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ACETAL - BEARING REARRANGED VIBSANE-TYPE DITERPENOIDS FROM *VIBURNUM AWABUKI*¹

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Abstract – Neovibsanin J (**1**), neovibsanin K (**2**), and neovibsanin P (**3**), unique
vibsane-type diterpenoids bearing an acetal moiety at the C-7 position, were
isolated from the leaves of *Viburnum awabuki* and their structures were elucidated
by NMR spectral analysis using 2D techniques.

INTRODUCTION

Vibsane-type diterpenes are very rare diterpenoids, whose occurrence is limited to a few *Viburnum* species such as *Viburnum awabuki*, *V. odoratissimum*, and *V. suspensum*, and they have not been found in other *Viburnum* species.^{2,3,7-8} The carbon skeletons of these diterpenoids are further classified into three subtypes: 11-membered ring, 7-membered ring, and rearranged types, which are represented by vibsantin B (**4**),^{4,5} vibsantin C (**5**),^{4,5} and neovibsanin A (**6**),⁶ respectively. Additionally, we have established the chemical correlations vibsantin B to vibsantin C and neovibsanins, which has allowed us to propose a plausible biosynthetic route to three subtypes from vibsantin B.² Some of them have attracted considerable synthetic attention because of their unique structures and wide-ranging biological activities.⁹⁻¹⁵ With an extensive background like the aforementioned facts we have continued to study the chemical constituents of the leaves of *V. awabuki*, resulting in the isolation of three new diterpenoids named neovibsanin J (**1**), neovibsanin K (**2**), and neovibsanin P (**3**).

Herein, we report the isolation and structural elucidation of three new rearranged vibsane-type diterpenes **1** – **3**, which are unique in bearing an acetal ring at the C-7 position as their common feature.

