Cytotoxic Constituents of the Stem Bark of *Neolitsea acuminatissima*

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Three new eudesmanolide sesquiterpenes, neolitacumone A–C (**1**–**3**), and one new benzylisoquinoline alkaloid, neolitacumonine (**5**), along with 27 known compounds were isolated from the stem bark of *Neolitsea acuminatissima*. The structures of compounds **1**–**3** and **5** were established on the basis of spectral and chemical evidence. Compounds **2**, **3**, and **20** were selectively inhibitory to Hep 2,2,15 cells with IC$_{50}$ values in the range 0.24–0.04 μg/mL. Compound **20** was marginally cytotoxic to Hep G$_2$ cells.

Constituents of the *Neolitsea* genus (Lauraceae) have been reported to possess diverse bioactivities.[1–7] Thaliporphine, isolated from *N. konishii*, has been found to possess vasoconstricting action and selectively inhibits expression of inducible, but not constitutive, nitric oxide synthase.[1–4] Several cytotoxic and antiplatelet aggregation sesquiterpenes and alkaloids were also isolated and characterized from *N. konishii*,[5,6] *N. villosa*,[7] *N. parvigemma*,[5] *N. variabillima*,[5] and *N. aurata*.[5] In our continuing search for novel plant bioactive agents from Lauraceous plants, *N. acuminatissima* (Hayata) Kanehira & Sasaki, an evergreen shrub that is distributed in part of the highest mountain, Mountain Yu, and the middle of Taiwan,[8] was chosen for investigation. An alkaloid, (+)-laurotetanine, had been isolated from the stem bark of this plant in a previous study.[9] The current investigation led to the isolation of four eudesmanolide sesquiterpenes, neolitacumone A–C (**1**–**3**) and 1β-acetoxyeudesman-4(15),7(11),8(9)-trien-8,12-olide (**4**),[10–13] and 15 alkaloids, neolitacumonine (**5**), (−)-norushisunine (**6**),[14] (+)-norboldine (**7**),[6] (+)-*N*-methylactinodaphnine (**8**),[6] (+)-cassythine (**9**),[15] (−)-thalicsimidine (**10**),[6] liriodenine (**11**),[6] (+)-*O*-methylflavinantine (**12**),[6] (+)-reticuline,[14] (−)-anonaine,[14] (+)-boldine,[6] (−)-boldine,[6] (+)-isoboldine,[15] (+)-actinodaphnine,[15] and oxoglaucine.[6] Three lignans, (+)-lyoniresinol (**13**),[16] (+)-syringaresinol (**14**),[16] and (±)-glaberide I (**15**),[17] four benzoids, vanillin (**16**),[18] isovanillin (**17**),[18] *p*-methoxybenzoic acid (**18**),[19] and methylparaben (**19**),[19] and four steroids, mixtures of β-sitosterol and stigmasterol[19] and mixtures of β-sitosteryl-D-glucoside and stigmasteryl-β-D-glucoside,[19] together with one para-quinone, 2,6-dimethoxy-*p*-benzoquinone (**20**),[19–21] were also isolated from the stem bark of this species. Among them, **1**–**3** and **5** are new compounds, and their structures were established on the basis of interpretation of NMR and MS spectral data. Compounds **4** and **6**–**20** were isolated for the first time from this genus. Compounds **1**–**3**, **13**, **15**, and **20** were evaluated for their cytotoxicity to two human hepatocarcinoma cell lines, Hep G$_2$ and Hep 2,2,15. Compounds **2**, **3**, and **20** were significantly and selectively inhibitory to the Hep 2,2,15 cell line with IC$_{50}$ values in the range 0.24–0.04 μg/mL. Compound **20** showed marginal cytotoxicity to the Hep G$_2$ cell line.
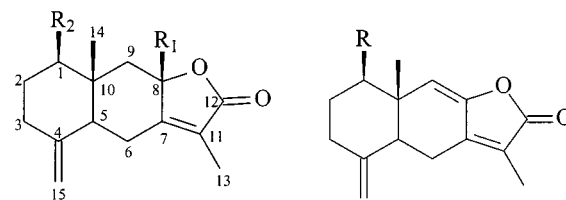
	R$_1$	R$_2$			R
1	OH	OH		**3**	OH
1a	OH	OAc		**4**	OAc
2	H	OH			
2a	H	OAc			
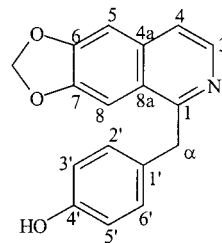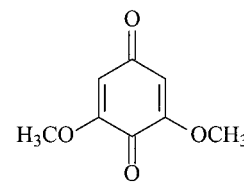
Results and Discussion

