Studies on the Constituents of *Viburnum* Species. VIII.[1] γ-Lactone Glycosides from the Leaves of *Viburnum wrightii* MIQ.[2]

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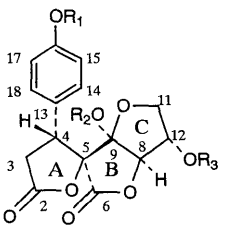
Three new γ-lactone glycosides, named viburnolides A (1), B (4) and C (6), were isolated from the leaves of *Viburnum wrightii* MIQ., and their structures have been determined on the basis of spectral analysis and chemical evidence.

Keywords *Viburnum wrightii*; Caprifoliaceae; γ-lactone glycoside; viburnolide A; viburnolide B; viburnolide C

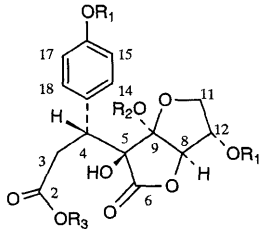
As a continuation of our studies on the glycosides in *Viburnum* species,[1] we have recently reported on phenylpropanoid glycosides isolated from the leaves of *V. wrightii* MIQ.[2] Further investigation of the constituents of the leaves of *V. wrightii* led to the isolation of γ-lactone glucosides, named viburnolides A(**1**), B(**4**), and C (**6**). This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the experimental section.

Viburnolide A (**1**) was obtained as prisms, mp 158—160 °C, $[\alpha]_D$ −18.6° (MeOH) with the molecular formula $C_{21}H_{24}O_{13}$. The infrared (IR) spectrum showed absorption bands of hydroxy groups (3392 cm^{-1}), γ-lactone (1801 cm^{-1}) and aromatic ring (1616, 1520 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** in DMSO-d_6 showed signals of *p*-hydroxyphenyl protons at δ 6.77 and 7.17 (each 2H, d, J=8.5 Hz), an anomeric proton at δ 4.71 (1H, d, J=7.6 Hz), a methylene group adjacent to a γ-lactone carbonyl [δ 2.91 (1H, dd, J=17.2, 9.2 Hz, 3-H_A), 3.07 (1H, dd, J=17.2, 12.2 Hz, 3-H_B)], and a methine proton at δ 4.81 (1H, dd, J=12.2, 9.2 Hz, 4-H) which was evidently coupled with the methylene protons described above. Further, the ^{1}H-NMR spectrum of **1** exhibited an ABM signal [δ 3.97 (1H, dd, J=10.0, 4.9 Hz), 4.22 (1H, dd, J=6.9, 4.9 Hz), 4.32 (1H, dd, J=10.0, 6.9 Hz)] and an oxygenated methine signal [δ 3.94 (1H, s)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum suggested the presence of two carbonyl groups [A ring (δ 174.0, C-2), B ring (δ 170.7, C-6)], a *p*-hydroxyphenyl group and a glucosyl moiety. Detailed analyses of the ^{1}H- and ^{13}C-NMR spectra of **1** were undertaken with the aid of ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), ^{13}C–^{1}H COSY and ^{1}H-detected multiple-bond connectivity (HMBC) spectra. Chart 2 shows multiple-bond interactions found by the HMBC experiment. These findings allowed us to connect the two γ-lactone (A ring and B ring) through a quaternary carbon at C-5 (spiro carbon), and indicated the locations of the *p*-hydroxyphenyl and glucosyl groups to be at C-4 and C-9, respectively. In addition, it was proved that the hydrofuran ring (C ring) was fused to the γ-lactone (B ring). Thus, the planar structure of **1** was established.

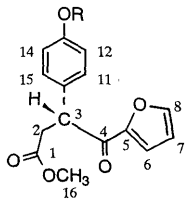
The stereochemistry of **1** was determined as follows. Methanolysis of **1** under condition A (see Experimental) afforded **8**, and subsequent CH_2N_2 treatment afforded **9**, which was identified as methyl (3*R*)-3-(α-furoyl)-3-(*p*-methoxyphenyl)propionate by comparison of the spectral data, including $[\alpha]_D$, with reported values.[3,4] Thus, the configuration of C-4 has been established as *R*. The configurations at C-5, 8, 9 and 12 were determined as *S*, *R*, *R* and *S*, respectively, on the basis of the following evidence. Methanolysis of **1** under condition B (see Experimental) afforded **3**. In a difference nuclear Overhauser effect (NOE) experiment on **3**, positive NOE's were observed at 8-H and 9-OCH_3 upon irradiation of 4-H. The ^{1}H-NMR signal of 8-H in each of **1** and **3** appeared as a singlet, indicating that the dihedral angle



1:R_1=R_3=H, R_2=Glc
1a:R_1=R_3=$COCH_3$, R_2=Glc(Ac)
1':R_1=H, R_2=H, R_3=Glc
2:R_1=R_2=R_3=H
2a:R_1=R_2=R_3=$COCH_3$
3:R_1=R_3=H, R_2=CH_3
3a:R_1=R_3=$COCH_3$, R_2=CH_3



