Neovibsanin F and Its Congeners, Rearranged Vibsane-Type Diterpenes from *Viburnum suspensum*

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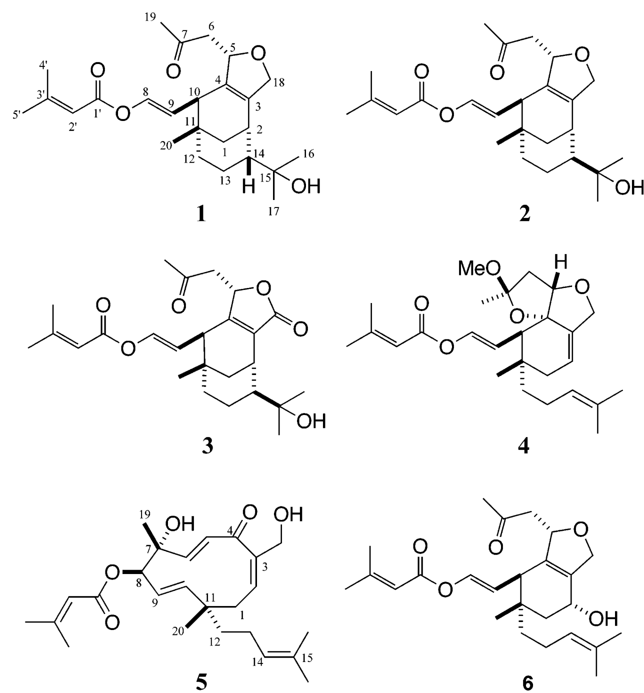
Three new rearranged vibsane-type diterpenes, neovibsanin F (**1**), 14-*epi*-neovibsanin F (**2**), and 14-*epi*-18-oxoneovibsanin F (**3**), have been isolated from the leaves of *Viburnum suspensum*, and their structures were elucidated on the basis of spectroscopic data and comparison with those of previously reported vibsane-type diterpenes.

Only two *Viburnum* species, *V. awabuki*[1−6] and *V. odoratissisium*,[7−10] elaborate biosynthetically unique vibsane-type diterpenes, although about 150 species are known around the world. Vibsane-type diterpenes can be further categorized into two subtypes with 11- and seven-membered ring types, as well as a rearranged type such as vibsanins B (**5**) and C[11,12] and neovibsanin A (**4**),[13] respectively. Over 60 vibsane diterpenes have been so far isolated exclusively from these two species, in addition to the liverwort, *Odontoschisma denudatum*.[14] Some vibsane-type diterpenes have attracted considerable synthetic attention[15] because of their unique structures, combined with wide-ranging biological activities.[12,16] As part of our chemical and biological studies on vibsane-type diterpenes, we have investigated the chemical components of *V. suspensum*, thus resulting in the isolation of three new rearranged vibsane-type diterpenes, **1**−**3**. In this paper, we report the structure elucidation and biological activity of these compounds.

The methanol extract of the leaves of *Viburnum suspensum* was subjected to a variety of chromatographic separations and was finally purified by HPLC to give the new compounds **1**−**3** along with the previously known vibsanin B (**5**)[12] and neovibsanin I (**6**).[17]



Neovibsanin F (**1**) was obtained as colorless and amorphous. The molecular formula of **1** was established as $C_{25}H_{36}O_5$ by HRFABMS. The NMR (Tables 1 and 2) and IR data of **1** showed the presence of a hydroxy group (3455 cm^{-1}), three tertiary methyl groups [δ_H 0.81 (H_3-20), 0.95 (H_3-16), and 0.96 (H_3-17)], a disubstituted double bond with an *E* geometry [δ_H 5.17 (dd, J = 12.6, 11.0 Hz, H-9), 7.42 (d, J = 12.6 Hz, H-8); δ_C 115.0 (C-9), 136.0 (C-8)], an oxymethylene [δ_H 4.43 (ddd, J = 11.8, 3.3, 3.3 Hz, H-18), 5.26 (ddd, J = 11.8, 3.0, 3.0 Hz, H-18), δ_C 77.6 (C-18)] having long-range couplings with H-2 at δ_H 2.35 (dddd, J = 3.3, 3.0, 2.7, 2.5 Hz) and H-5 at δ_H 5.19 (dddd, J = 5.6, 4.4, 3.3, 3.0 Hz), a methyl ketone [1715 cm^{-1}; δ_H 1.99 (H_3-19); δ_C 207.1 (C-7)], and a β,β-dimethyl acrylate group (partial unit **A** shown in Figure 1), as is usual for vibsane-type diterpenes. Analysis of the

