A Novel Secobetulinic Acid 3,4-Lactone from *Viburnum aboricolum*

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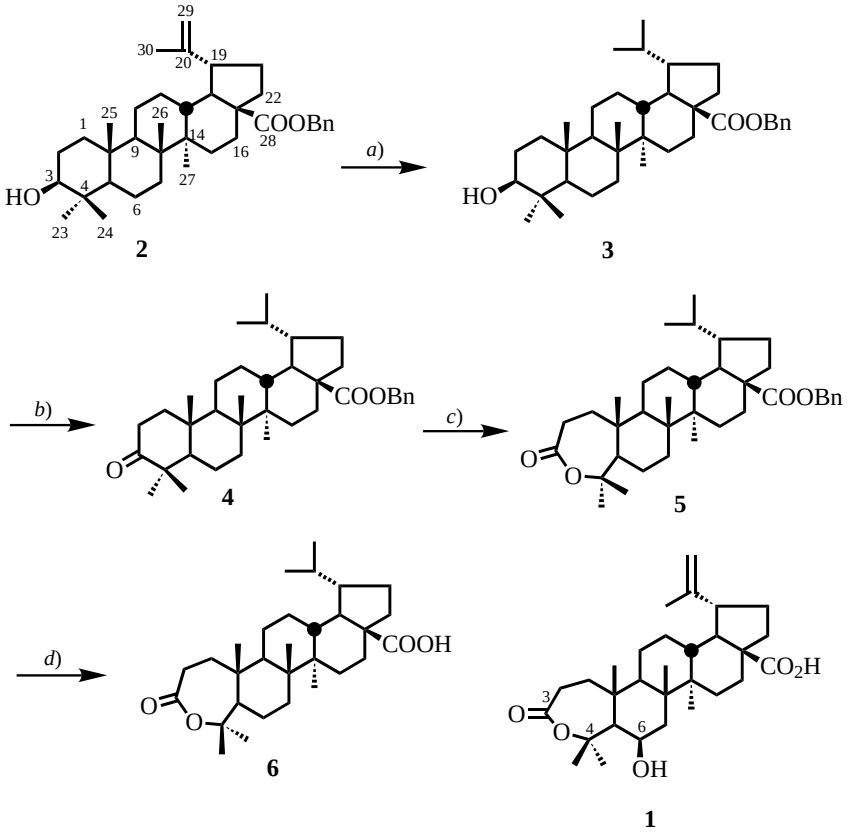
Bio-assay-guided fractionation of the $CHCl_3$-soluble extract from the leaves of *Viburnum aboricolum* led to the isolation of a novel secobetulinic acid 3,4-lactone, viburolide (=(6β)-4,6-dihydroxy-3,4-secolup-20(29)-ene-3,28-dioic acid 3,4-lactone; **1**). This is the first lupane-type compound possessing such a lactone skeleton from natural products. Its structure was elucidated by spectral analysis and comparison with 6-dehydroxy-20,29-dihydroviburolide (**6**) prepared from benzyl betulinate (**2**). Compound **6** was found to inhibit androgen-independent human prostate cancer cells (PC-3) with an IC_{50} of 12.3 μM.

1. Introduction. – *Viburnum aboricolum* HAYATA (Caprifoliaceae) is a shrub or small tree endemic to the northern and central parts of Taiwan [1]. During our biological-activity screening of Taiwan flora, the MeOH extract of its leaves was found to possess significant cytotoxic and antiviral activities against NUGC and HONE-1. This prompted us to investigate the active components from this plant, which, to our knowledge, has never been studied for its constituents. Bio-assay-guided fractionations traced the activity in the $CHCl_3$-soluble fraction. The latter was separated by repetitive column chromatography (silica gel) to yield eight components, including ursolic acid [2], five betulinic acid derivatives, 6β-hydroxy-3,*O*-didehydrobetulinic acid [3], compound **1**, 6α-hydroxy-3,*O*-didehydrobetulinic acid [3], betulinic acid [4][5], 6β-hydroxybetulinic acid [6][7], and two oleanolic acid derivatives, sumaresinolic acid [8] and maslinic acid, the latter being isolated in its diacetate form [9]. Of these, compound **1**, a secobetulinic acid 3,4-lactone, was found to be a novel natural product. In the following, we describe the structure elucidation of this compound, including chemical correlation and cytotoxicity testing of related compounds.

