Notes

Vibsane Diterpenoids from the Leaves and Flowers of *Viburnum odoratissimum*

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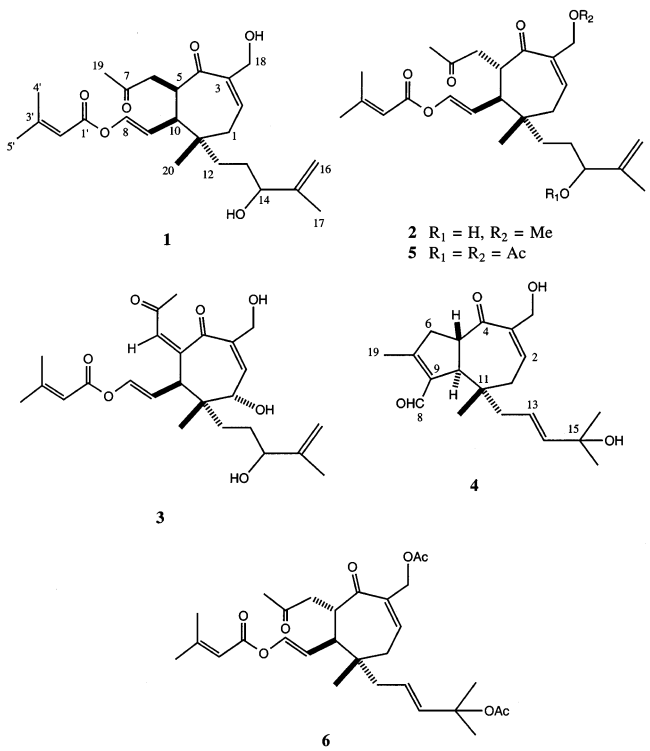
In addition to the five known compounds 5-*epi*-vibsanin H, vibsanins C, H, and G, and aldovibsanin B, four new diterpenes, 5-*epi*-vibsanin G (**1**), 18-*O*-methylvibsanin G (**2**), vibsanin M (**3**), and aldovibsanin C (**4**), were isolated from an acetone extract of the leaves and flowers of *Viburnum odoratissimum* by bioassay-directed fractionation. In addition, two acetyl derivatives **5** and **6** were obtained from the naturally occurring diterpenes. The structures of the new compounds were established on the basis of NMR spectral analysis, including COSY, HMQC, HMBC, and NOESY correlations. The compounds were evaluated for cytotoxicity against human nasopharyngeal carcinoma (HONE-1) tumor cells and human gastric cancer (NUGC-3) cells.

Viburnum odoratissimum Ker. (Caprifoliaceae) is a poisonous plant that grows in the southern part of Taiwan. It has been reported to contain several di- and triterpenoids.[1−4] Its vibsane-type diterpenoid constituents, such as vibsanins A−C, exhibited piscicidal, plant growth regulatory,[1] and cytotoxic activities.[4,5] In our search for antitumor natural products,[4,6] we have investigated this plant, from which we have previously reported two vibsane diterpenes[4] and four triterpenoids.[7] Bioassay-directed fractionation of the acetone extract of the same plant has resulted in the isolation of five known and four new vibsane diterpenes with a seven-membered ring. However, we did not detect any vibsane diterpenes with an 11-membered ring in the present investigation. Herein we report the isolation, structural elucidation, and biological activity of new vibsane diterpenes **1**−**4** together with two of their acetyl derivatives (**5**, **6**).

Bioassay-guided fractionation of the acetone extract of the leaves and flowers of *V. odoratissimum* by silica gel column chromatography, using the HONE-1 (human nasopharyngeal carcinoma and NUGC-3 (gastric tumor) cell lines, yielded four new diterpenes, 5-*epi*-vibsanin G (**1**), 18-*O*-methylvibsanin G (**2**), vibsanin M (**3**), and aldovibsanin C (**4**), and 5-*epi*-vibsanin H,[5] vibsanins C,[8] H,[11] and G,[11] and aldovibsanin B.[2]

