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NGF-potentiating vibsane-type diterpenoids from *Viburnum sieboldii*

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ABSTRACT

Six new vibsane-type diterpenoids, named neovibsanin O (**1**), neovibsanin M (**2**), neovibsanin L (**3**), (8*Z*)-neovibsanin M (**4**), 15-*O*-methylvibsanin H (**5**), and 5-*epi*-15-*O*-methylvibsanin H (**6**), were isolated from the leaves of *Viburnum sieboldii* by bioassay-guided fractionation using NGF-differentiated PC12 cells. The structures of **1**–**6** were established by analyzing their spectroscopic data and comparing their NMR data with those of previously reported vibsane-type diterpenoids. Compounds **3** and **4**, and the known vibsane-type diterpenoids neovibsanins A (**7**) and B (**8**) significantly enhanced the neurite outgrowth of NGF-mediated PC12 cells at concentrations ranging from 20 to 40 μM.

Vibsane-type diterpenoids are quite rare natural products and their occurrence has been limited to *Viburnum awabuki*, *Viburnum odoratissimum* and *Viburnum suspemsum*.[1,2,6–8] The carbon skeletons of these diterpenoids are further classified into three subtypes: those with an eleven-membered ring, those with a seven-membered ring, and the rearranged types, which are represented by vibsanin B (**9**),[3,4] vibsanin C (**10**),[3,4] and neovibsanin A (**7**),[5] respectively. Additionally, we have established the chemical correlations between vibsanin B and vibsanin C, and neovibsanins, which has allowed us to propose a plausible biosynthetic route to the two other subtypes from vibsanin B (**9**).[1] Some vibsane-type diterpenoids have attracted increasing attention in recent years because of their unique structures and wide-ranging biological activities.[9–17] As part of our chemical and biological studies on vibsane-type diterpenes, we have investigated the MeOH extract of the leaves of *Viburnum sieboldii*, which exhibited neurite outgrowth-promoting activity in NGF-mediated PC12 cells at 25 μg/mL, resulting in the isolation of six new vibsane-type diterpenenoids, which were named neovibsanin O (**1**), neovibsanin M (**2**), neovibsanin L (**3**), (8*Z*)-neovibsanin M (**4**), 15-*O*-methylvibsanin H (**5**), and 5-*epi*-15-*O*-methylvibsanin H (**6**). In this Letter, we report the structures of **1**–**6** and the NGF-potentiating effects of vibsane-type diterpenoids on PC12 cells (Fig. 1).

The MeOH extract of the dried leaves (420 g) of *V. sieboldii* was fractionated in order of increasing polarity by column chromatography on silica gel with dichloromethane/ethyl acetate in order of increasing polarity to give seven fractions. The active fractions were further purified by a combination of silica gel column chromatography and HPLC, furnishing six new compounds, neovibsanin O (**1**), neovibsanin M (**2**), neovibsanin L (**3**), (8*Z*)-neovibsanin M (**4**), 15-*O*-methylvibsanin H (**5**), and 5-*epi*-15-*O*-methylvibsanin H (**6**) together with the previously known neovibsanins A (**7**) and B (**8**).[5]

Compound **1** had a molecular formula, $C_{24}H_{32}O_6$, as established by high resolution (HR)-FABMS. The spectroscopic data of **1** showed the presence of a β,β-dimethyl acrylate group [*m*/*z* 83; λ_{max} 229 nm; ν_{max} 1733 cm^{-1}; δ_H 1.34 (d, *J* = 1.1 Hz), 2.02 (d, *J* = 1.1 Hz) and 5.63 (qq, *J* = 1.1, 1.1 Hz), δ_C 163.0], a trisubstituted olefin (δ_H 5.27, m), a disubstituted olefin [δ_H 7.67 (d, *J* = 12.4 Hz), 5.07 (dd, *J* = 12.4, 11.1 Hz)], an oxymethylene [δ_H 3.96 (ddt, *J* = 13.2, 3.0, 3.0 Hz), 4.33 (ddt, *J* = 13.2, 3.0, 3.0 Hz); δ_C 68.3], two oxymethines [δ_H 4.02 (dddd, *J* = 7.3, 5.9, 2.9, 1.5 Hz) and 4.46 (dd, *J* = 7.7, 4.8 Hz); δ_C 66.2 and 80.3], three methyl groups [δ_H 0.71, 1.78, and 1.88], and two carbonyl groups [δ_C 204.8 and 205.1; ν_{max} 1716 and 1704 cm^{-1}]. These spectral data of **1** were found to be inconsistent with those of the previously known vibsane-type diterpenoids. Extensive analysis of 2D ^1H–^1H COSY and HMQC gave five partial structures (**A**–**E**) as shown in Figure 2 and six quaternary carbons (δ_C 33.0, 80.5, 135.7, 163.0, 204.8, and 205.1).

In order to determine the connectivities between these five partial structures (**A**–**E**) and the quaternary carbons, HMBC experiments were carried out (Fig. 2). The HMBC correlation of H_3-20 with C-1 (δ_C 36.2), C-10 (δ_C 45.8), C-11 (δ_C 33.0), and C-12 (δ_C 47.3) indicated that the quaternary carbon C-11 was connected to C-20, as well as C-12, C-10 and C-1 in **A**, **D,** and **C**, respectively.

