Two New Benzofuran-Type Lignans from the Wood of *Viburnum awabuki*

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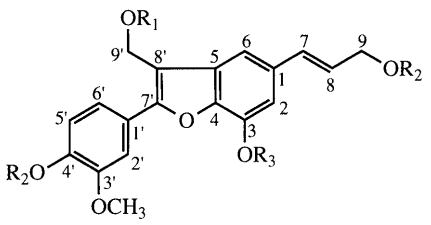
New benzofuran-type lignans, vibsanol (1) and 9′-*O*-methylvibsanol (2), along with dihydrodehydrodiconiferyl alcohol (3), have been isolated from the wood of *Viburnum awabuki* (Caprifoliaceae). Their structures have been elucidated mainly on the basis of spectroscopic data. The antioxidant property of new compounds has been evaluated.

Key words *Viburnum awabuki*; benzofuran; lignan; vibsanol; 9′-*O*-methylvibsanol; dihydrodehydrodiconiferyl alcohol

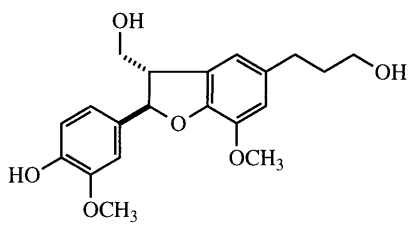
The leaves of *Viburnum awabuki* (Caprifoliaceae) are known to have been used as fish poison for the purpose of catching fish in Okinawa islands.[1] Kawazu already reported that its piscicidal principle was vibsanine A,[2] which belongs to an unprecedented humulene-type diterpene.[3] Phytochemical studies on the leaves of *V. awabuki* have documented the occurrence of triterpenes and coumarine glucosides.[4] On the other hand, few chemical studies of its wood have been done so far.[5] As part of our search for antioxidant natural products we have examined the chemical constituents of the methanol extract of the title plant, which our screening system[6] revealed to contain an antilipid peroxidative chemical. In this paper, we report the isolation and structural elucidation of two new lignans named vibsanol (**1**) and 9′-*O*-methylvibsanol (**2**), along with dihydrodehydrodiconiferyl alcohol (**3**), which was isolated without attaching any sugars for the first time.

The methanol extract of the wood of *V. awabuki* was partitioned between ethyl acetate and water, and the ethyl acetate soluble portion was fractionated by repeated column chromatography on silica gel to give two new lignans, **1** and **2**, together with **3**.

Vibsanol (**1**) has the molecular formula $C_{19}H_{18}O_6$, established by high resolution electron impact mass spectrum (HR-EIMS), indicating the equivalent of eleven double bonds. Its UV and IR showed the presence of hydroxyl groups (3368 cm^{-1}) and aromatic rings (228 and 271 nm; 1516 and 1603 cm^{-1}). The usual acetylation of **1** gave the tetraacetate **1a**, in which two (δ_H 2.12 and 2.13) of the four acetyl groups were bonded to an aliphatic carbon and the others (δ_H 2.35 and 2.42) were located on a benzene ring judging from their chemical shifts. The ^{1}H-NMR spectrum (Table 1) of **1** contained a typical ABX aromatic proton system at δ_H 6.93 (1H, d, J=8.0 Hz), 7.28 (1H, dd, J=8.0, 1.8 Hz) and 7.43 (1H, d, J=1.8 Hz) and a set of *meta*-coupled aromatic proton signals at δ_H 6.82 (1H, d, J=1.8 Hz) and 7.17 (1H, d, J=1.8 Hz), indicating the presence of a 1,3,4-trisubstituted benzene and a 1,3,4,5-tetrasubstituted benzene rings, respectively. In addition to nine quaternary sp^2 carbons counted by distortionless enhancement by polarization transfer (DEPT), the presence of a methoxy group (δ_H 3.87; δ_C 55.7), an oxymethylene (unit D) at δ_H 4.68 (2H, d, J=4.7 Hz) and 5.23 (1H, t, J=4.7 Hz, OH), δ_C 53.6 (C-9′), and a 2-propenol moiety (unit A) at δ_H 4.12 (2H, dd, J=5.4, 5.1 Hz), 6.23 (1H, dt, J=15.7, 5.1 Hz), 6.56 (1H, d, J=15.7 Hz), δ_C 61.7 (C-9), 129.0 (C-8) and 129.4 (C-7) was clarified by analyses of the ^{1}H- and ^{13}C-NMR (Table 2) data, including the ^{1}H-detected multiple quantum coherence spectrum (HMQC). The methoxy signal showed the sole nuclear Overhauser effect (NOE) on one (δ_H 7.43) of the ABX aromatic proton signals, suggesting the presence of a 4-hydroxy-3-methoxybenzene ring (ring E). This was also supported by the observation of a fragment ion peak at m/z 151, as shown in Fig. 2. In the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectrum (HMBC), one (δ_H 9.99) of the two phenolic hydroxyl proton signals correlated to C-2 (δ_C 108.4) and C-4 (δ_C 141.4) through three bonds, and thereby the ring B, as shown in Fig. 3, was constructed as a 1,3,4,5-tetrasubstituted benzene ring. Therefore, the remaining quaternary carbons (δ_C 114.7 and 153.0) must comprise unit C, to which the oxymethylene and ring E should be bonded at the C-8′ and C-7′ positions, respectively, by the HMBC correlations, as shown in Fig. 3. Further