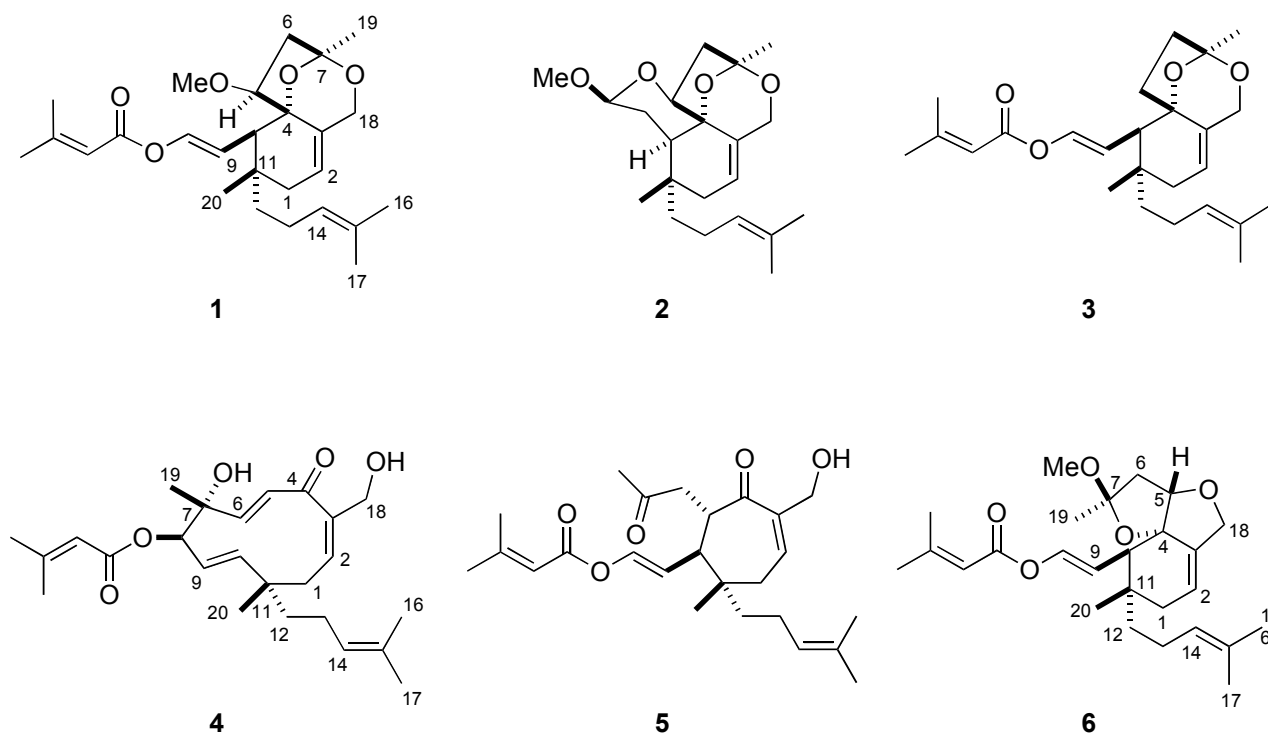


Figure 1. The structures of the vibsanine-type diterpenes isolated from *V. awabuki*.

RESULTS AND DISCUSSION

The dried leaves of *V. awabuki* collected in Tokushima, Japan were extracted with MeOH. Repeated purification of the MeOH extract by a combination of silica gel column chromatography and HPLC furnished neovibsanin J (**1**, 0.00054 %), neovibsanin K (**2**, 0.0013 %) and neovibsanin P (**3**, 0.0029 %) as the new compounds.

Neovibsanin J (**1**) had the molecular formula, $C_{25}H_{38}O_5$, which was established by HR-FABMS at m/z 453 ($M + Na$)⁺. The 1H NMR (Table 1) and physical data of **1** showed the presence of a methoxy group [δ_H 2.95 (3H, s); δ_C 57.4], two tertiary methyl groups [δ_H 0.95 and 1.47 (each s, 3H)], two trisubstituted double bonds [δ_H 5.25 (brt, $J = 7.1$ Hz), 5.35 (brdd, $J = 3.6, 0.8$ Hz)], one disubstituted double bond [δ_H 5.67 (dd, $J = 12.4, 11.3$ Hz), 7.57 (d, $J = 12.4$ Hz)], and an oxymethine [δ_H 3.68 (dd, $J = 9.6, 4.3$ Hz)], as well as a β,β -dimethylacrylate group [m/z 83; λ_{max} 229 nm; ν_{max} 1732 cm^{-1} ; δ_H 1.36 (d, $J = 1.4$ Hz, 3H), 2.05 (d, $J = 1.4$ Hz, 3H), 5.69 (qq, $J = 1.4, 1.4$ Hz)] that is typical of the vibsanine-type diterpenoids. Analyses of H-H COSY and HMQC spectra provided five partial structures A–E, among which the sole partial fragment C was different from those of neovibsanin A (Figure 2). Next, 1H -heteronuclear multiple-bond correlation (HMBC) experiments were carried out in order to determine the connectivity between the partial structures A–E and the quaternary carbons. As shown in Figure 2, an enol ester moiety was formed by the units A and B, and the units D and E were arranged on the cyclohexene ring in the same manner as in neovibsanin A (**6**), whereas the unit C (C5 – C6 – C7) was not consistent with that

of **6** since the coupling constants ($J_{5,6\beta} = 9.6$ Hz, $J_{5,6\alpha} = 4.3$ Hz) between H-5 and H-6 in **1** were quite different from those of **6** ($J_{5,6\beta} = 4.4$ Hz, $J_{5,6\alpha} = 0$ Hz). The HMBC correlation of a methoxy signal to the C-5 oxymethine resonating at δ_C 83.0 as well as of H₃-19 at δ_H 1.47 to the C-7 acetal carbon resonating at δ_C 105.0 indicated that the methoxy and C-19 methyl groups were connected to C-5 on the unit C and the C-7 acetal carbon, respectively. Moreover, H₂-6, H-18 and H-5 showed HMBC correlations to C-7, and thereby C-6 was connected to C-7, which in turn formed a cyclic acetal through the C-4 and C-18 oxygen atoms. The HMBC correlation of H-5 to the C-4 quaternary carbon allowed us to connect between C-4 and C-5, with considering 8 degrees of unsaturation, thus leading to propose the tricyclic plane structure **1** as shown in Figure 2. The relative stereochemistry of **1** was elucidated by NOESY as shown in Figure 3. Namely, H₃-20 showed NOE correlation to H-9, indicating that both the methyl group at C-11 and the enol ester side chain at C-10 should have *R**-orientations. Additionally, H-10 α and H-9 showed NOE correlations to H-5, suggesting that the methoxy group at C-5 has a *S**-configuration. Finally, the C-19 methyl group was defined as α on the basis of a series of sequential NOE correlations of H-2/H-18 α , H-18 β /H-6 β /OMe, and H-5/H-6 α /H₃-19 as shown in Figure 3. Thus, on the basis of the aforementioned spectra data, the structure of neovibsanin J was elucidated as **1**.