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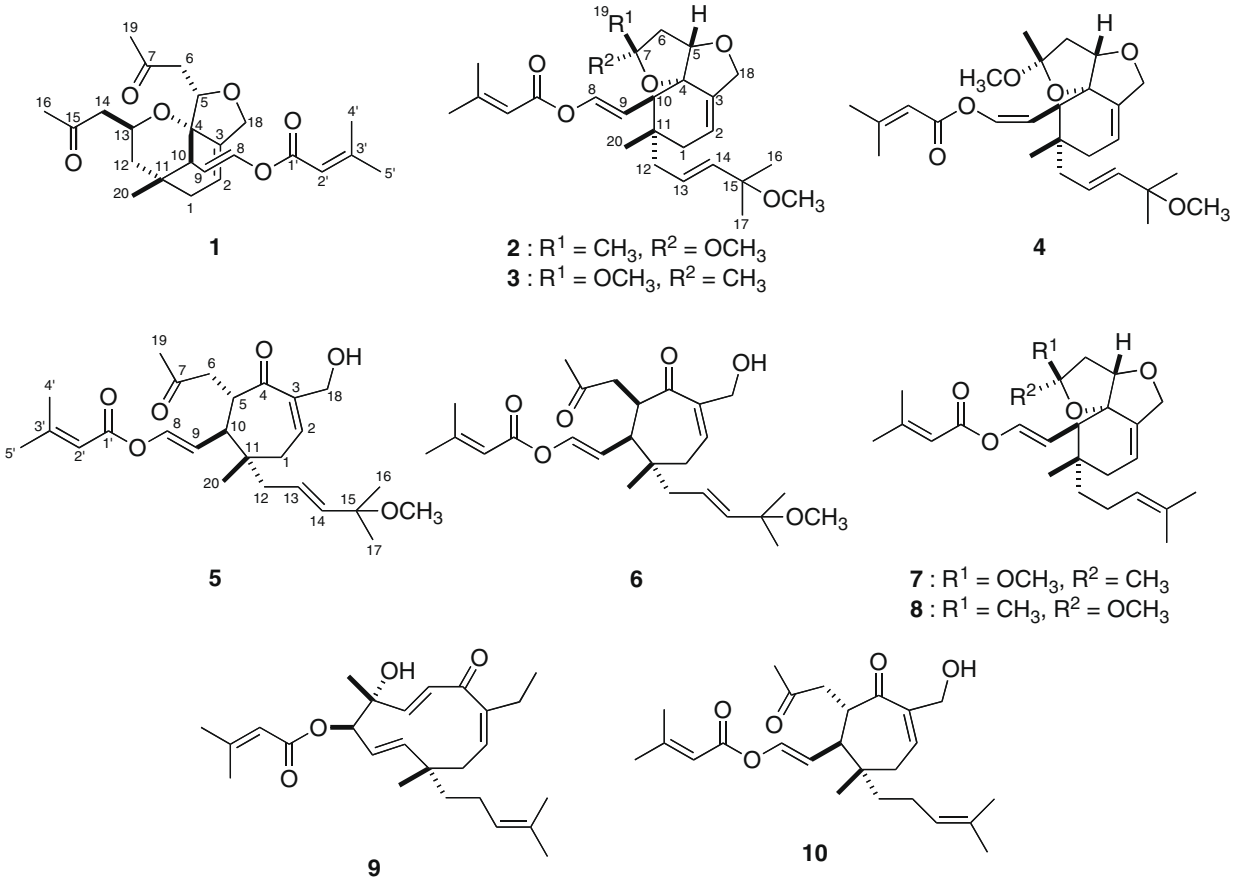


Figure 1. Structures of compounds **1**–**10**.

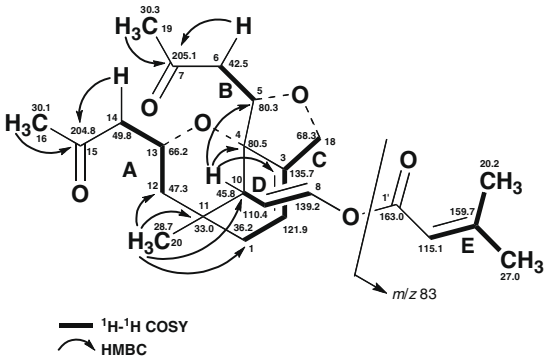


Figure 2. HMBC correlations of **1**.

