Cytotoxic Constituents from the Stem Bark of *Dichapetalum gelonioides* Collected in the Philippines[⊥,1]

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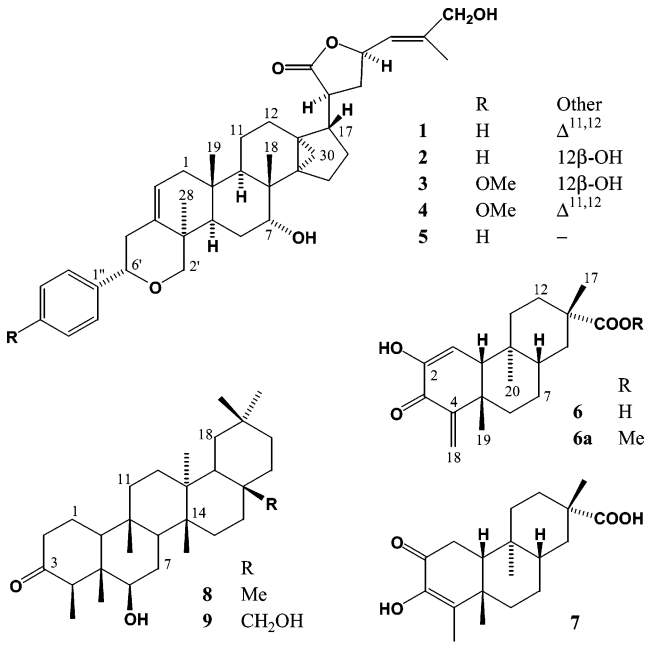
Received July 15, 2005

Fractionation of an ethyl acetate-soluble extract of the stem bark of *Dichapetalum gelonioides*, collected in the Philippines, using the LNCaP (hormone-dependent human prostate) cell line as a monitor, led to the purification of three dichapetalin-type triterpenoids [dichapetalins A (**1**), I (**2**), and J (**3**)], along with two dolabrane norditerpenoids (**6**, **7**), and the additional triterpenoids zeylanol (**8**), 28-hydroxyzeylanol (**9**), and betulinic acid. Since compounds **1**−**3** exhibited promising selectivity against the SW626 (human ovarian cancer) cell line, a re-collection of the plant material was carried out, to obtain more of these compounds for additional biological testing. Two further phenylpyranotriterpenoids [dichapetalins K (**4**) and L (**5**)] were isolated from the re-collected plant material. The structures of the new compounds **2**−**5** and **9** were determined on the basis of spectroscopic data interpretation, and the relative configuration of **6** was confirmed using X-ray crystallography. Compounds **4**−**6** and the methyl ester, **6a**, exhibited broad cytotoxic activity when tested against a panel of human tumor cell lines. Dichapetalin A (**1**) was not active when evaluated in an in vivo hollow fiber assay in the dose range 1−6 mg/kg.

Dichapetalum Thouars represents the major genus in the family Dichapetalaceae (syn. Chailletiaceae) and comprises 124 species, of which the majority (86 species) are found in Africa, with the others occurring in tropical Asia (19 species) and South America (19 species).[2] Many *Dichapetalum* species are poisonous to livestock due to the presence of fluorinated compounds such as fluoroacetic acids and ω-fluorinated fatty acids.[3−6] The leaves of *D. gelonioides* have been used to treat amenorrhea in the Philippines.[7] Several phenylpyranotriterpenoids (dichapetalins A−H) have been isolated and structurally characterized from the roots of *D. madagascariense* Poir.[8−10] The absolute configuration of dichapetalin A was determined by single-crystal X-ray diffraction analysis.[9] While this compound was reported to show potent activity in the brine shrimp lethality bioassay, exceeding that of podophyllotoxin by 7-fold, and also exhibited cytotoxicity for L1210 murine leukemia cells (EC_{90} < 0.0001 μg/mL), the KB cell line and an unspecified murine bone marrow cell line were not sensitive to this triterpenoid.[8]

As a part of our program on the discovery of new anticancer agents from plants, an ethyl acetate-soluble extract has been investigated of the stem bark of *D. gelonioides* (Roxb.) Engl., collected in 1994 on Palawan Island in the Philippines. Only one phytochemical investigation has been reported previously on this species, resulting in the isolation of the triterpenoid friedelin.[11] Activity-guided fractionation using the LNCaP (hormone-dependent human prostate cancer) cell line led to the isolation of two new dichapetalin-type triterpenoids [dichapetalins I (**2**) and J (**3**)] and the known compound, dichapetalin A (**1**), along with two dolabrane norditerpenoids (**6**, **7**) and zeylanol (**8**), 28-hydroxyzeylanol (**9**), and betulinic acid. Despite the above-mentioned strong cytotoxicity of dichapetalin A for L1210 cells, highly functionalized triterpenoids of this class have not been subjected to evaluation against a human cancer cell panel previously. Thus, it was found in our work that dichapetalins A, I, and J all exhibited promising selective activity in a human tumor panel against the SW626 (human ovarian adenocarcinoma) cell line. Therefore, these compounds were prioritized for a more detailed biological evaluation of their antitumor potential.



Since all supplies of dichapetalins A, I, and J (**1**−**3**), along with the plant material of origin, were exhausted in the initial phy-

⊥ Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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10.1021/np058083q CCC: $33.50
Published on Web 12/17/2005

tochemical and biological work, it was decided to obtain a re-collection of the stem bark of *D. gelonioides* in order to perform follow-up biological testing on one or more of these compounds. However, after the initial collection of the plant in January 1994, bioprospecting in the Philippines has been restricted under Executive Order 247 (EO247), effective from May 18, 1995.[12,13] To comply with these new regulations, a four-party Memorandum of Agreement for this specific plant re-collection had to be signed (see Experimental Section). Further to this agreement, 12 kg of the stem bark of *D. gelonioides* was collected in Palawan Island in August 2003, and an ethyl acetate extract was prepared at the University of the Philippines, Manila, and shipped to the United States for compound purification work. Altogether, 47 mg of dichapetalin A (**1**) was isolated from the re-collected plant material. However, dichapetalins I (**2**) and J (**3**) were absent from the re-collection, although two additional analogues were purified, namely, dichapetalins K (**4**) and L (**5**). In the present contribution, we describe the isolation, characterization, and biological testing of the constituents of *D. gelonioides* stem bark in a human tumor panel, as well as the evaluation of the known phenylpyranotriterpenoid, dichapetalin A (**1**), in an in vivo hollow fiber test.[14]

Results and Discussion

Compound **1** was identified as dichapetalin A, a substance of previously established structure and absolute configuration, by comparing its spectroscopic data to values reported in the literature.[8]

