Three New Diterpenoids from the Fruit of *Vitex agnus-castus*

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Three new labdane-type diterpenoids, viteagnusins F, G, and H, were isolated from the hexane extract of fruit (chasteberry) of *Vitex agnus-castus* L. (Verbenaceae) along with seven known compounds including four labdane-type diterpenoids, one norlabdane-type diterpenoid, one aromadendrane-type sesquiterpenoid, and one flavonoid. The chemical structures of the three new labdane-type diterpenoids were determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Vitex agnus-castus*; Verbenaceae; chasteberry; diterpenoid; labdane; viteagnusin

Vitex agnus-castus L. (Verbenaceae) is a shrub that grows widely throughout Central Asia, the Mediterranean region, and Southern Europe. The fruit (chasteberry) of this plant is used as a dietary supplement for treating the hormone-imbalance syndrome in women.[1)] This fruit has been reported to contain essential oils, iridoids, flavonoids, and diterpenoids.[1—9)] Further, linoleic acid isolated from this fruit was identified as an estrogenic compound.[10)] In a previous paper,[11)] we reported the isolation and structural elucidation of seven diterpenoids from the hexane extract of *V. agnus-castus*. As part of an ongoing study of this plant, we presently describe the isolation and structural characterization of three new labdane-type diterpenoids along with seven known compounds including four labdane-type diterpenoids, one norlabadane-type diterpenoid, one aromadendrane-type sesquiterpenoid, and one flavonoid from the hexane extract.

The hexane extract of the fruit of *V. agnus-castus* was successively subjected to silica gel, Chromatorex octadecyl silica (ODS), and HPLC on ODS to yield 10 compounds (**1**—**10**).

Compounds **4**—**10** were identified as (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,15*R*)-6-acetoxy-9,13;15,16-diepoxy-15-methoxy-labdane (**4**),[12)] (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,15*S*)-6-acetoxy-9,13;15,16-diepoxy-15-methoxy-labdane (**5**),[12)] (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*S*)-6-acetoxy-9,13;15,16-diepoxy-15-methoxy-labdane (**6**),[12)] (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*R*)-6-acetoxy-9,13;15,16-diepoxy-15-methoxy-labdane (**7**),[12)] *iso*-ambreinolide (**8**),[13)] 4β,10β-dihydroxyaromadendrane (**9**),[14)] and 5-hydroxy-3,4′,6,7-tetramethoxyflavone (**10**),[15)] respectively, based on comparison of their physical and spectral data with authentic samples or those already reported (Fig. 1).

Compound **1**, tentatively named viteagnusin F, was obtained as a colorless syrup. The positive FAB-MS of **1** exhibited an $[M+Na]^+$ ion peak at *m/z* 449, and the molecular formula of **1** was determined to be $C_{23}H_{38}O_7$ by high-resolution (HR)-positive FAB-MS. The ^{1}H-NMR spectrum (in $CDCl_3$) of **1** exhibited signals generated by three tertiary methyl groups (δ 1.20, 0.99, 0.97), one secondary methyl group [δ 0.95 (d, J=6.5 Hz)], one methoxy group (δ 3.40), one acetyl group (δ 2.04), and four oxygenated methine protons [δ 5.63 (s), 5.40 (ddd, J=2.5, 2.5, 2.5 Hz), 4.86 (s), 3.66 (s)]. The ^{13}C-NMR spectrum (in $CDCl_3$) of **1** yielded 23 carbon signals including those corresponding to one carbonyl carbon (δ 170.7), two acetal carbons (δ 108.7, 107.1), two oxygenated quaternary carbons (δ 82.8, 74.6), two oxygenated methine carbons (δ 80.1, 71.0), and one methoxy carbon (δ 55.4). These ^{1}H- and ^{13}C-NMR signals were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) techniques, as represented in Tables 1 and 2. Further, the planar structure of **1**—a labdane-type diterpenoid possessing one methoxy group, one acetyl group, two hydroxy groups, one six-membered cyclic acetal group, and one five-membered cyclic acetal group—could be deduced using the above-mentioned techniques, as shown in Fig. 2. In order to confirm an epoxy bridge between C-9 and C-16, ordinary acetylation of **1** with acetic anhydride and pyridine was attempted, and two acetates (**1a**, **1b**) were obtained. The ^{1}H-NMR spectrum of **1a** was compared

