Cytotoxic Terpenes from the Stems of *Dipterocarpus obtusifolius* Collected in Cambodia

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From the stems of *Dipterocarpus obtusifolius*, five new triterpenes, 3-oxo-20-hydroxy-30*α*-methyl,17(29)-*α*-epoxy-28-norlupane (1), 3-oxo-20-hydroxy-30*β*-methyl-17(29)*α*-epoxy-28-norlupane (2), 3,20-dioxo-28,29-norlupan-17*α*-ol (3), 27-demethyl-20(*S*)-dammar-23-ene-20-ol-3,25-dione (4), and 3-*epi*-cecropic acid (5) together with 13 known compounds including diterpene, sesquiterpenes and triterpenes were isolated and characterized. All isolates were tested for their cytotoxicities against a small panel of human cancer cell lines. Of the tested compounds, compounds 4–11 were found to be cytotoxic against one or more human cancer cell lines.

Key words Dipterocarpaceae; *Dipterocarpus obtusifolius*; terpene; cytotoxicity

Dipterocarpus is a plant genus which belongs to the family Dipterocarpaceae, which consists of approximately 75 species distributed in the tropical regions.[1)] This family of plant is known to contain sesquiterpenes,[2)] triterpenes,[3,4)] flavonoids,[5)] and resveratrol oligomers,[1,6)] and exert diverse biological activities, such as anticancer, anti-human immunodeficiency virus (HIV),[1,4,7)] antibacterial,[8,9)] and antioxidant activities.[9)]

As part of our ongoing project in search for biologically active small molecules from Cambodian medicinal plants,[10)] *Dipterocarpus obtusifolius* Teijsm. ex Miq. was selected for phytochemical investigation since its MeOH extract was found to be cytotoxic against a small panel of human cancer cell lines in the initial screening test. *D. obtusifolius* is a tree, the usual height of 10–15 m, which grows in clear forests in low regions and widely distributed in countries such as Cambodia, Laos, Vietnam, and Thailand.[11,12)] Traditionally, the resin of this plant has been used to relieve abdominal discomfort in Cambodia, Laos and Vietnam.[12)]

To our knowledge, there has been no report of pharmacological and phytochemical investigation for *D. obtusifolius* so far. Hence, the present study described the structure characterization and cytotoxicity evaluation of five new triterpenes and 13 known compounds, using spectroscopic methods and *in vitro* cell based cytotoxic assay.

Results and Discussion

Structure Elucidation Compound **1** was obtained as a white powder and the high resolution-electrospray ionization (HR-ESI)-MS of **1** provided a protonated peak at *m/z* 443.3562 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525), corresponding to a molecular formula of $C_{29}H_{46}O_3$. The ^{1}H-NMR spectroscopic data of **1** exhibited resonances for six singlet methyl groups at δ_H 0.92, 0.96, 0.97, 1.01, 1.06, and 1.35, and an oxygenated methylene signals at δ_H 3.39 (1H, dd, *J*=10.8, 0.9 Hz) and 3.46 (1H, d, *J*=10.8 Hz). The ^{13}C-NMR spectroscopic data (Table 2) of **1** revealed 29 carbon signals consisting of six methyls, eleven methylenes, five methines, seven quaternary carbons including a carbonyl group (δ_C 218.4) and two quaternary oxygenated carbons (δ_C 70.7, 71.0), sorted by distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence (HMQC) spectroscopic data. The observed ^{1}H- and ^{13}C-NMR (Tables 1, 2) chemical shifts of **1** were similar to those of 20*S*-17*β*,29-epoxy-28-norlupan-3*β*-ol[13)] except for the presence of a carbonyl group and an additional hydroxyl group. The location of the carbonyl group (δ_C 218.4) was confirmed to be at C-3 by HMBC correlations (Fig. 2A) of H-2, H-23, and H-24 to C-3 (δ_C 218.4). In addition, the additional hydroxyl group was placed on C-20 (δ_C 71.0) by the observed heteronuclear multiple bond connectivity (HMBC) correlations of H-19, H-29 and H-30 to C-20 (δ_C 71.7).

The relative configurations of compound **1** were assigned on the basis of the rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum (Fig. 2B). The ROESY correlations of H-18 to H-27 and H-30, and H-9 to both of H-5 and H-27, and H-5 to H-23 were observed, being suggestive of the same *α*-orientation. The ROESY correlations of H-26 to H-25 and H-13, H-25 to H-24 and H-26 indicated that these groups are *β*-oriented. Furthermore, the absolute configuration of **1** was determined by measuring the circular dichroism (CD) spectrum. The CD spectrum of **1** showed a positive Cotton effect at 290 nm, the same as ekeberins A,[14)] suggesting that the structure of compound **1** was as described in Fig. 1. Consequently, compound **1** was elucidated as 3-oxo-20-hydroxy-30*α*-methyl,17(29)*α*-epoxy-28-norlupane.