Results and Discussion. – Compound **1** has a molecular formula $C_{30}H_{46}O_5$, based on HR-EI-MS analysis. The spectral data suggested that **1** was a betulinic acid derivative. Based on the ^{1}H- and ^{13}C-NMR, HMBC, and NOESY data, compound **1** was identified (6β)-4,6-dihydroxy-3,4-secolup-20(29)-ene-3,29-dioic acid 3,4-lactone and was named viburolide after its plant origin. Viburolide (**1**) represents the first natural occurrence of such a 3,4-lactone structure among the lupane-type triterpenes.

In the NMR spectra ($CDCl_3$) of **1**, signals for an allylic H-atom at δ(H) *ca.* 3.40 (H–C(19)), 6*s*, for six Me groups, one appearing at *ca.* 1.67 (Me(30)), two broad *s* for two coupled olefinic H-atoms (CH_2(29)) at 4.70 and 4.60, the latter three signals corresponding to the isopropylidene group at C(19), and signals for a carboxyl C-atom at δ(C) 180.3 (C(28)) and for two olefinic C-atoms at 150.0 (*s*, C(20)) and 109.8 (*t*, C(29)) were typical for a betulinic acid skeleton [4]. In addition, the ^{13}C-NMR spectrum revealed signals for a lactone C-atom at 175.5 (C(3)) and an O-linked quaternary C-atom at 86.7 (C(4)), but the corresponding signal for the C(3) oxymethine was lacking. These data of **1** are consistent with 3,4-secobetulinic acid 3,4-lactone structure.

Scheme



a) H_2, 10% Pt/C, AcOH/dioxane 1:3. *b*) PCC, CH_2Cl_2. *c*) *m*CPBA, $NaHCO_3$, CH_2Cl_2. *d*) H_2, 10% Pd/C, AcOEt.

The ^{1}H-NMR spectrum (*Table*) of **1** showed a broad *s* for an oxymethine H-atom at 4.44. The NOESY plot displayed NOE interactions between this H-atom and two Me *s* at 1.49 and 1.73, the former being larger in magnitude (see *Table*). The coupling patterns and NOE interactions among these groups allowed the assignment of H_α–C(6), Me(23) and Me(24) to these signals at δ(H) 4.44, 1.49, and 1.73, respectively, and suggested a 6β-OH function. The assignment of the Me(24) signal was confirmed by its NOE correlations to Me(23) (δ 1.49) and Me(25) (δ 1.31), and the interactions of the latter to H_β–C(2) (δ 2.89) and Me(26) (δ 1.27). In comparison with the corresponding signals for Me(23) and Me(24) (δ 0.95 and 0.74, resp., in $(CD_3)_2CO$) in betulinic acid [5], the downfield-shifted signals for these two Me groups in **1** suggested C(4) to be oxygenated. Its HMBC spectrum (*Table*) also suggested this connection by displaying the two-bond connections between this C(4) (δ 86.7) and the H-atoms of Me(23) and Me(24). The complete assignment of the ^{13}C-NMR data of **1** (*Table*) was made by analysis of the HMBC spectrum and comparison with the ^{13}C-NMR assignment of betulinic acid [4].

