

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

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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 ¹H 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 ¹H 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.

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 ¹H NMR spectrum of semisynthetic **1** matched that of the natural product.² The ¹³C 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 SeO₂ and *tert*-butyl hydroperoxide, has been previously discussed.⁷ Mild oxidation of the allylic alcohol **1** gave the conjugated aldehyde **2** (73% yield), the ¹H NMR spectrum of which matched that of the aldehyde from *M. minima*.² In the ¹³C NMR spectrum of **2**, the chemical shift

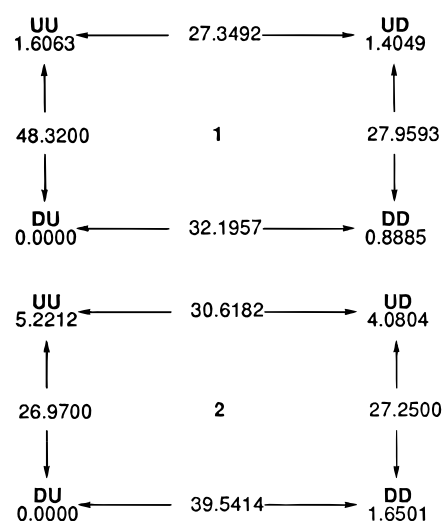
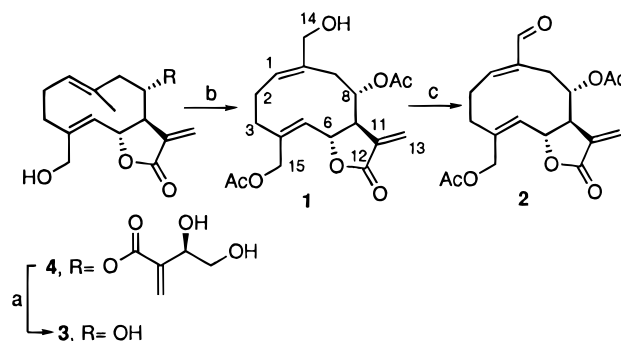


Figure 1. Relative energies (kcal/mol) of the four conformers of **1** and **2** and calculated energy barriers (kcal/mol) between them (MM2).

Scheme 1



a) K₂CO₃. b) i: Ac₂O/Py; ii: SeO₂, *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 ¹H NMR spectral features.² Inasmuch as the absolute stereochemistry of (+)-cnicin (**4**) was previously established by X-ray

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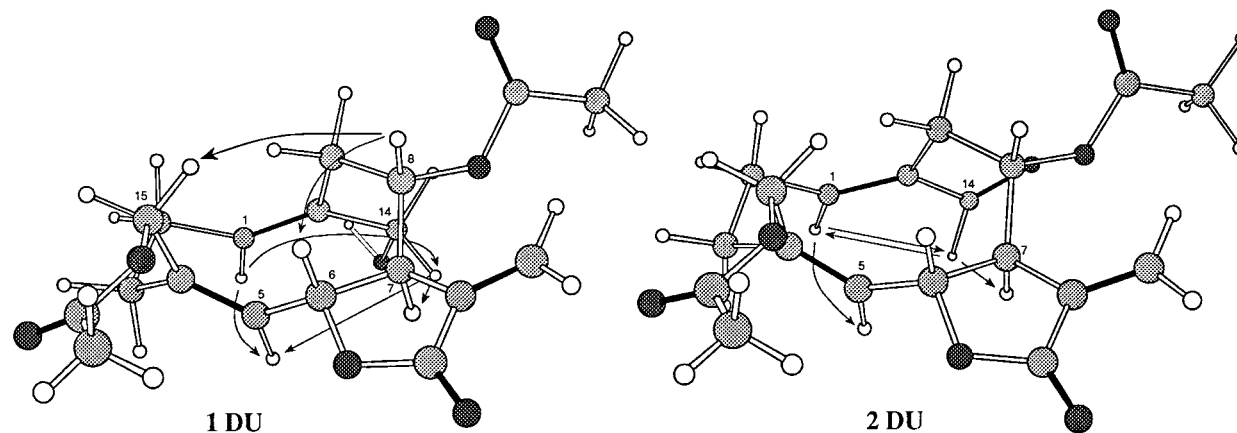


Figure 2. Minimum energy conformers of compounds **1** and **2**. Arrows show the observed NOEs and start at the irradiated hydrogens.

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 ^1H 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, ^1H 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 ^1H NMR spectra of **1** and **2** (see Table 1). Furthermore, the NOEs observed in a series of NOE-dif¹⁰ 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 (+)-cinicn (**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 salonitenolide¹⁴ (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; $[\alpha]_D^{20} +89.4^\circ$ (c 1.02, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 205 (4.33) nm; IR (film) ν_{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) δ 170.7 (s, C-12), 169.9 (s, MeCOO), 169.5 (s, MeCOO), 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 (CH_3COO), 20.9 (CH_3COO); CIMS m/z 365 $[\text{M} + \text{H}]^+$ (27), 347 $[\text{365} - \text{H}_2\text{O}]^+$ (41), 305 $[\text{365} - \text{HOAc}]^+$ (60), 287 $[\text{305} - \text{H}_2\text{O}]^+$ (66), 245 $[\text{305} - \text{HOAc}]^+$ (100), 227 $[\text{245} - \text{H}_2\text{O}]^+$ (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; $[\alpha]_D^{20} +178.6^\circ$ (c 0.99, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.67) nm; IR (film) ν_{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 δ ($CDCl_3$, 75 MHz) 193.6 (d, C-14), 170.5 (s, C-12), 170.4 (s, $MeCOO$), 169.3 (s, $MeCOO$), 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 (CH_3COO), 20.8 (CH_3COO); 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.

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