Compound **2** had the same molecular formula based on a pseudomolecular ion peak at *m/z* 443.3597 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525) in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR (Tables 1, 2) spectroscopic data of **2** were closely identical to those of **1**, suggesting that **2** had the same nortriterpene skeleton. There were distinctive differences at C-18, C-20, C-21 and C-30 in the ^{13}C-NMR chemical shifts of **2** compared to those of **1**, which indicated that structure **2** was assumed to be slightly different from structure **1**. The ROESY spectrum of **2** (Fig. 2C) revealed the absence of ROESY correlations

The authors declare no conflict of interest.

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Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**, **2**, **3**, **4** and **5** ($CDCl_3$)

H	**1**[a)]	**2**[b)]	**3**[b)]	**4**[a)]	**5**[b)]
1a	1.91, m	1.91, m	1.90, m	1.89, m	1.64, m
1b	1.41, m	1.41, m	1.44, m	1.43, m	1.40, m
2a	2.47, m	2.47, m	2.46, m	2.48, m	—
2b	2.42, m	2.42, m	2.42, m	2.41, m	5.21, m
3	—	—	—	—	3.74, d (1.6)
5	1.34, dd (10.8, 3.6)	1.35, dd (9.2, 3.6)	1.35, dd (8.0, 4.0)	1.34, m	1.27, m
6a	1.45, m	1.48, m	1.46, 2H, m	1.52, m	1.42, m
6b	1.42, m	1.44, m	—	1.45, m	1.28, m
7a	1.44, m	1.45, 2H, m	1.47, m	1.53, m	1.49, m
7b	1.41, m	—	1.38, dd (8.4, 3.2)	1.29, m	1.28, m
9	1.40, m	1.41, m	1.45, m	1.39, dd (11.7, 2.7)	1.65, m
11a	1.52, m	1.50. m	1.55, m	1.49, m	1.90, 2H, dd (8.4, 2.8)
11b	1.26, m	1.24, m	1.26, m	1.28, t (3.6)	—
12a	1.58, m	1.58, m	1.61, m	1.83, t (3.6)	5.20, m
12b	0.94, m	0.96, m	1.02, m	1.26, t-like (2.7)	—
13	1.20, dt(11.6, 3.6)	1.18,dt (10.4, 3.2)	1.11 (dt, 11.2, 3.2)	1.66, td (11.7, 2.7)	—
15a	1.48, m	1.49, d (4.0)	1.40, d (3.2)	1.09, m	1.83, dt (11.9, 4.0)
15b	1.28, m	1.28, dd (11.2, 4.0)	1.27, m	1.44, m	1.05, m
16a	1.66, dd (12.5, 4.5)	1.71, dd (11.2, 4.0)	1.80, td (11.2, 3.2)	1.75, m	1.97, dt (11.6, 3.2)
16b	1.61, m	1.60, m	1.73, dt (12.0, 4.0)	1.46, m	1.63, m
17	—	—	—	1.72, m	—
18	1.50, m	1.92, m	1.68, br d (9.6)	0.97, s	2.15, d (9.6)
19	1.97, d (6.3)	2.01, d (5.6)	2.88, t-like (6.0)	0.92, s	1.30, m
20	—	—	—	—	0.92, m
21a	1.89, m	1.74, m	1.95, m	1.15, s	1.48, m
21b	1.62, t (4.5)	1.50, t (4.0)	—	—	1.28, m
22a	1.87, t (4.5)	1.89, br s	1.93, m	2.42, m	1.69, td (11.2, 2.4)
22b	1.53, m	1.55, m	1.53, m	2.33, dd (12.4, 8.1)	1.63, m
23	1.06, s	1.06, s	1.06, s	6.88,dt-like (16.2, 8.1)	1.00, s
24	1.01, s	1.00, br s	1.01, s	6.09, d (16.2)	0.87, s
25	0.92, s	0.91, s	0.91, s	—	1.01, s
26	0.97, s	0.97, s	0.95, s	2.24, s	0.73, s
27	0.96, s	1.01, br s	0.94, s	—	1.06, s
28	—	—	—	1.05, s	—
29a	3.46, d (10.8)	3.57, d (11.2)	—	1.01, s	0.81, d (5.6)
29b	3.39, dd (10.8, 0.9)	3.37, d (11.2)	—	—	—
30	1.35, s	1.09, s	2.20, s	0.86, s	0.92, d (5.6)
2′	—	—	—	—	2.04, s

a) Measured at 900 MHz. *b*) Measured at 800 MHz.