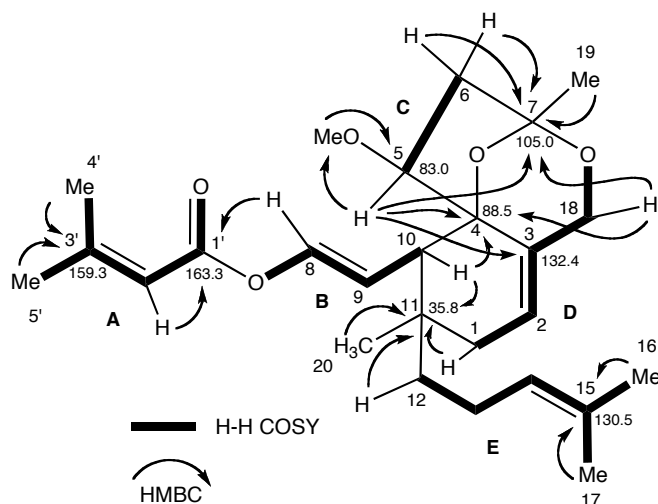


Figure 2. HMBC correlations of **1**.

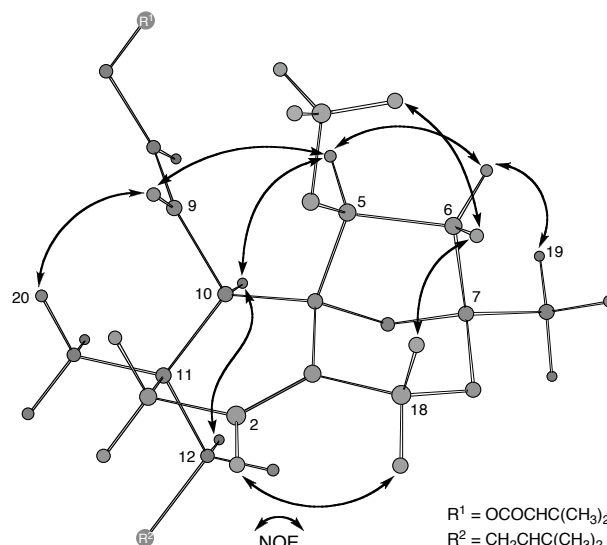


Figure 3. NOESY correlations of **1**.

Neovibsanin K (**2**) had the molecular formula $C_{21}H_{32}O_4$, which was established by HR-FABMS at m/z 371 ($M + Na$)⁺, and indicated 6 degrees of unsaturation. The NMR data (Table 1) of **2** showed the presence of four tertiary methyl groups [δ_H 0.79, 1.52, 1.68, and 1.70 (each 3H, s)], a methoxy group [δ_H 3.30 (3H, s); δ_C 54.8], two trisubstituted double bonds [δ_H 5.06 (brd, $J = 6.5$ Hz), 5.32 (brt, $J = 6.6$ Hz)], an oxymethylene [δ_H 3.90 (2H, s, H-18); δ_C 65.1, C-18], one oxymethine [δ_H 4.15 (dd, $J = 7.4, 3.0$ Hz, H-5); δ_C 74.0, C-5], an acetal carbon (δ_C 105.9, C-7), and a methyl acetal moiety [δ_H 4.61 (dd, $J = 8.5, 6.0$