The H-6 and H_3-19 signals showed a cross-peak to the C-7 carbonyl carbon at δ_C 205.1 and the H-14 signal correlated to the C-15 carbonyl signal at δ_C 204.8, suggesting that a methyl ketone was bonded to C-6 in **B** and that another methyl ketone was bonded to C-14 in **A**. The type of the linkage between the C-18 oxymethylene in **C** and the C-5 oxymethine in **B** failed to be confirmed by HMBC experiments. However, in NOESY experiments (Fig. 3), H-18β showed a clear NOE correlation to H-5, which indicated that C-5 was connected to C-18 through an oxygen atom, demonstrating the presence of a tetrahydrofuran ring. The HMBC correlations of H-10 in **D** to C-3 (δ_C 135.7), C-4 (δ_C 80.5), and C-5 (δ_C 80.3) demonstrated the presence of the formation of a cyclohexene ring fused with a tetrahydrofuran, which is the typical core structure of the neovibsanin skeleton. Due to the absence of a HMBC correlation between H-13 and C-4 (δ_C 80.5), the type of linkage between the C-13 oxymethine in **A** and the C-4 quaternary carbon remains unsolved. However, taking the eight degrees of unsaturation into account, **1** must have a tricyclic structure. This allowed us to connect C-13 and C-14 through an oxygen atom. Thus, the above-mentioned spectroscopic data culminated in our proposing the plane structure **1,** which is comprised of a 2-oxa-bicyclo[3,3,1]nonane skeleton. The relative stereochemistry of **1** was elucidated based on the NOESY data, as shown in Figure 3. The proposed bicyclic framework automatically fixes the stereochemistry of C-4 and C-11. The NOE correlations of H-12β/H-10, H-10/H-5, and H-6/H-14 indicated that the methyl ketone side chain at C-5 has an α-configuration. Additionally, the C-13 side chain was defined to be in the β from sequential NOE correlations of H-12α/H-1α, H-13/H-1α, H-13/H-2, and H-13/H-12α. Additionally, the most stable conformation obtained by MM2 calculations using MacroModel® was able to account for the observed NOEs. Hence, the structure of neovibsanin O was elucidated to be **1**. It should be noted that neovibsanin O (**1**) is the second example of a *nor*-vibsane-type diterpenoids.[8]

Neovibsanin O (**1**) can be derived from neovibsanin as outlined in Scheme 1. Namely, an auto-oxidation of neovibsanin occurs at the C-15 position to give hydroperoxy ***a***, which subsequently undergoes a β-cleavage to lose one carbon, giving rise to methyl ketone ***b***. The resultant unsaturated ketone is then attacked in a 1,4-addition manner by the hydroxy group at the C-4 position to produce **1**.

Compound **2** had a molecular formula $C_{27}H_{40}O_6$, as deduced by HR-FABMS at *m*/*z* 483 $[M+Na]^+$. The NMR data of **2** showed the presence of four tertiary methyl groups [δ_H 0.89 (H_3-20), 1.27 (H_3-16), 1.27 (H_3-17), 1.34 (H_3-19)], two methoxy groups (δ_H

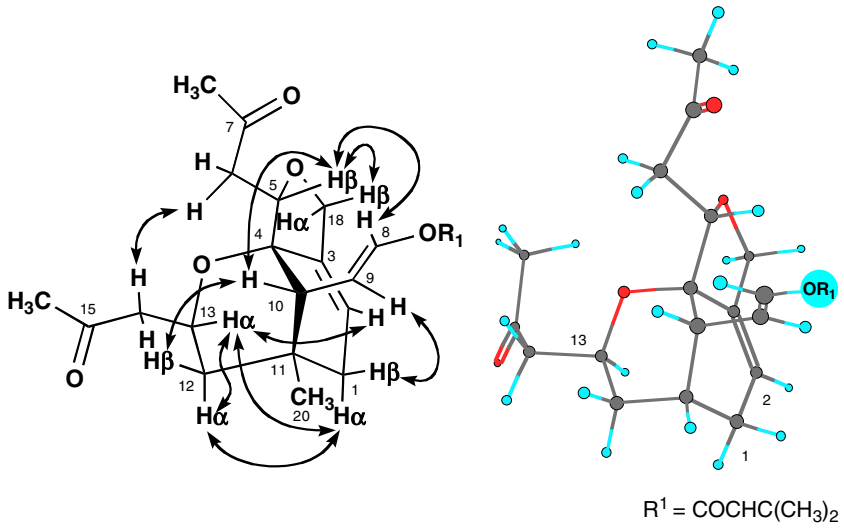
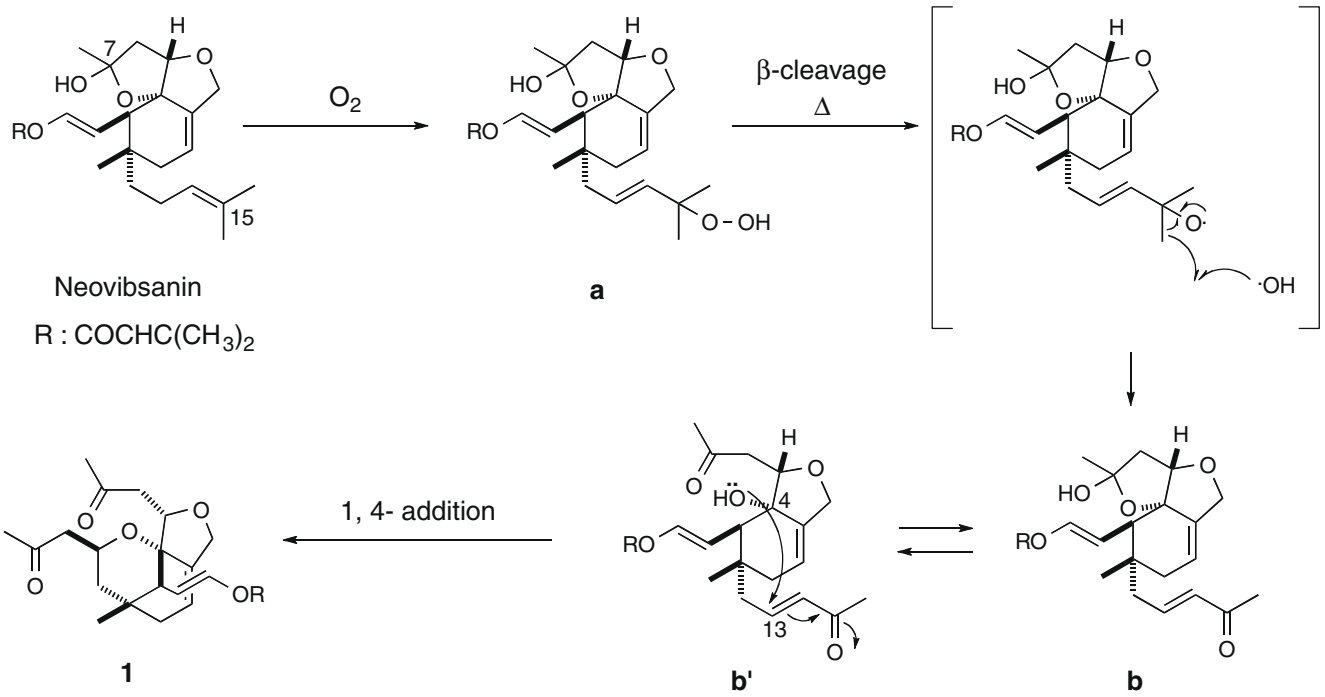