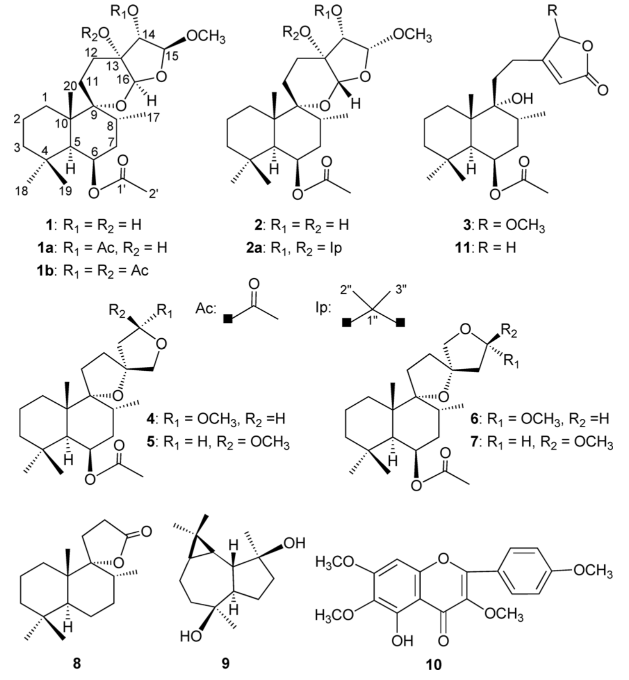

Fig. 1. Structures of **1**—**11**, **1a**, **1b**, and **2a**

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Table 1. ^{1}H-NMR Spectral Data for **1**—**3** (500 MHz)

	1$^{a)}$	**1**$^{b)}$	**2**$^{a)}$	**2**$^{b)}$	**3**
1	*ca.* 1.68	*ca.* 1.97	*ca.* 1.87	*ca.* 2.19	*ca.* 1.54
	ca. 1.31	*ca.* 1.61	*ca.* 1.25	*ca.* 1.34	*ca.* 1.45
2	1.60 dddd (3.0, 3.0, 13.5, 13.5)	*ca.* 1.63	*ca.* 1.59	1.63 m	*ca.* 1.64
	1.49 m	*ca.* 1.46	1.47 m	1.51 m	*ca.* 1.50
3	*ca.* 1.31	*ca.* 1.26	*ca.*1.28	*ca.* 1.29	1.35 br d (13.0)
	ca. 1.17	*ca.* 1.10	1.12 m	*ca.* 1.29	1.17 ddd (3.0, 13.0, 13.0)
5	1.83 d (2.5)	1.97 d (2.5)	1.91 d (3.0)	2.14 d (2.5)	*ca.* 1.57
6	5.40 ddd (2.5, 2.5, 2.5)	5.66 ddd (2.5, 2.5, 2.5)	5.41 ddd (3.0, 3.0, 3.0)	5.64 ddd (2.5, 2.5, 2.5)	5.39 ddd (2.5, 2.5, 2.5)
7	1.87 ddd (2.5, 13.5, 13.5)	*ca.* 2.14	1.91 ddd (3.0, 14.0, 14.0)	2.00 ddd (2.5, 13.5, 13.5)	*ca.* 1.56
	1.37 ddd (2.5, 2.5, 13.5)	*ca.* 1.59	1.56 ddd (3.0, 3.0, 14.0)	1.66 ddd (2.5, 2.5, 13.5)	*ca.* 1.58
8	*ca.* 2.04	*ca.* 2.15	*ca.* 2.08	*ca.* 2.15	2.13 m
11	2.07 ddd (3.5, 14.0, 14.0)	2.46 ddd (3.5, 14.5, 14.5)	*ca.* 1.84	1.92 ddd (5.5, 6.5, 14.0)	1.94 ddd (5.5, 9.0, 15.5)
	1.44 ddd (3.5, 3.5, 14.0)	*ca.* 1.47	1.72 ddd (5.0, 9.0, 14.0)	1.83 ddd (5.0, 9.0, 14.0)	1.75 m
12	2.22 ddd (3.5, 14.0, 14.0)	2.42 ddd (3.5, 14.5, 14.5)	*ca.* 2.06	2.34 ddd (5.5, 9.0, 14.0)	2.52 m
	ca. 1.68	1.90 m	*ca.* 1.81	*ca.* 2.11	2.39 m
14	3.66 s	4.08 s	3.88 d (1.0)	4.39 d (2.0)	5.87 s
15	4.86 s	5.27 s	4.93 d (1.0)	5.43 d (2.0)	
16	5.63 s	6.11 s	5.47 s	5.92 s	5.65 s
17	0.95 d (6.5)	1.14 d (6.0)	1.07 d (7.5)	1.16 d(7.0)	0.90 d(7.0)
18	0.97 s	0.91 s	0.94 s	0.97 s	0.97 s
19	0.99 s	1.06 s	0.98 s	1.06 s	1.01 s
20	1.20 s	1.28 s	1.23 s	1.26 s	1.26 s
2′	2.04 s	2.08 s	2.04 s	2.06 s	2.06 s
OCH_3	3.40 s	3.46 s	3.43 s	3.53 s	3.57 s

