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New sesquiterpene lactones from *Ambrosia cumanensis* Kunth

Nora del Socorro Jimenez-Usuga [a], Nicola Malafronte [b], Roberta Cotugno [b], Marinella De Leo [c], Edison Osorio [a], Nunziatina De Tommasi [b,*]

[a] Grupo de Investigacion en Sustancias Bioactivas, Facultad de Ciencias Farmaceuticas y Alimentarias, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellin, Colombia
[b] Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II, 84084 Fisciano, Salerno, Italy
[c] Dipartimento di Farmacia, Università di Pisa, Pisa, Via Bonanno 33, 56126 Pisa, Italy

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ABSTRACT

Eleven sesquiterpene lactones, including three new natural products (**1**–**3**), were isolated from the *n*-butanolic extract of *Ambrosia cumanensis* Kunth. aerial parts. The structure of all isolated compounds was elucidated by 1D- and 2D-NMR, and MS analyses. All compounds were tested for their antiproliferative activity on HeLa, Jurkat, and U937 cell lines. Compound **3**, 2,3-dehydropsilostachyn C, showed cytotoxic activity with different potency in all cell lines. By means of flow cytometric studies, compound **3** was demonstrated to induce in Jurkat cells a G_2/M cell cycle block, while in U937 elicited both cytostatic and cytotoxic responses.

1. Introduction

Ambrosia cumanensis Kunth. (Asteraceae) is a perennial, very aromatic plant, up to 2 m high, alternate or opposite leaves, covered at the bottom of long villi and deeply bipinnatifid, native to Central America, where is known as "Altamisa" and "Artemis". Its male flowers are green in terminal chapters, while female flowers are clustered in the upper leaf axils; the fruits are ovoid, angles, prickly, 3–4 mm long [1]. The plant is used in the Marinilla region of Colombia for chronic pain treatment [2] and as antimicrobial [3] and spasmolytic remedy [4]. Previous phytochemical studies on *A. cumanensis* reported the presence of sesquiterpene lactones [5], and recently an investigation on several Colombian medicinal plants demonstrated that the polar *A. cumanensis* extracts were moderate antioxidant [2]. In the course of our studies on Marinilla region medicinal plants [6], herein we performed a phytochemical investigation on *A. cumanensis* aerial parts. Eleven sesquiterpene lactones, including three new natural products **1**–**3** (Fig. 1), were isolated and elucidated by 1D- and 2D-NMR and MS analyses. Sesquiterpene lactones are a wide group of natural compounds showing a large spectrum of bioactivities, including antitumor property [7]. The activity of these compounds has been correlated mainly to the α-β-unsaturated carbonyl group, which acting as Michael acceptor may compromise protein structures and activities [8]. These molecules display different antitumor potency depending on other structure features [9–10]. Hence, the anti-proliferative potential of all the isolates was tested in vitro on Jurkat, U937, and HeLa cell lines. The effect of the most active compound **3** on cell cycle progression, cell death, and apoptosis was investigated.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Rudolph Research Analytical Autopol IV polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Thermo scientific MULTISKAN spectrophotometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbe at 300 K. All 2D NMR spectra were acquired in methanol-d_4 (99.95%, Sigma-Aldrich), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. ESI-MS were obtained using a Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. HR-ESIMS spectra were acquired in positive ion mode on a Q-TOF premier spectrometer (Waters-Milford). TLC were performed on precoated Kieselgel 60 F_{254} plates (Merck); compounds were detected by spraying with $Ce(SO_4)_2/H_2SO_4$ solution. Column chromatography was performed over silica gel (70–220 mesh, Merck); reversed-phase (RP) HPLC separations were conducted on a Shimadzu LC-20AT series pumping system equipped with a Shimadzu RID10A refractive index detector and a Shimadzu injector, using a C_{18}μ-Bondapak column (30 cm × 7.8 mm, 10 μm, Waters-Milford) and a mobile phase consisting of MeOH-H_2O mixtures at a flow rate of 2 mL/min.