Figure 3. NOESY correlations and most stable conformation of **1** (calculated by Macromodel®).



Scheme 1. Plausible biosynthesis of **1**.

3.11, 3.13), an oxymethylene [δ_H 4.17 (br d, J = 10.2 Hz), 4.80 (ddt, J = 10.2, 3.0, 3.0 Hz)], an oxymethine [δ_H 4.65 (d, J = 5.5 Hz)], two disubstituted double bonds with an *E*-geometry [δ_H 5.23 (dd, J = 12.4, 11.4 Hz, H-9), 7.48 (d, J = 12.4 Hz, H-8); δ_C 137.9 (C-8), 112.8 (C-9), and δ_H 5.52 (d, J = 16.2 Hz, H-14), 5.77 (ddd, J = 16.2, 8.5, 6.0 Hz, H-13); δ_C 127.4 (C-13), 139.1 (C-14)], a trisubstituted double bond [δ_H 5.31 (m)], and a β,β-dimethylacrylate group. These spectral features indicated that **2** is a typical rearranged vibsane-type diterpenoid. In fact, the ^{1}H and ^{13}C NMR data of **2** were found to be similar to those of neovibsanin B (**8**) except for the C-12–C-17 side chain and the presence of an extra methoxy group. The HMBC correlations between a methoxy signal (δ_H 3.13) and C-7 (δ_C 108.9), and between another methoxy signal (δ_H 3.11) and C-15 (δ_C 74.8) indicated that two methoxy groups were located at C-7 and C-15, respectively (Fig. 4). The above spectroscopic data suggested the plane structure **2** for neovibsanin M. The relative stereochemistry of **2** was found to be the same as that of neovibsanin B (**8**) on the basis of the NOESY (Table 1, Supplementary data, Fig. 7).

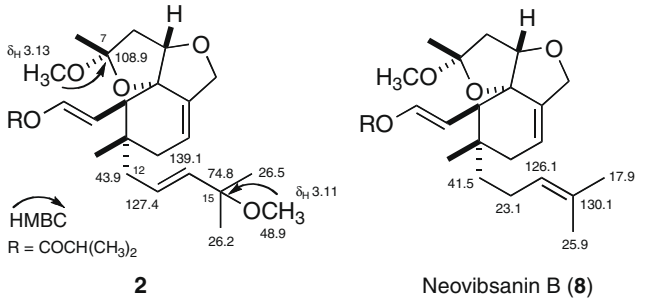


Figure 4. Comparison of C12–C17 side chains between **2** and neovibsanin B (**8**).

Compound **3** had a molecular formula ($C_{27}H_{40}O_6$) as established by HR-FABMS. The spectroscopic data of **3** were similar to those of **2**. 2D NMR analyses of **3** gave the same plane structure as **2**. Thus, compound **3** is an epimer of **2** with regard to C-7. The relative stereochemistry of **3** was defined by NOESY (Supplementary data, Fig. 8). Thus, on the basis of spectral data, the structure of neovibsanin L was elucidated as **3**.