Hz, H-8), 3.30 (3H, s); δ_C 100.8, C-8] which was verified by HMBC correlation between C-8 and OMe, but no signal was found to be corresponded to the β,β -dimethylacrylate group that commonly exists in the vibsane-type diterpenoids. Extensive analyses of H-H COSY and HMQC of **2** provided a new partial structure **A** including a methyl acetal carbon (δ_C 100.8) instead of the β,β -dimethylacrylate group, in addition to the same three partial structures **B** – **D** as **1** had (Figure 4). In HMBC, the acetal H-8 correlated to C-5 (δ_C 74.0), and also H-9 showed a cross-peak to the oxygen-bearing quaternary C-4 (δ_C 83.8). Considering the 6 degrees of unsaturation and the other HMBC correlations, **2** should contain another six-membered acetal ring that includes the unit **A** at C-5 and C-4. Thus, the above spectral data culminated in giving the tetracyclic plane structure **2**. The relative stereochemistry of **2** was elucidated by NOESY as shown in Figure 5. The configurations of C-4, C5 and C-7 were conceivably identical with those of the corresponding stereogenic centers in **1** according to the NOEs. Additionally, it was suggested from the NOESY correlations between H₃-20 and H-9 α as well as between H-10 and H-8 that the methoxy group at the C-8 position has a *S**-configuration and that H₃-20 has a β and equatorial orientation. Conformational searches of **2** using Macro Model[®] (v. 6.0) provided the most stable conformer, which exactly corresponded to the conformation conceived by the NOESY experiments. In fact, the observed *J* values (8.5 and 6.0 Hz for H-8, and 14.3 and 2.7 Hz for H-10) were comparable with the calculated ones (8.2 and 6.7 Hz for H-8, and 12.3 and 2.4 Hz for H-10). Hence, on the basis of above spectral data, the structure of neovibsanin K was elucidated as **2**.

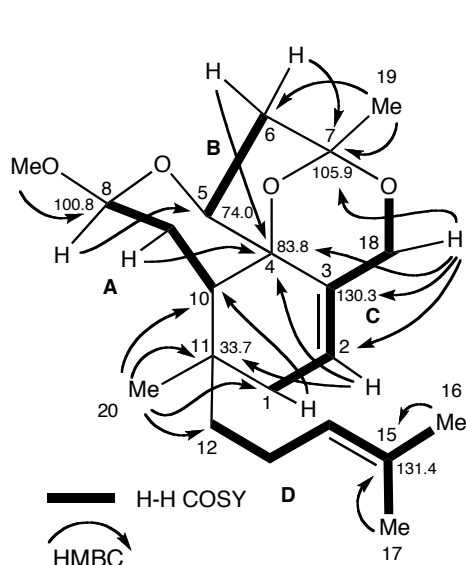


Figure 4. HMBC of **2**.

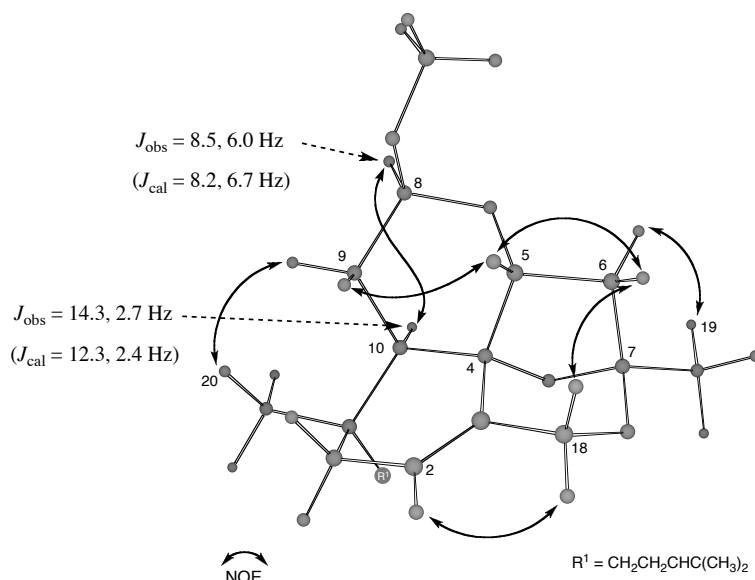


Figure 5. NOESY correlations of **2** and calculated *J* values for the most stable conformation of **2** obtained by MacroModel[®].

The ¹H NMR data of neovibsanin P (**3**) were very similar to those of **1** except for the lack of a methoxy group existing at the C-5 position in **1**. The molecular formula (C₂₅H₃₆O₄) obtained from HR-FABMS

at m/z 423 ($M + Na$)⁺ indicated that **3** is a demethoxy derivative of **1**. In a comparison of the NMR data (Table 1) of **3** with those of **1**, compound **3** was found to have an extra methylene resonating at δ_C 31.7, which was assignable to C-5 by 2D H-H COSY. Additionally, 2D NOESY concluded that the relative stereochemistry of **3** to be the same as **1**. Thus, the structure of **3** was determined to be 5-demethoxy-neovibsanin K.