4:R_1=H, R_2=Glc, R_3=CH_3
4a:R_1=$COCH_3$, R_2=Glc(Ac), R_3=CH_3
5:R_1=H, R_2=R_3=CH_3
6:R_1=R_3=H, R_2=Glc
7:R_1=R_2=R_3=H



8: R=H
9: R=CH_3

Chart 1

between 8-H and 12-H is nearly 90°. Finally, **3** was proved to be identical ($[\alpha]_D$, IR, ^{1}H-NMR comparisons)[3,4] with the 9-*O*-methyl ether of the aglycone derived from methanolysis of dilaspirolactone,[3] in which the relative configuration between C-4 and C-5 is known.[3,4] Consequently, the stereochemistry of **1** has been elucidated as 4*R*, 5*S*, 8*R*, 9*R* and 12*S*. The structure of viburnolide A (**1**)[5] is, therefore, as shown in Chart 1.

A similar compound, dilaspirolactone, has been reported by Iwagawa and Hase[3] from the leaves of *V. dilatatum* THUNB., and the isomeric structure (**1'**) has been proposed. However, our reinvestigation[6] revealed that all spectral data including $[\alpha]_D$ of viburnolide A (**1**) and dilaspirolactone are identical. Thus, the structure of dilaspirolactone may be represented by **1** and not by **1'**.

Viburnolide B (**4**) was obtained as an amorphous powder, mp 145—146 °C, $[\alpha]_D$ −4.3° (MeOH) with the molecular formula $C_{22}H_{28}O_{14}$. In the ^{1}H(270 MHz)- and ^{13}C(67.8 MHz)-NMR spectra of **4** at room temperature (296 K) in CD_3OD, signal patterns were similar to those of viburnolide A (**1**), except for the presence of a methoxyl group (δ_H 3.50; δ_C 52.1). The ^{13}C-NMR signal at C-5 of **4** was shifted up field by 8.6 ppm in comparison with that of **1**. This upfield shift was considered to be caused by the opening of the γ-lactone (A ring) of **1**. As shown in Fig. 1, the ^{1}H-NMR spectrum showed a broad singlet at δ 7.15, corresponding to 14,18-H of the *p*-hydroxyphenyl group. Similarly, the ^{13}C-NMR signal of C-14,18 was hardly observed (Fig. 1). These broadenings result from an exchange between 14-H (C-14) and 18-H (C-18), as indicated by the spectra at various temperatures (193—333 K, Fig. 2) and at low magnetic field (^{1}H, 60 MHz, ^{13}C, 15 MHz, Fig. 1), in which the peaks are sharpened.[7,8] The HMBC spectrum demonstrated the planar structure to be as shown in Chart 1. The stereochemistry of **4** was estimated as *R*, *S*, *R*, *R* and *S*, respectively, because **1** afforded **4** on methanolysis. Consequently, the structure of **4** is as shown in Chart 1.

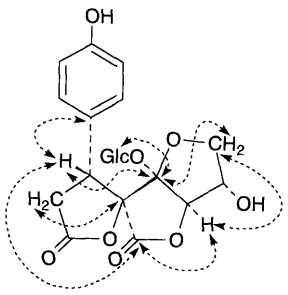


Chart 2. The Main HMBC Correlations of Viburnolide A (**1**)

Viburnolide C(**6**) was obtained as an amorphous powder, and positive ion fast atom bombardment mass spectrometry (FAB-MS) showed an $[M+Na]^+$ ion at *m/z* 525. The ^{1}H- and ^{13}C-NMR spectra resembled those of viburnolide B (**4**) except for the absence of carbomethoxyl signals. The shape and line width of 14,18-H and C-14,18 signals of **6** were similar to those of **4** (Fig. 1). Finally, the methyl ester derived from **6**[9] was proved to be identical ($[\alpha]_D$, IR and ^{1}H-NMR) with **4**. Consequently, the structure of **6** is as shown in Chart 1.

Viburnolide B (**4**) may be an artifact formed from viburnolide C(**6**) during the extraction and isolation processes. In fact, treatment of **6** with MeOH at room temperature give **4**. It is interesting that the *p*-hydroxyphenyl signal of **4** and **6** exhibited dynamic phenomena.

Experimental

Melting points were determined on a Yanagimoto MP-S3 micro-melting points apparatus and uncorrected. Optical rotations were

TABLE I. ^{13}C-NMR Chemical Shifts (296 K)