Compound **4** had the same molecular formula ($C_{27}H_{40}O_6$) as **2** and **3**. The spectroscopic data of **4** were also similar to those of **2**. In the ^{1}H NMR spectrum of **4**, the coupling constant ($J_{8,9}$ = 6.6 Hz) between H-8 and H-9 in **4** was small in contrast to the large *J* value of **2** ($J_{8,9}$ = 12.4 Hz). NOESY and the small $J_{8,9}$ value of **4** indicated that the $\Delta_{8,9}$ double bond takes a *Z*-geometry. Thus, **4** was assigned as (8*Z*)-neovibsanin M.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **1**–**3** in C_6D_6

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.2	1.50 (ddt, 19.2, 3.0, 3.0) 1.58 (br d, 19.2)	36.1	1.70 (m) 1.82 (br dt, 18.6, 3.0)	33.0	1.58 (br d, 18.4) 1.96 (br d, 18.4)
2	121.9	5.27 (m)	120.8	5.31 (m)	120.3	5.22 (m)
3	135.7		138.2		137.8	
4	80.5		91.6		90.6	
5	80.3	4.46 (dd, 7.7, 4.8)	86.5	4.65 (d, 5.5)	87.6	4.50 (d, 4.1)
6	42.5	2.61 (dd, 16.2, 4.8) 2.80 (dd, 16.2, 7.7)	46.5	1.68 (dd, 14.0, 5.5) 2.43 (d, 14.0)	45.4	2.25 (d, 14.3) 2.31 (dd, 14.3, 4.1)
7	205.1		108.9		110.8	
8	139.2	7.67 (d, 12.4)	137.9	7.48 (d, 12.4)	137.4	7.52 (d, 12.1)
9	110.4	5.07 (dd, 12.4, 11.1)	112.8	5.23 (dd, 12.4, 11.4)	112.7	5.14 (dd, 12.1, 12.1)
10	45.8	1.99 (d, 11.1)	48.5	2.34 (d, 11.4)	48.5	2.68 (d, 12.1)
11	33.0		36.3		35.7	
12	47.3	0.85 (dd, 13.0, 1.5) 1.15 (dd, 13.0, 2.9)	43.9	2.09 (dd, 13.7, 8.5) 2.71 (dd, 13.7, 6.0)	42.1	2.36 (dd, 13.7, 6.5) 2.96 (dd, 13.7, 8.7)
13	66.2	4.02 (dddd, 7.3, 5.9, 2.9, 1.5)	127.4	5.77 (ddd, 16.2, 8.5, 6.0)	128.0	5.75 (ddd, 15.7, 8.7, 6.5)
14	49.8	2.01 (dd, 15.5, 5.9) 2.32 (dd, 15.5, 7.3)	139.1	5.52 (d, 16.2)	139.4	5.52 (d, 15.7)
15	204.8		74.8		74.7	
16	30.1	1.78 (s)	26.5	1.27 (s)	26.2	1.24 (s)
17			26.2	1.27 (s)	26.4	1.25 (s)
18[a]	68.3	3.96 (ddt, 13.2, 3.0, 3.0) 4.33 (ddt, 13.2, 3.0, 3.0)	70.5	4.17 (br d, 10.2) 4.80 (ddt, 10.2, 3.0, 3.0)	70.1	4.16 (d, 10.8) 4.56 (ddt, 10.8, 3.0, 3.0)
19	30.3	1.88 (s)	23.3	1.34 (s)	23.9	1.44 (s)
20	28.7	0.71 (s)	25.7	0.89 (s)	26.2	0.91 (s)
1'	163.0		163.2		163.1	
2'	115.1	5.63 (qq, 1.1, 1.1)	115.1	5.66 (qq, 1.4, 1.4)	115.1	5.66 (qq, 1.1, 1.1)
3'	159.7		159.9		159.6	
4'	20.2	2.02 (d, 1.1)	20.2	2.04 (d, 1.4)	20.2	2.03 (d, 1.1)
5'	27.0	1.34 (d, 1.1)	27.0	1.37 (d, 1.4)	27.0	1.36 (d, 1.1)
CH_3O-7			50.2	3.13 (s)	49.9	3.20 (s)
CH_3O-15			48.9	3.11 (s)	50.2	3.10 (s)

[a] Having long-range couplings with H-2 and H-1 determined by ^{1}H–^{1}H COSY.

Compound **5** had a molecular formula $C_{26}H_{38}O_6$ (*m*/*z* 469.2583 $[M+Na]^+$). The NMR data of **5** were similar to those of vibsanin H[1] except for the presence of a methoxy group (δ_H 3.10; δ_C 50.2). These spectral data disclosed that the hydroxy group at the C-15 or C-18 position in vibsanin H is methylated in **5**. In HMBC, the methoxy signal (δ_H 3.10) showed a cross-peak to C-15 (δ_C 74.7), suggesting that it was located on C-15 (Supplementary data, Fig. 9). The relative stereochemistry of **5** was found to be the same as that of vibsanin H on the basis of NOESY (Supplementary data, Fig. 10). Thus, **5** was named 15-*O*-methylvibsanin H (Table 2).