The amount of **1** obtained was scarcely enough to allow any biological testing. To provide a large enough amount of similar analogues for a biological study and to confirm the assigned structure of **1**, we prepared a 6-dehydroxy-20,29-dihydro

derivative of **1** as shown in the *Scheme*. Selective catalytic hydrogenation of benzyl betulinate (**2**) obtained by chemical separation from *Paliurus ramossismus* [10] yielded benzyl 20,29-dihydrobetulinate (**3**). It was observed that this reaction could be accelerated under acidic conditions. The reaction mixture was monitored by ^{1}H-NMR analysis (disappearance of the olefinic CH_2(29) (δ(H) 4.70, 4.57) and allylic Me(30) signals (δ(H) 1.67), appearance of 2*d* of Me(29) and Me(30) (δ(H) 0.82 and 0.72)). Oxidation of the 3-hydroxy function by pyridinium chlorochromate (PCC) [11] yielded the 3-oxo derivative (34%); no H–C(3) at δ(H) 3.15 (*dd*, $J = 11.2, 5.1$; *cf.* **3**); C(3)=O at δ(C) 218.1). *Baeyer-Villiger* reaction with 3-chloroperbenzoic acid (*m*CPBA) [12] was then performed with **4** to yield the lactone **5** (65%) as the sole product C(3) at δ(C) 175.1 and lactonic O-linked quaternary C(4) at δ(C) 86.1, both almost superimposable to those of **1** (*Table*)). Removal of the C(28) benzyl protecting group by 10% Pd/C and H_2 yielded 6-dehydroxy-20,29-dihydroviburolide (**6**), having a molecular formula $C_{30}H_{48}O_4$ (HR-FAB-MS). The ^{1}H- and ^{13}C-NMR (*Table*) of **6** were assigned by analysis of 2D NMR spectral data (COSY, NOESY, HMQC, and HMBC) and confirmed the proposed structure.

The key NOE relationships of **6** include H_β–C(2)/Me_β(24) (δ 1.36) and Me(25), Me(25)/Me(26), Me(26)/H–C(13), and Me(27)/H–C(18), all supporting the assignments of related chemical shifts. The signal for Me_α(23) (δ 1.45) was assigned by the observation of a two-bond correlation of C(4) (δ 86.1) to Me_α(23) and Me_β(24). The downfield-shifted signals of these two groups, caused by the effect of the lactonic O-linkage, are consistent with what was observed for **1**. Some differences in the ^{13}C-NMR data of **1** and **6** are caused by the CH_2(6) group of **6** (*vs.* OH–CH(6) of **1**) and the saturation of the C=C bond between C(20) and C(29). For instance, C(8) of **6** is downfield shifted relative to that of **1** (δ(C) 40.6 *vs.* 30.3), due to the relief of the γ-effect from OH–C(6), while C(21) of **6** is upfield shifted relative to that of **1** (δ(C) 22.7 *vs.* 30.3), due to an additional γ-effect from Me(29).

Cytotoxic activity of betulinic acid and some synthetic derivatives, especially *anti*-HIV activity, has been reported [13][14]. Since prostate cancer is a very troublesome disease for which no effective drug has been developed yet, we chose this as a target. Bio-assay of the prepared compounds and of betulinic acid against androgen-independent human prostate cancer cells (PC-3) [15] was undertaken. The result indicated that 6-dehydroxy-20,29-dihydroviburolide (**6**), having an IC_{50} of 12.3 μM, is the most active one. For comparison, the IC_{50} of betulinic acid is >30 μM in the same experiment. Furthermore, the cytotoxic effects induced by these agents were also examined by the LDH release reaction, an indicator of the necrotic cell death. Interestingly, there was little necrosis in the cells in response to the mentioned agents. These data combined with the microscopic observation that these agents induce typical apoptotic and not necrotic morphology suggest that the cytotoxic effects induced in PC-3 cells by betulinic acid and all of these derivatives is apoptotic cell death.

Experimental Part

General. RPMI-1640 Medium and all of the other cell culture reagents were from *Gibco*, Grand Island, NY, USA) and 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-2*H*-tetrazolium bromide (MTT) from *Sigma Chemical Co.*, St. Louis, MO, USA. M.p.: in open capillaries; *Fisher-Johns* melting-point apparatus; uncorrected. FT-IR Spectra: *Jasco IR-Report-100* spectrophotometer; KBr pellets. 1D and 2D NMR Spectra: *Bruker AMX-400* spectrometer in CD_3OD (δ(H) 3.30, δ(C) 49.0) or $CDCl_3$ (δ(H) 7.24, δ(C) 77.0) with *Bruker*'s standard pulse programs; δ in ppm, J in Hz; in the HMQC and HMBC experiments, $\Delta = 1$ s and $J = 140$, 8 Hz, resp., the

Table. *^{1}H- and ^{13}C-NMR* (δ/ppm), *HMBC, and NOESY Data for* **1** ($CDCl_3$). δ in ppm, J in Hz.