between H-18 and H-30 in compound **2** as opposed to **1**, and this observation led to a conclusion that H-30 is β-oriented. In addition, a positive Cotton effect at 292 nm was observed in the CD spectrum of **2**, implying the same configurations as **1** at C-4 and C-10. Based on all data, the structure of **2** was confirmed as 3-oxo-20-hydroxy-30β-methyl,17(29)α-epoxy-28-norlupane.

Compound **3** was isolated as a white powder and its molecular formula was determined to be $C_{28}H_{44}O_3$ by a protonated molecular ion peak at *m/z* 429.3384 $[M+H]^+$ (Calcd for $C_{28}H_{44}O_3$+H, 429.3369) in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectroscopic data of **3** (Tables 1, 2) exhibited the presence of five singlet methyl signals at δ_H 0.91, 0.94, 0.95, 1.01 and 1.06, and an acetyl group at δ_H 2.20. Total of 28 carbon signals were observed, consisting of six methyls, ten methylenes, five methines, seven quaternary carbons, including two carbonyl groups at δ_C 214.6 and 218.3. The ^{1}H- and ^{13}C-NMR (Tables 1, 2) chemical shifts of **3** closely resembled those of 3,20-dioxo-29-norlup-28-ol,[15)] except for the presence of an hydroxyl group instead of the methyl alcohol group at C-28. This was also supported by the observed HMBC correlations (Fig. 3A) of H-16, H-18, and H-21 to C-17 (δ_C 80.9). The relative configuration of **3** was resolved by interpretation of the ROESY experiment (Fig. 3B). ROESY correlations of H-5 to both of H-23 and H-27, and H-27 to both of H-9 and H-18 were able to prove that H-5 is α-oriented. Moreover, the ROESY correlations of H-25 to both H-24 and H-26, and H-13 to both of H-19 and H-26 were observed, which suggested that H-13 and H-19 are β-oriented. Compound **3** was dissolved in DMSO-d_6 and its ROESY experiment was run to determine the relative configuration of OH-17. The ROESY correlations of H-18 to both H-27 and OH-17 indicated that OH-17 is α-oriented. Therefore, the structure of **3** was determined as 3,20-dioxo-28,29-norlupan-17α-ol as depicted in Figure 1.

The molecular formula of **4** was assigned as $C_{29}H_{46}O_3$ based on the observed protonated molecular ion peak at *m/z* 443.3599 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525) in the HR-ESI-MS. The ^{1}H-NMR spectroscopic data of **4** displayed

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**, **2**, **3**, **4** and **5** ($CDCl_3$)

Carbon	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]
1	39.9	39.9	39.9	40.1	38.3
2	34.3	34.3	34.2	34.3	71.4
3	218.4	218.4	218.3	218.3	77.0
4	47.5	47.5	47.5	47.6	38.7
5	55.1	55.0	55.0	55.5	48.3
6	19.8	19.9	19.8	19.8	18.1
7	33.5	33.4	33.4	34.7	32.9
8	40.7	40.8	40.6	40.5	39.9
9	50.3	50.2	50.6	50.1	47.5
10	37.1	37.1	37.1	37.0	38.6
11	21.7	21.8	22.1	22.1	23.5
12	25.5	25.4	27.3	27.7	125.7
13	35.9	35.9	42.1	42.8	138.3
14	40.9	41.1	41.4	50.5	42.2
15	28.3	28.3	28.3	31.3	28.1
16	28.1	28.2	30.8	25.1	24.3
17	83.5	83.4	80.9	50.7	48.3
18	49.5	46.7	52.4	15.4	52.7
19	49.4	48.5	56.1	16.2	39.2
20	71.0	72.5	214.6	75.5	39.0
21	22.4	24.5	25.5	26.5	30.8
22	28.9	28.5	37.2	43.8	36.9
23	27.0	27.1	27.1	144.7	28.6
24	21.1	21.2	21.1	134.7	22.1
25	16.5	16.5	16.6	198.6	16.6
26	15.7	15.7	15.5	27.2	17.3
27	13.7	13.9	14.3	—	23.9
28	—	—	—	26.9	183.7
29	70.7	70.8	—	21.2	17.2
30	26.8	23.9	29.8	16.5	21.4
1′	—	—	—	—	170.4
2′	—	—	—	—	21.6

a) Measured at 900 MHz. *b*) Measured at 800 MHz.