Compound **6** had a molecular formula $C_{26}H_{38}O_6$, as established by HR-FABMS (*m*/*z* 469.2546 $[M+Na]^+$). The NMR and physical data of **6** were similar to those of **5**. Analyses of the 2D NMR (HMQC, ^{1}H–^{1}H COSY, HMBC) data of **6** gave the same plane structure as

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **4**–**6** in C_6D_6

Position	4		5		6	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.0	1.69 (br d, 18.4) 2.27 (br d, 18.4)	36.5	1.81 (m) 1.90 (m)	38.7	1.82 (dd, 16.5, 8.1) 2.10 (dd, 16.5, 4.8)
2	120.7	5.33 (m)	136.8	6.19 (dddd, 9.2, 6.6, 6.0, 5.4)	138.9	6.13 (dddd, 8.2, 6.5, 5.9 4.8)
3	139.6		142.4		144.0	
4	90.6		204.7		203.4	
5	89.1	4.54 (d, 5.8)	47.7	2.88 (ddd, 9.9, 6.3, 5.1)	48.4	3.50 (ddd, 8.8, 4.4, 4.4)
6	47.5	1.79 (dd, 13.7, 5.8) 2.44 (d, 13.7)	43.9	2.53 (dd, 17.9, 5.1) 2.75 (dd, 17.9, 6.3)	44.1	1.91 (dd, 18.1, 4.4) 3.03 (dd, 18.1, 8.8)
7	109.5		206.3		205.6	
8	136.2	7.50 (d, 6.6)	138.0	7.20 (d, 12.4)	137.7	7.38 (d, 12.4)
9	110.0	5.00 (dd, 10.6, 6.6)	112.8	5.15 (dd, 12.4, 11.5)	111.9	5.33 (dd, 12.4, 11.8)
10	47.7	3.00 (d, 10.6)	44.3	2.29 (dd, 11.3, 9.9)	47.6	2.09 (dd, 11.8, 4.4)
11	36.0		40.9		41.1	
12	37.4	2.33 (dd, 14.2, 7.7) 2.70 (dd, 14.2, 7.8)	43.1	1.76 (dd, 13.7, 6.6) 1.82 (dd, 13.7, 8.2)	45.9	2.02 (m) 2.05 (m)
13	127.8	5.67 (ddd, 12.1, 7.8, 7.7)	125.6	5.37 (ddd, 15.7, 8.2, 6.6)	124.8	5.51 (dt, 13.2, 5.5)
14	139.1	5.55 (d, 12.1)	139.9	5.44 (d, 15.7)	140.8	5.51 (d, 13.2)
15	74.7		74.7		74.7	
16	26.2	1.26 (s)	25.9	1.21 (s)	26.2	1.26 (s)
17	26.4	1.26 (s)	26.1	1.22 (s)	26.2	1.27 (s)
18[a]	70.1	4.22 (d, 9.9) 4.89 (ddt, 9.9, 3.0, 3.0)	63.6	4.29 (dd, 13.2, 6.6) 4.43 (dd, 13.2, 6.0)	64.1	4.20 (dd, 12.9, 5.9) 4.30 (dd, 12.9, 6.5)
19	23.9	1.26 (s)	29.5	1.78 (s)	29.7	1.72 (s)
20	26.2	0.98 (s)	23.7	0.68 (s)	24.5	0.66 (s)
1'	163.1		163.1		163.2	
2'	115.1	5.57 (qq, 1.4, 1.4)	114.8	5.65 (qq, 1.4, 1.4)	114.9	5.62 (s)
3'	159.6		160.4		160.3	
4'	20.2	2.04 (d, 1.4)	20.2	2.02 (d, 1.4)	20.3	2.02 (s)
5'	27.0	1.36 (d, 1.4)	27.0	1.35 (d, 1.4)	27.0	1.34 (s)
CH_3O-7	49.9	3.12 (s)				
CH_3O-15	50.2	3.12 (s)	50.2	3.10 (s)	50.2	3.14 (s)
OH-18				2.33 (dd, 6.6, 6.0)		2.09 (dd, 6.5, 5.9)

[a] Having long-range couplings with H-2 and H-1 determined by ^{1}H–^{1}H COSY.

