JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Isolation, Structure Elucidation, and Biological Evaluation of 16,23-Epoxycucurbitacin Constituents from *Eleaocarpus chinensis*

Li Pan,[†] Yeonjoong Yong,[‡] Ye Deng,[†] Daniel D. Lantvit,[§] Tran Ngoc Ninh,[⊥] Heebyung Chai,[†] Esperanza J. Carcache de Blanco,[†,‡] Djaja D. Soejarto,[§,∥] Steven M. Swanson,[§] and A. Douglas Kinghorn*[,†]

[†]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States

[‡]Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States

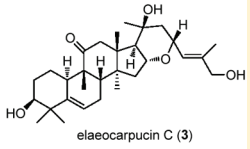
[§]Program for Collaborative Research in the Pharmaceutical Science and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States

[⊥]Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

[∥]Department of Botany, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, Illinois 60605, United States

Supporting Information

ABSTRACT: Eight new 16,23-epoxycucurbitacin derivatives, designated as elaeocarpucins A–H (**1**–**8**), and five known cucurbitacins (**9**–**13**) were isolated from the chloroform-soluble partitions of separate methanol extracts of the fruits and stem bark of *Elaeocarpus chinensis* collected in Vietnam. Isolation work was facilitated using a LC/MS dereplication procedure, and bioassay-guided fractionation was monitored using HT-29 human cancer cells. The structures of compounds **1**–**8** were determined on the basis of spectroscopic data interpretation, with the absolute configurations of isomers **1** and **2** established by the Mosher ester method. Compounds **1**–**13** were evaluated in vitro against the HT-29 cell line and using a mitochondrial transmembrane potential assay. Elaeocarpucin C (**3**), produced by partial synthesis from 16α,23α-epoxy-3β,20β-dihydroxy-10α*H*,23β*H*-cucurbit-5,24-dien-11-one (**13**), was found to be inactive when evaluated in an in vivo hollow fiber assay using three different cancer cell types (dose range 0.5–10 mg/kg/day, ip).



elaeocarpucin C (**3**)

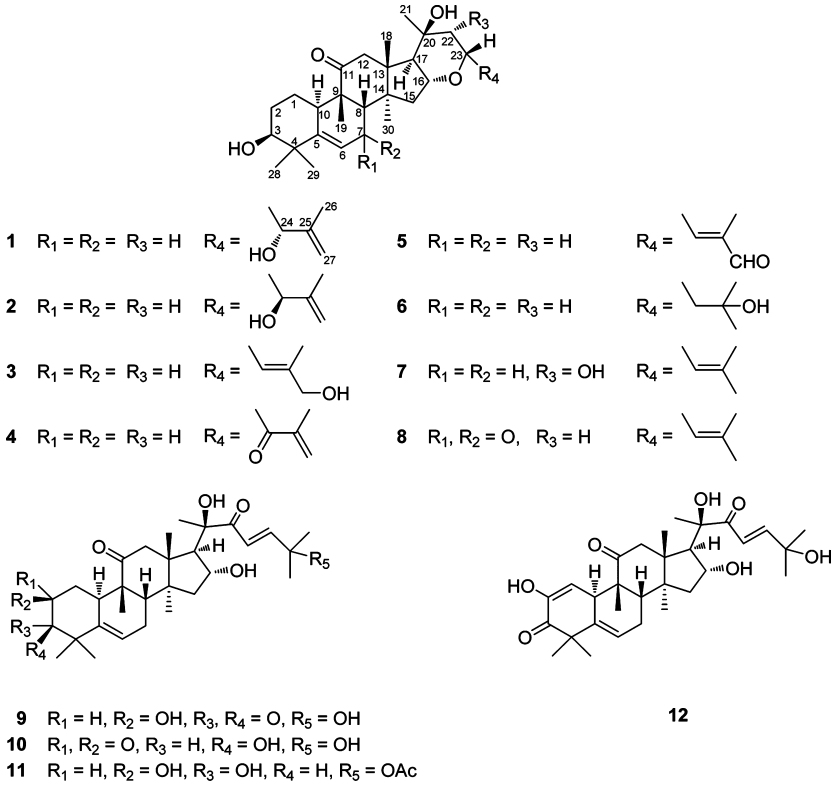
Elaeocarpus chinensis (Gardner & Champ.) Hook.f. ex Benth. (syn.: *Friesia chinensis* Gardner & Champ.), an evergreen tree of the family Elaeocarpaceae, is distributed mainly in subtropical or tropical areas of Asia, including southern mainland China, Laos, and Vietnam.[1] Besides being grown for ornamental purposes, *E. chinensis* is used also as a traditional Chinese herbal medicine for the treatment of emmeniopathy as well as extravasated blood and inflammatory edema caused by traumatic injury.[2] *Elaeocarpus* is a large genus comprising 350–360 species distributed from Madagascar to Oceania, with the highest concentration occurring in Borneo and Papua New Guinea.[3,4] Previous phytochemical work has resulted in the isolation of anthocyanins,[5] cucurbitacin-type triterpenoids,[6−12] flavonoids,[13,14] other phenolic derivatives,[13] and indolizidine alkaloids.[15−17] Among these compounds, cucurbitacins and their derivatives are tetracyclic triterpenoids obtained initially from plants of the family Cucurbitaceae and are reported to have anticancer, antifertility, anti-inflammatory, cytotoxic, and purgative activities.[18,19] Although the development of cucurbitacins as anticancer drug candidates has been hindered by their nonspecific cytotoxicity, there is much interest in the relationship of structure to cytotoxicity within this compound class.[18,19] Some cucurbitacins have been found to affect JAK-STAP and MAPK signaling pathways in cancer cells and to show synergistic effects in combination with certain known anticancer therapeutic agents, such as doxorubicin and gemcitabine.[20,21] Thus far, there have been no studies on the phytochemical constituents of *E. chinensis*.

As part of our ongoing program to discover new anticancer agents from varied natural sources,[22,23] a $CHCl_3$ extract of the fruits of *E. chinensis* was found to exhibit cytotoxic activity (IC_{50} 0.4 μg/mL) against human colon cancer (HT-29) cells. Scrutiny of the NAPRALERT[SM] (Natural Products Alert) database[24] indicated that more than 150 compounds have been isolated from the genus *Elaeocarpus*, with most of the cytotoxic compounds known being cucurbitacin-type triterpenes. In order to decide whether or not to further pursue this lead,