δ in ppm from tetramethylsilane (TMS) (coupling constants (*J*) in Hz are given in parentheses). *a*) In $CDCl_3$. *b*) In C_5D_5N.

Table 2. ^{13}C-NMR Data for **1**, **1a**, **1b**, **2**, **2a**, **3** and **11** (125 MHz)

	1$^{a)}$	**1**$^{b)}$	**1a**$^{a)}$	**1b**$^{a)}$	**2**$^{a)}$	**2**$^{b)}$	**2a**$^{a)}$	**3**$^{a)}$	**11**$^{a,c)}$
1	35.5	35.8	35.5	35.3	33.5	33.8	34.1	33.7	33.6
2	18.9	19.3	18.9	19.1	18.9	19.2	18.7	18.6	18.6
3	44.2	44.5	44.3	44.1	44.0	44.3	44.0	43.6	43.6
4	34.2	34.3	34.2	34.2	33.9	34.1	33.8	34.0	34.0
5	47.6	48.0	47.6	47.5	46.5	46.9	46.4	47.7	47.7
6	71.0	70.7	70.9	70.7	70.9	70.7	70.8	69.9	69.8
7	35.9	36.7	36.0	35.9	36.8	37.4	36.9	36.1	36.1
8	34.4	34.8	34.4	34.4	32.8	33.4	32.1	31.9	32.1
9	82.8	82.6	83.0	83.1	81.9	81.9	81.1	76.6	76.5
10	45.9	46.1	45.9	45.7	45.1	45.4	45.2	43.8	43.8
11	21.8	22.6	21.5	21.5	20.3	21.2	19.7	31.2	31.6
12	29.6	29.7	29.9	24.5	30.7	31.1	31.0	24.5	25.4
13	74.6	74.2	74.4	80.6	76.2	75.9	87.9	168.1	171.1
14	80.1	80.6	81.9	78.9	78.8	78.9	88.7	117.8	115.0
15	108.7	109.8	106.4	107.0	109.8	111.0	107.7$^{d)}$	170.4	174.0
16	107.1	107.6	106.9	105.4	105.6	106.5	107.4$^{d)}$	104.4	73.2
17	16.6	17.2	16.6	16.5	19.6	19.9	19.2$^{e)}$	16.0	16.1
18	33.3	33.4	33.4	33.4	33.3	33.3	33.4	33.6	33.6
19	24.1	24.2	24.1	24.0	23.7	23.9	23.7	23.7	23.7
20	18.8	19.0	18.8	18.8	19.1	19.3	19.0$^{e)}$	19.0	19.0
1′	170.7	170.3	170.7	170.5	170.5	170.2	170.5	170.4	170.4
2′	22.0	21.8	22.0	22.0	22.0	21.8	22.0	21.9	21.9
OCH_3	55.4	55.2	55.5	55.7	55.3	55.5	54.5	57.0	
1″							112.6		
2″							27.3		
3″							26.5		
CO			170.5	169.3					
CO				169.1					
$COCH_3$			20.9	21.2					
$COCH_3$				20.7					

δ in ppm from TMS. *a*) In $CDCl_3$. *b*) In C_5D_5N. *c*) Previous report (ref. 16). *d*, *e*) Assignments may be interchangeable.

with that of **1**. A signal due to an acetyl group was observed in the spectrum of **1a**, in addition to the signal at C-6. Furthermore, the signal obtained due to H-14 in **1a** was shifted downfield by 1.01 ppm. The ^{1}H-NMR spectrum of **1b** was compared with that of **1**. The ^{1}H-NMR spectrum of **1b** revealed signals generated by three acetyl groups; however, the acetylation shift was observed only in the case of the signal due to H-14. Further, comparison of the ^{13}C-NMR data of **1b**

