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

Miwa Kubo, Yuka Minoshima, Daiki Arimoto, Hiroyuki Minami, Kenichi Harada, Hideaki Hioki, and Yoshiyasu Fukuyama*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

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 vibsanin B (**4**),[4,5] vibsanin C (**5**),[4,5] and neovibsanin A (**6**),[6] respectively. Additionally, we have established the chemical correlations vibsanin B to vibsanin C and neovibsanins, which has allowed us to propose a plausible biosynthetic route to three subtypes from vibsanin 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.

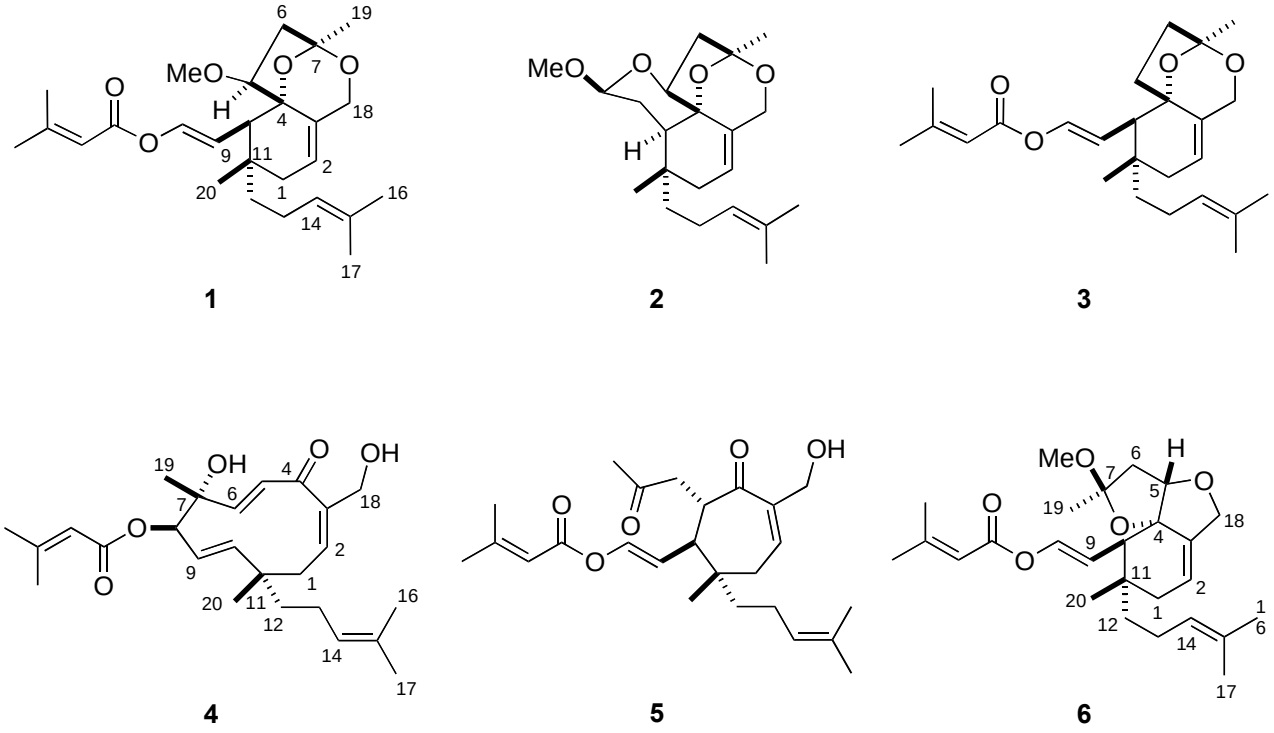


Figure 1. The structures of the vibsane-type diterpenes isolated from *V. awabuki.*

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 vibsane-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 H_3-19 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, H_2-6, 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, H_3-20 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-10α 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-18α, H-18β/H-6β/OMe, and H-5/H-6α/H_3-19 as shown in Figure 3. Thus, on the basis of the aforementioned spectra data, the structure of neovibsanin J was elucidated as **1**.