The molecular formula of **2** was confirmed by HREIMS ($[M]^+$ at *m*/*z* 602.3607) as $C_{38}H_{50}O_6$. Prominent IR absorption bands at 3454 and 1753 cm^{-1} indicated the presence of one or more hydroxy function(s) and a five-membered lactone ring. The ^1H NMR spectroscopic data of **2** demonstrated an unsubstituted phenyl ring (δ_H 7.27−7.38, m, 5H), two double bonds (δ_H 5.51, 5.38), four methyl groups (δ_H 1.06, 1.18, 1.31, 1.74), and a cyclopropyl methylene (δ_H 0.43, 0.76). Also, there were observed four oxymethine signals (δ_H 5.14, 4.26, 4.31, 3.84) and two oxymethylene groups [δ_H 4.05 (2H), 3.57, 3.77]. The ^{13}C NMR spectrum of **2** showed 35 signals, which supported the presence of a phenyl ring, a cyclopropyl ring, two double bonds, and all the other structural elements mentioned above. The assignment of the two overlapping symmetric carbons of the phenyl ring at δ_C 125.8 and 128.3 and two methylene signals at δ_C 25.7 were clarified from their HMQC NMR spectrum. These data and other 2D NMR experiments conducted (COSY, HMBC) revealed a phenylpyranotriterpenoid skeleton with a γ-lactone side chain. Compared to **1**, **2** was found to lack a $\Delta^{11,12}$-double bond, but could be assigned one more secondary hydroxyl group. The position of this hydroxy group was determined at C-12 because of the long-range heteronuclear coupling of H-12 ($J = 5$ Hz) in a selective INEPT NMR experiment. The configuration of the hydroxy group at C-12 was defined as β on the basis of a ROESY NMR experiment, which demonstrated a cross-peak between H-12 and H-30. Therefore, the structure of **2** was assigned as (4α,6′α,7α,12β,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-tetrahydro-7,12,23,26-tetrahydroxy-6′-phenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone, and has been named dichapetalin I, according to a previous convention.[8]

The protonated molecular ion ($[M + H]^+$) of a second new dichapetalin-type triterpenoid (**3**) was indicated by CIMS at *m*/*z* 633 and confirmed by both positive- and negative-FABMS, consistent with an elemental formula of $C_{39}H_{52}O_7$. Attempts to obtain high-resolution mass spectral data for this compound were unsuccessful. The IR spectrum displayed hydroxy (3391 cm^{-1}) and a γ-lactone (1746 cm^{-1}) absorption bands. The ^1H NMR spectrum of **3** exhibited a pattern very similar to that of **2**, except in the aromatic region as a result of the presence of a methoxy singlet at δ_H 3.79. The phenyl protons appeared as a pair of doublets (δ_H 6.87, d, $J = 8.6$ Hz; 7.29, d, $J = 8.6$ Hz) integrating for four protons, a typical pattern for a *para*-substituted aromatic ring. The ^{13}C NMR spectrum of **3** exhibited one more resonance at δ_C 55.3 compared to **2**. Several 2D NMR experiments (COSY, HMQC, HMBC) were also performed on **3** to permit the unambiguous interpretation of its ^1H and ^{13}C NMR spectra. Compound **3** (dichapetalin J) was assigned the structure (4α,6′α,7α,12β,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-tetrahydro-7,12,23,26-tetrahydroxy-6′-*p*-methoxyphenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone.

Compound **4** exhibited a molecular formula of $C_{39}H_{50}O_6$ from its HREIMS. The ^1H NMR spectrum of **4** was very similar to that of **1**, except for the presence of signals consistent with a methoxy group at δ_H 3.80 (3H, s) at C-4″. In the ^{13}C NMR spectrum of **4**, when compared with that of **1**, a methoxyl carbon at δ_C 55.3 was observed, associated with C-4″. Thus, a HMBC correlation between the methoxy methyl proton signal and C-4″ was apparent. Other COSY and HMBC correlations were consistent with the structure proposed. Thus, compound **4** was determined as (4α,6′α,7α,17α,-20*S*,23*R*,24*E*)-2′,3′,5′,6′-tetrahydro-7,23,26-trihydroxy-6′-*p*-methoxyphenyl-13,30-cyclo-29-nordammara-2,11,24-trieno[4,3-*c*]pyran-21-oic acid γ-lactone (dichapetalin K).

The spectroscopic data of compound **5** were similar to those of **2**. Compound **5** exhibited a molecular formula of $C_{38}H_{50}O_5$ from its HREIMS, suggesting the loss of a hydroxy group. In the ^{13}C NMR spectrum of **5**, a methylene carbon (δ_C 25.0) was observed instead of an oxygen-bearing methine carbon (δ_C 66.3) at C-12, indicating a loss of the hydroxy group at C-12. Also upfield shifts were observed at C-11 and C-13 (11.6 and 7.4 ppm, respectively). The HMBC spectrum showed cross-peaks between H-11 and C-9 and C-12, between H-12 and C-11 and C-13, between H-30 and C-13, and between H-18 and C-7, C-9, and C-14. Other correlations in the HMBC and COSY spectra supported the structure proposed and the NMR spectroscopic assignments made. Therefore, compound **5** was characterized as (4α,6′α,7α,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-tetrahydro-7,23,26-trihydroxy-6′-phenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone (dichapetalin L).

Thus, it was readily apparent that compounds **3** and **4** represent methoxylated variants of dichapetalins I (**2**) and A (**1**), respectively. While it is possible that these are extraction artifacts due to the initial use of methanol as a solvent, samples of compounds **1** and **2** were not available to attempt chemical interconversion experiments by storage of these substances in methanol, since their supplies were consumed in the biological testing aspects of this study.

