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Structures of furanovibsanins A–G from *Viburnum awabuki*

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Abstract—The structures of nine new 7-membered vibsane-type diterpenes named furanovibsanin A (**1**), 3-*O*-methylfuranovibsanin A (**2**), furanovibsanin F (**3**), furanovibsanin B (**4**), 7-*epi*-furanovibsanin B (**5**), furanovibsanin C (**6**), furanovibsanin D (**7**), furanovibsanin E (**8**), and furanovibsanin G (**9**), isolated from the leaves of *Viburnum awabuki*, were elucidated by intensive analyses of 2D NMR data and comparison of NMR data of the previously known vibsanins C and E. Their structures feature in a furan or a cyclic enol acetal ring forming on the C-4 and C-5 positions in vibsanin C (**12**).

1. Introduction

In 1980, Kawazu reported the first isolation of unique vibsane-type diterpenes, vibsanins A–F from the leaves of *Viburnum awabuki*,[1] but much more studies of these diterpenes remained to be done. Since then, neither of chemical and biological studies on this type of diterpenes had been documented until we reported the structure of neovibsanins A and B in 1996.[2] An unprecedented structure of vibsane has attracted us to exhaustive chemical studies of these diterpenes, thereby resulting in vibsane-type diterpenes being further classified into three subtypes, 11-membered ring, 7-membered ring and rearranged type, which vibsanin B (**11**),[1,3] vibsanin C (**12**)[1,3] and neovibasnin A (**13**)[2] are representative of, respectively. Additionally, chemical correlation of vibsanin B to vibsanin C and neovibsanin A has been successfully established by us, which allowed us to propose a plausible biosynthetic pathway of three subtypes of vibsane-type diterpenes.[2,3] Our continuing studies on *Viburnum* species has resulted in the isolation of a variety of three subtypes,[4–9] indicating that vibsane-type diterpenes can be regarded as quite rare natural products because their occurrence has been limited to only two kinds of *Viburnum* plants, *V. awabuki*[1–9] and *Viburnum odoratissimum*[10,11] and they have not been isolated from other *Viburnum* species.[12–19]

In this paper, we describe the structural elucidation and biological activity of nine new 7-membered ring vibsane-type diterpenes **1**–**9**, which feature in a furan ring and/or an acetal ring formed between the 2-propanone unit attached on the C-5 position and the C-4 ketone in vibsanin C (**12**), isolated from the methanol extract of the leaves of *V. awabuki* (Fig. 1).

2. Results and discussion

Furanovibsanin A (**1**) had the molecular formula, $C_{25}H_{34}O_5$, established by high-resolution (HR) fast atom bombardment mass spectrometry (FABMS). Its infrared spectroscopy (IR) spectrum showed the presence of a hydroxyl group (3380 cm^{-1}) and a carbonyl group (1725 cm^{-1}). Routine analysis of ^1H–^1H correlated spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) of **1** indicated the presence of the same partial structures **A**–**D** as those of the previously known 3-hydroxyvibsanin E (**10**) as shown in Fig. 2a, but showed that the 2-propanone unit attached on the C-5 position and the C-4 ketone existing in **10** were missing in **1**. On the other hand, a methylfuran ring **E** newly occurring in **1** was deduced form the signals due to δ_H 1.91 (d, J=1.1 Hz) and 5.58 (q, J=1.1 Hz), and δ_C 18.3, 110.2, 126.1, 149.8 and 150.1, and then its presence was substantiated by the HMBC correlations of H-6 (δ_H 5.58) to C-5 (δ_C 126.1) and C-4 (δ_C 149.8). This methylfuran ring is presumably formed through an enol acetalization between the C-4 ketone and the 2-propanone unit at C-5 in **10** followed by dehydration. Thus, the methyl furan ring could compensate for lack of two functional groups in **10**. Additionally, the other HMBC correlations as shown in Fig. 2a were consistent with the same structure framework as that of **10**. The relative stereochemistry of **1** as shown in Fig. 2c was elucidated by rotating frame nuclear Overhauser and exchange spectroscopy (ROESY) to be identical with **10**. On the basis of the above spectral data, the structure of furanovibsanin A was represented as **1**.

Compound **2** had the molecular formula, $C_{26}H_{36}O_5$,

Keywords: *Viburnum awabuki*; 7-membered vibsane-type diterpene; furanovibsanin; brine shrimp test (BST); cytotoxicity.

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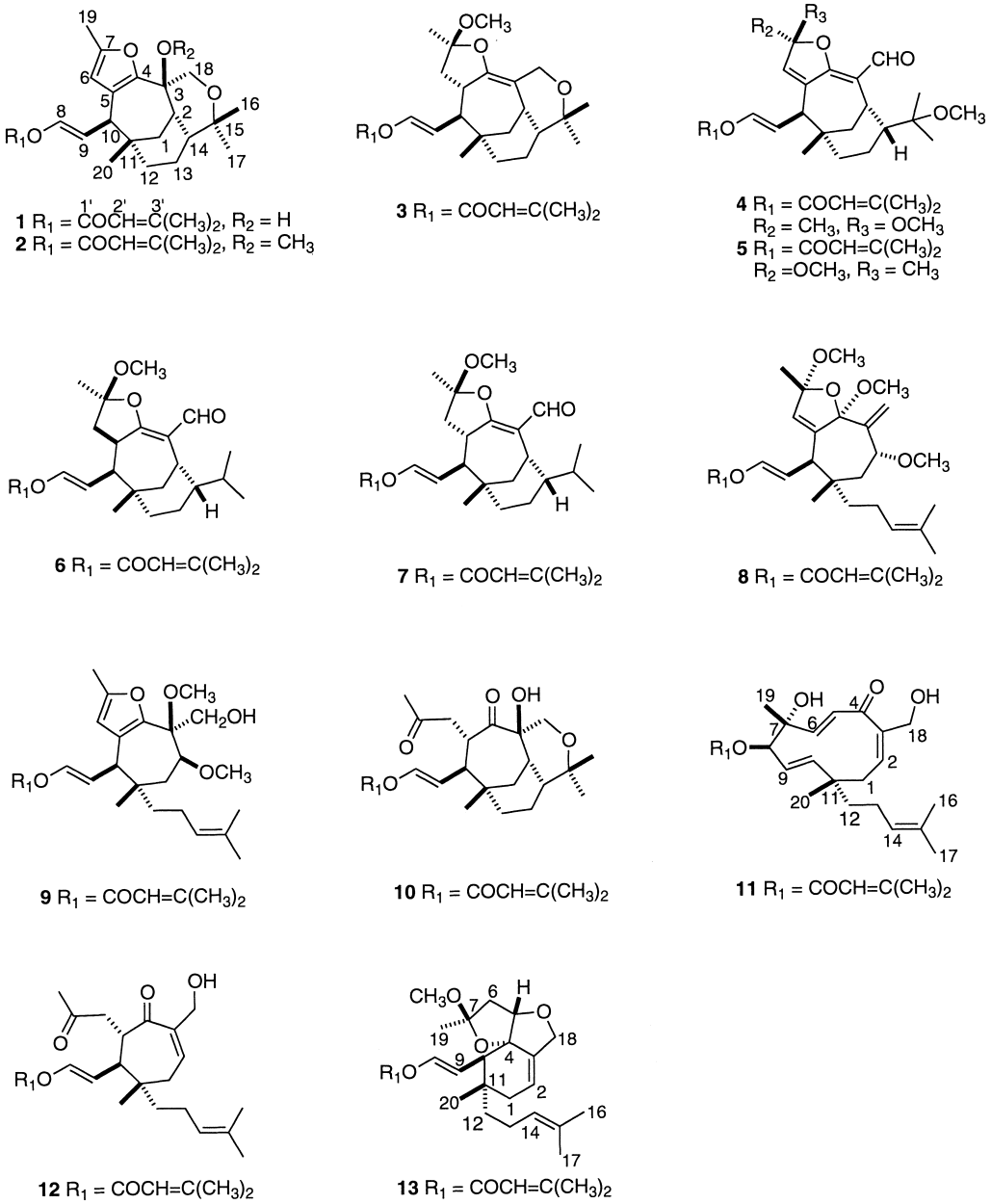


