Supporting Information I

Chemical Constituents from Eryngium Agavifolium L.

Eryngium is a genus in the family Apiaceae of about 230 species of annuals and perennials with hairless and usually spiny leaves, and dome-shaped umbels of flowers resembling those of thistles [1]. Some of the species, such as *E. maritimun*, *E. campestre*, and *E. foetidum* have been used in folk medicine [2]. *E. agavifolium*, a species that belongs to the Apiaceae family, is a grassland plant. To our knowledge, there is no chemical constituent reported from this specie. As part of our ongoing program to screen cytotoxic compounds from plants in east Texas, seven known compounds were isolated from the EtOH extract of the whole plant of *E. agavifolium*, and were identified as (8*E*)-1,8-Heptadecadiene-4,6-diyne-3,10-diol (**1**) [3], (*Z*)-15-hydroxy-9,16-Heptadecadiene-11,13-diyn-8-one (**2**) [4], (*E*)-15-hydroxy-9,16-Heptadecadiene-11,13-diyn-8-one (**3**) [5], Hexadecanoic acid (**4**) [6], (9*Z*,11*Z*)-9,11-Octadecadienoic acid, methyl ester (**5**) [7], (7*Z*)-7-Hexadecen-1-ol, 1-acetate (**6**) [8], (3*β*,22*Z*)-Ergosta-5,22-dien-3-ol (**7**) [9] by spectroscopic methods (**Fig. 1**).

EXPERIMENTAL SECTION

General Experimental Procedures

Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh), TLC: Precoated silica gel GF_{254} Semi-prep. HPLC: Agilent 1100 HPLC system with an Agilent 1100 diode array detector using a Hypersis ODS column (250 × 4.6 mn, 5 μm, Supelco).

Plant Material

Whole plants of *E. agavifolium* were collected in Nacogdoches, Texas on Auguest 10, 2011, and were identified by Dr. Shiyou Li. A voucher specimen (NCPC-GH-20101004-004-wp) was deposited at the National Center for Pharmaceutical Crops, Arghur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, Texas, USA.

Extraction and Isolation

The dried, powdered whole plants (430 g) were macerated for 48 h with 6 L of 95% EtOH for 2 times. The filtrate was concentrated under reduced pressure, and the residue (50 g) was suspended in H_2O and then partitioned with EtOAc. The EtOAc extract (10 g) was subjected to a silica gel column, eluted with a gradient of hexane/acetone (20:1-2:1), to yield five fractions (A-E). Fraction B (1.8 g) was subjected to silica gel column, eluted with hexane/EtOAc (from 6:1 to 0:1), to yield compounds **4** (18 mg), **5** (9 mg) and **6** (11 mg). Fraction C (2 g) was subjected to an ODS column, eluted with a gradient of MeOH/H_2O (from 40:60 to 90:10), to give three subfractions, B1-B3. Fraction B2 (51 mg) was separated by preparative HPLC using the mobile phase CH_3CN/H_2O (65:35, v:v) to yield **1** (37.5 mg), **2** (5.7 mg), and **3** (271 mg). Fraction B3 (95 mg) was isolated with hexane/EtOAc (from 6:1 to 2:1), to yield compound **7** (45mg).

ACKNOWLEDGEMENTS

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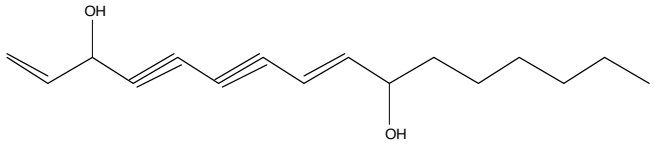

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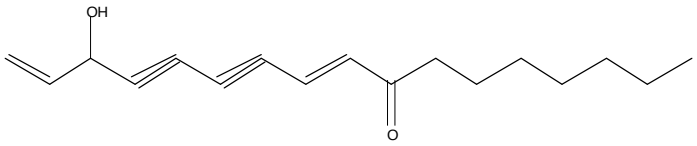