Compound **6** was isolated and identified as a known dolabrane norditerpenoid,[15−17] possessing a diosphenol system in the A ring, which has been found in both diterpenes[15,17,18] and triterpenes.[19] This isolate from *D. gelonioides* exhibited the same ^1H and ^{13}C NMR and MS data as "*ent*-16-nor-5α,3-oxodolabr-1,4(18)-dien-2-ol-15-oic acid", isolated from *Endospermum diadenum* Airy Shaw (Euphorbiaceae).[15] However, no optical rotation was published for the *E. diadenum* isolate, making it unclear whether **6** and this known compound are the same or are enantiomeric.[20] The "dolabrane" (8,-14-*freido*-pimarane) diterpenoid nomenclature was first proposed by Ansell et al. in 1993[21] and is based on dolabradiene, a diterpenoid isolated from *Thujopsis dolabrata* Sieb. et Zucc.[22] In earlier literature, the same type of skeleton was named "dolabradane".[23] Dolabradiene and a related compound, erythroxydiol Y, have the same carbon skeleton and the same configuration at C-13, but are enantiomeric at C-5, C-8, C-9, and C-10.[24] They have opposite specific rotations, with dolatradiene being negative (−70) and erythoxydiol Y positive (+87).[22,25] Fagonone, an analogue whose structure was proved by X-ray crystallography, has the same skeleton as erythroxydiol Y, but with an A/B *cis* junction. The structure of fagonone was proposed by comparison of its CD spectrum to 7-oxosteroids with the same absolute configuration at C-5 and C-10, and it was shown to exhibit a positive optical rotation (+26).[26] Therefore, because of its observed positive optical rotation

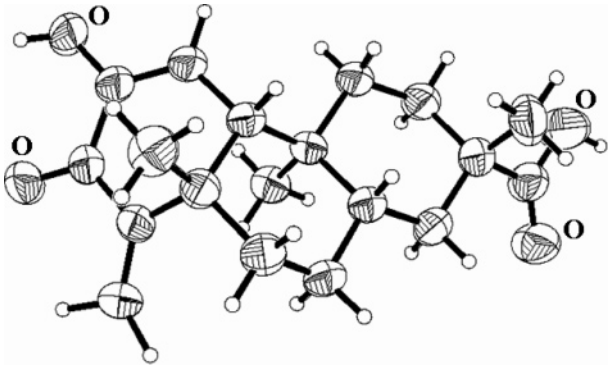

Figure 1. ORTEP drawing of **6** with thermal ellipsoids at 50% probability for non-H atoms and open circles for H atoms.

($[\alpha]_D$ +158; *c* 0.46, MeOH), it was concluded that **6** is the same compound as "*ent*-16-nor-5α,3-oxodolabr-1,4(18)-dien-2-ol-15-oic acid" from *E. diadenum*.[15] On methylation using diazomethane, **6** afforded a monomethyl ester (**6a**). The structure and relative stereochemistry of **6** were confirmed in the present investigation by single-crystal X-ray diffraction. The two carbon−carbon double bonds are 1.340 Å ($\Delta^{1,2}$) and 1.318 Å ($\Delta^{4,18}$), and rings B and C adopt a normal chair conformation while the A ring is a half boat (Figure 1). It appears that many of the semisystematic names of dolabrane diterpenoids are inconsistent in the literature, since they do not specify the configuration at the C-13 position. Therefore, **6** was identified as *ent*-16-nor-5α,13α(methyl)-3-oxodolabra-1,4(18)-dien-2-ol-15-oic acid.

Since it also exhibited a positive optical rotation ($[\alpha]_D$ +47.4; *c* 0.29, $CHCl_3$), compound **7** was identified as another known constituent of *E. diadenum*, namely, "*ent*-16-nor-5α,2-oxodolabr-3-en-3-ol-15-oic acid",[15] which was also published without any optical rotation. However, the semisystematic name of this compound should be changed to *ent*-16-nor-5α,13α(methyl)-2-oxodolabra-3-en-3-ol-15-oic acid.

Zeylanol (**8**) was identified by comparing its spectroscopic data to values reported in the literature,[27,28] but its ^{13}C NMR data were assigned for the first time in this investigation.

The molecular formula of compound **9** was indicated as $C_{30}H_{50}O_3$ by CIMS, with a $[M + H]^+$ peak occurring at *m/z* 459. This was supported by HREIMS on the basis of the $[M - CH_2OH]^+$ peak at *m/z* 427 ($C_{29}H_{47}O_2$). The IR spectrum suggested the presence of hydroxyl group(s) (br 3453 cm^{-1}) and a cyclic ketone group (1715 cm^{-1}) similar to those of **8**. The presence of primary and secondary hydroxyl groups was suggested from the ^1H, ^{13}C, and DEPT NMR spectra of **9**. Although the ^1H NMR signals of the H-6 and the CH_2-28 overlapped at δ_H 3.87−3.90, they could be correlated to the two hydroxyl proton signals (δ_H 5.64, d, J = 5.3 Hz; 5.99, br s) in the COSY NMR spectrum. The secondary hydroxyl group was assigned as C-6β, because the ^1H NMR data were in good agreement with the analogous data of **8**. Confirmation was obtained from a ROESY NMR experiment, which showed spatial interaction between OH-6 and Me-24, OH-6 and Me-23, and H-4 and H-6. The primary hydroxyl group was assigned to C-28 because of the downfield shift of the C-17 resonance (+5.8 ppm) and the upfield shifts of the C-16 (−6.2 ppm), C-18 (−3.3 ppm), and C-22 (−7.0 ppm) resonances compared to those of **8**. The presence of a C-28 hydroxymethyl group was confirmed by comparing the data to those published for model compounds[29] and by a HMBC NMR experiment, indicating long-range correlations between C-28 and H-15 and between C-28 and H-16. Compound **9** was therefore assigned as 6β,28-dihydroxyfriedelan-3-one.

Betulinic acid was identified by direct comparison to the standard compound[30] and by comparing its spectroscopic data (MS, ^1H NMR, ^{13}C NMR, UV, IR) to published values.[31−33]

All compounds isolated (**1**−**9** and betulinic acid) and the methylated derivative of **6** (**6a**) were screened for cytotoxicity against a panel of human cancer cell lines according to established protocols.[34,35] The dolabrane diterpenoid **6** and its methyl ester **6a** were broadly cytotoxic against all cell lines tested. The cytotoxic potency of **6** was increased by methylation, with the methyl ester **6a** being 2- to 11-fold more active than the parent compound. In contrast, the second dolabrane diterpenoid isolated (**7**), with a modified A ring when compared to **6**, was not significantly active in any of the cell lines tested. Dolabrane diterpenoids have been found to inhibit mouse ear edema induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and to inhibit mouse paw edema induced by carrageenan.[36] They also inhibited myeloperoxidase activity, an index of leukocyte migration, suggesting a mechanism of blocking the recruitment of neutrophils into inflammatory lesions.[36] Cytotoxicity against cancer cell lines with this type of compound is reported here for the first time. The triterpenoids dichapetalins A (**1**), I (**2**), and J (**3**) showed selective and significant cytotoxicity (IC_{50}: 0.2−0.5 μg/mL) against the SW626 human ovarian cancer cell line, while dichapetalins K (**4**) and L (**5**) showed broader cytotoxicity against the cell lines tested. Compounds **8** and **9** did not show any cytotoxicity for any of the cancer cell lines tested. Betulinic acid was reported to be a melanoma-specific cytotoxic agent in cell culture against the MEL-1 (ED_{50}: 1.1 μg/mL) and MEL-2 (ED_{50}: 2.0 μg/mL) cell lines.[30,37] In a series of studies performed with athymic mice injected subcutaneously with MEL-1 and MEL-2 cells, betulinic acid completely inhibited tumor development and growth in vivo.[30] In the current study, this isolate did not exhibit any cytotoxicity against the cancer cell lines tested, owing to the omission of a melanoma cell line from the panel.