Carbon	**1**[a] (DMSO-d_6)	**1**[a] (CD_3OD)	**3**[a] (CD_3OD)	**4**[a] (CD_3OD)	**4**[b] (CD_3OD)	**5**[a] (CD_3OD)	**6**[a] (CD_3OD)
2	174.0	176.3	175.4	175.3	175.7	176.8	176.7
3	32.3	34.2	34.2	36.4	36.6	36.4	34.5
4	42.8	45.0	46.2	45.9	44.8	46.3	46.2
5	88.1	91.1	91.2	82.5	82.7	82.4	83.0
6	170.7	172.2	172.5	175.7	175.7	175.0	176.7
8	88.0	89.0	89.7	88.2	88.3	88.0	87.6
9	107.1	109.1	109.1	111.4	111.7	111.1	111.8
11	74.8	77.5	77.2	76.4	76.8	76.0	76.4
12	73.2	74.6	74.9	75.1	75.3	75.5	75.3
13	122.6	124.3	123.8	128.5	128.9	128.8	131.1
14, 18	129.8	131.3	131.1	*ca.* 132 (br)	132.6	132.0 (br)	Unidentified
16	157.4	159.1	159.3	158.3	158.5	158.2	157.9
15, 17	115.4	116.7	116.7	115.9	116.3	115.8	115.8
1'	95.9	97.5	—	97.1	97.3	—	97.1
2'	73.0	74.6	—	75.0	75.3	—	75.1
3'	76.6	77.8	—	78.5	78.7	—	79.2
4'	68.9	70.6	—	72.4	72.6	—	72.0
5'	76.9	78.1	—	78.1	78.4	—	78.0
6'	60.0	61.4	—	63.5	63.7	—	62.9
OCH_3	—	—	51.5	—	—	51.4	—
$COOCH_3$	—	—	—	52.1	52.0	52.0	—

Assignments were confirmed by ^{1}H–^{1}H and ^{13}C–^{1}H COSY, and HMBC methods. *a*) Measured at 67.8 MHz. *b*) Measured at 15 MHz. *ca.*, *circa.* br, broad.

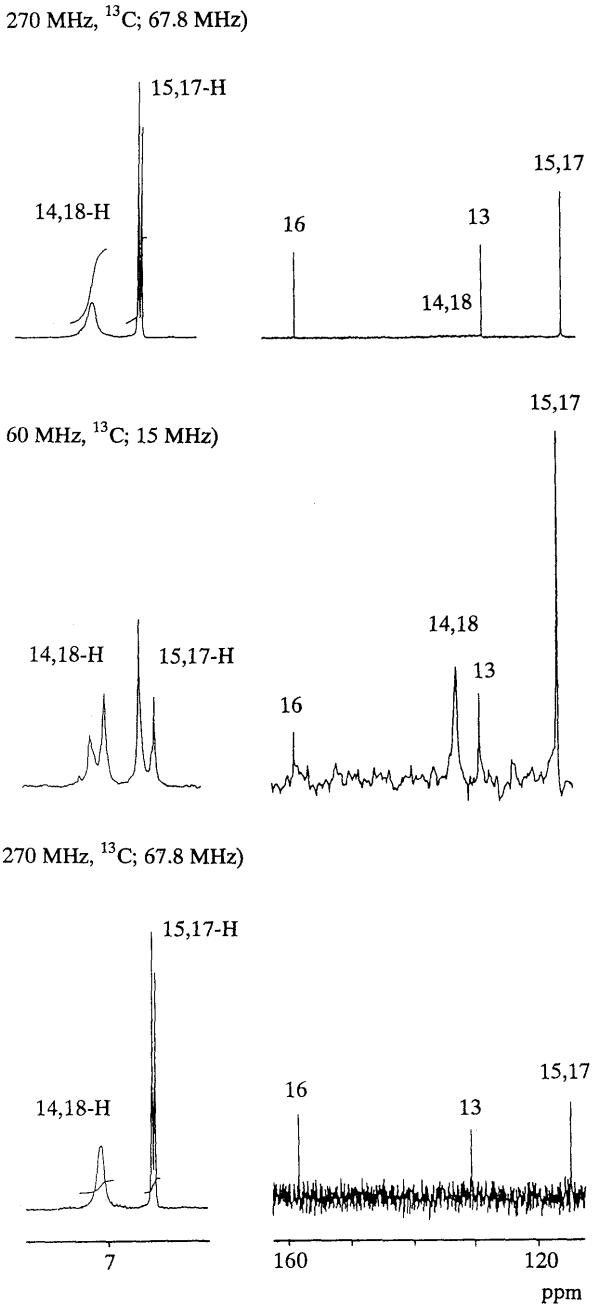


Fig. 1. ^{1}H-, ^{13}C-NMR Spectra in CD_3OD at 296 K

determined with a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin–Elmer 1725 FT-IR instrument and ultraviolet (UV) spectra with a Beckman DU-64 spectrometer. The circular dichroism (CD) spectra were obtained with a JASCO J-700 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JMX-EX 270 [^{1}H (270 MHz, PA[10] = $\pi/4$, PR[11] = 7.0 s), ^{13}C (67.8 MHz, PA = $\pi/4$, PR = 3.0 s)] and JEOL FX-60 [^{1}H (60 MHz, PA = $\pi/2$, PR = 4.5 s), ^{13}C (15 MHz, PA = $\pi/2$, PR = 1.5 s)] spectrometers. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet; br, broad). Electron impact mass spectra (EI-MS), chemical ionization mass spectra (CI-MS) and positive ion FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck, 70—230 and 230—400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying 50% H_2SO_4 followed by heating. Preparative high-performance liquid chromatography (prep-HPLC) was carried out on a Tosoh HPLC system (pump, CCPD; detector, UV-8000) using TSK gel ODS-120A, ODS-120T and Silica-60 (Tosoh, column size: 7.8 mm i.d. × 30 cm) columns.