two olefin protons (δ_H 6.88, 6.09 ppm) in a *trans* relationship (J=16.2 Hz), and seven singlet methyl signals at δ_H 0.86, 0.92, 0.97, 1.01, 1.05, 1.15, and 2.24. The ^{13}C-NMR spectroscopic data of **4** indicated 29 carbon resonances, which were classified by DEPT and HMQC experiments as one double bond (δ_C 134.7, 144.7), seven methyls, nine methylenes, four methines, seven quaternary carbons including an oxygenated quaternary carbon (δ_C 75.5) and two carbonyls groups (δ_C 198.6, 218.3), suggesting that structure **4** was very similar to that of rhombenone,[16,17] except for the absence of the hydroxyl group at C-6. By the detailed inspection of **4** using ^{1}H–^{1}H correlation spectroscopy (COSY) and HMQC technique, the location of C-6 was assigned through observation of the correlations between H-6 and H-5, as well as H-6 and H-7 in the ^{1}H–^{1}H COSY spectrum. This was also supported by observation of the HMBC correlations between H-5 to C-6 and C-7. Moreover, the locations of the double bond (C-23 and C-24, δ_C 144.6 and 134.1, respectively) and the carbonyl group at C-25 were determined on the basis of the HMBC correlations between both H-23 and H-24 to C-25, and H-26 to C-25. The relative configuration of compound **4** was characterized by the interpretation of ROESY spectrum, which was consistent with the configuration of rhombenone.[16,17] Thus, compound **4** was elucidated as 27-demethyl-20(S)-dammar-23-ene-20-ol-3,25-dione.

The HR-ESI-MS of compound **5** provided a protonated molecular ion peak at m/z 515.3784 $[M+H]^+$ (Calcd for $C_{32}H_{50}O_5$+H, 515.3736), corresponding to a molecular formula of $C_{32}H_{50}O_5$. The ^{1}H-NMR spectroscopic data of **5** showed eight methyl signals at δ_H 0.73 (s), 0.87 (s), 1.01 (s), 1.06 (s), 0.81 (d, J=5.6 Hz), 0.92 (d, J=5.6 Hz), and 2.04 (s), two oxygenated methine signals at δ_H 3.47 (1H, d, J=1.6 Hz) and 5.21 (1H, m), and a vinyl signal at δ_H 5.20 (1H, m). The ^{13}C-NMR spectroscopic data of **5** exhibited 32 carbon signals consisting of eight methyls, eight methylenes, eight methines, and eight quaternary carbons. The observed ^{1}H- and ^{13}C-NMR chemical shifts of **5** suggested that this structure bears a close resemblance to the known cecropic acid[18] except for a small coupling constant (1.6 Hz) of H-3 at δ_H 3.47, implying that H-3 has a different configuration in contrast to cecropic acid.[19] The relative configurations of H-2 and H-3 in structure **5** were resolved using the ROESY experiment. As described in Fig. 4, the correlations of H-2 to H-3, H-24 and H-25 were observed, suggesting that H-2 and H-3 are positioned in β-axial and β-equatorial orientations, respectively. Moreover, the HMBC correlations of both H-2 and H-2′ to C-1′ (δ_C 170.4) confirmed the location of an acetyl group at C-2 *via* an ester linkage. Hence, the structure of **5** was assigned as 3-*epi*-cecropic acid.

Additionally, 13 known compounds were identified as ursolic acid (**6**),[20] asiatic acid (**7**),[21] corosolic acid