Figure 1. Structures of vibsane-type diterpenes isolated from *V. awabuki*.

determined by HR-FABMS. The spectral data of **2** were very similar to those of **1** except for the presence of additional signals at δ_H 3.04 and δ_C 50.9 due to a methoxy group in the ^1H and ^{13}C NMR spectra. These suggest that **2** is a 3-*O*-methyl derivative of **1**. In fact, the methoxy signal correlated with δ_C 73.7 (C-3) in HMBC spectrum, thereby indicating that the C-3 hydroxyl group of **1** was methylated in **2**. The relative configuration of **2** was elucidated on the basis of 2D NOESY to be the same as **1**. Thus, the structure of **2** was determined to be 3-*O*-methylfuranovibsanin A.

Furanovibsanin F (**3**) had the molecular formula, $C_{26}H_{38}O_5$, established by HR-FABMS. The NMR data of **3** (Table 1) were found to resemble those of **1** except for the presence of the signals due to a methoxy group (δ_H 3.08; δ_C 48.9) and an acetal carbon (δ_C 106.1), showing a modification of the furan ring existing in **1**. The analysis of $^1H-^1H$ COSY and HMQC provided a new partial structure **B** extendable to C-6 which was involved in the furan ring in **1**. The other partial structures **A**, **C** and **D** were identical with those of **1** as shown in Fig. 2b. This spectral feature suggested that the furan ring in **1** was just modified into an enol acetal ring with adding methanol on C-7. In HMBC, the C-7 acetal carbon (δ_C 106.1) showed the cross-peaks to the methoxy (δ_H 3.08), and 19-Me (δ_H 1.22) signals which correlated to C-6 (δ_C 44.9), and H-6 showed further correlation to one carbon (C-4; δ_C 153.6) of the tetrasubstituted double bound **E**. Additionally, the other sp^2 carbon (C-3; δ_C 106.0) had the HMBC correlations with H-1 and H-18, and together with considering ^{13}C NMR chemical shifts for C-3 and C-4 (Table 1), C-3 and C-4 should be involved into an enol double bond and thus a 5-membered enol acetal ring must be placed at the C-4 and C-5 positions. Moreover, the other HMBC correlations, as shown by arrows in Fig. 2b, allowed us to propose the plane structure **3**. The relative stereochemistry of **3** was elucidated by ROESY as shown in

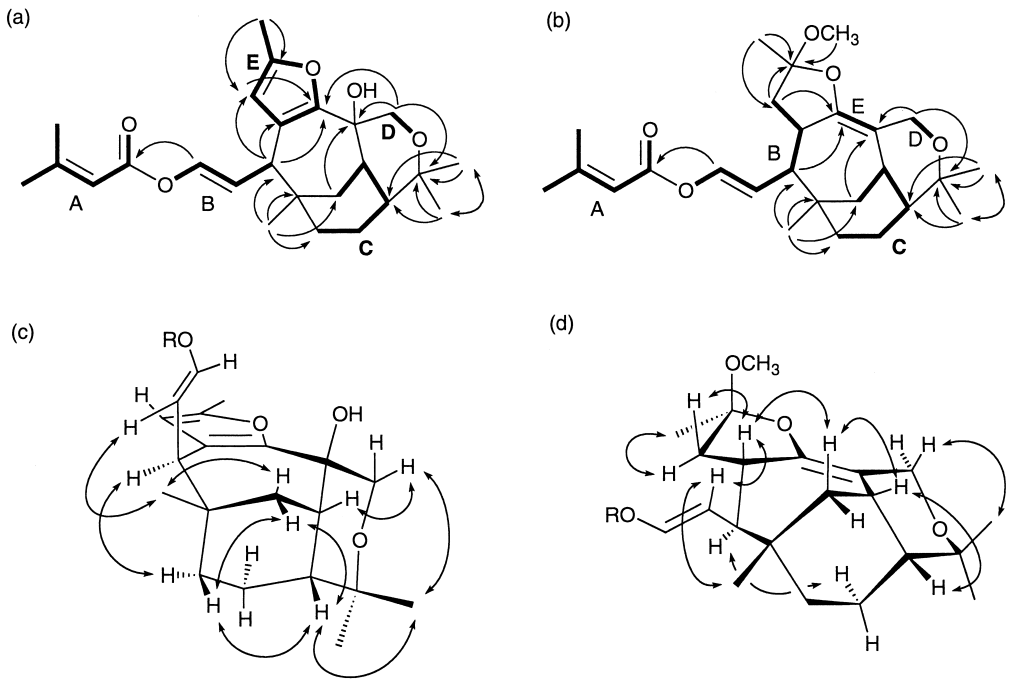


Figure 2. Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrow): (a) for **1** and (b) for **3**, and ROSEY correlations: (c) for **1** and (d) for **3**.

Fig. 2d. Namely, H-9 which had a ROESY correlation to 20-Me showed the distinct cross-peak to H-5, indicating that 20-Me, the side chain at C-10 and H-5 should take the same β-orientations. Additionally, ROESY correlations not only between H-5 and H-6β [δ_H, 1.51 (dd, J=12.6, 10.2 Hz)] but also between 19-Me and H-6α [δ_H, 2.08 (dd, J=12.6, 8.8 Hz)] suggested that the methoxy and methyl groups at C-7 took a β-configuration and an α-configuration, respectively. On the basis of the other ROESY correlations, the relative stereochemistry of **3** was verified to be the same