Dichapetalin A (**1**) was evaluated in the in vivo hollow fiber model (i.p. and s.c.),[14] at doses of 1, 2, 4, and 6 mg/kg. However, no significant growth inhibition was observed for any of the LNCaP (hormone-dependent prostate), Lu1 (lung), MCF-7 (breast adenocarcinoma), or SW626 (ovarian adenocarcinoma) human cell types used (data not shown). As a result of these negative data, dichapetalin A (**1**) and its phenylpyranotriterpenoid analogues do not seem to be very promising for their potential anticancer activity. Accordingly, dichapetalin A (**1**) will not be subjected to any more advanced biological testing through our plant anticancer drug discovery program.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were taken on a Midac Collegian FT-IR spectrometer, an ATI Mattson Genesis Series FT-IR spectrometer, and a JASCO FT/IR-410 spectrophotometer. ^1H and ^{13}C NMR data (including DEPT, HMQC, HMBC, NOESY, and $^1H-^1H$ COSY spectra) were measured on a Bruker DRX-500 (500 MHz) instrument, a Bruker DRX-360 (360 MHz) instrument, a Bruker DRX-300 (300 MHz) instrument, or a Nicolet-360 (360 MHz) instrument, using tetramethylsilane (TMS) as internal standard. ^{13}C NMR multiplicity was determined using DEPT experiments. Low- and high-resolution mass spectra were recorded on a Finnigan MAT-90 spectrometer or a JEOL GC Mate II instrument.

Plant Material. The stem bark of *Dichapetalum gelonioides* Engl. was collected in a tropical rainforest at Irawan, in the Municipality of Puerto Princesa, Palawan Province, Philippines, in January 1994. A second collection of the stem bark of *D. gelonioides* was made at the same Irawan forest, in August 2003. The first collection in 1994 was made as part of the performance of a U.S. National Cancer Institute (NCI) Plant Collection Contract to the University of Illinois at Chicago (UIC), under a Letter of Collection between the NCI and the Philippine National Museum, and the sample was transferred to UIC under a Materials Transfer Agreement dated May 25, 1999. The second 2003 collection was made under a Memorandum of Agreement further to an agreement between the Philippine National Herbarium of the National Museum, Manila, Philippines; the University of the Philippines Manila Development Foundation, Inc., Manila, Philippines; the Palawan

Council for Sustainable Development, Puerto Princesa, Palawan, Philippines; and the Board of Trustees of the University of Illinois at Chicago. Voucher specimens (A2175 and AA2175) have been deposited at the Philippine National Herbarium of the National Museum, Manila, Philippines, and the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The air-dried, ground stem bark of *D. gelonioides* (2.35 kg, initial collection) was extracted with MeOH twice at room temperature. The combined MeOH extract was concentrated under reduced pressure and diluted with H_2O to afford a MeOH−H_2O (9:1) extract, which was then defatted by hexane twice. The MeOH layer was combined, concentrated, and diluted with H_2O, and then partitioned between 10% aqueous MeOH solution and EtOAc. The EtOAc-soluble extract (23.7 g) was chromatographed over a large silica gel column into 13 pooled fractions (F001−F013) by elution with mixtures of $CHCl_3$−MeOH of increasing polarity. The cytotoxic activity toward the LNCaP cell line was associated with fractions F002, F004, and F006−F009 (IC_{50}: 1.3−5.4 μg/mL). Fraction F004 (1.66 g, IC_{50} 2.0 μg/mL), eluted from the initial column with $CHCl_3$−MeOH (98:2), was further purified by passage over a silica gel column using a gradient of $CHCl_3$−EtOAc (1:4), into eight subfractions. Combined subfractions 4−8 were finally purified using a RP-18 reversed-phase silica gel column, by elution with MeOH−H_2O (3:2, 4:1), to afford compound **6** (60 mg). Fraction F006 (1.73 g, IC_{50} 1.7 μg/mL), eluted from the initial column with $CHCl_3$−MeOH (97:3), was further fractionated into 13 subfractions (F020−F032) by normal-phase column chromatography (CC) over silica gel, eluting with a gradient of hexane−acetone. F023 (342 mg), eluted with hexane−acetone (3:1), was purified by RP-18 reversed-phase silica gel CC using MeOH−H_2O (3:2) as eluent to afford compound **7** (5.4 mg). Compound **9** (9.5 mg) was obtained as white crystals from F025, eluted with hexane−acetone (2:1), after washing with MeOH, and was purified by recrystallization in MeOH. Compounds **2** (13 mg) and **3** (5.0 mg) were isolated from F008 (1.5 g, IC_{50} 2.2 μg/mL), eluted from the initial column with $CHCl_3$−MeOH (97:3). Fraction F008 was divided into 16 subfractions (F049−F064) by normal-phase silica gel CC using gradient mixtures of $CHCl_3$−acetone as eluents. Combined subfractions F053−F055 (524 mg) were applied to reversed-phase RP-18 silica gel CC eluted with MeOH−H_2O mixtures (starting from 50% MeOH to 100% MeOH). Altogether 76 subfractions were obtained, each 10 mL in volume. Subfractions 50−56 (47 mg) were found to contain a mixture of compounds **2** and **3**. These two compounds were separated successfully via normal-phase CC over silica gel eluted with mixtures of hexane−EtOAc−MeOH (1:1:0.001 to 1:1:0.005). Fraction F007 (1.64 g, IC_{50} 3.2 μg/mL), eluted from the initial column with $CHCl_3$−MeOH (97:3), was purified into seven subfractions (F042−F048) by normal-phase silica gel CC using a gradient hexane−acetone mixture as eluent. F046 (129 mg), eluted with hexane−acetone (3:1), was then separated into 21 subfractions by normal-phase silica gel CC eluted with gradient $CHCl_3$−acetone mixtures of increasing polarity, and compound **1** (10 mg) was afforded after silica gel CC separation of combined subfractions 19−20 eluted with hexane−EtOAc (3:1 to 1:1). Finally, compound **8** and betulinic acid were isolated from F002 (1.18 g, IC_{50} 1.3 μg/mL), eluted from the initial column with $CHCl_3$−MeOH (98:2). Normal-phase CC over silica gel eluted with gradient $CHCl_3$−EtOAc solvent mixtures of increasing polarity permitted the separation of F002 into six subfractions (F014−F019). Purification by silica gel CC of F016 (85 mg), obtained initially with $CHCl_3$−EtOAc (3:1), afforded compound **8** (11 mg) by elution with hexane−acetone (95:5). Fraction F018 (531 mg), eluted with $CHCl_3$−EtOAc (3:2), was purified over silica gel by CC to afford betulinic acid (75 mg) by elution with hexane−acetone (85:15).