Isolation Fresh leaves of *V. wrightii* (2.0 kg) collected in August 1989 in Sendai, Japan, were extracted with MeOH at room temperature for two months. The MeOH extract was concentrated under reduced pressure and the residue was suspended in a small excess of water. This suspension was successively extracted with $CHCl_3$, Et_2O, AcOEt and *n*-BuOH. The AcOEt-soluble fraction was concentrated under reduced pressure to produce a residue (8.0 g). This residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and the eluate was separated into twelve fractions (frs. 1—12). Fraction 11 was rechromatographed on a Sephadex LH-20 column using MeOH–H_2O (1 : 1) and the eluate was separated into nine fractions. Fraction 11-3 was subjected to prep-HPLC (ODS-120T, MeOH : H_2O = 2 : 3, 1.2 ml/min) to give compounds **1** (760 mg), **4** (40 mg) and **6** (3 mg).

Viburnolide A (1) Prisms, mp 158—160 °C, $[\alpha]_D$ −18.6° (c = 3.9, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3392, 1801, 1616, 1520,1229, 1157, 1079, 899, 839. UV λ_{max}^{MeOH} nm (log ε): 276 (3.13), 227 (3.94). ^{1}H-NMR (270 MHz, DMSO-d_6, 296 K) δ: 2.91 (1H, dd, J = 17.2, 9.2 Hz, 3-H_A), 3.07 (1H, dd, J = 17.2, 12.2 Hz, 3-H_B), 3.09 (1H, m, 5′-H), 3.23 (3H, m, 2′,3′,4′-H), 3.56 (2H, m, 6′-H), 3.94 (1H, s, 8-H), 3.97 (1H, dd, J = 10.0, 4.9 Hz, 11-H_A), 4.22 (1H, dd, J = 6.9, 4.9 Hz, 12-H), 4.32 (1H, dd, J = 10.0, 6.9 Hz, 11-H_B), 4.45 (1H, t, J = 5.3 Hz, 6′-OH), 4.71 (1H, d, J = 7.6 Hz, 1′-H), 4.81 (1H, dd, J = 12.2, 9.2 Hz, 4-H), 4.94, 4.98, 5.07 (each 1H, each d, J = 5.3, 4.3, 3.3 Hz, respectively, 2′,3′, 4′-OH), 5.58 (1H, d, J = 4.9 Hz, 12-OH), 6.77 (2H, d, J = 8.5 Hz, 15,17-H), 7.17 (2H, d, J = 8.5 Hz, 14,18-H), 9.59 (1H, s, 16-OH). ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.91 (1H, dd, J = 17.3, 8.9 Hz, 3-H_A), 3.12 (1H, dd, J = 17.3, 12.5 Hz, 3-H_B), 3.29 (1H, m, 5′-H), 3.45 (2H, m, 2′,3′-H), 3.54 (1H, m, 4′-H), 3.78 (2H, m, 6′-H), 4.01 (1H, s, 8-H), 4.06 (1H, m, 11-H_A), 4.36 (2H, m, 11-H_B, 12-H), 4.80 (1H, dd, J = 12.5, 8.9 Hz, 4-H), 4.89 (1H, d, J = 7.9 Hz, 1′-H), 6.78 (2H, d, J = 8.9 Hz, 15,17-H), 7.25 (2H, d, J = 8.9 Hz, 14,18-H). ^{13}C-NMR [(67.8 MHz, DMSO-d_6, 296 K), (67.8 MHz, CD_3OD, 296 K)]: Table I. CD (MeOH)] [θ] (nm): -1.6×10^3 (278.2), $+3.1 \times 10^4$ (232.9), -1.4×10^4 (218). *Anal.* Calcd for $C_{21}H_{24}O_{13} \cdot 3/2H_2O$: C, 49.32; H, 5.32. Found: C, 49.61; H, 5.32.

Acetylation of 1 Compound **1** (20 mg) was acetylated with Ac_2O–pyridine in the usual manner to give **1a** (18 mg). An amorphous powder, mp 107—109 °C, $[\alpha]_D$ −10.4° (c = 2.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1812, 1757, 1510, 1433, 1372, 1224, 1039, 911. UV λ_{max}^{MeOH} nm (log ε): 279 (1.87), 260 (2.47), 217 (3.85). ^{1}H-NMR (270 MHz, $CDCl_3$, 296 K) δ: 2.03, 2.052, 2.055, 2.06, 2.08 (each 3H, s, CH_3COO), 2.31 (3H, s, CH_3COO), 2.93 (1H, dd, J = 17.3, 8.8 Hz, 3-H_A), 3.15 (1H, dd, J = 17.3, 13.2 Hz, 3-H_B), 3.83 (1H, m, 5′-H), 3.93 (1H, dd, J = 10.3, 4.4 Hz, 11-H_A), 4.00 (1H, s, 8-H), 4.20 (1H, dd, J = 13.0, 3.3 Hz, 6′-H_A), 4.30 (1H, dd, J = 13.0, 6.3 Hz, 6′-H_B), 4.64 (1H, dd, J = 10.3, 7.0 Hz, 11-H_B), 4.67 (1H, dd, J = 13.2, 8.8 Hz, 4-H), 5.15 (1H, dd, J = 7.0, 4.4 Hz, 12-H), 5.22 (4H, m, 1′, 2′, 3′, 4′-H), 7.15 (2H, d, J = 8.4 Hz, 15,17-H), 7.38 (2H, d, J = 8.4 Hz, 14,18-H). *Anal.* Calcd for $C_{33}H_{36}O_{19} \cdot 1/2H_2O$: C, 53.16; H, 5.00. Found: C, 53.35; H, 5.22.