1 $R_1 = R_2 = R_3 = H$
1a $R_1 = R_2 = R_3 = Ac$
2 $R_1 = CH_3$, $R_2 = R_3 = H$
2a $R_1 = CH_3$, $R_2 = R_3 = Ac$
4 $R_1 = R_2 = H$, $R_3 = CH_3$



3

Fig. 1

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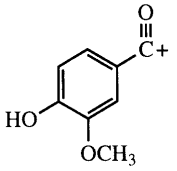
Table 1. ^{1}H-NMR Data of Compounds **1**—**3**

	1[a]	**2**[a]	**3**[b]
2	6.82 (1H, d, J=1.8 Hz)	6.86 (1H, d, J=1.5 Hz)	6.67 (1H, s)
6	7.17 (1H, d, J=1.8 Hz)	7.16 (1H, d, J=1.5 Hz)	6.67 (1H, s)
7	6.56 (1H, d, J=15.7 Hz)	6.55 (1H, d, J=16.0 Hz)	2.67 (2H, t, J=7.3 Hz)
8	6.23 (1H, dt, J=15.7, 5.1 Hz)	6.25 (1H, dt, J=16.0, 5.5 Hz)	1.88 (2H, tt, J=7.3, 6.6 Hz)
9	4.12 (2H, dd, J=5.4, 5.1 Hz)	4.12 (2H, bt, J=5.5 Hz)	3.69 (2H, t, J=6.6 Hz)
3-OCH_3	—	—	3.88 (3H, s)
3-OH	9.99 (1H, s)		—
9-OH	4.83 (1H, t, J=5.4 Hz)	4.84 (1H, t, J=5.5 Hz)	
2′	7.43 (1H, d, J=1.8 Hz)	7.37 (1H, d, J=1.8 Hz)	6.94 (1H, d, J=1.7 Hz)
5′	6.93 (1H, d, J=8.0 Hz)	6.94 (1H, d, J=8.8 Hz)	6.87 (1H, d, J=8.1 Hz)
6′	7.28 (1H, dd, J=8.0, 1.8 Hz)	7.26 (1H, dd, J=8.8, 1.8 Hz)	6.91 (1H, dd, J=8.1, 1.7 Hz)
7′	—	—	5.54 (1H, d, J=7.6 Hz)
8′	—	—	3.60 (1H, q, J=7.6 Hz)
9′	4.68 (2H, d, J=4.7 Hz)	4.60 (2H, s)	3.90 (2H, d, J=7.6 Hz)
3′-OCH_3	3.87 (3H, s)	3.86 (3H, s)	3.86 (3H, s)
9′-OCH_3	—	3.48 (3H, s)	—
4′-OH	9.46 (1H, s)		
9′-OH	5.23 (1H, t, J=4.7 Hz)	—	

a) In DMSO-d_6. *b*) In $CDCl_3$.