Special Issue: Special Issue in Honor of Gordon M. Cragg

Received: November 1, 2011
Published: January 12, 2012

Chart 1



the $CHCl_3$-soluble extract of *E. chinensis* fruits was subjected to an LC-MS dereplication procedure, which revealed the probable presence of the known cytotoxic cucurbitacins cucurbitacin D (**9**), 3-*epi*-isocucurbitacin D (**10**), and 25-*O*-acetylcucurbitacin F (**11**). In addition, certain unknown cytotoxic compounds corresponding to possible molecular formulas of $C_{30}H_{44}O_5$ and $C_{30}H_{46}O_5$ were evident. Accordingly, subsequent bioassay-guided fractionation was conducted using HT-29 cancer cells to monitor purification and led to the isolation of six new 16,23-epoxycucurbitacins, elaeocarpucins A–F (**1**–**6**), together with five known cucubitacins inclusive of cucurbitacin D (**9**),[25,26] 3-*epi*-isocucurbitacin D (**10**),[26] 25-*O*-acetylcucurbitacin F (**11**),[9,27] cucurbitacin I (**12**),[28] and 16α,23α-epoxy-3β,20β-dihydroxy-10α*H*,23β*H*-cucurbit-5,24-dien-11-one (**13**).[11] Moreover, from the less potently cytotoxic $CHCl_3$ extract of the stem bark of the same plant, two additional new cucurbitacins, elaeocarpucins G (**7**) and H (**8**), were purified. Herein, we report the isolation and structure elucidation of the eight new compounds, **1**–**8**, as well as the biological assessment of all isolates obtained in this investigation.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Its molecular formula was assigned as $C_{30}H_{46}O_5$ on the basis of the $[M + Na]^+$ ion peak at m/z 509.3225 (calcd 509.3243) in the HRESIMS. Observed in the ^{1}H NMR spectrum were signals for seven tertiary methyl groups at δ_H 0.93 (3H, s, H-18), 1.02 (3H, s, H-28), 1.14 (3H, s, H-19), 1.17 (3H, s, H-29), 1.22 (3H, s, H-30), 1.32 (3H, s, H-21), and 1.72 (3H, s, H-26), while resonances at δ_H 3.48 (1H, brs, H-3), 4.40 (1H, ddd, J = 10.4, 10.4, 3.6 Hz, H-16), 4.01 (1H, ddd, J = 12.0, 3.0, 3.0 Hz, H-23), and 4.24 (1H, d, J = 2.8 Hz, H-24) were attributed to proton signals attached to four oxygenated methine carbons. In addition, three olefinic protons were recognized in the ^{1}H NMR spectrum at δ_H 4.91 (1H, s, H-27$_a$), 5.04 (1H, s, H-27$_b$), and 5.67 (1H, d, J = 5.8 Hz, H-6). The ^{13}C NMR spectrum of **1** showed 30 carbon signals, which were classified from DEPT and HSQC data as seven methyls, six methylenes, three methines, four quaternary carbons, five oxygen-bearing carbons (including four secondary and one tertiary), a trisubstituted double bond, a disubstituted terminal double bond, and a carbonyl group. The characteristic NMR data of compound **1** were comparable to those of 16α,23α-epoxy-3β,20β-dihydroxy-10α*H*,23β*H*-cucurbit-5,24-dien-11-one (**13**), a known 16α,23α-epoxycucurbitane analogue first isolated from *Eleaocarpus hainanensis*[11] that was also identified in the present investigation. Comparison of the 1D- and 2D-NMR data of **1** with those of **13** revealed a major change evident in the side chain located at C-23, with a 2-methylprop-1-ene group in **13** being replaced by a 2-methylprop-2-en-1-ol moiety in **1**. The signals of the latter unit occurred at δ_H 4.24 (1H, d, J = 2.8 Hz, H-24), 4.91 (1H, s, H$_a$-27), 5.04 (1H, s, H$_b$-27), and 1.72 (3H, s, H-26) in the ^{1}H NMR spectrum as well as at δ_C 75.8 (CH, C-24), 142.7 (C, C-25), 19.5 (CH_3, C-26), and 111.7 (CH_2, C-27) in the ^{13}C NMR spectrum. Moreover, key HMBC correlations from the terminal olefinic methylene protons of H-27 to C-24, C-25, and C-26, as well as H-24 and H-26 to C-27, supported the structure assigned for the side-chain moiety. Thus, the planar structure of **1** could be proposed.

The Mosher ester procedure was employed to determine the absolute configuration of the OH groups located at C-3 and C-24 in compound **1**. After treatment with (*R*)- and (*S*)-MTPA chloride, the secondary OH groups at C-3 and C-24 were both esterified, to afford the (*S*)- and (*R*)-MTPA derivatives, respectively. By analyzing the observed ^{1}H NMR chemical

dx.doi.org/10.1021/np200879p | *J. Nat. Prod.* 2012, 75, 444–452

shift difference values ($\Delta\delta_{S-R}$) of certain diagnostic key protons assigned unambiguously, the absolute configurations of C-3 and C-24 were both assigned as *S* (Figure 1). Furthermore, the

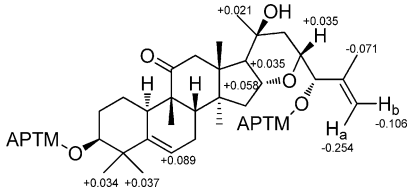


Figure 1. $\Delta\delta_{S-R}$ values of MTPA esters of **1**.

observed NOESY cross-peaks of H-10/H_3-28 and H_3-30, H-8/H_3-18 and H_3-19, H-17/H_3-30, H-16/H_3-18, H-23/H-15β, and H_3-21/H-17 and H-12α provided evidence that the relative configurations of the remaining chiral carbons of compound **1** were identical with those of previously reported related compounds.[6−12] Hence, the structure of compound **1** was determined to be (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*R*,24*S*)-16,23-epoxy-3,20,24-trihydroxycucurbit-5,25(27)-dien-11-one, and this substance has been accorded the trivial name elaeocarpucin A.

Compound **2** gave the same molecular formula, $C_{30}H_{46}O_5$, as that of **1** by analysis of the HRESIMS. The NMR spectra of **1** and **2** were closely comparable, with the only differences evident in signals for the side chain at C-23. In the ^{1}H NMR spectrum of **2**, the H-23 and H-24 resonances appeared at δ_H 3.84 (ddd, J = 10.7, 8.0, and 2.7 Hz) and 3.87 (d, J = 7.2 Hz), respectively. Both were shifted upfield and showed a change of coupling pattern when compared with **1**. Correspondingly, in the ^{13}C NMR spectrum, a downfield shift of approximately 3.0 ppm for the carbon signal of C-24 (δ_C 78.8) was discernible. These observed differences suggested that the absolute configuration of C-24 might be *R*, which was confirmed subsequently by calculation of the ^{1}H NMR chemical shift differences for the (*S*)- and (*R*)-MTPA esters of **2** produced as a result of the Mosher ester reaction (Figure 2). Thus, compound **2** (elaeocarpucin B) was determined structurally as the C-24 epimer of **1**.

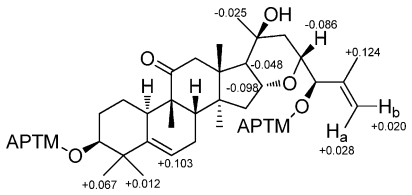


Figure 2. $\Delta\delta_{S-R}$ values of MTPA esters of **2**.

Compound **3** was obtained as a pale yellow powder. The HRESIMS gave a sodiated molecular ion peak at m/z 509.3255 $[M + Na]^+$, consistent with a molecular formula of $C_{30}H_{46}O_5$, the same as those of both compounds **1** and **2**. The ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of compound **13**. On comparison of the ^{1}H NMR data of these two compounds, the H_3-27 signal at δ_H 1.68 in compound **13** was absent, while an oxygenated methylene resonance appeared at δ_H 4.02 (2H, s). This suggested that the C-27 methyl group in compound **13** is substituted by a primary alcohol group in compound **3**. Correspondingly, in the ^{13}C NMR spectrum of **3**, the observed downfield shift of 2.7 ppm for C-25 and an upfield shift of 4.0 ppm for C-26 were consistent with the substitution of an OH group at C-27. The HMBC spectrum of **3** showed key correlations of H-24 with C-23, C-25, and C-27, as well as H-27 and H-26 with C-25 and C-24, which supported the above functional group assignment. The *trans*-configuration of the C-24, C-25 double bond was deduced on the basis of the key NOESY correlation between H-24 and H-27. Other observed NOE effects indicated the relative configuration of the remaining part of the molecule of **3** to be identical with that of **1** and other known 16α,23α-epoxycucurbitacins. Thus, the structure of compound **3** (elaeocarpucin C) was determined as (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*R*,24*E*)-16,23-epoxy-3,20,27-trihydroxycucurbit-5,24-dien-11-one.