Compound **1** was assigned a molecular formula of $C_{25}H_{36}O_6$, as established by HRFABMS, indicating eight degrees of unsaturation. The IR spectrum displayed absorption bands characteristic of a hydroxyl functionality, as well as for ester and carbonyl groups, which were supported by three ^{13}C NMR signals at δ 203.9 (C-4), 207.5 (C-7), and 163.2 (C-1′). The ^1H NMR spectrum (Table 1) showed signals of two trisubstituted olefins at δ 6.60 (H-2) and 5.68 (H-2′) in addition to a *trans*-disubstituted olefin at δ 7.08 (d, J = 12 Hz, H-8) and 5.08 (t, J = 12 Hz, H-9). A third disubstituted double bond with an exomethylene group was indicated by two mutually coupled proton signals at δ 4.87 and 4.97 and confirmed by the CH_2 signal



at δ 111.5 in the ^{13}C NMR spectrum. The latter spectrum also showed an oxygenated methylene group at δ 64.8 (C-18) and an oxygenated methine carbon at δ 76.1 (C-14). The ^{13}C NMR signals (Table 2) at δ 163.2 (C-1′), 114.6 (C-2′), 160.4 (C-3′), 20.5 (C-4′), and 27.6 (C-5′), along with a fragment ion at *m*/*z* 83 in the EIMS, confirmed the presence of a β,β-dimethylacrylate moiety, which is common in vibsane diterpenes.[3,8−10] To satisfy the unsaturation number of eight and taking into account the presence of four double bonds and three ketone groups, it was assumed that **1** possesses only one ring. With the aid of HMQC and COSY spectra, it was possible to assign the proton and carbon signals of the 2-methyl-3-hydroxy-1-pentenyl group (C-12 to C-17) as well as the 2-oxopropyl moiety (C-6, C-7, and

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Table 1. ^{1}H NMR Data ($CDCl_3$, 300 MHz) of Compounds **1**−**4**[a,b]

position	1	2	3	4
1	2.30 m	2.30 dd (5.0, 16)	4.44 s	2.32 d (7.7)
	2.49 m	2.40 dd (5.0, 16)		
2	6.60 br s	6.59 dd (5.0, 9.0)	6.87 d (3.5)	6.84 t (7.4)
5	3.51 m	3.09 m		3.15 m
6	2.29 m	2.52 dd (5.0, 18)	6.14 s	2.92 dd (5.4, 13.5)
	3.12 dd (7.7, 18)	2.95 dd (7.5, 18)		3.06 m
8	7.08 d (12)	7.00 d (12.5)	7.19 d (12)	10.05 s
9	5.08 t (12)	5.17 t (12)	5.44 t (11)	
10	2.19 m	2.08 dd (10, 11)	2.78 d (11)	3.12 m
12	1.32 m	1.13 m	1.60 m	2.56 m
	1.71 m	1.29 m	1.62 m	
13	1.26 m	1.27 m	1.26 m	5.69 m
	1.61 m	1.45 m	1.28 m	
14	4.03 br s	3.95 t (6.0)	4.05 br d (10)	5.69 d (16)
16	4.87 br s	4.82 s	5.02 s	1.31 s
	4.97 br s	4.90 s	4.88 s	
17	1.75 s	1.69 s	1.78 s	1.27 s
18	4.24 br s	4.05 d (13)	4.34 d (13)	4.17 d (13.3)
		4.12 d (13)	4.45 d (13)	4.41 d (13.3)
19	2.18 s	2.16 s	2.27 s	2.19 s
20	0.78 s	0.91s	1.14 s	0.86 s
2′	5.68 br s	5.68 br s	5.69 s	
4′	2.20 s	2.20 s	2.21 s	
5′	1.94 s	1.94 s	1.96 s	
OCH_3		3.40 s		

[a] Chemical shifts in ppm, *J* values in Hz are in parentheses. [b] Assignments were made using HMQC and HMBC techniques.

Table 2. ^{13}C NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**−**4**[a]

