Evelynin, a Cytotoxic Benzoquinone-Type *retro*-Dihydrochalcone from *Tacca chantrieri*

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A new benzoquinone-type *retro*-dihydrochalcone, named evelynin, was isolated from the roots and rhizomes of *Tacca chantrieri.* The structure was elucidated on the basis of the analysis of spectroscopic data and confirmed by a simple one-step total synthesis. Evelynin exhibited cytotoxicity against four human cancer cell lines, MDA-MB-435 melanoma, MDA-MB-231 breast, PC-3 prostate, and HeLa cervical carcinoma cells, with IC_{50} values of 4.1, 3.9, 4.7, and 6.3 μM, respectively.

Natural products have long been an important source for new drug discovery. When considering anticancer drugs, natural products continue to play a prominent role. On the basis of recent reviews from the Developmental Therapeutics Program of the National Cancer Institute (USA), 63% of FDA-approved anticancer drugs are natural products, semisynthetic compounds derived from a natural product, and/or a synthetic product designed after a natural product pharmacophore.[1,2] During our search for new anticancer agents from tropical plants, we found that the lipophilic extract from the roots of *Tacca chantrieri* caused microtubule bundling similar to the cellular effects of Taxol. We isolated the taccalonolides A and E, highly acetylated steroids from the roots and rhizomes of *T. chantrieri,* and identified them as new microtubule-stabilizing agents.[3] Plants of the *Tacca* genus are native to tropical areas, and *T. chantrieri* is distributed throughout tropical Asiatic countries including Malaysia, Thailand, and southern China. The rhizomes of various *Tacca* species have been used by many cultures in traditional folk medicine to treat ailments including ulcers, high blood pressure, and hepatitis.[4] *T. plantaginea* is purported to have analgesic and anti-inflammatory properties,[5] and extracts of *T. leontopetaloides* were used in Africa to control slugs, snails, and roundworms.[6]

Over the last few decades, many classes of molecules have been isolated from *T. chantrieri* including diarylheptanoid glucosides,[4] steroidal saponins,[7] steroidal glycosides,[8–10] and withanolide glucosides.[11] Perhaps the most unique and well-studied class of compounds isolated from *Tacca* sp. *chantrieri, plantaginea*, *leontopetaloides, and integrifolia* are the appropriately named taccalonolides.[5,12,13] The taccalonolides are pentacyclic steroidal compounds with microtubule-stabilizing properties and antitumor activity.[3,14]

In the process of isolating and purifying the white, amorphous solid of the taccalonolides from *T. chantrieri*, we noticed a yellow component that coextracted with the taccalonolides through the process of supercritical fluid extraction and in subsequent hexane and CH_2Cl_2 extractions. Solid-phase extraction chromatography using successive CH_2Cl_2 and EtO_2 extractions allowed successful separation of the taccalonolides from the yellow compound. The structure of the new compound was identified through spectroscopic analysis, and biological evaluations show that it has cytotoxic activity at low micromolar concentrations.

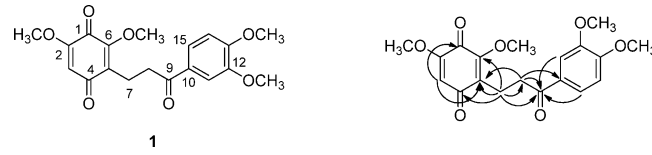

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Figure 1. Structure of evelynin (**1**) and key HMBC correlations shown with arrows.

Roots and rhizomes of *T. chantrieri* were extracted using supercritical CO_2 with MeOH. The extract was subjected to column chromatography followed by SPE to yield taccalonolides A and E and evelynin (**1**).