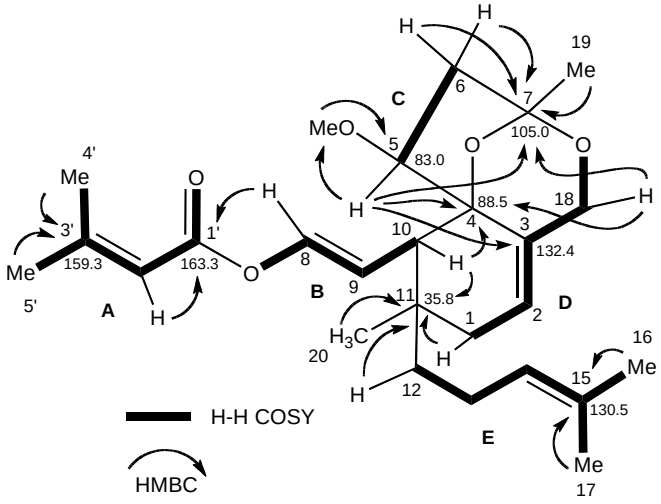


Figure 2. HMBC correlations of **1**.

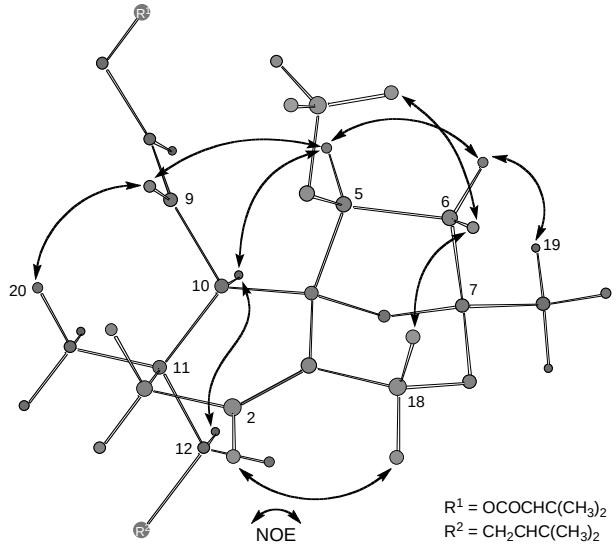


Figure 3. NOESY correlations of **1**.

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 form the NOESY correlations between H_3-20 and H-9α as well as between H-10 and H-8 that the methoxy group at the C-8 position has a *S**-configuration and that H_3-20 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**.

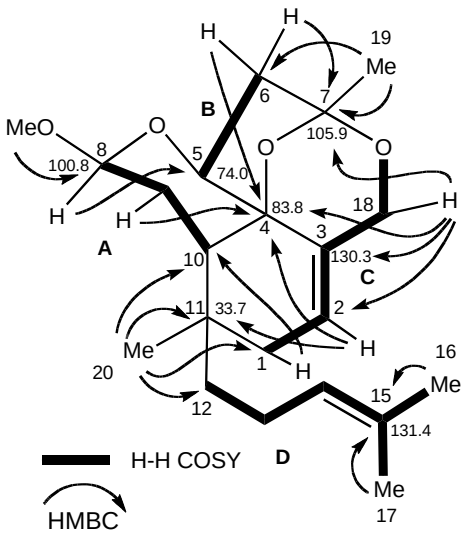


Figure 4. HMBC of **2**.

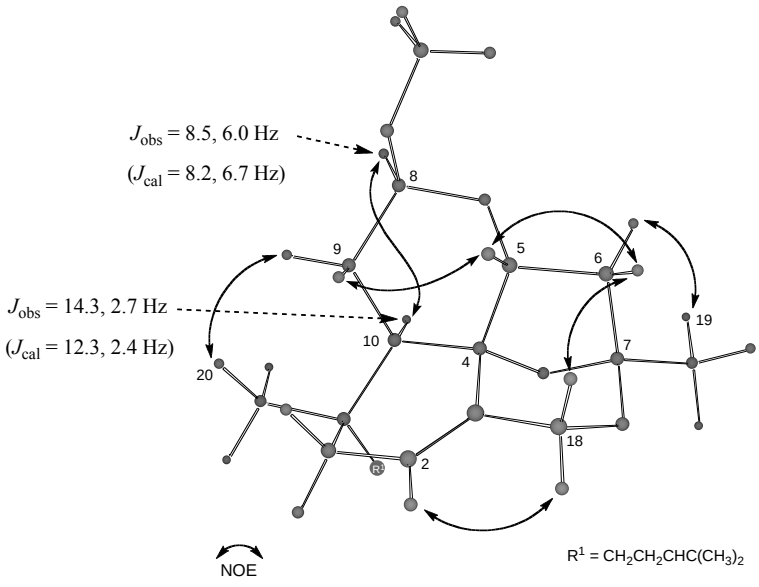


Figure 5. NOESY correlations of **2** and calculated *J* values for the most stable conformation of **2** obtained by MacroModel®.