carbon	1		2		3		4	
1	39.2	CH_2	36.1	CH_2	78.4	CH	38.1	CH_2
2	140.6	CH	138.3	CH	143.9	CH	139.9	CH
3	142.9	C	139.6	C	137.6	C	140.8	C
4	203.9	C	203.4	C	195.8	C	201.0	C
5	48.0	CH	48.3	CH	149.0	C	51.0	CH
6	44.1	CH_2	43.7	CH_2	134.0	CH	42.3	CH_2
7	207.5	C	207.8	C	200.1	C	164.5	C
8	137.5	CH	137.2	CH	138.1	CH	189.2	CH
9	110.9	CH	112.7	CH	107.9	CH	144.5	C
10	47.2	CH	46.4	CH	48.0	CH	52.0	CH
11	40.2	C	39.8	C	38.9	C	41.6	C
12	38.0	CH_2	35.5	CH_2	33.0	CH_2	43.9	CH_2
13	29.0	CH_2	29.4	CH_2	29.7	CH_2	125.3	CH
14	76.1	CH	76.2	CH	74.3	CH	140.8	CH
15	147.2	C	146.9	C	145.2	C	70.8	C
16	111.5	CH_2	111.6	CH_2	111.1	CH_2	29.9	CH_3
17	17.5	CH_3	17.3	CH_3	18.8	CH_3	29.9	CH_3
18	64.8	CH_2	71.1	CH_2	62.8	CH_2	63.0	CH_2
19	30.4	CH_3	30.2	CH_3	30.0	CH_3	15.2	CH_3
20	24.8	CH_3	24.2	CH_3	17.1	CH_3	24.4	CH_3
1′	163.2	C	163.2	C	162.9	C		
2′	114.6	CH	114.6	CH	114.3	CH		
3′	160.4	C	160.3	C	161.0	C		
4′	20.5	CH_3	20.5	CH_3	20.6	CH_3		
5′	27.6	CH_3	27.6	CH_3	27.7	CH_3		
OCH_3			58.5	CH_3				

[a] Assignments were made using HMQC and HMBC techniques.

C-19) (Tables 1 and 2). The HMBC spectrum was used to determine the position of each functional group and revealed correlations between H-2/C-4, H-19/C-7, H-8/C-1′, and H-14/C-16. Furthermore, the attachment of the alkyl groups was indicated by correlations between H-2/C-18, H-5/C-7, H-20/C-12, H-10/C-8, and H-12/C-10. The spectral data of **1** were very close to those of vibsanin G,[11] with a relatively significant downfield shift of H-5 (+0.61 ppm). A further comparison was made between the ^{1}H NMR spectra of several vibsanin isomers, especially vibsanin C, with H-5β and H-10α substituents,[8] and 5-*epi*-vibsanin C, with H-5α and H-10α configurations.[5] It was observed that the chemical shift of H-5α is invariably around δ 3.50 and shifted downfield by ca. 0.61 ppm compared to the case with a H-5β substituent in related

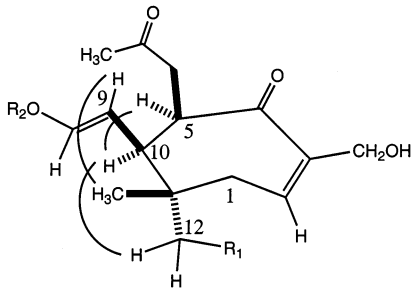

Figure 1. Key NOESY interactions of compound **1**.

vibsanins. The configurations at C-5, C-10, and C-11 were determined through the NOESY spectrum, which revealed correlations between proton signals at δ 0.78/3.12 (H-20/H-6), δ 0.78/5.08 (H-20/H-9), and δ 2.19/1.32 (H-10/H-12) (Figure 1). On the basis of the analysis of all available data, the structure of **1** was established as 5-*epi*-vibsanin G.

The molecular formula of **2** was proved to be $C_{26}H_{38}O_6$ as deduced from the FABMS and the DEPT NMR spectrum. The NMR data (Tables 1 and 2) were similar to those of **1** except for the significant chemical shift of H-5, which was shifted to a higher field at δ 3.09. This suggested a different configuration at C-5 (H-5β), as was verified by the NOESY correlation peaks between H-5 (δ 3.09) and both H-9 (δ 5.17) and H-20 (δ 0.91). The increase of the molecular weight of **2** over that of **1** by a CH_2 unit indicated the replacement of a hydroxyl group by a methoxyl group (δ_H 3.40 and δ_C 58.5). The position of the methoxyl group was determined to be at C-18, as evidenced from the correlation peak between the singlet at δ 3.40 (OCH_3) and the methylene carbon signal at δ 71.1 (C-18) in the HMBC spectrum. Thus, **2** was characterized as 18-methoxyvibsanin G.

Compound **3** has a molecular formula $C_{25}H_{34}O_7$, as established by NMR spectra and FABMS, indicating nine degrees of unsaturation, that is, one degree more than the case of **1**. Comparative analysis of the NMR data (Tables 1 and 2) of **3** indicated the presence of the same structural skeleton of **1** with the disappearance of two methylene signals at C-1 and C-6 and a methine signal at C-5 with

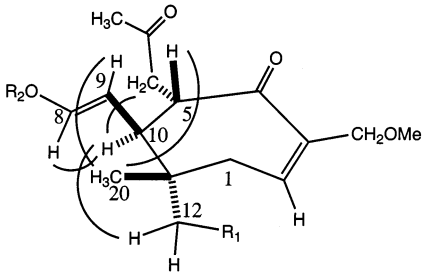

Figure 2. Key NOESY interactions of compound **2**.