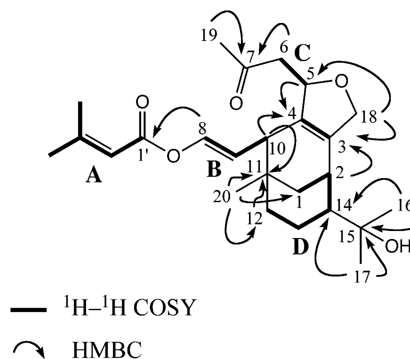


Figure 1. ^{1}H−^{1}H COSY and HMBC correlations of **1**.

COSY and HMQC spectra of **1** provided the additional partial structures **B**−**D** depicted in Figure 1. These spectroscopic data disclosed that **1** is a rearranged vibsane-type diterpene without an acetal ring and similar to neovibsanin I (**6**).[17]

In the HMBC spectrum of **1** (Figure 1), the H_3-17 and H_3-16 signals showed correlations to the quaternary C-15 signal (δ_C 71.2), indicating the presence of a 2-hydroxyisopropyl unit, which was proved to be attached to C-14 in unit **D**, on the basis of a HMBC correlation between H_3-16 and/or H_3-17 and C-14. The H-6 signal showed a cross-peak to the C-7 carbonyl resonance and the H-8 signal correlated to the C-1′ ester carbonyl signal at δ_C 163.4 in unit **A**, suggesting that the methyl ketone was bonded to C-6 and unit **A** linked to C-8 via an ester bond. Moreover, one (δ_H 4.43) of the H_2-18 signals showed an HMBC correlation with the C-5 (δ_C 84.2) signal. In addition to the HMBC data, long-range couplings between H-5 and H-18 and low-field chemical shifts observed for C-5 and C-18 allowed us to propose an ether bond between C-5 and C-18. From the additional HMBC correlations of H_3-20 to C-1 (δ_C 40.0), C-12 (δ_C 43.1), and the quaternary carbon C-11 (δ_C 32.8), the unit **D** led to the formation of a cyclohexane ring including the C-11 quaternary carbon. Finally, another six-membered ring was obtained by the HMBC correlations of H-10 and H-2 to C-11 and C-3 (δ_C 136.2), respectively. These HMBC data culminated in the

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Table 1. ^{1}H NMR (600 MHz, C_6D_6) Data of **1**−**3**