Table 1. ^{1}H and ^{13}C NMR data for compounds **1**–**3** in C_6D_6 (J values in parentheses)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1β	1.02 dd (14.0, 5.0)	33.5	1.06 ddd (14.6, 5.5, 1.4)	33.8	0.92 dd (14.3, 2.2)	40.1
α	2.71 dd (14.0, 2.0)		2.90 ddd (14.6, 2.2, 2.2)		2.16 ddd (14.3, 3.0, 2.0)	
2	2.24 ddd (5.0, 4.0, 2.0)	39.0	2.73 ddd (5.5, 4.7, 2.2)	38.5	2.64 m	34.3
3		69.0		73.7		106.0
4		149.8		147.9		153.6
5		126.1		127.7	3.28 ddd (11.5, 10.2, 8.2)	38.8
6	5.58 q (1.1)	110.2	5.61 q (0.8)	110.2	1.51 dd (12.6, 10.2, 6β)	44.9
					2.08 dd (12.6, 8.2, 6α)	
7		150.1		150.5		106.1
8	7.47 d (12.4)	136.1	7.57 d (12.4)	136.0	7.36 d (12.4)	135.5
9	5.80 dd (12.4, 9.9)	117.5	5.87 dd (12.4, 10.2)	116.6	5.22 dd (12.4, 11.5)	115.8
10	2.71 d (9.9)	47.7	2.72 dd (10.2, 1.4)	47.7	2.30 dd (11.5, 11.5)	50.8
11		32.6		32.5		34.3
12β	1.28 m	41.2	1.27 ddd, (13.8, 13.8, 5.8)	40.9	1.32 m	38.9
α	1.58 m		1.58 dddd (13.8, 4.7, 4.7, 2.2)		1.44 m	
13β	1.54 m	22.2	1.49 dddd (13.8, 13.8, 13.2, 4.7)	22.7	1.27 m	19.5
α	1.24 m		1.22 dddd (13.8, 5.8, 4.7, 4.7)		1.79 m	
14	1.08 m	48.0	1.10 ddd (13.2, 4.7, 4.7)	43.9	0.93 m	45.9
15		73.6		73.6		73.6
16	1.00 s	27.4	1.01 s	27.4	1.16 s	27.9
17	1.17 s	24.0	1.20 s	24.1	1.24 s	24.0
18β	3.54 d (11.3)	67.7	3.55 d (10.8)	65.3	4.02 d (11.4)	60.9
α	4.63 d (11.3)		4.89 d (10.8)		4.92 d (11.4)	
19	1.91 d (1.1)	18.3	1.94 d (0.8)	13.5	1.22 s	20.2
20	0.89 s	33.5	0.92 s	33.5	0.74 s	28.9
1′		163.5		163.4		163.3
2′	5.66 qq (1.1, 1.1)	115.3	5.61 qq (1.9, 1.4)	115.4	5.67 qq (1.4, 1.4)	115.3
3′		159.2		158.9		59.4
4′	2.04 d (1.1)	20.2	2.03 d (1.9)	20.2	2.05 d (1.4)	20.2
5′	1.34 d (1.1)	27.0	1.33 d (1.4)	26.9	1.36 d (1.4)	27.0
OCH_3			3.04 s	50.9	3.08 s	48.9

Table 2. ^{1}H and ^{13}C NMR data for compounds **4**–**7** in C_6D_6 (J values in parentheses)

Position	**4**		**5**		**6**		**7**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1β	1.32 dd (13.7, 4.5)	39.3	1.26 dd (14.8, 3.8)	39.2	1.10 dd (14.3, 4.9)	37.6	1.14 dd (14.3, 5.5)	42.1
α	1.48 dd (13.7, 2.5)		1.78 dd (14.8, 2.2)		1.71 dd (14.3, 2.7)		1.70 dd (14.3, 2.5)	
2	3.73 m	28.7	3.81 ddd (4.5, 3.8, 2.7)	28.8	3.72 ddd (4.9, 4.9, 2.7)	29.4	3.49 ddd (5.5, 4.0, 2.5)	32.9
3		119.4		118.7		118.9		117.1
4		168.0		168.0		175.0		175.4
5		146.6		146.3	4.01 ddd (10.4, 8.5, 4.4)	44.8	3.27 ddd (11.5, 11.3, 7.4)	41.7
6	5.57 s	138.8	5.42 s	136.7	1.76 dd (12.4, 10.4) 1.81 dd (12.6, 8.5)	41.4	1.33 dd (12.4, 11.5) 1.90 dd (12.6, 7.4)	43.3
7		112.7		113.5		109.5		108.2
8	7.32 d (12.2)	136.3	7.36 d (12.4)	136.5	7.26 d (12.4)	138.9	7.37 d (12.4)	135.8
9	5.49 dd (12.2, 10.5)	116.9	5.49 dd (12.4, 9.9)	116.8	5.34 dd (12.4, 11.0)	112.0	5.11 dd, (12.4, 11.3)	114.3
10	2.73 d (10.5)	49.1	2.71 d (9.9)	48.8	1.61 dd (11.0, 4.4)	47.5	2.38 dd (11.5, 11.3)	46.1
11		33.5		33.7		34.1		34.8
12β	1.29 m	41.9	1.30 ddd (12.9, 12.9, 4.7)	42.0	1.30 ddd (11.0, 11.0, 2.5)	28.7	0.78 m	38.1
α	1.76 m		1.52 ddd (12.9, 2.2, 2.2)		1.43 ddd (11.0, 4.4, 4.4)		1.47 m	
13β	1.24 m	20.2	1.46 dddd (12.9, 12.9, 12.9, 3.6)	20.1	1.30 m	26.6	1.28 m	18.8
α	1.65 m		1.68 m		1.51 m		1.51 m	
14	1.44 m	54.0	1.35 ddd (12.9, 4.5, 3.6)	54.4	1.13 m	49.9	1.29 m	48.2
15		76.5		76.5	1.46 dqq (13.2, 6.9, 6.6)	30.4	2.38 dqq (11.5, 6.6, 6.6)	28.8
16	1.08 s	23.5	1.17 s	24.0	0.83 d (6.9)	20.3	0.82 d (6.6)	18.8
17	1.20 s	21.8	1.19 s	21.8	1.21 d (6.6)	22.8	1.08 d (6.6)	23.3
18	10.41 s	189.1	10.55 s	189.4	10.66 s	189.5	10.69 s	190.9
19	1.45 s	24.2	1.42 s	24.8	1.14 s	20.2	1.11 s	20.1
20	0.70 s	32.8	0.71 s	33.1	0.74 s	34.1	0.60 s	27.0
1′		163.3		163.3		163.3		163.3
2′	5.62 qq (1.1, 1.1)	114.9	5.64 qq (1.4, 1.1)	115.0	5.66 qq (1.4, 1.4)	115.1	5.66 qq (1.4, 1.1)	115.4
3′		160.1		160.0		160.0		160.0
4′	2.03 d (1.1)	20.2	2.08 d (1.4)	20.3	2.04 d (1.4)	20.2	2.04 d (1.1)	20.3
5′	1.38 d (1.1)	27.0	1.35 d (1.1)	27.0	1.37 d (1.4)	27.0	1.36 d (1.4)	27.0
OCH_3	2.94 s	50.6	3.17 s	50.8	2.94 s	49.3	2.91 s	49.4
	2.94 s	48.5	2.92 s	48.5				

as that of **1**. Thus, these spectral data collaborated furanovibsanin F to be **3**.