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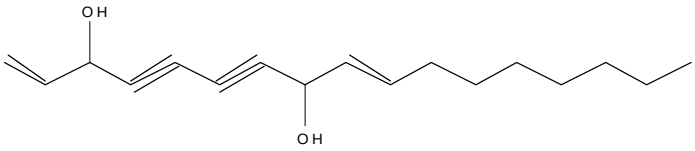

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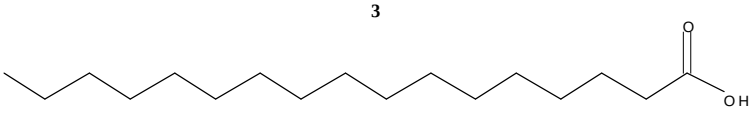

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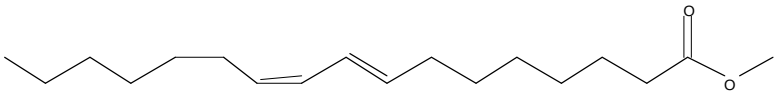

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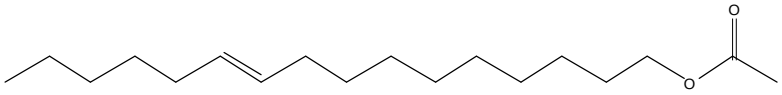

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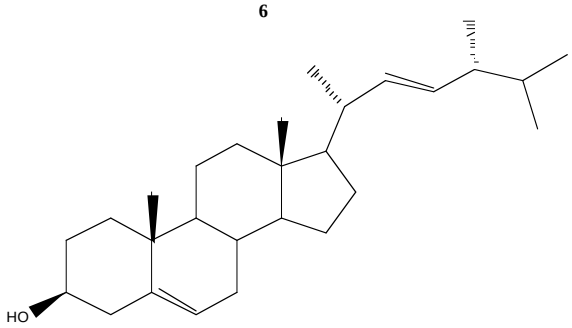

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Fig. (1). The structures of compounds 1-7 isolated from *Eryngium agavifolium.*

Supporting Information II

Experimental Section

Chemistry

11 Erygium saponins used in this study were pure compounds (>95% in purity, by HPLC) isolated from the whole plants of *Eryngium yuccifolium* (Zhang, Z.Z. et al., 2008) in our laboratory.

Chemicals and Reagents

Supercoiled pHOT-1 DNA, 10X TGS buffer, and recombinant human DNA TOP1 and TOP2 enzymes were obtained from TopoGEN, Inc. (Port Orange, FL). pHOT-1 DNA was purchased at a concentration of 250 ng/μL in 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and was used as is after dilution in 1X TGS buffer. TOP1 and TOP2 were purchased in solution at 2 U/μL and 4 U/μL, respectively. Proteinase K was obtained from Promega Corp. (Madison, WI). Agarose (molecular biology grade) was purchased from Midwest Scientific (St. Louis, MO) and sodium dodecyl sulfate (SDS) was purchased from EMD Biosciences (San Diego, CA). Dimethyl sulfoxide (DMSO; ACS grade), bromophenol blue (electrophoresis grade), and ethidium bromide (EtBr; electrophoresis grade) were purchased from Fisher Scientific (Fair Lawn, NJ). CPT (>98% in purity) was isolated from *C. acuminata* in our laboratory[62] and luteolin (≥98%) was purchased from Aldrich (St. Louis, MO).