Methanolysis of 1 (Condition A) Compound **1** (70 mg) was refluxed with concentrated HCl–MeOH (1 : 10) (8 ml) for 3 d. The solvent was removed, and the residue was diluted with H_2O and extracted with Et_2O. The Et_2O layer was evaporated to dryness *in vacuo* and the residue was separated by prep-HPLC (ODS-120A, MeOH : H_2O = 2 : 3, 1.2 ml/min) to give **2**, **3** and **8** (10 mg). For structural elucidation, small amounts of **2** and **3** were purified after acetylation. After usual work-up, each crude product was purified by prep-HPLC (Silica-60, hexane : acetone = 3 : 2, 1.25 ml/min) to give **2a** (1 mg) and **3a** (1 mg), respectively. **2a**: An amorphous solid, $[\alpha]_D$ +44.4° (c = 0.1, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1815, 1753, 1606, 1511, 1217, 1138, 797. UV λ_{max}^{MeOH} nm (log ε): 261 (2.72), 217 (3.90). ^{1}H-NMR (270 MHz, $CDCl_3$, 296 K) δ: 2.12, 2.31, 2.32 (each 3H, s, CH_3COO), 2.93 (1H, dd, J = 17.2, 8.9 Hz, 3-H_A), 3.25 (1H, dd, J = 17.2, 12.5 Hz, 3-H_B), 3.87 (1H, dd, J = 12.5, 8.9 Hz, 4-H), 4.13 (1H, dd, J = 10.9, 3.6 Hz, 11-H_A), 4.24 (1H, s, 8-H), 4.60 (1H, dd, J = 10.9, 6.0 Hz, 11-H_B), 5.17 (1H, dd, J = 6.0, 3.6 Hz, 12-H), 7.17 (2H, d, J = 8.6 Hz, 15,17-H), 7.31 (2H, d, J = 8.6 Hz, 14,18-H). CI-MS m/z: 449 $(M+H)^+$. **3a**: An amorphous solid, $[\alpha]_D$ +18.6° (c = 0.1, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1812, 1752, 1606, 1510, 1216, 1131, 797. UV λ_{max}^{MeOH} nm (log ε): 260 (2.69), 217 (3.81). ^{1}H-NMR (270 MHz, $CDCl_3$, 296 K) δ: 2.09, 2.31 (each 3H, s, CH_3COO), 2.93 (1H, dd, J = 17.3, 8.6 Hz, 3-H_A), 3.28 (1H, dd, J = 17.3, 13.6 Hz, 3-H_B), 3.60 (3H, s, 9-OCH_3), 3.94 (1H, s, 8-H), 3.94 (1H, dd, J = 10.6, 4.3 Hz, 11-H_A), 4.08 (1H, dd, J = 13.6, 8.6 Hz, 4-H), 4.57 (1H, dd, J = 10.6, 6.6 Hz, 11-H_B), 5.09 (1H, dd, J = 6.6, 4.3 Hz, 12-H),

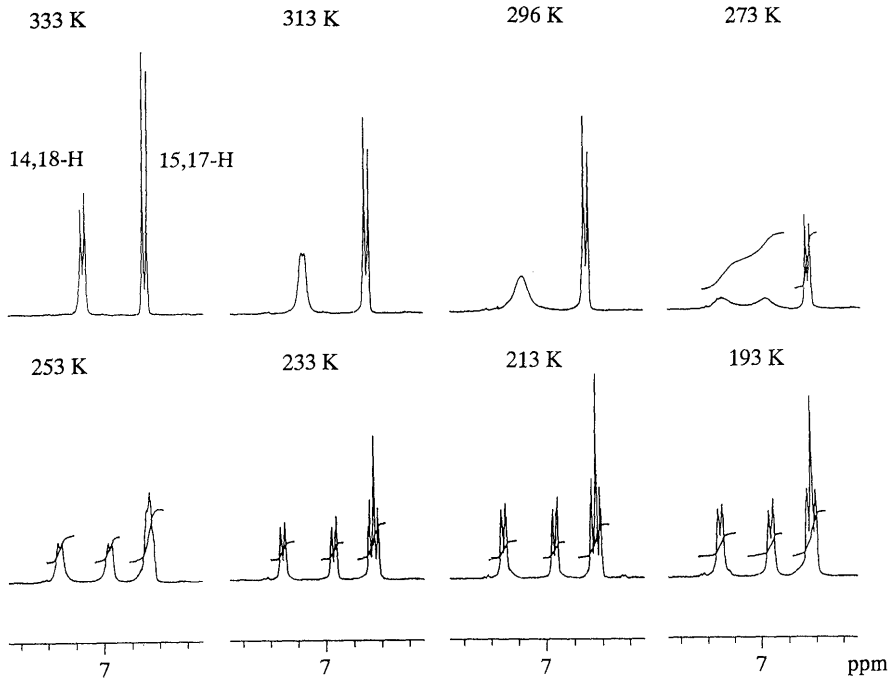


Fig. 2. ^{1}H-NMR Spectra of **4** at 193 K-333 K (CD_3OD, 270 MHz)