The re-collected dried stem bark of *D. gelonioides* (12 kg) was extracted four times with MeOH at room temperature. The resultant extracts were combined, concentrated under reduced pressure, and diluted with H_2O to afford a 75% aqueous MeOH extract, and washed with hexane. The lower layer was concentrated to dryness under reduced pressure and partitioned between 5% MeOH−H_2O and EtOAc. The EtOAc-soluble extract [42 g, ED_{50} 3.4 μg/mL against the Lu1 cell line (human lung cancer cell line)] was subjected to silica gel CC and eluted with a gradient mixture of $CHCl_3$−MeOH to give 12 pooled fractions. Additional chromatographic separation of fraction 6 over silica gel using gradient mixtures of $CHCl_3$−acetone as eluents yielded seven subfractions (fractions 6A−6G). Further purification of subfraction 6C, using HPLC with an ODS-AQ column (250 × 20 mm; YMC, Inc., Willington, NC) using 83% CH_3CN−H_2O as eluant at a flow rate of 6 mL/min, afforded compounds **1** (47 mg, t_R 29.1 min) and **5** (19 mg, t_R 42.4 min). Compound **4** (21 mg) was obtained from subfraction 6E using HPLC with 77% CH_3CN−H_2O (t_R 29.9 min).

Table 1. ^{13}C NMR Spectroscopic Data of Dichapetalins I−L (**2**−**5**) ($CHCl_3$, 90 MHz)

C	2	3	4	5
1	40.0 t	40.0 t	40.6 t	40.2 t
2	118.0 d	117.9 d	117.7 d	118.0 d
3	139.5 s	139.5 s	140.1 s	139.6 s
4	38.3 s	38.2 s	38.2 s	38.3 s
5	43.9 d	43.8 d	43.7 d	43.7 d
6	24.0 t	24.0 t	24.1 t	23.8 t
7	73.8 d	73.8 d	72.3 d	73.9 d
8	38.8 s	38.8 s	36.4 s	38.4 s
9	41.2 d	41.2 d	45.7 d	42.6 d
10	36.5 s	36.5 s	36.2 s	36.7 s
11	27.8 t	27.7 t	124.1 d	16.2 t
12	66.3 d	66.2 d	128.9 d	25.0 t
13	34.0 s	34.0 s	30.0 s	26.6 s
14	38.5 s	38.4 s	35.1 s	34.0 s
15	25.7 t	25.7 t	24.9 t	26.2 t
16	25.7 t	25.8 t	22.7 t	21.1 t
17	39.9 d	39.9 d	40.9 d	44.5 d
18	19.4 q	19.4 q	17.4 q	19.6 q
19	16.5 q	16.5 q	18.2 q	16.7 q
20	44.8 d	44.9 d	42.1 d	41.5 d
21	180.2 s	180.2 s	178.3 s	178.7 s
22	35.0 t	35.1 t	31.3 t	30.8 t
23	75.5 d	75.5 d	75.1 d	75.1 d
24	121.5 d	121.4 d	121.9 d	122.0 d
25	142.3 s	142.3 s	141.8 s	141.7 s
26	67.1 t	67.1 t	67.1 t	67.1 t
27	14.1 q	14.1 q	14.2 q	14.2 q
28	23.8 q	23.8 q	23.8 q	23.8 q
30	11.4 t	11.4 t	15.0 t	14.0 t
2′	72.6 t	72.5 t	72.5 t	72.6 t
5′	40.7 t	40.6 t	40.1 t	40.7 t
6′	81.8 d	81.4 d	81.5 d	81.8 d
1″	142.6 s	134.8 s	134.8 s	142.6 s
2″,6″	125.8 d	127.0 d	127.1 d	125.8 d
3″,5″	128.3 d	113.7 d	113.7 d	128.4 d
4″	127.5 d	158.9 s	159.0 s	127.5 d
OMe		55.3 q	55.3 q	

Dichapetalin A, (4α,6′α,7α,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-Tetrahydro-7,23,26-trihydroxy-6′-phenyl-13,30-cyclo-29-nordammara-2,11,-24-trieno[4,3-*c*]pyran-21-oic acid γ-lactone (1): crystals; mp 213−214 °C; $[\alpha]_D^{20}$ +31.5 (*c* 1.0, $CHCl_3$); lit.[8] colorless crystals, mp 211−214 °C, $[\alpha]_D^{20}$ +35 (*c* 1.5, $CHCl_3$); exhibited spectroscopic (UV, IR, ^1H NMR, ^{13}C NMR) data comparable to published values.[8] CIMS and 2D-NMR experiments (COSY, HETCOR, NOESY) were performed to confirm the structural identity of **1**.

Dichapetalin I, (4α,6′α,7α,12β,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-Tetrahydro-7,12,23,26-tetrahydroxy-6′-phenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone (2): crystals; mp 192−193 °C; $[\alpha]_D^{20}$ +13.1 (*c* 0.11, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 239 (2.95), 282 (2.90) nm; IR (dry film) ν_{max} 3454, 3017, 2926, 2854, 1753, 1585, 1216, 758 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (2H, m, H-2″, H-6″), 7.34 (2H, m, H-3″, H-5″), 7.27 (1H, m, H-4″), 5.51 (1H, dd, *J* = 8.5, 1.4 Hz, H-24), 5.38 (1H, d, *J* = 6.9 Hz, H-2), 5.14 (1H, m, H-23), 4.31 (1H, m, H-12), 4.26 (1H, dd, *J* = 11.6, 2.5 Hz, H-6′), 4.05 (2H, s, H-26), 3.84 (1H, br s, H-7), 3.77 (1H, d, *J* = 10.7 Hz, H-2′), 3.57 (1H, d, *J* = 10.7 Hz, H-2′), 2.85−2.86 (2H, overlapped, H-17, H-20), 2.39 (1H, m, H-22), 2.18 (2H, dd, *J* = 13.4, 2.5 Hz, H-5′), 1.95−2.00 (4H, overlapped, H-1, H-5, H-11, H-15), 1.78−1.83 (2H, overlapped, H-6, H-22), 1.74 (3H, d, *J* = 0.8 Hz, H-27), 1.58−1.64 (3H, overlapped, H-1, 6, 15), 1.41 (2H overlapped, H-9, H-11), 1.31 (3H, s, H-28), 1.18 (3H, s, H-18), 1.06 (3H, s, H-19), 0.98 (2H, m, H-16), 0.76 (1H, d, *J* = 5.4 Hz, H-30), 0.43 (1H, d, *J* = 5.4 Hz, H-30); ^{13}C NMR (90 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* $[M]^+$ 602 (22), 447 (61), 260 (50), 131 (63), 105 (100); HREIMS *m*/*z* $[M]^+$ 602.3607 (calcd for $C_{38}H_{50}O_6$, 602.3600).