Furanovibsanin B (**4**) and 7-*epi*-furanovibsanin B (**5**) had the same molecular formula $C_{27}H_{38}O_6$ established by HR-FABMS and their spectral data were very similar to each other. The NMR data (Table 2) of **4** and **5** showed the presence of an aldehyde group, two trisubstituted olefins, one disubstituted olefin, one tetrasubstituted olefin, an acetal carbon (δ_C 112.7 for **4** and 113.5 for **5**), six tertiary methyl groups and two methoxy groups. The $^{1}H-^{1}H$ COSY and HMQC for **4** and **5** gave the partial structure units (bold line) as shown in Fig. 3a. The aldehyde proton showed a HMBC correlation to C-3 (δ_C 119.4 for **4** and 118.7 for **5**), which in order correlated to H-2. This means that the C-18 oxymethylene occurring in most of the 7-membered ring vibsanes is oxidized to an aldehyde function, and the ether ring formed between the C-18 and C-15 positions is not present in the case of **4** and **5**. In fact, an *O*-methyldimethylcarbinol group was clarified to be linked to the C-14 position by HMBC (Fig. 3a). The 19-Me signal, which showed an additional correlation to the C-6 olefinic carbon signal, and the remaining methoxy proton signal had HMBC correlations to the C-7 acetal carbon. In addition to these spectral data, HMBC correlations of the H-6 olefinic proton to C-4 and C-10 led **4** and **5** to the same plane structure as shown in Fig. 3a. Thus, compounds **4** and **5** are most likely to be an epimer to each other with regard to C-7. This was defined by ROESY experiments (Fig. 3b and c). The NOE observed between H-9 and the methoxy signal at C-7 for **4** could not be detected for **5**, whereas all of the other NOEs for **4** were identical with those of **5**. Accordingly, the methoxy group on the C-7 position should take a β-configuration for **4** and an α-configuration for **5**, respectively. On the basis of the above spectral data, the structure of furanovibsanin B was represented as **4** and compound **5** was assigned as 7-*epi*-furanovibsanin B.

Furanovibsanin C (**6**) had the molecular formula, $C_{26}H_{38}O_5$, established by HR-FABMS. Its ^{1}H NMR data showed the presence of an aldehyde group [δ_H 10.66 (s)], a trisubstituted olefin [δ_H 5.66 (qq, J=1.4, 1.4 Hz)], a disubstituted olefin [δ_H 5.34 (dd, J=12.4, 11.0 Hz), 7.26 (d, J=12.4 Hz)], four tertiary methyl groups (δ_H 0.74, 1.14, 1.37, 2.04), a methoxy group (δ_H 2.94), and an isopropyl group [δ_H 0.83 (d, J=6.9 Hz), 1.21 (d, J=6.6 Hz), 1.46 (dqq, J=13.2, 6.9, 6.6 Hz)] which was found to be involved in the partial structure **C** by COSY. The ^{1}H NMR data of **6** were similar to those of **4** and **5**. Comparison of ^{13}C NMR data between **6** and **4** or **5** implied that the $\Delta^{5,6}$ double bond was reduced to

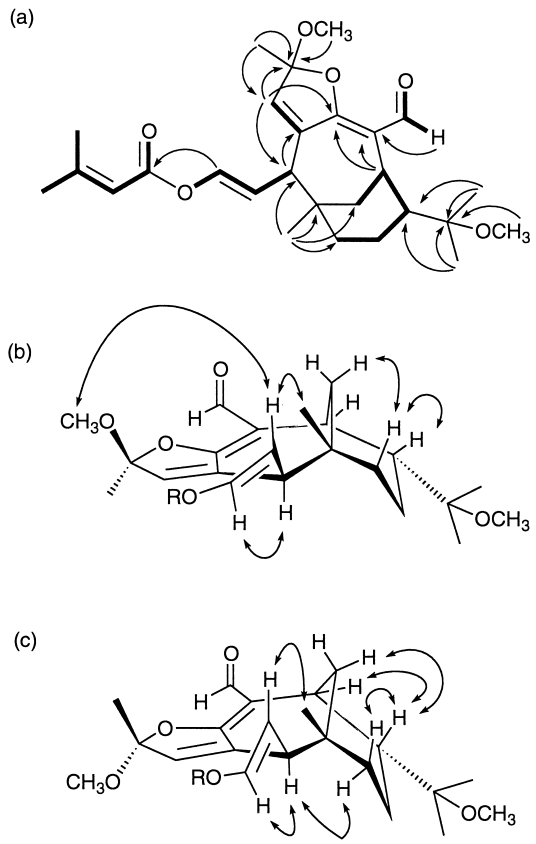


Figure 3. (a) Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrow) for **4** and **5**, and ROSEY correlations: (b) for **4** and (c) for **5**.

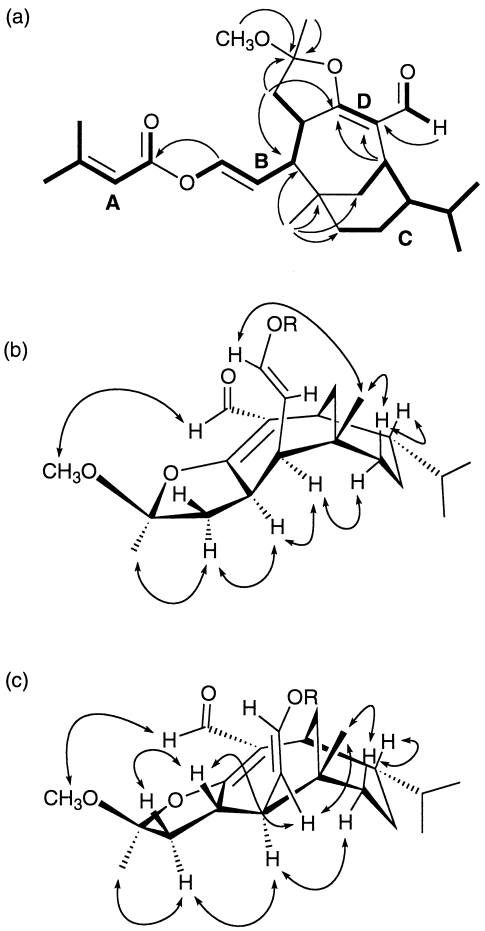
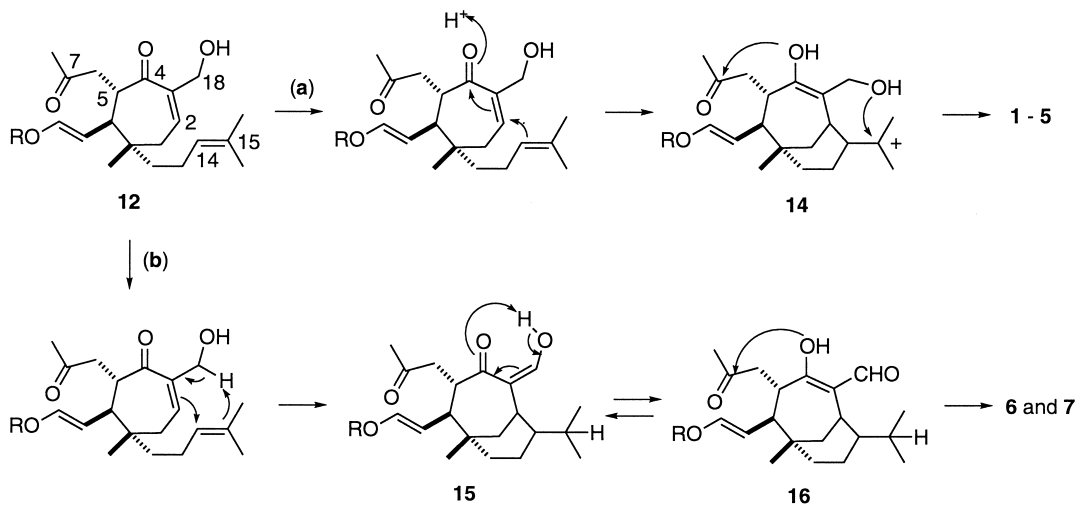