7.15 (2H, d, J=8.6 Hz, 15,17-H), 7.41 (2H, d, J=8.6 Hz, 14,18-H). CI-MS m/z: 421 $(M+H)^+$. **8**: An amorphous powder, mp 113 °C, $[\alpha]_D$ −89.4° (c=0.3, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3596, 1733, 1613, 1597, 1569, 1515, 967, 902, 885, 836, 811. UV λ_{max}^{MeOH} nm (log ε): 358 (1.68), 271 (3.96), 222 (3.81). ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.66 (1H, dd, J=16.8, 5.3 Hz, 2-H_A), 3.23 (1H, dd, J=16.8, 10.2 Hz, 2-H_B), 3.61 (3H, s, 10-CH_3), 4.81 (1H, dd, J=10.2, 5.3 Hz, 3-H), 6.56 (1H, dd, J=3.6, 2.0 Hz, 7-H), 6.71 (2H, d, J=8.6 Hz, 12,14-H), 7.13 (2H, d, J=8.6 Hz, 11,15-H), 7.32 (1H, dd, J=3.6, 0.7 Hz, 6-H), 7.71 (1H, dd, J=2.0, 0.7 Hz, 8-H). ^{13}C-NMR (67.8 MHz, CD_3OD, 296 K) δ: 38.2 (C-2), 49.9 (C-3), 52.2 (10-CH_3), 113.5 (C-7), 116.8 (C-12, 14), 120.0 (C-6), 129.8 (C-10), 130.4 (C-11, 15), 148.6 (C-8), 153.3 (C-5), 158.1 (C-13), 174.0 (C-1), 189.8 (C-4). EI-MS m/z: 274 $(M)^+$.

Compound **8** (4 mg) was methylated with ethereal CH_2N_2 to give **9** (2 mg). **9**: An oil. $[\alpha]_D$ −60.0° (c=0.2, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1733, 1673, 1610, 1569, 1512, 1254, 885, 834. UV λ_{max}^{MeOH} nm (log ε): 270 (4.08), 221 (3.98). ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.68 (1H, dd, J=17.0, 5.3 Hz, 2-H_A), 3.24 (1H, dd, J=17.0, 10.3 Hz, 2-H_B), 3.61 (3H, s, 10-CH_3), 3.74 (3H, s, 13-OCH_3), 4.87 (1H, m, 3-H), 6.56 (1H, dd, J=3.6, 1.7 Hz, 7-H), 6.85 (2H, d, J=8.7 Hz, 12,14-H), 7.23 (2H, d, J=8.7 Hz, 11,15-H), 7.33 (1H, dd, J=3.6, 0.7 Hz, 6-H), 7.72 (1H, dd, J=1.7, 0.7 Hz, 8-H). EI-MS m/z: 288 $(M)^+$.

The H_2O layer, after removal of Et_2O under reduced pressure, was neutralized with Amberlite IR-120 (Na^+ form), concentrated *in vacuo* and separated by prep-HPLC (ODS-120A, MeOH : H_2O = 1 : 3, 1.5 ml/min) to give unreacted **1** (10 mg) and **4** (5 mg).