Dichapetalin J, (4α,6′α,7α,12β,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-Tetrahydro-7,12,23,26-tetrahydroxy-6′-*p*-methoxyphenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone (3): crystals; mp 210−212 °C; $[\alpha]_D^{20}$ +14.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 240 (3.49), 275 (3.29), 281 (sh, 3.24) nm; IR (dry film) ν_{max} 3391, 2921, 2847, 1746, 1613, 1514, 1035 cm^{-1}; ^1H NMR (300

Table 2. Cytotoxic Activity of Compounds Obtained from the Stem Bark of *Dichapetalum gelonioides*[a−c]

compound	BC1	Lu1	Col2	KB	KB-V$^+$	KB-V$^-$	LNCaP	SW626	SKNSH	MCF-7	HUVEC
1	>20	4.1	6.1	3.6	11.6	>20	7.0	0.2	6.9	2.1	5.5
2	>20	7.6	4.1	16.6	>20	>20	>20	0.5	9.9		
3	>20	14.5	2.1	16.4	>20	>20	16.4	0.4	4.0		
4		1.0					2.6	3.0		1.0	2.2
5		1.0					1.1	8.3		2.1	4.2
6	4.0	3.8	2.7	3.7	4.3	5.0	3.7	5.0	9.0		
6a	0.6	1.6	0.8	0.7	1.7	1.8	0.9	1.6	0.8		
7	12.2	>20	11.2	16.2	>20	>20	10.8	15.8	10.4		
8	>20	10.9	>20	>20	>20	>20	ND	>20	16.8		
9	>20	>20	>20	>20	>20	>20	>20	>20	13.0		

[a] Results are expressed as ED_{50} values (μg/mL). [b] Key to cell lines used: BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human oral epidermoid carcinoma; KB-V$^+$ = drug-resistant KB (human oral epidermoid carcinoma) in the presence of vinblastine; KB-V$^-$ = drug-resistant KB (human oral epidermoid carcinoma) in the absence of vinblastine; LNCaP = hormone-dependent human prostate cancer; SW626 = human ovarian adenocarcinoma; SKNSH = human neuroblastoma; MCF-7 = breast adenocarcinoma; HUVEC = human umbilical vein endothelial cells. [c] Betulinic acid was inactive in all cell lines (ED_{50} > 20 μg/mL).

MHz, $CDCl_3$) δ 7.29 (2H, d, J = 8.6 Hz, H-2″, H-6″), 6.87 (2H, m, H-3″, H-5″), 5.51 (1H, dd, J = 7.9, 1.4 Hz, H-24), 5.37 (1H, d, J = 6.8 Hz, H-2), 5.13 (1H, m, H-23), 4.31 (1H, m, H-12), 4.20 (1H, dd, J = 11.6, 2.5 Hz, H-6′), 4.06 (2H, s, H-26), 3.81 (1H, br s, H-7), 3.79 (3H, s, OMe-4″), 3.76 (1H, d, J = 10.7 Hz, H-2′), 3.55 (1H, d, J = 10.7 Hz, H-2′), 2.84 (2H, overlapped, H-17, H-20), 2.40 (1H, m, H-22), 2.15 (2H, dd, J = 13.4, 2.5 Hz, H-5′), 1.96−2.00 (4H, overlapped, H-1, H-5, H-11, H-15), 1.78−1.83 (2H, overlapped, H-6, H-22), 1.74 (3H, s, H-27), 1.55−1.64 (3H, overlapped, H-1, H-6, H-15), 1.41 (2H overlapped, H-9, H-11), 1.31 (3H, s, H-28), 1.18 (3H, s, H-18), 1.05 (3H, s, H-19), 0.98 (2H, m, H-16), 0.76 (1H, d, J = 5.3 Hz, H-30), 0.43 (1H, d, J = 5.3 Hz, H-30); ^{13}C NMR (90 MHz, $CDCl_3$), see Table 1; EIMS [M] 448 (13), 330 (37), 315 (85), 285 (79), 117 (100); CIMS m/z [M + H]$^+$ 633 (1); FABMS m/z [M − H]$^-$ 631 (51), [M + H]$^+$ 633 (1).

Dichapetalin K, (4α,6′α,7α,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-Tetrahydro-7,23,26-trihydroxy-6′-*p*-methoxyphenyl-13,30-cyclo-29-nordammara-2,11,24-trieno[4,3-*c*]pyran-21-oic acid γ-lactone (4): crystals; mp 116−118 °C; $[\alpha]_D^{20}$ +34.1 (*c* 0.5, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 241 (3.65), 265 (3.50), 318 (2.94) nm; IR (dry film) ν_{max} 3400, 2918, 2850, 1734, 1457, 1214 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.30 (2H, d, J = 8.7 Hz, H-2″, H-6″), 6.88 (2H, d, J = 8.7 Hz, H-3″, H-5″), 6.14 (1H, dd, J = 10.0, 2.9 Hz, H-12), 5.53 (1H, dq, J = 8.4, 1.3 Hz, H-24), 5.46 (1H, dd, J = 10.0, 2.1 Hz, H-11), 5.39 (1H, br d, J = 7.0, H-2), 5.14 (1H, ddd, J = 14.0, 7.2, 5.5 Hz, H-23), 4.22 (1H, dd, J = 11.7, 2.4 Hz, H-6′), 4.05 (2H, br s, H-26), 3.94 (1H, dd, J = 2.3, 2.3 Hz, H-7), 3.80 (3H, s, OMe), 3.76 (1H, d, J = 10.7 Hz, H-2′), 3.58 (1H, d, J = 10.7 Hz, H-2′), 3.10 (1H, ddd, J = 13.0, 8.2, 4.9 Hz, H-20), 2.59−2.66 (2H, overlapped, H-17, H-5′), 2.39 (1H, ddd, J = 13.0, 8.2, 5.5 Hz, H-22), 2.17 (1H, dd, J = 13.4, 2.5 Hz, H-5′), 2.10 (1H, dd, J = 16.4, 7.1 Hz, H-1), 2.01−2.04 (2H, overlapped, H-5, 15), 1.97 (1H, m, H-9), 1.76−1.86 (3H, overlapped, H-6, H-16, H-22), 1.74 (3H, d, J = 0.8 Hz, H-27), 1.64−1.73 (3H, overlapped, H-1, H-6, H-15), 1.32 (3H, s, H-28), 1.19 (1H, d, J = 5.2 Hz, H-30), 1.14 (1H, m, H-16), 1.08 (3H, s, H-19), 0.91 (3H, s, H-18), 0.78 (1H, d, J = 5.2 Hz, H-30); ^{13}C NMR (90 MHz, $CDCl_3$), see Table 1; EIMS m/z [M]$^+$ 614 (29), 612 (38), 596 (100), 584 (57); HREIMS m/z [M]$^+$ 614.3607 (calcd for $C_{39}H_{50}O_6$, 614.3607).