Figure 4. (a) Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrow) for **6** and **7**, and ROSEY correlations: (b) for **6** and (c) for **7**.

make up the C-5 methine (δ_C 44.8) and the C-6 methylene (δ_C 41.4). Moreover, intensive analyses of $^1H-^1H$ COSY and HMQC gave the partial structures **A**–**D** as depicted by bold lines in Fig. 4a. It turns out that **6** is regarded as a 5,6-dihydro-analogue of **4** and/or **5** bearing no methoxy group on the C-15 position. This was substantiated by the following HMBC correlations: the methoxy (δ_H 2.94) and 19-Me (δ_H 1.14) signals correlated to the C-7 acetal carbon resonated at δ_C 109.5, and H-6 showed cross-peaks to C-7 as well as to the sp^2 C-4 carbon (δ_C 175.0) connected to an oxygen atom, indicating the formation of a 5-membered acetal ring on the C-4 and C-5 positions in the same fashion as furanovibsanin F (**3**). Thus, the above spectral data culminated in giving the plane structure **6** (Fig. 4a). The relative stereochemistry of **6** was elucidated by ROESY as shown in Fig. 4b. Namely, H-8 showed a cross-peak to 20-Me, indicating that the 20-Me and the side chain attached on C-10 took the same β-configurations. The observation of cross-peaks from H-10 to H-12α and H-5, and H-6α to H-5 and 19-Me suggested that the methyl group at C-19 and H-5 took α-configurations and the methoxy group at C-7 took a β-configuration. Furthermore, the observation of cross-peaks between H-12β and 20-Me as well as H-12β and H-14 indicated that a cyclohexane ring adopts a chair conformation with an equatorial isopropyl group on the C-14 position. Hence, on the basis of the above data, the structure of furanovibsanin C was elucidated as **5**.

Furanovibsanin D (**7**) was obtained as a colorless needle and its spectral data were found to be very similar to those of **6**. Analysis of 2D NMR for **7** gave the same plane structure as **6**. This result suggests that compound **7** may be a stereoisomer on some chiral carbon. In order to define the relative stereochemistry of **7**, ROESY experiment was carried out. As shown in Fig. 4c, its ROESY indicates that the methyl group at C-11 and the side chain at C-10 take β-configurations and a cyclohexane ring adopts a chair conformation with an equatorial isopropyl group on the C-14 position in the same manner as **6**. However, H-5 showed cross-peaks to H-6β but not to H-10, which showed successive NOE interactions from H-6α to 19-Me indicating that the methoxy group on the C-7 acetal carbon and H-5 disposed in the direction of a β-orientation. Thus, the structure of furanovibsanin D (**7**) was elucidated as a C-5 epimer of **6**.

We have already proposed such a plausible biogenetic pathway for three subtypes of vibsane-type diterpenes as vibsanin B (**11**) could be transformed into vibsanin C (**12**) and neovibasnin A (**13**) by a Cope-type reaction and a sequential retro-aldol/aldol reaction, respectively, based on the results of thermal and photochemical reactions of **11**.[2,4] Additional isolation of these furanovibsanins compel us to elaborate their biosynthetic process after **12** is produced. Thus, our proposed biosynthetic sequences leading to the furanovibsanins from **12** are outlined in Scheme 1.



Scheme 1. Possible biosynthetic pathway of furanovibsanins **1**–**7**.

Biogenetic conversion of compounds **1**–**5** from vibsanin C (**12**) can be rationalized by a cationic process like (**a**) in Scheme 1 followed by an acetal formation between C-4 and C-7 ketones and intramolecular addition of oxygen nucleophiles. This is based on the fact that this type of tricyclic formation can be readily realized by $BF_3{\cdot}OEt_2$-mediated conversion of **12** to a tricyclic vibsanin E.[5] On the other hand, compounds **6** and **7** are not likely to follow a cationic cyclization (**a**) since there is no proof where an isopropyl group on the C-14 position originates. One possible way is that a protonation onto the Δ^{14} double bond in **12** may produce less stable secondary cation on C-14, which can trigger a cyclization to result in the formation of cyclohexane ring having a isopropyl group on the C-14 position, but the polarization of the Δ^2 double bond which must participate in this cyclization is reverse and thus this type of cationic process is not likely to occur. As vibsanin C is produced from vibsanin B by the Cope-type reaction, compounds **6** and **7** are also postulated to be formed via a non-ionic process. Namely, intramolecular ene reaction might involve in the formation of a cyclohexane ring between C-2 and C-14 as well as of an aldehyde function at C-18. Following pathway (**b**) as outlined in Scheme 1, it is postulated that the prenyl side chain of