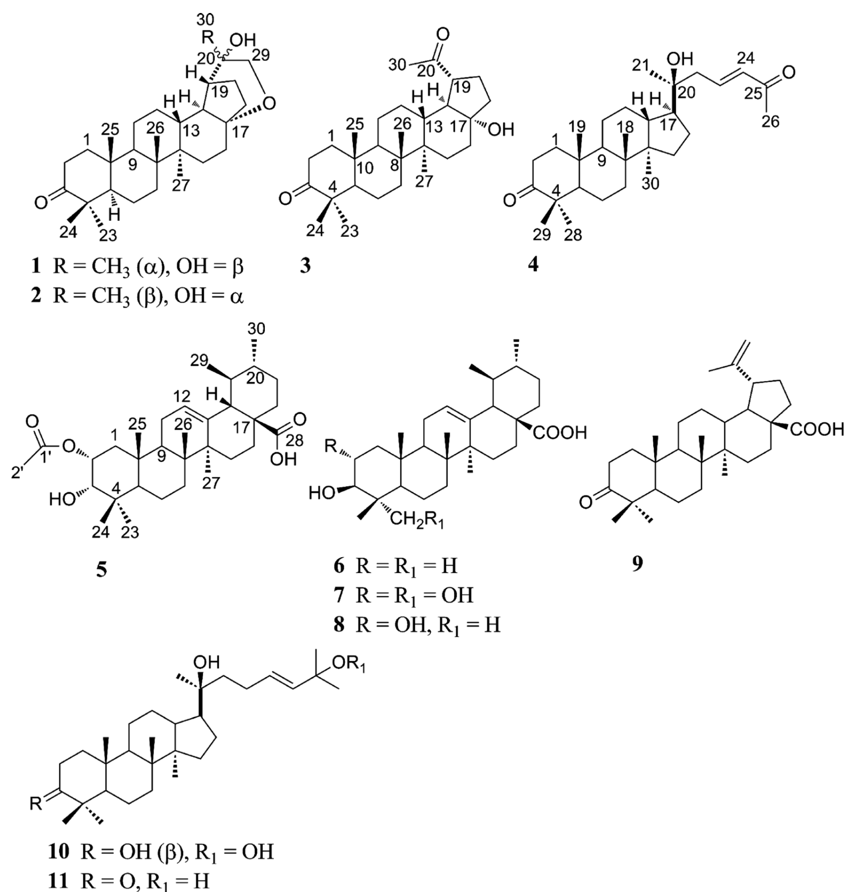

Fig. 1. Structures Isolated from *D. obtusifolius*

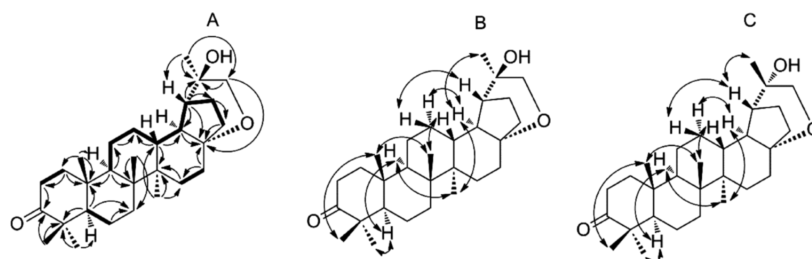

Fig. 2. (A) HMBC (H→C) and COSY (H–H) Correlations of Compound **1**; (B) ROESY (↔) Correlations of Compound **1** and (C) ROESY (↔) Correlations of Compound **2**

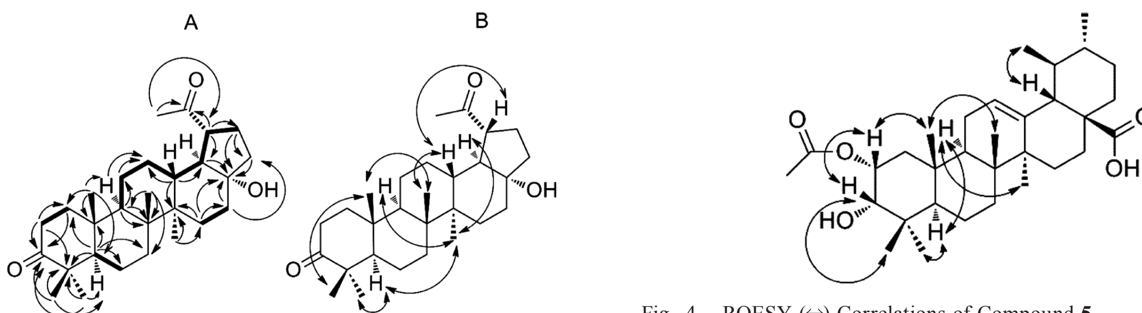

Fig. 3. (A) HMBC (H→C) and COSY (H–H) Correlations of Compound **3** and (B) ROESY (↔) Correlations of Compound **3**

Fig. 4. ROESY (↔) Correlations of Compound **5**

(**8**),[21] betulonic acid (**9**),[22] isofouquierone peroxide,[23] (*E*)-25-hydroperoxydamma-23-ene-3*β*,20-diol (**10**),[24] isofouquierone (**11**),[25] 20-hydroxy-damma-24-ene-3-one,[26] 7-*α*-hydroxysitosterol,[27] 7-oxysitosterol,[28] hollongdione,[29] 14(5)-eudesmen-1*β*-6*α*-diol,[30] and kobusone,[31] by comparison with published values.