Compound **4** was obtained as white, amorphous powder. The HRESIMS of **4** gave a sodiated molecular ion peak at m/z 507.3073 $[M + Na]^+$, suggesting an elemental formula of $C_{30}H_{44}O_5$, representing one more degree of unsaturation than in compounds **1**−**3**. In the ^{1}H NMR spectrum of **4**, the proton signal of an oxygenated CH, assigned as H-24 in compound **1**, was absent, and the corresponding oxygenated carbon was substituted by a carbonyl group at δ_C 198.9 in the ^{13}C NMR spectrum. These differences suggested that the OH group at C-24 in **1** is oxidized to a carbonyl group in **4**. Moreover, downfield shifts of 0.97 ppm for H-23, 1.09 ppm for H_a-27, 0.82 ppm for H_b-27, and 0.17 ppm for H-26, due to the deshielding effect caused by the nearby carbonyl group at C-24, were observed in the ^{1}H NMR spectrum. Data from the HSQC, HMBC, and NOESY 2D-NMR spectra were consistent with the above deduction. Thus, the structure of compound **4** (elaeocarpucin D) was elucidated as (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*R*)-16,23-epoxy-3,20-dihydroxycucurbit-5,25(27)-diene-11,24-dione.

The molecular formula of compound **5** was deduced as $C_{30}H_{44}O_5$ on the basis of its HRESIMS, the same as that of compound **4**. In the ^{1}H NMR spectrum, a proton singlet appeared at δ_H 9.40, and this signal exhibited a correlation in the HSQC spectrum with a carbonyl group signal at δ_C 195.2, which implied the presence of a formyl group. In the HMBC spectrum, key correlations were observed from the aldehyde proton to the methyl group carbon at δ_C 9.9 (C-26) and two carbon signals of a double bond at δ_C 151.8 and 139.0 (C-24 and C-25), respectively, which suggested that the formyl group was at C-25. Comparison of the NMR data of **5** with those of compound **13** showed a downfield ^{1}H NMR shift of 0.54 ppm for H-24 and downfield ^{13}C NMR shifts of 26.7 and 3.1 ppm for C-24 and C-25, respectively. These were consistent with the substitution of a formyl group at C-27 in **5**. The *trans*-configuration of the C-24−C-25 double bond was deduced from the key NOE correlation between the aldehyde proton (H-27) and H-24. Thus, the structure of compound **5** (elaeocarpucin E) was deduced as (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*R*,24*E*)-16,23-epoxy-3,20-dihydroxycucurbit-27-aldehyde-5,24-dien-11-one.

Compound **6** gave a molecular formula of $C_{30}H_{48}O_5$, as determined by HRESIMS, with one degree of unsaturation less than compound **1**. In comparison of the NMR spectra with those of compound **1**, besides one trisubstituted double bond ascribed to C-5−C-6, no other double-bond signal was found, which implied that the side chain of **6** is saturated. This inference was confirmed by the observed ^{1}H−^{1}H COSY correlations of H-23 (δ_H 4.22, 1H, dddd, J = 11.1, 11.1, 2.4, and 2.4 Hz) with two CH_2 groups, H-22 (δ_H 1.31 and 1.45, each 1H) and H-24 (δ_H 1.42 and 1.87, each 1H). In addition to the ^{13}C NMR signal at δ_C 72.3 of C-20, another quaternary

dx.doi.org/10.1021/np200879p | J. Nat. Prod. 2012, 75, 444−452

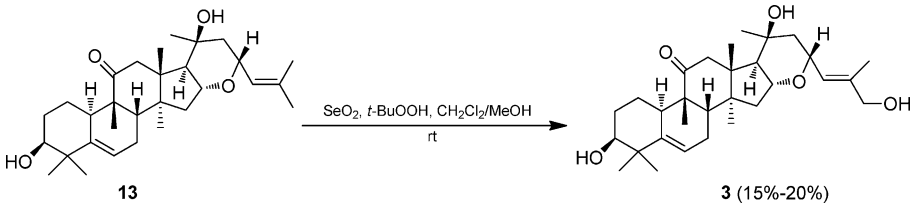


Figure 3. Semisynthesis of compound **3** from compound **13**.

oxygenated carbon appeared at δ_C 70.5 and exhibited HMBC correlations with the H_3-26 (δ_H 1.20, 3H, s) and H_3-27 (δ_H 1.27, 3H, s) signals, respectively, which indicated a tertiary OH group at C-25. Analysis of the NOESY spectrum suggested the relative configuration of the remainder of the molecule of **6** to be identical with that of **1**. Thus, the structure of **6** (elaeocarpucin F) was determined as (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*S*)-16,23-epoxy-3,20,25-trihydroxycucurbit-5-en-11-one.

Compound **7** was obtained as a white, amorphous powder, and its molecular formula was deduced as $C_{30}H_{46}O_5$ from the HRESIMS. The NMR spectra of **7** were very similar to those of compound **13**, with the only difference being the replacement of the CH_2 group at C-22 by an oxygenated CH. In the COSY spectrum, a broad singlet of an oxygenated proton at δ_H 2.95 (H-22) exhibited a weak (due to the small *J* value of the coupling constant between H-22 and H-23) but discernible enhancement with H-23 at δ_H 4.67 (1H, d, *J* = 7.8 Hz), and its corresponding carbon signal at δ_C 77.6 showed a HMBC correlation with the methyl proton signal at δ_H 1.38 (3H, s, H-21). Thus, it was inferred that an OH group is positioned at C-22 in compound **7**. Correspondingly, a shift to higher field of around 6 ppm was observed for C-17 in the ^{13}C NMR spectrum due to the γ-effect caused by this substituent. In the NOESY spectrum, key NOE cross-peaks of H-22/H-23, H-23/H-16, H-16/H-18, and H-21/H-17 were observed, which indicated the α-orientation of the OH group. Accordingly, the structure of compound **7** (elaeocarpucin G) was assigned as (3*S*,8*S*,9*R*,10*R*,13*R*,14*S*,16*R*,17*R*,21*R*,22*R*,23*S*)-16,23-epoxy-3,20,22-trihydroxycucurbit-5,24-dien-11-one.

The molecular formula of compound **8** was determined as $C_{30}H_{44}O_5$ from the protonated molecular ion peak at *m*/*z* 485.3261 $[M + H]^+$ in the HRESIMS. The NMR data of **8** were again similar to those of **13**. When comparing the ^{13}C NMR spectra of these two compounds, the signal of a CH_2 group at δ_C 24.0 ascribed to C-7 in compound **13** was absent, with a carbonyl group signal appearing at δ_C 199.6 instead, thus suggesting a carbonyl group at C-7 in **8**. In the ^{1}H NMR spectrum, downfield shifts of approximately 0.5 ppm for H-6 and 0.6 ppm for H-8 were observed due to deshielding effects caused by the carbonyl group at C-7. These were consistent with the downfield shifts of around 27 and 5 ppm for C-5 (δ_C 167.0) and C-6 (δ_C 125.6), respectively, as well as ca. 15 ppm for C-8 (δ_C 58.1) in the ^{13}C NMR spectrum. Key HMBC correlations of H-3, H-10, H-28, and H-29 to C-5, and H-8 to C-7, were observed to support the structure proposed. Further analysis of the NOESY experiment revealed the consistent relative configuration of **8** with other cucurbitacin analogues isolated in this investigation. Thus, the structure of compound **8** (elaeocarpucin H) was deduced as (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,16*R*,17*R*,21*S*,23*R*)-16,23-epoxy-3,20-dihydroxycucurbit-5,24-diene-7,11-dione.