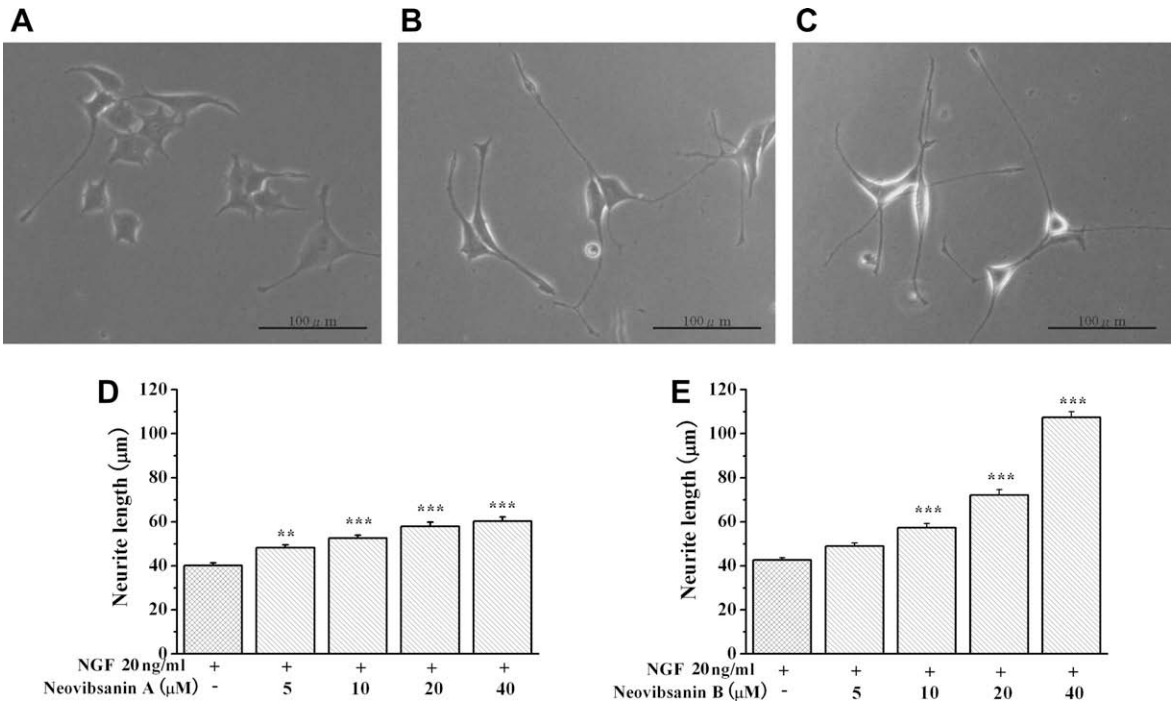


Figure 5. Neurite outgrowth-promoting activities of neovibsanins A (**7**) and B (**8**) in PC12 cells. (A) Morphology of PC12 cells in the control groups. (B) Morphology of PC12 cells in 40 μM neovibsanin A + 20 ng/mL NGF. (C) Morphology of PC12 cells in 40 μM neovibsanin B + 20 ng/mL NGF. (D) and (E) Quantitative analysis of neurite outgrowth. PC12 cells were cultured in DMEM/2% HS + 1% FBS with or without 20 ng/mL NGF and different concentrations of neovibsanins A and B for 48 h. PC12 cells were fixed and then the percentage of cells bearing neurites was quantified, and the primary neurite length was measured. Over 40 fields were randomly selected under a microscope for analysis of the percentage of cells bearing neurites. At least 200 cells were selected for calculating the neurite length. Data are expressed as mean ± SE. $^{***}P$ <0.001 compared with NGF only by one-way ANOVA followed by Bonferroni's post hoc means comparison. ###P <0.001 versus control according to the Student's t-test.