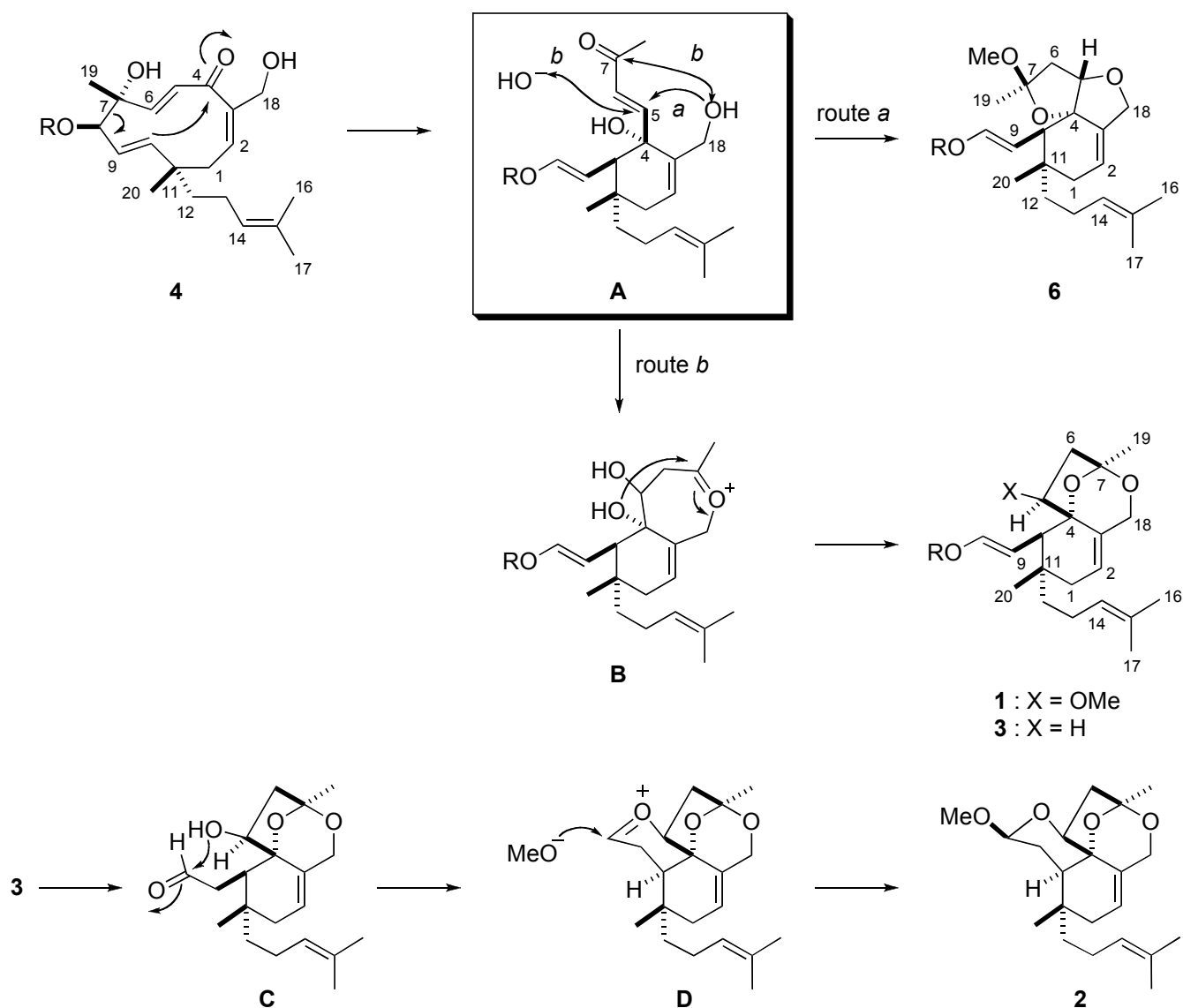
Table 1. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for compounds **1** – **3** in C₆D₆