Methanolysis of 1 (Condition B) Compound **1** (70 mg) was refluxed with concentrated HCl–MeOH (1 : 15) (8 ml) for 6 h. The solvent was removed and the residue was diluted with H_2O (10 ml). The reaction mixture was neutralized with Amberlite IR-120 (Na^+ form), concentrated *in vacuo* and separated by silica gel column chromatography. Elution with hexane–acetone (3 : 2) yielded a mixture of **2**, **3**, **5** and **7**. Further elution with MeOH afforded unreacted **1** (20 mg) and **4** (3 mg). The mixture was purified by prep-HPLC (Silica-60, hexane : acetone = 3 : 2, 1.5 ml/min) to give a mixture of **2** and **7** (3 mg), **3** (4 mg) and **5** (1.5 mg). The structures of **2** and **7** were estimated to be as shown in Chart 1 analysis of the ^{1}H-NMR spectrum. Mixture of **2** and **7**: ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.71 (1H, dd, J=15.8, 10.6 Hz, 3-H_A of **7**), 2.86 (1H, dd, J=17.5, 8.6 Hz, 3-H_A of **2**), 3.11 (1H, s, 8-H of **7**), 3.19 (1H, dd, J=17.5, 13.2 Hz, 3-H_B of **2**), 3.30 (1H, dd, J=15.8, 4.9 Hz, 3-H_B of **7**), 3.75 (1H, dd, J=10.6, 4.9 Hz, 4-H of **7**), 3.85 (1H, s, 8-H of **2**), 3.92 (1H, dd, J=9.6, 3.3 Hz, 11-H_A of **7**), 4.02 (1H, dd, J=8.9, 3.0 Hz, 11-H_A of **2**), 4.09 (1H, dd, J=9.6, 5.9 Hz, 11-H_B of **7**), 4.15—4.27 (3H, m, 4-H, 11-H_B and 12-H of **2**), 4.25 (1H, dd, J=5.9, 3.3 Hz, 12-H of **7**), 6.67 (2H, d, J=8.9 Hz, 15,17-H of **7**), 6.67 (2H, d, J=8.9 Hz, 15,17-H of **2**), 7.14 (2H, d, J=8.9 Hz, 14,18-H of **7**), 7.25 (2H, d, J=8.9 Hz, 14,18-H of **2**). **3**: An amorphous powder, mp 263—265 °C, $[\alpha]_D$ −25.5° (c=0.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3569, 1797, 1614, 1596, 1218, 1070, 898, 844. UV λ_{max}^{MeOH} nm (log ε): 275 (3.06), 227 (3.88). ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.90 (1H, dd, J=17.5, 8.6 Hz, 3-H_A), 3.20 (1H, dd, J=17.5, 13.2 Hz, 3-H_B), 3.58 (3H, s, 9-OCH_3), 3.81 (1H, s, 8-H), 3.89 (1H, dd, J=9.5, 3.6 Hz, 11-H_A), 4.17 (1H, dd, J=13.2, 8.6 Hz, 4-H), 4.26 (1H, dd, J=6.5, 3.6 Hz, 12-H), 4.33 (1H, dd, J=9.5, 6.5 Hz, 11-H_B), 6.78 (2H, d, J=8.6 Hz, 15,17-H), 7.24 (2H, d, J=8.6 Hz, 14,18-H). ^{13}C-NMR (67.8 MHz, CD_3OD, 296 K): Table I. EI-MS m/z: 336 $(M)^+$. CD (MeOH) [θ] (nm): -7.8×10^2 (278), $+2.9\times10^4$ (234), -1.4×10^4 (218). **5**: An amorphous solid, $[\alpha]_D$ −5.6° (c=0.1, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3377, 1809, 1733, 1616, 1518, 1232, 1073, 905, 838. UV λ_{max}^{MeOH} nm (log ε): 276 (3.09), 226 (3.93). ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.66 (1H, dd, J=15.9, 9.8 Hz, 3-H_A), 3.06 (1H, s, 8-H), 3.33 (1H, dd, J=15.9, 5.0 Hz, 3-H_B), 3.51 (3H, s, $COOCH_3$), 3.59 (3H, s, 9-OCH_3), 3.74 (1H, dd, J=9.8, 5.0 Hz, 4-H), 3.79 (1H, dd, J=9.3, 3.3 Hz, 11-H_A), 4.20 (2H, m, 11-H_B, 12-H), 6.68 (2H, d, J=8.8 Hz, 15,17-H), 7.11 (2H, d, J=8.8 Hz, 14,18-H). ^{13}C-NMR (67.8 MHz, CD_3OD, 296 K): Table I. EI-MS m/z: 368 $(M)^+$.

Viburnolide B (4) An amorphous powder, mp 145—146 °C, $[\alpha]_D$ −4.3° (c=3.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3395, 1795, 1720, 1620, 1525, 1226, 1158, 1072, 900, 842. UV λ_{max}^{MeOH} nm (log ε): 275 (3.11), 226 (3.87). ^{1}H-NMR (270 MHz), CD_3OD, 296 K) δ: 2.65 (1H, dd, J=15.9, 10.8 Hz, 3-H_A), 3.08 (1H, s, 8-H), 3.30 (1H, dd, J=15.9, 4.4 Hz, 3-H_B), 3.38—3.54 (4H, m, 2′, 3′, 4′, 5′-H), 3.50 (3H, s, $COOCH_3$), 3.72 (1H, dd, J=11.5, 7.7 Hz, 6′-H_A), 3.83 (1H, dd, J=9.1, 3.7 Hz, 11-H_A)3.98 (1H, dd, J=11.5, 2.3 Hz, 6′-H_B), 4.23 (1H, dd, J=6.6, 3.7 Hz, 12-H), 4.30 (1H, dd, J=9.1, 6.6 Hz, 11-H_B), 4.38 (1H, dd, J=10.8, 4.4 Hz, 4-H), 4.80 (1H, d, J=7.6 Hz, 1′-H), 6.69 (2H, d, J=8.7 Hz, 15,17-H), 7.15 (2H, brs, 14,18-H). ^{1}H-NMR (60 MHz, CD_3OD, 296 K) δ: 2.61 (1H, dd, J=15.8, 10.7 Hz, 3-H_A), 3.07 (1H, s, 8-H), 3.32 (1H, m, 3-H_B), 3.40—4.49 (10H, m, 4, 11, 12, 2′, 3′, 4′, 5′, 6′-H), 3.47 (3H, s, $COOCH_3$), 6.66 (2H, d, J=8.5 Hz, 15,17-H), 7.13 (2H, d, J=8.5 Hz, 14,18-H), 1′-H (overlapped). ^{13}C-NMR [(67.8 MHz, CD_3OD, 296 K), (15.0 MHz, CD_3OD, 296 K)]: Table I. CD (MeOH) [θ] (nm): -9.0×10^2 (275.7), $+1.3\times10^4$ (225.2). *Anal.* Calcd for $C_{22}H_{28}O_{14}\cdot1/2H_2O$: C, 50.29; H, 5.56. Found: C, 50.04; H, 5.53.