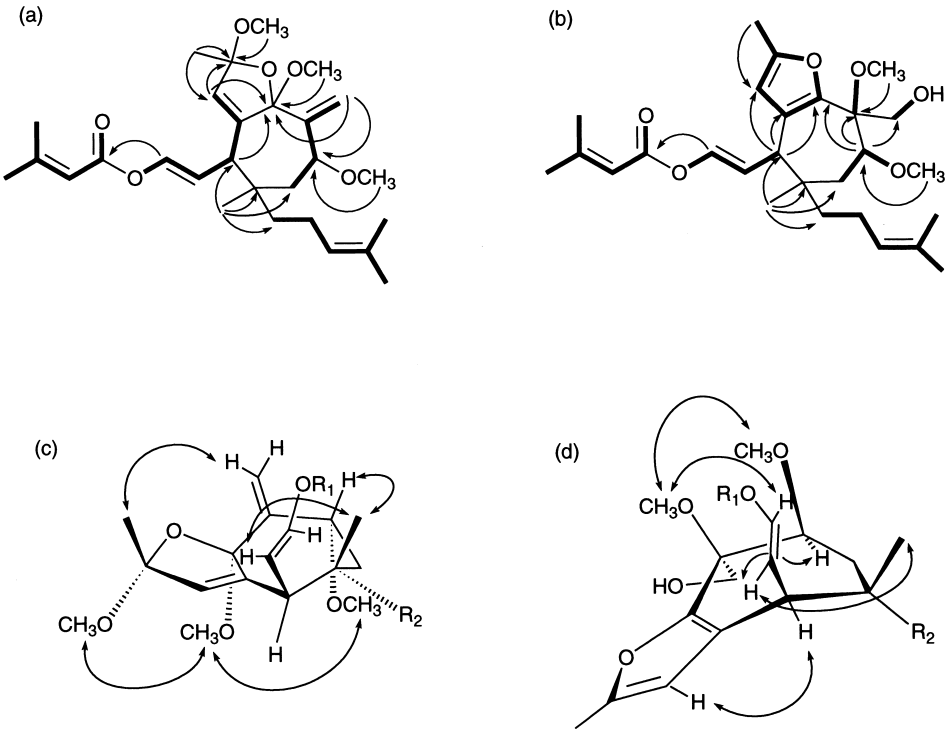
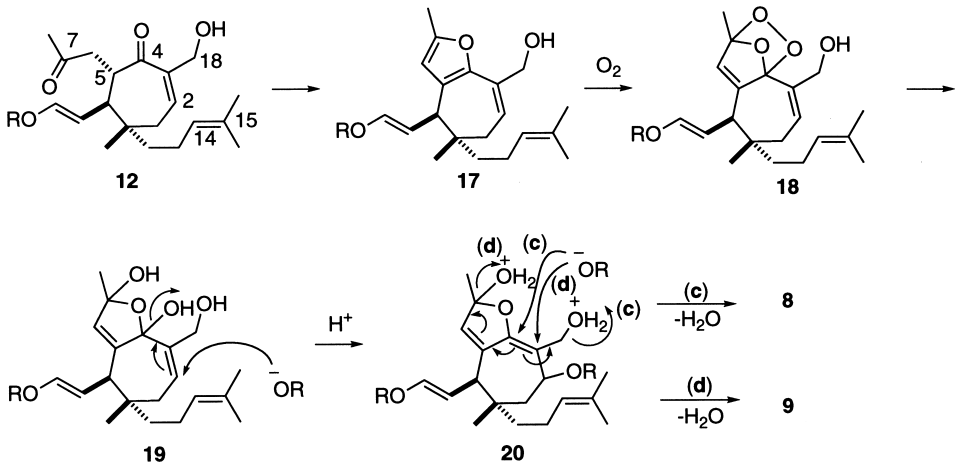


Figure 5. Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrow): (a) for **8** and (b) for **9**,and ROSEY correlations: (c) for **8** and (d) for **9**.



Scheme 2. Possible biosynthetic pathway of furanovibsanins **8** and **9**.

vibsanin C could be cyclized onto C-2 by an intramolecular ene reaction, resulting in the formation of a bicyclic aldehyde **15**, and then its tautomer **16** could make an acetal giving rise to **6** and **7**.[20] This non-ionic process can rationalize not only where an isopropyl group originates but also why bicyclic 7-membered ring vibsanins bearing an isopropyl group such as **6** and **7** accompany an aldehyde group on the C-18 position.

Furanovibsanin E (**8**) had the molecular formula, $C_{28}H_{42}O_6$, deduced from HR-FABMS at *m/z* 497.2848 $[M+Na]^+$, indicating eight degrees of unasaturation. The spectral data of **8** showed the presence of three methoxy groups (δ_H 3.17, 3.26, 3.41), an exomethylene [δ_H 5.33 and 6.07 (each d, J=2.2 Hz); δ_C 115.9 and 147.6], and a β,β-dimethylacryl ester group (229 nm, 1732 cm^{-1}, *m/z* 83) which was found to be linked to a *trans* double bond [δ_H 5.54 (dd, J=12.4, 11.0 Hz, H-9) and 7.53 (d, J=12.4 Hz, H-8)] by HMBC correlation (Fig. 5a) between H-8 and the ester carbonyl resonated at δ_C 163.0, typical of vibsane-type diterpenes. The NMR of **8**, however, showed neither oxymethylene nor aldehyde signal corresponding to C-18 existing in most of vibsanes. The newly appeared exomethylene should allocate to C-3 and C-18 since these protons had not only long-range couplings but also clear HMBC correlations with the C-2 adjacent methine bearing a methoxy group (δ_H 3.17) which connected to the C-1 methylene on the basis of COSY, which further showed the HMBC correlation with 20-Me (δ_H 0.76). Following additional HMBC correlations from the H-18 exomethylene and 20-Me to each carbon of the structural fragments as shown by bold line in Fig. 5 as well as to the C-4 (δ_C 111.4) and C-11 (δ_C 36.9) quaternary carbons, a 7-membered ring was constructed, and then the C-12–C-18 carbon unit and the second methoxy group (δ_H 3.41) were placed on its C-4 quaternary carbons, respectively. Moreover, the HMBC data (Fig. 5a) indicated that the third methoxy group (δ_H 3.26) and 19-Me could connect to C-7 (δ_C 109.6), which was regarded as an acetal carbon. On account of eight degrees of unsaturation and the HMBC correlation of H-6 with C-4, the final ring must be an acetal ring fused between the C-4 and C-5 positions. Thus, the plane structure of furanovibsanin E turns out to be **8**. All the methoxy groups deposited down in the α-direction and the relative configurations for the other chiral centers were assigned as shown in Fig. 5c.

Furanovibsanin G (**9**) had the molecular formula $C_{27}H_{40}O_6$ and its spectral data suggested that structure of **9** resembled that of **8**, but differed in the presence of a methylfuran ring [δ_H 1.86 (3H, d, J=0.8 Hz) and 5.59 (1H, d, J=0.8 Hz); δ_C 110.3, 127.1, 145.8, 150.7] and an hydroxyl methylene [3448 cm^{-1}; δ_H 4.29 and 4.42 (each d, J=12.1 Hz); δ_C 58.2] as well as in missing one methoxy group and an exomethylene existing in **8**. The HMBC as shown in Fig. 5b indicated that one (δ_H 2.88) of two methoxy groups bonded to C-3 (δ_C 80.8), to which the hydroxyl methylene also connected, whereas the other methoxy group (δ_H 3.20) linked to C-2 (δ_C 79.5). These spectral data and similarity of **8** led to the conclusion that **9** was a furan-version of vibsanin C (**12**) with dimethoxyl groups on the 2 and 3 positions. It was determined on the basis of NOESY as shown in Fig. 5d that two methoxy groups took the same β-configurations and the other structure units on C-10 and C-11 deposited in the same direction as **12**.

We postulate that compounds **8** and **9** may be formed from vibsanin C (**12**) by oxidation process. Since vibsane-type diterpenes bearing a hydroperoxide group were frequently isolated from *V. awabuki*,[8,9] we envisaged biogenetic pathway involving an oxidation step as outlined in Scheme 2. Namely, intramolecular acetal formation between C-4 and C-7 ketones leads to furan **17**, and then autoxidation of furan ring occurs to give endoperoxide **18**, followed by a series of ring opening reaction, dehydration and addition of alkoxyl groups, giving rise to **8** and **9** via pathway (**c**) and (**d**), respectively. However, we have no evidence to support this biosynthetic route leading to **8** and **9**.