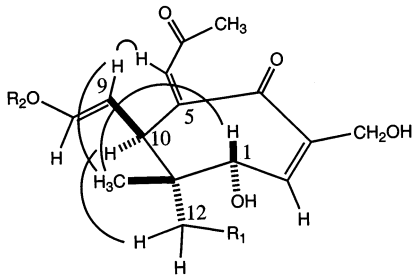

Figure 3. Key NOESY interactions of compound **3**.

the concomitant appearance of a quaternary carbon at δ 149.0, an olefinic methine at δ 134.0, and an oxymethine at δ 78.4. The HMQC spectrum revealed that the latter oxymethine carbon signal at δ 78.4 was correlated to a proton signal at δ 4.44 (1H, s, H-1), while the olefinic methine carbon signal at δ 134.0 was correlated to a proton signal at δ 6.14 (1H, s, H-6). The HMBC spectrum showed that the olefinic proton at δ 6.14 was correlated to each of the signals at δ 195.8 (C-4) and 48.0 (C-10), while the oxymethine proton at δ 4.44 was correlated to a quaternary olefinic carbon signal at δ 137.6 (C-3) and to a methine signal at δ 48.0 (C-10). This indicated the presence of the oxopropenyl side chain at C-5 and an additional hydroxyl group at C-1. The NOESY spectrum of **3** (Figure 3) displayed correlations between H-10 and H-12, H-20 and H-1, H-20 and H-9, and H-9 and H-6, confirming that both H-1 and the side chain at C-10 possess a β-configuration, while H-10 has an α-configuration. In addition, the same spectrum confirmed that the side chain at C-10 is placed between C-5 and C-11. Thus the structure of compound **3** was established and assigned the name vibsanin M.

The FABMS of compound **4** exhibited quasi-molecular ion peaks at *m/z* 355 $[M + Na]^+$ and 333 $[M + H]^+$, appropriate for a molecular formula of $C_{20}H_{28}O_4$. The IR spectrum of **4** showed the presence of hydroxyl (3425 cm^{-1}), carbonyl (1716 cm^{-1}), and α,β-unsaturated ketone (1685 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) indicated the presence of an aldehydic downfield signal at δ 10.05 (1H, s), signals of a cycloheptenone moiety, and signals that matched those of a 2-hydroxy-2-methyl-3-pentenyl group. Signals attributable to either a 2-oxopropyl or a β,β-dimethyl acrylate unit were absent. The HMBC spectrum revealed correlations between the tertiary methyl signal at δ 2.19 (s, Me-19) and the quaternary carbon at δ 144.5 (C-9) and between the proton signal at δ 3.12 (H-10) and the aldehyde carbon signal at δ 189.2 (C-8) and the quaternary carbon signal at δ 164.5 (C-7). Consequently, **4** was proposed as containing a five-membered ring attached to the heptenone ring, in addition to three olefinic bonds and two ketones, with one belonging to the aldehyde group, to satisfy seven degrees of unsaturation. The stereochemistry of **4** was determined by comparison of its NMR data with those of aldovibsanin B.[2] The structure of **4** was determined to be aldovibsanin C.

Two diacetyl derivatives (**5** and **6**) were prepared and analyzed. All naturally occurring and acetylated compounds were tested for their cytotoxic activity at concentrations of 20 μg/mL. Compound **1** exhibited a weak cytotoxic activity against the NUGC-3 cell line (58% survival), while compounds **5** and **6** possessed a moderate activity against both the HONE-1 and NUGC-3 cell lines (0−1% survival).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. The ^1H, ^{13}C, DEPT, COSY, HMBC, HSQC, and NOESY NMR spectra were recorded either on a Varian Inova 500 or a Bruker Avance 300 spectrometer. The chemical shifts are given in δ (ppm) and coupling constants in Hz. EIMS and FABMS were measured with VG Quattro 5022 and JEOL JMS-SX 102 mass spectrometers.