m/z 151

Fig. 2

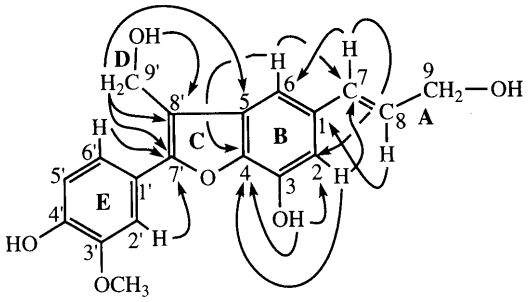


Fig. 3. Structural Units **A**—**E** and HMBC Correlations (J_{CH}=8.1 Hz) of **1**

Table 2. ^{13}C-NMR Data of Compounds **1**, **1a**, **2**, **2a** and **3**

Carbon No.	**1**[a]	**1a**[b]	**2**[a]	**2a**[b]	**3**[b]
1	132.7	132.8	133.1	132.6	133.0
2	108.4	116.2	108.1	115.8	112.4
3	142.1	135.1	142.3	135.2	144.2
4	141.4	144.9	141.3	144.8	146.6
5	131.8	131.2	131.9	132.4	127.7
6	108.6	116.3	108.5	116.2	116.0
7	129.4	133.5	129.3	133.8	32.0
8	129.0	123.4	129.2	123.2	34.6
9	61.7	64.9	61.7	65.0	62.3
3-OCH_3	—	—	—	—	56.0
1′	121.4	128.1	121.1	128.5	135.4
2′	111.0	111.8	111.2	111.8	108.8
3′	147.8	151.4	147.8	151.3	146.6
4′	147.6	140.8	147.5	140.6	145.6
5′	115.8	123.3	115.9	123.3	114.3
6′	120.3	120.5	120.2	120.4	119.4
7′	153.0	155.4	154.3	155.1	87.9
8′	114.7	111.5	110.8	113.2	53.8
9′	53.6	56.7	63.9	64.5	63.9
3′-OCH_3	55.7	56.1	55.6	56.0	56.0
9′-OCH_3	—	—	57.4	—	—

a) In DMSO-d_6. *b*) In $CDCl_3$. $OCO\underline{CH_3}$: for **1a** δ_C 20.6, 20.8, 20.9, 21.0; for **2a** δ_C 20.7 (×2), 20.8, 21.0. $O\underline{C}OCH_3$: for **1a** δ_C 170.9 (×2), 168.8, 168.4; for **2a** 171.6 (×2), 168.9, 168.4.

HMBC correlation was detected between H-9′ and C-5 (δ_C 131.8) on ring B, resulting in the formation of a benzofuran ring at the C-4 and C-5 positions in ring B. Additionally, the 2-propenol unit (A) was compelled to attach to the C-1 position of ring B by HMBC correlations, as shown in Fig. 3. The *E* geometry on the $\Delta^{7,8}$ double bond was evident from a large *J* value (15.7 Hz). Thus, the structure of vibsanol, belonging to a benzofuran-type lignan, was represented as **1** and closely related to herpetol (**4**)[7] isolated from *Herpetospermum caudigerum*.

Compound **2** has the molecular formula $C_{20}H_{20}O_6$ [*m/z* 356.1260 (M^+); Calcd 356.1260], suggesting the addition of an extra methyl group to vibsanol (**1**). Acetylation of **2** yielded the triacetate **2a**, the ^{1}H-NMR spectrum of which was found to be very similar to that of **1a** except for the loss of an aliphatic acetyl group and the appearance of a methoxy group at δ_H 3.48. The above similarity and difference suggest that **2** should be also a benzofuran-type lignan having an extra methoxy group on C-9 or C-9′ in **1**. A HMBC experiment for **2a** was carried out to determine the location of the extra methoxy group. The methoxy signal at δ_H 3.48 showed correlation with an isolated oxymethylene (C-9′) at δ_C 64.5, the proton signal (δ_H 4.67) of which correlated to C-5, C-7′ and C-8′ quaternary carbon signals at δ_C 132.4, 155.1 and 113.2, respectively. Thereby, the methoxy group was placed at the C-9′ position, and the structure of **2** was elucidated as 9′-*O*-methylvibsanol.

