Biomimetic Cyclization of Cnicin to Malacitanolide, a Cytotoxic Eudesmanolide from *Centaurea malacitana*

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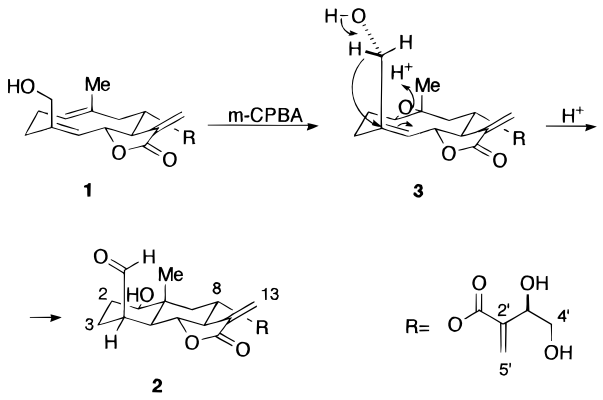
Malacitanolide (**2**), a new eudesmanolide isolated from the aerial parts of *Centaurea malacitana*, was characterized spectroscopically. The synthesis of **2** from cnicin (**1**), via the epoxide **3**, confirmed the structure and stereochemistry of malacitanolide, as well as its biogenetic relationship with **1**. Cytotoxic activity values for **2** are significantly higher than for **1**.

Centaurea malacitana Boiss. (Compositae) contains cnicin (**1**) and other cytotoxic and antimicrobial germacranolides[1] that are useful for the semisynthesis of (+)-vernolepin related compounds.[2] Further investigation of the extract of *C. malacitana* led to the isolation of malacitanolide (**2**), a new eudesmanolide with potent cytotoxic activity, and 8-*O*-(4-acetoxyangeloyl)salonitenolide,[3] here described for the second time in nature.

The molecular formula of malacitanolide (**2**) is $C_{20}H_{26}O_8$ as deduced from its HRCIMS. Its IR spectrum showed bands due to hydroxyl, aldehyde, δ-lactone, and α,β-unsaturated ester groups. In the CIMS, peaks at m/z 281 $[M + H - C_5H_6O_3]^+$, 263 $[M + H - C_5H_8O_4]^+$, and 115 $[C_5H_7O_3]^+$ were indicative of a five-carbon-atom dihydroxylated-ester side chain. The ^{1}H-NMR spectrum showed signals confirming the presence, at C-8, of the same side chain that occurs in **1**.[1] However, the chemical shift of H-14 (0.93 ppm) was the usual one for eudesmanolides.[4] The ^{13}C-NMR spectrum confirmed the eudesmanolide skeleton.[5] Additionally, the ^{1}H-NMR spectrum showed signals (δ 3.42 and 2.82), which could be justified if an equatorial hydroxyl group was located at C-1 and an axial formyl group was present at C-4. Chemical shift and multiplicity of H-15 confirmed the β axial orientation of the aldehyde.[4] The coupling constants between H-5, H-6, H-7, and H-8 were the ones expected if they all had axial orientations. These data suggested structure **2** for malacitanolide. Several NOE experiments confirmed the relative configuration of **2** and the position and preferential conformation of the formyl group (Scheme 1).

A biogenetic precursor of malacitanolide (**2**) could be cnicin (**1**), which, after enzymatic epoxidation, stereospecific transannular cyclization of the 1,10-epoxide, and hydride shift, would yield **2**. In order to support this hypothesis and to confirm the structure of malacitanolide, chemical synthesis of **2** from **1** was performed (Scheme 1). Treatement of **1** with *m*-CPBA, in the presence of pyridine, led to the oxirane **3**. In the ^{1}H-NMR spectrum of **3**, H-1 appeared at δ 2.89 (dd) and the Me-14 at δ 1.25 (s), the expected values considering the presence of epoxide carbons at C-1 and C-10.[6] The $1\beta,10\alpha$ stereochemistry of the epoxide was proposed

Scheme 1. Biomimetic cyclization of **1** to **2**, via the epoxide **3**.



considering the preferential conformation of germacranolides,[7] the reaction mechanism of *m*-CPBA, and the coupling constants of H-1. When **1** was treated with *m*-CPBA without pyridine, malacitanolide was directly obtained. Apparently, *m*-chlorobenzoic acid, formed during the epoxidation reaction, was responsible for the electrophilic opening and subsequent rearrangement of epoxide **3** (Scheme 1). As the absolute configuration of (+)-cnicin (**1**) has been reported,[8] the synthesis of (+)-malacitanolide (**2**) from **1** confirms the absolute configuration of **2** and supports its biogenetic relationship with cnicin.