Plant Material. The leaves and flowers of *V. odoratissimum* were collected in Ping-tong County, Taiwan, in May 2000. A voucher specimen (TPG8-2) was deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation. The leaves and flowers (12.7 kg) were dried at room temperature and ground. The obtained powder was extracted three times with acetone. The combined acetone extract was concentrated under vacuum, and the residue was successively extracted with *n*-hexane (10 L) and *n*-hexane/EtOAc (1:1, 10 L) to give a *n*-hexane extract (215 g) and a *n*-hexane/EtOAc extract (280 g), respectively. A part of the *n*-hexane/EtOAc-soluble extract (240 g) was chromatographed on a silica gel column (1.7 kg) using a gradient of *n*-hexane/EtOAc and then EtOAc/MeOH mixtures to give 29 fractions. Cytotoxic screening of these fractions revealed that fractions 16−29 were active against HONE-1 and NUGC-3 cells (<50% inhibition at 20 μg/mL). Fractions 21−22 were combined (17 g) and on further chromatography over silica gel (200 g, *n*-hexane/CH_2Cl_2/MeOH, 100:100:1) yielded vibsanin C (37 mg). Fractions 24−26 (31 g) were further chromatographed on a silica gel column (600 g, *n*-hexane/CH_2Cl_2/MeOH, 100:100:1 to 8:8:1) to yield three fractions, 24A, 24B, and 24C. Fraction 24B (11.5 g) was further chromatographed on a silica gel column (300 g, *n*-hexane/CH_2Cl_2/MeOH, 20:20:1) to yield three fractions, $24B_1$, $24B_2$, and $24B_3$. Fraction $24B_1$ (1.5 g) was chromatographed on a silica gel column (45 g, *n*-hexane/*n*-BuOH, 40:1) to yield two fractions, $24B_{1-1}$ and $24B_{1-2}$. Fraction $24B_{1-2}$ (85 mg) was subjected to reversed-phase HPLC using MeOH/CH_3CN/H_2O (1:1:1) to give **1** (17 mg) and **4** (5 mg). Fraction $24B_2$ (2.3 g) was chromatographed on a silica gel column (60 g) using a mixture of *n*-hexane/EtOAc. Elution with *n*-hexane/EtOAc (2:1) afforded **2** (2 mg), while elution with *n*-hexane/EtOAc (3:1) yielded **3** (2 mg). Fraction $24B_3$ (0.8 g) was chromatographed on a silica gel column (25 g, *n*-hexane/*n*-BuOH (30:1) to give three fractions, $24B_{3-1}$, $24B_{3-2}$, and $24B_{3-3}$. Fraction $24B_{3-1}$ (100 mg) was further separated using preparative TLC on a silica gel column using *n*-hexane/*n*-butanol (4:1) for development to yield vibsanin H (25 mg) and vibsanin G (27 mg). In addition, fraction $24B_{3-2}$ (30 mg) was further separated using preparative TLC on a silica gel column using *n*-hexane/*n*-butanol (4:1) to yield vibsanin G (13 mg). Finally, fraction $24B_{3-3}$ (105 mg) was subjected to reversed-phase HPLC using MeOH/CH_3CN/H_2O (1:1:1) to afford 5-*epi*-vibsanin H (39 mg) and vibsanin G (9 mg) and a third fraction, which yielded aldovibsanin B (1.5 mg) after purification using preparative TLC on a silica gel column with *n*-hexane/CH_2Cl_2/MeOH (10:10:1).

5-*epi*-Vibsanin G (1): colorless amorphous solid; $[\alpha]^{26}_D$ +5.0° (*c* 3.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 244 (4.25) nm; IR ($CHCl_3$) ν_{max} 3435, 1723, 1645, 1446, 1383, 1223, 1140, 756 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 433 $[M + H]^+$, 455 $[M + Na]^+$; EIMS (70 eV) *m/z* 432 ($[M]^+$, 0.5), 414 ($[M - H_2O]^+$, 1.5), 396 ($[M - 2H_2O]^+$, 2), 378 ([M −

$3H_2O]^+$, 2), 315 ($[M - H_2O - C_6H_{11}O]^+$, 2), 215 (3), 83 (100); HRFABMS *m/z* $[M + Na]^+$ 455.2416 ($C_{25}H_{36}O_6Na$ requires 455.2410).

18-Methoxyvibsanin G (2): colorless amorphous solid; $[\alpha]^{26}_D$ +38.1°(*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 244 (3.56) nm; IR ($CHCl_3$) ν_{max} 3432, 1717, 1647, 1456, 1379, 1223, 1140, 849, 756 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 429 $[M - H_2O + H]^+$, 447 $[M + H]^+$; EIMS (70 eV) *m/z* 345 (1), 328 (1), 314 (2), 271 (2), 257 (2), 243 (3), 215 (3), 162 (5), 125 (6), 83 (100).