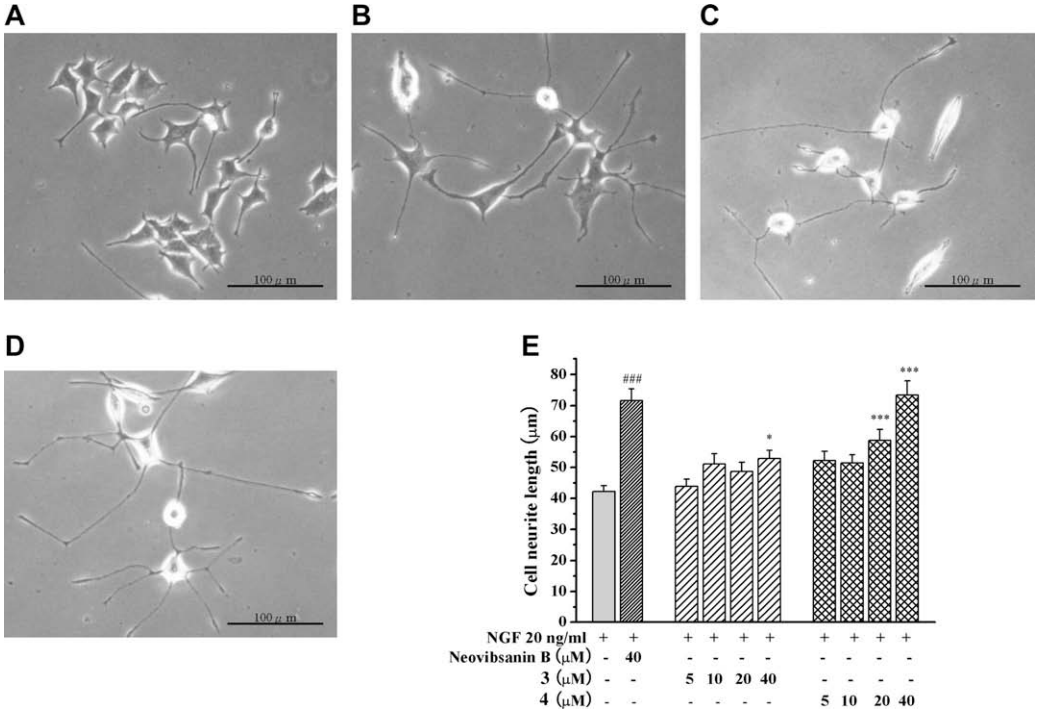


Figure 6. Neurite outgrowth-promoting activities of compounds **3** and **4** in PC12 cells. (A) Morphology of PC12 cells in 20 ng/mL NGF. (B) Morphology of PC12 cells in 40 μM neovibsanin B (**8**) + 20 ng/mL NGF. (C) Morphology of PC12 cells in 40 μM neovibsanin L (**3**) + 20 ng/ml NGF. (D) Morphology of PC12 cells in 40 μM (8*Z*)-neovibsanin M (**4**) + 20 ng/mL NGF. (E) Quantitative analysis of neurite outgrowth. PC12 cells were cultured in DMEM/2% HS + 1% FBS with or without 20 ng/mL NGF and different concentrations of **3** and **4** for 48 h. PC12 cells were fixed and the percentage of cells bearing neurites quantified and the primary neurite length was observed. Over 40 fields were randomly selected under a microscope for analysis of the percentage of cells bearing neurites. At least 200 cells were selected for calculating the neurite length. Data are expressed as mean ± SE. $^{***}P$ <0.001, $^{**}P$ <0.01, $^{*}P$ <0.05 compared with NGF only by one-way ANOVA followed by Bonferroni's post hoc means comparison. $^{***}P$ <0.001 versus NGF only, ###P <0.001 versus control according to the Student's t-test.

5. In a comparison of ^{1}H NMR spectra between **5** and **6**, **6** had a smaller vicinal H-5/H-10 coupling constant (4.4 Hz) than **5** (9.9 Hz), indicating that compound **6** is the C-5 epimer of **5**. The relative stereochemistry of **6** was defined by NOESY. Namely, the NOE correlations of H_3-20/H-6 and H_3-20/H-9 indicated that the methyl ketone side chain at C-5 takes a β-configuration. Accordingly, the structure of **6** was determined to be 5-*epi*-15-*O*-methylvibsanin H.

Since compounds **1**–**8** were isolated from the active fractions that promoted neurite outgrowth from NGF (20 ng/mL)-mediated PC12 cells,[18] they were evaluated for their ability to induce neurite outgrowth in PC12 cells according to a previously reported experimental procedure.[19–22] None of the compounds had morphological effects on PC12 cells in the absence of NGF, whereas, in the presence of NGF (20 ng/mL), neovibsanins A (**7**) and B (**8**), neovibsanin L (**3**), and (8*Z*)-neovibsanin M (**4**) significantly promoted neurite outgrowth from PC12 cells in a dose-dependent manner at concentrations ranging from 20 to 40 μM. The degree of their effects on neurite outgrowth in PC12 cells was demonstrated by morphological observations and quantitative analysis of the neurite length extending from the cell bodies (Figs. 5 and 6). The mean neurite length of the NGF-mediated PC12 cells treated with **7**, **8**, **3** or **4** increased dose-dependently. The seven-membered vibsane-type diterpenes **5** and **6** showed no activity at concentrations ranging from 0.1 to 40 μM in the presence of 20 ng/mL NGF. In a comparison of the mean neurite length between **7** and **8**, **8** seemed to be a more potent NGF-potentiator than **7**. Likewise, in a comparison of the mean neurite length between **3** and **4**, **4** seemed to be more potent NGF-potentiator than **3**. It should be noted that both **4** and **8** carry an α-methoxy group on their C-7 acetal carbon. These results suggest that in addition to the neovibsanin skeleton, which is regarded as a structural requirement for neurite outgrowth-promoting activity, the stereochemistry of the methoxy group at the C-7 position plays an important role in the enhancement of NGF-potentiating activity in PC12 cells.

In conclusion, six novel diterpenoids, neovibsanin O (**1**), neovibsanin M (**2**), neovibsanin L (**3**), (8*Z*)-neovibsanin M (**4**), 15-*O*-methylvibsanin H (**5**), and 5-*epi*-15-*O*-methylvibsanin H (**6**), were isolated from *V. sieboldii*.[23] Neovibsanin O (**1**) is the second example of a vibsane-type 17-norditerpenoid and has a unique 2-oxabicyclo[3,3,1]nonane structure. This is the first time type a natural product has been reported to have such a structure. Compounds **3**, **4**, **7**, and **8**, all of which possess a neovibsanin skeleton, had significant NGF-potentiating effects on PC12 cells, and therefore were identified to be responsible for the neurite outgrowth-promotion of NGF-mediated PC12 cells induced by the MeOH extract of *V. sieboldii*. Among the subtypes of vibsane-type diterpenoids, the present study demonstrates, for the first time, that only neovibsanins with an acetal group in their molecule show potent NGF-potentiating activity in PC12 cells. Compounds with a neovibsanin structure are expected to be investigated with the aim of developing drugs for the treatment of neurodegenerative diseases such as Alzheimer's disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.085.

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23. Compounds **2**, **3**, **4**, **7**, and **8** might be artificial products since methanol was used for the extraction procedure. As shown in Scheme 1, it is assumed that neovibsanin which has a free hydroxy group at the acetal C-7 position is the intact natural product.