Compound **1** was obtained as white prisms from CHCl$_3$. The molecular formula, C$_{15}$H$_{20}$O$_4$, was confirmed by the HREIMS. Peaks at *m*/*z* 264 [M]$^+$ and 246 [M − H$_2$O]$^+$ in the EIMS suggested the presence of a hydroxyl group. The presence of a hydroxyl group and an α,β-unsaturated lactone was indicated by its IR (ν_{max} at 3439 and 1749 cm^{-1}, respectively) and UV (λ_{max} at 218 nm) spectra.[10] The ^{1}H NMR spectrum exhibited signals for two methyl groups at δ 0.97 (3H, s) and 1.79 (3H, d, *J* = 1.6 Hz), which are typical of Me-14 and Me-13 of eudesmanolides, along with one terminal double bond at δ 4.86 (1H, d, *J* = 1.6 Hz) and 4.89 (1H, d, *J* = 1.6 Hz) for H-15.[10–13] The ^{1}H NMR patterns of **1** are similar to those of a known eudesmanolide, 1β-acetoxy-8β-hydroxyeudesman-4(15),7(11)-dien-8α,12-olide,[10] except for the signal for an acetoxy group instead of a hydroxyl group at C-1, and the H-1 proton signal at δ 4.62 of 1β-acetoxy-8β-hydroxyeudesman-4(15),7(11)-dien-8α,12-olide was shifted upfield to δ 3.39. The ^{13}C NMR
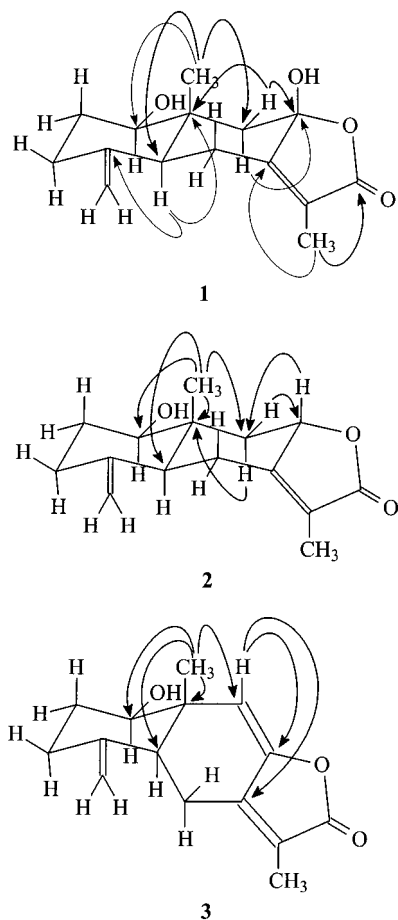
Figure 1. Long-range HETCOR correlations of **1**–**3**.

spectrum and DEPT experiments showed 15 carbons consisting of two methyls at δ 8.0 and 11.0, five methylenes at δ 25.0, 31.5, 34.7, 49.4, and 108.0 (a terminal double bond CH_2 carbon), two methines at δ 50.6 and 79.1 (a carbinol carbon), and six quaternary carbons at δ 42.2, 105.5, 122.5, 148.7, 162.7, and 174.4 (a carbonyl carbon), consistent with the skeleton of eudesmanolides. The signals at δ 8.0 (C-13), 105.5 (C-8), 122.5 (C-11), 162.7 (C-7), and 174.4 (C-12) are characteristic of a 5-hydroxy-3-methyl-5-hydrofuran-2-one functional moiety of eudesmanolides.[10] Complete unambiguous assignments for the ^{1}H and ^{13}C NMR signals were made by combination of COSY, HET-COR, long-range HETCOR, and NOESY spectra. In the long-range HETCOR spectrum (Figure 1) Me-14 (δ 0.97) shows [2]J correlation to C-10 (δ 42.2) and [3]J correlations to C-9 (δ 49.4), C-5 (δ 50.6), and C-1 (δ 79.1). The methylene protons H-9α (δ 1.41) and H-9β (δ2.64) display [2]J correlations to C-10 (δ 42.2) and C-8 (δ 105.5). Therefore, the planar structure of **1** was confirmed. The *trans*-A/B ring junction of **1** was confirmed by the observation of NOESY cross-peaks between δ 0.97 (Me-14)/1.60 (H-2β), 0.97 (Me-14)/2.47 (H-6β), and 0.97 (Me-14)/2.64 (H-9β), as well as between δ 1.60 (H-2β)/2.35 (H-3β) (Figure 1).[10] Thus, **1** was determined to be 1β,8β-dihydroxyeudesman-4(15),7(11)-dien-8α,12-olide and was named neolitacumone A. Treatment of **1** with acetic anhydride and pyridine failed to yield the expected acetoxy derivative **1a**,[10] providing instead of a compound with mp, UV, IR, and ^{1}H and ^{13}C NMR data identical to known compound **4**,[12] thus confirming the stereochemistry of **1** as 1β-OH, 5α-H, and 10β-CH$_3$.