Acetylation of 4 Compound **4** (8 mg) was acetylated with Ac_2O–pyridine in the usual manner to give **4a** (4 mg). An amorphous powder, mp 142—144 °C, $[\alpha]_D$ 0° (c=0.2, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3501, 1799, 1757, 1654, 1607, 1559, 1438, 1371, 1232, 1041, 910. UV λ_{max}^{MeOH} nm (log ε): 259.5 (2.53), 216 (3.89). ^{1}H-NMR (270 MHz, $CDCl_3$, 296 K) δ:

2.00, 2.01, 2.05, 2.08, 2.10 (each 3H, s, CH_3COO), 2.28 (3H, s, CH_3COO), 2.63 (1H, dd, $J=17.0$, 6.9 Hz, 3-H_A), 2.98 (1H, s, 8-H), 3.48 (1H, dd, $J=17.0$, 6.0 Hz, 3-H_B), 3.57 (3H, s, $COOCH_3$), 3.78 (1H, dd, $J=10.4$, 4.6 Hz, 11-H_A), 3.90 (1H, m, 5'-H), 4.24 (1H, dd, $J=12.2$, 7.2 Hz, 6'-H_A), 4.30 (1H, t, $J=6.9$ Hz, 4-H), 4.45 (1H, dd, $J=12.2$, 2.3 Hz, 6'-H_B), 4.54 (1H, dd, $J=10.4$, 6.9 Hz, 11-H_B), 5.03 (1H, d, $J=8.3$ Hz, 1'-H), 5.07 (1H, dd, $J=6.9$, 4.6 Hz, 12-H), 5.21 (1H, m, 4'-H), 5.31 (2H, m, 2',3'-H), 7.04 (2H, d, $J=8.6$ Hz, 15,17-H), 7.33 (2H, br s, 14,18-H).

Viburnolide C (6) An amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3393, 3200, 1785, 1690, 1625, 1575, 1230, 1158, 1074, 900, 845. ^{1}H-NMR (270 MHz, CD_3OD, 296 K) δ: 2.43 (1H, dd, $J=16.0$, 5.3 Hz, 3-H_A), 3.03 (1H, s, 8-H), 3.22—3.44 (5H, m, 3-H_B, 2', 3', 4', 5'-H), 3.76 (1H, dd, $J=12.0$, 5.4 Hz, 6'-H_A), 3.89 (1H, dd, $J=8.6$, 2.6 Hz, 11-H_A), 3.94 (1H, m, 6'-H_B), 4.19—4.38 (3H, m, 4-H, 11-H_B, 12-H), 6.67 (2H, d, $J=8.6$ Hz, 15,17-H), 7.16 (2H, br s, 14,18-H). 1'-H (overlapped). ^{13}C-NMR (67.8 MHz, CD_3OD, 296 K): Table I. Positive ion FAB-MS m/z: 525 $(M+Na)^+$.

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References and Notes

1) Part VII: K. Machida, M. Kikuchi, *Tohoku Yakka Daigaku Kenkyu Nempo*, **39**, 61 (1992).
2) K. Machida, M. Kikuchi, *Chem. Pharm. Bull.*, **41**, 248 (1993).
3) T. Iwagawa, T. Hase, *Phytochemistry*, **23**, 2299 (1984).
4) A. J. Poss, R. K. Belter, *Tetrahedron Lett.*, **28**, 2555 (1987).
5) We examined the *in vitro* antibacterial activities (the minimum inhibitory concentration values) of **1** toward several bacterial strains gram-positive bacterial strains: *B. subtilis* ATCC 6633, *B. cereus* ATCC 11778, *M. luteus* ATCC 10240, *S. aureus* ATCC 6538. Gram-negative bacterial strains: *Escherichia coli* NIHJ, *E. coli* IID 953, *E. coli* IID 958, *P. aeruginosa* IFO 3456) by the agar dilution method. Compound **1** exhibited no activity against any of the bacteria tested.
6) We have isolated viburnolide A (**1**) from the leaves of *V. dilatatum* THUNB., but dilaspirolactone (**1'**) was not found in this plant.
7) H. Nakanishi, *Kagaku No Ryoiki*, **31**, 226, 374 (1977).
8) J. K. M. Sanders, B. K. Hunter (eds.), "Modern NMR Spectroscopy," Oxford University Press, London, 1987: [Y. Arata, U. Sakata (trans.), "NMR Guidebook," Hirokawa Publishing Co., Tokyo, 1992, pp. 268—336].
9) The methyl ester derivative of **6** was obtained by treatment of **6** with MeOH at room temperature overnight.
10) PA: pulse angle.
11) PR; pulse repetition time.