DNA Topoisomerase I Assays

Pure compounds were evaluated for their ability to inhibit TOP1 inhibitory activity following the protocol described by Webb and Ebeler.[65] This protocol allows for the detection of compounds with the ability to inhibit TOP1 through intercalation, inhibition of relaxation activity, or by poisoning. For the poisoning section of the assay, the known TOP1 poison CPT was used as a control. Luteolin was used as an enzyme inhibition control, and ethidium bromide was used as a DNA intercalation control. A dilution of supercoiled DNA was prepared in 1X TGS buffer to give a concentration of 4.4 ng/μL. Fully relaxed DNA was prepared from an aliquot of this solution by the addition of the amount of TOP1 calculated to produce full relaxation when incubated at 37ºC for 2 h. To 17 μL of either the relaxed or supercoiled DNA dilution was added 2 μL of test compound dilution, control compound, or blank solution. For the quantitative determination of TOP1 inhibitory activity, photographic negatives were densitometrically scanned by Molecular Imager® Gel Doc™ XR System (Bio-Rad Laboratories Inc., Hercules, CA). The area representing supercoiled DNA, migrating as a single band at the bottom of the gel was determined. The IC_{50} values were determined by extrapolating the concentration of the inhibitor at which it prevented 50% of the substrate (supercoiled DNA) from being converted into the reaction product (relaxed DNA) by plotting the data. Mean IC_{50} values were determined in this manner from three independent experiments. The compounds with negative result at 125 μM were considered inactive.

DNA Topoisomerase II Assays

Relaxation activity of TOP2 was analyzed in the same manner described above except that the reaction was performed in 20 μL of reaction mixtures containing 17 μL of either the relaxed, linear, or supercoiled DNA (0.75 ng), 2 μL of the test compound, and 4X amount of TOP2.

Cytotoxicity Assays

Human pancreatic cancer (PANC-1), non-small cell lung cancer (A549), prostate cancer (PC-3), and leukemia (HL-60) were obtained from Dr. Peiying Yang (The University of Texas M. D. Anderson Cancer Center, Houston, TX), and Normal lung cells (MRC-5) was purchased from the American Type Culture Collection (ATCC). PC-3, A549 and HL-60cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and antibiotics (1,000 units/mL penicillin- streptomycin solution, Hyclone). PANC-1 and MRC-5 was cultured in complete growth mediums according the ATCC protocols. Cells were grown at 37°C and 5% CO_2 in humidified air. The isolated pure compounds were tested in each of the cultured human tumor and normal cell lines at concentrations of 0.1, 1, and 10 μM with three replications. Human tumor and normal cells were inoculated into 96 well microtiter plates in 90 μL at plating densities ranging from 5,000 to 40,000 cells/well (measured by Z2 Coulter Counter of Beckman) depending on the doubling time of individual cell lines. Cell growth and viability was measured by WST-8 (water soluble tetrazolium) assays following a standard protocol (Cell Counting Kit-8, CCK-8, Dojindo Molecular, Maryland). The WST-8 assay measured the relative activity of mitochondrial reductase enzymes in viable cells. A control WST reading (T_0) before treatment was measured. Cells were continuously exposed to isolates for 48 h. WST was added and incubated for 2 h, and the amount of formazan was measured by using a microplate reader. T_C or T_D represents the readings of the untreated control or with tested compounds. Percent cell-growth (G) and percent cell-growth inhibition (GI) was calculated as: $G = (T_D\text{-}T_0/T_C\text{-}T_0) \times 100$; $GI = [1\text{-}(T_D\text{-}T_0/T_C\text{-}T_0)] \times 100$ for which $T_D \geq T_0$; and $G = (T_D\text{-}T_0/T_0) \times 100$; $GI = [1\text{-}(T_D\text{-}T_0/T_0)] \times 100$ for which $T_D < T_0$. To determine the inhibitory potency of active compounds (GI_{50}, 50% cell-growth inhibition), tests were expanded to additional concentrations (varying from 0.01 to 100 μM). Cancer drug Doxorubicin was used as the positive controls in the cell-based assays.

Cell Proliferation Assays

CCK-8 was used to measure the proliferation response of Eryngioside H and Eryngioside I, according to the manufacturer's instructions. Cells were grown in growth medium plus 10% FBS and 5% antibiotics in 96-well plates and then were treated with various concentrations of test compounds and incubated for 12, 24, 48, and 72 h, respectively. The end of the incubation

period, 10% CCK-8 solution (Dojindo Molecular, Maryland) was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured. Also cell suspensions (100 μL/well) that contain known numbers of viable cells were inoculated, CCK-8 assay was performed. A calibration curve was prepared using the absorbance observed known numbers of viable cells.