All isolates (**1**−**13**) were evaluated their cytotoxic activity against HT-29 human colon cancer cells. The known cucurbitacins, cucurbitacin D (**9**), 3-*epi*-isocucurbitacin D (**10**), 25-*O*-acetylcucurbitacin F (**11**), and cucurbitacin I (**12**), were found to be the most active in inhibiting the proliferation of HT-29 cancer cells, with IC_{50} values ranging from 0.039 to 0.54 μM. Of the eight new 16,23-epoxycucurbitacins, elaeocarpucin C (**3**) was found to display potent cytotoxicity against HT-29 cells with an IC_{50} value of 0.41 μM, while elaeocarpucins D (**4**), G (**7**), and H (**8**) were less active against this same cell line. Thus, a 24(25)-en-27-ol functionality on the side chain of these new compounds seems to be required for potent cytotoxicity. In general, when the C-17−C-23 unit is contained in an epoxide ring, the resultant cytotoxicity is reduced when compared with known compounds such as 3-*epi*-isocucurbitacin D (**10**) (Table 3).

Compounds **1**−**3**, **6**, **9**, **10**, and **13** were also evaluated in a HT-29 cell-based mitochondrial transmembrane potential assay, but none of these substances were found to be active (IC_{50} >10 μM).

The initial cytotoxic activity of compound **3** encouraged further biological evaluation of this compound. A sufficient amount of **3** (>25 mg) was generated from the known inactive compound **13** by selectively oxidizing the allylic methyl group (C-27) into a primary alcohol (Figure 3), for evaluation in the in vivo hollow fiber assay. This method may be used as a secondary discriminator to prioritize compounds possessing promising in vitro activity for potential further testing in a relevant in vivo xenograft model.[29−32] The human cancer cell lines evaluated using ip administration comprised MDA-MB-435 (melanoma), MCF-7 (breast), and HT-29 (colon) for the in vivo hollow fiber assay. However, no inhibition of proliferation by **3** was observed over the course of the study for any of the cancer cell types, which were administrated at a dose range of 0.5 to 10 mg/kg/day.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using a Fisher Scientific melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 343 automatic polarimeter. UV spectra were measured with a Perkin-Elmer Lambda 10 UV/vis spectrometer. IR spectra were obtained on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. NMR spectroscopic data were run at room temperature on Bruker Avance DRX-400 or 600 MHz spectrometers, and the data were processed using MestReNova 6.0 software (Mestrelab Research SL, Santiago de Compostela, Spain). Accurate mass values were performed on a Micromass LCT ESI spectrometer. Sodium iodide was used for mass calibration for a calibration range of *m*/*z* 100−2000. LC-MS experiments were performed on a liquid chromatographic/autosampler system that consisted of a Waters Alliance 2690 Separations Module (Waters, Milford, MA, USA) and a Micromass LC-TOF II mass spectrometer (Micromass, Wythenshawe, UK) equipped with an orthogonal electrospray source (Z-spray). Column chromatography was carried out with silica gel (230−400 Mesh; Sorbent Technologies,

dx.doi.org/10.1021/np200879p | *J. Nat. Prod.* 2012, 75, 444−452

Table 1. ^{1}H NMR Chemical Shifts of Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
1	1.47, m	1.47, m	1.42, m	1.43, m	1.49, m	1.49, m	1.48, m	1.66[b] 1.78[b]
2α	1.73, m	1.74, m	1.73, m	1.73, m	1.74, m	1.74, m	1.75, m	1.78, m
2β	1.67, m	1.66, m	1.68, m	1.68, m	1.68, m	1.65, m	1.68, m	
3	3.48, brs	3.48, brs	3.48, brs	3.48, brs	3.48, brs	3.48, brs	3.48, brs	3.65, brs
6	5.67, d (5.8)	5.67, d (5.8)	5.67, d (5.8)	5.67, d (5.6)	5.68, d (6.0)	5.67, d (5.8)	5.67, d (5.6)	6.17, d (brs)
7α	1.88, m	1.89, m	1.91, m	1.90, m	1.91, m	1.90, m	1.93, m	
7β	2.46, m	2.44, m	2.44, m	2.44, m	2.44, m	2.44, m	2.44, m	
8	1.96[b]	1.94[b]	1.96[b]	1.92, d (8.3)	1.96, d (8.0)	1.91[b]	1.94[b]	2.54, brs
10	2.25, m	2.25, m	2.25, m	2.25, m	2.25, m	2.24, m	2.24, m	2.67, m
12α	3.03, d (14.8)	3.03, d (14.8)	3.03, d (14.8)	3.03, d (14.8)	3.04, d (14.4)	3.01, d (14.4)	3.04, d (14.8)	3.04, d (14.8)
12β	2.43, d (14.8)	2.42, d (14.8)	2.43, d (14.8)	2.42, d (14.8)	2.44, d (14.4)	2.42, d (14.4)	2.42 (14.8)	2.55 (14.8)
15α	1.48, m	1.48, m	1.47, m	1.50, m	1.48, m	1.42, m	1.48, m	1.36, m
15β	1.85, m	1.84, m	1.85, m	1.84, m	1.85, m	1.82, m	1.85, m	2.40, m
16	4.40, ddd (10.4, 10.4, 3.6)	4.33, ddd (10.4, 10.4, 3.7)	4.38, ddd (10.1, 10.1, 3.9)	4.42, ddd (10.1, 10.1, 3.9)	4.43, ddd (10.4, 10.4, 3.8)	4.37, ddd (10.4, 10.4, 3.6)	4.37, ddd (10.0, 10.0, 3.6)	4.37, ddd (10.4, 10.4, 3.6)
17	1.95[b]	1.95[b]	1.99, d (10.5)	2.00, d (10.5)	1.95, d (10.1)	1.95, d (10.4)	2.34, d (10.1)	1.90, d (10.6)
18	0.93, s	0.93, s	0.94, s	0.94, s	0.96, s	0.93, s	0.95, s	0.98, s
19	1.14, s	1.14, s	1.13, s	1.14, s	1.15, s	1.13, s	1.14, s	1.17, s
21	1.32, s	1.31, s	1.32, s	1.36, s	1.36, s	1.31, s	1.38, s	1.32, s
22α	1.57, dd (12.0, 14.0)	1.36, m	1.52, m	1.67, m	1.52, m	1.45, m	2.95, brs	1.49, m
22β	1.25, m	1.26, m	1.38, m	1.52, m	1.41, m	1.31, m		1.43, m
23	4.01, ddd (12.0, 3.0, 3.0)	3.84, ddd (10.7, 8.0, 2.7)	4.61, ddd (11.1, 8.5, 2.8)	4.98, dd (11.8, 2.8)	4.86, ddd (11.1, 7.2, 2.7)	4.22, dddd (11.1, 11.1, 2.4, 2.4)	4.67, d (7.8)	4.22, ddd (11.2, 8.0, 2.4)
24	4.24, d (2.8)	3.87, d (7.2)	5.44, d (8.3)		5.66, dd (7.6, 1.4)	1.87, m 1.42, m	5.34, brd (8.0)	5.12, d (8.0)
26	1.72, s	1.72, s	1.72, s	1.89, s	1.77, d (1.2)	1.20, s	1.72 (brs)	1.69, s
27a	4.91, s	4.92, s	4.02, s	6.10, s	9.40, s	1.27, s	1.77 (s)	1.71, s
27b	5.04, s	5.04, s		5.86, s				
28	1.02, s	1.02, s	1.02, s	1.01, s	1.02, s	1.01, s	1.01, s	1.14, s
29	1.17, s	1.17, s	1.17, s	1.17, s	1.18, s	1.17, s	1.17, s	1.28, s
30	1.22, s	1.22, s	1.21, s	1.24, s	1.25, s	1.20, s	1.24, s	1.24, s

[a]Measured at 400 MHz; obtained in $CDCl_3$ with TMS as internal standard; *J* values (Hz) are given in parentheses. Assignments are based on ^{1}H−^{1}H COSY, HSQC, and HMBC spectroscopic data. [b]Overlapping signals.

Atlanta, GA, USA). Analytical TLC was conducted on precoated 250 μm thick silica gel UV_{254} aluminum-backed plates (Sorbent Technologies). Waters Atlantis (4.6 × 150 mm) and semipreparative (10 × 150 mm) C_{18} (5 μm) columns were used for analytical and semipreparative HPLC, respectively, as conducted on a Waters system comprised of a 600 controller, a 717 Plus autosampler, and a 2487 dual-wavelength absorbance detector.

