemical structure and configuration of **1** and **2** were confirmed, a conformational analysis was conducted using molecular mechanics calculations, $^1$H NMR coupling constants, and NOE experiments. In solution, *trans,trans*-germacra-1(10),4-dien-6,12-olides show four main conformations, called UU, UD, DU, and DD depending on the orientation "up" or "down" of C-14 and C-15, respectively. The UU conformer is the most stable and predominant one at room temperature.[1] Our molecular mechanics calculations (MM2)[9] also predict four possible conformers for melampolides **1** and **2**, but, in contrast to germacrolides, the conformer DU is the most stable. The relative energies calculated for the four conformers in both compounds **1** and **2** and the energy barriers calculated between them are given in Figure 1. Figure 2 shows the conformers DU of **1** and **2** calculated by molecular mechanics.[9] Calculated values (MM2) of the vicinal coupling constants of the conformers DU are in agreement with those measured in the $^1$H NMR spectra of **1** and **2** (see Table 1). Furthermore, the NOEs observed in a series of NOE-dif[10] experiments support the predominant DU conformation of both compounds **1** and **2** in solution (see Figure 2). In these melampolides, the energy barriers surrounding the DU conformer (Figure 1) are high enough to prevent any appreciable conformational interconversion at room temperature; therefore, NMR spectra show signals only for this conformer (it is known that some medium-size ring sesquiterpenoids exist as two or more conformers in equilibrium at room temperature).[1,6,11] On the basis of limited data from X-ray and neutron diffraction studies, Fischer et al. proposed that an arrangement like the DU conformation is typical for the melampolides,[12] and this proposal has been assumed later by other authors. Our results provide the first theoretical−experimental combined evidence confirming Fischer's hypothesis and extending it to melampolides in solution.

Finally, the cytotoxic activities of **1** and **2** for three tumor cell lines were tested.[13] $IC_{50}$ values in the order of $10^{-6}$ M were observed (see Experimental Section). Melampolide **1** shows an activity level similar to that of salonitenolide diacetate,[14] a germacrolide that probably exists as a preferred UU conformer in solution.[1] It seems that the additional OH group of **1**, as well as the configurational and conformational changes, do not seriously affect the cytotoxicity of these germacranolides. On the other hand, aldehyde **2** showed higher activity than alcohol **1**. It is generally accepted that the cytotoxic activity of the sesquiterpene lactones basically lies in the α-methylene-γ-lactone and is enhanced by an additional Michael acceptor group.[15] Hence, the higher activity of **2** may be attributed to the conjugated aldehyde.

## Experimental Section

**General Experimental Procedures.** These procedures were described previously.[16]

***(6S,7R,8S)*-8,15-Diacetoxy-14-hydroxymelampa-1(10),4,11(13)-trien-12,6-olide (1).** (+)-Salonitenolide (**3**) (380 mg, 1.44 mmol), obtained from (+)-cnicin (**4**) with $K_2CO_3$, as previously described,[14] was treated with acetic anhydride (3.7 mL) and pyridine (3.7 mL). After the usual workup, the diacetate of salonitenolide[14] (410 mg, 1.18 mmol, 82%) was obtained and oxidized as follows. A 3 M solution of *tert*-butyl hydroperoxide in isooctane (0.8 mL, 2.4 mmol) was added to a suspension of $SeO_2$ (67 mg, 0.60 mmol) in anhydrous $CH_2Cl_2$ (1 mL) and stirred until a clear solution was obtained. Then, a solution of the diacetate (410 mg, 1.18 mmol) in anhydrous $CH_2Cl_2$ (9 mL) was added and stirred under argon for 6 h between 5 and 10 °C. The mixture was then diluted with $CH_2Cl_2$ (25 mL), washed with $H_2O$ (2 × 25 mL), and dried with anhydrous $Na_2SO_4$. The solvent was removed in vacuo, and the residue was chromatographed over a Si gel column ($CHCl_3$−$Me_2CO$, 9:1) yielding (**1**) (370 mg, 1.02 mmol, 86%): colorless oil; $[\alpha]^{20}_D$ +89.4° (*c* 1.02, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 205 (4.33) nm; IR (film) $\nu_{max}$ 3482, 2937, 1765, 1737, 1657, 1377, 1235, 1142, 1029, 983 cm$^{-1}$; NOE experiments, proton irradiated (NOE observed) H-8 (H-6, H-15), H-1 (H-5, H-14), H-14 (H-5, H-7); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 170.7 (s, C-12), 169.9 (s, Me*C*OO), 169.5 (s, Me*C*OO), 139.1 (s, C-10), 138.2 (s, C-4), 136.6 (s, C-11), 128.0 (d, C-5), 127.6 (d, C-1), 122.8 (t, C-13), 75.4 (d, C-6), 72.5 (d, C-8), 67.9 (t, C-14), 62.1 (t, C-15), 48.1 (d, C-7), 34.0 (t, C-3), 30.2 (t, C-9), 26.0 (t, C-2), 21.3 (*C*$H_3$COO), 20.9 (*C*$H_3$COO); CIMS *m*/*z* 365 [M + H]$^+$ (27), 347 [365 − $H_2O$]$^+$ (41), 305 [365 − HOAc]$^+$ (60), 287 [305 − $H_2O$]$^+$ (66), 245 [305 − HOAc]$^+$ (100), 227 [245 − $H_2O$]$^+$ (51),

215 (38), 199 (6); HRCIMS *m/z* 365.1599 $[M + H]^+$ (calcd for $C_{19}H_{25}O_7$ 365.1600).