Evelynin (**1**) was obtained as a yellow powder, and the molecular formula was determined as $C_{19}H_{20}O_7$ by HRMS (calcd 360.1209, measd 360.1229). The ^{1}H NMR spectrum showed signals for four methoxy groups at δ 3.98, 3.94, 3.93, and 3.80, two vicinal methylene groups at δ 3.08 (2H, J = 7.8 Hz) and 2.85 (2H, J = 7.8 Hz), and four aromatic protons at δ 7.59 (dd, J = 8.4, 1.9 Hz), 7.53 (d, J = 1.9 Hz), 6.87 (d, J = 8.4 Hz), and 5.85 (s). The spin pattern of the aromatic protons indicated a 1,3,4-trisubstituted phenyl ring. The HMBC correlations of the two methoxy groups at δ 3.94 and 3.93 with the aromatic carbons at δ 153.4 (C-13) and 149.1 (C-12), respectively, indicated two of the substitutions are *O*-methyl groups. The additional substitution was assigned as a carbonyl functionality at δ 197.6 (C-9) by its HMBC correlations with H-15 (δ 7.59) and H-11 (δ 7.53) (see Figure 1). A *p*-benzoquinone moiety was suggested by two carbonyl signals at δ 178.2 (C-1) and 187.4 (C-4) and four aromatic signals at δ 157.5 (C-2), 154.8 (C-6), 132.3 (C-5), and 107.1 (C-3) in the ^{13}C NMR spectrum and was verified by the HMBC correlations shown in Figure 1. Two methoxy substituents on the *p*-benzoquinone ring were readily assigned by the HMBC correlations of CH_3O/C-2 and CH_3O/C-6. The connection of the *p*-benzoquinone and the 3,4-dimethoxylbenzoyl moiety via a CH_2-CH_2 bridge was evidenced by the HMBC correlations between H_2-7/C-4, C-5,C-6, C-9 and H_2-8/C-9, C-10. The structure of **1** was determined as depicted in Figure 1, and a trivial name, evelynin, was given in honor of Evelyn M. Jackson for her many years' contribution to this program and her recent retirement. Evelynin may, thus, be classified as a unique benzoquinone-type *retro*-dihydrochalcone.

To confirm the chemical structure and biological activities, **1** was synthesized via a simple one-step procedure as outlined in Scheme 1. The approach involves the addition of 3-(3,4-dimethoxybenzoyl)propionic acid (**2**) to 2,6-dimethoxy-1,4-benzoquinone (**3**) via the well-known silver(I)/persulfate-mediated decarboxylative radical alkylation of quinones and related compounds.[16,17] The

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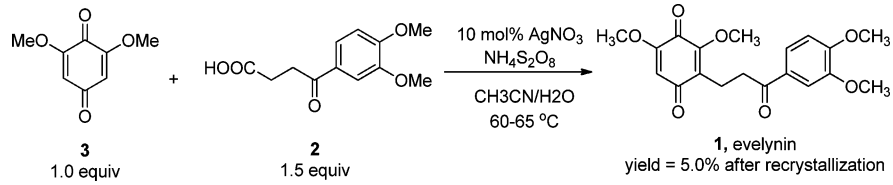
Scheme 1. Synthesis of Evelynin via a Catalytic Radical Alkylation Approach



synthetic evelynin obtained via this procedure is spectroscopically identical to the authentic sample and can be readily obtained in high purity (>98.5% by LC/MS) via recrystallization from EtOH. While the overall yield of this unoptimized approach is low (5%), the simplicity of the one-step procedure from two commercially available starting materials and its ability to provide multigram quantities in a relatively short period of time (<1 day) more than compensate for its inefficiency.

The IC_{50} value for synthetic evelynin in the MDA-MB-435 human melanoma cell line was determined to be 4.1 ± 0.3 μM. Other cancer cell lines, including the MDA-MB-231 breast cancer cell line, the PC-3 prostate cancer cell line, and the HeLa cervical cancer cell line, showed similar sensitivities to evelynin, with IC_{50} values of 3.9 ± 0.1, 4.7 ± 0.4, and 6.3 ± 0.7 μM, respectively. Synthesized evelynin was evaluated side by side with the taccalonolides for effects on cellular microtubules in A-10 smooth muscle cells and in HeLa cells. Unlike the taccalonolides, evelynin had no observable effects on microtubule density or structure at concentrations up to 40 μM, demonstrating that the cytotoxicity exhibited by this compound was not a result of disruption of cellular microtubules (Figure 1 in the Supporting Information).

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker Avance 600 MHz or a Varian QE 300 MHz instrument. All spectra were measured and reported in ppm by using the residual solvent ($CDCl_3$) as an internal standard. The HRMS was measured using a Thermo Scientific LTQ Orbitrap mass spectrometer. IR data were obtained on a Bruker Vector 22 using a Specac Golden Gate ATR sampler. The UV spectrum was measured on a Varian Cary 5000 UV−vis−NIR spectrophotometer. TLC was performed on aluminum sheets (silica gel 60 F_{254}, Merck KGaA, Germany). HPLC was performed on a Waters Breeze HPLC system, and LC/MS was recorded on an Agilent 1200 series HPLC connected to a 6130 series single-quad mass spectrometer.

Biological Material. *Tacca chantreiri* plants were purchased from a commercial grower. The roots and rhizomes were collected from living plants grown in greenhouses and stored at 80 °C until lyophilized. A voucher specimen was used for identification of *T. chantrieri* and was deposited in the herbarium of the University of Hawaii.