The ^{1}H 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 ($C_{25}H_{36}O_4$) 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.

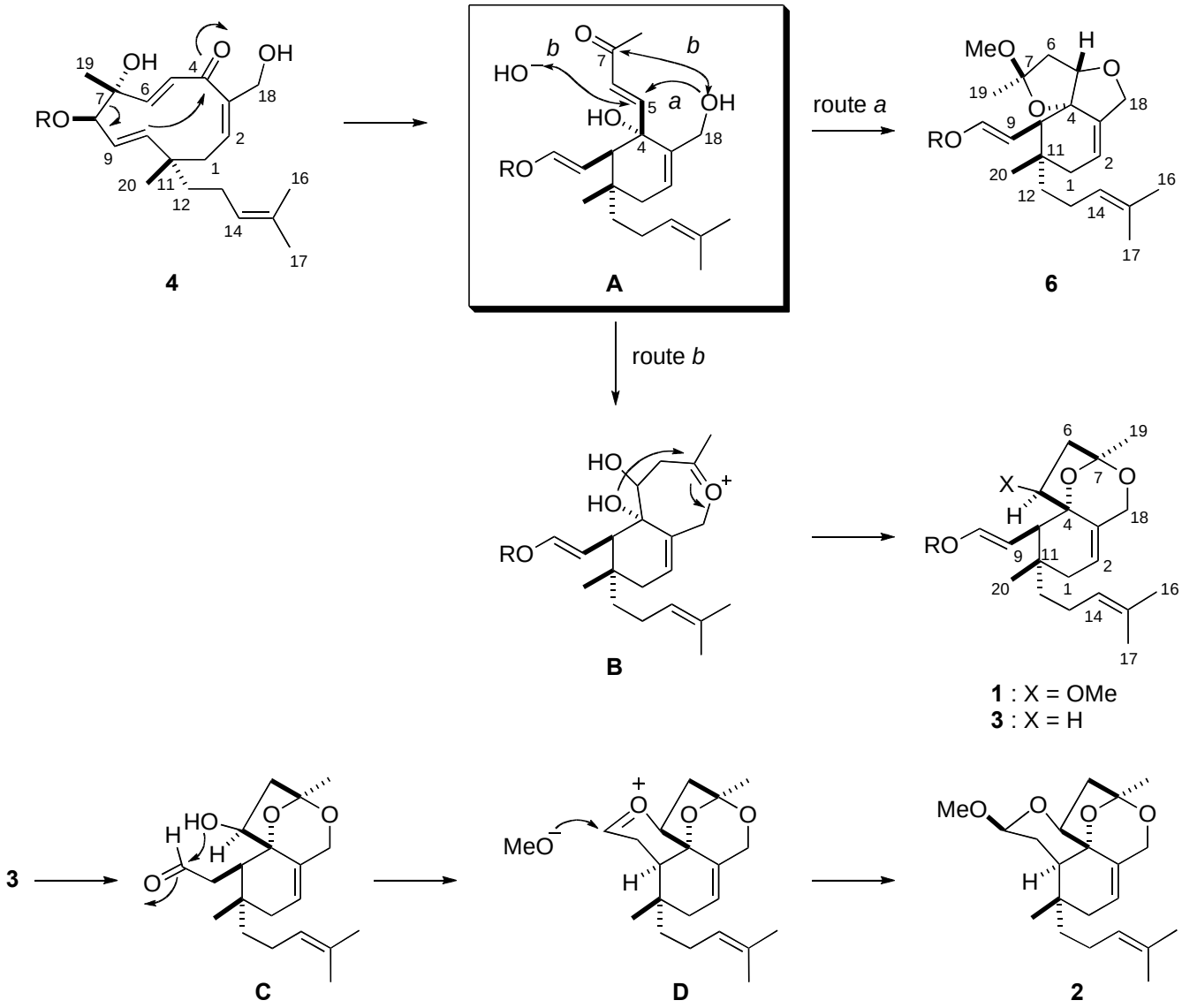
Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for compounds **1** – **3** in C_6D_6