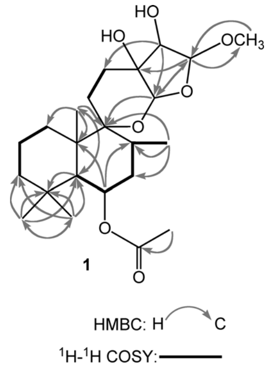

Fig. 2. Partial Structure Elucidated by the ^{1}H–^{1}H COSY Spectrum (Bold Lines) and HMBC Correlations (Arrow) Observed for **1**

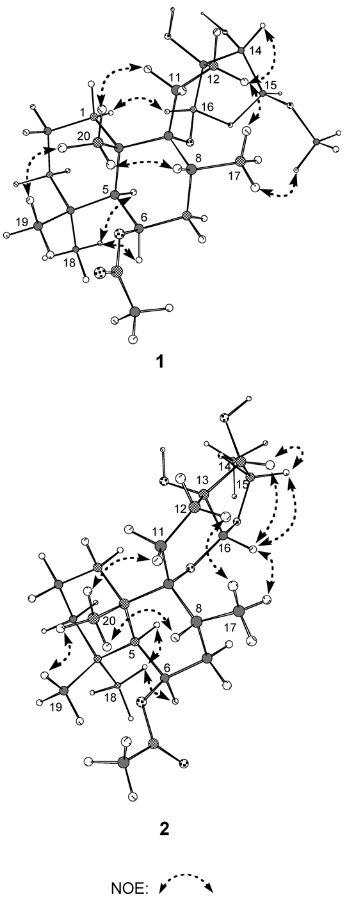

Fig. 3. Key NOE Correlations Observed for **1** and **2**

with those of **1a** revealed significant difference in chemical shift [$\Delta\delta$ (δ**1b**$-\delta$**1a**)]: -5.4 (C-12), $+6.2$ (C-13), -3.0 (C-14), and -1.5 (C-16) ppm. Thus, the structures of **1a** and **1b** were defined as 14-*O*-acetyl and 13,14-di-*O*-acetyl derivatives of **1**, respectively. Furthermore, the presence of an epoxy bridge between C-9 and C-16 was established.

On the basis of nuclear Overhauser and exchange spectroscopy (NOESY) spectra in $CDCl_3$ and C_5D_5N, the relative stereochemistry in **1** was defined. In the NOESY spectra of **1**, the key nuclear Overhauser effects (NOEs) were observed between Hb-1 and H-16; H-5 and H_3-18; H-6 and H_3-18; H-8 and H_3-20; Ha-11 and H_3-20; Ha-12 and H-14; Ha-12 and H_3-17; H_3-17 and OCH_3; H_3-19 and H_3-20 (Fig. 3). These correlations suggested that the configurations at C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-15, and C-16 were *S**, *R**, *R**, *R**, *S**, *R**, *S**, *S**, and *R**, respectively. Thus, the structure of **1** was concluded to be (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,14*S*,15*S*,16*R*)-6-acetoxy-9,16;15,16-diepoxy-13,14-dihydroxy-15-methoxy-labdane (Fig. 1).

Compound **2**, tentatively named viteagnusin G, was obtained as a colorless syrup, and its positive FAB-MS indicated the same $[M+Na]^+$ ion peak as that of **1**. Further, the ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**. These NMR signals were assigned with the help of 2D-NMR techniques as in the case of **1**, and the planar structure of **2** was determined to be the same as that of **1**. The stereostructure of **2** was characterized on the basis of the NOESY and difference NOE spectra of **2**, in which the key NOEs were observed between H-5 and H_3-18; H-6 and H_3-18; H-8 and H_3-20; Ha-11 and H_3-20; H-14 and H-15; H-14 and H-16; H-15 and H-16; H-16 and H_3-17; H_3-19 and H_3-20 (Fig. 3). From these data, the configurations at C-5, C-6, C-8, C-9, C-10, C-14, C-15, and C-16 were determined to be *S**, *R**, *R**, *R**, *S**, *S**, *R**, and *S**, respectively. However, the configuration at C-13 could not be elucidated. Finally, the configuration at C-13 in **2** was confirmed by the following evidence. Treatment of **2** with 2,2-dimethoxypropane and pyridium *p*-toluene sulfonic acid afforded corresponding 13,14-diol acetonide (**2a**); this indicated that the hydroxy groups at C-13 and C-14 were *cis*-configuration. Therefore, the structure of **2** was concluded to be (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,14*S*,15*R*,16*S*)-6-acetoxy-9,16;15,16-diepoxy-13,14-dihydroxy-15-methoxy-labdane (Fig. 1).