Compound **3**, $[\alpha]_D^{20}$ −3.8°, has the molecular formula $C_{20}H_{24}O_6$ obtained from HR-EIMS at *m/z* 360.1574 (M^+), and was suggested to comprise the same aromatic rings as vibsanol (**1**) by the NMR data (Tables 1 and 2). Additionally, the proton-proton correlation spectroscopy

(^{1}H–^{1}H COSY) and HMQC indicated the presence of two types of C 3 units as follows: $HOCH_2CH_2CH_2$–; $HOCH_2CHCH$–. The HMBC could allow the above four partial units to assemble so that **3** was found to be identical with dihydrodehydrodiconifenyl alcohol.[8] Although dihydrodehydrodiconifenyl alcohol was obtained as the aglycone of several glycosides,[9] the configurations at C-7′ and C-8′ have not been determined. The optical rotation of **3** suggested that **3** has the same stereochemistry as the aglycones ($[\alpha]_D$ −3.2°) obtained by Yamaguchi *et al.*[10] and Kouno *et al.*[11] A trans relationship on the phenyl and hydroxymethylene groups at the C-7′ and C-8′ positions was evident from the observation of NOEs, not only between H-7′ and H-9′ but also between H-8′ and H-2′. In light of the CD study on 2-aryl-3-methyl-2,3-dihydrobenzofuran derivatives by H. Aschenbach *et al.*,[12] the absolute configurations at C-7′ and C-8′ may be represented as *R* and *S*, respectively, since **3** showed a negative Cotton effect at 290 nm.

The compounds **1**—**3** were tested for their antioxidative properties using three *in vitro* assays.[6] As a result, vibsanol (**1**) exhibited moderate inhibitory activity (68% inhibition at 10 μg ml^{-1}) of lipid peroxidation in rat brain homogenates,[13] whereas the others showed no inhibitory activity, even at concentrations higher than 10 μg ml^{-1}.

Experimental

UV spectra were recorded on a Hitachi 340 spectrophotometer. IR spectra were measured on a Jasco FT-IR 5300 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained at 400 MHz (^{1}H-NMR) and 100.16 MHz (^{13}C-NMR) using a JEOL GX-400 instrument. Chemical shift values were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. The MS were recorded on a JEOL AX-500 instrument. Silica gel (Wako, C-300) was used for column chromatography. Silica gel F_{254} (Merck) was used for analytical (0.25 mm) and preparative (0.5 mm) thin-layer chromatographies, and spots were visualized under UV (254 nm) light and by spraying with 40% $CeSO_4$–H_2SO_4 followed by heating.

Extraction and Purification The dried and powdered wood (15 kg) of *V. awabuki*, collected in October, 1993 in Tokushima, was immersed in methanol at room temperature for 2 weeks. The MeOH extract was evaporated *in vacuo* to give a gummy extract, which was partitioned between EtOAc and water. The EtOAc soluble portion (50 g) was chromatographed on silica gel in turn with *n*-hexane, *n*-hexane–EtOAc (9 : 1; 7 : 3; 4 : 6), EtOAc and EtOAc–MeOH (9 : 1) to give 6 fractions (frs. 1—6). Fraction 3 (7.6 g) was chromatographed on Sephadex LH-20 with MeOH to divide into frs. 7—11. Fraction 9 (425 mg) was rechromatographed on silica gel with CH_2Cl_2–MeOH (15 : 1) to give vibsanol (**1**) (70 mg) and 9′-*O*-methylvibsanol (**2**) (18 mg). Fraction 2 (2.4 g) was chromatographed on silica gel with CH_2Cl_2–MeOH (15 : 1) to fractionate into frs. 12—17. Fraction 14 (190 mg) was subjected to reversed-phase chromatography using Cosmosil 75C_{18}-OPN and eluted with MeOH–H_2O (1 : 1) to give frs. 18—23. Fraction 22 (45 mg) was purified by HPLC [Cosmosil 5C_{18}-AR, i.d. 10 × 280 mm; MeOH–CH_3CN–H_2O (1 : 1 : 4, 2.5 ml min^{-1})] to afford dihydrodehydrodiconiferyl alcohol (**3**) (12.5 mg).