Compound **2** was isolated as white prisms from CHCl$_3$. The molecular formula was established as $C_{15}H_{20}O_3$ by HREIMS, which is an oxygen atom less than **1**. Its UV and IR spectra are characteristic of an α,β-unsaturated

lactone.[10–13] The[1]H NMR spectrum of **2** exhibits signals for two methyl groups and one terminal double bond, as in **1**.[10] Inspection of the ^{13}C NMR and DEPT data for **2** showed 15 resonances consistent with a eudesmanolide skeleton. The signal for C-8, which occurred at δ 105.5 in **1**, is replaced by a signal at δ 78.3 in **2**, indicating that C-8 is unsubstituted in **2**. Signals at δ 7.8 (C-13), 78.3 (C-8), 119.9 (C-11), 162.9 (C-7), and 175.3 (C-12) represent the 3-methyl-5-hydrofuran-2-one moiety of eudesmanolides. The ^{1}H and ^{13}C NMR data of **2** are similar to those of a known eudesmanolide, 1β-acetoxyeudesman-4(15),7(11)-dien-8α,-12-olide (**2a**),[10] except the signal for the C-1 acetoxyl group in **2a** is replaced by a signal for the C-1 hydroxyl group in **2**. HETCOR and long-range HETCOR spectra (Figure 1) were employed for determining the planar structure of **2**. In the long-range HETCOR spectrum, Me-14 (δ 0.69) shows [2]J correlation to (C-10) (δ 41.2) and [3]J correlations to C-9 (δ 43.5), C-5 (δ 47.6), and C-1 (δ 77.6). The methylene protons, H-9α (δ 0.82) and H-9β (δ 2.65), display [2]J correlations to C-10 (δ 41.2) and C-8 (δ 78.3). The *trans*-A/B ring junction of **2** was confirmed by NOESY cross-peaks between δ 0.69 (Me-14)/1.42 (H-2β), 0.97 (Me-14)/2.22 (H-6β), 0.97 (Me-14)/2.65 (H-9β), and 0.97 (Me-14)/4.66 (H-8β), as well as between 1.42 (H-2β)/2.35 (H-3β). These results suggested a β-orientation of H-8. Accordingly, **2** was determined to be 1β-hydroxyeudesman-4(15),7(11)-dien-8α,12-olide and was given the trivial name neolitacumone B. Compound **2** was acetylated to obtain the known acetoxy derivative **2a**, and the physical data of mp, UV, IR, and ^{1}H and ^{13}C NMR spectra were consistent with those of the known compound 1β-acetoxyeudesman-4(15),7(11)-dien-8α,12-olide.[10]