All eighteen isolates were evaluated for their cytotoxicities

against HepG2, SK-OV-3, A-549, MCF-7, and SNU-1 human cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in Table 3, only compounds **4**–**11**were found to be cytotoxic against one or more cell lines tested in the present study, while other compounds deemed inactive (>40 μM). Interestingly, compounds **4**, **6**–**11** displayed the comparable cytotoxicities to adriamycin against either HepG2 or/and SK-OV-3 cells. The Dipterocarpaceaeous plants including *Dipterocarpus* genus have been reported to contain oligostibenoids, sesquiterpenes and triterpenes.[32] A few oligomeric stilbenoids were isolated as cytotoxic constituents from *Dipterocarpus* genus.[1,33] The present study found that triterpenoids which are frequently occurring compounds in this genus *Dipterocarpus* as a chemotoxonomic marker were also present in this plant.[32] Triterpenes in this plant might also be responsible for cytotoxic activities.

Experimental

General Procedures Melting points were determined on a Kofler micro-hotstage and were uncorrected. Optical rotation was measured with a Jasco P-2000 polarimeter. CD spectra were obtained on a Jasco J-710 spectropolarimeter, and FT-IR spectrum was taken using a Jasco FT/IR-4200. NMR spectra were recorded on a Varian UNITY 400, Bruker DMX-800 and DMX-900 MHz FT-NMR spectrometers with tetramethylsilane as an internal standard. HR-ESI-MS was performed with a Waters Q-Tof Premier spectrometer. HPLC separation was conducted on a Gilson, pump 305 and UV/VIS-155 detector.

Plant Material The stems of *D. obtusifolius* were collected in Kampongkdey district, Siem Reap province, Cambodia, in February 2011. The plant samples were identified by Prof. Thao Sokunthia, Head of the Department of Biology, Faculty of Science, and Royal University of Phnom Penh, Cambodia. A voucher specimen (RUPP0011) has been deposited at the herbarium of Royal University of Phnom Penh, Cambodia.

Extraction and Isolation The dried stems of *D. obtusifolius* (3.0 kg) were extracted with MeOH at room temperature three times to obtain 130 g of solid extract. The MeOH extract was suspended in water and then partitioned with *n*-hexane and $CHCl_3$ successfully to afford 43.0 g of *n*-hexane-soluble fraction and 14.0 g of $CHCl_3$-soluble fraction. Since the *n*-hexane-soluble fraction was found to be cytoxtoxic at a concentration of 50 μg/mL against SNU-1 (2% of cell viability), A-549 (20% of cell viability), and HepG2 (6% of cell viability), this fraction (43.0 g) was subjected to a silica gel column chromatography with a stepwise gradient mixture of *n*-hexane–EtOAc (40 : 1 to 1 : 1, v/v), followed by $CHCl_3$–MeOH (5 : 1 to 1 : 1, v/v) to yield nine fractions (DH-1–DH-9). Fraction DH-3 (8.0 g) was chromatographed on a column of RP C-18 (3.5×40 cm, 40C_{18}-PREP) in a medium pressure liquid chromatography system (MPLC), eluted with MeOH–H_2O (3.5 : 1 to 4.5 : 1, then 100% MeOH) to obtain five fractions (DH-3-1–DH-3-5). Fraction DH-3-2 (35.0 mg) was purified by preparative TLC (PTLC) using PTLC silica gel 60 F_{254}, 0.5 mm, $CHCl_3$–MeOH (20 : 1, v/v) to afford kobusone (4.0 mg) and hollongdione (6.6 mg). From fraction DH-3-3 (900 mg), 20-hydroxy-damma-24-ene-3-one (720 mg) was precipitated. Fraction DH-3-4 (200 mg) was chromatographed by MPLC on an RP C-18 column [(250×20 mm)×8, 40C_{18}-PREP)], eluted with 95% MeOH in H_2O to obtain compounds **6** (10.0 mg) and **9** (12.0 mg). Fraction DH-4 (980 mg) was chromatographed on a MPLC column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (2 : 1 to 4.5 : 1, then 100% MeOH) to obtain four fractions (DH-4-1–DH-4-4). Fraction DH-4-2 (43.0 mg) was purified by PTLC using PTLC silica gel 60 F_{254}, 0.5 mm, petroleum ether–acetone (4 : 1, v/v) to give 14(5)-eudesmen-1β-6α-diol (12.0 mg). Fraction DH-5 (1.0 g) was chromatographed on a MPLC column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (2 : 1 to 4.5 : 1, followed by 100% MeOH) to obtain four fractions (DH-5-1–DH-5-4). Fraction DH-5-2 (444 mg) was subjected to a silica gel column chromatography, eluted with *n*-hexane–EtOAc (2 : 1, v/v) and then PTLC using PTLC silica gel 60 F_{254}, 0.5 mm thickness, *n*-hexane–EtOAc (1 : 1, v/v) was conducted to obtain isofouqueirone peroxide (26.0 mg). Fraction DH-6 (768 mg) was chromatographed on a column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (3.5 : 1, 4.5 : 1, then 100% MeOH) to obtain three fractions (DH-6-1–DH-6-3). Fraction DH-6-1 (118 mg) was chromatographed by MPLC on an RP C-18 column [(250×20 mm)×8, 40C_{18}-PREP], eluted with 95% MeOH in H_2O and then PTLC using PTLC silica gel 60 F_{254}, 0.5 mm, *n*-hexane–EtOAc (1 : 1, v/v, 2 times development) was carried out to obtain compounds **3** (6.7 mg), **4** (11.0 mg), and **10** (5.0 mg). Fraction DH-6-2 (30.0 mg) was separated by preparative HPLC using a reversed-phase column [YMC J'sphere ODS-H80 column (250×20 mm, 4 μm particle size); mobile phase MeOH–H_2O (9 : 1); flow rate 10 mL/min; UV detection at 210 nm] to afford compound **1** (7.0 mg, t_R 15.1 min). Fraction DH-6-3 (98.0 mg) was subjected to a silica gel column chromatography with a