position	**1**	**2**	**3**
1	0.98 (dd, 12.5, 2.5)	1.13 (dd, 12.5, 2.2)	0.91 (dd, 10.2, 3.3)
	1.44 (dd, 12.5, 3.3)	1.18 (dd, 12.9, 3.6)	1.25 (dd, 10.2, 2.5)
2	2.35 (dddd, 3.3, 3.0, 2.7, 2.5)	2.10 (br dd, 3.6, 2.2)	2.81 (ddd, 3.6, 2.5, 2.5)
5	5.19 (dddd, 5.6, 4.4, 3.0, 3.0)	5.27 (br dd, 8.0, 4.1)	4.91 (dd, 7.4, 4.4)
6	2.39 (dd, 13.6, 5.6)	2.29 (dd, 14.3, 8.0)	1.92 (dd, 16.2, 7.4)
	2.61 (dd, 13.6, 4.4)	2.38 (dd, 14.3, 4.1)	2.10 (16.2, 4.4)
8	7.42 (d, 12.6)	7.47 (d, 12.4)	7.33 (d, 12.5)
9	5.17 (dd, 12.6, 11.0)	5.33 (dd, 12.4, 9.8)	5.00 (dd, 12.5, 10.4)
10	2.42 (d, 11.0)	2.10 (d, 9.8)	2.06 (d, 10.4)
12	1.12 (ddd, 13.5, 13.4, 4.9)	1.23 ddd, 11.5, 11.5, 3.0)	0.97 (ddd, 13.7, 13.5, 3.6)
	1.55 (dddd, 13.4, 4.9, 4.4, 3.3)	1.36 (ddd, 11.5, 3.8, 2.8)	1.32 (ddd, 13.7, 4.3, 4.1)
13	1.38 (dddd, 13.5, 4.9, 4.9, 2.7)	1.06 (dddd, 12.1, 11.5, 11.4, 3.8)	1.13 (2H, m)
	1.63 (dddd, 13.5, 13.4, 12.5, 4.4)	1.25 (dddd, 12.1, 5.3, 3.0, 2.8)	
14	1.05 (ddd, 12.5, 2.7, 2.7)	1.02 (ddd, 11.4, 5.3, 2.2)	1.35 (dd, 10.4, 3.6)
16	0.95 (s)	0.87 (s)	1.24 (s)
17	0.96 (s)	0.88 (s)	1.11 (s)
18	4.43 (ddd, 11.8, 3.0, 3.0)	4.41 (dd, 12.1, 3.3)	
	5.26 (ddd, 11.8, 3.0, 3.0)[a]	4.63 (ddd, 12.1, 2.5, 2.5)	
19	1.99 (s)	1.95 (s)	1.64 (s)
20	0.81 (s)	0.85 (s)	0.75 (s)
2′	5.64 (qq, 1.4, 1.4)	5.66 (qq, 1.4, 1.1)	5.67 (qq, 1.4, 1.1)
4′	2.04 (d, 1.4)	2.04 (d, 1.1)	2.04 (d, 14.1, 1.1)
5′	1.36 (d, 1.4)	1.36 (d, 1.4)	1.38 (d, 1.4)

[a] The chemical shift of one of the H-18 protons is shifted downfield presumably due to the electronegative effect of the α-oriented 2-hydroxylisopropyl group at the C-14 position.

Table 2. ^{13}C NMR Data (150 MHz, C_6D_6)[a] of **1**−**3**

position	**1**	**2**	**3**
1	40.0	29.2	28.7
2	30.2	28.5	27.1
3	136.2	141.2	134.7
4	136.6	133.3	161.9
5	84.6	82.9	77.3
6	47.5	48.4	44.1
7	207.1	205.3	202.5
8	136.0	137.5	138.1
9	115.0	112.9	111.5
10	42.9	44.7	45.7
11	32.8	32.2	32.6
12	43.1	36.0	35.8
13	20.0	20.0	19.9
14	50.1	47.2	47.7
15	71.2	72.5	72.6
16	30.3	26.1	29.3
17	28.5	28.1	25.8
18	77.6	74.9	171.9
19	31.4	30.4	30.4
20	29.7	27.9	27.5
1′	163.4	163.2	163.1
2′	115.2	115.2	114.8
3′	159.5	159.6	160.7
4′	20.2	20.3	20.3
5′	27.0	27.1	27.1

[a] Assignment made from DEPT, HMQC, and HMBC NMR spectra.

proposal of a planar structure for **1** with a bicyclo[3.3.1]nonane ring. The relative stereochemistry of **1** was elucidated by NOESY experiments as summarized in Figure 2a. According to the NOESY correlations of H-1α to H-14β and H-12β, and the large J values of H-13α [δ_H 1.63 (dddd, J = 13.5, 13.4, 12.5, 4.4 Hz)] and H-14β [δ_H 1.05 (ddd, J = 12.5, 2.7, 2.7 Hz)], the cyclohexane ring in **1** adopts a chair conformation and has an α and equatorial 2-hydroxyisopropyl group at the C-14 position. The other NOESY correlations supported the relative configurations on C-5 and C-10. Hence, on the basis of the above spectroscopic data, the structure of neovibsanin F could be represented as **1**.