Compound **3** was obtained as white prisms from CHCl$_3$. The HREIMS confirmed the molecular formula, $C_{15}H_{18}O_3$. The presence of a hydroxyl group and a 5-methylenefuran-2-one conjugated system was revealed by its IR (ν_{max} at 3443 and 1763 cm^{-1}) and UV (λ_{max} at 276 nm) spectra, respectively.[12] The ^{1}H NMR spectrum of **3** exhibits signals for two methyl groups and one terminal double bond, as in **1**.[12] Due to the existence of a 5-exo-double bond of the unsaturated lactone ring moiety, it is reasonable to assign a proton signal at δ 6.17 to H-9.[12] In addition, the ^{13}C NMR and DEPT spectra show 15 resonance lines consistent with a eudesmanolide skeleton. The olefinic carbon signals at δ 148.6 and 115.7 of **3** were assigned to C-8 and C-9.[12] Thus, signals at δ 8.4 (C-13), 115.7(C-9), 120.4 (C-11), 147.6 (C-7), 148.6 (C-8), and 171.5 (C-12) further confirmed the presence of a 3-methyl-5-methylenefuran-2-one functional moiety.[12] In the long-range HETCOR spectrum (Figure 1), Me-14 (δ 0.89) shows [2]J correlation to C-10 (δ 42.8) and [3]J correlations to C-9 (δ 115.7), C-5 (δ 45.9), and C-1 (δ 74.4). The proton H-9 (δ 6.17) displays J correlations to C-7 (δ 147.6) and [2]J correlation to C-8 (δ 148.6). The *trans*-A/B ring junction of **3** was also confirmed by the NOESY spectrum. The molecular weight of **3** was 2 amu less than **2**, which indicated a single/double bond difference between them. Compound **2** was 18 amu less than **1**, which indicated they are biogenetically correlated through dehydration. Thus, the structure of **3** was determined to be 1β-hydroxyeudesman-4(15),7(11),8(9)-trien-8,12-olide and was named neolitacumone C. Compound **3** was acetylated to obtain the synthetic compound **4**,[12] which also confirmed the structure of **3**.

Compound **5** was obtained as a white amorphous powder from CHCl$_3$ and was positive to Dragendorff's reagent. The molecular formula $C_{17}H_{13}O_3N$ was confirmed by the HRE-IMS. The UV spectrum shows intense absorption bands at λ_{max} 261, 298, and 330 nm, consistent with a 6,7,4'-oxygenated benzylisoquinoline skeleton.[22,23] The IR spec-

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1**−**3** in CDCl$_3$

position	δ_H, mult. (J in Hz) 1	2	3	δ_C, mult. 1	2	3
1	3.39, dd (11.6, 4.4)	3.24, dd (11.4, 4.4)	3.62, dd (11.6, 4.4)	79.1, d	77.6, d	74.4, d
2α	1.83, ddd (13.2, 5.4, 1.2)	1.66, ddd (13.2, 4.4, 1.0)	1.93, ddd (13.6, 5.0, 2.0)	31.5, t	30.1, t	31.3, t
2β	1.60, ddd (13.2, 4.6, 1.2)	1.42, ddd (13.2, 4.8, 1.0)	1.61, ddd (13.6, 4.6, 1.2)			
3α	2.08, td (13.8, 5.4)	1.90, td (13.6, 4.4)	2.36, ddd (13.8, 5.0, 2.0)	34.7, t	33.5, t	33.5, t
3β	2.35, ddd (13.8, 5.4, 2)	2.35, td (13.6, 4.4)	2.35, td (13.8, 5.0)			
4				148.7, s	146.8, s	146.0, s
5	1.84, m	1.66, m	2.24, dd (4.6, 4.0)	50.6, d	47.6, d	45.9, d
6α	2.65, dd (13.0, 3.2)	2.56, dd (13.9, 3.2)	2.71, dd (16.8, 4.0)	25.0, t	24.9, t	22.1, t
6β	2.47, t (13.0)	2.22, d (13.9)	2.57, t (16.8)			
7				162.7, s	162.9, s	147.6, s
8		4.66, dd (10.4, 6.4)		105.5, s	78.3, d	148.6, s
9			6.17, s	49.4, t	43.5, t	115.7, d
9α	1.41, d (13.6)	0.82, t (12.0)				
9β	2.64, d (13.6)	2.65, dd (12.0, 6.4)				
10				42.2, s	41.2, s	42.8, s
11				122.5, s	119.9, s	120.4, s
12				174.4, s	175.3, s	171.5, s
13	1.79, d (1.6)	1.62, d (1.6)	1.88, d (2.0)	8.0, q	7.8, q	8.4, q
14	0.97, s	0.69, s	0.89, s	11.0, q	10.1, q	12.7, q
15a	4.86, d (1.6)	4.48, br s	4.72, d (1.2)	108.0, t	107.9, t	108.6, t
15b	4.89, d (1.6)	4.72, br s	4.98, d (1.2)			