Vibsanol (1) Colorless oil. EIMS *m/z* (rel. int.): 342 (M^+, 36), 328 (45), 151 (20). UV λ_{max}^{EtOH} nm: 204 (ε 25000), 228 (ε 17000), 271 (ε 20000), 305 (ε 17600). IR ν_{max} cm^{-1}: 3368 (OH), 1603, 1516 . ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-EIMS *m/z*: 342.1102 (M^+), Calcd 342.1103 for $C_{19}H_{18}O_6$.

Acetylation of 1 Vibsanol (**1**) (10 mg) was acetylated with acetic anhydride (0.4 ml) and pyridine (0.6 ml) to give the acetylated derivative **1a** (9.2 mg). EIMS *m/z* (rel. int.): 510 (M^+, 48), 468 (100), 426 (95). IR ν_{max} cm^{-1}: 1767, 1736, 1601, 1508. ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.13, 2.35, 2.42 (each 3H, s, Ac), 3.92 (3H, s, OCH_3), 4.75 (2H, d, J = 6.5 Hz, H_2-9), 5.38 (2H, s, H_2-9′), 6.29 (1H, dt, J = 15.9, 6.5 Hz, H-8), 6.75 (1H, d, J = 15.9 Hz, H-7), 7.16 (1H, d, J = 8.1 Hz, H-5′), 7.17 (1H, d, J = 1.5 Hz, H-2), 7.37 (1H, dd, J = 8.1, 1.9 Hz, H-6′), 7.45 (1H, d, J = 1.9 Hz, H-2′), 7.59 (1H, d, J = 1.5 Hz, H-6). ^{13}C-NMR: see Table 2. HR-EIMS *m/z*: 510.1513 (M^+), Calcd 510.1526 for $C_{27}H_{26}O_{10}$.

9′-*O*-Methylvibsanol (2) Colorless oil. EIMS *m/z* (rel. int.): 356 (M^+, 100), 338 (35). UV λ_{max}^{EtOH} nm: 203 (ε 19100), 224 (ε 15000), 278 (ε 13300), 306 (ε 14600). IR ν_{max} cm^{-1}: 3347 (OH), 1603, 1516. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-EIMS *m/z*: 356.1260 (M^+), Calcd 356.1260 for $C_{20}H_{20}O_6$.

Acetylation of 2 Compound **2** (10 mg) was acetylated with acetic anhydride (0.4 ml) and pyridine (0.6 ml) to give the acetylated derivative **1a** (8 mg). EIMS *m/z* (rel. int.): 482 (M^+, 50), 440 (100), 398 (100). IR ν_{max} cm^{-1}: 1767, 1738, 1601, 1508. ^{1}H-NMR ($CDCl_3$) δ: 2.13, 2.35, 2.43 (each 3H, s, Ac), 3.48 (3H, s, 9′-OCH_3), 3.92 (3H, s, 3′-OCH_3), 4.67 (2H, s, H_2-9′), 4.75 (1H, d, J = 6.0 Hz, H_2-9), 6.28 (1H, dt, J = 16.0, 6.0 Hz, H-8), 6.74 (1H, d, J = 16.0 Hz, H-7), 7.16 (1H, d, J = 8.0 Hz, H-5′), 7.16 (1H, d, J = 1.5 Hz, H-2), 7.38 (1H, dd, J = 8.1, 1.9 Hz, H-6′), 7.46 (1H, d, J = 1.9 Hz, H-2′), 7.54 (1H, d, J = 1.5 Hz, H-6). ^{13}C-NMR: see Table 2. HR-EIMS *m/z*: 482.1570 (M^+), Calcd 482.1577 for $C_{26}H_{26}O_9$.

Dihydrodehydrodiconifenyl Alcohol (3) Colorless oil. EIMS *m/z* (rel. int.): $[\alpha]_D^{20}$ −3.8° (c = 0.65, MeOH). CD (MeOH): 290 nm ($\Delta\varepsilon$ −0.59). EIMS *m/z*: 360 (M^+, 60), 342 (100), 327 (45). UV λ_{max}^{EtOH} nm: 225 (ε 34000), 278 (ε 13300). IR ν_{max} cm^{-1}: 3362 (OH), 1610, 1518. ^{1}H-NMR and ^{13}C-NMR: see Tables 1 and 2. HR-EIMS *m/z*: 360.1574 (M^+), Calcd 360.1572 for $C_{20}H_{24}O_6$.

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