Vibsanin M (3): colorless amorphous solid; $[\alpha]^{26}_D$ +9.1° (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 244 (4.25) nm; IR ($CHCl_3$) ν_{max} 3448, 1730, 1647, 1541, 1456, 1381, 1223, 1138, 1086, 943, 849, 756 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 447 $[M + H]^+$; EIMS (70 eV) *m/z* 430 (0.1), 429 ($[M - H_2O + H]^+$, 0.1), 416 (1), 385 (1), 328 (1), 299 (1), 239 (1), 231 (1), 229 (1), 175 (2), 162 (10), 135 (4), 109 (7), 83 (100).

Aldovibsanin C (4): colorless amorphous solid; [α] +0.9° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 235 (3.5) nm; IR ($CHCl_3$) ν_{max} 3425, 1725, 1716, 1685, 1616, 1471 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 355 $[M + Na]^+$, 333 $[M + H]^+$.

Vibsanin G 14,18-Diacetate (5). Acetylation (Ac_2O/pyridine, 1:1; room temperature) of vibsanin G (20 mg) gave after workup a solid (**5**, 16 mg): ^1H NMR (300 MHz, $CDCl_3$) δ 2.24 (2H, m, H-1), 6.63 (1H, dd, J = 8, 4 Hz, H-2), 3.01 (1H, m, H-5), 2.52 (1H, dd, 17.5, 4.3 Hz, H-6a), 2.98 (1H, dd, J = 17.5, 7.8 Hz, H-6b), 7.01 (1H, d, J = 12 Hz, H-8), 5.14 (1H, t, J = 12 Hz, H-9), 2.15 (1H, m, H-10), 1.12 (2H, m, H-12), 1.45 (2H, m, H-13), 5.01 (1H, t, J = 6 Hz, H-14), 4.86 (1H, s, H-16a), 4.89 (1H, s, H-16b), 1.67 (3H, s, H-17), 4.74 (2H, d, J = 13 Hz, H-18), 2.15 (3H, s, H-19), 0.89 (3H, s, H-20), 5.67 (1H, br s, H-2′), 2.19 (3H, s, H-4′), 1.93 (3H, s, H-5′), 2.04, 2.07 (each 3H, s, $OCOCH_3$).

Vibsanin H 15,18-Diacetate (6). Vibsanin H (10 mg) was dissolved in pyridine and Ac_2O (each 1 mL), and the solution was heated at 60 °C in a water bath for 10 h to give a diacetate (**6**): ^1H NMR (300 MHz, $CDCl_3$) δ 2.11 (1H, m, H-1a), 2.45 (1H, dd, J = 13, 4.5 Hz, H-1b), 6.67 (1H, dd, J = 7.5, 4 Hz, H-2), 3.11 (1H, m, H-5), 2.51 (1H, dd, J = 18, 6 Hz, H-6a), 2.95 (1H, dd, J = 18, 7.1 Hz, H-6b), 7.02 (1H, d, J = 12.3 Hz, H-8), 5.20 (1H, t, J = 12 Hz, H-9), 2.25 (1H, dd, J = 9.1, 11.2 Hz, H-10), 1.69 (1H, m, H-12), 5.73 (1H, m, H-13), 5.51 (1H, d, J = 13 Hz, H-14), 1.48 (1H, s, H-16), 1.25 (3H, s, H-17), 4.69 (1H, d, J = 13 Hz, H-18a), 4.78 (1H, d, J = 13 Hz, H-18b), 2.16 (3H, s, H-19), 0.93 (3H, s, H-20), 5.68 (1H, br s, H-2′), 2.20 (3H, s, H-4′), 1.92 (3H, s, H-5′), 1.98, 2.08 (3H each, s, $OCOCH_3$).

Cytotoxicity Assay. The cytotoxic activities of **1**−**6** against HONE-1 (human nasopharyngeal carcinoma) and NUGC (gastric tumor) cell lines were assayed by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl tetrazolium salt (MTS) colorimetric method to measure the mitochondrial NADPH dehydrogenase activity as previously described.[12] The percent survival of the NUGC-3 or HONE-1 cells at a concentration of 20 μg/mL of each test compound was determined and compared to untreated cells. Antinomycin D (5 μM) was used as a positive control.

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