* Corresponding author.
E-mail address: detommasi@unisa.it (N. De Tommasi).

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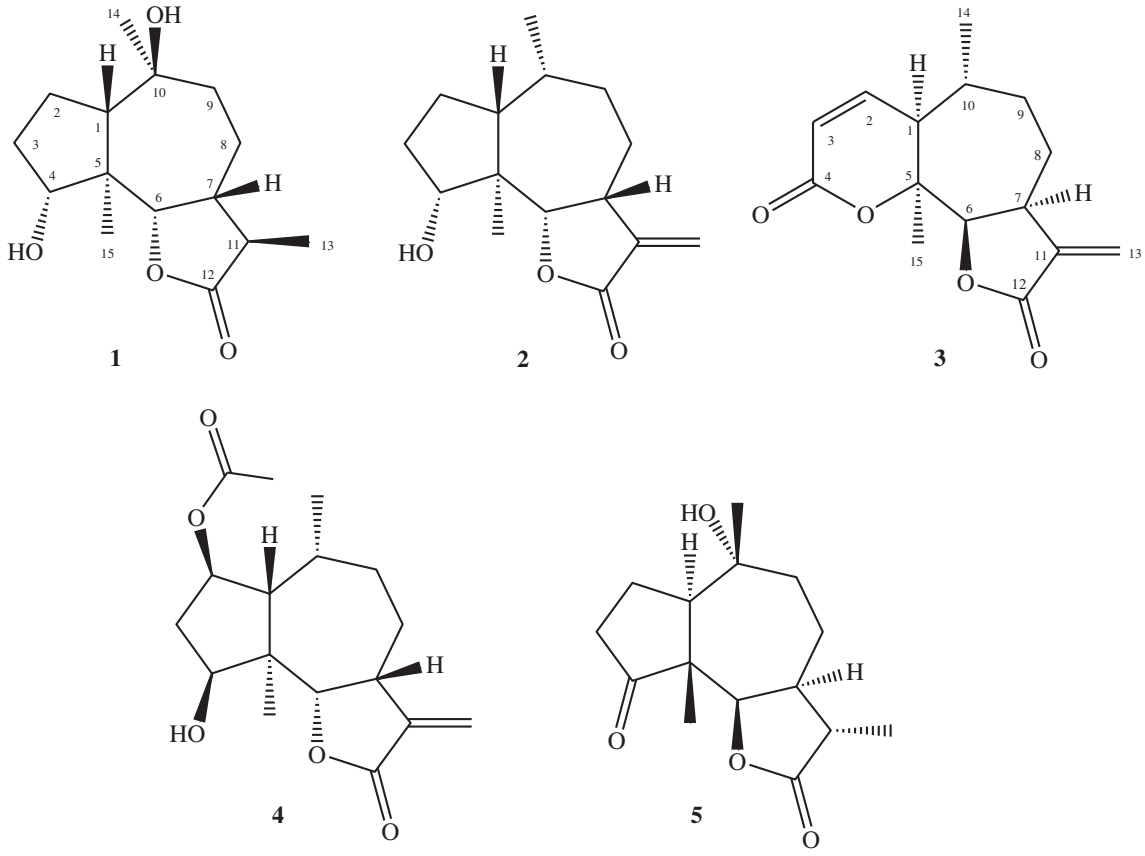

Fig. 1. Structures of compounds **1**–**5**.

2.2. Plant material

Aerial parts of *A. cumanensis* were collected in Marinilla region, Colombia, in April 2011. The plant was identified by Dr. Julio Cesar Jaramillo of the University of Antioquia's Herbarium, where a voucher specimen (JCJ 25) was deposited.