	6	**1**			
	δ(C)[a])	δ(C)[a])	δ(H)[b])	Key HMBC	Key NOESY
CH_2(1)	39.9 (*t*)	37.6 (*t*)	1.21 (*m*, H_α), 1.83 (*m*, H_β)	C(10)	
CH_2(2)	32.3 (*t*)	31.6 (*t*)	2.89 (br. *t*, $J = 14.2$, H_β)		H_β–C(1), H_α–C(2), Me(24), Me(25)
			2.57 (br. *dd*, $J = 6.8$, 14.2, H_α)	C(10)	H_β–C(2), H_α–C(1), H_β–C(1)
C(3)	175.1 (*s*)	175.5 (*s*)			
C(4)	86.1 (*s*)	86.7 (*s*)			
CH(5)	53.0 (*d*)	57.1 (*d*)			
CH_2(6) or H–C(6)	23.5 (*t*)	69.6 (*d*)	4.44 (br. *s*)		Me(23), Me(24)
CH_2(7)	33.3 (*t*)	41.5 (*t*)			
C(8)	40.6 (*s*)	30.3 (*s*)			
CH(9)	50.8 (*d*)	49.7 (*d*)			
C(10)	39.5 (*s*)	40.0 (*s*)			
CH_2(11)	22.2 (*t*)	20.9 (*t*)			
CH_2(12)	27.1 (*t*)	25.3 (*t*)			
CH(13)	38.5 (*d*)	37.0 (*d*)	2.25 (*m*)		Me(26)
C(14)	42.7 (*s*)	42.6 (*s*)			
CH_2(15)	29.6 (*t*)	29.6 (*t*)			
CH_2(16)	31.9 (*t*)	31.9 (*t*)			
C(17)	56.8 (*s*)	56.1 (*s*)			
CH(18)	48.6 (*d*)	49.1 (*d*)	1.60 (*m*)		Me(27)
CH(19)	44.0 (*d*)	46.7 (*d*)	2.99 (*dt*, $J = 4.4$, 10.8)		CH_2(29), Me(30)
CH(20) or C(20)	29.7 (*d*)	150.0 (*s*)			
CH_2(21)	22.7 (*t*)	30.3 (*t*)			
CH_2(22)	37.3 (*t*)	36.8 (*t*)			
Me(23)	31.1 (*q*)	33.4 (*q*)	1.49 (*s*)	C(4), C(5), C(6), C(24)	CH_2(6), Me(24)
Me(24)	26.6 (*q*)	26.7 (*q*)	1.73 (*s*)	C(4), C(5), C(23)	H_β–C(2), Me(23), Me(25)
Me(25)	18.3 (*q*)	17.1 (*q*)	1.31 (*s*)	C(1), C(5), C(9), C(10)	H_β–C(2), Me(24), Me(26)
Me(26)	15.7 (*q*)	16.9 (*q*)	1.27 (*s*)	C(7), C(8), C(9), C(14)	CH(13)
Me(27)	14.4 (*q*)	14.7 (*q*)	0.91 (*s*)	C(8), C(13), C(14), C(15)	CH(18)
C(28)	181.3 (*s*)	180.3 (*s*)			
Me(29) or CH_2(29)	14.6 (*q*)	109.8 (*t*)	4.70 (br. *s*, H_{cis} to C(19), 4.60 (br. *s*, H_{trans} to C(19))	C(20)	CH(19), Me(30)
Me(30)	22.9 (*q*)	19.2 (*q*)	1.67 (*s*)	C(19), C(20), C(30)	CH(19), CH_2(29)

[a]) Multiplicities were obtained from DEPT experiments. [b]) Signals without multiplicity were assigned from NOESY or HMBC plots.

correlation maps consisted of 512×1K data points per spectrum, each composed of 16 to 64 transients. HR-EI-MS: *Finnigan MAT-95S* spectrometer. HR-FAB-MS: *Jeol JMS-SX102A* spectrometer.