Plant Material. The fruits and stems of *E. chinensis* were collected in Honba Forest Reserve (12°06.953′ N; 109°00.072′E; Alt. 275 m), Khanh Hoa Province, Vietnam, by T.N.N., Vuong Tan Tu, and D.D.S. in November 2008, who also identified this plant. A voucher specimen (original collection DDS et al. 13583; re-collection 114330) has been deposited in the John G. Searle Herbarium of the Field Museum of Natural History (under accession number FM 2287877), Chicago, Illinois.

LC-MS Dereplication Procedure. *LC-UV Conditions.* Sample concentration, 10 mg/mL MeOH solution; mobile phase, gradient elution of MeOH−H_2O (0−10 min, from 50:50 to 70:30; 11−30 min, 100% MeOH); UV detection wavelength, 220 nm; flow rate, 0.75 mL/min; injection volume, 45 μL for the 96-well plate with sample concentration of ca. 20 μg/mL, and 11.3 μL for the 96-well plate with sample concentration of ca. 5 μg/mL, respectively.

Cytotoxicity Assay Screening. Fractions were collected into two 96-well plates (250 μL/well × 90 and negative control/well × 6) with sample concentrations of 20 and 5 μg/mL, respectively, and were tested for HT-29 cell growth inhibition activity, according to an established protocol.[33]

LC-MS Conditions. HPLC conditions: mobile phase, a gradient elution of MeOH−H_2O (0−10 min, from 50:50 to 70:30; 11−30 min, 100% MeOH); injection volume, 45 μL (10 mg/mL). The mobile phase flow rate was maintained at 0.75 mL/min and was split post-column using a microsplitter valve (Upchurch Scientific, Oak Harbor, WA, USA) to ca. 20 μL/min for introduction to the ESI source. Optimal ESI conditions: capillary voltage, 3000 V; source temperature, 110 °C; cone voltage, 55 V. Q1 was set to optimally pass ions from *m/z* 100−2000, and all ions transmitted into the pusher region of the TOF analyzer were scanned over *m/z* (100−1000 range) with a 1 s integration time. Data were acquired in a continuum mode during the LC run.

Data Analysis. Using a combination search of proposed molecular formulas corresponding to the major active peaks and the key word "Eleaocarpus" in the SciFinder database (Chemical Abstracts Service, Columbus, OH, USA), the peaks with unknown molecular formulas were designated for further fractionation.

Extraction and Isolation. The air-dried and milled fruits (480 g) of *E. chinensis* were extracted by maceration with MeOH (3 × 2 L) at room temperature for two days each. After removing the solvent under reduced pressure, the combined and concentrated MeOH extract was suspended in a mixture of 80% MeOH−H_2O (1 L), then partitioned with hexane and $CHCl_3$ in turn, to afford hexane- (20 g) and $CHCl_3$ (7 g)-soluble extracts. The $CHCl_3$-soluble extract, with an IC_{50} value of 0.4 μg/mL against HT-29 cells, was subjected to a LC-MS dereplication procedure, in which the effluent from the HPLC chromatography was split, with part passed into a mass spectrometer and part collected in a 96-well plate. The latter was subjected to

Table 2. ^{13}C NMR Chemical Shifts of Compounds 1−8[a,b]

position	1	2	3	4	5	6	7	8
1	20.5	20.5	20.5	20.5	20.7	21.0	20.5	20.5
2	28.4	28.5	28.5	28.6	28.7	28.6	28.5	28.3
3	76.0	76.1	76.0	76.1	76.2	76.0	76.1	76.1
4	42.0	41.7	41.7	41.7	41.8	42.0	41.7	43.0
5	139.6	139.7	139.6	139.6	139.8	139.6	139.6	167.0
6	120.3	120.4	120.4	120.3	120.5	120.4	120.3	125.6
7	23.9	23.9	23.9	24.0	24.3	24.9	23.9	199.6
8	42.7	42.7	42.7	42.7	42.8	42.7	42.7	58.1
9	49.3	49.4	49.3	49.3	49.5	49.3	49.3	49.5
10	35.1	35.2	35.1	35.2	35.3	35.1	35.2	37.4
11	213.4	213.4	213.4	213.2	213.5	213.4	213.5	211.0
12	48.2	48.3	48.3	48.2	48.4	48.2	48.2	48.3
13	48.5	48.5	48.3	48.4	48.6	48.4	48.5	47.8
14	47.9	48.0	48.1	47.9	48.2	47.9	47.5	47.5
15	40.6	40.6	40.7	40.3	40.7	40.7	40.5	40.5
16	76.7	76.6	77.3	77.0	76.6	76.5	76.6	76.5
17	55.2	55.3	54.9	54.9	55.1	55.0	48.7	54.7
18	19.8	19.8	19.9	19.9	20.1	19.8	20.3	19.9
19	20.3	20.3	20.3	20.4	20.5	20.3	20.4	21.1
20	72.0	72.1	72.4	72.3	72.5	72.3	74.8	72.2
21	29.3	29.3	29.3	29.2	29.4	29.2	25.7	29.7
22	41.1	45.0	48.7	45.3	47.2	49.5	77.6	49.1
23	76.5	76.7	72.5	77.2	73.2	74.2	75.4	72.8
24	75.8	78.8	124.8	198.9	151.8	46.9	120.8	124.8
25	142.7	143.7	138.6	143.0	139.0	70.5	138.2	136.6
26	19.5	17.7	14.2	18.1	9.9	28.0	18.8	18.5
27	111.7	114.7	67.9	126.4	195.2	31.0	26.0	25.8
28	27.2	27.2	27.2	27.2	27.3	27.1	27.1	27.8
29	25.3	25.4	25.3	25.4	25.6	25.3	25.3	24.8
30	21.2	21.1	21.1	21.2	21.3	21.1	21.3	21.1

[a]Measured at 100 MHz; obtained in $CDCl_3$ with TMS as internal standard. Assignments are based on HSQC and HMBC NMR spectra. [b]Multiplicity obtained from the DEPT spectrum.

cytotoxicity screening using HT-29 cancer cells. ESIMS analysis indicated that the active peaks were at m/z 560, 516, 484, and 486, of which those at 516 and 560 amu corresponded to the presence of three known cytotoxic cucurbitacins, cucurbitacin D (**9**), 3-*epi*-isocucurbitacin D (**10**), and 25-*O*-acetylcucurbitacin F (**11**). However, peaks at m/z 484 and 486, with possible molecular formulas of $C_{30}H_{44}O_5$ and $C_{30}H_{46}O_5$, respectively, did not seem to match those of any known cucurbitacin triterpenes. Accordingly, bioassay-guided fractionation was used to facilitate the isolation process.