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)	34.9	1.47 (m)	36.6
			1.63 (brd, 17.4)		1.88 (m)	
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)	31.7
					1.88 (m)	
6	1.84 (dd, 13.7, 4.3)	40.8	2.02 (dd, 14.0, 3.0)	42.2	1.66 (m)	35.4
	1.88 (dd, 13.7, 9.6)		2.26 (dd, 14.0, 7.4)		1.93 (m)	
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)	27.8	5.46 (dd, 12.4, 10.7)	109.6
			1.78 (ddd, 14.3, 6.0, 2.7)			
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)	40.9	1.38 (m)	39.6	1.25 (m)	41.7
	1.87 (m)		2.12 (m)		1.47 (m)	
13	1.94 (m)	22.7	1.90 (m)	23.0	1.93 (m)	22.2
	2.12 (m)		2.35 (m)		1.93 (m)	
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)	66.8	3.90 (2H, s)	65.1	4.03 (d, 12.9)	64.6
	4.77 (brdd, 12.4, 0.8)				4.23 (brdd, 12.9, 2.1)	
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 neovinsanin 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 vibsane-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 vibsane-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 CH_2Cl_2 (2 L), CH_2Cl_2–EtOAc (3 : 2, 2 L), CH_2Cl_2–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 : H_2O (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 : H_2O (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 CH_2Cl_2–MeOH (99 : 1), and finally purified by HPLC [Cosmosil 5C18 AR-II, i.d. 10 x 250 mm; MeOH : H_2O (4 : 1; 2.0 mL/min); det. 254 nm] to give neovibsanin P (**3**, 2.4 mg).

neovibsanin J (**1**): colorless oil; $[a]_D^{22}$ +91.3 (*c* 0.22, EtOH); IR ν_{max} 1732, 1645 cm^{-1}, UV (EtOH) λ_{max} 229 (ε 14700) nm; ^1H and ^{13}C 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 $C_{26}H_{38}O_5Na$.

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

data (Table 1); FABMS *m/z* 371 $(M + Na)^+$; HR-FABMS *m/z* 371.2183, calcd 371.2199 for $C_{21}H_{32}O_4Na$. neovibsanin P (**3**): colorless oil; $[a]_D^{21}$ +2.15 (*c* 0.62, MeOH); IR ν_{max} 1732, 1645 cm^{-1}; UV (EtOH) λ_{max} 226 (ε 20161) nm; ^1H and ^{13}C NMR data (Table 1); FABMS *m/z* 423 $(M + Na)^+$; HR-FABMS *m/z* 423.2521, calcd 423.2511 for $C_{25}H_{36}O_4Na$.

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REFERENCES AND NOTES

1. Dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.
2. Y. Fukuyama and T. Esumi, *J. Org. Synth. Chem. Jpn.*, 2007, **65**, 585 and references cited therein.
3. Y. Fukuyama, M. Kubo, T. Fujii, A. Matsuo, Y. Minoshima, H. Minami, and M. Morisaki, *Tetrahedron*, 2002, **58**, 10033.
4. Y. Fukuyama, H. Minami, S. Takaoka, M. Kodama, K. Kawazu, and H. Nemoto, *Tetrahedron Lett.*, 1997, **38**, 1435.
5. K. Kawazu, *Agric. Biol. Chem*., 1980, **44**, 1367.
6. Y. Fukuyama, H. Minami, K. Takeuchi, M. Kodama, and K. Kawazu, *Tetrahedron Lett.*, 1996, **37**, 6767.
7. Y.-C. Shen, C.-L. Lin, S.-C. Chien, A. T. Khalil, C.-L. Ko, and C.-H. Chin, *J. Nat. Prod*., 2004, **67**, 74.
8. Y.-C. Shen, C. V. S. Prakash, L.-T. Wang, C.-T Chien, and M.-C. Hung, *J. Nat. Prod*., 2002, **65**, 1052.
9. B. D. Schwartz, C. M. Williams, E. Anders, and P. V. Bernhardt, *Tetrahedron*, 2008, **64**, 6482.
10. M. J. Gallen and C. M. Williams, *Org. Lett*., 2008, **10**. 713.
11. J. Nikolai, O. Loe, P. M. Dominiak, O. O. Gerlitz, J. Autschbach, and H. M. L. Davies, *J. Am. Chem. Soc*., 2007, **129**, 10763.
12. B. D. Schwartz, D. P. Tilly, R. Heim, S. Wiedemann, C. M. Williams, and P. V. Bernhardt, *Eur. J. Org. Chem*., 2006, 3181.
13. H. M. L. Davies, O. Loe, and D. G. Stafford, *Org. Lett.*, 2005, **7**, 5561.
14. R. Heim, S. Wiedemann, C. M. Williams, and P. V. Bernhardt, *Org. Lett*., 2005, **7**, 1327.
15. H. Yuasa, G. Makado, and Y. Fukuyama, *Tetrahedron Lett*., 2003, **44**, 6235.