Plant Material. The leaves of *Viburnum aboricolum* were collected from the suburban mountain of I-Lan county, Taiwan, in April 2000. A voucher specimen has been deposited in the School of Pharmacy, NTU.

Extraction and Isolation. Dried leaf powder (4 kg) was macerated with MeOH ($20\,l \times 4$) at 25°. The MeOH extract (1.18 kg, 29.5%), obtained after evaporation, was suspended in 90% aq. MeOH (4 l), and the suspension was extracted with hexane ($4\,l \times 5$), to give the hexane-soluble fraction (96 g, 2.4%). The 90% MeOH layer was evaporated, the residue suspended in H_2O (4 l), and the suspension extracted with $CHCl_3$ ($4\,l \times 5$) and BuOH ($2\,l \times 5$) to give fractions soluble in $CHCl_3$ (177 g, 4.4%), BuOH (188 g, 4.7%), and H_2O (721 g).

Part of the $CHCl_3$-soluble fraction (15 g out of 177 g) was separated by column chromatography (silica gel) (600 g, 230–400 mesh), gradient hexane/AcOEt → AcOEt/MeOH 9:1): seven fractions. Repeated column chromatography (silica gel or *Sephadex LH-20*) of *Fr. 2* (40% AcOEt/hexane), *Fr. 3* (40% AcOEt/hexane), *Fr. 4* (50% AcOEt/hexane), and *Fr. 5* and *Fr. 6* (75% AcOEt/hexane) yielded betulinic acid [13] (26 mg) (*Fr. 2*), 6β-hydroxy-3,*O*-didehydrobetulinic acid [3] (106 mg) (*Fr. 3*), ursolic acid [2] (1.20 g) (*Fr. 3–6*), compound **1** (1.2 mg) (*Fr. 3*), 6β-hydroxybetulinic acid [6][7] (135 mg), 6α-hydroxy-3,*O*-didehydrobetulinic acid [3] (35 mg), sumaresinolic acid [8] (23 mg) (*Frs. 4* and *5*), and 2,3-di-*O*-acetylmaslinic acid [9] (10 mg).

(6β)-4,6-Dihydroxy-3,4-secolup-20(29)-ene-3,28-dioic Acid 3,4-Lactone (= *Viburolide*; **1**). Colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): *Table.* ^{13}C-NMR (50 MHz, $CDCl_3$): *Table.* MS: 486 (13.8, M^+), 468 (48.7), 395 (48), 259 (64.5), 201 (49.2), 187 (64.3), 175 (67.9), 147 (57), 133 (77.7), 107 (100), 81 (69), 69 (58.8), 43 (27.5), 41 (51.6). EI-HR-MS: 486.3335 (M^+, $C_{30}H_{46}O_3^+$; calc. 486.3335).

Benzyl Betulinate (= *Benzyl (3β)-3-Hydroxylup-20(29)-en-28-oate*; **2**). ^{1}H-NMR ($CDCl_3$): 7.28–7.34 (*m*, *Ph*CH_2); 5.07, 5.13 (each *d*, *J* = 12.3, PhCH_2); 4.70 (*d*, *J* = 2.0, H_b–C(29)); 4.57 (br. *s*, H_a–C(29)); 3.15 (*dd*, *J* = 11.2, 5.1, H_α–C(3)); 3.00 (*ddd*, *J* = 11.2, 11.2, 4.6, H_β–C(19)); 1.65 (br. *s*, Me(30)); 0.93, 0.92, 0.78, 0.74, 0.73 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)). HR-FAB-MS: 547.4151 ($[M+H]^+$, $C_{37}H_{55}O_3^+$; calc. 547.4172).