Position	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.74 (2H, m)	35.4	1.48 (dd, 17.4, 6.5) 1.63 (brd, 17.4)	34.9	1.47 (m) 1.88 (m)	36.6
2	5.35 (brdd, 3.6, 0.8)	121.7	5.06 (brd, 6.5)	119.4	5.02 (brt, 4.9)	117.1
3		132.4		130.3		134.9
4		88.5		83.8		83.3
5	3.68 (dd, 9.6, 4.3)	83.0	4.15 (dd, 7.4, 3.0)	74.0	1.44 (m) 1.88 (m)	31.7
6	1.84 (dd, 13.7, 4.3) 1.88 (dd, 13.7, 9.6)	40.8	2.02 (dd, 14.0, 3.0) 2.26 (dd, 14.0, 7.4)	42.2	1.66 (m) 1.93 (m)	35.4
7		105.0		105.9		104.5
8	7.57 (d, 12.4)	137.6	4.61 (dd, 8.5, 6.0)	100.8	7.69 (d, 12.4)	138.9
9	5.67 (dd, 12.4, 11.3)	112.2	1.24 (ddd, 14.3, 14.3, 8.5) 1.78 (ddd, 14.3, 6.0, 2.7)	27.8	5.46 (dd, 12.4, 10.7)	109.6
10	2.41 (d, 11.3)	48.1	1.78 (dd, 14.3, 2.7)	39.7	2.64 (d, 10.7)	47.3
11		35.8		33.7		35.2
12	1.34 (ddd, 13.2, 13.2, 4.4) 1.87 (m)	40.9	1.38 (m) 2.12 (m)	39.6	1.25 (m) 1.47 (m)	41.7
13	1.94 (m) 2.12 (m)	22.7	1.90 (m) 2.35 (m)	23.0	1.93 (m) 1.93 (m)	22.2
14	5.25 (brt, 7.1)	125.9	5.32 (brt, 6.6)	125.9	5.13 (brt, 7.1)	125.5
15		130.5		131.4		130.7
16	1.68 (3H, s)	25.9	1.68 (3H, s)	25.9	1.65 (3H, s)	25.8
17	1.65 (3H, s)	17.8	1.70 (3H, s)	17.9	1.60 (3H, s)	17.7
18	4.14 (d, 12.4) 4.77 (brdd, 12.4, 0.8)	66.8	3.90 (2H, s)	65.1	4.03 (d, 12.9) 4.23 (brdd, 12.9, 2.1)	64.6
19	1.47 (3H, s)	25.2	1.52 (3H, s)	24.4	1.59 (3H, s)	24.4
20	0.95 (3H, s)	24.2	0.79 (3H, s)	25.3	0.73 (3H, s)	21.4
1'		163.3				163.0
2'	5.69 (qq, 1.4, 1.4)	115.3			5.64 (qq, 1.4, 1.1)	115.3
3'		159.3				159.2
4'	2.05 (3H, d, 1.4)	20.2			2.01 (3H, d, 1.1)	20.2
5'	1.36 (3H, d, 1.4)	27.0			1.33 (3H, d, 1.4)	26.9
OCH ₃ -5	2.95 (3H, s)	57.4				
OCH ₃ -8			3.30 (3H, s)	54.8		

We have already a proposed plausible biogenetic pathway from vibsanin B (**4**) to the neovibsanin skeleton (**A**) and have successfully transformed from **4** to both neovibsnains A (**6**) and B by

photochemical reactions.^{2,3} Neovibsanins **1** – **3** could presumably be converted from **A** as outlined in Scheme 1. Namely, the hydroxyl group at the C-18 position would undergo a 1,4-addition to the α,β -unsaturated ketone, followed by acetalization to give neovibsanin A (**6**) via route *a*. On the other hand, according to route *b*, two hydroxy groups at the C-4 and C-18 positions would make a bicyclic acetal after 1,4-addition of an oxygen nucleophile or reduction of the Δ^5 double bond to produce **1** or **3** through **B**. In the case of hydrolyzing the enol ester, the liberated aldehyde **C** would undergo an acetalization to give **2** through **D**. Considering the plausible biosynthesis, **1** – **3** should take the same absolute configuration as that of **6**, but we have no evidence to confirm it.