In vitro cytotoxic activity of **2** was assayed[9] towards the P-388, SCHABEL, A-549, HT-29, and MEL-28 tumor cell lines. Malacitanolide (**2**) showed $IC_{50} = 3.05 \times 10^{-7}$ M in the five cases. Previously reported IC_{50} values for cnicin (**1**) were 6.6×10^{-6} M against the P-388 cells and 1.32×10^{-5} M towards both the A-549 and HT-29 lines.[1] It is generally accepted that the cytotoxic activity of the sesquiterpene lactones resides chiefly on their Michael acceptor groups.[10] However, although **2** and **1** have identical Michael acceptor groups, **2** has an activity twenty to forty times higher than **1** towards the P-388, A-549, and HT-29 lines. This increase in activity could be due either to the new functionalization pattern or to the higher molecular rigidity.

Eperimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 141 polarim-

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eter. IR spectra were obtained, in liquid film between NaCl plates, on a 983 G Perkin-Elmer apparatus. HRMS were measured on a Autospec-Q VG-Analytical (FISONS) mass spectrometer, and LRMS were determined on a 5988A Hewlett-Packard instrument. NMR spectra were recorded on a Bruker AM 300 spectrometer. Chemical shifts are reported in parts per million (δ) relative to TMS, and coupling constants (J) are in Hertz. Carbon substitution degrees were established by DEPT multipulse sequence. TLC was performed on precoated 0.25-mm thick Merck plates of Si gel 60 F_{254}, using a 7% phosphomolybdic acid solution (EtOH) to visualize the spots. Gravity column chromatography was carried out on Merck Si gel 60 (70−230 mesh), and flash chromatography was performed as described previously.[11]

Plant Material

C. malacitana was colleted in Carataunas, Granada, Spain, in June 1996, and was taxonomically identified by Prof. G. Blanca (Departamento de Biología Vegetal, Universidad de Granada, Spain). A voucher specimen (no. 40128) is deposited at the Herbarium of the Faculty of Sciences of the University of Granada.

Extraction and Isolation

The aerial parts of the plants were air-dried, ground, and extracted with *t*-BuOMe in a Soxhlet apparatus (5.4 kg furnished 157.5 g of extract). A portion (10 g) of the extract was subjected to column chromatography over 110 g Si gel using a $CHCl_3$−Me_2CO gradient. The following sesquiterpene lactones were isolated: 8-*O*-(4-acetoxyangeloyl)salonitenolide[3] (10 mg, $CHCl_3$−Me_2CO 9:1), cnicin 4′-*O*-acetate[12] (1.85 g, $CHCl_3$ −Me_2CO 9:1), stenophyllolide[13] (1.45 g, $CHCl_3$−Me_2CO 5:5), cnicin (**1**)[1] (2.06 g, $CHCl_3$−Me_2CO 4:6), and malacitanolide (**2**) (90 mg, $CHCl_3$−Me_2CO 35:65).

Malacitanolide (2): $[\alpha]^{25}_D$ +96° (*c* 1.02, MeOH); IR (dry film) υ_{max} 3412, 2733, 1766, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.96 (1H, s, H-15), 6.39 (1H, s, H-5′a), 6.20 (1H, d, J = 3.1 Hz, H-13a), 6.08 (1H, s, H-5′b), 5.59 (1H, d, J = 2.9 Hz, H-13b), 5.31 (1H, td, J = 11.0, 4.4 Hz, H-8), 4.65 (1H, m, H-3′), 4.55 (1H, dd, J = 11.8, 11.0 Hz, H-6), 3.85 (1H, dd, J = 11.1, 3.3 Hz, H-4′a), 3.61 (1H, dd, J = 11.1, 6.7 Hz, H-4′b), 3.42 (1H, dd, J = 10.9, 4.2 Hz, H-1), 2.90 (1H, tt, J =11.0, 3.0 Hz, H-7), 2.82 (1H, td, J = 5.6, 1.5 Hz, H-4), 2.50 (1H, dd, J = 12.8, 4.4 Hz, H-9), 2.05 (1H, dd, J = 11.8, 5.6 Hz, H-5), 0.93 (3H, s, H-14); NOE-difference, proton irradiated (NOEs observed) H-1 (H-5), H-4 (H-5, H-15), H-5 (H-1, H-4, H-7), H-6 (H-8, H-14, H-15), H-7 (H-5), H-8 (H-6, H-14), H-15 (H-4, H-6, H-14); ^{13}C NMR [$(CD_3)_2$-SO, 75 MHz] δ 203.7 (d, C-15), 169.3 (s, C-12), 165.2 (s, C-1′), 141.9 (s, C-2′), 137.5 (s, C-11), 125.3 (t, C-5′), 118.7 (t, C-13), 76.0 (d, C-6), 75.9 (d, C-1), 70.2 (d, C-3′), 69.5 (d, C-8), 65.4 (t, C-4′), 52.3 (d, C-7), 47.2 (d, C-5), 44.7 (d, C-4), 43.4 (t, C-9), 41.0 (s, C-10), 26.7 (t, C-2), 21.8 (t, C-3), 13.4 (q, C-14); the ^{13}C-NMR data were assigned through analysis of 2D NMR spectra (HETCOR and HMBC) of **2**; CIMS m/z 395 $[M + H]^+$ (1), 281 (23), 263 (42), 245 (44), 115 (100), 97 (98), 55 (53); HRCIMS m/z 395.1705 (calcd for $C_{20}H_{27}O_8$ 395.1706).