Table 2. In Vitro Cytotoxicity Data of Compounds **1**−**3**, **13**, **15**, and **20**[a]

	cell lines[b]/IC$_{50}$ (μg/mL)	
compound	Hep G$_2$	Hep 2,2,15
1	8.4 ± 0.74	8.4 ± 0.26
2	7.6 ± 0.22	0.24 ± 0.04
3	8.5 ± 0.43	0.08 ± 0.02
13	13.0 ± 0.52	12.9 ± 0.96
15	8.5 ± 0.84	8.6 ± 0.64
20	4.2 ± 0.51	0.04 ± 0.01
adriamycin	0.05 ± 0.01	0.45 ± 0.06

[a] The values represents means ± SEM from three determinations. [b] Hep G$_2$ is human hepatoma cell line, and Hep 2,2,15 is Hep G$_2$ cell line transfected with hepatitis B virus (HBV).

trum absorption bands at ν_{max} 3400, 1051, and 949 cm^{-1} indicated the presence of hydroxyl and methylenedioxy groups, respectively.[22,23] The chemical shifts at δ 8.54 (1H, d, J = 5.6 Hz) and 7.48 (1H, d, J = 5.6 Hz) in the ^{1}H NMR are characteristic of the H-3 and H-4 of benzylisoquinoline alkaloids. Additionally, the 6,7,4′-oxygenated benzylisoquinoline skeleton was suggested by the signals at δ 7.17 (1H, s), δ 7.71 (1H, s), and an A$_2$B$_2$ coupling system at δ 7.11 (2H, d, J = 8.4 Hz) and 7.42 (2H, d, J = 8.4 Hz) in the aromatic region, along with a signal at δ 4.69 (2H, s). On the basis of the pattern of ^{1}H NMR, a methylenedioxy signal at δ 6.05 (2H, s) was assigned to the substitution of C-6 and C-7; thus the hydroxyl group should be located at C-4′.[23] The chemical shift of the benzyl protons at δ 4.69 indicated that the benzyl group was bonded to the isoquinoline moiety.[22,23] In the ^{13}C NMR spectrum, 15 aromatic signals between δ 159.3 and 102.2, a methylenedioxy carbon at δ 103.3, and a signal for a methylene carbon at δ 41.7 were evident and further supported the structure of **5**.[24] The complete assignments of the aromatic protons of **5** were made by COSY, HETCOR, and NOESY experiments. Correlations between H-3/H-4, H-4/H-5, H-8/α, H-2′/α, and H-6′/α were observed in the NOESY spectrum. From a consideration of all the above data, **5** was elucidated as 1-(*p*-hydroxy-benzyl)-6,7-methylenedioxyisoquinoline and given the trivial name neolitacumonine.

The new constituents **1**−**3** and known compounds **13**, **15**, and **20** were chosen for evaluating cytotoxicity to human hepatocarcinoma cells (Table 2). The other compounds were not investigated for their cytotoxicity because they were either previously evaluated for cytotoxicity or because they were not available in sufficient quantity. Compounds **2** and **3** show significant inhibition against Hep 2,2,15 cells with

IC$_{50}$ 0.24 and 0.08 μg/mL, respectively, while compound **1** is only weakly active at IC$_{50}$ 8.4 μg/mL. Thus, it appears that the presence of the 8-hydroxyl decreases the cytotoxicity to this cell line. It is interesting that simple known molecule **20** showed strong inhibition against Hep 2,2,15 cells (IC$_{50}$ 0.04 μg/mL), but was only marginally inhibitory (IC$_{50}$ 4.2 μg/mL) to Hep G$_2$ cells. In a previous study, Handa et al. also reported **20** to be cytotoxic to different cell lines.[20] Furthermore, Gunatilaka et al. isolated and synthesized a series of paraquinone analogues,[21] which also exhibited significant cytotoxicity toward other cell lines.

Known compounds were characterized by comparison of their spectral data (UV, IR, ^{1}H and ^{13}C NMR) with those reported in the literature.[10−21]

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H (400 MHz, using CDCl$_3$ or C$_5$D$_5$N as solvents for measurement), ^{13}C, DEPT, HETCOR, COSY, NOESY, and long-range HETCOR NMR spectra were obtained on a Varian NMR spectrometer (Unity Plus). FABMS and EIMS were collected on a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC−MS instrument having a direct inlet system. HREIMS and HR-FABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC, and precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. Spots were detected by spraying with Dragendorff's reagent or 50% H$_2$SO$_4$ and then heating on a hot plate.