Compound **2** was assigned the same molecular formula, $C_{25}H_{36}O_5$, as **1**, on the basis of its HRFABMS. The NMR data (Tables 1 and 2) of **2** resembled those of **1**. Analysis of the 2D NMR spectra for **2** gave the same planar structure as that of **1**. These spectroscopic similarities suggested that **2** is a stereoisomer of **1** at the C-14 position. The relative stereochemistry of **2** was defined at the same stereogenic centers as those of **2** except for C-14 by the NOESY experiments summarized in Figure 2b. The observation of cross-peaks between H-1α and H-13β as well as H-12α and H-10/H-14α suggested that in the case of **2** the cyclohexane ring adopts a boat conformation with a pseudoequatorial 2-hydroxyisopropyl group at the C-14 position. Additionally, this was supported by the fact that the chemical shift of C-1 shifted significantly upfield by ca. 10 ppm as compared with that of **1**, presumably because of a steric effect between C-1 and C-13. Thus, the structure of **2** was elucidated as 14-*epi*-neovibsanin F.

The molecular formula of compound **3** was assigned as $C_{26}H_{36}O_6$ by HRFABMS. The IR spectrum displayed an absorption at 1750 cm^{-1} characteristic of a conjugated five-membered lactone ring, and the ^{13}C NMR data (Table 2) were very similar to those of **2** except for the absence of a H_2-18 oxymethylene and the presence of an additional ester carbonyl at δ_C 171.9. Accordingly, the C-18 oxymethylene occurring in most vibsane-type diterpenes was oxidized to a carbonyl function in **3**. Analysis of the $^{1}H-^{1}H$ COSY and HMQC spectra of **3** gave the same partial structures **A**−**D** as **1** except for the missing C-18 oxymethylene. A replacement carbonyl group (C-18) exhibited HMBC spectroscopic correlations with H-2 (δ_H 2.81) and H-5 (δ_H 4.91), and the C-3 (δ_C 134.7) and C-4 (δ_C 161.9) signals showed HMBC correlations to the H-14 (δ_C 1.35) signal and to H-5 and H-10 (δ_H 2.06), respectively, thus supporting the presence of a conjugated γ-lactone ring. The other HMBC correlations allowed the planar structure of **3** to be proposed. The relative configuration of **3** was elucidated on the basis of the NOESY spectrum as being identical with that of **2**. On the basis of the above-mentioned consideration, the structure of **3** was assigned as 14-*epi*-18-oxoneovibsanin F.

In conclusion, we have isolated for the first time three new rearranged vibsane-type diterpenes **1**−**3** together with the 11-membered-ring-containing compound, vibsanin B (**5**), and neovibsanin I (**6**) from the leaves of *V. suspensum*. In addition to *V. awabuki* and *V. odoratissisium*, *V. suspensum* can be regarded as the third member of the genus *Viburnum* to produce vibsane-type diterpenoids. The tricyclic neovibsanins **1**−**3** are presumably derived from neovibsanin A (**4**) via neovibsanin I (**6**) or in one step by dehydration of the hydroxy group at the C-14 position.[18,19]

In the brine shrimp lethality assay,[20,21] vibsanin B (**5**) and neovibsanin F (**1**) showed activity with LD_{50} values of 17.5 and 46.0 μg/mL, respectively, but compounds **2** and **3** had no activity at 100 μg/mL. Compounds **1**−**6** were also evaluated for cytotoxicity against the KB cell line,[22] and compounds **5** and **6** exhibited IC_{50} values of 3.5 and 18.0 μM, respectively.

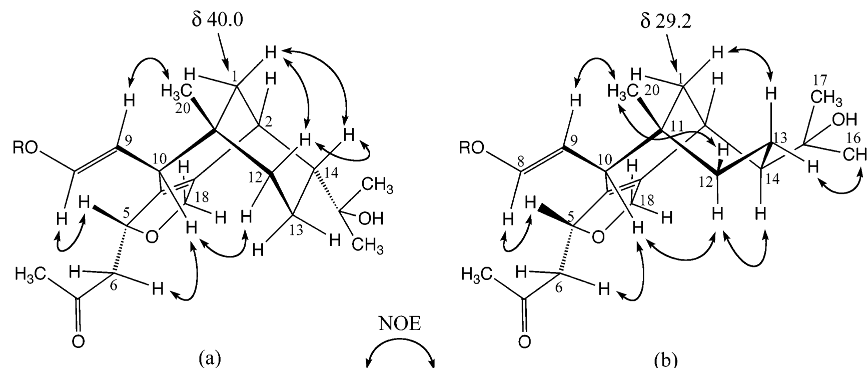

Figure 2. NOESY correlations for **1** (a) and **2** (b).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR and UV spectra was measured on a JASCO FT-IR 5300 infrared and a Shimazu UV-300 spectrophotometer, respectively. NMR spectra were recorded on a Varian Unity 600 instrument. Chemical shifts are given as δ (ppm) with TMS as internal standard. The HRFABMS were recorded on a JEOL AX-500 instrument. Column chromatography was carried out on Kiselgel 60 (70−230 mesh and 230−400 mesh) and Sephadex LH-20.