Epoxidation Reactions of Cnicin (1). *m*-CPBA (160 mg) was added to a solution of **1** (200 mg) in 5 mL of THF and 0.1 mL of pyridine. The mixture was stirred for 30 min at room temperature, and the solvent was removed *in vacuo*; then H_2O (10 mL) was added to the residue, and the mixture was extracted with EtOAc. Evaporation of the organic solvent gave 103 mg of epoxide **3**: ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 6.32 (1H, s, H-5′a), 6.10 (1H, s, H-5′b), 6.09 (1H, d, J = 3.1 Hz, H-13a), 5.75 (1H, d, J = 3.3 Hz, H-13b), 5.49 (1H, d, J = 9.8 Hz, H-5), 5.41 (1H, dd, J = 9.8, 8.5 Hz, H-6), 5.17 (1H, br t, J= 8.5 Hz, H-8), 4.55 (1H, m, H-3′), 4.42 (1H, d, J = 14.0 Hz, H-15a), 4.26 (1H, d, J = 14.0 Hz, H-15b), 3.76 (1H, dd, J = 11.1, 3.3 Hz, H-4′a), 3.48 (1H, dd, J = 11.1, 6.7 Hz, H-4′b), 3.31 (1H, tt, J = 8.5, 3.2 Hz, H-7), 2.89 (1H, dd, J =11.3, 2.4 Hz, H-1), 1.25 (3H, s, H-14); ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ 170.1 (s, C-12), 165.6 (s, C-1′), 147.6 (s, C-4), 141.8 (s, C-2′), 137.3 (s, C-11), 126.8 (t, C-5′), 125.5 (d, C-5), 123.8 (t, C-13), 76.7 (d, C-6), 72.3 (d, C-8), 71.5 (d, C-3′), 67.4 (d, C-1), 66.6 (t, C-4′), 60.9 (t, C-15), 58.9 (s, C-10), 54.1 (d, C-7), 48.1 (t, C-9), 32.2 (t, C-3), 25.6 (t, C-2), 17.5 (q, C-14); HR-FABMS m/z 395.1696 (calcd for $C_{20}H_{27}O_8$, 395.1706).

m-CPBA (160 mg) was added to **1** (200 mg) in 5 mL of THF and the mixture was stirred for 30 min at room temperature. Evaporation of the solvent generated 360 mg of a residue containing *m*-chlorobenzoic acid and **2** (^1H NMR). The residue was flash chromatographed ($CHCl_3$−Me_2CO 6:4), giving 58 mg of pure **2**, identical in all respects to natural **2**, including optical rotation.

Cytotoxicity Assays. The in vitro cytotoxic activities of 8-*O*-(4-acetoxyangeloyl)salonitenolide and **2** were assayed[9] towards P-388 and SCHABEL mouse lymphomas and towards the A-549 (lung carcinoma), HT-29 (colon carcinoma), and MEL-28 (melanoma) human cell lines. 8-*O*-(4-Acetoxyangeloyl)salonitenolide showed IC_{50}= 2.5 μg/mL against both mouse lymphomas, and IC_{50}= 5 μg/mL towards the three human cell lines. Compound **2** showed IC_{50}= 0.12 μg/mL in all cases.

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