Plant Material. The stem barks of *N. acuminatissima* (Hay.) Kanehira & Sasaki were collected from Tong-Po Village, Nan-Tou County, Taiwan, in August 1971. A voucher specimen was prepared by the late Professor Sheng-Teh Lu and deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The stem bark (3.1 kg) of *N. acuminatissima* was extracted repeatedly with EtOH at room temperature for 24−48 h. The combined EtOH extracts were evaporated and partitioned to yield CHCl$_3$ and aqueous extracts. The CHCl$_3$ solution was extracted with 3% HCl to leave the acidic portion and CHCl$_3$ solution (part A). The acidic portion was basified with NH$_4$OH and extracted with CHCl$_3$, and we gathered the CHCl$_3$ portion (part B). The CHCl$_3$

solution (part B), which was positive to alkaloidal test with Dragendorff's reagent, was dried and evaporated to leave a brownish viscous residue (1.69 g). The crude alkaloid portion (part B) was chromatographed over Si gel and eluted with *n*-hexane−CHCl$_3$−MeOH mixtures of increasing polarities to obtain 22 fractions. Fractions 4−6, eluted with CHCl$_3$−MeOH (20:1), were further separated by Si gel column chromatography to afford compounds **11** (11.0 mg), oxoglaucine (3.5 mg), and **12** (6.0 mg). Compounds (−)-anonaine (5.5 mg) and (+)-isoboldine (2.0 mg) were isolated from fraction 7 by Si gel column chromatography using CHCl$_3$−MeOH (15:1) as eluting solvent systems. Fractions 8−11, eluted with CHCl$_3$−MeOH (10:1), were purified by Si gel column chromatography to afford compounds **5** (2.5 mg), **6** (6.3 mg), **9** (14.0 mg), and **10** (8.5 mg). Fraction 12, eluted from CHCl$_3$−MeOH (8:1), was separated over Si gel to obtain compounds **7** (2.6 mg), (+)-boldine (2.4 mg), (−)-boldine (3.0 mg), and (+)-actinodaphnine (12.0 mg). Fractions 13−17 were separated on Si gel eluted with CHCl$_3$−MeOH (8:1) to afford compounds (+)-reticuline (3.2 mg) and **8** (22.0 mg). The CHCl$_3$ layer (part A) was concentrated and chromatographed over Si gel and eluted with *n*-hexane−CHCl$_3$−MeOH mixtures of increasing polarities to obtain 8 fractions. Fraction 2 was rechromatographed over Si gel eluting with a gradient of CHCl$_3$−MeOH (40:1) to obtain compounds **2** (126.0 mg) and **4** (18.0 mg), successively. Compound **3** (78.0 mg) was isolated by Si gel column chromatography from fraction 3 with CHCl$_3$−MeOH, 40:1.5, as elute solvent. Fraction 5, eluted with CHCl$_3$−MeOH (20:0.5), was purified over Si gel to afford compounds **1** (65.0 mg), **13** (13.0 mg), **14** (11.0 mg), **15** (7.0 mg), and a mixture of *β*-sitosterol and stigmasterol (11.0 mg). Fraction 6 was purified by Si gel column chromatography (CHCl$_3$−MeOH, 9:1) to afford compounds **16** (18.0 mg), **17** (12.0 mg), **18** (3.4 mg), and **19** (4.5 mg). Fraction 7 was further separated by Si gel column chromatography eluting with CHCl$_3$−MeOH (10:1) to give compounds **20** (16.0 mg) and a mixture of *β*-sitosterol-*β*-D-glucoside and stigmasterol-*β*-D-glucoside (38.0 mg).

Neolitacumone A (1): colorless prisms (CHCl$_3$); mp 112−114 °C; [α]$^{25}_{D}$ +158° (*c* 0.14, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 218 (3.94) nm; IR (neat) ν$_{max}$ 3439, 1749, 1645 cm$^{−1}$; ^{1}H (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) spectral data, see Table 1; EIMS *m*/*z* 264 [M]$^+$ (12), 246 (80), 213 (93), 185 (70), 162 (100), 134 (57), 109 (55), 77 (53); HREIMS *m*/*z* 264.1364 (calcd for C$_{15}$H$_{20}$O$_4$, 264.1361).