In conclusion, we have isolated nine 7-membered ring vibsanins **1**–**9**[21] with a furan ring and its diversity from *V. awabuki*. They are regarded as another subtype of vibsane-type diterpenes, in particular, furanovibsanins C (**6**) and D (**7**) differ from other polycyclic 7-membered ring vibsanins in terms of the biosynthetic route. The present results suggest that vibsane-type diterpenes are rich in diversity and occupy a unique group of the diterpenes.

Further chemical and biological studies on vibsane-type diterpenes are under way.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzs UV-300 or Hitachi-U-3000 spectrophotometer. IR spectra were recorded on a Jasco FT-IR 5300 or 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. Silica gel (Merck, 70–230, 230–400 mesh, Wacogel C-300) and were used for column chromatography. Sephadex LH-20 was used for gel filtration chromatography. Precoated silica gel $60F_{254}$ and RP-8 F_{254} plates were used for analytical or preparative thin-layer chromatography, and spots were visualized by UV (254 nm) light and 2% $CeSO_4$ in H_2SO_4 after heating.

3.2. Plant material

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

3.3. Extraction and isolation

Air-dried and powdered leaves (1.3 kg) of *V. awabuki* were immersed in 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 (600 g)] and then the MeOH was removed under reduced pressure. The obtained solids were pulverized, packed into a glass column, and eluted in order with CH_2Cl_2 (2 L), CH_2Cl_2–EtOAc (9:1, 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 1–9.

Fraction 3 (16.7 g) was divided by silica gel column chromatography eluted with hexane–EtOAc (15:1) to give fractions 10–18. Fraction 10 was purified by silica gel chromatography with hexane–EtOAc (8:1), and then finally purified by preparative TLC on silica gel with $CHCl_3$–EtOAc (8:1) to give furanovibsanin A (**1**) (1.7 mg) and 3-*O*-methylfuranovibsanin A (**2**) (7.6 mg). Fraction 11 was subjected to silica gel column chromatography eluted with hexane–EtOAc (8:1), and finally purified by HPLC [Cosmosil $5C_{18}$-AR, i.d. 10×250 mm; MeOH–H_2O (4.5:1; 2.0 mL/min); det. 254 nm] to give furanovibsanin B (**4**) (2.1 mg) and 7-*epi*-furanovibsanin B (**5**) (2.1 mg). Fraction 13 was purified by preparative TLC on RP-18 with CH_3CN–H_2O (4:1)] to give furanovibsanin C (**6**) (5.0 mg). Fraction 16 (4.3 mg) was purified by HPLC [Cosmosil $5C_{18}$-ARII, i.d. 10×250 mm; CH_3CN–H_2O (77:23; 2.0 mL/min; det. 254 nm) to give furanovibsanin D (**7**) (2.0 mg).

Table 3. ^1H and ^{13}C NMR data for compounds **8** and **9** in C_6D_6 (*J* values in parentheses)

Position	**8**		**9**	
	δ_H	δ_C	δ_H	δ_C
1β	1.98 dd (13.5, 3.8)	47.0	1.77 dd (15.5, 2.2)	33.0
α	2.12 dd (13.5, 10.7)		2.11 dd (15.5, 5.2)	
2	3.51 dd (10.7, 3.8)	79.4	3.68 dd (5.2, 5.2)	79.5
3		147.6		80.8
4		111.4		145.8
5		146.3		127.1
6	5.48 d (1.6)	130.7	5.59 q (0.8)	110.3
7		109.6		150.7
8	7.53 d (12.4)	137.2	7.65 d (12.4)	136.3
9	5.54 dd (12.4, 11.0)	115.9	6.14 dd (12.4, 11.0)	115.3
10	2.98 dd (11.0, 1.6)	43.6	2.88 d (11.0)	45.9
11		36.9		38.4
12	1.25 ddd (13.7, 12.1, 4.7)	43.9	1.61 m	39.7
	1.31 ddd (13.7, 12.1, 5.2)		1.77 m	
13	1.90 dddd (16.4, 12.1, 7.1, 5.2)	22.1	2.03 m	23.2
	1.98 dddd (16.4, 12.1, 7.1, 4.7)			
14	5.09 tqq (7.1, 0.8, 0.8)	125.1	5.18 tqq (5.5, 0.8, 0.8)	126.2
15		131.0		130.0
16	1.53 d (0.8)	17.7	1.69 d (0.8)	25.8
17	1.61 q (0.8)	25.8	1.59 q (0.8)	17.7
18	5.33 d (2.2)	115.9	4.29 d (12.1)	58.2
	6.07 d (2.2)		4.42 d (12.1)	
19	1.50 s	23.8	1.86 d (0.8)	13.3
20	0.76 s	19.6	0.95 s	28.1
1′		163.0		163.0
2′	5.67 qq (1.4, 1.1)	115.1	5.66 qq (1.1, 1.1)	115.4
3′		159.6		159.0
4′	2.02 d (1.4)	20.2	2.05 d (1.1)	20.2
5′	1.36 d (1.1)	27.0	1.35 d (1.1)	26.9
OCH_3	3.17 s	55.9	2.88 s	50.0
	3.26 s	49.8	3.20 s	58.1
	3.41 s	50.8		
OH			4.31 s	

Fraction 4 was divided by silica gel column chromatography eluted with benzene–EtOAc (15:1) to give fractions 19–30. Fraction 26 (78.5 mg) was purified by HPLC [Cosmosil $5C_{18}$-AR, i.d. 10×250 mm; CH_3CN–H_2O (4:1; 2.0 mL/min); det. 220 nm] to give furanovibsanin F (**3**) (2.1 mg). Fraction 28 was subjected to silica gel chromatography eluted with benzene–EtOAc (15:1) to give three factions. The third one was purified by HPLC [Cosmosil $5C_{18}$-AR, i.d. 10×250 mm; CH_3CN–H_2O (77:23; 2.0 mL/min); det. 254 nm] to give furanovibsanin E (**8**) (2.1 mg) and furanovibsanin G (**9**) (2 mg).

3.3.1. Furanovibsanin A (1). Colorless oil, $[\alpha]_D^{22}$=+230.7° (*c* 0.03, EtOH). IR ν_{max}^{FT}: 3380 (OH), 1725 C=O), 1640 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 235 (ε 15000) nm; FABMS *m/z* 437 $[M+Na]^+$, 453 $[M+K]^+$; HR-FABMS found 437.2341, calcd 437.2304 for $C_{25}H_{34}O_5Na$; ^1H and ^{13}C NMR: Table 1.

3.3.2. 3-*O*-Methylfuranovibsanin A (2). Colorless oil, $[\alpha]_D^{22}$=+92.0° (*c* 0.31, EtOH). IR ν_{max}^{FT}: 1730 (C=O) cm^{-1}; UV λ_{max}^{EtOH}: 238 (ε 17300) nm; FABMS *m/z* 428 $[M]^+$, 137 (100%); HR-FABMS found 428.2578, calcd 428.2563 for $C_{26}H_{36}O_5$; ^1H and ^{13}C NMR: Table 1.