Table 3. Cytotoxic Activities of Compounds **4**–**11** against Human Cancer Cell Lines

Compounds	IC_{50} (μM)[a]				
	HepG2	SK-OV-3	A-549	MCF-7	SNU-1
4	19.2±0.09	>40	25.3±0.24	19.5±0.09	24.9±0.13
5	>40	>40	24.1±0.21	19.4±0.03	24.3±0.03
6	24.5±0.07	24.5±0.20	19.8±0.17	18.3±0.12	17.4±0.10
7	>40	27.2±0.23	26.5±0.21	25.1±0.21	26.8±0.21
8	17.9±0.18	18.3±0.13	18.5±0.12	18.9±0.07	16.6±0.06
9	39.4±0.20	38.4±0.21	29.9±0.28	19.5±0.08	22.9±0.02
10	23.7±0.20	>40	>40	22.9±0.02	20.2±0.04
11	25.1±0.21	39.9±2.78	23.9±0.18	29.5±0.04	23.9±0.19
Adriamycin[b]	26.4±0.35	48.9±2.09	6.43±0.08	0.13±0.07	4.32±0.18

a) Data are mean±S.D. from three different experiments. *b*) Positive control. Other compounds are found in active.

stepwise gradient mixture of *n*-hexane–EtOAc (4 : 1 to 1 : 1, v/v) and then PTLC using PTLC silica gel 60 F_{254}, 0.5 mm thickness, *n*-hexane–EtOAc (2 : 1, v/v) was performed to obtain 7-oxysitosterol (6.6 mg). Fraction DH-7 (2.0 g) was subjected to a MPLC column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (4 : 1, 4.5 : 1, and 100% MeOH) to give four fractions (DH-7-1–DH7-4). Fraction DH7-3 (200 mg) was chromatographed on a MPLC column of silica gel [(3.0×40 cm)×2, 230–400 mesh], eluted with *n*-hexane–EtOAc (2 : 1, v/v) to afford compounds **2** (4.0 mg), **5** (13.0 mg), and **11** (88.0 mg). Fraction DH-8 (500 mg) was chromatographed on a MPLC column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (2 : 1 to 4.5 : 1, and 100% MeOH) to obtain three fractions (DH-8-1–DH-8-3). Fraction DH-8-2 (171 mg) was chromatographed on a MPLC column of silica gel, eluted with $CHCl_3$–MeOH (30 : 1, v/v) to give compound **8** (28.0 mg). Fraction DH-8-3 (30.0 mg) was chromatographed on a MPLC column of RP C-18 [(250×20 mm)×8, 40C_{18}-PREP)], eluted with 95% MeOH in H_2O to give 7-*α*-hydroxysitosterol (6.0 mg). Fraction DH-9 (1.2 g) was chromatographed on a MPLC column of silica gel, eluted with $CHCl_3$–MeOH (30 : 1–1 : 1, v/v, 3.5×40 cm) to give four fractions (DH-9-1–DH-9-4). Fraction DH-9-3 (900 mg) was chromatographed on a MPLC column of RP C-18 (3.5×40 cm, 40C_{18}-PREP), eluted with MeOH–H_2O (4 : 1 to 4.5 : 1, then 100% MeOH) to obtain compound **7** (540 mg).