2.3. Extraction and isolation

The dried aerial parts of *A. cumanensis* were exhaustively extracted with methanol (4 × 3 L) at room temperature for 48 h to give 17.8 g of dried residue. Methanol extract was partitioned between *n*-butanol and H_2O to afford a *n*-butanol (7.6 g) soluble portion. Part of *n*-butanolic extract (5.0 g) was dissolved in chloroform and chromatographed over silica gel column (5 × 150 cm) using a gradient elution with mixtures of $CHCl_3$-MeOH (99:1, 49:1, 97:3, 9:1, 4:1, 7:3, 1:1), to obtain ten major fractions (A–L). Fraction D (609.2 mg) was analyzed by RP-HPLC with MeOH-H_2O (1:1) to give parthenin (0.7 mg, t_R = 9 min), psilostachyn A (0.5 mg, t_R = 12 min) and damsin (4 mg, t_R = 27.5 min). Fraction E (300.0 mg) was analyzed by RP-HPLC with MeOH-H_2O (47:53) to give pure psilostachyn A (1.1 mg, t_R = 11 min), ambrosin (5.4 mg, t_R = 13 min), and **2** (1.3 mg, t_R = 45 min). Fraction F (130.5 mg) was analyzed by RP-HPLC with MeOH-H_2O (45:55) to give compounds **3** (1.1 mg, t_R = 20 min) and **4** (1.3 mg, t_R = 62.0 min). Fraction G (86.2 mg) was analyzed by RP-HPLC with MeOH-H_2O (45:55) to give psilostachyn A (3.4 mg, t_R = 17 min). Fraction H (111.0 mg) was analyzed by RP-HPLC with MeOH-H_2O (1:1) to give bipinnatin (0.9 mg, t_R = 15 min). Fraction I (463.7 mg) was analyzed by RP-HPLC with MeOH–H_2O (45:55) to give compounds **5** (1.1 mg, t_R = 8 min), **1** (0.8 mg, t_R = 11 min) and hymenolin (0.8 mg, t_R = 15 min).

2.3.1. 4α,10β-dihydroxypseudoguaian-12,6-olide (**1**)

Amorphous powder; $[\alpha]_D^{20}$ −4.95° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 289; ^1H and ^{13}C NMR data, see Table 1; ESI MS *m/z* 291 $[M + Na]^+$, 273 $[M + Na\text{-}18]^+$, 247 $[M + Na\text{-}44]^+$; HR ESIMS *m/z* 291.1594 $[M + Na]^+$ (calcd for $C_{15}H_{24}NaO_4$ 291.1572).

2.3.2. 4α-hydroxy-11(13)-pseudoguaien-12,6-olide (**2**)

Amorphous powder; $[\alpha]_D^{20}$ −78.26° (*c* 0.023, MeOH); UV (MeOH) λ_{max} (log ε): 298; ^1H and ^{13}C NMR data, see Table 1; ESI MS *m/z* 273 $[M + Na]^+$; HR ESIMS *m/z* 273.1531 $[M + Na]^+$ (calcd for $C_{15}H_{22}NaO_3$ 273.1467).

2.3.3. 2,3-dehydropsilostachyn C (**3**)

Amorphous powder; $[\alpha]_D^{20}$ −25.75° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 294; ^1H and ^{13}C NMR data, see Table 1; ESI MS *m/z* 285 $[M + Na]^+$; HR ESIMS *m/z* 285.1087 $[M + Na]^+$ (calcd for $C_{15}H_{18}NaO_4$ 285.1103).

2.4. Cytotoxicity assay

2.4.1. Reagents and antibodies

Fetal bovine serum (FBS) was from GIBCO (Life Technologies, Grand Island, NY, USA). The antibodies anti-Cdc2 (mouse monoclonal, sc-8395) and anti-phospho (Thr161)-Cdc2 p34 (rabbit polyclonal, sc-101654), were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); appropriate peroxidase-conjugated secondary antibodies were from Jackson Immuno Research (Baltimore, PA, USA).

2.4.2. Cells and treatment

HeLa (cervical carcinoma), Jurkat (T-cell leukemia), and U937 (monocytic leukemia) cell lines were obtained from the American