3.3.3. Furanovibsanin F (3). Colorless oil, $[\alpha]_D^{25}=-29.9°$ (*c* 0.54, EtOH). IR ν_{max}^{FT}: 1723 (C=O), 1645 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 232 (ε 11000) nm; FABMS *m/z* 453 $[M+Na]^+$; HR-FABMS found 453.2607, calcd 453.2617 for $C_{26}H_{38}O_5Na$; ^1H and ^{13}C NMR: Table 1.

3.3.4. Furanovibsanin B (4). Colorless oil, $[\alpha]_D^{21}=+55.6°$ (*c* 0.20, $CHCl_3$). IR ν_{max}^{FT}: 1732 (C=O), 1651 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 225 (ε 15100) nm, 317 (ε 6300). FABMS *m/z* 459 $[M+H]^+$, HR-FABMS found 459.2749, calcd 459.2746 for $C_{27}H_{39}O_6$; ^1H and ^{13}C NMR: Table 2.

3.3.5. 7-*epi*-Furanovibsanin B (5). Colorless oil, $[\alpha]_D^{20}=+102.8°$ (*c* 0.32, $CHCl_3$). IR ν_{max}^{FT}: 1731 (C=O), 1650 (C=C) cm^{-1}. UV λ_{max}^{EtOH}: 226 (ε 16500), 315 (ε 8600) nm; FABMS *m/z* 481 $[M+Na]^+$, 459 $[M+H]^+$; HR-FABMS found 459.2736, calcd 459.2746 for $C_{27}H_{39}O_6$; ^1H and ^{13}C NMR: Table 2.

3.3.6. Furanovibsanin C (6). Colorless oil, $[\alpha]_D^{21}=+230.3°$ (*c* 0.23, EtOH). IR ν_{max}^{FT}: 1730 (C=O), 1660 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 229 (ε 15700), 276 (ε 12500) nm; FABMS *m/z* 431 $[M+H]^+$, 453 $[M+Na]^+$; HR-FABMS found 453.2592, calcd 453.2617 for $C_{26}H_{38}O_5Na$; ^1H and ^{13}C NMR: Table 2.

3.3.7. Furanovibsanin D (7). White needles, mp 150–152°C. $[\alpha]_D^{20}=-3.9°$ (*c* 0.34, EtOH); IR ν_{max}^{FT}: 1728 (C=O), 1665 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 229 (ε 15400), 274 (ε 14400) nm; FABMS *m/z* 431 $[M+H]^+$, 453 $[M+Na]^+$; HR-FABMS found 453.2592, calcd 453.2617 for $C_{26}H_{38}O_5Na$; ^1H and ^{13}C NMR: Table 2.

3.3.8. Furanovibsanin E (8). Colorless oil, $[\alpha]_D^{20}=+70.3°$ (*c* 0.05, EtOH). IR ν_{max}^{FT}: 1732 (C=O), 1645 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 229 (ε 14000) nm; FABMS *m/z* 497 $[M+Na]^+$; HR-FABMS found 497.2848, calcd 497.2879 for $C_{28}H_{42}O_6Na$; ^1H and ^{13}C NMR: Table 3.

3.3.9. Furanovibsanin G (9). Colorless oil, $[\alpha]_D^{20}=+66.0°$ (*c* 0.2, EtOH). IR ν_{max}^{FT}: 1730 (C=O), 1647 (C=C) cm^{-1}; UV λ_{max}^{EtOH}: 225 (ε 11000) nm; FABMS *m/z* 483 $[M+Na]^+$, 136 (100%); HR-FABMS found 483.2685, calcd 483.2723 for $C_{27}H_{40}O_6Na$; ^1H and ^{13}C NMR: Table 3.

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References

1. Kawazu, K. *Agric. Biol. Chem.* **1980**, *44*, 1367–1372.
2. Fukuyama, Y.; Minami, H.; Takeuchi, K.; Kodama, M.; Kawazu, K. *Tetrahedron Lett.* **1996**, *37*, 6767–6776.
3. Fukuyama, Y.; Minami, H.; Takaoka, S.; Kodama, M.; Kawazu, K.; Nemoto, H. *Tetrahedron Lett.* **1997**, *38*, 1435–1438.
4. Minami, H.; Anzaki, S.; Kubo, M.; Kodama, M.; Kawazu, K.; Fukuyama, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1194–1198.
5. Fukuyama, Y.; Minami, H.; Kagawa, M.; Kodama, M.; Kawazu, K. *J. Nat. Prod.* **1999**, *62*, 337–339.
6. Fukuyama, Y.; Minami, H.; Yamamoto, I.; Kodama, M.; Kawazu, K. *Chem. Pharm. Bull.* **1998**, *46*, 545–547.
7. Kubo, M.; Minami, H.; Hayashi, E.; Kodama, M.; Kawazu, K.; Fukuyama, Y. *Tetrahedron Lett.* **1999**, *40*, 6261–6265.
8. Kubo, M.; Fujii, T.; Hioki, H.; Tanaka, M.; Kawazu, K.; Fukuyama, Y. *Tetrahedron Lett.* **2001**, *42*, 1081–1083.
9. Fukuyama, Y.; Minami, H.; Matsuo, A.; Kitamura, K.; Akizuki, M.; Kubo, M.; Kodama, M. *Chem. Pharm. Bull.* **2002**, *50*, 368–371.
10. Kubo, M.; Chen, I.-S.; Minami, H.; Fukuyama, Y. *Chem. Pharm. Bull.* **1999**, *47*, 295–296.
11. Kubo, M.; Chen, I.-S.; Fukuyama, Y. *Chem. Pharm. Bull.* **2001**, *49*, 242–245.
12. Kikuchi, M.; Matsuda, N.; Sugimoto, T. *Nat. Med.* **1995**, *49*, 219–221.
13. Machida, K.; Kikuchi, M. *Tetrahedron Lett.* **1996**, *37*, 4157–4160.
14. Iwagawa, T.; Yaguchi, S.; Hase, T.; Okubo, T.; Kim, M. *Phytochemistry* **1992**, *31*, 1311–1315.
15. Hase, T.; Iwagawa, T. *Chem. Lett.* **1982**, 13–14.
16. Hase, T.; Takao, H.; Iwagawa, T. *Phytochemistry* **1983**, *22*, 1977–1982.
17. Jensen, S. R.; Nielsen, B. J.; Norn, V. *Phytochemistry* **1985**, *24*, 487–489.
18. Baker, W.; Finch, A. C. M.; Ollis, W. D.; Robinson, K. W. *J. Chem. Soc.* **1963**, 1477–1490.
19. Hase, T.; Iwagawa, T. *Bull. Chem. Soc. Jpn* **1982**, *55*, 3363–3664.
20. To be precise, compound **7** should be derived through process (**b**) in Scheme 1 from 5-*epi*-vibsanin C which has been already isolated from the title plant[9].
21. Biological activities of all compounds were evaluated by BS (brine shrimp) test, KB cell line and primary neuronal culture. Among them, 3-*O*-methylfuranovibsanin A (**2**) solely exhibited lethal BS activity at IC_{50} 22 μg/mL and weak cytotoxicity against KB cell.