Dichapetalin L, (4α,6′α,7α,17α,20*S*,23*R*,24*E*)-2′,3′,5′,6′-Tetrahydro-7,23,26-trihydroxy-6′-phenyl-13,30-cyclo-29-nordammara-2,24-dieno[4,3-*c*]pyran-21-oic acid γ-lactone (5): crystals; mp 110−112 °C; $[\alpha]_D^{20}$ +16.8 (*c* 0.2, $CHCl_3$); UV($CHCl_3$) λ_{max} (log ϵ) 240 (2.77), 267 (2.61), 318 (2.31) nm; IR (dry film) ν_{max} 3466, 2916, 2848, 1772, 1457, 1215, 1029 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.38 (2H, m, H-2″, H-6″), 7.34 (2H, m, H-3″, H-5″), 7.27 (1H, m, H-4″), 5.51 (1H, dd, J = 8.4, 1.1 Hz, H-24), 5.37 (1H, d, J = 6.8 Hz, H-2), 5.12 (1H, ddd, J = 14.1, 8.7, 5.6 Hz, H-23), 4.26 (1H, dd, J = 11.7, 2.4 Hz, H-6′), 4.05 (2H, br s, H-26), 3.83 (1H, dd, J = 2.3, 2.3 Hz, H-7), 3.76 (1H, d, J = 10.7 Hz, H-2′), 3.59 (1H, d, J = 10.7 Hz, H-2′), 2.98 (1H, ddd, J = 12.7, 8.4, 4.3 Hz, H-20), 2.62 (1H, m, H-5′), 2.55 (1H, m, H-17), 2.31 (1H, ddd, J = 13.1, 8.3, 4.8 Hz, H-22), 2.18 (1H, dd, J = 13.4, 2.5 Hz, H-5′), 1.93−2.00 (4H, overlapped, H-1, H-5, H-12, H-15), 1.83 (1H, m, H-22), 1.74 (3H, s, H-27), 1.57−1.65 (4H, overlapped, H-1, H-6, H-15, H-16), 1.50 (1H, dd, J = 13.5, 1.6 Hz, H-6), 1.36−1.44 (2H overlapped, H-11, H-12), 1.32 (3H, s, H-28), 1.25−1.30 (2H, overlapped, H-9, H-11), 1.07 (3H, s, H-18), 1.03 (3H, s, H-19), 0.96 (2H, m, H-16), 0.67 (1H, d, J = 4.6 Hz, H-30), 0.47 (1H, d, J = 4.6 Hz, H-30); ^{13}C NMR (90 Hz, $CDCl_3$), see Table 1; EIMS m/z [M]$^+$ 586 (31), 584 (35), 574 (17), 566 (100), 562 (19); HREIMS m/z [M]$^+$ 586.3673 (calcd for $C_{38}H_{50}O_5$, 586.3658).

***ent*-16-Nor-5α,13α(methyl)-3-oxodolabra-1,4(18)-dien-2-ol-15-oic acid (6):** pale yellow crystals; mp 87−89 °C; $[\alpha]_D^{20}$ +157.8 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ϵ) 305 (3.75) nm; IR (dry film) ν_{max} 3416 (br), 2932, 2863, 1698, 1661, 1414, 1223 cm^{-1}; ^{1}H and ^{13}C NMR data, consistent with literature values;[15] EIMS m/z [M]$^+$ 318 (44), 300 (6), 257 (4), 181 (19), 151 (79), 150 (54), 137 (80), 135 (100), 107 (28); HREIMS m/z [M]$^+$ 318.1806 (calcd for $C_{19}H_{26}O_4$, 318.1831). This compound was obtained previously as a gum, with no $[\alpha]_D$, UV, IR, or HRMS data obtained.[15]

Methylation of 6. Compound **6** (3 mg) was dissolved in 1 mL of EtOAc, and 0.2 mL of diazomethane in ether was added at room temperature. The solution was stirred and allowed to stand for about 10 min. Evaporation of the solvent and reagent gave 2 mg of the methyl ester of **6** (**6a**): yellow gel-like solid; $[\alpha]_D^{20}$ +154.9 (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 303 (2.94) nm; IR (dry film) ν_{max} 3415, 2932, 2863, 1728, 1662, 1604, 1413, 755 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.25 (1H, s, H-18), 6.18 (1H, d, J = 6.7 Hz, H-1), 5.41 (1H, s, H-18), 3.66 (3H, s, COOMe-15), 2.04 (1H, d, J = 6.7 Hz, H-10), 1.27 (3H, s, Me-17), 1.12 (3H, s, Me-19), 0.63 (3H, s, Me-20); ^{13}C NMR (90 MHz, CD_3OD) δ 186.3 (s, C-3), 180.6 (s, C-15), 151.5 (s, C-4), 149.7 (s, C-2), 119.4 (d, C-1), 118.6 (t, C-18), 56.4 (d, C-10), 52.4 (q, COOMe-15), 43.4 (s, C-13), 42.1 (s, C-5), 41.5 (s, C-9), 41.4 (s, C-8), 37.7 (t, C-14), 37.4 (t, C-6), 35.7 (t, C-11), 34.3 (q, C-19), 29.9 (t, C-12), 26.5 (t, C-7), 21.7 (q, C-17), 12.5 (q, C-20); EIMS m/z [M]$^+$ 332 (20), 272 (14), 195 (22), 151 (58), 150 (27), 138 (51), 137 (43), 136 (30), 135 (100), 121 (21), 107 (30).