The $CHCl_3$-soluble extract was subjected to chromatography over a silica gel column and eluted with a CH_2Cl_2−acetone gradient to afford 10 fractions (F1−F10). Fractions F3, F4, and F5 were active against HT-29 cells with IC_{50} values of 0.3, <0.16, and 0.2 μg/mL, respectively. Fraction F3 (220 mg) was chromatographed over an open C_{18} column (2.2 × 20 cm) using MeOH−H_2O mixtures (70:30 to 100% MeOH) for elution, to give three subfractions (F301−F303). F302 was purified by HPLC on a semipreparative RP-18 column, using MeOH−H_2O (60:40) as solvent, to afford **1** (10 mg), **2** (9.0 mg), **6** (7.0 mg), and a mixture of two compounds, which was subjected to further separation by HPLC, using CH_3CN−H_2O (33:67) for elution, to give **4** (4.0 mg) and **5** (1.0 mg), respectively. Fraction F4 (250 mg) was fractionated over an open C_{18} column (2.2 × 20 cm), eluted with MeOH−H_2O (70:30 to 100% MeOH), to afford five subfractions (F401−F405). Cucurbitacin D (**9**, 35 mg) was obtained as crystals from a MeOH−H_2O (ca. 70:30) solution of F401. Further purification of combined fractions F402−4 was conducted on a semipreparative RP-18 HPLC column, using MeOH−H_2O (60:40) as solvent, to yield 3-*epi*-isocucurbitacin D (**10**, 2.5 mg), cucurbitacin I (**12**, 1.0 mg), and 25-*O*-acetylcucurbitacin F (**11**, 3.5 mg). Elaeocarpucin C (**3**, 4.0 mg) was purified from fraction F5 (270 mg) by repeated separation on a semipreparative RP-18 HPLC column, using MeOH−H_2O (60:40) and CH_3CN−H_2O (35:65) sequentially for elution. In addition, compound **13** (10 mg) was recrystallized from the inactive fraction F2 using acetone as solvent. In order to obtain a sufficient amount of **13** as starting material to support the semisynthesis of elaeocarpucin C (**3**), the residue of F2 was chromatographed over a silica gel column and eluted with CH_2Cl_2−acetone mixtures (20:1 to 5:1) to afford an additional 200 mg quantity of this compound.

The stems of *E. chinensis* were also investigated in the present study. A $CHCl_3$-soluble extract (11 g) was prepared from the air-dried and then powdered stems (900 g) by following the extraction and partition procedures described above for the fruits. However, this was less cytotoxic (IC_{50} 10.1 μg/mL, HT-29 cells) than the $CHCl_3$-soluble extract of the fruits. This extract was fractionated over a silica gel column using CH_2Cl_2−acetone mixtures of increasing polarity to yield eight fractions (F1′−F8′). All fractions were analyzed using HPLC and TLC, and F4′ was found to be rich in cucurbitacins and was determined to contain cucurbitacin D (**9**), 3-*epi*-isocucurbitacin D (**10**), 25-*O*-acetylcucurbitacin F (**11**), and unknown cucurbitacin analogues. Separation of F4′ over a semipreparative RP-18 HPLC column using MeOH−H_2O (60:40) led to the purification of compounds **7** (2.0 mg) and **8** (1.8 mg).

*Elaeocarpucin A (**1**):* white powder; mp 258−260 °C; $[\alpha]^{20}_D$ +144.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.73) nm; IR (film) ν_{max} 3445, 1687, 1456, 1375, 1215, 1097, 1075, 1028, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS m/z 509.3225 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

*Elaeocarpucin B (**2**):* white powder; mp 270−272 °C; $[\alpha]^{20}_D$ +94.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.63) nm; IR (film)

Table 3. Cytotoxicity of Compounds Isolated from *E. chinensis*[a]

compound	HT-29[b]
3	0.41
4	5.6
7	2.9
8	2.7
9	0.12
10	0.039
11	0.54
12	0.19
paclitaxel[c]	0.006

[a]Compounds **1**, **2**, **5**, **6**, and **13** were not cytotoxic against HT-29 cells (IC_{50} > 10 μM), using a standard protocol.[31] [b]Results are expressed as IC_{50} values (μM). [c]Used as a positive control substance.

ν_{max} 3442, 1687, 1457, 1375, 1216, 1097, 1075, 1022, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 509.3264 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

*Elaeocarpucin C (**3**):* pale yellow, amorphous powder; $[\alpha]^{20}_D$ +91.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.87), 219 (3.48) nm; IR (film) ν_{max} 3435, 1685, 1465, 1375, 1215, 1067, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 509.3255 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

*Elaeocarpucin D (**4**):* white, amorphous powder; $[\alpha]^{20}_D$ +136.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.84) nm; IR (film) ν_{max} 3471, 1685, 1462, 1375, 1096, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 507.3073 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_5Na$, 507.3086).

*Elaeocarpucin E (**5**):* white, amorphous powder; $[\alpha]^{20}_D$ +82.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.12) nm; IR (film) ν_{max} 3458, 1703, 1688, 1460, 1213, 1376, 1072, 1021, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 507.3112 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_5Na$, 507.3086).

*Elaeocarpucin F (**6**):* white, amorphous powder; $[\alpha]^{20}_D$ +79.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.57) nm; IR (film) ν_{max} 3432, 1688, 1462, 1213, 1391, 1376, 1162, 1072, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 511.3399 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_5Na$, 511.3388).

*Elaeocarpucin G (**7**):* white, amorphous powder; $[\alpha]^{20}_D$ +55.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.50) nm; IR (film) ν_{max} 3476, 2948, 2917, 2845, 1687, 1462, 1380, 1059, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 487.3423 $[M + H]^+$ (calcd for $C_{30}H_{47}O_5$, 487.3423).

*Elaeocarpucin H (**8**):* white powder; mp 244−246 °C; $[\alpha]^{20}_D$ +68.0 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.95) nm; IR (film) ν_{max} 3429, 2968, 2925, 2855, 1695, 1647, 1458, 1377, 1026, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 485.3261 $[M + H]^+$ (calcd for $C_{30}H_{45}O_5$, 485.3267).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compound 1. Portions of compound **1** (1.0 mg of each) were added into two NMR tubes and dried under a vacuum overnight at room temperature. Deuterated pyridine (1 mL) was transferred to each tube to give a clear solution. (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride (10 μL) or (*R*)-MTPA chloride (10 μL) was injected into the NMR tubes separately under a N_2 gas steam and mixed quickly with the dissolved sample. The NMR tubes with reagents were sealed and stored overnight in a dryer until the reaction was completed, with ^1H NMR spectroscopy used to monitor the reaction. The ^1H NMR chemical shifts of the (*R*)-MTPA ester and the (*S*)-MTPA ester of **1** were recorded directly after each reaction and were assigned on the basis of COSY and NOESY experiments, with ambiguous and overlapping signals not used for the $\Delta\delta_{S-R}$ calculation.[34,35] ^1H NMR data of (*R*)-MTPA ester of **1** (400 MHz, pyridine-d_5): δ 5.923 (1H, d, *J* = 4.4 Hz, H-24), 5.547 (1H, d, *J* = 5.6 Hz, H-6), 5.255 (1H, s, H-27a), 5.139 (1H, brs, H-3), 5.069 (1H, s, H-27b), 4.752 (1H, ddd, *J* = 9.4, 9.4, 2.3 Hz, H-16), 4.512 (1H, m, H-23), 1.830 (3H, s, H-26), 2.072 (1H, d, *J* = 9.4 Hz, H-17), 1.442 (3H, s, H-21), 1.282 (3H, s, H-30), 1.258 (3H, s, H-18), 1.216 (3H, s, H-29), 1.132 (3H, s, H-19), 1.097 (3H, s, H-28). ^1H NMR data of (*S*)-MTPA ester of **1** (400 MHz, pyridine-d_5): δ 5.889 (1H, d, *J* = 2.7 Hz, H-24), 5.636 (1H, d, *J* = 5.6 Hz, H-6), 5.077 (1H, brs, H-3), 5.001 (1H, s, H-27a), 4.963 (1H, s, H-27b), 4.810 (1H, ddd, *J* = 9.4, 9.4, 2.3 Hz, H-16), 4.547 (1H, m, H-23), 2.107 (1H, d, *J* = 9.4 Hz, H-17), 1.759 (3H, s, H-26), 1.463 (3H, s, H-21), 1.327 (3H, s, H-30), 1.253 (3H, s, H-29), 1.221 (3H, s, H-18), 1.131 (3H, s, H-28), 1.123 (3H, s, H-19).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compound 2. The (*R*)-MTPA ester and the (*S*)-MTPA ester of **2** were produced by following the same Mosher reaction procedure applied to compound **1**. ^1H NMR data of (*R*)-MTPA ester of **1** (400 MHz, pyridine-d_5): δ 5.855 (1H, d, *J* = 8.2 Hz, H-24), 5.546 (1H, d, *J* = 5.2 Hz, H-6), 5.216 (1H, s, H-27a), 5.140 (1H, brs, H-3), 5.014 (1H, s, H-27b), 4.745 (1H, m, H-16), 4.467 (1H, m, H-23), 2.119 (1H, d, *J* = 9.6 Hz, H-17), 1.615 (3H, s, H-26), 1.483 (3H, s, H-21), 1.271 (3H, s, H-29), 1.265 (3H, s, H-30), 1.229 (3H, s, H-18), 1.138 (3H, s, H-19), 1.109 (3H, s, H-28). ^1H NMR data of (*S*)-MTPA ester of **1** (400 MHz, pyridine-d_5): δ 5.924 (1H, d, *J* = 8.0 Hz, H-24), 5.649 (1H, d, *J* = 4.8 Hz, H-6), 5.244 (1H, s, H-27a), 5.082 (1H, brs, H-3), 5.034 (1H, s, H-27b), 4.647 (1H, m, H-16), 4.381 (1H, m, H-23), 2.071 (1H, d, *J* = 9.7 Hz, H-17), 1.739 (3H, s, H-26), 1.458 (3H, s, H-21), 1.283 (3H, s, H-29), 1.264 (3H, s, H-30), 1.176 (3H, s, H-28), 1.161 (3H, s, H-18), 1.198 (3H, s, H-19).