Acetylation of 1. Compound **1** (10 mg) was dissolved in a mixture of dry pyridine (2 mL) and acetic anhydride (2 mL). The reaction mixture was stirred overnight at room temperature. After aqueous workup, the reaction mixture was extracted with CHCl$_3$ (5 mL × 3), and the CHCl$_3$ extract was washed with water, dried over anhydrous MgSO$_4$, and evaporated under reduced pressure to yield the monoacetate **4**. The mass and spectral data were consistent with the literature data.[12]

Neolitacumone B (2): colorless prisms (CHCl$_3$); mp 159−161 °C; [α]$^{25}_{D}$ +254° (*c* 0.12, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 218 (4.42) nm; IR (neat) ν$_{max}$ 3449, 1738, 1648 cm$^{−1}$; ^{1}H (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) spectral data, see Table 1; EIMS *m*/*z* 248 [M]$^+$ (100), 220 (21), 202 (8), 187 (23), 159 (40), 135 (41), 121 (38), 91 (62); HREIMS *m*/*z* 248.1417 (calcd for C$_{15}$H$_{20}$O$_3$, 248.1412).

Acetylation of 2. Compound **2** was acetylated as described above to obtain **2a**. The mass and spectral data were consistent with the literature data.[10]

Neolitacumone C (3): white needles (CHCl$_3$); mp 122−124 °C; [α]$^{25}_{D}$ +78° (*c* 0.06, CHCl$_3$), UV (MeOH) λ$_{max}$ (log ε) 276 (4.65) nm; IR (neat) ν$_{max}$ 3443, 1763, 1649 cm$^{−1}$; ^{1}H (CDCl$_3$, 400 MHz) and ^{13}C NMR (CDCl$_3$, 100 MHz) spectral data, see Table 1; EIMS *m*/*z* 246 [M]$^+$ (26), 228 (13), 213 (38), 200 (22), 185 (40), 162 (48), 91(100), 77 (58), 53 (86); HREIMS *m*/*z* 246.1255 (calcd for C$_{15}$H$_{18}$O$_3$, 246.1255).

Neolitacumonine (5): amorphous powder; UV (MeOH) λ$_{max}$ (log ε) 261 (4.56), 298 (4.45), and 330 (4.38) nm; IR (neat) ν$_{max}$ 3400, 1051, 949 cm$^{−1}$; ^{1}H NMR (400 MHz, C$_5$D$_5$N) δ 8.54

(1H, d, *J* = 5.6 Hz, H-3), 7.71 (1H, s, H-8), 7.48 (1H, d, *J* = 5.6 Hz, H-4), 7.42 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 7.17 (1H, s, H-5), 7.11 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 6.05 (2H, s, OCH$_2$O), 4.69 (2H, *s*, H-α); ^{13}C NMR (100 MHz, C$_5$D$_5$N) δ 159.3 (C-1), 157.4 (C-4′),151.0 (C-6), 149.0 (C-7), 141.8 (C-3), 134.9 (C-4a), 130.7 (C-1′), 130.2 (C-2′, 6′), 124.0 (C-8a), 119.5 (C-4), 116.3 (C-3′, 5′), 103.3 (OCH$_2$O), 102.3 (C-5), 102.2 (C-8), 41.7 (C-α); EIMS *m*/*z* 278 [M − 1]$^+$ (100), 261 (12), 248 (9), 220 (10), 191 (8); HREIMS *m*/*z* 279.0887 (calcd for C$_{17}$H$_{13}$NO$_3$ 279.0895).

Cytotoxicity Assay. Hep G2 (human hepatoma cell) and Hep 2,2,15 (Hep G2 cell transfected with HBV), provided by G. Acs (Mount Sinai Medical Center, New York, NY), were cultured in RPMI 1640 and DMEM (Dulbecco's minimal essentail medium) containing 10% FCS and 100 units/mL penicillin and 100 μg/mL streptomycin. All the cell lines were maintained in an incubator at 37 °C in humidified air containing 5% CO$_2$. The activity of test compounds on various cancer cells were assayed by the methylene blue colorimetric method previously reported.[25] The plates were read immediately on an enzyme-linked immunosorbant microplate reader (ELISA Biokinetics Reader) at a wavelength of 592 nm. The 50% inhibition concentration (IC$_{50}$) was defined as 50% reduction of absorbance in the control assay without drugs.[26,27] The data represent means ± SEM, from three independent experiments. The positive control drug, adriamycin, showed an IC$_{50}$ value of 0.05 and 0.45 μg/mL against Hep G$_2$ and Hep 2,2,15, respectively.

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