Compound **3**, tentatively named viteagnusin H, was obtained as a colorless syrup. The HR-positive FAB-MS indicated the molecular formula of **3** to be $C_{23}H_{36}O_6$. The ^{13}C-NMR spectrum of **3**, which contained signals corresponding to two carbonyl carbons (δ 170.4, 168.1), one tri-substituted double bond (δ 170.4, 117.8), one acetal carbon (δ 104.4), one oxygenated quaternary carbon (δ 76.6), one oxygenated methine carbon (δ 69.9), and one methoxy carbon (δ 57.0), displayed 23 carbon signals. The ^{1}H-NMR spectrum of **3**, which was similar to those of **1** and **2**, indicated the signals generated by three tertiary methyl groups (δ 1.26, 1.01, 0.97), one secondary methyl group [δ 0.90 (d, $J=7.0$ Hz)], one acetyl group (δ 2.06), one methoxy group (δ 3.57), two oxygenated methine protons [δ 5.87 (s), 5.39 (ddd, $J=2.5$, 2.5, 2.5 Hz)], and one olefinic proton [δ 5.63 (s)]. The NMR signals were examined in detail, and the planar structure of **3** was characterized as in the case of **1**. The assigned NMR data were imposable on those of vitexilactone (**11**)[16]; however, with the exception to the signals generated by one methoxy group and one acetal proton appeared, and the signals generated by one oxygenated methylene group disappeared. In particular, the ^{13}C-NMR signals generated by C-1—C-11 and C-17—C-20 were almost superimposable. In addition, NOEs were observed between H-5 and H_3-18; H-6 and H_3-18; H-8 and H_3-20; H_3-19 and H_3-20. Consequently, **3** was elucidated to be (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-16-methoxy-13(14)-labden-15,16-olide (Fig. 1). However, the configuration of the methoxy group at C-16 was not determined.

Compounds **1**, **2**, and **3** are new diterpenoids, and the isolation of **4**—**9** from *V. agnus-castus* has been described here for the first time. However, the absolute configurations of **1**—**3** have not yet been confirmed.

Experimental

All instruments and materials used were the same as cited in previous reports[17] unless otherwise specified.

Plant Material The fruit of *Vitex agnus-castus* L. was purchased in May 2006 from Charis Seijo Co., Ltd., a commercial supplier of herbs in Tokyo, Japan and identified by Prof. Toshihiro Nohara, Faculty of Pharmaceutical Sciences, Sojo University. A voucher specimen has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Extraction and Isolation The powdered fruit of *V. agnis-castus* (1994 g) was percolated with hexane, acetone, and MeOH at room temperature, and each solvent was removed under reduced pressure to yield hexane extract (188.4 g), acetone extract (36.9 g), and MeOH extract (113.4 g), respectively. The hexane extract was subjected to silica gel column chromatography (Merck, Art. 1.07734) with a gradient of mixtures of hexane–acetone (20 : 1, 10 : 1, 3 : 1, 1 : 1, 0 : 1) to yield fractions 1—9. Fraction 4 (11.0 g) was successively subjected to Chromatorex ODS column chromatography (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, and 100% MeOH) and HPLC [column 1 (COSMOSIL 5C18 AR-II, 250 mm× 20 mm i.d., Nacalai Tesque Inc.), 80% MeOH] to yield **8**. Fraction 7 (20.6 g) was chromatographed on Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, and 100% MeOH) to yield **10** (28 mg) and fractions 7.1—7.11. The successive chromatography of fraction 7.5 (2927 mg) over silica gel column [Merck, Art. 1.09385, hexane–acetone (20 : 1, 15 : 1, 10 : 1, 5 : 1, 3 : 1, 1 : 1, 0 : 1)] and HPLC (column 1, 80% acetonitorile) gave **9** (2 mg), **2** (13 mg), **1** (23 mg), and **3** (4 mg). Fraction 7.6 (4915 mg) was successively chromatographed over silica gel column [Merck, Art. 1.09385, hexane–acetone (20 : 1, 15 : 1, 10 : 1, 5 : 1, 3 : 1, 1 : 1, 0 : 1)], Chromatorex ODS column (70% MeOH, 80% MeOH, 90% MeOH, and 100% MeOH) and HPLC (column 1, 80% MeOH) to yield **4** (23 mg), **6** (8 mg), **7** (16 mg), and **5** (18 mg).