Generation of Compound 3 from Compound 13. Selenium dioxide (SeO_2, 500 mg) was dissolved in 2.5 mL of distilled water, and then 12.5 mL of MeOH was added to give a clear solution. Next, 10 g of silica gel was added to this solution to form a slurry, with the solvent evaporated under reduced pressure to afford a silica gel powder containing 5% selenium dioxide.[36] A portion of this pretreated silica gel (1 g) was suspended in 7 mL of CH_2Cl_2 with 0.5 mL of *t*-BuOOH (5.0−6.0 M in decane) and stirred for 15 min at room temperature. Compound **13** (200 mg) was dissolved in a mixture of CH_2Cl_2−MeOH (4:1, 20 mL), and the solution obtained was added dropwise to the above-mentioned oxidizing reagent. The mixture was sealed and stirred overnight at room temperature, with the product analyzed by TLC (CH_2Cl_2−acetone, 5:1; R_f 0.2). After the reaction, the mixture was filtered and the residue was washed with $CHCl_3$. The filtrate was partitioned with water, and the organic phase was evaporated under reduced pressure after washing with saturated NaCl water, to give a mixture of compound **3** and unchanged compound **13**. This mixture was subjected to chromatography on an open reversed-phase C_{18} column, using a gradient of MeOH−H_2O (70:30 to 100% MeOH) for elution, to afford 32 mg of **3** and 150 mg of **13**. The yield of this reaction was around 15−20%, and the unchanged **13** could be recycled (Figure 3). When performing this selective oxidation procedure, modification of the reaction by raising the temperature used, prolonging the reaction time, or increasing the amount of oxidant did not increase the yield of the desired primary alcohol (**3**), but led to the generation of an α,β-unsaturated aldehyde derivative, as an undesired side product, which was identified as compound **5**.

Cytotoxicity Assay. Compounds **1**−**13** were evaluated against human colon cancer cells (HT-29), according to a previously described protocol.[33]

Mitochondrial Transmembrane Potential Assay. A JC-1 mitochondrial membrane potential assay kit obtained from Cayman Chemicals was used to detect the $\Delta\Psi$. Experiments were conducted according to the protocol established previously.[37,38]

In Vivo Hollow Fiber Assay. The hollow fiber assay was conducted as described previously,[30−32,39] and is summarized here. Human cancer cell lines designated HT29, MCF-7, and MDA-MB-435

were propagated in RPMI-1640 medium supplemented with fetal bovine serum (5% v/v) and 2 mM glutamine at 37 °C in a 5% CO_2 atmosphere. Monolayer cultures in late log-phase growth were released by digestion with trypsin, and suspended in medium. Sterile conditioned[30] polyvinylidene fluoride hollow fibers perforated with 500 kDa molecular weight exclusion pores were filled with the cells (HT29: 1×10^6; MCF-7: 5×10^6 and MDA-MB- 435: 1×10^6 per fiber). The fibers were then heat sealed at two-cm intervals and cut to generate the fibers used for the study. Prior to implantation, the fibers were cultured overnight at 37 °C in a 5% CO_2 atmosphere. On the following day (day zero) a set of fibers representative of each cell line was evaluated for viable cell mass by a modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[40] Another set of fibers remained in culture to confirm sterility. The remaining fibers were transplanted into immunodeficient female NCr *nu/nu* mice. For intraperitoneal (ip) implants, a small incision was made through the skin and musculature of the dorsal abdominal wall, the fiber samples were inserted into the peritoneal cavity in a craniocaudal direction, and the incision was closed with skin staples. On day three, the mice were treated with elaeocarpucin C (**3**) at 0.5, 1, 5, and 10 mg/kg in four daily ip injections on days 3, 4, 5 and 6 followed by fiber retrieval on day 7. Elaeocarpucin C (**3**) was initially dissolved in EtOH and subsequently diluted (1:1) with Tween 80. This mixture was then diluted with saline to its final injection state which consisted of only 5% EtOH. Paclitaxel was administered at 2 mg/kg in a 10% EtOH-Tween 80 (1:1) solution. The vehicle group received the 10% EtOH-Tween 80 (1:1) vehicle. On day 7 of the study, all mice were sacrificed and the fibers were retrieved and viable cell mass was evaluated by MTT assay. The percent net growth for each cell line in each treatment group was calculated by subtracting the day-zero absorbance from the day 7 absorbance and dividing this difference by the net growth in the day 7 vehicle-treated controls minus the day-zero values.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, ^{13}C, and 2D NMR spectra of compounds **1**–**8**, ^{1}H NMR of (*R*)- and (*S*)-MTPA esters of **1** and **2**, and hollow fiber assay data for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-614-247-8094. Fax: +1-614-247-8119. E-mail: kinghorn@pharmacy.ohio-state.edu.

■ ACKNOWLEDGMENTS

This study was supported by grant P01 CA125066 (awarded to A.D.K.) from NCI, NIH. *E. chinensis* samples were collected under the terms of agreement between the University of Illinois at Chicago and the Institute of Ecology and Biological Resources of the Vietnam Academy of Science and Technology, Hanoi, Vietnam. We acknowledge Mr. J. Fowble, College of Pharmacy, The Ohio State University (OSU), and Dr. C.-H. Yuan, Campus Chemical Instrument Center, OSU, for facilitating the acquisition of the 400 and 600 MHz NMR spectra. We thank Ms. N. Kleinholz, Mr. M. Apsega and Dr. K. Green-Church, Campus Chemical Instrument Center, OSU, for the mass spectrometric measurements. We are very grateful to Dr. G. M. Cragg, formerly of NCI-Frederick, for serving for several years as NCI Program Director of a forerunner of the above-cited program project, namely, grant U19 CA52956 from NCI, NIH.

■ DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

■ REFERENCES

(1) Flora of China Editorial Committee. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, 1989; Vol. *49*, p 22.
(2) Institute of Botany, the Chinese Academy of Sciences. *Iconographia Cormophytorum Sinicorum*; Science Press: Beijing, 1989; Vol. *2*, p 787.
(3) Zmarzty, S. *Kew Bull.* **2001**, *56*, 405–447.
(4) Tang, Y.; Phengklai, C. Elaeocarpaceae. In *Flora of China*; Wu, Z.; Raven, P. H.; Hong, D. Eds.; 2007; Vol *12*, pp 223–239. Available online at http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10299. Accessed on 10/27/2011.
(5) Lowry, J. B. *Phytochemistry* **1970**, *9*, 2411.
(6) Bittner, M.; Poyser, K. A.; Poyser, J. P.; Silva, M.; Weldt, E.; Sammes, P. G. *Phytochemistry* **1973**, *12*, 1427–1431.
(7) Schenkel, E. P.; Farias, M. R.; Mayer, R.; Breitmaier, E.; Rücker, G. *Phytochemistry* **1992**, *31*, 1329–1333.
(8) Achenbach, H.; Horn, K.; Dominguez, X. A.; Rombold, C.; Gómez López, E. G. *Phytochemistry* **1993**, *33*, 437–435.
(9) Fang, X.; Phoebe, C. H. Jr.; Pezzuto, J. M.; Fong, H. H. S.; Farnsworth, N. R.; Yellin, B.; Hecht, S. M. *J. Nat. Prod.* **1984**, *47*, 988–993.
(10) Ito, A.; Chai, H.-B.; Lee, D.; Kardono, L. B. S.; Riswan, S.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **2002**, *61*, 171–174.
(11) Meng, D. H.; Qiang, S. G.; Lou, L. G.; Zhao, W. M. *Planta Med.* **2008**, *74*, 1741–1744.
(12) Zhang, S.; Tao, Z.-M.; Zhang, Y.; Shen, Z.-W.; Qin, G.-W. *Chin. J. Nat. Med.* **2010**, *8*, 21–24.
(13) Chand, L.; Dasgupta, S.; Chattopadhyay, S. K.; Ray, A. B. *Planta Med.* **1977**, *32*, 197–199.
(14) Ray, A. B.; Dutta, S. C.; Dasgupta, S. *Phytochemistry* **1976**, *15*, 1797–1798.
(15) Carroll, A. R.; Arumugan, G.; Quinn, R. J.; Redburn, J.; Guymer, G.; Grimshaw, P. *J. Org. Chem.* **2005**, *70*, 1889–1892.
(16) Katavic, P. L.; Venables, D. A.; Rali, T.; Carroll, A. R. *J. Nat. Prod.* **2007**, *70*, 866–868.
(17) Katavic, P. L.; Venables, D. A.; Rali, T.; Carroll, A. R. *J. Nat. Prod.* **2007**, *70*, 872–875.
(18) Chen, J. C.; Chiu, M. H.; Nie, R. L.; Cordell, G. A.; Qiu, S. X. *Nat. Prod. Rep.* **2005**, *22*, 794–795.
(19) Rios, J. L.; Escandell, J. M.; Recio, M. C. In *Studies in Natural Product Chemistry: Bioactive Natural Products (Part L)*; Atta-ur-Rahman., Ed.; Elsevier, B.V.: Amsterdam, 2005; Vol. *32*, pp 429–469.
(20) Saba, B. A.; Oridupa, A. O. *J. Med. Plant Res.* **2010**, *4*, 2821–2826.
(21) Lee, D. H.; Iwanski, G. B.; Thoennissen, N. H. *Sci. World J.* **2010**, *10*, 413–418.
(22) Kinghorn, A. D.; Carcache-Blanco, E. J.; Chai, H.-B.; Orjala, J.; Farnsworth, N. R.; Soejarto, D. D.; Oberlies, N. H.; Wani, M. C.; Kroll, D. J.; Pearce, C. J.; Swanson, S. M.; Kramer, R. A.; Rose, W. C.; Fairchild, C. R.; Vite, G. D.; Emanuel, S.; Jarjoura, D.; Cope, F. O. *Pure Appl. Chem.* **2009**, *81*, 1051–1063.
(23) Orjala, J.; Oberlies, N. H.; Pearce, C. J.; Swanson, S. M.; Kinghorn, A. D. In *Bioactive Compounds from Natural Sources, Second Edition. Natural Products as Lead Compounds in Drug Discovery*; Tringali, C., Ed.; Taylor & Francis: London, 2012; pp 37–63.
(24) Loub, W. D.; Farnsworth, N. R.; Soejarto, D. D.; Quinn, M. L. *J. Chem. Inf. Comput. Sci.* **1985**, *25*, 99–103.
(25) Seger, C.; Sturm, S.; Haslinger, E.; Stuppner, H. *Monatsh. Chem.* **2005**, *136*, 1645–1649.
(26) Halaweish, F. T. *J. Chem. Ecol.* **1993**, *19*, 29–37.
(27) Abd El-Fattah, H. *Phytochemistry* **1994**, *36*, 159–161.

(28) Wu, P.-L.; Lin, F.-W.; Wu, T.-S.; Kuoh, C.-S.; Lee, K.-H.; Lee, S.-J. *Chem. Pharm. Bull.* **2004**, *52*, 345−349.
(29) Alley, M. C.; Pacula-Cox, C. M.; Hursey, M. L.; Rubinstein, L. R.; Boyd, M. R. *Cancer Res.* **1991**, *51*, 1247−56.
(30) Hollingshead, M. G.; Alley, M. C.; Camalier, R. F.; Abbott, B. J.; Mayo, J. G.; Malspeis, L.; Grever, M. R. *Life Sci.* **1995**, *57*, 131−141.
(31) Mi, Q.; Lantvit, D.; Reyes-Lim, E.; Chai, H.; Zhao, W.; Lee, I. S.; Peraza-Sanchez, S.; Ngassapa, O.; Kardono, L. B.; Riswan, S.; Hollingshead, M. G.; Mayo, J. G.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D.; Pezzuto, J. M. *J. Nat. Prod.* **2002**, *65*, 842−850.
(32) Mi, Q.; Pezzuto, J. M.; Farnsworth, N. R.; Wani, M. C.; Kinghorn, A. D.; Swanson, S. M. *J. Nat. Prod.* **2009**, *72*, 573−580.
(33) Pan, L.; Kardono, L. B. S.; Riswan, S.; Chai, H.-B.; Carcache de Blanco, E. J.; Pannell, C. M.; Soejarto, D. D.; McCloud, T. G.; Newman, D. J.; Kinghorn, A. D. *J. Nat. Prod.* **2010**, *73*, 1873−1878.
(34) Rieser, M. J.; Hui, Y. H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203−10213.
(35) Su, B.-N.; Park, E. J.; Mbwambo, Z. H.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 1278−1282.
(36) Chhabra, B. R.; Hayano, K.; Ohtsuka, T.; Shirahama, H.; Matsumoto, T. *Chem. Lett.* **1981**, *10*, 1703−1706.
(37) Deng, Y.; Balunas, M. J.; Kim, J.-A.; Lantvit, D. D.; Chin, Y.-W.; Chai, H.; Sugiarso, S.; Kardono, L. B. S.; Fong, H. H. S.; Pezzuto, J. M.; Swanson, S. M.; Carcache de Blanco, E. J.; Kinghorn, A. D. *J. Nat. Prod.* **2009**, *72*, 1165−1169.
(38) Pan, L.; Matthew, S.; Lantvit, D. D.; Zhang, X.; Ninh, T. N.; Chai, H.-B.; Carcache de Blanco, E. J.; Soejarto, D. D.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2011**, *74*, 2193−2199.
(39) Pan, L.; Lantvit, D. D.; Riswan, S.; Kardono, L. B. S.; Chai, H.-B.; Carcache de Blanco, E. J.; Farnsworth, N. R.; Soejarto, D. D.; Swanson, S. M.; Kinghorn, A. D. *Phytochemistry* **2010**, *71*, 635−640.
(40) Alley, M. C.; Pacula-Cox, C. M.; Hursey, M. L.; Rubinstein, L. R.; Boyd, M. R. *Cancer Res.* **1991**, *51*, 1247−1256.