Benzyl 20,29-Dihydrobetulinate (= *Benzyl (3β)-3-Hydroxylupan-28-oate*; **3**). To a soln. of **2** (1.00 g) in AcOH/dioxane 1:3 (20 ml) was added 10% Pt/C (200 mg). After the usual degassing, the mixture was heated to 50° and hydrogenated (H_2, 1 atm) for 48 h. The mixture was filtered through a *Celite* pad and the residue washed with $CHCl_3$. The filtrate and washings were evaporated: essentially pure **3** (998 mg, quant.). Colorless solid. M.p. 201–202°. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.28–7.34 (*m*, *Ph*CH_2); 5.10, 5.06 (each *d*, *J* = 12.3, PhCH_2); 3.16 (*dd*, *J* = 11.2, 4.9, H_α–C(3)); 0.94, 0.90, 0.81, 0.79, 0.73 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.733 (*d*, *J* = 5.6) and 0.719 (*d*, *J* = 5.8, Me(29), Me(30)). HR-FAB-MS: 549.4343 ($[M+H]^+$, $C_{37}H_{57}O_3^+$; calc. 549.4308).

*Benzyl 3,*O*-Didehydro-20,29-dihydrobetulinate* (= *Benzyl 3-Oxolupan-28-oate*; **4**). To a soln. of **3** (500 mg) in CH_2Cl_2 (15 ml) was added PCC (350 mg). After stirring for 16 h, the mixture was diluted with Et_2O and filtered through a *Celite* pad. The residue was washed several times with Et_2O. The filtrate and washings were evaporated, and the residue was chromatographed (silica gel (15 g, 230–400 mesh), AcOEt/hexane 1:49): **4** (171 mg, 34.4%). Colorless needles (from MeOH). M.p. 148–150°. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.28–7.34 (*m*, *Ph*CH_2); 511, 5.06 (each *d*, *J* = 12.3, PhCH_2); 1.09, 1.00, 0.91, 0.89, 0.75 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.82, 0.72 (each *d*, *J* = 6.8, Me(29), Me(30)). ^{13}C-NMR (100 MHz, $CDCl_3$; key signals): 218.1 (*s*, C(3)); 176.0 (*s*, C(28)); 65.6 (*t*, Ph*C*H_2); 47.3 (*s*, C(4)). HR-FAB-MS: 547.4167 ($[M+H]^+$, $C_{37}H_{55}O_3^+$; calc. 547.4152).

4-Hydroxy-3,4-secolupane-3,28-dioic Acid 28-(Benzyl Ester) 3,4-Lactone (**5**). To a soln. of **4** (30 mg) in CH_2Cl_2 (10 ml) was added *m*CPBA (50 mg) and $NaHCO_3$ (30 mg). The mixture was stirred for 36 h at r.t. until a white cloudy suspension formed. The latter was filtered through a *Celite* pad, and the residue was washed with CH_2Cl_2. The combined filtrate and washings were washed with 10% $NaHSO_3$ soln., dried ($MgSO_4$), and evaporated, and the residue chromatographed (silica gel (5.0 g, 230–400 mesh), $CHCl_3$): **5** (20 mg, 65%). Colorless solid. M.p. 192–194° (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.28–7.34 (*m*, *Ph*CH_2); 5.11, 5.06 (each *d*, *J* = 12.3, PhCH_2); 2.59 (*ddd*, *J* = 14.3, 12.6, 4.4, H_α–C(2)); 2.46 (*ddd*, *J* = 14.3, 6.2, 3.3, H_β–C(2)); 2.24 (*m*, H–C(19)); 1.44, 1.35, 1.02, 0.89, 0.76 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.82, 0.72 (each *d*, *J* = 6.8, Me(29), Me(30)). ^{13}C-NMR (100 MHz, $CDCl_3$): 175.9 (*s*, C(28)); 175.1 (*s*, C(3)); 86.1 (*s*, C(4)); 65.6 (*t*, Ph*C*H_2). HR-FAB-MS: 563.4115 ($[M+H]^+$, $C_{37}H_{55}O_4$; calc. 563.4101).