***(6S,7R,8S)*-8,15-Diacetoxy-14-oxomelampa-1(10),4,11-(13)-trien-12,6-olide (2).** Pyridinium dichromate (178 mg, 0.47 mmol) was added to a solution of **1** (107 mg, 0.29 mmol) in anhydrous $CH_2Cl_2$ (4 mL) and the mixture stirred under argon for 6 h. The reaction was diluted with $Et_2O$ (4 mL), filtered, and the solid residue washed with $Et_2O$ (4 × 10 mL). Combined organic filtrates were distilled in vacuo to give a residue that was chromatographed over a Si gel column ($CHCl_3$−$Me_2CO$, 9:1) yielding **2** (77 mg, 0.21 mmol, 73%): colorless oil; $[\alpha]^{20}_D$ +178.6° (*c* 0.99, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 204 (4.67) nm; IR (film) $\nu_{max}$ 2938, 2872, 2722, 1767, 1736, 1688, 1655, 1377, 1239, 1141, 1031, 985, 956 $cm^{-1}$; NOE experiments, proton irradiated (NOE observed) H-1 (H-5, H-14), H-14 (H-1, H-7); $^{13}C$ NMR $\delta$ ($CDCl_3$, 75 MHz) 193.6 (d, C-14), 170.5 (s, C-12), 170.4 (s, Me*C*OO), 169.3 (s, Me*C*OO), 150.4 (d, C-1), 143.6 (s, C-10), 138.1 (s, C-4), 136.0 (s, C-11), 129.3 (d, C-5), 123.8 (t, C-13), 75.2 (d, C-6), 72.6 (d, C-8), 62.0 (t, C-15), 48.6 (d, C-7), 32.9 (t, C-3), 28.6 (t, C-9), 27.6 (t, C-2), 21.3 (*C*$H_3$COO), 20.8 (*C*$H_3$COO); CIMS *m/z* 363 $[M + H]^+$ (3), 303 $[363 - HOAc]^+$ (11), 261 $[303 - CH_2CO]^+$ (3), 243 $[303 - HOAc]^+$ (18), 215 $[243 - CO]^+$ (4), 85 (44), 61 (100); HRCIMS *m/z* $[M + H]^+$ 363.1437 (calcd for $C_{19}H_{23}O_7$ 363.1444).

**Cytotoxicity Assays.** The in vitro cytotoxic activities of **1** and **2** were assayed against P-388 mouse lymphoma and against the A-549 (lung carcinoma) and HT-29 (colon carcinoma) human cell lines, as previously described.[13] Compound **1** showed $IC_{50} = 2.7 \times 10^{-6}$M, $IC_{50} = 5.5 \times 10^{-6}$M, and $IC_{50} = 5.5 \times 10^{-6}$M, respectively. Compound **2** showed $IC_{50} = 1.4 \times 10^{-6}$M, $IC_{50} = 2.8 \times 10^{-6}$M, and $IC_{50} = 2.8 \times 10^{-6}$M, respectively.

**Acknowledgment.** Our thanks go to the Spanish CICYT for the Research Program PB 95/1192, to the Junta de Andalucía for the grant provided to A. Barragán, to the Spanish Ministerio de Educación y Cultura for the grant provided to M. Álvarez, and to Martin J. Keane for his contribution in the translation into English.

## References and Notes

(1) Watson, W. H.; Kashyap, R. P. *J. Org. Chem.* **1986**, *51*, 2521−2524.
(2) Cuenca, M. R.; Borkosky, S.; Catalán, C. A. N.; Goedken, V. L.; Díaz, J. G.; Herz, W. *Phytochemistry* **1993**, *32*, 1509−1513.
(3) Barrero, A. F.; Oltra, J. E.; Morales, V.; Álvarez, M. *J. Nat. Prod.* **1997**, *60*, 1034−1035.
(4) Barrero, A. F.; Oltra, J. E.; Álvarez, M. *Tetrahedron Lett.* **1998**, *39*, 1401−1404.
(5) Barrero, A. F.; Oltra, J. E.; Barragán, A.; Álvarez, M. *J. Chem. Soc., Perkin 1* **1998**, 4107−4113.
(6) Barrero, A. F.; Molina, J.; Oltra, J. E.; Altarejos, J.; Barragán, A.; Lara, A.; Segura, M. *Tetrahedron* **1995**, *51*, 3813−3822.
(7) Haruna, M.; Ito, K. *J. C. S. Chem. Commun.* **1981**, 483−485.
(8) Adekenov, S. M.; Gatilov, Y. W.; Bagrianskaya, I. Y.; Raldugin, V. A. *Sib. Khim. Zh.* **1993**, 76−79. *Chem. Abstr.* **1993**, *119*, 181139p.
(9) PC MODEL. Serena Software.
(10) Bruker software library.
(11) Yoshioka, H.; Mabry, T. J. *Tetrahedron* **1969**, *25*, 4767−4779.
(12) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. In *Progress in the Chemistry of Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: Weinheim, 1979; Vol. 38, p 90.
(13) Berengeron, R. I.; Davanaugh, Jr., P. F.; Kline, S. J.; Hughes, Jr., R. G.; Elliot, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 848−854.
(14) Barrero, A. F.; Oltra, J. E.; Rodríguez, I.; Barragán, A.; Grávalos, D. G.; Ruiz, P. *Fitoterapia* **1995**, *66*, 227−230.
(15) Hoffmann, H. M. R.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 94−110.
(16) Barrero, A. F.; Oltra, J. E., Raslan, D. S.; Saúde, D. A. *J. Nat. Prod.* **1999**, *62*, 726−729.

NP990436W