3-Oxo-20-hydroxy-30*α*-methyl,17(29)*α*-epoxy-28-norlupane (**1**): White powder. mp 290–291°C. IR (film) ν_{max} cm^{-1}: 3437, 2952, 2866, 1701, 754. CD (EtOH) λ_{max} ($\Delta\varepsilon$): 290 (+3.8) nm. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data see Tables 1 and 2. HR-ESI-MS *m/z* 443.3562 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525). $[\alpha]_D^{20}$ +34.0 (*c*=0.30, $CHCl_3$).

3-Oxo-20-hydroxy-30*β*-methyl,17(29)*α*-epoxy-28-norlupane (**2**): White powder; mp 251–252°C. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data see Tables 1 and 2. IR (film) cm^{-1}: 3453, 2952, 2866, 1701, 754. CD (EtOH) λ_{max} ($\Delta\varepsilon$): 292 (+5.7) nm. HR-ESI-MS *m/z* 443.3597 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525). $[\alpha]_D^{20}$ +57.4 (*c*=0.13, $CHCl_3$).

3,20-Dioxo-28,29-norlupan-17*α*-ol (**3**): White powder; mp 236–237°C. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data see Tables 1 and 2. IR (film) ν_{max} cm^{-1}: 3460, 2946, 2868, 1702, 754. CD (EtOH) λ_{max} ($\Delta\varepsilon$): 273 (−2.1), 302 (+2.3) nm. HR-ESI-MS *m/z* 429.3384 $[M+H]^+$ (Calcd for $C_{28}H_{44}O_3$+H, 429.3369). $[\alpha]_D^{20}$ +19.7 (*c*=0.25, $CHCl_3$).

27-Demethyl-20(S)-dammar-23-ene-20-ol-3,25-dione (**4**): Colorless gum. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data see Tables 1 and 2. IR (film) ν_{max} cm^{-1}: 3471, 2951, 2870, 1701, 1669, 755. CD (EtOH) λ_{max} ($\Delta\varepsilon$): 229 (−11.1), 291 (+5.6) nm. HR-ESI-MS *m/z* 443.3599 $[M+H]^+$ (Calcd for $C_{29}H_{46}O_3$+H, 443.3525). $[\alpha]_D^{20}$ +41.6 (*c*=0.28, $CHCl_3$).

3-*epi*-Cecropic Acid (**5**): White powder; mp 158–159°C. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) spectroscopic data see Tables 1 and 2. IR (film) ν_{max} cm^{-1}: 3490, 2946, 2871, 1721, 1697, 1248, 756. HR-ESI-MS *m/z* 515.3784 $[M+H]^+$ (Calcd $C_{32}H_{50}O_5$+H, 515.3736). $[\alpha]_D^{20}$ +21.6 (*c*=0.23, $CHCl_3$).

Cell Lines and Cell Culture HepG2 (human liver hepatocellular carcinoma) cells were maintained in Dulbecco's modified Eagle's medium in the presence of 10% (v/v) fetal bovine serum (FBS) and 1% (w/v) penicillin/streptomycin. SK-OV-3 (ovary adenocarcinoma) were cultured in McCoy's 5A medium with supplement of 10% (v/v) FBS and 1% (w/v) penicillin/streptomycin. A-549 (human lung carcinoma), MCF-7 (breast carcinoma), and SNU-1 (human gastric carcinoma) cells were cultured in RPMI-1640 with 10% (v/v) FBS and 1% (w/v) penicillin/streptomycin. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. All cells were obtained from the American Type Culture Collection (ATCC, Manassas, U.S.A.).

Cytotoxicity Assay Cell viability was measured using MTT assay.[34] In brief, HepG2 cells (3×10^3), SK-OV-3, A-549, and MCF-7 cells (10^4) were seeded in each 96-well plates (0.1 mL). The SNU-1 cells (2×10^4) were seeded in each 96-well plates (0.2 mL). Test samples were dissolved in a small amount of dimethyl sulfoxide (DMSO) and diluted in the appropriate culture medium (final concentration of DMSO $<0.5\%$). After incubation with compounds for 24 h, 10 μL of MTT solution (5 mg/mL in PBS) was added to each well and re-incubated for 4 h at 37°C. The formazan crystals were produced and dissolved in 100 μL of DMSO. Absorption was measured by an enzyme-linked immunosorbant assay reader (ELISA, Bio-Rad) at 570 nm. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (IC_{50}) and the given values were calculated from the mean of three different experiments.

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