6-Dehydroxy-20,20-dihydroviburolide (= *4-Hydroxy-3,4-secolupane-3,28-dioic Acid 3,4-Lactone*; **6**). To a soln. of **5** (10 mg) in AcOEt (10 ml) was added 10% Pd/C (10 mg). After the usual degassing, the mixture was hydrogenated (H_2, 1 atm) overnight. The mixture was filtered through a *Celite* pad and the residue washed with $CHCl_3$. The filtrate and washings were evaporated, and the residue was chromatographed (silica gel (5.0 g, 230–400 mesh), $CHCl_3$): **6** (8.3 mg, 98%). Colorless solid. M.p. 216–218° (MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): 2.61 (*ddd*, *J* = 14.1, 10.0, 4.4, H_α–C(2)); 2.47 (*ddd*, *J* = 14.3, 6.1, 3.4, H_β–C(2)); 2.24 (*m*, H–C(13), CH_2(16), H–C(19)); 1.87 (br. *dd*, *J* = 7.1, 3.6, H_β–C(22)); 1.82 (*m*, H_β–C(1)); 1.80 (*m*, H–C(20)); 1.71 (*m*, H–C(5)); 1.70 (*m*, H_β–C(12)); 1.62 (*m*, CH_2(21)); 1.55 (*m*, H_α–C(1)); 1.50 (*m*, H_α–C(11)); 1.48 (*m*, H_α–C(15)); 1.45 (*s*, Me(23)); 1.42–1.40 (*m*, 5 H, CH_2(6), CH_2(7), H–C(9), H_β–C(11)); 1.39 (*m*, H–C(18)); 1.36 (*s*, Me(24)); 1.30 (*m*, H_α–C(22)); 1.22 (*m*, H_β–C(15)); 1.20 (*m*, H_α–C(12)); 1.05 (*s*, Me(25)); 0.96 (*s*, Me(26)); 0.94 (*s*, Me(27)); 0.84 (*d*, *J* = 6.8, Me(29)); 0.74 (*d*, *J* = 6.7, Me(30)). Key NOESY ($CDCl_3$): H_β–C(2)/H_β–C(1), H_α–C(2), Me(24), and Me(25), Me(23)/H–C(5), H–C(13)/H_β–C(12) and Me(26), Me(24)/Me(25), Me(27)/H_α–C(16) and H–C(18), Me(29)/H–C(19) and Me(30), Me(30)/H–C(19) and Me(29). Major HMBC: C(1)/

H_α–C(2), H_β–C(2), and Me(25), C(2)/H_α–C(1), C(3)/H_α–C(1), H_β–C(1), H_α–C(2), H_β–C(2), and Me(24), C(4)/H–C(5), Me(23), and Me(24), C(5)/H–C(6), Me(23), Me(24), and Me(25), C(6)/H–C(7), C(7)/Me(26), C(8)/H–C(9), Me(26), and Me(27), C(9)/H–C(11), Me(25), and Me(26), C(10)/H_α–C(1), H–C(5), and Me(25), C(12)/H–C(11) and H–C(13), C(13)/H–C(18) and Me(27), C(14)/H–C(15), H–C(16), Me(26), and Me(27), C(15)/H–C(16) and Me(27), C(17)/H–C(15), H–C(16), H–C(18), and H_β–C(22), C(18)/H–C(13), H–C(19), and H_β–C(22), C(19)/H–C(18), H_β–C(22), Me(29), and Me(30), C(20)/H–C(18), Me(29), and Me(30), C(21)/H–C(19), C(23)/H–C(5) and Me(24), C(24)/H–C(5) and Me(23), C(28)/H–C(16), H–C(18), and H–C(22), Me(29)/Me(30), Me(30)/Me(29). ^{13}C-NMR (100 MHz, $CDCl_3$): *Table*. HR-FAB-MS: 473.3654 ($[M+H]^+$, $C_{30}H_{49}O_4^+$; calc. 473.3632).

Cell Cultures. Human prostate adenocarcinoma PC-3 cells were cultured in RPMI-1640 medium supplemented with 10% FBS (*v*/*v*) and penicillin (100 units ml^{-1})/streptomycin (100 µg ml^{-1}). Cells were incubated at 37° in a humidified atmosphere of 5% CO_2 in air. The medium was changed every 2 days, and cells were passaged by treatment with 0.05% trypsin/0.02% EDTA soln.