***ent*-16-Nor-5α,13α(methyl)-2-oxodolabra-3-en-3-ol-15-oic acid (7):** crystals; mp 73−74 °C; $[\alpha]_D^{20}$ +47.4 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 283 (3.29) nm; IR (dry film) ν_{max} 3423, 3190, 2930, 2865, 1698, 1665, 1638, 1395, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, consistent with literature values;[15] EIMS m/z [M]$^+$ 320 (33), 305 (58), 151 (18), 137 (100), 121 (23); HREIMS m/z [M]$^+$ 320.1979 (calcd for $C_{19}H_{28}O_4$, 320.1986). This compound was obtained previously as a semisolid, with no $[\alpha]_D$, UV, IR, or HRMS data obtained.[15]

Zeylanol [6β-Hydroxyfriedelan-3-one] (8): crystals; mp 254 °C [lit.[21] 274−276 °C]; $[\alpha]_D^{20}$ +9.5 (*c* 0.16, pyridine) [lit.[21] $[\alpha]_D^{20}$ +17 ($CHCl_3$)]; IR and ^{1}H NMR data consistent with literature values;[21] ^{13}C NMR (75 MHz, pyridine-d_5) δ 212.0 (s, C-3), 78.7 (d, C-6), 58.5 (d, C-4), 58.4 (d, C-10), 49.7 (d, C-8), 47.9 (s, C-5), 42.8 (d, C-18), 41.5 (t, C-2), 39.7 (s, C-13), 39.2 (t, C-22), 38.0 (s, C-14), 37.3 (s, C-9), 36.0 (t, C-16), 35.5 (t, C-11), 35.3 (t, C-19), 34.8 (q, C-30), 32.8 (t, C-21), 32.3 (t, C-15), 32.0 (q, C-28), 31.7 (q, C-29), 30.5 (t, C-12), 29.9 (s, C-17), 29.8 (t, C-1), 28.1 (s, C-20), 22.0 (t, C-7), 20.1 (q, C-27), 18.6 (q, C-26), 17.5 (q, C-25), 11.1 (q, C-23), 9.7 (q, C-24); EIMS m/z [M]$^+$ 442 (95), 370 (48), 318 (79), 273 (100), 205 (88), 123 (93).

28-Hydroxyzelanol [6β,28-Dihydroxyfriedelan-3-one] (9): crystals; mp 282 °C; $[\alpha]_D^{20}$ +2.8 (*c* 0.84, pyridine); UV (MeOH) λ_{max} (log ϵ) 251 (3.40), 257 (3.43), 263 (3.27) nm; IR (dry film) ν_{max} 3453, 2942, 2872, 1715, 1443, 1389, 1026, 710 cm^{-1}; ^{1}H NMR (300 MHz, pyridine-d_5) δ 5.99 (1H, br s, OH-28), 5.64 (1H, d, J = 5.3 Hz, OH-16), 3.90 (2H, overlapped, H-28), 3.87 (1H, overlapped, H-6), 1.60 (3H, d, J =

6.7 Hz, H-23), 1.21 (3H, s, H-27), 1.08 (3H, s, H-29), 1.08 (3H, s, H-30), 1.03 (3H, s, H-24), 0.99 (3H, s, H-26), 0.86 (3H, s, H-25); ^{13}C NMR (90 MHz, pyridine-d_5) δ 212.0 (s, C-3), 78.8 (d, C-6), 67.2 (t, C-28), 58.5 (d, C-4), 58.5 (d, C-10), 49.2 (d, C-8), 47.9 (s, C-5), 41.5 (t, C-2), 39.5 (s, C-13), 39.5 (d, C-18), 38.0 (s, C-14), 37.5 (s, C-9), 35.7 (s, C-17), 35.5 (t, C-11), 34.8 (t, C-19), 34.3 (q, C-30), 33.6 (t, C-21), 32.9 (q, C-29), 32.2 (t, C-22), 31.6 (t, C-15), 30.2 (t, C-12), 29.8 (t, C-1), 29.7 (t, C-16), 28.2 (s, C-20), 22.0 (t, C-7), 19.4 (q, C-27), 19.0 (q, C-26), 17.7 (q, C-25), 11.1 (q, C-23), 9.7 (q, C-24); EIMS *m*/*z* $[M]^+$ 472 (41, $M^+ - CH_2OH$), 409 (32), 289 (29), 137 (100); CIMS *m*/*z* $[M + H]^+$ 459; HREIMS *m*/*z* 427 ($M^+ - CH_2OH$, calcd for $C_{29}H_{47}O_2$, 427.3576).

Betulinic acid: crystals; mp 281−283 °C; $[\alpha]_D^{20}$ +5.7 (*c* 0.84, pyridine) [lit.[31] mp 275−278 °C; $[\alpha]_D^{20}$ +7.9 (*c* 0.057, pyridine)]; UV, IR, ^{1}H, ^{13}C NMR, and EIMS data, consistent with literature values.[31−33]

Single-Crystal X-ray Crystallographic Analysis[38] of 6. A colorless plate crystal of **6**, size 0.45 × 0.38 × 0.15 mm, obtained from a mixture of EtOH−H_2O, was used for X-ray diffraction experiments. The intensity data were measured on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Cu Kα radiation ($\lambda = 1.5418$ Å) at room temperature. The crystal is monoclinic, space group $P2_12_12_1$, with cell constants $a = 8.2239(4)$ Å, $b = 10.2867(3)$ Å, $\beta = 90°$, $V = 2015.6(1)$ Å, with 4 molecules in the unit cell; calculated crystal density $D_x = 1.200$ g/cm^3, and absorption coefficient $\mu = 0.65$ mm^{-1}. The structure was solved by the direct method and refined by a full-matrix least-squares procedure.[39] The refinement converged to a final $R(F) = 0.051$, $wR(F) = 0.060$, where $w = 1/(\sigma^2 + 0.01F^2)$, $S = 3.084$ for 1912 reflections with $I \geq 3\sigma$ and 235 variable parameters. The absolute configuration of **6** was not determined, and the enantiomorph used in the reference is based on the known stereochemistry of dolabrane diterpenoids.[15]

Bioassay Evaluation. Compounds **1**−**9** and **6a** and betulinic acid were evaluated for cytotoxicity against a panel of human cancer cell lines according to established protocols.[34,35] Dichapetalin A (**1**) was evaluated in the in vivo hollow fiber testing, according to established protocols.[14]

Acknowledgment. This investigation was supported by grant U19 CA-52956, funded by the National Cancer Institute (NCI), NIH, Bethesda, MD. We acknowledge the NCI for the transfer of plant material and Palawan Council for Sustainable Development, Puerto Princesa, Palawan, Philippines, for the re-collection of the plant sample. We thank Ms. C. A. Crot, Research Resources Center, University of Illinois at Chicago, and Mr. R. B. Dvorak, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, for the mass spectral data. We are grateful to the Nuclear Magnetic Resonance Laboratory of the Research Resources Center, University of Illinois at Chicago, for the provision of certain NMR spectroscopic facilities used in this investigation.

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NP058083Q