Viteagnusin F (**1**): Colorless syrup, $[\alpha]_D^{21}$ +14.8° (c=2.6, $CHCl_3$). Positive FAB-MS *m/z*: 449 $[M+Na]^+$, HR-FAB-MS *m/z*: 449.2523 (Calcd for $C_{23}H_{38}O_7Na$: 449.2516). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Viteagnusin G (**2**): Colorless syrup, $[\alpha]_D^{28}$ −33.7° (c=5.6, acetone). Positive FAB-MS *m/z*: 449 $[M+Na]^+$. HR-FAB-MS *m/z*: 449.2461 (Calcd for $C_{23}H_{38}O_7Na$: 449.2516). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Viteagnusin H (**3**): Colorless syrup, $[\alpha]_D^{21}$ −24.3° (c=0.4, $CHCl_3$). Positive FAB-MS *m/z*: 331 $[M+Na]^+$, HR-FAB-MS *m/z*: 331.2615 (Calcd for $C_{20}H_{36}O_2Na$: 331.2613). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Acetylation of 1 Compound **1** (13 mg) in Ac_2O–pyridine (1 : 1, 1 ml) was left to stand at room temperature for 12 h. The solvent was removed under a N_2 stream to afford a residue, which was subjected to HPLC (column 1, 70% MeOH) to yield **1a** (5 mg) and **1b** (2 mg).

1a: Colorless syrup, ^{1}H-NMR spectral data (in $CDCl_3$, 500 MHz) δ: 5.63 (1H, s, H-16), 5.41 (1H, ddd, J=2.5, 2.5, 2.5 Hz, H-6), 4.86 (1H, s, H-15), 4.67 (1H, s, H-14), 3.41 (3H, s, OCH_3), 2.16 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 1.20 (3H, s, H_3-20), 0.99 (3H, s, H_3-19), 0.97 (3H, s, H_3-18), 0.96 (3H, d, J=6.5 Hz, H_3-17). ^{13}C-NMR spectral data: see Table 2.

1b: Colorless syrup, ^{1}H-NMR spectral data (in $CDCl_3$, 500 MHz) δ: 5.82 (1H, s, H-16), 5.42 (1H, ddd, J=2.5, 2.5, 2.5 Hz, H-6), 5.23 (1H, s, H-14), 4.78 (1H, s, H-15), 3.41 (3H, s, OCH_3), 2.08 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 1.20 (3H, s, H_3-20), 1.00 (3H, s, H_3-19), 0.98 (3H, s, H_3-18), 0.96 (3H, d, J=6.5 Hz, H_3-17). ^{13}C-NMR spectral data: see Table 2.

Preparation of Acetonide (2a) of 2 2,2-Dimethoxypropane (30 μl) and pyridium *p*-toluene sulfoic acid (13 mg) were added to a solution of **2** (10 mg) in DMF (0.5 ml) under N_2 atmosphere, and the mixture was stirred at room temperature for 48 h. The solvent was removed *in vacuo*, and the residue was subjected to silica gel column chromatography (Merck, Art. 1.09385) with a gradient of mixtures of hexane–EtOAc (10 : 1, 3 : 1) to yield **2a** (7 mg) and **2** (3 mg).

2a: Colorless syrup, ^{1}H-NMR spectral data (in $CDCl_3$, 500 MHz) δ: 5.54 (1H, s, H-16), 5.41 (1H, ddd, J=2.5, 2.5, 2.5 Hz, H-6), 5.02 (1H, s, H-15), 4.13 (1H, s, H-14), 3.40 (3H, s, OCH_3), 2.03 (3H, s, H_3-2′), 1.94 (1H, d, J= 2.5 Hz, H-5), 1.43 (3H, s, H_3-2″), 1.36 (3H, s, H_3-3″), 1.25 (3H, s, H_3-20), 1.07 (3H, d, J=7.0 Hz, H_3-17), 0.98 (3H, s, H_3-19), 0.94 (3H, s, H_3-18). ^{13}C-NMR spectral data: see Table 2.

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