Extraction and Isolation. Dried and pulverized rhizome and root material (168.15 g) was extracted in several batches with supercritical CO_2 and MeOH. The crude extracts were washed with hexanes and extracted with CH_2Cl_2. The extracts were applied to silica gel columns (Biotage) for flash chromatography, and the yellow fractions, containing evelynin and the taccalonolides, were dried. Extracts were dissolved in CH_2Cl_2 for solid-phase extraction on silica cartridges (Burdick and Jackson). Evelynin was eluted with CH_2Cl_2; 59 mg of material was recovered and analyzed as described below.

Evelynin (1): yellow powder; IR (film) ν_{max} 3069, 2950, 1682, 1664, 1596, 1514, 1417, 1274, 1082, 1023 cm^{-1}; UV λ_{max} (log ε) 274 (3.97) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 7.59 (dd, 8.4, 1.9, H-15), 7.53 (d, 1.9, H-11), 6.87 (d, 8.4, H-14), 5.85 (s, H-3), 3.98 (3H, s, C_6-OCH_3), 3.94 (3H, s, C_{13}-OCH_3), 3.93 (3H, s, C_{12}-OCH_3), 3.80 (3H, s, C_2-OCH_3), 3.08 (2H, d, 7.8, H-8), 2.85 (2H, d, 7.8, H-7); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.6 (C-9), 187.4 (C-4), 178.2 (C-1), 157.5 (C-2), 154.8 (C-6), 153.4 (C-13), 149.1 (C-12), 132.3 (C-5), 129.9 (C-10), 122.9 (C-15), 110.3 (C-11), 110.1 (C-14), 107.1 (C-3), 61.2 (C_2-OCH_3), 56.6 (C_6-OCH_3), 56.2 (C_{13}-OCH_3), 56.1 (C_{12}-OCH_3), 37.2 (C-8), 19.2 (C-7).

Synthesis of 1. To a three-neck, 250 mL, round-bottom flask were added 2,6-dimethoxy-1,4-benzoquinone (1.7 g, 10.0 mmol), 3-(3,4,-dimethoxybenzoyl)propionic acid (3.6 g, 15 mmol), and $AgNO_3$ (17 mg, 1.0 mmol). The flask was fitted with an addition funnel (125 mL), a thermocouple, and a nitrogen inlet valve. The flask was purged with N_2 for ~20 min followed by the addition of degassed MeCN (75 mL) and H_2O (15 mL). The heterogeneous mixture was heated to 60 °C, at which point a solution of $NH_4S_2O_8$ (2.7 g, 12 mmol) in H_2O (10 mL) was added dropwise via the addition funnel over a period of ~25 min. After complete addition, the reaction was monitored by LC/MS for product formation. The reaction was heated for a total of 16 h and then cooled to room temperature. The mixture was extracted with EtOAc (3 × 50 mL), and the combined organic extracts were washed with H_2O (2 × 50 mL) and 10% brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a crude dark yellow solid (3.1 g). The product was partially purified via silica gel chromatography using a mixture of EtOAc/CH_2Cl_2 (3:97) as eluent to give synthetic evelynin (350 mg) in ~90% purity. This was further purified via recrystallization from hot EtOH to give pure evelynin as a light yellow crystalline solid (180 mg, 5.0% yield). All spectroscopic data matched that of natural evelynin (see above). Additional data on synthetic evelynin: mp =165−168 °C; ESIMS (*m*/*z*) 361 (M + H), 383 (M + Na).

Biological Assays. The MDA-MB-435 human melanoma cancer cell line was obtained from the Lombardi Cancer Center (Georgetown University, Washington, DC). The MDA-MB-231 breast, PC-3 prostate, and HeLa cervical human cancer cell lines as well as the A-10 embryonic rat aortic smooth muscle cell line were purchased from American Type Culture Collection (Manassas, VA). MDA-MB-435 and MDA-MB-231 cells were cultured in Richter's IMEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% FBS and 25 μg/mL gentamicin. HeLa and A-10 cells were cultured in Basal Medium Eagle (Sigma, St. Louis, MO) with 10% FBS and 50 μg/mL gentamicin. PC-3 cells were grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% FBS and 50 μg/mL gentamicin. The SRB assay[15] was used to determine the sensitivity of the cancer cell lines to evelynin as previously described.[14] Briefly, a range of concentrations of synthetic evelynin was added to cells in triplicate and incubated for 48 h. The concentration of evelynin that caused 50% inhibition of cell proliferation (IC_{50}) was calculated from the linear portion of the log dose−response curves. The mean IC_{50} value with standard deviation was calculated from three independent experiments, each performed in triplicate. Cellular microtubules were evaluated as previously described.[3]

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Supporting Information Available: NMR spectra of compound **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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