In conclusion, we have isolated three unique rearranged vibsanine-type diterpenoid **1**–**3** bearing a cyclic acetal moiety at the C-7 position from *V. awabuki*. The isolation of **1** – **3** provides additional evidence



Scheme 1. Plausible biosynthesis of **1**–**3** from vibsanin B (**4**)

to support the presence of intermediate **A** in the course of neovibsanin biosynthesis. The present studies suggest that vib sane-type diterpenoids are rich in structural diversity and occupy a unique position in the diterpenoid family.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzu UV-300 or Shimadzu UV-1650PC or Hitachi U-3000 spectrophotometer. IR spectra were recorded on a Jasco FT-IR 5300 or a FT-IR 410 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on a Varian Unity 600. MS were recorded on a JEOL AX-500 instrument.

Plant Material. The leaves of *V. awabuki* K. Koch were collected in Tokushima city in September, 1999. A voucher sample has been preserved in the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and Isolation. Air-dried and powdered leaves of *V. awabuki* (1.3 kg) were extracted with MeOH at room temperature for 30 days. The MeOH extract was concentrated *in vacuo* to give a gummy extract (421 g). The MeOH extract was mixed with silica gel [Merck silica gel 70-230 mesh (360 g)], and then the solvent was removed under reduced pressure. The obtained solids were pulverized, and the resultant powders were packed into a glass column and then eluted in order with CH₂Cl₂ (2 L), CH₂Cl₂–EtOAc (3 : 2, 2 L), CH₂Cl₂–EtOAc (2 : 3, 2 L), EtOAc (2 L), EtOAc–MeOH (9 : 1, 2 L), EtOAc–MeOH (3 : 2, 2 L), EtOAc–MeOH (1 : 1, 2 L), and MeOH (2 L) to give fractions 1–10.

Fraction 3 (16.7 g) was separated by silica gel column chromatography with hexane–EtOAc (4 : 1 to 3 : 2) to give fractions 11–22. Fractions 11–12 were subjected to silica gel column chromatography with hexane–EtOAc (9 : 1) to give fractions 23–32. Fraction 25 was separated by silica gel column chromatography with benzene–EtOAc (15 : 1) to give fractions 32–40, and finally purified by HPLC [Cosmosil 5C18 AR II, i.d. 10 x 250 mm; MeCN : H₂O (82 : 18; 2.0 mL/min); det. 254 nm] to give neovibsanin J (**1**, 2.3 mg). Fraction 33 was purified by HPLC [Cosmosil 5C18 AR, i.d. 10 x 250 mm; MeCN : H₂O (4 : 1; 2.0 mL/min); det. 220 nm] to give neovibsanin K (**2**, 5.7 mg). Fraction 32 was separated by silica gel column chromatography with CH₂Cl₂–MeOH (99 : 1), and finally purified by HPLC [Cosmosil 5C18 AR-II, i.d. 10 x 250 mm; MeOH : H₂O (4 : 1; 2.0 mL/min); det. 254 nm] to give neovibsanin P (**3**, 2.4 mg).

neovibsanin J (**1**): colorless oil; $[\alpha]_D^{22} +91.3$ (*c* 0.22, EtOH); IR $\nu_{\max}$ 1732, 1645 cm⁻¹, UV (EtOH) $\lambda_{\max}$ 229 (ϵ 14700) nm; ¹H and ¹³C NMR data (Table 1); FABMS *m/z* 431 (M + H)⁺, 453 (M + Na)⁺, 469 (M + K)⁺; HR-FABMS *m/z* 453.2617, calcd 453.2617 for C₂₆H₃₈O₅Na.

neovibsanin K (**2**): colorless oil; $[\alpha]_D^{19} +51.1$ (*c* 0.50, EtOH); IR $\nu_{\max}$ 1464, 1385 cm⁻¹; ¹H and ¹³C NMR

data (Table 1); FABMS m/z 371 ($M + Na$)⁺; HR-FABMS m/z 371.2183, calcd 371.2199 for C₂₁H₃₂O₄Na. neovibsanin P (**3**): colorless oil; $[\alpha]_D^{21} +2.15$ (c 0.62, MeOH); IR $\nu_{\max}$ 1732, 1645 cm⁻¹; UV (EtOH) $\lambda_{\max}$ 226 (ϵ 20161) nm; ¹H and ¹³C NMR data (Table 1); FABMS m/z 423 ($M + Na$)⁺; HR-FABMS m/z 423.2521, calcd 423.2511 for C₂₅H₃₆O₄Na.

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