Plant Material. The leaves of *Viburnum suspensum* Lindl. were collected in the medicinal plant garden of Tokushima Bunri University (TBU) in September 2001 and identified by Dr. Manabe (Naruto Educational College). A voucher specimen (1719LF) has been deposited in the Institute of Pharmacognosy, TBU.

Extraction and Isolation. The dried leaves of *V. suspensum* (1.3 kg) were powdered and extracted with methanol at room temperature to give 20 g of a MeOH extract. A portion of the extract (8 g) was chromatographed over 100 g of silica gel (70−230 mesh), eluted successively with CH_2Cl_2, CH_2Cl_2−EtOAc (9:1, 1:1), EtOAc, EtOAc−MeOH (7:3), and MeOH, to yield five fractions (A−E). Fraction B (1.3 g) was further separated by chromatography on a Cosmosil $75C_{18}$ (ODS) column, with MeOH−H_2O (3:1) as eluent, to give five fractions. The fifth fraction (48 mg) was further purified by HPLC on a Cosmosil $5C_{18}$ column (10 × 250 mm) with MeOH−H_2O (7:3, at a flow rate of 2.5 mL/min), to afford neovibsanin F (**1**, 6.9 mg) and 14-*epi*-neovibsanin F (**2**, 3.5 mg). The fourth fraction (31 mg) was purified by HPLC on a Cosmosil $5C_{18}$-AR column (10 × 250 mm) with MeOH−MeCN−H_2O (12:7:1, at a flow rate of 2.0 mL/min), to give neovibsanin F (**1**, 3.9 mg) and neovibsanin I (**6**, 6.0 mg). The third fraction (79 mg) was purified by HPLC on a Cosmosil $5C_{18}$-AR column (10 × 250 mm) with MeOH−MeCN−H_2O (12:7:1, at a flow rate of 2.0 mL/min), to give 14-*epi*-18-oxoneovibsanin F (**3**, 5.5 mg) and vibsanin B (**5**, 12 mg).

Neovibsanin F (1): colorless, amorphous solid; $[\alpha]^{23}_{D}$ +136.7 (*c* 1.99, $CHCl_3$); IR ν_{max} 3455 (OH), 1715 (C=O), 1644 cm^{-1}; UV λ_{max} (EtOH) 234 (ϵ 10 100) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 439.2451 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_5Na$, 439.2460).

14-*epi*-Neovibsanin F (2): colorless, amorphous solid; $[\alpha]^{23}_{D}$ +128.8 (*c* 0.71, $CHCl_3$); IR ν_{max} 3414 (OH), 1722 (C=O), 1643 cm^{-1}; UV λ_{max} (EtOH) 236 (ϵ 9500) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 439.2454 (calcd for $C_{25}H_{36}O_5Na$, 439.2460).

14-*epi*-18-Oxoneovibsanin F (3): colorless, amorphous solid; $[\alpha]^{23}_{D}$ +126.3 (*c* 1.13, $CHCl_3$); IR ν_{max} 3457 (OH), 1750, 1728, 1643 (C=O) cm^{-1}; UV λ_{max} (EtOH) 229 (ϵ 16 000) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 453.2248 (calcd for $C_{25}H_{34}O_6Na$, 453.2253).

Bioassay Evaluation. The brine shrimp lethality assay was carried out according to the previously established protocol.[23] Berberine chloride (LD_{50} 66.9 μM) was used as a positive control. The cytotoxic activity was evaluated against the KB cell line according to the established protocol.[22] Adriamycin (IC_{50} 0.007 μM) was used as a positive control.

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