Cytotoxic Assessment. The cytotoxic reaction was assessed by the MTT assay method. MTT was dissolved in phosphate-buffered saline (PBS) at a conc. of 5 mg ml^{-1} and filtered through a *Millipore* membrane. From the stock soln., 10 µl per 100 µl of medium was added to each well, and plates were gently shaken and incubated at 37° for 3 h. Treatment of living cells with MTT produces a dark blue formazan product, whereas no such staining is observed in dead cells. After the loading of MTT, the medium was replaced with 100 µl acidified ^{i}PrOH and was left for 20–30 min at r.t. for color development, and then the 96-well plate was read by the enzyme-linked immunosorbent-assay (ELISA) reader (570 nm) to obtain the absorbance density values.

Assessment of Lactate Dehydrogenase (LDH) Release [16]. The necrotic cell death was measured by the release of LDH into the culture medium, which indicates the loss of membrane integrity and cell necrosis. LDH Activity was measured using a commercial assay kit (cytotoxicity assay kit, *Promega*, Madison, WI, USA), where the released LDH in culture supernatants is measured with a coupled enzymatic assay in which a tetrazolium salt is converted to a red formazan product. The necrotic percentage was expressed as (sample value/maximal release) × 100%, where the maximal release was obtained following the treatment of control cells with 0.5% *Triton X-100* for 10 min at r.t.

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REFERENCES

[1] K. C. Yang, S. T. Chiu, in 'Flora of Taiwan' 2nd edn., Editorial Committee of the Flora of Taiwan, Dept. Botany, National Taiwan University, Taipei, 1998, Vol. 4, p. 752.
[2] Y. K. Kim, S. K. Yoon, S. Y. Ryu, *Planta Medica* **2000**, *66*, 485.
[3] M. Kuroyanagi, M. Shiotsu, T. Ebihara, H. Kawai, A. Ueno, S. Fukushima, *Chem. Pharm. Bull*. **1986**, *34*, 4012.
[4] M. Scholichin, K. Yamasaki, R. Kasai, D. Tanaka, *Chem. Pharm. Bull*. **1980**, *28*, 1006.
[5] S. S. Lee, B. F. Lin, K. C. S. Chen, *Chin. Pharm. J.* **1995**, *47*, 511.
[6] A. P. Dantanarayana, N. S. Kumar, M. U. S. Sultanbawa, S. Balasubramaniam, *J. Chem. Soc., Perkin Trans. 1* **1981**, 2717.
[7] A. R. Bilia, I. Morelli, J. Mendez, *J. Nat. Prod.* **1996**, *59*, 297.
[8] W. R. Chan, V. Sheppard, K. A. Medford, W. F. Tinto, W. F. Reynolds, S. McLean, *J. Nat. Prod*. **1992**, *55*, 963.
[9] A. Yagi, N. Okamura, Y. Haraguchi, K. Noda, I. Nishioka, *Chem. Pharm. Bull*. **1978**, *25*, 3075.
[10] S. S. Lee, W. C. Su, K. C. Liu, *J. Nat. Prod*. **1991**, *54*, 615.
[11] E. J. Corey, J. W. Suggs, *Tetrahedron Lett*. **1975**, *31*, 2647.
[12] K. Mori, H. Mori, *Tetrahedron* **1987**, *43*, 4097.
[13] W. N. Setzer, M. C. Setzer, R. B. Bates, B. R. Jackes, *Planta Medica* **2000**, *66*, 176.
[14] I. C. Sun, H. K. Wang, Y. Kashiwada, J. K. Shen, L. M. Cosentino, C. H. Chen, L. M. Yang, K. H. Lee, *J. Med. Chem*. **1998**, *41*, 4648, and ref. cit. therein.
[15] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[16] S. C. Chueh, J. H. Guh, M. K. Lai, C. M. Teng, *J. Urol*. **2001**, *166*, 347.

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